Stress effects on memory and emotion:

genetic differences in resilience and vulnerability

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Abbreviations

2-AG	2-arachidonoylglycerol
ACC	Anterior cingulate cortex
ACTH	Adrenocorticotropic hormone
ADRA2B	Adrenoceptor alpha 2B gene
AEA	*N*-arachidonyl ethanolamine (anandamide)
AR	Adrenergic receptor
AVP	Arginine vasopression
BLA	Basolateral amygdala
BMI	Body mass index
BOLD	Blood oxygenation level-dependent
CA	Cornu ammonis
CB1R	Cannabinoid receptor type 1
CNR1	Cannabinoid receptor type 1 gene
COMT	Catechol-O-methyltransferase
CRH	Corticotropin releasing hormone
d'	D-prime
DG	Dentate gyrus
dm	Dorsomedial
DNA	Deoxyribonucleic acid
DRD2	Dopamine receptor D2
EC	Entorhinal cortex
eCB	Endocannabinoid
EEG	Electroencephalography
ERP	Event-related potential
FAAH	Fatty acid amide hydrolase
fMRI	Functional magnetic resonance imaging
FRN	Feedback-related negativity
FWE	Family-wise error
GABA	Gamma aminobutyric acid

GC	Glucocorticoid
GR	Glucocorticoid receptor
GWAS	Genome wide association study
HD	Huntington's disease
HPA	Hypothalamus-pituitary-adrenal
IAPS	International affective picture system
MAGL	Monoacyglycerol lipase
MDBF	German mood questionnaire
mPFC	Medial prefrontal cortex
MR	Mineralocorticoid receptor
mRNA	Messenger ribonucleic acid
MTL	Medial temporal lobe
NA	Noradrenaline
NCBI	National center for biotechnology information
NR3C1	Nuclear receptor subfamily 3 group C member 1, glucocorticoid receptor gene
NR3C2	Nuclear receptor subfamily 3 group C member 2, mineralocorticoid receptor gene
PCL	Probabilistic classification learning
PD	Parkinson's disease
PFC	Prefrontal cortex
PPI	Psycho-physiological interaction
PTSD	Posttraumatic stress disorder
PVN	Paraventricular nucleus
ROI	Region of interest
SAM	Sympatho-adrenal medullary
SD	Standard deviation
SEM	Standard error of measurement
SNP	Single nucleotide polymorphism
S-R	Stimulus-response
SVC	Small volume correction

TR	Repetition time
TE	Echo time
TSST	Trier social stress test
vl	Ventrolateral
vm	Ventromedial
WPT	Weather prediction task

Glossary of terminology

Agonist	Pharmacological agent that binds to and activates a receptor, thereby increasing its natural response.
Allele	One of two variants from a gene that is located on a specific position on the chromosome. Two alleles represent the genotype of a specific gene, which can be homozygous (identical alleles) or heterozygous (different alleles).
Antagonist	Pharmacological agent that binds to and blocks a receptor, thereby reducing its natural response.
Chromatin	Substance of a nucleus that forms chromosomes during cell division and that is composed of DNA, RNA and proteins.
Consolidation	In memory, a stabilization process that helps to transfer information from short-term to long-term storage.
D-prime (d')	Sensitivity index from Signal Detection theory, which in memory research reflects the difference between hits (correct identification of an old item) and false alarms (correct rejection of a new item) and thus takes into account response biases.
Emotion regulation	Implementation of strategies that influence the intensity, duration and type of emotion experienced.
Encoding	In memory, conversion of perceived information into a structure that can be stored in the brain.
Endophenotype	Biological marker or intermediate phenotype that reflects an underlying phenotype with a clear genetic connection and is distinct from behavioral symptoms. The overt behavioral symptom in schizophrenia for example may be psychosis, but the underlying endophenotype may be a lack of sensory gating.

Epigenetics	Field of research that investigates changes in gene expression caused by gene × environment interactions, such as DNA methylation.
Epistasis	Gene × gene interactions, in which the phenotypic effect of one gene is dependent on the effects of one or more other genes. Epistatic statistics by definition are engaged with statistical deviation from additive genetic interaction effects.
Extinction	The process underlying exposure therapy used for treating fear-related disorders. A conditioned response to a stimulus previously paired with an aversive outcome is extinguished after the stimulus is repeatedly presented in absence of the aversive outcome.
Haplotype	Combinations of alleles that are inherited together.
Hardy-Weinberg equilibrium	Principle stating that the genetic variation in a population remains constant over generations when undisturbed by mutations, natural selection among others.
Heteroreceptor	Receptor that regulates the synthesis and/or release of neurotransmitters other than its own ligand.
Inverse agonist	Pharmacological agent that binds to the same receptor as an agonist but that induces the opposite response, thereby decreasing its activity.
Linkage disequilibrium	Nonrandom association of different alleles at nearby locations on the chromosome that together form a haplotype are said to be in high linkage disequilibrium.
Long-term potentiation	Process of strengthening between synapses thought to be one of the main mechanisms underlying learning and memory.
Phenotype	Physical appearance of a specific trait, such as eye color.

Pleiotropic	Pleiotropic in this case means that neurotransmitter, such as endocannabinoids, have multiple diverse effects. Endocannabinoids, for example, affect synthesis and release of GABA, glutamate, acetylcholine, noradrenaline and serotonin. Originally, pleiotropy denotes a gene which has several phenotypic effects.
Reconsolidation	The process of anew consolidation of memories that, after reactivation, have entered a labile state, in which they are susceptible to modulation.
Retrieval	In memory, the process of recovering information stored in memory.
Single Nucleotide Polymorphism	A difference in DNA sequence in which a nucleotide (A, T, C, G) differs between individuals.
Synonymous substitution	Substitution of a base pair in an exon of a gene coding for a protein in a way that does not change the amino acid sequence.

Publications

This dissertation is based on research experiments that have been published or are under revision in peer-reviewed journals. These research articles have been attached in the following order.

Appendix A

Wirz L, Wacker J, Felten A, Reuter M, Schwabe L (2017) A deletion variant of the alpha2b-adrenoceptor modulates the stress-induced shift from “cognitive” to “habit” memory. *J Neurosci* 37:2149-2160.

Appendix B

Wirz L, Reuter M, Wacker J, Felten A, Schwabe L (2017b) A haplotype associated with enhanced mineralocorticoid receptor expression facilitates the stress-induced shift from "cognitive" to "habit" learning. *eNeuro* 5:e0359-17.2017.

Appendix C

Wirz L, Reuter M, Felten A, Schwabe L (*under revision*) An endocannabinoid receptor polymorphism modulates affective processing under stress. *Soc Cogn Affect Neurosci*.

Im Grunde weiss jeder Mensch recht wohl, dass er nur einmal,
als ein Unicum, auf der Welt ist und dass kein noch so seltsamer Zufall
zum zweiten Mal ein so wunderlich buntes Mancherlei zum Einerlei,
wie er es ist, zusammenschütteln wird.

Friedrich Nietzsche

Unzeitgemäße Betrachtungen - Schopenhauer als Erzieher, 1874

Abstract

A stressful encounter sets a variety of processes in motion, all of which act on behalf of adaptive responding to the stressor. Importantly, individual differences influence these processes, contributing to relative resilience and vulnerability. In two large and independent experiments, we investigated genetic differences in stress effects on affective processing and memory. Carriers of an α2B-adrenergic receptor gene variant associated with enhanced noradrenaline availability (ADRA2B deletion) showed enhanced amygdala-hippocampus coupling, preventing a stress-induced shift toward dorsal striatum habit memory as indicated by reduced amygdala-dorsal striatum connectivity. A haplotype containing mineralocorticoid receptor gene variants associated with high receptor functionality facilitated a stress-induced shift toward the dorsal striatum, an effect mediated by reduced hippocampus activity and amygdala-hippocampus connectivity. During negative picture encoding, carriers of a cannabinoid receptor type 1 gene variant that may protect against dysfunctional affective processing, showed enhanced ventromedial prefrontal cortex activity under stress and stronger ventrolateral prefrontal cortex-amygdala coupling under no stress, a sign of appropriate regulation of negative affect. They also showed a positive correlation of the pattern of activation (hippocampus, insula, amygdala) and functional connectivity (hippocampus-basolateral amygdala) during negative picture encoding with memory performance 24 hours later, a mechanism which may be important for appropriate memory consolidation. Taken together, these findings suggest that in response to stress, enhanced noradrenergic activation of the amygdala promotes engagement of the hippocampus which may lead to overly strong encoding of the stressful experience, whereas enhanced mineralocorticoid receptor functioning promotes a shift toward the dorsal striatum in order to save cognitive resources and prevent performance deficits. Additionally, augmented endocannabinoid signaling may promote regulation of negative affect and appropriate incorporation of the emotional learning experience into autobiographical memory. These results suggest that engagement of prefrontal regions facilitates appropriate emotional responding and that a shift toward the dorsal striatum is an adaptive response to rescue performance. Therefore, the ADRA2B non-deletion, mineralocorticoid receptor haplotype and CNR1 polymorphism may confer some degree of resilience against stress-related psychopathologies.

1. General introduction

As different as humans can be, as diverse are their reactions to stress. Considering that stress affects every aspect of our being, including how we think (cognition), how we learn (memory), how we feel (emotion) and how we act (behavior), investigating individual differences is essential. More importantly, these individual differences determine whether someone will or will not be at risk of developing a psychiatric disorder in response to a highly aversive or chronically reoccurring stressor. In other words, some traits will make an individual more vulnerable, whereas others will increase resilience. These traits can be genetically determined, environmentally shaped, or – as will be discussed in chapter 4.2.3 – epigenetically (gene × environment interactions) controlled. In addition to providing answers to clinically relevant questions, researching individual differences will greatly expand our knowledge about the underlying brain systems that support certain functions, as well as the hormones and neurotransmitters and their respective receptors that are involved when these systems are challenged, for example by stress.

This dissertation investigated individual differences in highly relevant candidate genes and how they influence stress effects on memory and affective processing. Using electroencephalography (EEG) and functional magnetic resonance imaging (fMRI), the effects of single nucleotide polymorphisms (SNPs, changes of single nucleotides) in the genes coding for the α_{2B}-adrenergic receptor (AR; ADRA2B gene deletion) and the mineralocorticoid receptor (MR; Nuclear receptor subfamily 3 group C member 2 (NR3C2) gene haplotype) on the engagement of multiple memory systems after stress were examined. Additionally, the neural underpinnings of the influence of a SNP in the CNR1 gene coding for the cannabinoid receptor type 1 (CB1R) on affective processing in response to stress were investigated. In the following, I review relevant theories and empirical studies that laid the groundwork for these experiments. First, the concept of stress and its relevance for our health and well-being is introduced. Second, I elucidate the physiological effects of stress with a particular focus on molecular stress mediators (hormones and neurotransmitters), their receptors and where, when and how in the brain they exert their actions. Third, research on the effects of stress on affective processing, episodic memory und multiple memory systems is reviewed. Finally, I introduce the concept of individual differences, heritability and the SNPs that we investigated.

1.1 Stress in health and cognition

Stress is such a ubiquitous phenomenon in our daily lives that every woman and every man of any culture will be able to relate to its concept. While its cause – the stressor – as well as its severity and frequency of occurrence may differ, stress is generally understood as a state in which we evaluate something as aversive (stressful) and exceeding our currently available coping resources, a concept brought forward by Lazarus and Folkman in their transactional stress model (Lazarus, 1966; Lazarus and Folkman, 1984). The resulting imbalance leads to the initiation of an adaptive (stress) response with the goal of restoring homeostasis. In case of a highly traumatic event or chronically reoccurring stressors, this originally adaptive response can go awry, making us sick eventually. A highly elaborated model of the physiological reaction to stress and how it may cause disease has been proposed by Selye already in 1950 and developed further ever since. As defined by McEwen (1998, 2004), it is allostatic load, the wear and tear on the body resulting from a prolonged or constantly recurring initiation of the stress response, which can lead to disease.

A myriad of intelligently designed studies has since provided evidence for the multitude of cognitive and affective processes that are affected by stress. Higher-order cognitive functions, allowing for the flexible regulation of behavior and depending on prefrontal brain areas, seem to be particularly sensitive to the impairing effects of stress (Arnsten, 2009, 2015). These include inhibition, working memory and cognitive flexibility, the core executive control functions. Inhibition, a function dependent on the right inferior (ventral) prefrontal cortex (PFC; Aron et al., 2004), can be further subdivided into the ability to inhibit inappropriate motor actions and the cognitive inhibition of distracting thoughts and stimuli, which aids attentional focusing. Whereas the former was shown to be facilitated by acute stress exposure, an effect that disappeared following MR blockade (Schwabe et al., 2013a), stress had impairing effects on the latter. Specifically, stress disrupted selective attention by reducing attention allocation to task-relevant stimulus features, an effect reflected by increased error rates and decreased brain potentials related to top-down guided attention (Sänger et al., 2014). Similarly, chronic stress exposure compromised attentional control and frontoparietal network connectivity known to be important for attentional shifting (Liston et al., 2009). As is evident from the concordant results of a multitude of studies and the conclusion of a recent meta-analysis, an even clearer picture emerges for the influence of stress on working memory and set-shifting/cognitive flexibility. Consistently, working memory, defined as the ability to maintain and actively manipulate information in short-term

memory (Baddeley, 1992; D'Esposito and Postle, 2015), has been shown to be impaired by stress (for a review see Arnsten, 2009, 2015); for a meta-analysis see Shields et al., 2016). Interestingly, stress-induced working memory deficits could be prevented by stimulation of the dorsolateral PFC, emphasizing the causal role of this brain region and the potential therapeutic benefits of brain stimulation (Bogdanov and Schwabe, 2016). Similarly to working memory, stress also impaired performance on tasks requiring cognitive flexibility, the ability to detect change (e.g. in task rules) and to adapt our behavior accordingly (Alexander et al., 2007; Plessow et al., 2011; Plessow et al., 2012; Goldfarb et al., 2017).

In contrast to the impairing effects of stress on such higher-order cognitive functions, processes supported by the amygdala such as vigilance, threat detection and more reflexive behaviors are enhanced in response to stress, likely because they are highly relevant in the face of adversity (Hermans et al., 2014). Stress seems to shift the organism into a hypervigilant state, promoting stimulus-driven attentional mechanisms that prepare us for quick detection of salient stimuli in our environment (van Marle et al., 2009). Most importantly, enhanced vigilance and threat detection mechanisms facilitate the processing of emotionally relevant information (Weymar et al., 2012) and shift the organism into a memory formation mode (Smeets et al., 2008), leading to a stress-induced memory enhancement, a phenomenon that has frequently been replicated (for reviews see Cahill and McGaugh, 1998; McGaugh, 2000; Roozendaal et al., 2009).

What most likely drives these stress-induced changes in cognitive and affective processes, is a reconfiguration of brain networks that promotes vigilance and enhanced memory formation, processes supported by the salience network, but that impairs cognitive functions known to rely on the executive control network (Hermans et al., 2011; McEwen et al., 2015; van Oort et al., 2017).

1.2 The stress system: stress mediators and their receptors

An acute stressor sets highly coordinated and manifold operations in motion, some of which work in parallel, whereas others follow stringently timed sequences (Joëls and Baram, 2009). The goal of this evolutionary and biologically highly conserved stress response is to deal with the challenges that we encounter in our daily lives. *In vitro* as well as *in vivo* rodent studies have uncovered some of the mechanisms by which stress, in a brain region-dependent manner, exerts its effects (Ulrich-

Lai and Herman, 2009; Karst and Joels, 2016). Acute stress leads to the secretion of a broad range of monoamines, neuropeptides and steroids (Joëls and Baram, 2009). In this dissertation, I will focus on noradrenaline (NA), glucocorticoids (GCs; cortisol in humans, corticosterone in rodents), endocannabinoids (eCBs) and their respective receptors. Their actions are realized within different stress response phases that interact and partially overlap. Broadly, they consist of (1.) an initial and rapid arousal phase mainly mediated by NA and corticotropin releasing hormone (CRH), (2.) a slightly delayed non-genomic and mostly excitatory phase driven by GCs which interact with and potentiate noradrenergic signaling and (3.) an even further delayed stress response phase in which genomic GC actions reduce neuronal excitation and restore the brain's homeostasis. An additional stress mediator which has received much less attention but is highly important for the realization of GC effects, is the eCB system.

1.2.1 The rapid noradrenergic stress response

In response to threat, independent of whether it poses a real or just a potential danger, within seconds the first stress response phase is initiated. Set in motion by the hypothalamus, the sympatho-adrenal medullary (SAM) system through its sympathetic neuronal projection from the spinal cord to the adrenal medulla, induces several effects that are characteristic of the 'fight or flight' response. Both adrenergic release from the adrenal medulla and noradrenergic secretion from sympathetic nerves are rapidly enhanced, thereby accelerating heart rate, blood pressure, respiratory rate, sweat production and energy mobilization (Ulrich-Lai and Herman, 2009; **Figure 1**).

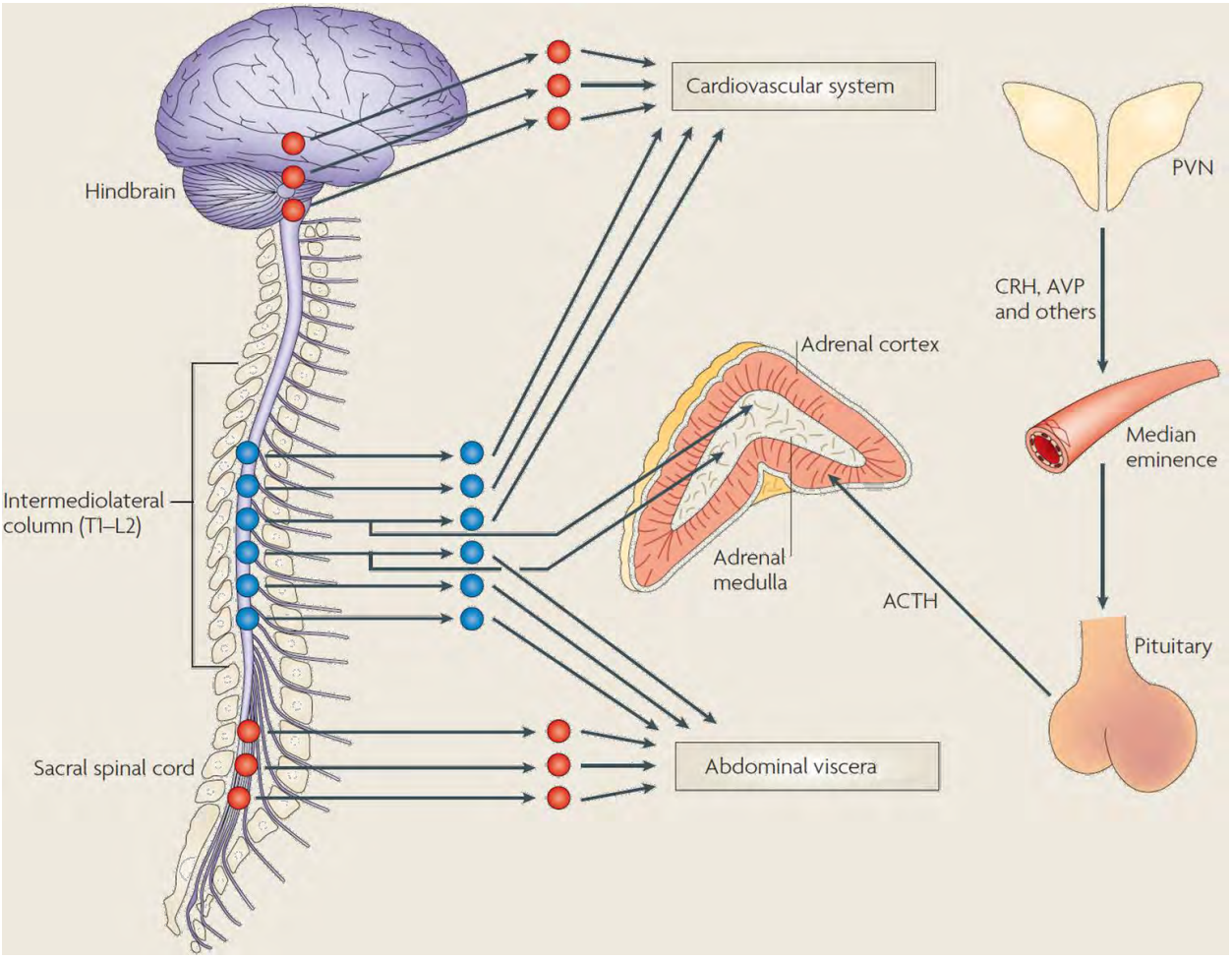


Figure 1. Sympatho-adrenal medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes activation during stress. Stress activates sympathetic neurons in the spinal cord, which ultimately results in increases in adrenaline from the adrenal medulla, noradrenaline (NA) from sympathetic nerves, heart rate, vasoconstriction and energy mobilization. Stress-induced release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from neurons in the paraventricular nucleus (PVN) of the hypothalamus into the median eminence act on the anterior pituitary to promote the secretion of adrenocorticotropic hormone (ACTH), which acts on the adrenal cortex to initiate the synthesis and release of glucocorticoids (GCs; from Ulrich-Lai and Herman, 2009).

Since adrenaline and NA cannot cross the blood-brain-barrier, they stimulate the vagus nerve, which in turn increases NA levels in the brain through its actions on brain stem nuclei, most importantly the nucleus of the solitary tract and the locus coeruleus (LC). Generally, α-ARs are widely expressed throughout the brain with particularly high levels in the brain stem (especially the LC), followed by the cerebral cortex, hypothalamus, hippocampus and amygdala (McCune et al., 1993; Scheinin et al., 1994; Day et al., 1997; **Figure 2**). The β-AR plays a vital role for the cardiac stress response but is also highly expressed and known for its memory-enhancing function in the hippocampus (Roozendaal et al., 2009; O'Dell et al., 2015), followed by a somewhat lower expression in the cerebellum, thalamus, basal ganglia and cerebral cortex (Reznikoff et al., 1986; **Figure 2**).

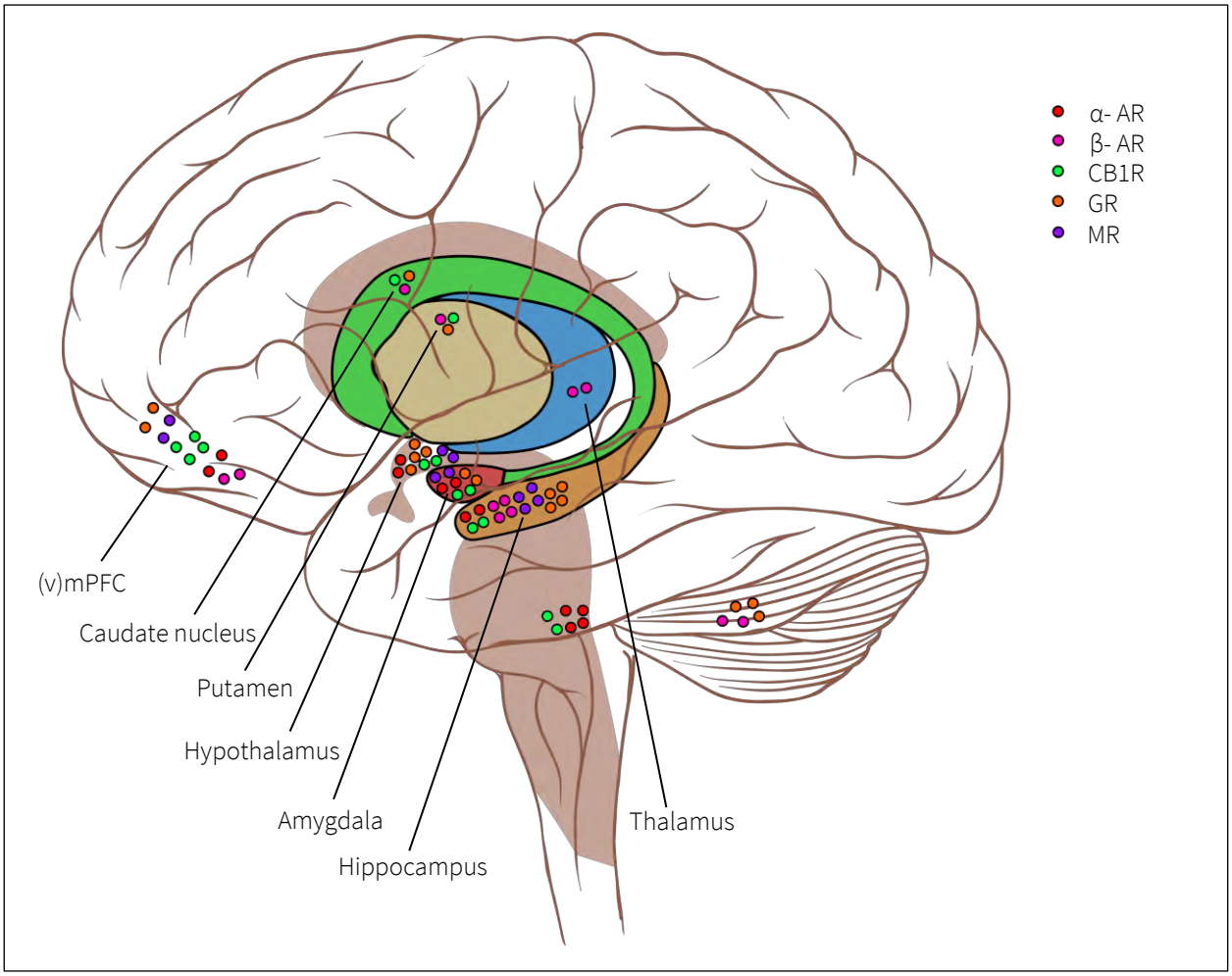


Figure 2. Schematic presentation of receptor distributions in the human brain as suggested by rodent studies. The adrenergic receptors (ARs) of the family α are highly expressed in the brain stem (locus coeruleus (LC)), followed by the cerebral cortex, hypothalamus, hippocampus and amygdala. Beta-ARs are highly expressed in the hippocampus, followed by the cerebellum, thalamus, basal ganglia and cerebral cortex. The cannabinoid CB1 receptor (CB1R) is prominently expressed in the forebrain, particularly in the (ventro)medial prefrontal cortex (v)mPFC but also in the brainstem, basal ganglia, hippocampus, amygdala and hypothalamus. The mineralocorticoid receptor (MR) is mainly restricted to limbic regions with high levels in the hippocampus and moderate levels in the amygdala, hypothalamus and PFC. In contrast, the glucocorticoid receptor (GR) is more widely expressed, with particularly high levels in the hypothalamus, especially in the PVN.

Importantly but beyond the purpose of this dissertation, regional and functional differences exist between the α- and β-AR subtypes. Under baseline conditions, NA follows a circadian rhythm (de Boer and van der Gugten, 1987) and mainly high-affinity α_2-ARs are occupied at moderate NA levels (**Figure 3A**), promoting PFC-dependent processes (Arnsten, 2009). In response to stress, however, NA binds to G-protein coupled α1- and β-ARs (**Figure 3B**), which, since it does not involve gene transcription, allows for rapid stimulation of limbic brain regions such as the amygdala, thereby increasing hypervigilance and attention to salient and relevant features in the environment (Hermans et al., 2014).

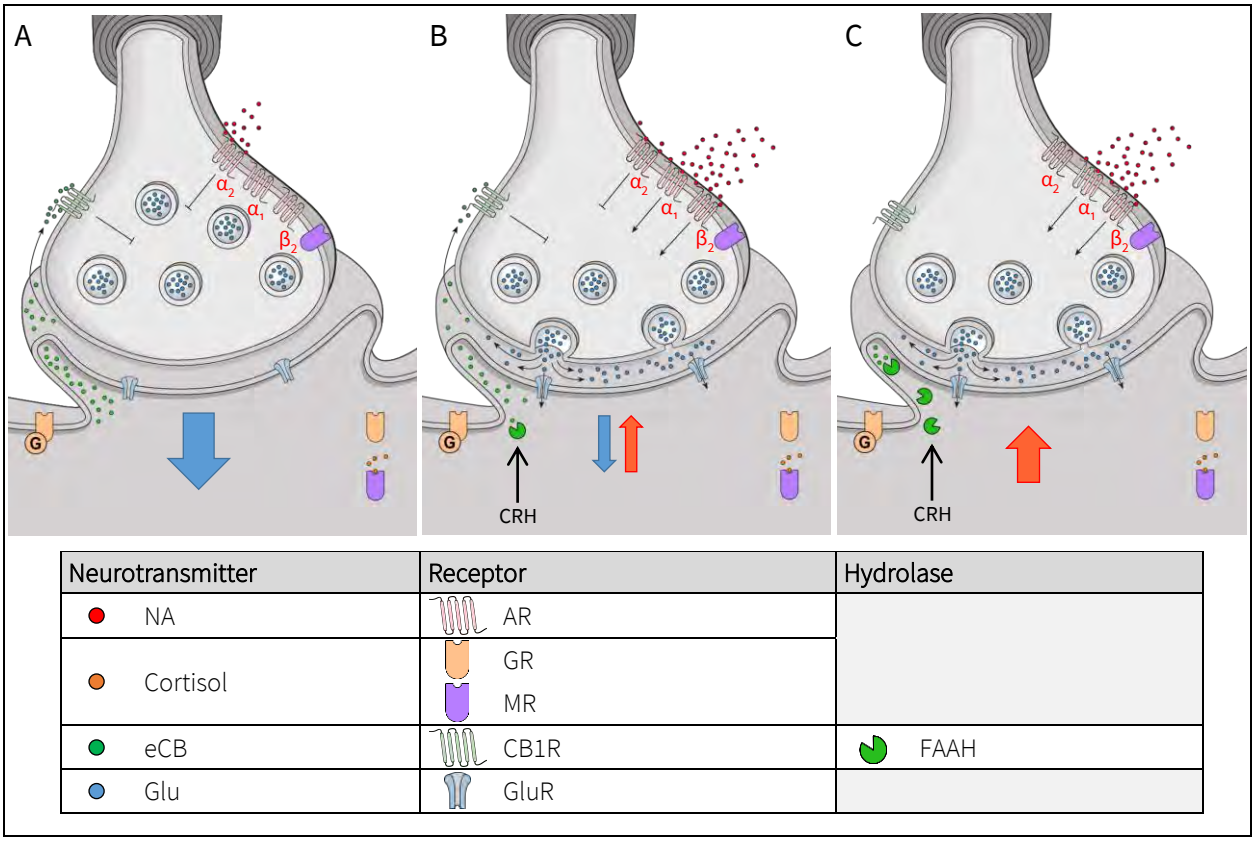


Figure 3. Schematic presentation of glutamatergic excitation at baseline and in the rapid noradrenergic stress response. (**A**) At baseline, α_2-ARs are occupied at moderate levels of NA, regulating glutamate (Glu) release as heteroreceptors and further NA release as autoreceptors. The endocannabinoid (eCB) anandamide (AEA) exerts tonic inhibitory effects on excitatory afferents to the PVN, thereby gating HPA axis activity (AEA also regulates gamma aminobutyric acid (GABAergic) signaling of inhibitory afferents to the PVN, see (McLaughlin et al., 2014). Moderate cortisol levels occupy MRs but not GRs to maintain homeostasis and to determine the stress response threshold. (**B**) In response to stress, NA is rapidly released from the LC and binds to α_2- and β-ARs, which quickly stimulate Glu release probability in limbic regions to increase vigilance. Simultaneously, CRH is released from the PVN, thereby increasing fatty acid amide hydrolase (FAAH) levels, which start degrading AEA. (**C**) The rising CRH-induced FAAH levels lead to a downregulation of the inhibitory influence of AEA, leading to even further Glu activation.

1.2.2 The non-genomic glucocorticoid stress response

Following this rapid surge of NA release, the second stress response phase is initiated. Specifically, neurons in the paraventricular nucleus (PVN) of the hypothalamus secrete CRH and vasopressin into the median eminence (Ulrich-Lai and Herman, 2009). CRH then leads to a quick reduction in levels of the endocannabinoid (eCB) *N*-arachidonyl ethanolamine (anandamide; AEA), an effect that may precede activation of the hypothalamic-pituitary-adrenal (HPA) axis (Morena et al., 2016). Presumably, under non-stressful conditions, AEA exerts tonic activation in the basolateral amygdala (BLA), hippocampus and mPFC with the function to gate HPA axis activity (Dubreucq et al., 2012; McLaughlin et al., 2014; **Figure 3A**). Through CRH, levels of fatty acid amide hydrolase (FAAH), the hydrolytic enzyme that degrades AEA, are increased, leading to the lowering of the tonic inhibitory tone of AEA, which then allows for the stress-induced activation of the HPA axis

response (**Figure 3C**). This claim is supported by the fact that AEA, despite its high-affinity for the G-protein coupled CB1R, shows only poor signal transduction efficacy, leading to more tonic, subtle CB1R stimulation (Hillard, 2000). In addition, inhibition of FAAH in the amygdala was shown to attenuate HPA axis activation in response to stress (Hill et al., 2009b) and CB1R antagonist administration into the mPFC prolonged the GC stress response (Hill et al., 2011). Both studies suggest a highly important role for eCB signaling in negative feedback control of the HPA axis, a factor which likely contributes to the anxiolytic effects of FAAH inhibitors (Haller et al., 2009). Importantly, at baseline AEA is synthesized on demand and, in a retrograde manner, leads to the suppression of glutamatergic excitation at the presynapse, thereby providing protection against damage caused by excessive excitation (Marsicano et al., 2003; Lutz et al., 2015; **Figure 3A**). Accordingly, stress-induced reductions in AEA result in disinhibition of excitatory projections from the BLA to the PVN, thereby leading to augmented secretion of CRH and vasopression, which then act on the anterior pituitary gland to promote the release of adrenocorticotropic hormone (ACTH; Ulrich-Lai and Herman, 2009). In turn, ACTH initiates the synthesis and secretion of GCs from the adrenal cortex (**Figure 1**). Whereas at baseline, cortisol concentrations show circadian rhythmicity with peak and nadir concentrations reflecting awake and asleep states, respectively (**Figure 3A**), stress can – depending on the stress induction paradigm – increase GC levels by a factor of 2 to 4 (Kirschbaum et al., 1992; Schwabe and Schachinger, 2018). Glucocorticoid levels begin to increase approximately 5-10 min after stress and reach peak levels around 20-30 min post stressor onset (Dickerson and Kemeny, 2004). Originally it was suspected that the sole purpose of GCs was to antagonize processes induced by the initial stress response via genomic mechanisms in order to restore homeostasis. However, this view dramatically changed in consequence of the discovery of membrane-bound receptors. Specifically pre- and postsynaptically located MRs have been discovered in the hippocampus (Karst et al., 2005) as well as in the BLA (Karst et al., 2010; Prager et al., 2010), where GCs exert rapid, reversible and more long-lasting effects, respectively. Evidence for the importance of membrane MRs comes from pharmacological experiments in rodents, showing the effectiveness of MR but not glucocorticoid receptor (GR) agonists, antagonists and gene knockout (Karst et al., 2005). It thus seems that rapid GC effects are mediated by membrane MRs, which have a much lower affinity for GCs than their cytosolic counterparts and therefore play a highly important role in mediating the effects of rising GC levels in response to stress (Groeneweg et al., 2011; **Figure 4A**).

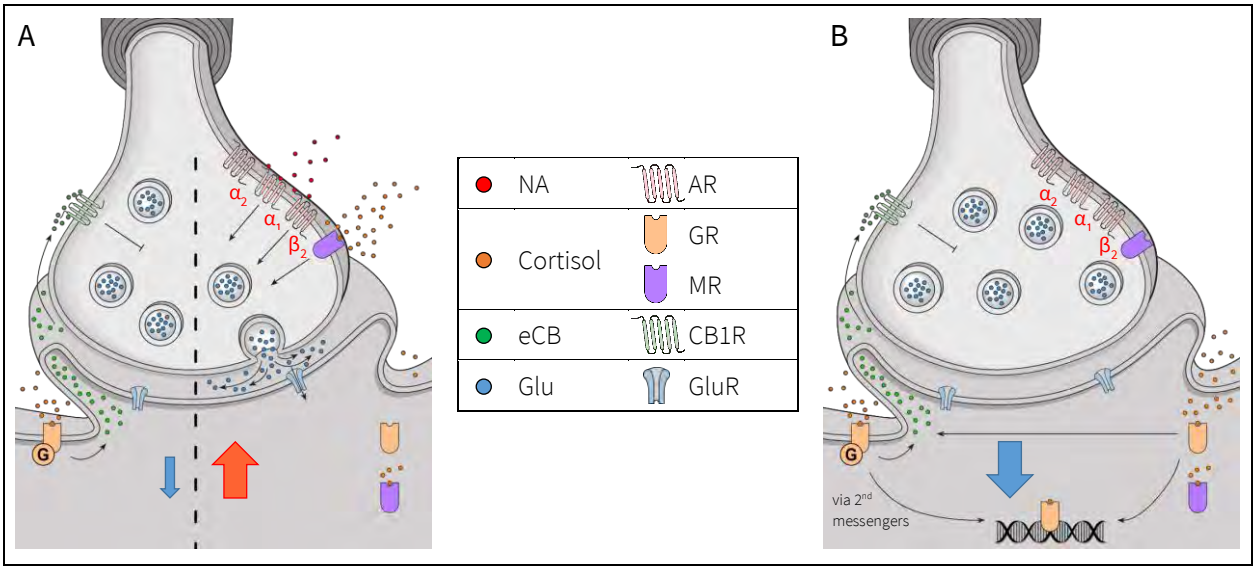


Figure 4. Schematic presentation of glutamatergic excitation in the non-genomic and genomic GC stress response. (A) Whereas NA binding to ARs decreases, rising CRH levels lead to augmented secretion of cortisol, which reaches peak levels around 20-30 min post stressor onset and quickly binds to membrane-bound MRs to increase Glu release probability. Cortisol also binds to membrane-bound GRs to increase levels of the eCB 2-arachidonylglycerol (2-AG), which exert rapid inhibitory feedback. (B) After about an hour post-stress, membrane-bound GRs regulate gene transcription indirectly through second messengers, whereas cytosolic GRs upon binding translocate to the nucleus to regulate gene transcription. Slow genomic GC actions provide further inhibitory feedback, facilitating recovery to baseline.

Particularly important seems to be the interaction between stress mediators in the brain (Joëls and Baram, 2009) and for the effects of non-genomic MRs in particular, interactions between GCs and NA in regions such as the amygdala, hippocampus and PFC (van Stegeren et al., 2008; van Stegeren et al., 2010; Krugers et al., 2012; Vogel et al., 2016). *In vitro* experiments were able to show that combined adrenergic and corticosteroid administration caused transient increases followed by later suppression of glutamatergic transmission in the rat BLA (Karst and Joels, 2016). Presumably, membrane MRs facilitate the initial stress response by increasing neuronal excitability, e.g. by augmenting glutamate release probability, whereas concomitant membrane and genomic GR-mediated feedback acts inhibitory (Groeneweg et al., 2011). In detail, whereas membrane MRs increase excitation in the BLA, subsequent pulses of GCs act via membrane GRs, which in turn induce synthesis and release of the eCB 2-arachidonylglycerol (2-AG) and potentially AEA. These eCBs then bind to CB1Rs at the presynapse, thereby inhibiting glutamate release probability (Karst et al., 2005; **Figure 4A**), a mechanism required for rapid inhibitory feedback of the HPA axis (Evanson et al., 2010; Hill and McEwen, 2010). Importantly, however, the mechanisms of the memory-enhancing effects of GC-induced eCB signaling via membrane GRs seem to be quite different (Atsak et al., 2015) and to require eCB-dependent inhibition of gamma aminobutyric acid (GABAergic) neurons, as will be discussed in more detail in chapter 1.3.2. In

general, stress-induced and GC-initiated eCB signaling through presynaptic CB1Rs, which are ubiquitously expressed throughout the forebrain, especially the vmPFC (Marsicano and Lutz, 1999), basal ganglia and the limbic system (McPartland et al., 2007; **Figure 2**), acts to confine neuronal activation in these structures and to suppress excitatory actions of CRH-producing cells, thereby terminating HPA axis activity (Hill and McEwen, 2010).

1.2.3 The genomic glucocorticoid stress response

Termination of HPA axis activity should not be equated with termination of GC effects. On the contrary, the genomic stress response phase mediated by cytosolic MRs and GRs is presumably not initiated until 60 min after stressor cessation and can last up to several hours (Hermans et al., 2014). Whereas membrane-bound GRs can only indirectly, via second messengers, regulate gene transcriptions, cytosolic GRs can do so directly. In contrast to membrane-bound receptors, intracellular GRs have a tenfold lower affinity for GCs than MRs, suggesting that genomic GR actions become particularly relevant when GC levels rise in response to stress (de Kloet et al., 1998). Upon binding, the receptor translocates to the nucleus, where it binds to GR response elements to regulates gene transcription, thereby changing transactivation and transrepression – the up- and downregulation of gene expression – of responsive genes (Datson et al., 2008; **Figure 4B**). In general, slow nuclear MR-mediated actions are important for maintaining neuronal integrity and stability and determining the stress response threshold (Joëls et al., 2008), whereas genomic GR processes, similarly to non-genomic GR effects (Zarzer et al., 2013), prevent overshooting of the initial stress response, facilitating recovery to baseline levels by providing inhibitory feedback in the PVN and pituitary gland (de Kloet and Reul, 1987). Interestingly, effects are bidirectional, with both receptor number and location influencing GC levels and GCs influencing receptor expression. Also, overlap between the genes that are affected by both MR and GR genomic effects amount to less than 30 %, indicating highly diverse consequences of these receptor actions (Datson et al., 2001). Expression of the MR is mainly confined to limbic regions, with high levels in the hippocampus and moderate levels in the amygdala and PFC (Reul and de Kloet, 1985). In contrast, the GR is expressed ubiquitously throughout the brain and shows highest expression in the PVN and hypothalamus (Reul and de Kloet, 1985; **Figure 2**). In addition, important regional and receptor-dependent differences of genomic GC actions exist. Whereas in

the hippocampus, low GC levels (via the MR) enhance and high GC levels (via the GR) inhibit plasticity, the opposite pattern is observed in the amygdala (Groeneweg et al., 2011).

1.2.4 Chronic and early life stress

Chronic stress augments some of these effects, whereas also additional and sometimes opposing consequences have been shown (Chattarji et al., 2015). Also, structural changes including adaptations in receptor density and secretion of their respective ligands are caused by chronic stress (Chattarji et al., 2015). Specifically, whereas dendrites and spine density are reduced in the hippocampus and PFC, dendritic branching in the BLA is increased. In combination with augmented CRH and vasopression messenger ribonucleic acid (mRNA) expression, this leads to region-specific up- or downregulation of GRs and MRs, as well as to reduced CB1R expression on GABAergic terminals and enhanced excitability of the SAM and HPA systems and their responses to stress, which is reflected in reduced AEA levels and increased GC and 2-AG secretion (Joëls and Baram, 2009; McLaughlin et al., 2014; McEwen et al., 2015). Stress during sensitive developmental stages may have similar consequences (Maccari et al., 2003; Benoit et al., 2015). Thus, both chronic and early life stress may have extensive and severe consequences, leading to enhanced vulnerability for stress-related disorders (Chrousos, 2009). Importantly, MR and eCB signaling may buffer some of these effects. Endocannabinoid signaling through CB1Rs also seems to limit excitatory neurotransmission in limbic structures in response to acute stress (Patel et al., 2005) as well as structural changes caused by chronic stress (McEwen et al., 2015). Further, MR overexpression in prefrontal areas or the BLA has been associated with reduced HPA axis activity in response to stress (Rozeboom et al., 2007; Mitra et al., 2009).

1.3 Stress, affective processing and memory

Rapid, non-genomic and slower genomic processes initiated in response to an acute stressor have important consequences. Briefly summarized, stress rapidly increases the release of NA, thereby augmenting attentional vigilance toward salient features in the environment. These effects are reinforced by rapid, non-genomic GC effects via membrane-bound MRs, whereas membrane and genomic GR processes mediated by eCB (mainly 2-AG) signaling via CB1Rs predominantly act to return the organism to homeostasis. Genomic MR mechanisms and AEA signaling have mainly regulatory functions and determine HPA axis responsivity. These different stress response phases

and the precisely timed actions of NA, GCs and eCBs, have important implications for affective processing and episodic memory (Roozendaal and McGaugh, 2011; Hermans et al., 2014).

1.3.1 Stress and affective processing

Affective stimuli activate a broad network of brain regions, with the amygdala likely being the most relevant and extensively studied structure. Although much research has investigated the role of the amygdala in the processing of fear (LeDoux, 2000), the amygdala has been shown to respond to both positive and negative stimuli (Sergerie et al., 2008). Several models haven been proposed to explain the preferential processing of emotional stimuli. A highly intriguing model brought forward by Pessoa and Adolphs (2010) suggests that the amygdala is more generally involved in processing salient, relevant and unexpected information and acts to prioritize features of information that are most important to the current goals. In addition, multiple streams of information processing are proposed that can be activated simultaneously. The amygdala can either directly, through regions of the visual cortex, or indirectly, via PFC areas, enhance sensory processing of affective stimuli. Indeed, compelling evidence suggests that the amygdala provides such direct and indirect top-down signals to potentiate early visual processing of affective stimuli (Vuilleumier et al., 2004; Vuilleumier, 2005). Time-sensitive EEG recordings of affective processing lend further support to the enhancing effects of affective stimuli on early visual and attentional processes (Olofson et al., 2008). Highly important for these modulatory influences driven by the amygdala, is noradrenergic activity since blockade of the noradrenergic system by means of the β-adrenergic antagonist propranolol decreases amygdala activation in response to aversive pictures (van Stegeren et al., 2005). Interestingly, enhanced amygdala activation in response to affective pictures was even more vigorous in subjects with high baseline cortisol levels (van Stegeren et al., 2008). The fact that propranolol abolished this effect suggests that cortisol likely interacted with NA in the amygdala. This finding immediately raises the question whether stress, known to increase NA and GC levels, may further enhance activation of the amygdala and thereby potentiate the preferential processing of affective stimuli even further. Indeed, such a stress-induced amplification is what several studies suggest. Affective face stimuli, embedded in stressful movie clips, led to enhanced amygdala activation as well as amplified sensory processing in early visual cortex and face-sensitive brain regions (van Marle et al., 2009). Interestingly, prolonged increases in functional connectivity of the amygdala with an attention network including the

dorsal anterior cingulate cortex (ACC) in the aftermath of stress, suggest that stress leads to an extended period of heightened attentional vigilance (van Marle et al., 2010). Also, an EEG study showed stress-induced amplitude increases of the late posterior potential in response to emotional pictures, an indication of enhanced attention allocation toward these stimuli (Weymar et al., 2012). Further evidence revealed that stress increases activation of and interconnectivity within the salience network, its core regions including the insular cortex, dorsal ACC, temporal pole and amygdala, and that the strength of this network connectivity correlated with stress-induced increases in cortisol and negative affect (Hermans et al., 2011; **Figure 5**). The role of this salience network seems to be the facilitation of attention toward salient features in the environment, which is highly relevant for immediate threat detection (Hermans et al., 2014). However, excessive noradrenergic signaling can also be detrimental, as is indicated by overly strong amygdala responses to negative stimuli in patients suffering from posttraumatic stress disorder (PTSD; Rauch et al., 2000).

In addition to activation of the amygdala and attention-related systems, emotional stimuli activate the dorsomedial (dm)PFC, which together with the dorsal ACC is involved in the appraisal and expression of negative affect (Etkin et al., 2011). Although a clear separation cannot be drawn, the ventral ACC and ventromedial (vm)PFC seem to be particularly important for the regulation of negative affect, which is achieved through their strong innervations of the limbic system. Direct causal evidence comes from a study showing that lesion-induced hypoactivation in the vmPFC led to elevated levels of amygdala activation and negative affect (Motzkin et al., 2015). A meta-analysis of 55 PET and fMRI affective processing studies identified the medial (m)PFC as the region most commonly activated (Phan et al., 2002), stressing the importance of this structure in affective processing. Whereas recruitment of prefrontal brain regions as part of the executive control network seems to be impaired in response to stress, activation within the default mode network may be enhanced and may reflect increased self-referential processing (van Oort et al., 2017; **Figure 5**). Particularly activation of the anterior part of the default mode network, including the mPFC, has been proposed to reflect attempts of regulating negative affect (van Oort et al., 2017).

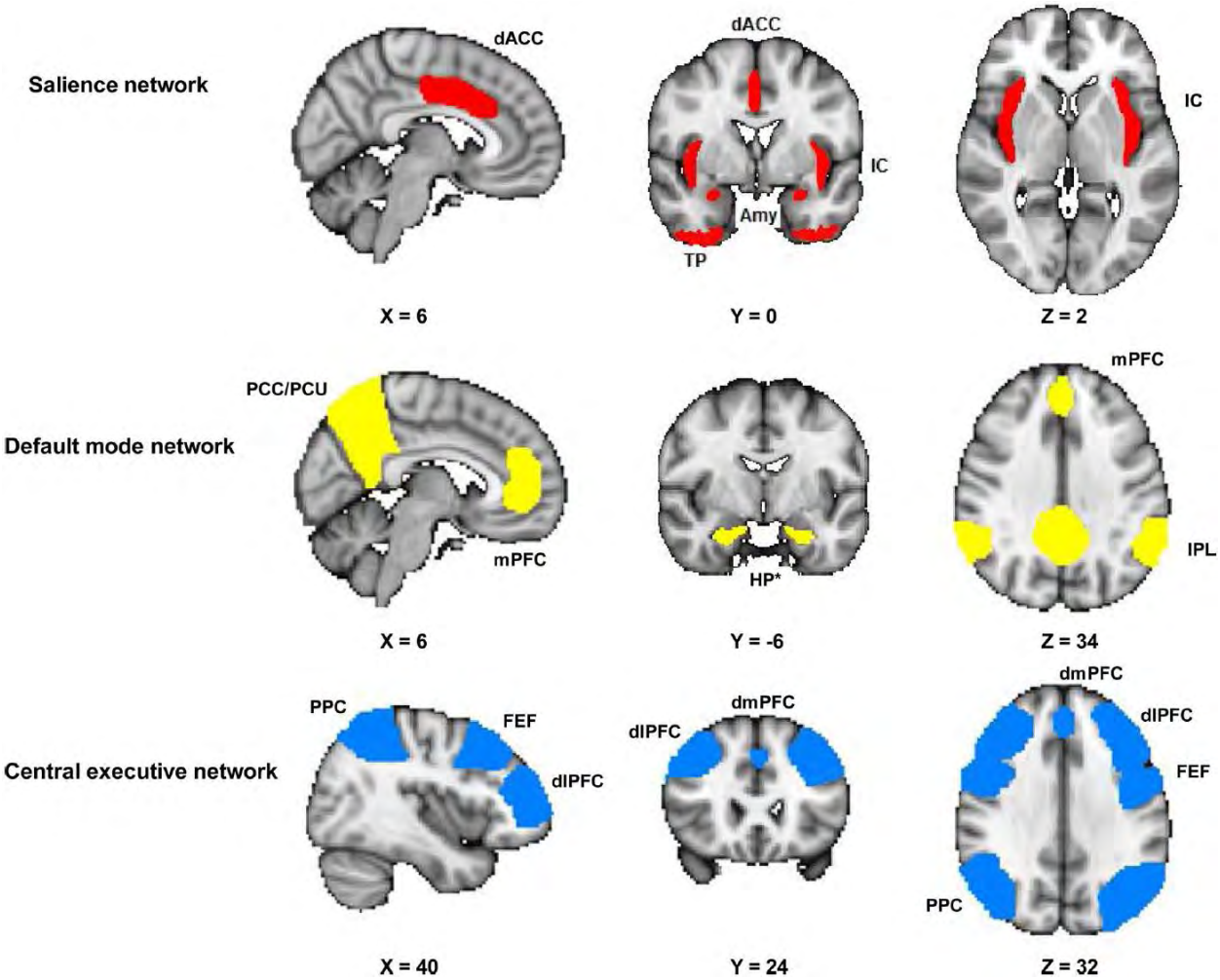


Figure 5. Functional connectivity networks in the acute stress phase. Core regions of the salience network are the insular cortex (IC), the dorsal anterior cingulate cortex (dACC), temporal pole (TP) and amygdala (Amy). The default mode network comprises the medial prefrontal cortex (mPFC), the posterior cinglutate cortex/precuneus (PCC/PCU) and the inferior parietal lobule (IPL). The parahippocampal gyrus and hippocampus (HP) are strongly related to the DMN. Core regions of the central executive network are the dorsolateral prefrontal cortex (dlPFC) and posterior parietal cortex (PPC), as well as parts of the dorsomedial prefrontal cortex (dmPFC) and frontal eye fields (FEF; from van Oort et al., 2017).

Functional connectivity of the amygdala with core default mode network structures (posterior cingulate cortex, precuneus, vmPFC) was increased even one hour after stress induction, suggesting adaptive processes in the recovery phase after stress (Veer et al., 2011). Prefrontal brain regions are highly important for emotion regulation in response to affective stimuli (Ochsner et al., 2012). In highly threatening situations such as stress, however, individuals seem to opt for maladaptive habitual strategies (Sheppes et al., 2014), likely because the use of more beneficial cognitive strategies is impaired under stress (Raio et al., 2013). Since effective emotional regulation skills have been proposed as the key mechanism for resilience (Kalisch et al., 2015), it is not surprising that psychopathologies such as PTSD, anxiety disorders and depression are proposed to be, at least partly, driven by stress-induced impairments in effective emotion regulation abilities

(Joormann and Gotlib, 2010; Campbell-Sills et al., 2011). Specifically, these disorders seem to be linked to a predominant use of avoidance, rumination or emotion suppression, maladaptive strategies that have been associated with enhanced sympathetic and subjective arousal, as well as sustained activation of emotion generating brain regions, such as the amygdala and insula (Goldin et al., 2008; Moore et al., 2008; New et al., 2009). Thus, under stress, subjects may show an enhanced bias toward emotional stimuli associated with augmented activation of the amygdala, and an inability to successfully engage prefrontal regions to downregulate negative affect.

The eCB system may be a particularly relevant for emotional processing and responding and has been referred to as the emotional buffer system (Morena and Campolongo, 2014). Specifically, CB1Rs are not only abundantly expressed in the amygdala and hippocampus; high density is also observed on inhibitory GABAergic neuron terminals in the mPFC (McLaughlin et al., 2014). Disinhibition of the vmPFC by eCBs may lead to suppression of the BLA in response to negative stimuli, thereby reducing excessive responding to affective stimuli (Morena and Campolongo, 2014). Pharmacological studies in healthy human subjects showed that eCB agonist Δ9-tetrahydrocannabinol administration led to reduced amygdala activity specifically in response to negative stimuli (Phan et al., 2008; Bossong et al., 2013). In contrast, in rodents, pharmacological or genetic CB1R inhibition has been associated with increased anxiety under stress (Lutz, 2009; Dubreucq et al., 2012) and eCB depletion in the BLA potentiated anxiety-like behaviors induced by intra-BLA administration of urocortin I, a structural analogue of CRH that stimulates ACTH release from the pituitary (Dono and Currie, 2012). These studies support a highly important role for eCB signaling in preventing exaggerated fear responding and maintaining proper functioning during stress exposure. In humans, administration of the CB1R antagonist rimonabant was associated with reduced activity in regions of the brain reward system in response to pleasant stimuli (Horder et al., 2010) and with a negative bias in memory recall (Horder et al., 2009; Horder et al., 2012). In the early 1990s rimonabant was used as a weight-reducing medication but had to be taken off the market due to its severe side effects. Specifically, rimonabant use was associated with significant increases in depressive symptoms and anxiety (Mitchell and Morris, 2007; Hill and Gorzalka, 2009), providing further evidence for the importance of eCB signaling in healthy affective processing and responding.

1.3.2 Stress and memory

Affective processing and episodic memory are inherently linked because attention and a certain level of processing/encoding are necessary for successful consolidation and later retrieval of the information. A key characteristic of adaptation is not only to rapidly detect and process emotional information but to remember it for future reference. Episodic memory can best be described by the phenomenon of mental time traveling: the time and place of the to-be-remembered information is recovered from memory, allowing for a detailed, vivid, and consciously accessible remembering of past experiences (Tulving, 2002). Linking temporal and spatial information of a certain event together to form an episodic memory is a process considerably dependent on the hippocampus (Squire, 1992a; Eichenbaum, 2000). Similarly to affective processing, memory for emotional information can be enhanced and has been shown to correlate with glucose metabolic rate of the amygdala (Cahill et al., 1996) and bilateral blood oxygenation level-dependent (BOLD) activity in the amygdala at encoding (Dolcos et al., 2004). Activity during successful encoding in the amygdala, hippocampus and entorhinal cortex (EC) was shown to be stronger and significantly correlated with memory retrieval for emotional pictures (Dolcos et al., 2004). In addition, in response to emotionally arousing information, independent of its valence, functional connectivity of the amygdala with the hippocampus quickly and strongly increased (Fastenrath et al., 2014), providing evidence for the modulatory role of the amygdala on memory storage regions such as the hippocampus (Hamann, 2001). The contribution of amygdala-medial temporal lobe connectivity to vivid remembering of affective pictures was even shown to increase over time (memory tested 20 minutes vs. 1 week after encoding; Ritchey et al., 2008). In another study, it was shown that retention of affective information was greater when memory was tested one day as opposed to one hour after learning, suggesting that arousal effects on memory benefit from a period of consolidation (Sharot and Phelps, 2004). Animal studies investigating the effects of post-training pharmacological injections into the amygdala supported the role of NA in mediating memory-enhancing effects of affective material by showing that whereas adrenergic agonists improved memory for the task, β-adrenergic antagonists abolished these effects (McGaugh et al., 1993; McGaugh and Cahill, 1997). In humans, administration of the β-adrenergic antagonist propranolol prevented amygdala activation during encoding and hippocampal activation during retrieval of emotional stimuli (Strange and Dolan, 2004), providing additional evidence for the role of NA and suggesting comparable mechanisms in animals and humans. To summarize, arousal or

rather noradrenergic activation induced by affective stimuli leads to enhanced memory consolidation which correlates with the strength of activation in and connectivity between the amygdala and hippocampus at encoding (LaBar and Cabeza, 2006).

Affective memory is highly sensitive to the effects of stress and GCs and similarly to the influence of stress on affective processing, GC actions seem to require concurrent noradrenergic activation in the BLA (Roozendaal et al., 2009). Importantly, stress effects can be quite diverse and depend on the emotional valence of the information, the timing of the stressor relative to the memory phase (encoding, consolidation, retrieval) and the timing of memory testing (Schwabe, 2017). Investigating the impact of stress solely on encoding, the conversion of perceived information into a construct that can be stored in memory, is a demanding task, because effects will inevitably be confounded with consolidation and retrieval mechanisms. To avoid the confound of retrieval, in an experiment by Payne and colleagues (2007), memory was tested a week after encoding, when cortisol levels had returned to baseline and genomic cortisol effects had likely ceased. Results show that stress before encoding improved long-term memory for visual episodes that had been complemented with an affective narrative, whereas memory for neutral narratives was impaired. Since noradrenergic signaling in the BLA might have already returned to baseline at the time of encoding and the neutral content of the material itself likely did not increase noradrenergic activation, increased cortisol levels may have disrupted encoding and consolidation processes. Generally, a recent meta-analysis of studies using healthy human subjects and inducing stress prior to or during encoding suggests that when the delay between the stress manipulation and encoding is very short and the study material is stress-related or otherwise relevant, stress improves encoding (Shields et al., 2017). Direct evidence for the modulation of hippocampal activation in response to stress hormones comes from an imaging study showing that concurrent hydrocortisone and reboxetine (NA reuptake inhibitor) administration enhanced hippocampal activity during encoding of affective stimuli (Kukolja et al., 2011).

Compelling evidence for the enhancing effects of stress on memory consolidation, the process by which information is transferred into long-term memory, and the importance of NA and GC interactions in the BLA is provided by pharmacological and lesion studies in rodents. It is

important to note that studies in rodents use mainly spatial memory tasks[1], which, similar to episodic memory tasks in humans, strongly depend on the hippocampus; as will be elucidated later, the effects of stress and stress hormones on memory involve very similar mechanisms in both rodents and humans (Cahill and McGaugh, 1998). The importance of noradrenergic arousal is demonstrated by several studies in which post-training administration of epinephrine and GCs improved memory performance, whereas amygdala lesions or β-adrenergic antagonist infusions into the amygdala prevented these effects (McGaugh et al., 1996). In addition to the effects of noradrenergic inhibitors, blockade of GC synthesis was shown to impair memory consolidation in stressed rats (Roozendaal et al., 1996b) and to abolish stress or epinephrine-induced memory enhancement (Roozendaal et al., 1996a), thereby demonstrating the importance of GCs. In addition, several experiments in rats showed that post-training corticosterone administration enhanced memory only in emotionally aroused animals, an effect prevented by concurrent systemic or intra-BLA administration of the β-AR antagonist propranolol, or in non-aroused animals that simultaneously received the α_2-AR antagonist yohimbine, thereby stimulating NA release (Roozendaal et al., 2006). Together, these studies convincingly illustrate the importance of concurrent NA and GC interactions in the BLA to facilitate memory consolidation. Presumably, such interactions modulate synaptic plasticity in the hippocampus to enhance memory. Indeed, post-training intra-BLA administration of a β-AR agonist improved memory consolidation and increased expression of the early-gene activity-regulated cytoskeletal-associated protein in the hippocampus, a protein which is known for its role in brain plasticity and memory (McIntyre et al., 2005). A similar effect was found in response to corticosterone administration and was blocked by concurrent intra-BLA propranolol injections (McReynolds et al., 2010). Human studies confirm the memory-enhancing effects of concurrent NA and GC actions, since the improving effects of stress or cortisol administration on memory are most pronounced for affectively arousing learning material, which leads to noradrenergic stimulation (Buchanan and Lovallo, 2001; Cahill et al., 2003). Direct evidence for the role of the amygdala comes from a study showing that in participants with high compared to low cortisol levels, amygdala activation in response to emotionally arousing stimuli was enhanced, an effect that was prevented by propranolol intake (van Stegeren et al., 2007).

[1] Spatial memory is frequently tested with versions of the Morris water maze task. In this task the animal is placed in a pool of water where it has to find a platform with the help of visual cues, allowing it to escape the water.

In contrast to the enhancing effects of stress on memory consolidation, stress prior to retention testing seems to impair retrieval of previously learned material. In rodents, 30 minutes after foot shock stress, rats showed impaired spatial memory retrieval, whereas this was not the case when shocks were given 2 minutes or 4 hours before testing (de Quervain et al., 1998). Interestingly, these time-dependent effects corresponded to corticosterone levels at the time of retrieval. This effect was also observed when GCs were administered before memory retrieval (de Quervain et al., 1998). Similarly to consolidation, evidence suggests that for stress effects on retrieval to occur, noradrenergic arousal and a functional BLA are required (Roozendaal, 2002). Studies using healthy human subjects showed analogous results. Specifically, exposure to a laboratory stressor before retention testing was shown to impair retrieval of affective words, whereas stress had no effects on neutral words (Kuhlmann et al., 2005). Importantly, retrieval was not impaired when tested immediately after the stressor but only when tested 25 minutes (elevated cortisol levels) and 90 minutes (cortisol levels returned to baseline) later, suggesting that rapid, non-genomic, as well as genomic GC actions impair memory retrieval (Schwabe and Wolf, 2014). A pharmacological study showed that cortisone administration prior to free recall impaired memory retrieval for affective words, an effect that was blocked by concurrent intake of propranolol (de Quervain et al., 2007b). Further, a PET study suggested that the impairing effects of cortisone administration on declarative memory retrieval are associated with reduced blood flow in the medial temporal lobe (MTL; de Quervain et al., 2003). In one behavioral study the effects of stress on all three memory phases were investigated (Smeets et al., 2008). Specifically, the authors tested memory 24 hours later when participants had been exposed to the stressor either shortly before (~5 minutes; encoding) or after (~5 minutes; consolidation) a word learning task, or it was tested approximately 10 minutes after the stressor when participants had learned the words 24 hours earlier. Compared to all other groups (encoding stress, retrieval stress, no-stress), stress shortly after learning improved recall for affective words. Stress before retrieval, however, impaired recall, especially for affective words. These effects correlated with stress-induced levels of salivary cortisol and alpha-amylase (sympathetic activity; Smeets et al., 2008).

The work of Marian Joëls and her colleagues has been very influential in this respect. According to their theory, stress will only enhance learning and memory when occurring around the time and context of learning and when stress hormones act on the same brain circuits as the learning episode itself (Joëls et al., 2006). This theory entails specific hypotheses: stress in the context of

learning will increase NA, CRH and GC levels, which will facilitate the learning process and suppress learning of unrelated information during the genomic effects of GCs; however, when stress comes to pass earlier and not within the learning context, the gene-mediated suppression will have developed by the time the learning episode occurs, thereby impairing memory. This theory, as well as the work of Roozendaal et al. (2006) with respect to the interaction of NA and GCs in the BLA, were integrated in an elegant model by Schwabe et al. (2012; **Figure 6**).

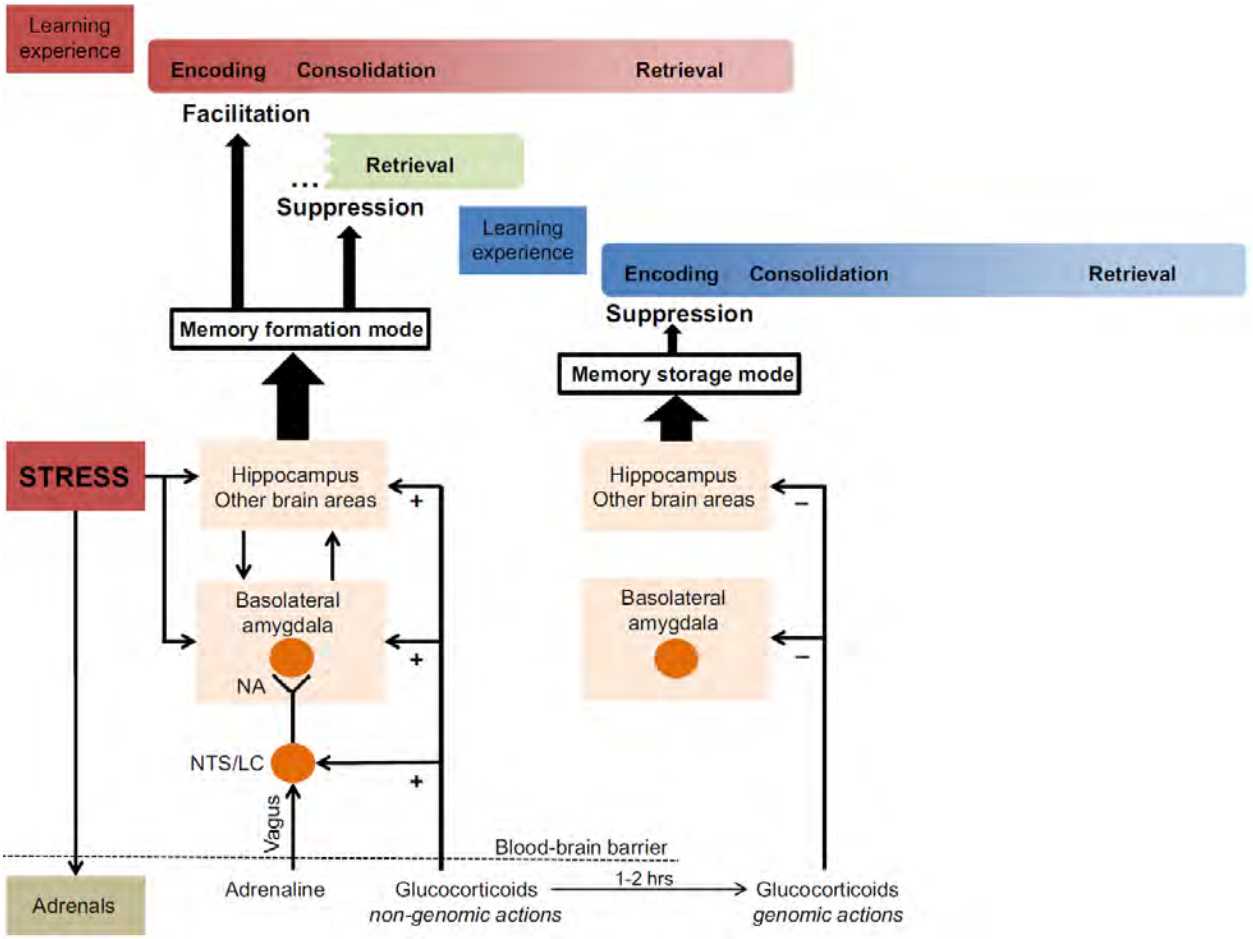


Figure 6. Integrative model of stress effects on memory. Rapid, non-genomic actions of NA and GCs interact in the basolateral amygdala (BLA) to shift the hippocampus into a memory formation mode, which facilitates encoding and early consolidation of the stressful event. Later genomic GC actions promote a memory storage mode, in which encoding of new information is suppressed to reduce interference with memory consolidation (from Schwabe, 2017).

This model suggests that rapid noradrenergic and non-genomic GC actions shift the brain into a memory formation mode, consequently augmenting perception, attention, encoding and early consolidation of information, whereas processes which may interfere are suppressed (e.g. retrieval of previously stored information). When genomic GC actions set in, the brain is shifted into a memory storage mode to promote long-term storage of the information experienced under stress. During this period, besides retrieval of stress-unrelated information, also encoding of new information is suppressed (Schwabe et al., 2012). A very insightful addition to this model are the

explications of Sandi (2011) who in detail describes the different pathways by which MRs and/or GRs can genomically or non-genomically change glutamatergic signaling and thereby affect memory processes. She also adds dosage as an important factor and concludes that moderate-to-high GC levels, induced by a stressful task or by GC administration around the time of training, will increase glutamate levels. Following a cascade of other processes, this will lead to the induction of long-term potentiation (strengthening of synapses), the cellular mechanism of memory consolidation, in synapses activated by the learning experience. In contrast, high-to-very high GC levels that do not coincide with the learning experience, will block glutamate reuptake. Excessive extracellular glutamate levels will then facilitate the induction of long-term depression (weakening of synapses), impair the effects of long-term potentiation and impede cognitive processing (Sandi, 2011).

Given this model, the question arises how the different stress hormone receptors and their functions might exactly fit in. It seems that although membrane-bound MRs have been shown to amplify glutamatergic excitation in the BLA and hippocampus – an important mechanism for the initial stress response including appraisal and coping to facilitate encoding (Joëls et al., 2008) – membrane and genomic GR actions seem to be particularly important for the stress-induced facilitation of memory consolidation in the hippocampus. Indeed, rodent studies suggest that non-genomic GR actions initiate early consolidation processes (Roozendaal et al., 2010) and gene-dependent GR activation facilitates long-term storage (Roozendaal and McGaugh, 1996). Evidence in humans comes from a pharmacological study showing that stress-induced cortisol levels enhanced long-term memory, even though the MR was blocked, which suggests that consolidation processes are augmented through GC actions via GRs (Cornelisse et al., 2011). An important role for the membrane-bound MR, however, has been proposed for memory retrieval processes. In several experiments on mice, intra-hippocampal injections of an MR antagonist were found to block the retrieval deficit caused by corticosterone conjugated to a 3-*O*-carboxymethyloxime-bovine serum albumin conjugate which cannot cross the cell membrane, thereby causing corticosterone to only bind to membrane-bound receptors (Dorey et al., 2011). Accordingly, membrane-bound MRs seem to mediate the rapid, non-genomic effects of acute stress on memory retrieval. Genomic MR effects are also important for memory retrieval, because MR blockade approximately 8 hours before retention testing – the learning episode had occurred 3 days earlier – was shown to impair free recall, especially of emotional learning material

(Rimmele et al., 2013). GR blockade, however, improved free recall, a finding which is in line with a study in rodents showing that GR blockade prevented retrieval impairments in rats with high hippocampal corticosterone levels (Dorey et al., 2012).

Accumulating evidence suggests that stress and GCs may also modulate memory dependent on the dorsal striatum. Specifically, as was shown by a study investigating the effects of amygdala lesions on memory for an aversive avoidance task[2], lesions restricted to the BLA blocked the memory-enhancing effects of post-training synthetic GC injections (Roozendaal and McGaugh, 1996). Similarly, post-training GC injections directly into the caudate nucleus improved inhibitory avoidance memory consolidation (Medina et al., 2007). In a water maze task, rats received training on a cued task version in which a rubber ball indicated the location of the platform, which changed every other trial, and on a spatial task version, in which the submerged platform remained at the same spatial location (quadrant) of the pool (Quirarte et al., 2009). Post-training corticosterone injections into the dorsal striatum dose-dependently increased performance on the cued version but had no influence on retention of the spatial training. Thus, increased corticosterone levels in the dorsal striatum after training strengthened simple stimulus-response (S-R) memory that is dependent on this brain region (Quirarte et al., 2009). Similar to corticosterone-induced impairments in hippocampus-dependent declarative memory retrieval (de Quervain et al., 2000), corticosterone injections were shown to impair retrieval of S-R memory, an effect blocked by concomitant metyrapone (corticosterone synthesis inhibitor) administration (Atsak et al., 2016).

Considering the abundance of CB1Rs in limbic areas such as the amygdala and hippocampus and the observation that eCB signaling is crucial for synaptic plasticity (Kano et al., 2009), it naturally followed that eCBs may play an important role in mediating the effects of stress on memory. Several studies, conducted mainly in rodents, suggest that as is the case with NA and GCs the effects of eCB signaling on learning and memory depend on the emotionality of the material, the memory phase that is being investigated and the brain regions involved (Akirav, 2011). Cannabinoid CB1Rs in the BLA have been proposed to regulate the effects of stress and of GCs on memory (Katona et al., 2001). This idea was investigated in a series of pharmacological

[2] During the training phase of the inhibitory avoidance task, the animal is placed in a brightly illuminated compartment (which the animal naturally wants to avoid) and is allowed to enter a dark compartment, where upon entering it receives a foot shock. During the retention phase the animal is placed again inside the bright compartment and the latency of entering the dark compartment is measured. Longer latencies are interpreted as indicating better retention. This type of memory is based on simple S-R learning supported by the dorsal striatum.

experiments conducted by Campolongo and colleagues (2009). Specifically, bilateral intra-BLA injections of a CB1R agonist after inhibitory avoidance training dose-dependently enhanced memory retrieval, whereas CB1R antagonist administration impaired retention. Interestingly, a usually non-impairing dose of the antagonist was sufficient to block the enhancing effects of both the agonist and the systemic corticosterone administration. In addition, effects of the CB1R agonist and antagonist were not observed when these drugs were administered 3 hours after training, when noradrenergic activity was likely to be low, or when injected into the central nucleus of the amygdala (Campolongo et al., 2009). This strongly suggests that concurrent noradrenergic activity is necessary and that CB1R activity in the BLA is required for the modulation of memory. CB1R antagonist injections into the hippocampus were also shown to block the memory-enhancing effects of synthetic GC administration (de Oliveira Alvares et al., 2010).

The rapidity with which eCBs were shown to increase in response to corticosterone administration in rats (Hill et al., 2010) leads to the assumption that non-genomic processes must be involved. Indeed, researchers were able to show that injections of a GR agonist or a GC ligand which cannot penetrate the cell membrane (corticosterone conjugated to bovine serum albumin) after inhibitory avoidance training, dose-dependently enhanced memory (Atsak et al., 2015). In contrast, intra-BLA CB1R antagonist administration prevented these effects, suggesting that eCB signaling is essential for the enhancing effects of GCs on memory consolidation. Interestingly, administration of a cannabinoid agonist was sufficient to enhance memory consolidation, an effect that was not abolished by GR blockade (Atsak et al., 2015). These findings support the notion that eCB signaling, induced by rapid actions of membrane-bound GRs, modulates synaptic transmission, likely by stimulating the release of NA from presynaptic terminals (Campolongo et al., 2009). The idea is that GC signaling through membrane-GRs rapidly increases eCB levels, which retrogradely act on GABAergic interneurons to disinhibit NA release and increases excitability of BLA neurons, which then facilitates encoding and/or early consolidation processes in the hippocampus (**Figure 7**).

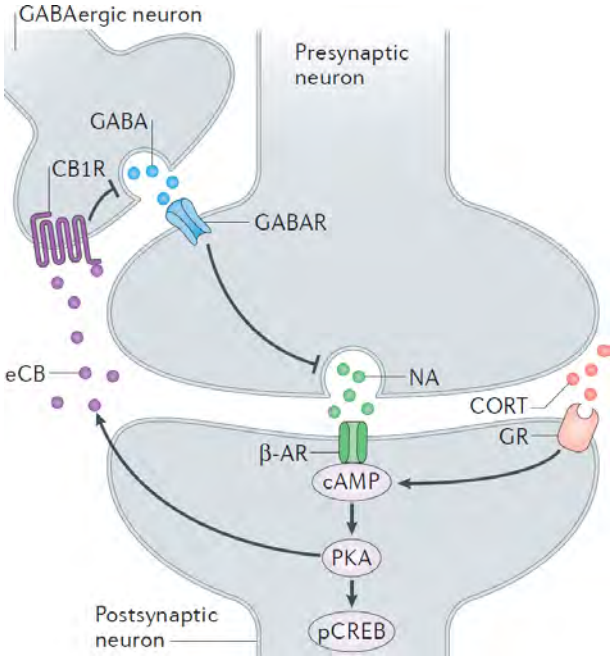


Figure 7. Facilitation of memory consolidation through eCBs. Glucocorticoid binding to membrane-bound GRs increases the release of eCBs, which travel back to the presynapse, where they bind to cannabinoid receptors type 1 (CB1Rs) on GABAergic interneurons, resulting in disinhibition of the presynaptic neuron. Ultimately, this may lead to increased NA release in the amygdala, which, through its connection to regions such as the hippocampus, may promote memory consolidation (from de Quervain et al., 2017).

Another very intriguing study was able to shed more light on the differential roles of the eCB ligands 2-AG and AEA in the stress-induced modulation of spatial memory (Morena et al., 2015). Specifically, rats were trained on a highly (19°C water temperature) or insignificantly (25°C water temperature) stressful version of a water maze task. Rats trained on the stressful version showed improved memory and heightened corticosterone levels as opposed to those in the low stress condition. Bilateral intra-hippocampal injection of either a CB1/CB2 receptor agonist or a 2-AG hydrolysis inhibitor 60 minutes before retention testing impaired this stress-induced memory enhancement, whereas injection of an AEA inhibitor was ineffective. At the time of retention testing, untreated rodents displayed greater 2-AG levels and diminished monoacylglycerol lipase (MAGL; the main 2-AG-degrading enzyme) activity, whereas hippocampal AEA levels and FAAH activity remained unchanged. These findings specifically suggest the importance of 2-AG signaling to mediate stress effects on hippocampus-dependent memory.

With respect to memory retrieval, several experiments by Atsak and colleagues (2012) revealed interesting findings. In a contextual fear conditioning paradigm[3], corticosterone administration before retention testing was shown to increase 2-AG levels and to impair contextual fear memory, an effect prevented by hippocampal CB1R blockade. Further, an intra-hippocampal propranolol injection abolished memory retrieval impairments induced by a CB1R agonist. These experiments provide strong evidence for an important role of eCB signaling in mediating NA-GC interactions to impair memory retrieval of arousing experiences.

1.4 Stress and multiple memory systems

While the previous paragraph highlighted the effects of stress on memory quantity – *how much* is learned and retrieved – multiple memory systems can be engaged during learning which qualitatively changes *how* something is learned. Importantly, stress can affect the relative engagement of these different memory systems. Whereas such stress effects have been demonstrated in many different types of learning (Wirz et al., 2018), my further explications will focus on research that has investigated the effects of stress on navigational (mostly studies in rodents) and category learning.

1.4.1 Multiple memory systems

Contrary to what was commonly believed, memory is not a unitary system that is responsible for all learning and storage operations and that is supported by a single neural structure. That such an oversimplification cannot be true was convincingly demonstrated with help from Henry Gustav Molaison, better known as patient H. M., who selflessly made himself available for years of highly informative research. Due to severe epileptic seizures, bilateral MTL resection was performed, leaving H. M. with considerably strong anterograde amnesia (Scoville and Milner, 1957). Whereas H. M. was unable to integrate new experiences into declarative long-term memory, his procedural memory capabilities were largely intact (Corkin, 2002). This supported the notion that declarative memory, including episodic and semantic memory, depends on the MTL (consisting of the

[3] On day 1 of the contextual fear conditioning paradigm, animals are habituated to the training context. During contextual fear conditioning on day 2, animals are placed in the training context and receive foot shocks. On day 3, animals are re-exposed to the fear conditioning context, whereas control animals are placed in a different but previously habituated context. Freezing behavior is analyzed to test whether animals retrieve the contextual fear memory.

hippocampal formation (dentate gyrus (DG), subiculum, CA1-CA3) and the entorhinal, perirhinal and parahippocampal cortices), whereas procedural skills as part of the non-declarative memory system are dependent on the striatum (consisting of a dorsal (dorsomedial: caudate nucleus, dorsolateral: putamen) and a ventral part; Squire and Zola, 1996). Particularly influential for the study of multiple memory systems are lesion studies in rodents (White and McDonald, 2002), studies of amnesic patients such as H. M., as well as investigations of neurodegenerative disorders, such as Parkinson's (PD) or Huntington's disease (HD; Squire and Zola, 1996; Squire, 2004). Although multiple memory systems perform different actions and depend on different structures in the brain, it is important to note that they also interact with each other and can work in parallel (Kim and Baxter, 2001), suggesting that it is the *relative* engagement of these memory systems rather than an-all-or-nothing principle that determines learning and memory processes.

Navigational learning

Research on navigational learning dates back to the early work of Edward Tolman (1938), who attempted to answer the question of "why rats turn the way they do". As suggested by his experiments, rats do so on the basis of different strategies, namely a hippocampus-dependent spatial strategy, in which the relationship of multiple cues forms a cognitive map, and a dorsal striatum-dependent strategy based on simple S-R learning (Tolman, 1948). These experiments stimulated a plethora of experimental studies in rodents using different tasks to dissociate the use of these strategies, the most common of which are versions of the Morris water, the plus maze or the radial maze tasks. In two versions of the radial maze task, animals were either reinforced for avoiding previously visited arms (win-shift) or for returning to them (win-stay; Gaffan and Davies, 1981). In the former, food rewards had to be collected at the end of each arm, requiring the animals to remember and avoid previously rewarded arms, so as to enter only those arms that still hold a reward. In the latter, reward was always placed in the same arm, requiring the animal to enter that arm only. Rats with damage to the fornix, the main output structure of the hippocampus, showed impaired learning of the win-shift version, whereas damage to the dorsal striatum impaired the win-stay version, suggesting that the fornix is necessary for the acquisition of a cognitive map, remembering the arms that were already rewarded, whereas learning dependent on the dorsal striatum leads to the formation of simple S-R associations (Packard et al., 1989). This finding was later replicated with direct damage to the hippocampus (McDonald and White, 1993). In another

study an adapted Morris water maze task was used with two cues, one leading to a platform (correct cue) and the other to a thin pedestal that the animal could not escape on (incorrect cue; Packard and McGaugh, 1992). In a spatial version of the task the correct cue was always located in the same quadrant of the maze, but the appearance of the cues alternated between trials. In a visual discrimination version of the task the correct cue always had a specific appearance, whereas its location was varied. The authors observed a double dissociation: lesions of the fornix but not of the caudate nucleus led to impairments in the spatial maze version, whereas the opposite was true for the visual discrimination version (Packard and McGaugh, 1992). In a similar adapted Morris water maze task with two versions, rats learned to swim to a visible platform and to a platform that was submerged into water but was located in the same quadrant as before (McDonald and White, 1994). On the final probe trials, the visible platform was placed in a different quadrant of the pool. The results showed that rats with dorsal striatal damage chose to swim to the spatial location of the platform that they had previously learned, instead of swimming to the visible platform in the new location. In contrast, rats with damage to the fornix acquired the visible platform version and swam to the visible platform in the new location but were unable to learn the spatial location of it, thereby failing the hidden platform version of the task (McDonald and White, 1994). Highly intriguing results came from another study by Packard and McGaugh (1996). Specifically, rats were trained on a plus maze task in which rewards were constantly given in a certain arm. On days 8 and 16 rats were placed in the arm opposite to the one they were usually put in. Rats which approached the place where they had been rewarded before were so-called place learners, whereas rats that made the same turn as in the trial before were response learners. Highly interesting to observe, control rats showed a preference for place learning on day 8, whereas they displayed response learning on day 16, suggesting that this task can be performed using different strategies and that there is a shift in those strategies with extended training. Further, rats with temporal inactivation of the caudate nucleus caused by lidocaine injections showed place learning on both probe trials, whereas hippocampal injections led to no differences in strategy use on day 8 but to a preference for response learning on day 16, indicating a blockade of response and place learning, respectively (Packard and McGaugh, 1996). These findings not only lent further support for independent and dissociable memory systems that can govern behavior but also indicated that hippocampus-dependent place learning is acquired faster than caudate-dependent response learning, that with learning expertise a shift toward the caudate nucleus

occurs, and that, should this system fail, the hippocampus-dependent strategy can still be used. In a navigational learning task in humans, video sequences of routes running through different rooms in a virtual environment were used and patients with HD, a neurodegenerative disorder characterized by early-stage caudate nucleus dysfunction, were compared to healthy control subjects (Voermans et al., 2004). Participants learned a fixed route through the different rooms and were, in a subsequent recognition task, asked to indicate where they thought the route had led them by pressing an arrow button at each intersection. The results showed that during route recognition, activity of the hippocampus positively and that of the caudate nucleus negatively correlated with disease severity in HD patients. Importantly, the patient group showed greater hippocampal and healthy controls greater caudate nucleus activity, an effect that had no influence on actual task performance (Voermans et al., 2004). This suggests that the hippocampus could compensate for dysfunctions in the caudate nucleus during route recognition, thereby rescuing performance, a finding that is in accordance with the previously reported animal data (Packard and McGaugh, 1996).

Probabilistic classification learning

Inspired by navigational learning tasks, a similar distinction between hippocampus-dependent, cognitive and dorsal striatum-dependent habit learning can be made in probabilistic classification learning (PCL). The weather prediction task (WPT) is likely the most famous PCL task and before presenting study results based on this task, I explain it in more detail, especially since we also used it in our experiments and the underlying concepts are quite complicated. Essentially, while performing a PCL task, participants learn a set of associations that are not obvious and difficult to memorize due to the probabilistic nature of the task. Consequently, single trials are not reliable indicators of what the true associations are, making it indispensable to acquire information across many trials. Because of its probabilistic nature, it mirrors real life decisions and learning processes very well. In case of the WPT, participants learn to predict the weather (‘sun’ or ’rain’) based on different cards (4 possible cards) and card combinations (14 possible combinations, each combination has no more than 3 cards) through trial-by-trial feedback. Every card and every combination has a certain probability for the two outcomes ‘sun’ and ’rain’, meaning that when the probability of a card for the outcome ‘sun’ is 85.7 % (e.g. the card with the triangles), the participant will receive positive feedback in this percentage of trials, whereas in the remaining 14.3

% of the trials depicting that card negative feedback is given when the participants responds with 'sun' (**Figure 8**). Interestingly, Gluck and colleagues (2002) investigated different strategies that participants performing the WPT may use. They distinguished between a singleton strategy, in which subjects learn to predict the outcome for trials with only a single card, a one-cue strategy, in which predictions are based on the presence or absence of one specific card, and a multi-cue strategy, in which participants focus on all 4 cards and their combinations. Which of these strategies a participant predominantly uses in a given set of trials can be determined by firstly generating ideal data for each strategy and secondly using a least mean squares approach to compare these ideal data with the participant's actual responses (**Figure 9**). The smaller the resulting fit-value for a certain strategy, the lower the error (the difference between the ideal and the actual data), and the better the fit for that strategy. Importantly, as I will review below, the one-cue and singleton strategies seem to depend on the hippocampus, whereas the multi-cue strategy is supported by the dorsal striatum (Shohamy et al., 2004; Schwabe and Wolf, 2012). Further, it has been demonstrated that the WPT can be equally well supported by both memory systems (Knowlton et al., 1996a; Poldrack et al., 2001). Only when participants use the singleton, one-cue, or multi-cue strategy throughout the task, respective performance levels would be 75 %, 87.5 % or 100% (see **Figure 8**). Evidence for multiple memory systems also comes from an EEG experiment showing that the use of a hippocampus-dependent learning strategy was associated with a more pronounced P300, an event-related potential (ERP) component that has first and foremost been associated with cognitive processes supporting memory (Ernst and Steinhauser, 2012; Rustemeier et al., 2013). In contrast, the feedback-related negativity (FRN) has often been shown to reflect dopaminergic feedback learning dependent on the striatum (Bellebaum and Daum, 2008; Ernst and Steinhauser, 2012; Glienke et al., 2015). Thus, also EEG measures suggest dissociable memory systems and may be advantageous in investigating their temporal dynamics.

Pattern	P(pattern)	P(rain)	Response single-cue strategy		Response multi-cue strategy
			Singleton	One-cue	
	0.14	0.857			
	0.08	0.625			
	0.09	0.889			
	0.08	0.375			
	0.06	0.833			
	0.06	0.500			
	0.04	0.750			
	0.14	0.143			
	0.06	0.500			
	0.06	0.167			
	0.03	0.667			
	0.09	0.111			
	0.03	0.333			
	0.04	0.250			
			Participant correctly resposnds to trials with one card and guesses on all other trials	Participant gives the same response to all trials containing a certain card (e.g. triangles)	Participant gives correct response on all trials
		Potential Performance	75%	87.5%	100%

Example trial

P(rain) = 85.7%

Single-cue learner: This card means rain

Multi-cue learner: The probability of this card is ~85%

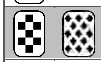
P(sun) = 66.7%

Single-cue learner: The rightmost card means rain

Multi-cue learner: The probability of this combination is ~66%

The single-cue learner only knows the correct response to single cues and either guesses on all other trials or ignores the other cards

Figure 8. Weather prediction task (WPT) probabilities and strategies. Probabilities of each card combination for 'sun' and 'rain' and the response pattern according to the singleton, one-cue or multi-cue strategy. In the example trials below, the difference between single- and multi-cue strategy users is depicted.

Pattern	P(pattern)	P(rain)	Ideal data for each strategy (sun response)		
			Singleton	One-cue: triangles	Multi-cue
	0.14	0.857	0	0	0
	0.08	0.625	0	8	0
	0.09	0.889	4.5	0	0
	0.08	0.375	0	8	8
	0.06	0.833	3	0	0
	0.06	0.500	3	3	3
	0.04	0.750	2	0	0
	0.14	0.143	0	14	14
	0.06	0.500	3	3	3
	0.06	0.167	3	6	6
	0.03	0.667	1.5	0	0
	0.09	0.111	4.5	9	9
	0.03	0.333	1.5	0	3
	0.04	0.250	2	4	4

Strategy calculation

$$\text{Strategy Model M} = \frac{\sum_P (\#\text{sun expected}_{P,M} - \#\text{sun actual}_P)^2}{\sum_P (\#\text{presentations})^2}$$

Fit-value for each strategy = For each pattern P, the difference between the number of the expected sun responses for that strategy and the number of the actual responses, relative to the number of presentations

Example calculation: fit-values for multi-cue strategy

Perfect fit $\frac{((0\text{-}0)+(0\text{-}0)+(0\text{-}0)+(8\text{-}8)+(0\text{-}0)+(0\text{-}0)+(14\text{-}14)+(6\text{-}6)+(0\text{-}0)+(9\text{-}9)+(3\text{-}3)+(4\text{-}4))^2}{(100)^2}$ = 0

Marginal fit $\frac{((0\text{-}0)+(0\text{-}0)+(0\text{-}0)+(7\text{-}8)+(1\text{-}0)+(0\text{-}0)+(13\text{-}14)+(4\text{-}6)+(1\text{-}0)+(6\text{-}9)+(2\text{-}3)+(2\text{-}4))^2}{(100)^2}$ = 0.0144

No fit $\frac{((0\text{-}0)+(0\text{-}0)+(4\text{-}0)+(0\text{-}8)+(3\text{-}0)+(2\text{-}0)+(0\text{-}14)+(3\text{-}6)+(2\text{-}0)+(5\text{-}9)+(1\text{-}3)+(2\text{-}4))^2}{(100)^2}$ = 0.1936

Instead $\frac{((0\text{-}0)+(0\text{-}0)+(4\text{-}4.5)+(0\text{-}0)+(3\text{-}3)+(2\text{-}2)+(0\text{-}0)+(3\text{-}3)+(2\text{-}1.5)+(5\text{-}4.5)+(1\text{-}1.5)+(2\text{-}2))^2}{(100)^2}$ = 0

= perfect fit for single-cue strategy

Figure 9. WPT strategy analysis. Ideal data for the singleton, one-cue and multi-cue strategy which can be compared to the participant's real data using a least mean squares approach. The example below shows how ideal responses lead to a fit value of 0 for a certain strategy. The more responses deviate from the ideal data, the worse becomes the fit. In that case, the fit of these data to ideal data from a different strategy may be better.

Before the calculation of hippocampus- versus dorsal striatum-dependent strategies was introduced, different versions of PCL tasks were used and patients were investigated to differentiate the two memory systems. Since learning dependent on the declarative memory system is accessible to conscious awareness, whereas procedural memory processes do not require or entail conscious recollection or awareness (Squire, 1992b), the degree of explicit task knowledge likely provides a clue about the predominant memory system. Specifically, a study investigating PCL in amnesic and PD patients, a neurodegenerative disease causing cell death in the substantia nigra and loss of neostriatal input, revealed that whereas amnesiacs were capable of learning the task, they showed severely impaired declarative knowledge about the learning episode (Knowlton et al., 1996a). In contrast, PD patients were able to recall this information but failed to perform the task. This double dissociation provided evidence for separate memory systems dependent on the hippocampus and the dorsal-striatum, supporting declarative memory and habitual learning, respectively. A similar finding that is in accordance with results from navigational learning research (Voermans et al., 2004) suggested that HD patients are also highly impaired in PCL task performance (Knowlton et al., 1996b). Another study replicated the finding that amnesic patients could learn the WPT but interestingly were impaired on transfer tests that required the flexible use of the acquired knowledge, suggesting that non-declarative habitual processes are relatively inflexible, whereas flexible knowledge acquisition may require a functional hippocampus (Reber et al., 1996).

Evidence for competition between the two memory systems in healthy subjects was provided by a neuroimaging study in which increased activation of the caudate nucleus during WPT learning was paralleled by MTL deactivation (Poldrack et al., 1999). Further, depending on whether declarative (paired-associative WPT version) or non-declarative (feedback-based WPT version) processes were emphasized, performance was supported by the MTL or caudate nucleus, respectively (Poldrack et al., 2001). In a second experiment using the feedback-based WPT, the authors demonstrated that initial learning was paralleled by enhanced MTL activation, whereas later on caudate nucleus activation predominated (Poldrack et al., 2001). In addition, by looking at the activation of individual trials within each subject, a negative correlation between MTL and caudate nucleus activity became apparent. Similar to the plus maze task findings (Packard and McGaugh, 1996), participants initially preferred hippocampus-dependent strategies, but eventually dorsal striatum-dependent learning took over (Poldrack et al., 2001). Possibly, in the

beginning of learning, when representations of the stimuli are being developed, participants select an outcome based on their declarative memory of the accuracy of their responses to the cue that was presented shortly before. With extended training, stimulus presentations are used by the dorsal striatum to develop and strengthen more complex S-R associations, which can guide behavior with potentially less effort.

In addition, whether and with how long a delay feedback is provided strongly influences the relative contribution of the hippocampus and dorsal striatum on PCL performance. Specifically, in a PCL task version with no feedback, PD patients were no longer impaired and an imaging study with healthy subjects revealed task-induced reductions in striatal activity (Shohamy et al., 2004). Similarly, when feedback was delayed by a few seconds, amnesic patients with MTL damage showed severe impairments in task performance, whereas they had no trouble learning the probabilistic associations when feedback was presented immediately (Foerde et al., 2013). The opposite pattern was observed in PD patients, again indicating a dissociation between the two memory systems and supporting a role of the MTL in PCL (Foerde et al., 2013).

Another factor that influenced the relative engagement of these memory systems is performance of a secondary task. Specifically, a neuroimaging study revealed that performing a demanding secondary task reduced the amount of declarative PCL knowledge, although it had no effect on overall task performance (Foerde et al., 2006). In addition, MTL activity correlated with single-task performance, whereas striatal activity correlated with dual-task performance. These data suggest that although distraction by a secondary task may not affect performance, it does seem to influence the use of more flexible, hippocampus-dependent learning processes. Another behavioral study reported similar findings, showing that whereas implicit cue-outcome associations could be learned, flexible and explicit knowledge of these relationships was impaired (Foerde et al., 2007)

Although there is still some debate about whether a multiple memory systems approach is superior to one that regards memory as a single system (Poldrack and Foerde, 2008), the evidence reviewed above clearly demonstrates the differential contributions of the hippocampus- and the dorsal striatum-dependent systems which support cognitive, flexible and rather rigid, habitual processes, respectively. The studies reviewed above suggest that certain factors can change the relative engagement of these memory systems as well as the cooperative or competitive nature of their interactions (feedback timing, task proficiency, performance of a secondary task; see data

from Dickerson et al., 2011 and reviews from Poldrack and Packard, 2003; Hartley and Burgess, 2005 on the subject of cooperation vs. competition). In the following paragraph, studies are reviewed that elaborate on stress as another highly relevant modulator of the relative engagement of multiple memory systems.

1.4.2 Stress-induced shift in multiple memory systems

Two highly crucial findings paved the way for research investigating the effects of stress on the engagement of multiple memory systems. First, another study by Packard (1999) with a design very similar to the plus maze study 3 years earlier (Packard and McGaugh, 1996) revealed that rats who had received glutamate infusions into the hippocampus or dorsolateral caudate on training days 3-5 displayed place or response learning on both testing days (days 8 and 16), respectively. This study provided strong evidence that glutamatergic strengthening of one system or the other can modulate the shift from place to response learning that is usually observed with extended training. A similar mechanism is likely induced by stress, because GCs act through glutamatergic pathways to modulate memory (Sandi, 2011). Second, intra-BLA injections of amphetamine, a potent central nervous system stimulant, enhanced performance on the caudate nucleus-dependent visible platform version of the Morris water maze task (Packard and Teather, 1998), suggesting that the BLA may modulate the engagement of multiple memory systems. Since ARs as well as MRs and GRs are abundantly expressed in the amygdala (Reul and de Kloet, 1985; McCune et al., 1993), stress hormones acting via these receptors may similarly stimulate the amygdala to modulate multiple memory systems.

Navigational learning

First evidence from navigational learning in rodents came from a maze task which could be performed by learning spatial cues outside the maze, a strategy dependent on the hippocampus, or by associating the correct arm with a proximal cue, thus by simple S-R learning known to depend on the dorsal striatum (Kim et al., 2001). In a probe trial, in which the proximal cue was relocated, rats that had been exposed to a foot shock stressor before training tended to rely more on the dorsal striatum-dependent S-R strategy. Evidence for the importance of noradrenergic activation in the BLA came from an experiment, in which an adapted version of the plus maze task from previous studies (Packard and McGaugh, 1996; Packard, 1999) was used (Packard and

Wingard, 2004). Specifically, the plus maze was filled with water and during training trials, rats learned to swim to a hidden platform in one of the arms. When placed inside the opposite arm of the one used during training, rats that had received systemic or intra-BLA injections of the α_2-AR antagonist yohimbine before training, showed a bias toward dorsal striatum-dependent response learning, indicated by the rat making the same turn that had previously been reinforced. Thus, an arousing state induced by enhanced noradrenergic activation was shown to modulate the use of multiple memory systems in favor of the more simple but rigid dorsal striatum-dependent memory system. Another experiment found that, similar to pre-training injections, systemic or intra-BLA infusion of an α_2-adrenoceptor antagonist before the probe trial also led to a bias toward the use of response learning dependent on the dorsal striatum (Elliott and Packard, 2008).

These results by Packard and Wingard (2004) were replicated in a study using two versions of a modified plus maze task (Wingard and Packard, 2008). Again, the maze was filled with water and animals had to find a hidden platform in one of the arms. During training, the starting arm in which animals were placed was pseudo-randomly alternated. In the place learning task, animals had to swim to the same goal arm to access the escape platform independent of their starting point, so that both left and right turns were reinforced. In the response learning task, rats had to turn consistently in the same direction to reach the platform. The results suggested that rats receiving post-training intra-BLA injections of an α_2-AR antagonist were impaired in the place task but showed superior performance in the response task. These findings support the notion that noradrenergic activation in the BLA, with efferent projections to the hippocampus and dorsal striatum, allows the BLA to modulate multiple memory system. Further, the authors concluded that the results pointed to injection-induced impairments of hippocampal memory, because if the dorsal striatal system was enhanced by the antagonist, no impairments should have been seen in the place learning task, since both left and right body turns had been reinforced (Wingard and Packard, 2008). Instead, however, the observed performance deficits were likely caused by BLA-mediated impairments of the hippocampus-dependent memory system, a finding supported by similar deficits observed after hippocampal inactivation (Schroeder et al., 2002). Evidence for the critical role of the BLA in mediating the influence of arousal on multiple memory systems was provided by another set of experiments, in which post-training intra-BLA injections of both an α_2-AR antagonist and a local anesthetic were given (Packard and Gabriele, 2009). Both the enhancing and the impairing effects of the α_2-AR antagonist on navigational response and place learning,

respectively, were blocked by the anesthetic-induced inactivation of the BLA. Another task designed to differentiate between hippocampus-dependent spatial and dorsal striatum-dependent S-R learning made use of a circular hole board, in which animals were trained to find an exit hole, either by memorizing spatial cues or by learning the association of a proximal cue with the correct escape hole (S-R learning; Schwabe et al., 2010b). In a final probe trial, the proximal cue was relocated. Animals that tried to enter the hole next to the proximal cue were dedicated S-R learners, whereas mice that had learned the spatial location of the cue and therefore entered the same hole in space as before, were identified as spatial learners. The results showed that both restraint stress and corticosterone injections before training induced a bias toward increased S-R learning (Schwabe et al., 2010b). This finding is highly relevant, since it suggests that increases in GCs, similarly to their importance for the quantitative aspect of memory, are crucial for the shift from hippocampus-dependent spatial toward dorsal striatum-dependent S-R memory.

Importantly, these results in rodents were also shown in healthy human subjects. Specifically, to investigate the effects of stress on the engagement of multiple memory systems, participants were randomly assigned to a stress (Trier Social Stress Test (TSST)) or control condition (Schwabe et al., 2007). The TSST is one of the most intensively researched and reliable stressors, leading to rapid increases in sympathetic arousal, indicated by increases in salivary alpha amylase, blood pressure and pulse, as well as to a reliable activation of the HPA axis, resulting in a 2- to 3-fold increase in salivary cortisol levels (Kirschbaum et al., 1993; Dickerson and Kemeny, 2004; Kudielka et al., 2007). After 5 min of preparation, the participant performs a mock job interview, during which he or she is asked to convince a reserved and non-reinforcing panel within 5 min that he or she is the ideal candidate for a job tailored to the participant's interests. In a subsequent mental arithmetic task, the participant is asked to count backwards from 2043 in steps of 17 for another 5 min. During the whole procedure, the participant is being videotaped and made believe that these recordings will serve to analyze their facial expressions later on. In contrast, participants in the control condition perform a non-stressful task, during which they are asked to freely talk about a self-chosen topic and then to count forward in steps of 15 while being alone in a room and no video recordings are taken. In the study by Schwabe et al. (2007), following stress induction and as soon as cortisol levels had reached their peak levels, participants were presented with a 3D model of a room in which 4 cards laid upside down on a table. In addition, a proximal cue (plant

on the table) and more distal spatial cues (door, window, picture, and clock) were present. Participants learned to identify the 'win' card by looking inside the room from various angles (a different wall was removed on each trial), whereas the 'win' card remained at the same location. Participants could either memorize the location of the 'win' card relative to the proximal cue (S-R learning) or learn its location in space with help from the distal cues (spatial learning). Similar to the hole board task, in a final probe trial the proximal cue was relocated. Results showed that stressed participants more often chose the card next to the proximal cue which was considered the S-R strategy, in comparison to participants in the control condition who predominantly used the spatial strategy and also had greater explicit task knowledge. Thus, also in humans enhanced sympathetic arousal, indicated by increased blood pressure during the stress manipulation and augmented cortisol levels at the time of task performance, seems to shift memory toward simple S-R strategies known to depend on the dorsal striatum at the expense of more cognitive and flexible learning (Schwabe et al., 2007). Interestingly, rats exposed to chronic restraint stress and human subjects assigned to a high chronic stress group based on an assessment by means of the Trier Inventory of Chronic Stress (TICS; Schulz and Schlotz, 1999; Schulz et al., 2004) both showed increased use of the S-R strategy in the hole board task and an adapted 2D version of the task by Schwabe et al. (2007), respectively (Schwabe et al., 2008). Thus, in addition to acute stress, chronic stress may reprogram the organism toward predominant engagement of rigid S-R learning.

To sum up, animal and human data indicate that increased noradrenergic and GC activation can bias the relative engagement of multiple memory systems toward the use of dorsal striatum-dependent S-R learning. Similar to stress effects on memory quantity, the BLA seems to be crucial. Likely, NA and GC interact in the BLA to increase competition between these memory systems, thereby orchestrating the stress-induced shift.

Probabilistic classification learning

The findings reviewed above stimulated further research and an intriguing neuroimaging study revealed that also in PCL a stress-induced modulation of the engagement of multiple memory systems can be observed (Schwabe and Wolf, 2012). More importantly, this study provided important insights into the neural underpinnings of these effects. In detail, in this study by Schwabe and Wolf (2012) participants were exposed to a laboratory stress or control manipulation

25 min before performing the WPT. As proposed by Shohamy et al. (2004), who found evidence showing that simple one-cue strategies are supported by the hippocampus and complex multi-cue strategies depend on the dorsal striatum, learning strategies were assessed (Gluck et al., 2002). Successful stress induction was verified by increased blood pressure, salivary cortisol and negative mood. Participants exposed to the stressor showed significantly less explicit task knowledge and used dorsal striatum-dependent, multi-cue strategies significantly more often than controls. Also, the use of multi-cue strategies positively correlated with salivary cortisol levels measured right before the WPT and 90 min after the stressor, as well as with increases in systolic blood pressure during the stress manipulation. Highly interesting results were provided by the neuroimaging data. Specifically, a predominant use of hippocampus-dependent, single-cue strategies was associated with increased activity in the hippocampus, whereas multi-cue strategy use positively correlated with dorsal striatal activity. Further, the larger the cortisol levels prior to and after the stress or control manipulations, the stronger the activity in the dorsal striatum. In control subjects, left hippocampal activity positively correlated with PCL task performance. In contrast, a positive correlation between dorsal striatal activity and task performance was observed in response to stress, whereas the association between task performance and hippocampal activity was negative. The fact that cortisol levels were associated with PCL task performance and increased activity in the dorsal striatum, in combination with stress-induced reductions in hippocampal activity suggest that stress may have disrupted the hippocampal system, thereby increasing the relative contribution of the dorsal striatum. Interestingly, the negative correlation between hippocampal activity and PCL task performance suggests that subjects who continued to rely on the hippocampus after stress suffered from performance deficits, a finding that is in line with previous studies (Kim et al., 2001; Schwabe et al., 2010b). This also suggests that a shift toward the dorsal striatum-dependent system may be beneficial after acute stress, rescuing performance at the cost of enhanced flexibility of the acquired knowledge (Schwabe and Wolf, 2012).

Role of the mineralocorticoid receptor

Although for the first time it was shown that stress modulates the engagement of multiple memory systems in PCL as well, in contrast to research on the quantitative aspect of memory, it remained unclear whether learning differences really depended on GC actions and which receptors were predominantly involved in mediating this stress-induced shift toward dorsal striatum-dependent

memory. For this reason, Schwabe et al. (2013b) conducted a similar experiment, this time blocking the MR before participants were exposed to a stressor or control manipulation and performed the WPT. The reasons why the researchers expected an involvement of the MR rather than the GR go back to earlier studies conducted in rodents and the general responsiveness of the MR to rising GC levels. As discussed previously, membrane-bound MRs are highly expressed in limbic areas and have a much lower binding affinity for cortisol than their cytosolic counterparts (Groeneweg et al., 2011). Accordingly, they are thought to be involved in the initial appraisal of stressful situations and to mediate the rapid effects of stress (Joëls et al., 2008). First evidence for the involvement of the MR in the engagement of multiple memory systems came from two experimental studies, in which mice with genetic deletion of MRs in the forebrain were tested on the hole board task (ter Horst et al., 2012; ter Horst et al., 2013). These rats were found to display impaired spatial learning performance under control conditions, whereas further impairments in response to stress were prevented by enhanced use of the S-R strategy (ter Horst et al., 2012). Interestingly, mice lacking the forebrain MRs were shown to require significantly more time in finding the exit hole when using the S-R strategy, suggesting the relevance of these receptors in successfully using this strategy (ter Horst et al., 2013). Further evidence came from a second experiment of the hole board study in rodents conducted by Schwabe et al. (2010b), in which an MR antagonist was administered 30 min before restraint stress or corticosterone injections and 60 min prior to behavioral testing. Similar to their first experiment, stress and corticosterone administration both significantly increased the use of S-R strategies. Blockade of the MR, however, prevented the stress-induced shift toward S-R learning, suggesting that GC actions via the MR are required for these effects to occur (Schwabe et al., 2010b).

Based on these data, the MR antagonist spironolactone was used 90 min prior to a stress or control manipulation and about 2 hours before PCL task performance in the study by Schwabe et al. (2013b). Whereas spironolactone administration lead to an increase in cortisol levels, this increase was augmented even further by implementation of the laboratory stressor. Blockade of the MR in stressed participants was associated with impaired PCL performance and increased use of single-cue, hippocampus-dependent strategies, a finding in contrast to the stress-induced shift toward multi-cue, dorsal striatum-dependent strategies under placebo conditions. The neuroimaging data provided highly important evidence for the neural correlates of these MR-dependent stress effects. In accordance with a stress-induced preference for the dorsal striatal memory system,

activity in the hippocampus was reduced. More importantly, only in participants receiving the placebo was the functional connectivity of the amygdala with the hippocampus reduced, whereas coupling of the amygdala and the dorsal striatum was enhanced. These effects, however, were abolished by concurrent spironolactone intake in stressed participants. In addition, in stressed participants who had taken the placebo, PCL task performance positively correlated with activation in the dorsal striatum and explicit task knowledge was reduced, whereas enhanced hippocampal activation was assocated with improved performance in control subjects. None of these effects were observed in participants receiving the MR antagonist. Importantly, the connectivity data suggested that it is indeed the amygdala that orchestrates the stress-induced shift in multiple memory systems. Although in contrast to the stress (hormone) studies on quantitative memory, there is no direct evidence for an interaction between NA and GCs in the amygdala, the finding that spironolactone-induced increases in cortisol did not lead to changes in the relative engagement of multiple memory systems is a first hint. Further, in line with GR-mediated impairments in hippocampal functioning, MR blockade did not diminish hippocampal activity directly but rather prevented the stress-induced changes in fuctional connectivity, suggesting that the stress-induced shift toward the dorsal striatum is mediated by GC binding to MRs in the amygdala. Despite this evidence for the critical involvement of the MR, the authors noted that because of its blockade, the MR:GR balance was disturbed and cortisol likely acted primarily via GRs, suggesting that more research is needed to elucidate the role of the GR in the modulation of multiple memory systems (Schwabe et al., 2013b).

Further evidence for the importance of the MR in the stress-induced shift subsequently came from a study by Vogel et al. (2015). The authors suggested that the stress- or GC-induced shift in memory systems proposed by Schwabe and Wolf (2013) was in line with the stress-induced network reorganization proposed by Hermans et al. (2014), in which resources are mainly allocated to the SN at the expense of the executive control network . In an attempt to bring these two lines of research closer together and to gain further insights into the role of the MR, participants were exposed to a laboratory stressor or control manipualtation inside an MRI scanner which was immediately followed by performance of an emotional face-matching task used to assess vigilance processing (Vogel et al., 2015). Although neither performance nor brain activity during the face-matching task was affected by stress or MR blockade, stress was shown to increase functional connectivity of the central amygdala with the striatum, an effect that positively

correlated with increases in cortisol levels and that was not observed when the MR was blocked. This increase in functional connectivity evolved within a few minutes after stress, when NA was presumably still active and cortisol levels were rising, suggesting that not only does stress rapidly enhance vigilance, but resources are similarly reallocated to the amygdala and dorsal striatum to promote simple, automatic and habitual functioning. Importantly, these effects seem to be mediated by rapid non-genomic MR actions (Vogel et al., 2015). In this same study, participants also performed a navigational learning task inside the MRI scanner, which was similar to previously presented tasks, in that boundary (spatial) cues as well as a landmark (proximal) cue were used (Vogel et al., 2017). In a first-person computer version of this task, participants navigated through an arena and were required to find and collect four everyday objects. Critically, between three learning blocks, the landmark was relocated but the objects maintained their location. The relative influence of the landmark cue or the boundary cues on the participant's response was calculated to differentiate spatial, hippocampus-dependent from S-R, dorsal striatum-dependent learning strategies. Results showed that the increase in cortisol in response to a laboratory stressor before task performance led to a significant increase in S-R learning strategy use, an effect accompanied by augmented activity in the amygdala as well as functional connectivity with the striatum. Importantly, these results were not seen after MR blockade, providing further evidence for that receptor's importance in mediating the stress-induced and likely amygdala-driven shift toward dorsal striatum-dependent memory (Vogel et al., 2017).

Together, the findings from navigational and classification learning suggest that stress- or pharmacologically-induced increases in stress hormones may act in the amygdala via membrane-bound MRs to strengthen connectivity of the amygdala with the dorsal striatum and/or to impair connectivity of the amygdala with the hippocampus to increase the competition between multiple memory systems in favor of the dorsal striatum, prompting a shift toward habitual processes, which may serve to rescue performance at the cost of increased flexibility (**Figure 10**; Vogel et al., 2016; Schwabe, 2017; Packard et al., 2018; see Goldfarb and Phelps (2017) for a discussion about possible modulation pathways).

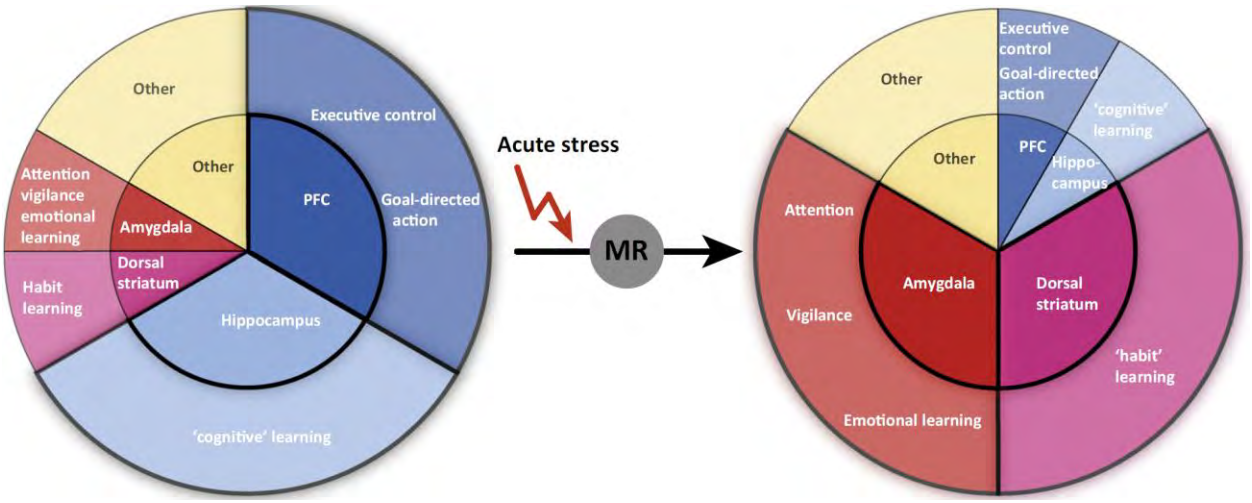


Figure 10. Schematic presentation of the distribution of cognitive resouces. Under baseline conditions (left), resources are mainly distributed among cognitive systems such as the hippocampus and prefrontal cortex (PFC), promotong flexible and goal-directed behavior. Under acute stress (right), stress hormones acting via the MR cause a reallocation of these resources toward brain regions of the salience network, such as the amygdala, which leads to increased attention, vigilance and emotional learning, and toward the dorsal striatum which suppports habitual forms of learning. This shift in resources also leaves fewer means for PFC- and hippocampus-dependent behavioral control. (from Vogel et al., 2016).

1.5 Stress resilience and vulnerability: individual differences

Although the findings reviewed above seem to generally hold true, they may not necessarily do so in some individuals. While it is neither realistic nor desirable to investigate how each individual by him- or herself will respond to stress, process affective information, or memorize that information, it is a highly relevant issue – and not merely an experimental irritant we want to get rid of by using larger samples – when it comes to resilience and vulnerability (Ebner and Singewald, 2017). It should be our ambition to discover why someone may suffer from the pathogenic consequences of stress while another does not. Why is it that not only Sapolsky's zebras do not get ulcers but also some of us do not suffer as much from the negative consequences of stress? Investigating the underlying traits and learning from those who are resilient will aid the development of preventative measures and treatment options for those who are vulnerable. A first step toward this goal is to investigate such individual difference.

Individual variability may come about through differences in our genetic makeup as well as our experiences and upbringing, which together determine how we respond to the stressor itself, as well as how we think and behave when confronted with a stressor. Particularly relevant for individual differences in stress effects are prenatal or early life stressors, as well as chronic or highly

traumatic stress experiences later in life. These stressors are highly relevant risk factors for the development of stress-related psychopathologies such as PTSD, anxiety disorders and depression and have been shown to cause sustained changes in cognitive and affective functioning (Lupien et al., 2009; Oitzl et al., 2010). Stress during sensitive developmental periods in particular is associated with a series of disturbances, ranging from hippocampus-dependent memory deficits to affective dysfunctions which cause symptoms such as anhedonia (Bolton et al., 2017). These are likely caused by stress-induced augmentation of excitatory signaling in stress-sensitive regions such as the hypothalamus, as well as by structural changes, including atrophy and altered connectivity, in the amygdala and hippocampus, promoting vulnerability to future stressors (Bolton et al., 2017). Prenatal stress in rats was found to increase the use of response compared to place learning strategies in a maze task, indicating increased dorsal striatum-dependent learning (Sutherland et al., 2000). Interestingly, two rodent studies suggest that despite these negative consequences, early life stress may actually prepare the organism toward superior performance under stress-like conditions (Champagne et al., 2008; Oomen et al., 2010). In the offspring of low-caring mothers, rats showed increased long-term potentiation in response to high corticosterone levels *in vitro*, and improved performance in a hippocampus-dependent contextual fear-conditioning task *in vivo* (Champagne et al., 2008). Further, postnatal maternal deprivation led to structural changes in the hippocampus and impaired water maze learning under no-threat conditions but facilitated spatial learning in a highly stressful environment, showing that early life stress may not develop into a general impairment of hippocampal functioning later in life but rather program the individual for optimal performance under stressful circumstances (Oomen et al., 2010). Although studying early life stress in humans is difficult, studies that do exist suggest that early life stress leads to a number of cognitive, higher-order (memory, executive functions), as well as affective (reward and affective processing, emotion regulation) deficits, especially when these functions depend on brain regions that undergo extensive changes during postnatal development (for a review see Pechtel and Pizzagalli, 2011). The amygdala appears to be particularly sensitive to the effects of early life stress and may, through dysfunctional affective processes, increase the risk for later psychopathologies (Pechtel and Pizzagalli, 2011). Similar to these early life stressors, chronic stress exposure is associated with structural and functional changes in brain networks which are determined by multiple factors of individual vulnerability and resilience (Sousa, 2016).

Independent of these individual life experiences, certain personality traits may confer risk for or protection against the consequences of stress. In a study that investigated the relationship between personality and the acute stress response, personality was able to explain 11 % of the variance observed in emotional stress responding (Childs et al., 2014). More specifically, high negative emotionality, defined as the tendency to experience negative emotions such as anxiety or anger, was associated with increased emotional distress in response to the TSST, whereas high positive emotionality, indicative of a strong tendency to experience positive emotions and to actively engage with the environment, was related to diminished cortisol and blood pressure responses to acute stress (Childs et al., 2014). Interestingly, with respect to coping, optimism, extraversion, conscientiousness and openness were shown to predict attempts to actively change and adapt to stressful events, whereas neuroticism was associated with disengagement coping (Carver and Connor-Smith, 2010). Thus, early life and chronic stress have detrimental functional and structural consequences but may also adaptively prepare the individual for future stressful events. In combination with certain personality characteristics, which influence the way we respond to and cope with a stressor, these factors have important implications for stress resilience and vulnerability.

Differences in certain genes are another highly relevant source of individual variability in the effects of stress. Although genes necessarily operate in interaction with the environment, especially when it comes to environmental stressors, twin studies can reveal the degree of genetic involvement and the extent to which individual differences can be attributed to our genome or to environmental factors (Thompson et al., 2001). It has been suggested that 30 to 80 % of the variability in cognitive phenotypes can be explained through an individual's genetic architecture (Scult and Hariri, 2018). Genetic rodent, as well as twin and adoption studies in humans have provided great advances concerning the heritability of human behaviors and suggest that multiple genes with small effects are involved rather than single major genes (Plomin, 1990). Advancements of molecular biology techniques, resulting for example in gene knock-out and knock-in mouse models, have been and still are greatly contributing to the investigation of highly complex phenotypes such as behavior (Plomin, 1990; Plomin et al., 1994).Particularly great advances with respect to the association of genetic differences and the development of stress-related psychopathologies such as depression, addiction, PTSD and anxiety disorders have been achieved (for reviews see Kreek et al., 2005; Arloth et al., 2015; Smoller, 2016), although the complexity of

these disorders and the myriad possible interactions between genes and environmental influences will require many more years of vigorous research. Accumulating evidence also points to the importance of epigenetic mechanisms, because of their ability to fundamentally change receptor expression and activity in reaction to stress (McEwen et al., 2015). Thus, neuroplastic adaptations take place, some of which seem to be only observed in resilient individuals (Russo et al., 2012). Heritability estimates revealed that 62 % of the variance seen in cortisol levels and 40-48 % of that observed in the cortisol awakening response are genetically determined (Wüst et al., 2000; Bartels et al., 2003). Twin studies estimated the heritability of episodic memory at up to 50-60 % (Rasch et al., 2010; Papassotiropoulos and de Quervain, 2011), whereas no data exists regarding heritability of the qualitative nature of memory. The strongest heritability of different neural pathways within the limbic system has been shown to amount up to 80 % and is found in a temporo-amygdala-orbitofrontal network which is associated with affective processing, semantic cognition and social behavior (Budisavljevic et al., 2016). This profound contribution of our genome has stimulated a lot of research and the study of SNPs in several candidate genes has evolved as a common method to investigate the association of genetic variants – often of known functionality – with certain traits of interest (Rasch et al., 2010).

As reviewed in chapters 1.2 and 1.3, the eCB system is highly important for negative feedback inhibition of the HPA axis, as well as for affective processing and memory under stress (Morena and Campolongo, 2014; Morena et al., 2016). Research on genetic differences in the cannabinoid CB1R gene (CNR1) has mainly been concerned with the receptor's protective role against anxiety disorders such as PTSD and major depression (Hillard et al., 2012), both of which are associated with dysfunctional affective processing (Etkin and Wager, 2007; Siegle et al., 2007). Specifically, in homo- and heterozygous A allele carriers of the rs1049353 SNP, the lifetime risk for anhedonia and depression after early life stress caused by physical abuse during childhood was significantly reduced (Agrawal et al., 2012). In contrast, G allele carriers showed increased antidepressant treatment resistance (Domschke et al., 2008). Further, neuroimaging data suggest diminished activation of the amygdala and striatum in response to happy faces in G allele carriers with major depression (Domschke et al., 2008) as well as healthy subjects (Chakrabarti et al., 2006), which may indicate reduced processing of positive stimuli and dysfunctional social reward responsivity. Since this SNP has been particularly relevant in depression but not in disorders such as ADHD (Hillard et al., 2012), this polymorphism may be especially important for healthy affective processing and

responding. Although the rs1049353 SNP is synonymous, meaning that it is not changing the amino acid sequence, it is positioned in the exogenic region of the CNR1 gene responsible for encoding part of the mature mRNA, which may have dramatic functional effects by altering mRNA stability or translation, an effect similar to that observed for synonymous SNPs of the dopamine receptor D2 gene (DRD2; Domschke et al., 2008). In addition, or alternatively to this direct mechanism, this SNP may be in high linkage disequilibrium with other polymorphisms with a so far unknown functionality, thereby possibly indirectly affecting CNR1 gene expression (Hill and Patel, 2013).

With respect to memory and the engagement of multiple memory systems, stress-induced increases in noradrenergic activation of the amygdala seem to be highly important (Packard and Wingard, 2004; Wingard and Packard, 2008; Roozendaal and McGaugh, 2011). In line with this, another set of studies has proposed a prominent role of a common functional deletion of the α_{2B}-AR gene (rs2900568; also called ADRA2B; de Quervain et al., 2007a; Rasch et al., 2009). This deletion of the presynaptic AR leads to the loss of 3 glutamatic acid residues (301-303) in the third intracellular loop of the receptor, which changes the receptor's negative feedback function (Cousijn et al., 2010). The ADRA2B deletion is present in about 30% of Caucasians and most likely acts as a loss-of-function variant, leading to increased availability of NA (Small et al., 2001; de Quervain et al., 2007a; Rasch et al., 2009). In line with the finding that administration of the α_2-AR antagonist yohimbine increased noradrenergic signaling in the amygdala, thereby potentiating the effects of affective arousal on memory (Roozendaal et al., 2006), a role for the ADRA2B deletion in memory was suggested. Specifically, participants were presented with positively and negatively valenced as well as neutral pictures, and delayed free recall was tested 10 min after presentation (de Quervain et al., 2007a). The results showed increased memory for affective pictures in homo- and heterozygous ADRA2B deletion carriers, an effect independent of differences in affective arousal, suggesting that the ADRA2B genotype influenced noradrenergic transmission and memory formation rather than arousal itself (de Quervain et al., 2007a). A functional imaging study investigated the neural mechanisms of these effects and could replicate the memory-enhancing influence of the ADRA2B deletion variant, although due to the lower statistical power, the *p*-value did not quite reach significance (Rasch et al., 2009). Importantly, amygdala activation during encoding of negative pictures, as well as functional connectivity between the amygdala and insula were enhanced in ADRA2B deletion carriers (Rasch et al., 2009). A very recent imaging study

revealed enhanced bilateral hippocampus activation, particularly for negative pictures, in ADRA2B deletion carriers (Schümann and Sommer, 2018). Increased activation of the amygdala and hippocampus are in line with the emotional memory enhancement found in ADRA2B deletion carriers. Interestingly, de Quervain and colleagues (2007a) also investigated whether the ADRA2B deletion variant may be associated with enhanced memory for traumatic experiences. They did so by assessing memory for traumatic experiences during the Rwandan civil war in survivors living in a refugee camp at the time of investigation. Deletion carriers displayed enhanced traumatic memory and greater re-experiencing symptoms than non-carriers, effects independent of an actual PTSD diagnosis. Another study which directly investigated the influence of the ADRA2B deletion in response to laboratory stress revealed that whereas slower, tonic changes in amygdala activity occurred independent of genotype, phasic amygdala responses to affective faces in response to acute stress were enhanced in deletion carriers (Cousijn et al., 2010). Thus, the augmented NA availability in deletion carriers may have caused NA to increase even further in response to affective stimuli, causing augmented phasic responses (Cousijn et al., 2010). A model proposed by Todd et al. (2011) suggests that the ADRA2B deletion genotype, through its influence on noradrenergic transmission in the amygdala, is particularly important for memory consolidation, leading to strengthening of the connections to sensory- and memory-related brain regions. Although evidence mainly implicated β-ARs in memory consolidation, α-ARs have been found to indirectly modulate their influence (Ferry et al., 1999). Another study showed that the ADRA2B deletion variant was associated with increased amygdala and inferior frontal gyrus activity during successful affective memory formation, whereas no activation differences in carriers and non-carriers were observed during retrieval, providing further support for the important role of the ADRA2B deletion in memory formation (Urner et al., 2011). Importantly, recent evidence also suggests that the ADRA2B deletion variant amplifies the memory-enhancing effects of a laboratory stressor to which participants were exposed to immediately before learning (Zoladz et al., 2017).

Considering the evidence suggesting that the MR is necessary for the stress-induced shift from cognitive, hippocampus-dependent toward habitual, dorsal striatum-dependent memory (Vogel et al., 2016), individual differences in the gene coding for the MR seem to be highly relevant. Two genetic variants of the MR gene have been extensively studied and associated with HPA axis reactivity (DeRijk, 2009). Specifically, one SNP which is characterized by a guanine (G) to cytosine

(C) change (rs2070951; also called MR-2G/C) revealed changes in transactivational activity using dexamethasone, an artificial GC, or cortisol as a ligand *in vitro* and has been associated with differences in basal and morning cortisol levels following dexamethasone administration the previous day, as well as with increased MR protein expression *in vivo* (DeRijk, 2009; van Leeuwen et al., 2010). Another SNP characterized by an isoleucine (A) to valine (G) change (rs5522; also called MRI180V) showed *in vitro* loss of function using cortisol as a ligand and has been associated with an increased cortisol response to the TSST (DeRijk et al., 2006; DeRijk, 2009). Both SNPs were also found to be associated with an increased autonomic stress response, indicated by a heightened heart rate (DeRijk and de Kloet, 2008). In addition, the val allele of the MRI180V SNP was related to increased threat-related amygdala reactivity in children with low childhood adversity (Bogdan et al., 2012) and predicted depression and depression-like HPA axis dysfunction (DeRijk and de Kloet, 2008; van Leeuwen et al., 2010; Klok et al., 2011). In contrast, increased MR functionality or expression (rs2070951 C allele, rs5522 A allele) has been associated with enhanced resilience to traumatic stress (ter Heegde et al., 2015). Another interesting study also pointed to an important role of the MRI180V SNP in striatal learning (Bogdan et al., 2010). In accordance with the importance of MRs in the stress-induced shift from hippocampus-dependent cognitive or spatial, toward dorsal striatum-dependent habit or S-R learning and memory (Schwabe et al., 2010b; Schwabe et al., 2013b), the val allele of this SNP which is associated with reduced MR functionality revealed deficits in stress-induced reward learning, which likewise depends on striatal functioning (Bogdan et al., 2010).

Together, these candidate gene studies show that hypothesis-driven testing of genetic differences in specific SNPs can be highly informative. They also suggest that polymorphisms in the α_{2B}-AR, MR and CB1R genes are particularly promising and may explain some of the individual differences regarding stress effects on affective processing and memory. These genetic variants may likely influence vulnerability or resilience to environmental stressors, leading to psychopathology in some individuals only.

1.6 Research goals

Advances in the identification of genetic polymorphism have opened the possibility of investigating genetic differences in complex phenotypes such as affective processing and memory. Associating individual differences in affective processing and the engagement of multiple memory systems with variants in genes relevant to these phenotypes is a powerful means to detect their molecular pathways. Particularly imaging genetics is a highly promising research area with the aim to identify the mediating mechanisms through which genetic variability shapes these individual differences. Since NA, GCs and eCBs are highly important stress mediators affecting emotion and cognition and individual differences in these domains are associated with relative vulnerability and resilience to stress-related psychopathologies, the overall goal of our experimental studies was to answer the following general question: **Can individual differences in the genes of important stress mediators (NA, GCs, eCBs) explain differences in stress effects on affective processing and memory?**

Given the evidence for the involvement of the ADRA2B deletion in memory and taking into account the findings from rodent studies which showed that pre-training systemic or intra-BLA administration of the α_2-AR antagonist yohimbine led to enhanced dorsal striatum-dependent S-R learning (Packard and Wingard, 2004; Elliott and Packard, 2008; Packard and Gabriele, 2009), the ADRA2B deletion may likewise be able to explain at least part of the individual differences in the engagement of multiple memory systems after stress. The first goal of our experimental studies was thus to answer the following question: **Is the stress-induced shift from hippocampus-dependent cognitive toward dorsal striatum-dependent habit memory modulated by the ADRA2B deletion?**

Considering the MR candidate gene studies, it seems likely that genetic variants in this gene may also modulate the engagement of multiple memory systems. Such a modulation may come to pass indirectly, by affecting cortisol levels and autonomic arousal in response to stress, which would be in line with studies suggesting that memory is affected by individual differences in the cortisol stress response (Buchanan et al., 2006), or by dose-dependent differences after corticosterone injections (Quirarte et al., 2009). This would suggest that the stress-induced shift toward the dorsal striatum is reduced in participants carrying MR SNPs associated with enhanced MR expression, since negative feedback inhibition of the HPA axis would be increased.

Alternatively, higher MR expression in the amygdala, may directly facilitate the stress-induced shift toward the dorsal striatum, for example by providing more binding sites for GCs, which would then, possibly in interaction with NA, strengthen connectivity with and activation of the dorsal striatum. The second goal of our experimental studies thus concerned the following question: **Is the stress-induced shift from hippocampus-dependent cognitive toward dorsal striatum-dependent habit memory modulated by MR gene variants?**

The evidence reviewed above suggests that the eCB system is highly important for stress (Lutz et al., 2015) and affective processing (Morena and Campolongo, 2014), and that genetic variants in the CB1R gene are associated with disorders characterized by dysfunctional affective processing (Hillard et al., 2012). The rs1049353 SNP specifically has been proposed to protect against stress-related psychopathologies (Hill and Patel, 2013). Particularly interesting since the effects of eCBs and CB1R gene differences with respect to affective processing are highly understudied, this led to the third question we aimed to investigate: **Are the effects of stress on affective processing modulated by the rs1049353 genotype?**

This dissertation investigated these questions in experimental studies with large sample sizes and by means of a genetics approach using EEG and fMRI to infer the underlying neural mechanisms of the genetic modulation of stress effects on affective processing and the engagement of multiple memory systems.

2. Genetic modulation of stress effects on multiple memory systems

2.1 ADRA2B deletion reduces the stress-induced shift from cognitive to habit memory

Wirz L, Wacker J, Felten A, Reuter M, Schwabe L (2017) A deletion variant of the alpha2b-adrenoceptor modulates the stress-induced shift from "cognitive" to "habit" memory. *J Neurosci* 37:2149-2160. (Appendix A pages 131-143)

2.1.1 Background

Multiple memory systems may work cooperatively or competitively (Hartley and Burgess, 2005). A factor that has been shown to increase competition between these systems, leading to a shift from hippocampus-dependent cognitive toward dorsal striatum-dependent habit memory, is stress (Schwabe et al., 2012). Particularly relevant for this shift to occur are, in addition to cortisol, stress-induced increases in noradrenergic activation of the amygdala, the structure which likely orchestrates this stress-induced shift from flexible to more rigid memory processes (Packard and Wingard, 2004; Vogel et al., 2016). However, not all individuals show this bias toward dorsal striatum-dependent habit memory after stress (Schwabe et al., 2007; Schwabe and Wolf, 2012). This is importance because the balance between cognitive and habit memory is highly relevant in the context of anxiety disorders such as PTSD (Schwabe et al., 2010a; Goodman et al., 2012; de Quervain et al., 2017). The ADRA2B deletion has been associated with enhanced episodic, hippocampus-dependent memory for emotional material in healthy subjects (de Quervain et al., 2007a; Rasch et al., 2009), as well as with traumatic experiences in Rwandan civil war survivors (de Quervain et al., 2007a). Neuroimaging data showed increased amygdala activation and connectivity with the insula for emotional material (Rasch et al., 2009), as well as increased stress-induced amygdala activation in deletion carriers (Cousijn et al., 2010). Considering the enhanced hippocampus-dependent memory for emotional material and the increased activation of the amygdala in deletion carriers, we hypothesized that carriers of the ADRA2B deletion genotype would show enhanced crosstalk between the amygdala and hippocampus, thereby attenuating the stress-induced shift toward dorsal striatum-dependent memory.

2.1.2 Methods

A total of 252 healthy individuals scanned for medication intake, history or current diagnosis of psychiatric disorders and other factors which may influence HPA axis responsiveness (e.g. hormonal contraceptives, smoking) participated in our first experiment (127 women; mean age 25.1 ± SD: 3.5 years; stress group: 74 carriers, 52 non-carriers; control group: 69 carriers, 57 non-carriers). For genetic analyses, deoxyribonucleic acid (DNA) was extracted from buccal cells and genotyping was performed by real-time PCR. In accordance with previous studies (de Quervain et al., 2007a; Rasch et al., 2009) homo- and heterozygous ADRA2B deletion carriers were compared to homozygous non-carriers (**Figure 11**).

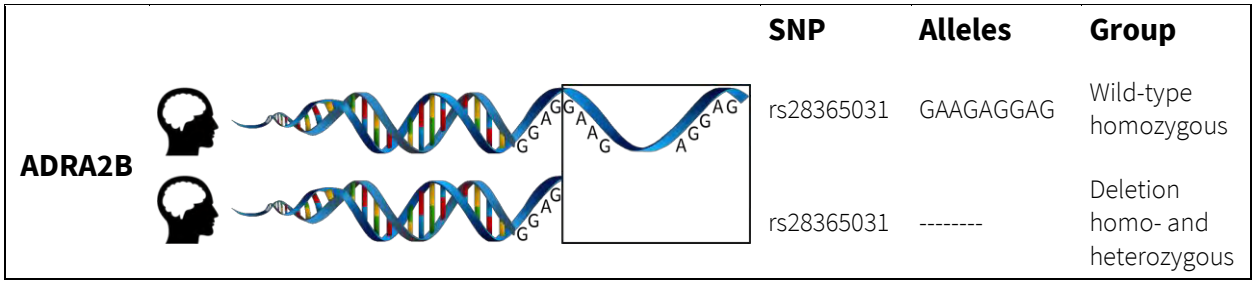


Figure 11. ADRA2B genotype. Homo- and heterozygous ADRA2B deletion carriers were tested against homozygous non-carriers (wild-type).

Participants performed a stress (TSST) or control condition around 15 minutes before performing 100 trials of the WPT while EEG data were recorded (**Figure 12**). The WPT was administered as described before, participants received trial-by-trial feedback in the form of a happy or sad face, and single- as well as multi-cue learning strategies were calculated. The EEG data were preprocessed, segmented into epochs from -200 to 800ms with respect to the feedback stimulus, baseline corrected and trials with artifacts were rejected before averaging, leaving data from 228 participants (stress group: 68 carriers, 46 non-carriers; control group: 64 carriers, 50 non-carriers). The FRN could provide direct evidence for group differences in striatum-dependent feedback processing (Nieuwenhuis et al., 2005; Hauser et al., 2014) and was calculated as the most negative peak in the time window between 200 and 350 ms following feedback presentation relative to the preceding positive peak between 150 ms and the latency of that negative peak (Rustemeier et al., 2013).

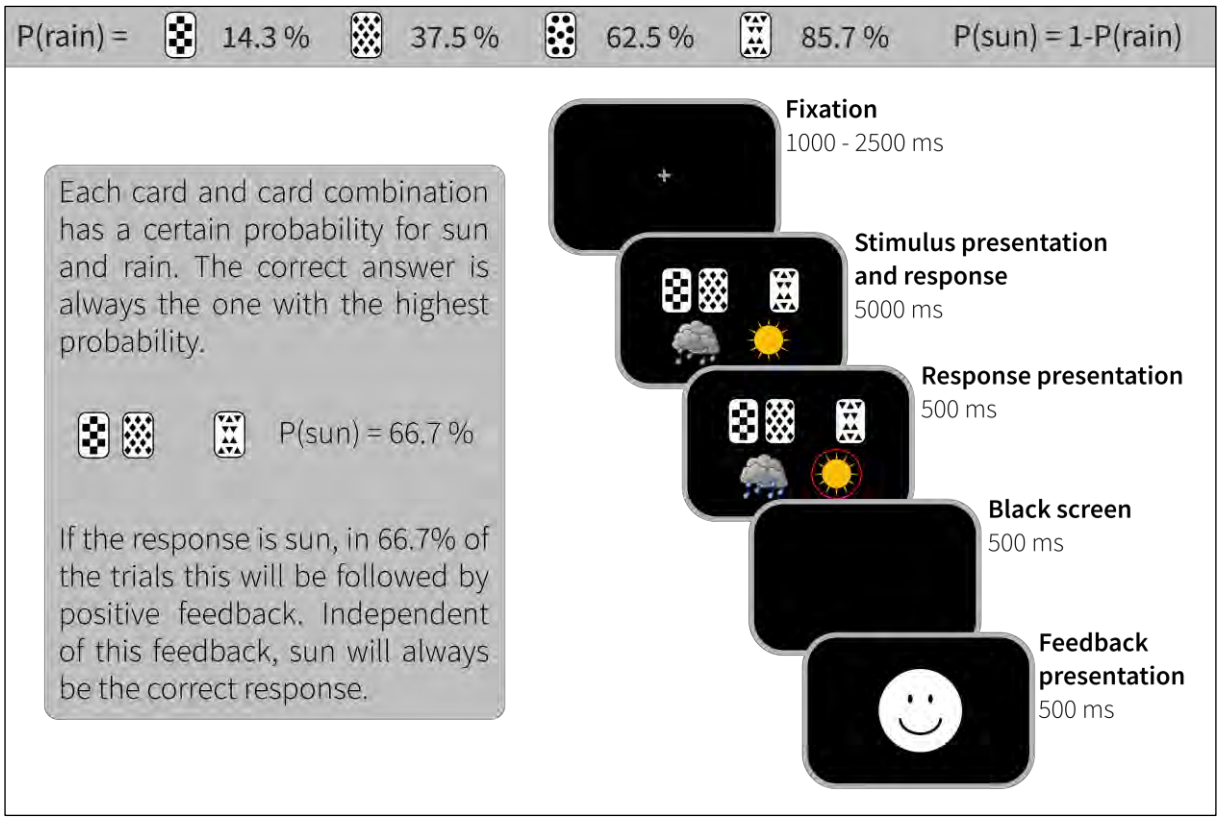


Figure 12. WPT card probabilities and design of the EEG experiment. After a fixation period, participants see 1 of 14 possible card combinations. After they choose 'sun' or 'rain', they will receive feedback in form of a happy or sad face. Event-related potentials (ERPs) with respect to feedback onset were analyzed.

Throughout the experiment, subjective mood and blood pressure were measured and saliva samples for cortisol analyses were sampled (**Figure 13**). Subjective and physiological measurements were analyzed using mixed-design ANOVAs with time as within-subject and experimental manipulation (TSST vs. control) as well as ADRA2B deletion genotype (carriers vs. non-carriers) as between-subjects factor. To assess PCL performance, another mixed ANOVA with 10-trial blocks as within factor was used. EEG data were analyzed using a mixed design ANOVA with electrode site (FC1, Fz, FCz, FC2) and feedback (positive vs. negative) as within-subject factors. Stress- and genotype-dependent differences in strategy use were analyzed by means of χ^2 tests.

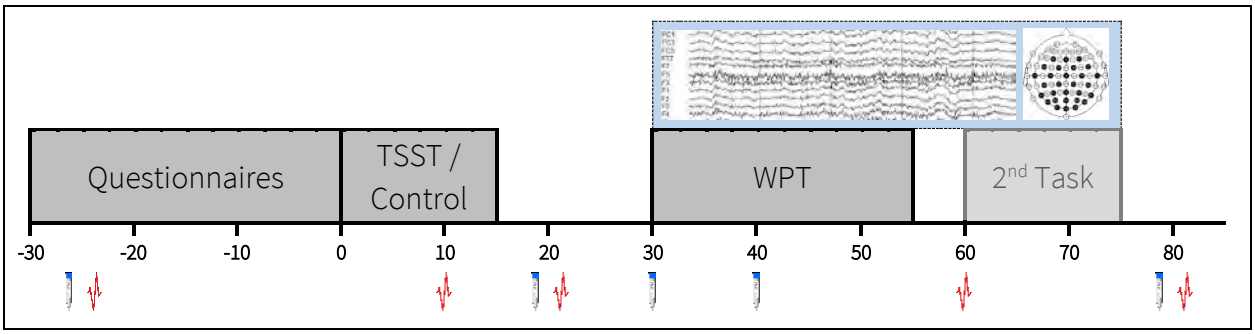


Figure 13. Experimental procedure of the EEG experiment. The WPT was performed 30 to 55 min after stress or control manipulation onset (T = 0 min). Saliva samples were taken 25 min before, and 20, 30, 40 and 80 min after manipulation onset. Blood pressure was measured 25 min before, during, as well as 20 and 80 min after manipulation onset.

Since replication studies are particularly important in genetics and with the aim to investigate group differences in activation and connectivity patterns during PCL performance, we conducted a second experiment. In this second experiment, 128 volunteers participated (62 women; mean age 25.1 ± SD: 3.5 years; stress group: 32 carriers, 33 non-carriers; control group: 31 carriers, 32 non-carriers), fulfilling the same inclusion criteria as in our first experiment with the addition of criteria relevant for fMRI measurements. After performing the stress or control manipulation, participants performed the WPT inside an MRI scanner. In addition to 100 WPT trials, participants completed 100 visuo-motor control trials, in which they were asked to indicate whether <2 or ≥2 cards were presented on the screen (Figure 14).

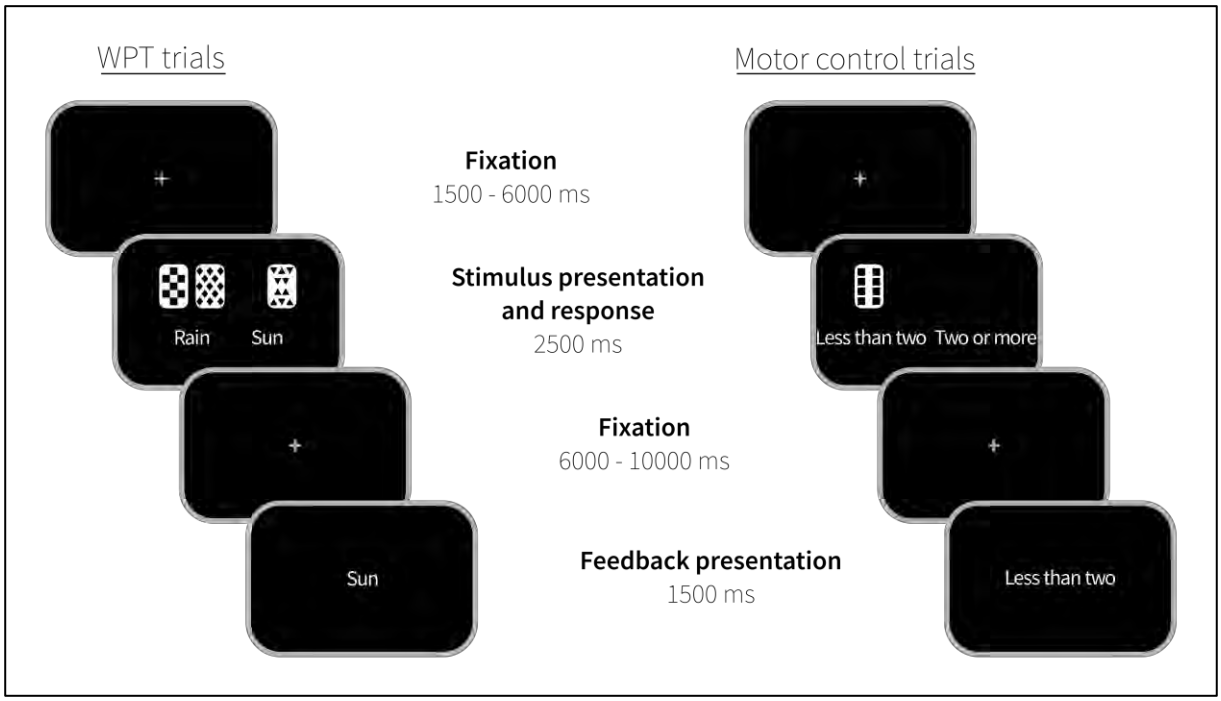


Figure 14. WPT and motor control trials in the fMRI experiment. WPT trials are similar to the EEG trials with the exceptions that the timing is adapted to the BOLD response and feedback is given by showing the correct answer. In motor control trials participants indicate whether <2 or ≥2 cards are presented.

Also, the timing of the task was adapted to the slow BOLD response. As before, subjective and physiological measures were taken throughout the experiment (Figure 15). Participants were genotyped for the ADRA2B deletion, their WPT strategy was calculated, and similar statistical analyses as in our first experiment were performed. The fMRI data acquired with a 3T Trio Scanner equipped with a 32-channel head coil (37 transversal slices, repetition time (TR) = 2000 ms, echo time (TE) = 30 ms) were preprocessed and analyzed using SPM12. Due to technical difficulties and excessive head motion, the final sample consisted of 120 participants (N = 30/group). Control and PCL trials were modeled and contrast images for group differences in activation (PCL minus

control) and functional connectivity by means of psycho-physiological interaction (PPI) analyses (PCL correct minus PCL incorrect) were generated. In addition to explorative whole-brain analysis ($p < 0.05$ threshold at cluster level, family-wise error (FWE) correction), region of interest (ROI) analyses were performed (amygdala, hippocampus, caudate nucleus, putamen; small volume correction (SVC) $p < 0.05$ uncorrected threshold followed by FWE correction).

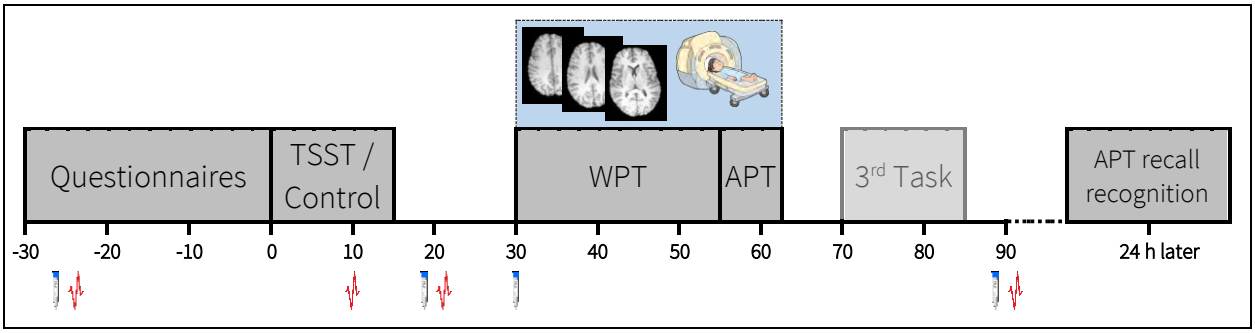


Figure 15. Experimental procedure of the fMRI experiment. The WPT was performed 30 to 55 min after stress or control manipulation onset (T = 0 min). Saliva samples were taken 25 min before, and 20, 30 and 90 min after manipulation onset. Blood pressure was measured 25 min before, during, as well as 20 and 90 min after manipulation onset.

2.1.3 Results

Verification of the successful stress induction was provided by increases in negative mood, blood pressure and salivary cortisol. These measures were not modulated by genotype. Peak cortisol concentrations were reached immediately before participants performed the WPT. Correct responses in the PCL task improved from 59 to 74 % and neither stress nor genotype showed any effects. The strategy analyses replicated previous findings (Schwabe and Wolf, 2012; Schwabe et al., 2013b) by showing that stressed compared to control participants used significantly more dorsal striatum-dependent multi-cue strategies. Importantly, these effects were modulated by genotype, in that only in ADRA2B deletion non-carriers did stress increase multi-cue strategy use (70 to 88 %; Figure 16), whereas the difference between stress and control participants was not significantly different in deletion carriers. Our EEG data revealed an augmented FRN for negative feedback in stressed participants, a finding that has been shown in another feedback-based learning task as well (Glienke et al., 2015) and that may point to increased dorsal striatum-dependent processing in stressed individuals, which is in line with increased multi-cue strategy use in these participants.

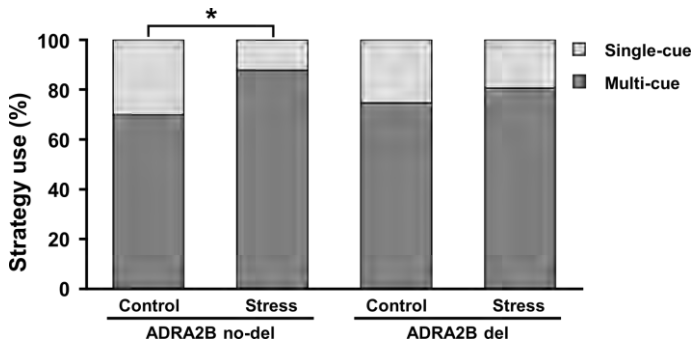


Figure 16. Strategy use in ADRA2B deletion carriers in exp. I. Stress increased the bias toward more multi-cue strategies in ADRA2B deletion non-carriers but not in ADRA2B deletion carriers (from Wirz et al., 2017a). *$p < 0.05$

Similar to our first experiment, subjective and physiological measures increased in response to stress and cortisol peak concentration were reached immediately prior to task performance in the MRI scanner. Participants' PCL performance increased from 37 to 63 % with no effects of stress or genotype. Strategy analyses showed that ADRA2B deletion carriers used overall more single-cue strategies. As shown before, stress was associated with a significant increase in multi-cue strategy use. More importantly, we replicated the results from our first experiment by showing that only in ADRA2B deletion non-carriers did we observe a significant increase in dorsal striatum-dependent multi-cue strategies (48 to 77 %; Figure 17A). The functional neuroimaging data shed more light on the underlying mechanisms of these effects. In response to stress, activation of the caudate nucleus during task performance was significantly increased. In line with the role of the amygdala in orchestrating the memory shift under stress, functional connectivity of the amygdala with the putamen was increased, whereas the crosstalk of the amygdala with the CA (cornu ammonis) subregion of the hippocampus was diminished. Importantly, functional connectivity of the amygdala with the dorsal striatum and hippocampus was modulated by ADRA2B genotype. Specifically, deletion carriers generally showed increased coupling of the amygdala with the EC, the main hippocampal input region (Figure 17B). In line with a genotype-dependent modulation of the effects of stress, we found that the functional connectivity of the amygdala with the putamen was enhanced only in stressed ADRA2B deletion non-carriers but not in deletion carriers (Figure 17C).

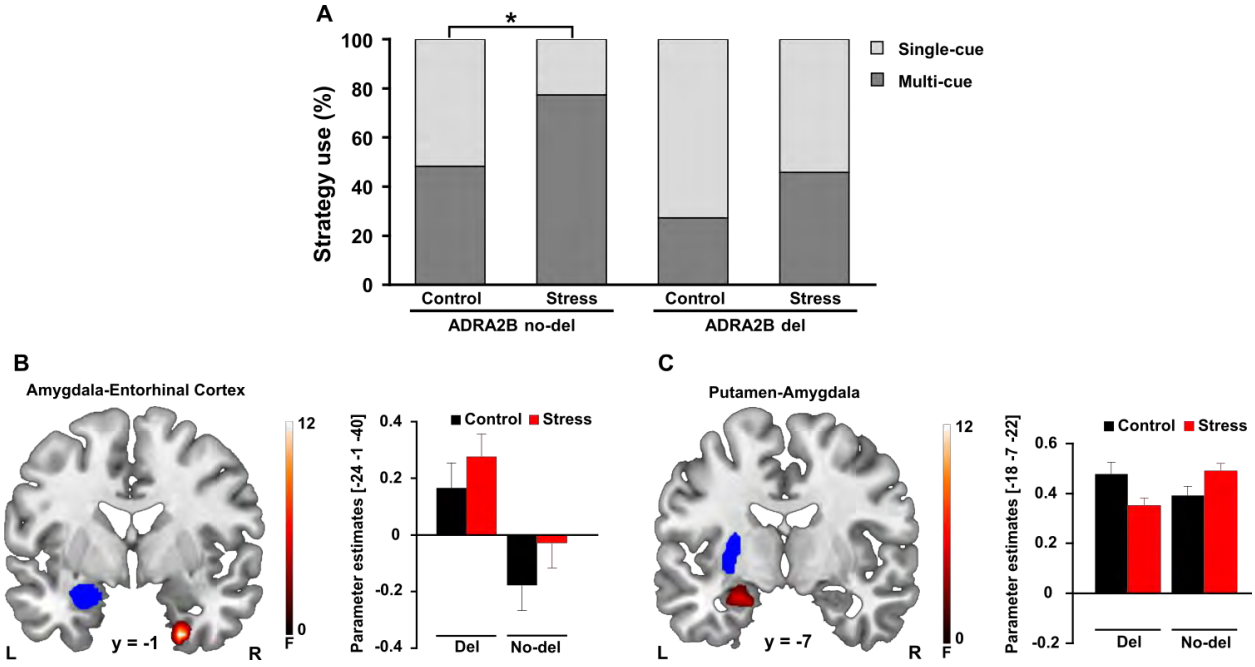


Figure 17. Strategy use and ADRA2B effects on functional brain connectivity in exp. II. (A) Stress biased learning toward multi-cue strategies only in ADRA2B deletion non-carriers but not in ADRA2B deletion carriers. (B) Independent of stress, ADRA2B deletion carriers showed enhanced functional connectivity of the left amygdala with the right entorhinal cortex $p_{FWE} < 0.05$). (C) Under stress, ADRA2B deletion non-carriers compared to carriers showed increased amygdala-putamen connectivity $p_{FWE} = 0.006$, whereas genotype groups did not differ under control conditions ($p_{FWE} = 0.352$; from Wirz et al., 2017a). Error bars indicate SEM. *$p < 0.05$, L left, R right

2.1.4 Discussion

For the first time and in two independent experiments, we showed that the ADRA2B deletion modulated stress effects on the engagement of multiple memory systems. In line with previous studies (Schwabe and Wolf, 2012; Schwabe et al., 2013b), stress induced a shift from hippocampus-dependent cognitive toward dorsal striatum-dependent habit memory. Increased recruitment of the dorsal striatum after stress was shown by an enhanced FRN and enhanced dorsal striatal activation during PCL. Also, functional connectivity data revealed an alleviated connectivity of the amygdala with the hippocampus, whereas the crosstalk of the amygdala with the putamen was increased after stress. This is in line with previous studies showing that the preferable engagement of habit memory can be realized by an impaired hippocampal as well as by a strengthened dorsal striatal system after stress (Schwabe and Wolf, 2012; Schwabe et al., 2013b; Vogel et al., 2015). Importantly, the shift toward dorsal striatum-dependent habit memory was reduced in deletion carriers, an effect which seems to be mediated by enhanced and reduced connectivity of the amygdala with the hippocampus and dorsal striatum, respectively. Generally, ADRA2B deletion carriers displayed a stronger connectivity of the amygdala with the EC, which may account for their superior episodic memory for emotional material (de Quervain et al., 2007a;

Rasch et al., 2009). This enhanced engagement of the hippocampus under no-stress conditions in deletion carriers may have prevented a shift toward the dorsal striatum after stress, so that in response to stress, coupling of the amygdala with the putamen only increased in deletion non-carriers, which is in accordance with increased dorsal striatum-dependent, multi-cue strategy use in these participants. The ADRA2B deletion has been linked with increased noradrenergic activation (Small et al., 2001), which in combination with animal data showing a bias toward the dorsal striatum after α_2-AR antagonist administration seems to be at odds with our findings. However, the α_{2B}-AR subtype may act differently than an unspecific α_2-AR antagonist, and acute changes induced by an antagonist can hardly be compared to genetic effects associated with a general adaptation of the NA system (Small et al., 2001). Considering the increased traumatic memory and re-experiencing symptoms observed in deletion carriers (de Quervain et al., 2007a), our findings may suggest that the impaired ability to switch to a more appropriate memory system in response to stress may render ADRA2B deletion carriers particularly vulnerable for developing PTSD (Liberzon et al., 1999; Liberzon et al., 2014; Schwabe, 2017).

2.2 NR3C2 haplotype facilitates the stress-induced shift from cognitive to habit memory

Wirz L, Reuter M, Wacker J, Felten A, Schwabe L (2017b) A haplotype associated with enhanced mineralocorticoid receptor expression facilitates the stress-induced shift from "cognitive" to "habit" learning. *eNeuro* 5:e0359-17.2017. (Appendix B pages 144-160)

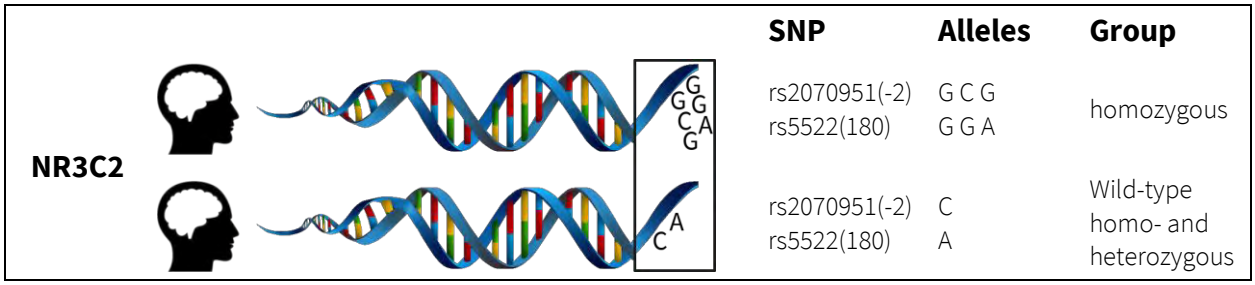
2.2.1 Background

Several pharmacological studies showed that GC binding to membrane-bound MRs is crucial for the stress-induced shift from hippocampus-dependent cognitive toward dorsal striatum-dependent habit memory (Schwabe et al., 2013b; Vogel et al., 2015; Vogel et al., 2016). It thus seems highly likely that, in addition to individual differences in the noradrenergic system (Wirz et al., 2017a), genetic variability in the MR gene contributes to differences in stress effects on the relative engagement of multiple memory systems. Two common variants in the MR gene are the MR-2G/C (rs2070951) and MRI180V (rs5522) SNPs. The major/wild-type alleles (rs2070951 C and rs5522 isoleucine (A)) have been associated with enhanced MR expression and transactivation activity *in vitro* (DeRijk and de Kloet, 2008; van Leeuwen et al., 2010), as well as altered HPA axis reactivity *in*

vivo (DeRijk, 2009). In contrast, the minor alleles (rs2070951 G and rs5522 valine (G)) showed, similar to blockade of the MR (Schwabe et al., 2013b; Vogel et al., 2015; Vogel et al., 2016), enhanced cortisol levels after stress (DeRijk et al., 2006). Since these two MR SNPs are in high linkage disequilibrium, they together form haplotypes, and the CA (rs2070951 C and rs5522 A) haplotype in particular has been associated with enhanced resilience against depression (Klok et al., 2011) and traumatic stress (ter Heegde et al., 2015; de Kloet et al., 2016). Considering the importance of the MR in the stress-induced shift toward dorsal striatum-dependent habit memory and the extensive differences in MR functionality as determined by several MR SNPs, we hypothesized that carriers of an MR haplotype, comprised of MR SNPs associated with enhanced MR expression and transactivation, would facilitate the stress-induced shift toward dorsal striatum-dependent memory.

2.2.2 Methods

For this purpose we reanalyzed the data from our two experiments and investigated group differences in MR haplotype carriers and non-carriers. In addition to the ADRA2B deletion, we genotyped participants for 7 MR gene SNPs (rs1512344, rs2070950, rs2070951, rs4835519, rs5522, rs5534, rs7658048) on which linkage analyses were performed and haplotypes were calculated. Homo- and heterozygous carriers of an MR haplotype containing SNP alleles associated with enhanced MR expression and transactivation (rs2070951 C and rs5522 A) were tested against homozygous non-carriers (**Figure 18**). We performed similar analyses as before to investigate group differences in stress induction, PCL performance and strategy use, and the neural correlates of these effects assessed by EEG and fMRI measurements. In addition to the FRN, we calculated the P3a (mean activity 235-425 ms post feedback at C1, Cz, C2) and P3b (mean activity 270-420 ms post feedback at P1, Pz, P2), ERP components known to facilitate attention and to promote memory processes (Polich, 2007). In our first experiment, behavioral analyses included 252 participants (stress group: 90 carriers, 36 non-carriers; control group: 101 carriers, 25 non-carriers), whereas EEG analyses were performed on 228 participants (stress group: 81 carriers, 33 non-carriers; control group: 91 carriers, 23 non-carriers). In our second experiment, the sample size for the behavioral analyses constituted 128 participants (stress group: 50 carriers, 15 non-carriers; control group: 48 carriers, 15 non-carriers), whereas fMRI data were available from 120 participants (stress group: 47 carriers, 13 non-carriers; control group: 45 carriers, 15 non-carriers).



SNP	Alleles	Group
rs2070951(-2) rs5522(180)	G C G G G A	homozygous
rs2070951(-2) rs5522(180)	C A	Wild-type homo- and heterozygous

Figure 18. MR haplotype. Homo- and heterozygous carriers of a haplotype containing the rs2070951 C and rs5522 A alleles were tested against homozygous non-carriers.

2.2.3 Results

The MR haplotype analyses showed significantly strong linkage between all but one (rs5534) MR SNP. The haplotype including the rs2070951 C and rs5522 A alleles was of particular interest (alleles in order of all SNPs: CCCTAG) and tested against carriers of the other haplotypes that were found not containing these high-functioning MR SNP alleles. In our first experiment, this MR haplotype did not affect any of the subjective or physiological measures, neither in the stress, nor in the control condition. Importantly, whereas MR genotype did not affect PCL performance, it modulated strategy use in response to stress, in that only MR haplotype carriers showed the shift toward dorsal striatum-dependent multi-cue strategy use (71 to 87 %; Figure 19A). Our EEG data did not reveal a modulatory effect of MR genotype on the FRN, but the P3a was shown to be generally reduced in MR haplotype carriers, an effect already observed at earlier ERP components (P2, N2; Figure 19B).

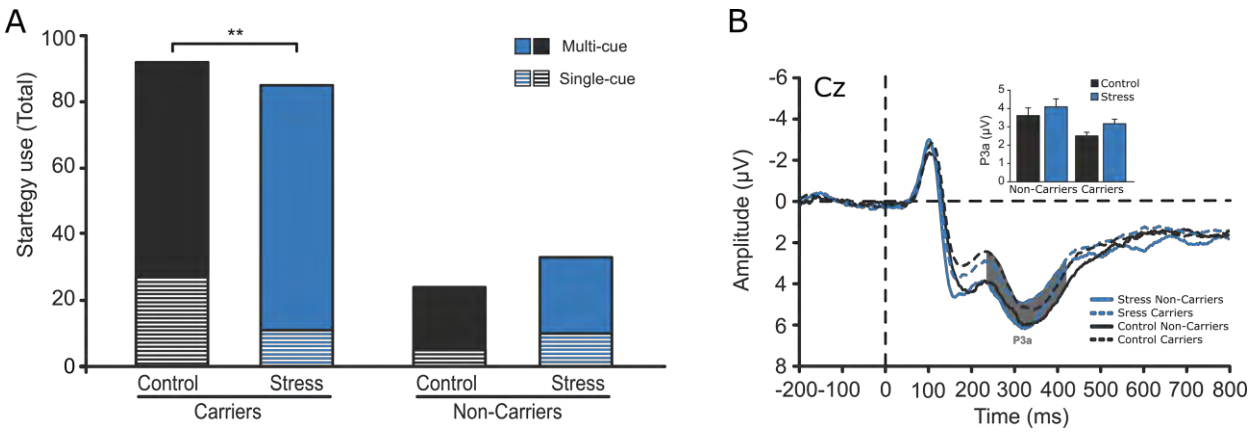


Figure 19. Strategy use and MR haplotype modulation of EEG data in exp. I. (A) Stress induced a bias toward dorsal striatum-dependent, multi-cue strategy use only in MR haplotype carriers but not in non-carriers. (B) Irrespective of stress, MR haplotype carriers showed a reduced P3a at central electrodes (C1, Cz, C2), calculated as the mean activity in the time window between 235-425 ms following feedback presentation (from Wirz et al., 2017b). Error bars indicate SEM. $^{**}p < 0.01$

In our second experiment, cortisol levels were modulated by the MR haplotype. Specifically, in line with enhanced cortisol levels in MRI180V A carriers after stress (DeRijk et al., 2006), cortisol levels were generally lower in MR haplotype carriers, an effect that tended to be stronger in response to the stress compared to the control condition (**Figure 20A**). Analysis of the behavioral data collected in our second experiment replicated the results from our first experiment. The MR haplotype did not affect PCL task performance but most importantly, the stress-induced shift toward dorsal striatum-dependent strategies was mainly confined to carriers of the MR haplotype (**Figure 20B**).

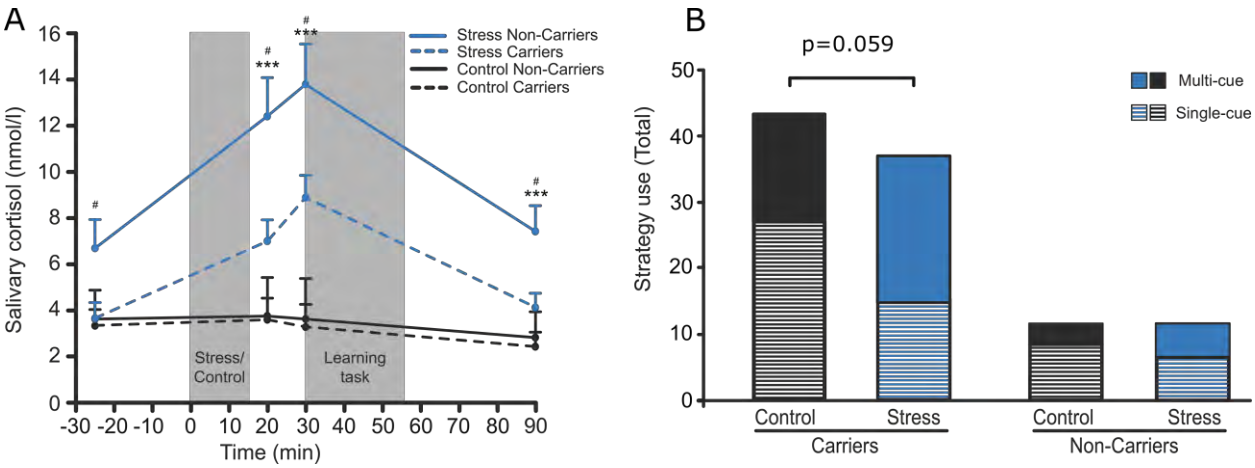


Figure 20. Cortisol levels and strategy use in MR haplotype carriers in exp. II. (**A**) Salivary cortisol levels increased in response to the Trier social stress test (TSST) but were generally reduced in MR haplotype carriers. (**B**) Stress induced a bias toward dorsal striatum-dependent, multi-cue strategy use only in MR haplotype carriers but not in non-carriers (from Wirz et al., 2017b). Error bars indicate SEM. Stress × time of measurement $^{***}p < 0.001$ MR haplotype $^{\#}p < 0.05$

The neuroimaging data showed stress-induced increases in caudate nucleus and amygdala activation, as well as reduced functional connectivity of the amygdala with both the CA and EC hippocampal subregions. Importantly, the MR haplotype modulated activation and connectivity patterns. Specifically, activation in the hippocampus was reduced in stressed MR haplotype carriers, whereas the stress-induced increase in amygdala activity was only observed in MR haplotype non-carriers (**Figure 21A**). Further, generally reduced activation in the caudate nucleus and putamen was found in MR haplotype carriers (**Figure 21B**). Interestingly, relative to non-carriers, MR haplotype carriers showed reduced amygdala-parahippocampus coupling after stress and enhanced amygdala-caudate nucleus connectivity under control conditions (**Figure 21C**). Due to MR haplotype-dependent changes in cortisol levels, we additionally tested for mediation and moderation effects but found no significant effects with respect to strategy use that depended on these group differences in cortisol levels, nor did our functional imaging data crucially change when cortisol was added as a covariate.

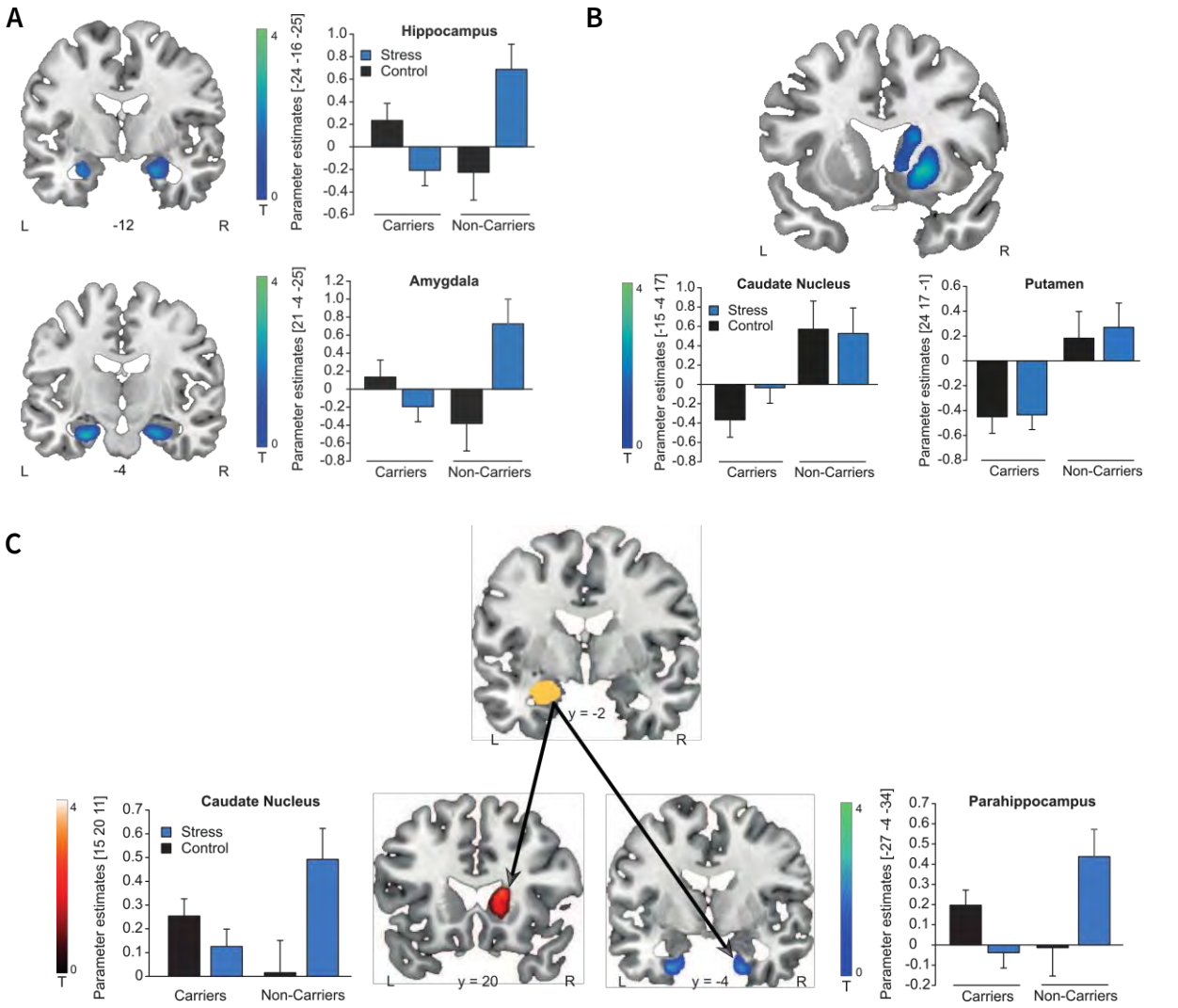


Figure 21. MR haplotype effects on brain activity and functional connectivity in exp. II. (A) Under stress, MR haplotype carriers showed reduced bilateral activation of the amygdala (both $p_{FWE} < 0.067$) and the hippocampus (both $p_{FWE} < 0.047$). (B) Independent of stress, MR haplotype carriers showed reduced activation of the right caudate nucleus ($p_{FWE} < 0.032$) and putamen ($p_{FWE} < 0.006$). (C) Under stress, MR haplotype carriers showed reduced amygdala-anterior parahippocampus connectivity (both $p_{FWE} < 0.063$), whereas under control conditions, amygdala-caudate nucleus coupling was increased ($p_{FWE} < 0.018$; from Wirz et al., 2017b). Error bars represent SEM. L left, R right

2.2.4 Discussion

In addition to the evidence provided by our first set of analyses showing that a deletion in the α_{2B}-AR gene modulated the effects of stress on the engagement of multiple memory system, a similar modulation was shown for a haplotype containing MR SNP alleles associated with increased MR expression and functionality. This MR haplotype was found to facilitate the stress-induced shift from hippocampus-dependent cognitive toward dorsal striatum-dependent habit memory, an effect mainly associated with impaired hippocampal processing and reduced amygdala-hippocampus coupling after stress. Membrane-bound MRs are particularly important mediators of rapid non-genomic GC effects (Joëls et al., 2008), and the necessity for a functioning MR system

in the stress-induced shift toward the dorsal striatum has previously been demonstrated (Schwabe et al., 2010b; Schwabe et al., 2013b). The findings that the P3a was generally reduced in MR haplotype carriers and that hippocampus activation was reduced in MR haplotype carriers after stress suggests reduced functioning of the hippocampal system, which may render these participants particularly vulnerable to the effects of stress. Together with the stress-induced decrease in the crosstalk of the amygdala with the parahippocampus, an MTL structure adjacent to the hippocampus and similarly involved in episodic memory (Hayes et al., 2007), these data fit very well with the existing literatures proposing a stress-induced shift toward dorsal striatum memory which may at least partly be caused by impaired hippocampal functioning (Schwabe and Wolf, 2012; Schwabe et al., 2013b). Increased connectivity of the amygdala with the caudate nucleus under no-stress conditions may also point to a general vulnerability for the stress-induced shift in MR haplotype carriers. Reduced amygdala activation after stress and reduced caudate and putamen activation under control conditions in MR haplotype carriers are less easy to interpret but may be understood in terms of enhanced cortical efficiency (Rypma et al., 2006), which may have facilitated the shift toward the dorsal striatum after stress. Importantly, our observations that differences in cortisol levels were only found in our second experiment and that results remained largely unchanged when these differences were accounted for suggest that MR haplotype effects do not simply come to pass through differences in cortisol concentrations. Upregulation of receptor density or increased binding efficiency may be involved, potential mechanisms which require further investigations by molecular studies. In line with a protective role of the high-functioning MR SNPs included in the MR haplotype (DeRijk et al., 2011; Klok et al., 2011; de Kloet et al., 2016), the shift toward the dorsal striatum in MR haplotype carriers may be an important adaptive response in the face of adversity. In the light of GC-based interventions that have been proposed for the treatment of PTSD, the investigation of genetically determined individual differences may aid the development of more personalized treatment strategies.

2.3 A Bayesian approach to gene × gene interactions

In addition to and as an extension of these findings, I present further analyses with the purpose of investigating gene × gene modulations of stress effects on multiple memory systems. Results will be discussed with respect to evidence for the alternative as well as the null hypothesis using a Bayesian approach.

2.3.1 Background

Previous studies suggest that anxiogenic drug injections into the amygdala as well as administration of GCs in animals are sufficient to induce a shift toward habit learning (Packard and Wingard, 2004; Schwabe et al., 2010b). The importance of MR-dependent GC actions in the stress-induced shift toward habits has been confirmed in humans as well (Schwabe et al., 2013b; Vogel et al., 2017). As opposed to the compelling evidence for the facilitation of noradrenergic effects on memory consolidation by GCs that interact in the BLA (Roozendaal et al., 2009), whether such interactions play a role in stress effects on multiple memory systems is still unknown. Since we showed evidence for the modulation of stress effects on multiple memory systems by a deletion of the α_{2B}-AR gene as well as by an MR haplotype, which supports the important influence of noradrenergic and MR-mediated GC effects, investigating potential interactions between these genetic variants allows pooling of the relative small effects of individual polymorphisms, may encourage future pharmacological studies and provide evidence for genetic interactions between these systems in their effects on the engagement of multiple memory systems under stress.

2.3.2 Methods

In behavioral genetics, studies require large sample sizes to reliably detect significant genotype-dependent differences in behavior (Rasch et al., 2010). Therefore and for the purpose of detecting possible gene × gene interactions, the raw data sets from our EEG and fMRI experiments were combined and re-analyzed using JASP (Version 0.8.5.1). Group differences in single- and multi-cue strategy use were investigated using χ^2 tests with the factors experimental manipulation (control vs. stress), ADRA2B (wild-type vs. deletion) and MR haplotype (carriers vs. non-carriers). Although the ADRA2B and NR3C2 genes are located on different chromosomes (2 and 4, respectively) which precludes them from being in high genetic linkage, this was additionally tested using a χ^2 tests. In

addition to p-values, Bayes factors (BFs) for the null as well as for the alternative hypothesis are reported.

2.3.3 Results

In accordance with the assumption that the ADRA2B and NR3C2 genes are unlinked, the proportions of ADRA2B deletion carriers and non-carriers are similar in MR haplotype carriers and non-carriers ($\chi^2_{(1)}$ = 0.140, p = 0.708). Replicating the results of our separate analyses, ADRA2B deletion non-carriers ($\chi^2_{(1)}$ = 9.512, p = 0.002) and MR haplotype carriers ($\chi^2_{(1)}$ = 10.938, p < 0.001) show a stress-induced increase in dorsal striatum-dependent multi-cue strategies. Bayes factors of 21.873 and 35.110, respectively, indicate very strong evidence for these differences in strategy use. Evidence for the null hypothesis in ADRA2B deletion carriers (BF = 2.532) and non-carriers of the MR haplotype (BF = 3.704), however, is only mediocre. Interestingly, our results suggest that ADRA2B deletion non-carriers only use more multi-cue strategies under stress when they also carry the MR haplotype ($\chi^2_{(1)}$ = 8.183, p = 0.004) but not when they do not carry the MR haplotype ($\chi^2_{(1)}$ = 1.815, p = 0.178; **Figure 22**). Given the small sample size of participants not carrying both the ADRA2B deletion and the MR haplotype (N = 33) and as is supported by a rather small Bayes factor (1.110) for the null hypothesis, there is, however, only very little evidence that there are indeed no differences in strategy use between the control and stress condition in participants carrying both of these gene variants. Contrary to our finding that ADRA2B deletion carriers do not show a stress-induced increase in multi-cue strategies, deletion carriers who also carry the MR haplotype show this shift after stress ($\chi^2_{(1)}$ = 4.065, p = 0.044; **Figure 22**), which indicates that the MR gene may overrule the effects that the ADRA2B genotype has by itself. Given a BF of only 1.485, however, further investigation of this possibility is required.

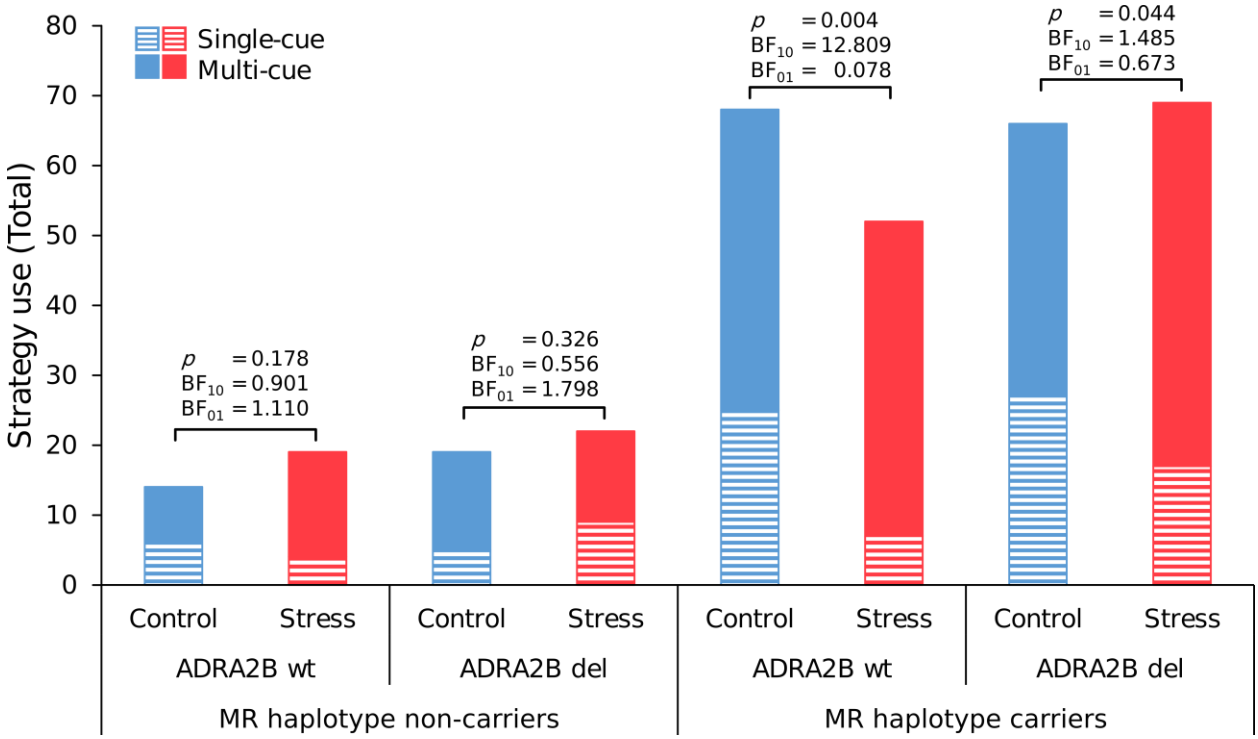


Figure 22. Combined data of strategy use in ADRA2B deletion and MR haplotype carriers in exp. I and II. Independent of the ADRA2B deletion, MR haplotype carriers use significantly more dorsal striatum-dependent, multi-cue strategies after stress, whereas the shift is not observed in MR haplotype non-carriers.

2.3.4 Discussion

Genes do not work in isolation but interact with one another. Instead of having additive effects, multiple genes that contribute to a single phenotype often show epistatic interactions. In such a gene × gene interaction, one gene may for example prevent another gene's phenotypic characteristic. Importantly, it has been suggested that negligence of these interactions is one of the reasons why candidate gene studies are often not successfully replicated (Moore and Williams, 2009). Naturally and importantly, gene × environment interactions as they were investigated in this dissertation by looking at genotype-dependent differences in behavior under stress and no-stress conditions, render the reality even more complex. The results presented here suggest that there may be epistatic interactions between the ADRA2B and NR3C2 genes and that the NR3C2 gene is dominant. Specifically, alleles of MR SNPs that are associated with enhanced MR expression and transactivational capacity may, if absent, prevent phenotypic effects of the ADRA2B genotype and at the same time, if present, may provoke those effects in the ADRA2B deletion carriers that are usually not seen when this genotype is investigated on its own. Thus, the MR haplotype may be associated with an increased shift toward dorsal striatum-dependent, habit memory even in ADRA2B deletion carriers, who, in isolation, usually do not show this shift under stress. This would

indicate that rather than noradrenergic activity, it is the efficiency of GC actions via MRs that drives the shift toward dorsal striatum-dependent habit memory under stress. Although this is an intriguing thought, Bayesian analyses lend only little support for such interactions, which may be owed to small sample sizes and the fact that due to relatively few homozygous ADRA2B deletion carriers, homo- and heterozygous carriers were grouped together. Although very common and necessary when investigating certain genetic variants with a very low probability for homozygocity, this may obscure pure effects of homozygous allele carriers. Independent of these interactions which require further research, the Bayes factors for the independent effects of the ADRA2B deletion and MR haplotype are in line with our findings presented before (Wirz et al., 2017b; Wirz et al., 2017a) and lend great support for our hypothesis that stress effects on the engagement of multiple memory systems are modulated by genetic variants known to be involved in stress and memory processes.

3. CNR1 polymorphism modulates affective processing under stress

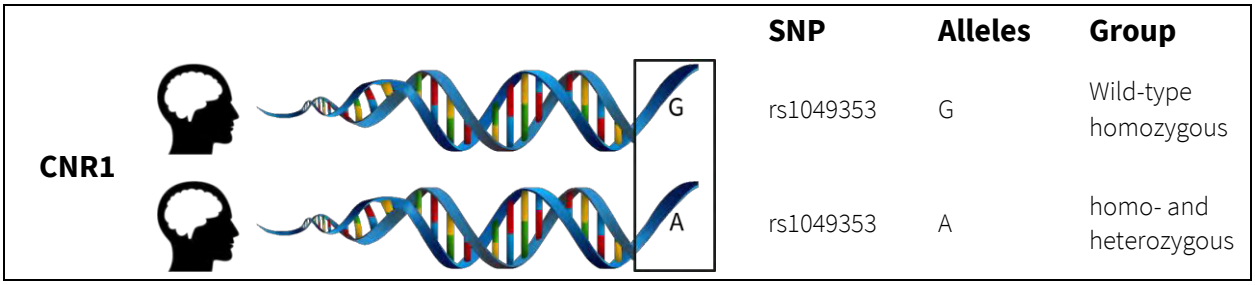
Wirz, L., Reuter, M., Felten, A., Schwabe, L. (*under revision*) An endocannabinoid receptor polymorphism modulates affective processing under stress. *Soc Cogn Affect Neurosci*. (Appendix C pages 161-190)

3.1 Background

The eCB system is a retrograde messenger system which can quickly inhibit glutamatergic and GABA-ergic cells via presynaptic CB1Rs, thereby being in a prime position to modulate stress effects on affective processing (McLaughlin et al., 2014). Through eCB-induced disinhibition of the medial PFC or direct actions in the BLA, eCBs may reduce GC secretion as well as excessive responding to affective stimuli, thereby acting as an emotional buffer system (Ganon-Elazar and Akirav, 2009; Hill et al., 2011; McLaughlin et al., 2014). This is in line with the anxiolytic properties of AEA (Lutz et al., 2015) and is supported by reduced amygdala activation in response to negative stimuli after eCB agonist administration (Phan et al., 2008; Bossong et al., 2013). Only relatively little evidence exists with respect to genetic variants in the CB1R gene. The A allele of the rs1049353 SNP has been associated with a reduced risk for depression after stressful events (Agrawal et al., 2012), suggesting that this allele may be a protective factor against dysfunctional affective processing. In line with this evidence, we hypothesized that the rs1049353 A allele may modulate affective processing after stress, possibly by changing mPFC functioning.

3.2 Methods

To investigate genotype-dependent differences in affective processing after stress, 139 participants from our second experiment were genotyped for the rs1049353 SNP and another task was administered (67 women; mean age 23.4 ± SD: 3.5 years). Due to technical difficulties, excessive head motion and missing data for the CNR1 SNP, 61 homo- and heterozygous A allele carriers were tested against 71 homozygous G allele carriers (stress group: 33 AA/AG genotype carriers, 38 GG genotype carriers; control group: 28 AA/AG genotype carriers, 33 GG genotype carriers; Figure 23).



SNP	Alleles	Group
rs1049353	G	Wild-type homozygous
rs1049353	A	homo- and heterozygous

Figure 23. CNR1 genotype. Homo- and heterozygous A allele carriers were tested against homozygous G allele carriers.

After participants had been exposed to the stress or control manipulation and had performed the WPT, 25 negatively and 25 neutrally valenced pictures were presented to them inside the MRI scanner (Figure 24). Each picture was presented for 2.5 sec and participants were asked to rate the emotionality of the picture on a scale ranging from negative, rather negative and rather neutral to neutral. Although our main goal was to investigate genetic modulations of stress effects on affective processing, we also investigated possible effects on emotional memory formation. Therefore, surprise free recall and forced-choice recognition tests (participants also indicated their certainty during recognition) were administered 24 h later. Subjective and physiological measures were analyzed as described before. Mixed design ANOVAs with emotionality (negative vs. neutral) as within-subject factor and experimental manipulation (stress vs. control) as well as CNR1 genotype (AA/AG genotype vs. GG genotype) as between-subjects factor were used to investigate group differences in picture and certainty ratings, free recall and recognition performance. With respect to memory performance, the number of correctly recalled pictures in the free recall as well as hits and false alarms in the recognition test were used as dependent variables. In addition, d-prime (d') was calculated for negative and neutral pictures. Our functional imaging data were preprocessed, negative and neutral images were modeled and contrast images for group differences in activation and functional connectivity (PPI analyses) patterns were generated. Explorative whole-brain and ROI analyses were performed with ROIs selected due to their importance for affective processing and memory formation (amygdala, insula, hippocampus, mPFC, vmPFC, ventrolateral (vl)PFC). Brain activity and functional connectivity of our ROIs were also correlated with participant's individual memory performance to investigate associations between activation/connectivity during encoding and subsequent memory performance. Correlation coefficients of separate experimental group correlations were z-transformed and statistically compared to assess whether the associations of brain activation and connectivity during encoding with memory performance differed between the groups.

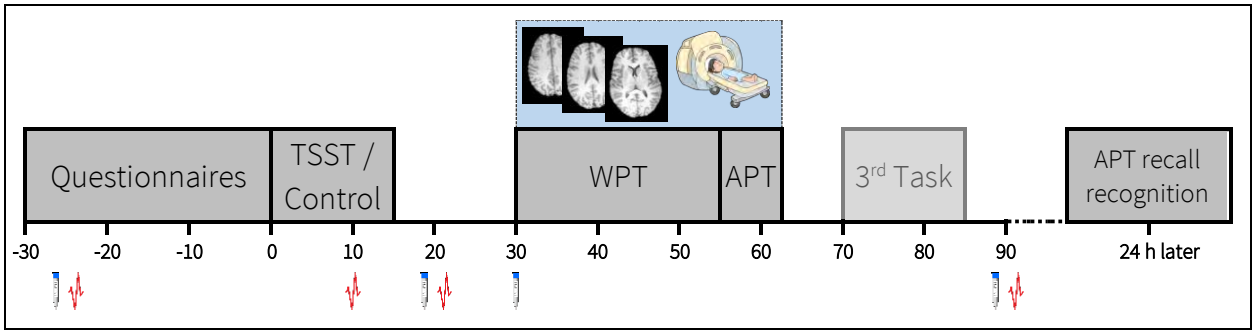


Figure 24. Experimental procedure of the fMRI experiment. The affective processing task (APT) was performed 55 to 63 min after stress or control manipulation onset (T = 0 min). Free recall and recognition tests were done 24 h later. Saliva samples were taken 25 min before, and 20, 30 and 90 min after manipulation onset. Blood pressure was measured 25 min before, during, as well as 20 and 90 min after manipulation onset.

3.3 Results

Subjective measurements, cortisol levels and systolic blood pressure were not generally nor in interaction with stress affected by CNR1 genotype. Irrespective of stress or rs1049353 genotype, negative compared to neutral picture presentation increased bilateral activation of the amygdala, vlPFC, vmPFC, insula as well as regions in the occipital and parietal cortex. Interestingly, in response to stress, AA/AG genotype compared to GG genotype carriers showed greater vmPFC activity for negative versus neutral pictures, whereas no group differences were observed in the control condition (**Figure 25A**). In addition, functional connectivity of the vlPFC with the amygdala was increased in AA/AG compared to GG genotype carriers in the control condition, whereas no genotype-dependent differences in connectivity were observed after stress (**Figure 25B**).

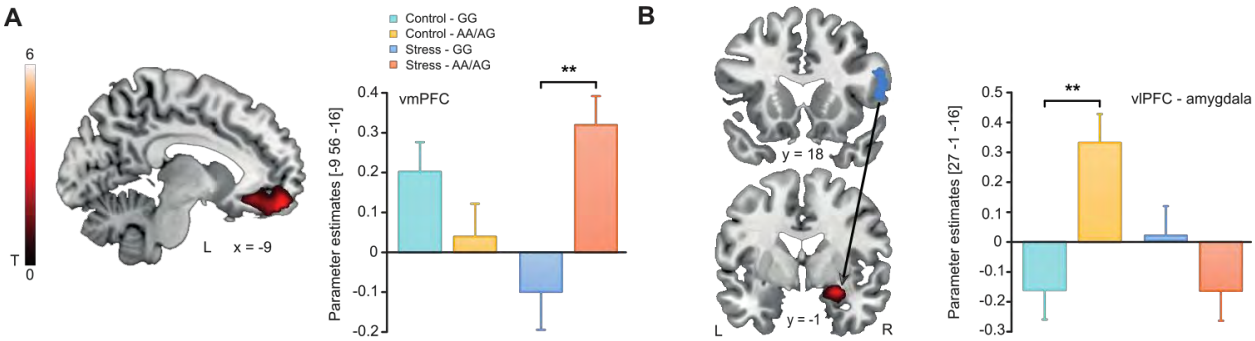


Figure 25. CNR1 genotype modulation of brain activity and functional connectivity during affective processing. (**A**) Under stress, activity in the vmPFC was increased in rs1049353 AA/AG compared to GG genotype carriers for negative compared to neutral picture processing. (**B**) Already under no-stress condition, functional connectivity of the ventrolateral prefrontal cortex (vlPFC) with the amygdala was enhanced in homo- and heterozygous A allele carriers while they processed negative compared to neutral pictures (from Wirz et al., under revision). Error bars indicate SEM. L left, R right, $^{**}p < 0.01$

The surprise free recall data were rather moderate, whereas recognition performance was overall very high. Both performance measures as well as the sensitivity index d' were improved for

negative compared to neutral images. Memory performance and confidence ratings were, however, unaffected by CNR1 genotype and stress. Our functional imaging data during picture encoding were correlated with d' and revealed a cluster including the amygdala, insula and hippocampus during negative compared to neutral picture encoding, which positively correlated with memory performance for negative items 24 h later. Importantly, in stressed AA/AG genotype carriers, emotional memory performance positively correlated with clusters in the amygdala, insula and hippocampus, whereas no such correlations were observed in GG genotype carriers (**Figure 26A**). Under control conditions, only in GG genotype carriers did we observe a significant correlation between emotional memory performance and activation of the insula, whereas no correlations were observed for AA/AG genotype carriers (**Figure 26B**). Importantly, these correlations between emotional memory performance and activation in limbic regions during negative compared to neutral picture encoding significantly differed between AA/AG and GG genotype carriers in the stress and control conditions. Interestingly, although correlations did not significantly differ between groups, functional connectivity of the hippocampus with the BLA was associated with enhanced emotional memory in stressed AA/AG but not GG genotype carriers, whereas no significant correlations were observed in the control condition (**Figure 25C**).

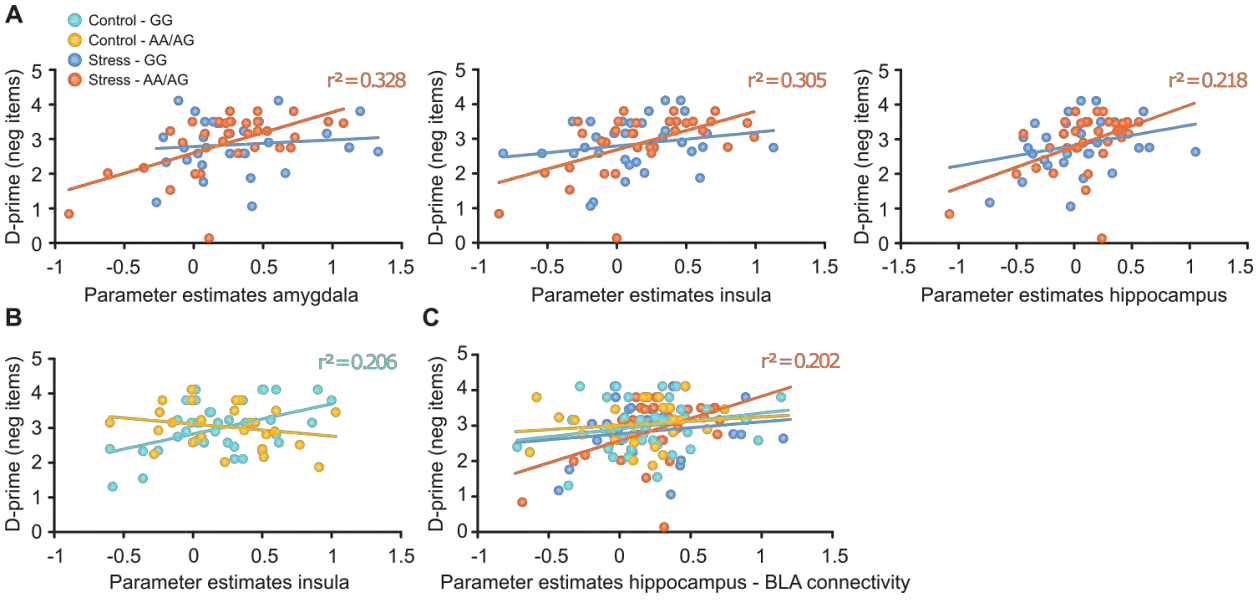


Figure 26. Correlations between brain activity/connectivity during encoding of negative compared to neutral pictures and emotional memory performance, indicated as sensitivity index d-prime (d') for negative pictures. (**A**) Under stress, activity of the amygdala, insula and hippocampus positively correlated with memory performance in AA/AG genotype carriers. (**B**) Under control conditions, insula activity positively correlated with memory performance in GG genotype carriers. (**C**) Enhanced functional connectivity of the hippocampus with the BLA during negative compared to neutral picture encoding was associated with enhanced memory performance for negative pictures only in stressed AA/AG genotype carriers (from Wirz et al., under revision). r^2 Pearson correlation coefficient squared

3.4 Discussion

In line with a crucial role for the eCB system in affective processing (Morena and Campolongo, 2014), we found modulations of brain activity and connectivity during processing of negative images by a CB1R gene variant. Specifically, stronger recruitment of the vmPFC during negative compared to neutral picture processing after stress was observed in AA/AG genotype carriers, suggesting that the rs1049353 A allele may be associated with more efficient affective processing and emotion regulation under stress. The vmPFC has been shown to regulate limbic brain regions involved in emotional response generation (Etkin et al., 2011) and enhanced vmPFC activation has been associated with reduced negative affect (Urry et al., 2006). This is in line with the importance of eCB signaling in appropriate emotional responding, possibly achieved through eCB-mediated disinhibition of the vmPFC (Morena and Campolongo, 2014). Indeed, eCB signaling, through regulation of GABAergic inhibition, is crucial for effective mPFC functioning (McLaughlin et al., 2014; Morena et al., 2016). This is consistent with the anxiolytic effects of FAAH inhibitor administration which increases AEA availability (Rubino et al., 2008) as well as with anxiety-reducing and antidepressant-like effects of CB1R agonists (Bambico et al., 2007; Akirav, 2011). Through eCB-induced increases in dopaminergic and serotonergic activation (Chiu et al., 2010; McLaughlin et al., 2012), mPFC-mediated self-focused emotion regulation and active stress coping may be achieved (Ochsner et al., 2004). These mechanisms may contribute to protective effects of the rs1049353 A allele against stress-related psychopathologies. Increased vlPFC-amygdala connectivity under control conditions in AA/AG genotype carriers may reflect increased vlPFC inhibition of the amygdala during negative stimulus processing (Banks et al., 2007; Wager et al., 2008), another mechanism through which beneficial coping in A allele carriers may be achieved.

As shown previously, negative compared to neutral information is better remembered (Hamann, 2001), an effect that may be caused by NA-GC interactions in the BLA, which then modulates episodic memory in the hippocampus (McGaugh, 2000; Roozendaal et al., 2009). Indeed, we found significant correlations between emotional memory performance and amygdala, insula and hippocampus activity. Endocannabinoids also play an important role in emotional memory formation, likely through GC-induced and GR-mediated increases in noradrenergic signaling in the amygdala (Campolongo et al., 2009; Atsak et al., 2015). Directly investigating the rs1049353 SNP, we observed a correlation of emotional memory with amygdala, insula and hippocampus only in stressed AA/AG but not in GG genotype carriers. Increased hippocampus-BLA connectivity for

negative pictures, as predicted by prominent emotional memory formation models (Roozendaal et al., 2009; Roozendaal and McGaugh, 2011; Roozendaal and Hermans, 2017), correlated with emotional memory performance only in stressed AA/AG genotype carriers, which is in line with improved emotional memory after intra-BLA CB1R agonist administration and the fact that intra-BLA CB1R blockade prevented corticosterone-induced memory enhancements, suggesting that eCBs act downstream from GCs and are required for memory effects to occur (Campolongo et al., 2009).

Thus, although no obvious behavioral differences between rs1049353 AA/AG and GG genotype carriers were observed, the genotype-induced changes in affective processing and memory formation may have important consequences for stress-related psychopathologies. Evidence comes from clinical studies (Domschke et al., 2008; Agrawal et al., 2012) and are in line with diminished striatal activation in response to happy faces in G allele carriers, which may indicate reduced social reward responsivity (Chakrabarti et al., 2006). The A allele may, through enhanced mRNA stability, improve CB1R functioning, thereby improving affect regulation in the face of stress and the incorporation of emotional information into hippocampus-dependent autobiographical memory. Although no genotype-dependent effects on cortisol levels were found, changes in other neurotransmitter systems such as serotonergic pathways may be important (McLaughlin et al., 2012). Evidence for targeting the eCB system as an effective treatment option (Ganon-Elazar and Akirav, 2013; Korem et al., 2016) in combination with proposed GC-based therapies (de Quervain et al., 2017), may hopefully advance treatment for disorders characterized by dysfunctional affective processing.

4. General discussion

Understanding the way we respond to stress and how we process affective information and engage multiple memory systems under stress will provide us with valuable information about the mechanisms of stress and the fundamental principles of vulnerability and resilience. Many factors contribute to individual differences in the effects of stress on cognition, emotion, and behavior. These are social and psychosocial factors, differences in the genetic makeup, as well as differences in upbringing and history with previous stressors, particularly when these occurred during highly sensitive developmental stages. Fully grasping each of these possible factors is likely impossible and would require endless resources and a multitude of compliant human and animal subjects. With respect to genetics, it thus seems very promising to focus on differences in highly relevant stress mediators and known polymorphisms to realize stringent hypothesis-driven testing. Although this approach will not bring forth enlightenment of everything there is to know, it will hopefully explain a profound amount of the individual variability. It will help to clarify the underlying mechanisms of stress effects on cognition, emotion and behavior, and aid the understanding of vulnerability for and resilience against stress-related psychopathologies, ultimately improving prevention and treatment options. This dissertation investigated SNPs in genes coding for the α_{2B}-AR, the MR and the CB1R, based on their highly relevant roles in stress effects on affective processing and memory. Due to the importance of noradrenergic and MR-mediated GC actions in stress effects on the engagement of multiple memory systems and due to previous research on the ADRA2B deletion and MR CA (rs2070951 C and rs5522 A) haplotype, our experimental investigations of PCL under stress focused on these polymorphisms. The eCB system just recently gained attention and is not only a highly relevant mediator of many GC-driven stress effects but also seems to be profoundly promising when it comes to resilience against stress-related psychopathologies such as PTSD and anxiety disorders. Due to the relevance of affective processing in these disorders, we investigated the influence of a CB1R gene variant on affective processing under stress. In the following, I first bring together the results of these experimental investigations, concentrating on possible mechanisms through which they may come about in the light of receptor distribution and their functions under stress. Second, I discuss implications for the development, maintenance and treatment of stress-related psychopathologies. Third, I address methodological issues and the importance of epigenetic mechanisms. Fourth, based on

our experimental results and the implications for stress-related psychiatric disorders, I propose a general model of our findings in the light of stress vulnerability and resilience. I finish my discussion by giving a short outlook for future research.

4.1 Brain systems under stress: role of genetic variability

In response to a stressful encounter, every process, ranging from perception, attention, learning, memory and executive control, happens on behalf of three crucial goals: rapid reaction to and coping with the stressor, restoration of homeostasis, and initiation of processes which will prepare the organism should the same or a similar situation reoccur. To realize these three goals, cognitive capacities available under stress are directed toward enhanced attentional vigilance (Hermans et al., 2014), the brain is shifted into a memory formation mode, which facilitates encoding and early consolidation but suppresses retrieval of unrelated information (Schwabe et al., 2012), and learning is shifted toward habitual and simpler but also more rigid strategies dependent on the dorsal striatum (Schwabe and Wolf, 2013). This also entails, however, that processes that do not aid immediate coping or that require too many of the available resources are suppressed. This shift therefore augments efficiency at the cost of the engagement of more elaborate and cognitively demanding processes dependent on the hippocampus and PFC. For the first time, our experimental studies show that polymorphisms in the genes coding for the α_{2B}-AR, the MR and the CB1R modulate the effects of stress on affective processing and the engagement of multiple memory systems.

In PCL, an ADRA2B deletion associated with enhanced NA availability and particularly strong episodic memories for emotional information (de Quervain et al., 2007a; Rasch et al., 2009) was associated with a reduced shift toward habitual, dorsal striatum-dependent memory, whereas an MR haplotype including SNPs associated with enhanced MR expression and functionality (Klok et al., 2011) was related to enhanced shifting toward habitual memory under stress. Interestingly, our neuroimaging data propose different underlying neural mechanisms. ADRA2B deletion carriers showed generally enhanced amygdala-EC connectivity, suggesting that in these participants the hippocampus-dependent system is preferentially engaged, which is in line with enhanced episodic memory formation in these individuals (de Quervain et al., 2007a; Rasch et al., 2009) and with the importance of NA in memory enhancement (McGaugh et al., 1993; McGaugh and Cahill, 1997). In contrast, MR haplotype carriers showed stronger amygdala-putamen connectivity,

indicating augmented recruitment of the dorsal striatum under no stress conditions. In response to stress, reduced amygdala-putamen connectivity in ADRA2B deletion carriers points to impaired dorsal striatal processes, whereas diminished coupling of the amygdala with the hippocampus and reduced hippocampal activity in MR haplotype carriers suggests impaired hippocampal processes. These findings are in accordance with previous studies (Schwabe and Wolf, 2012; Schwabe et al., 2013b; Vogel et al., 2015; Vogel et al., 2017) showing that the stress-induced shift toward dorsal striatum-dependent memory can be promoted either by enhanced amygdala-dorsal striatum coupling, as is seen in ADRA2B deletion non-carriers, or by reduced connectivity between the amygdala and the hippocampus, as is the case in MR haplotype carriers. Therefore, the system that is – in a genotype-dependent manner – preferably engaged is enhanced under no stress conditions, whereas the system that is less likely to be engaged even is impaired under stress. It is tempting to speculate that these differences in connectivity under no-stress conditions may actually prevent or facilitate the shift toward habit, dorsal striatum-dependent memory under stress in ADRA2B deletion and MR haplotype carriers, respectively. Previous studies showed that acute stress reduces hippocampus activity (Prüssner et al., 2008; Henckens et al., 2009; Schwabe and Wolf, 2012). In combination with a general preference for dorsal striatum memory in MR haplotype carriers, this may lead to disinhibition of the dorsal striatum, further augmenting suppression of the hippocampus which, as it was shown in MR haplotype carriers, will then lead to a stress-induced shift toward habit memory. This is also in line with the reciprocal inhibition found between dorsal striatum- and hippocampus-dependent memory (Lee et al., 2008). In contrast, the hippocampal system is strengthened in ADRA2B deletion carriers, possibly preventing stress-induced reductions in hippocampal activity which will then lead to overly strong engagement of the hippocampus-dependent memory under stress. The fact that we could show a modulation of stress effects on the engagement of multiple memory systems by an ADRA2B deletion and MR haplotype in two independent experiments is highly convincing and suggests that the modulation of memory systems under stress depends, at least to some extent, on genetic differences in known stress and memory modulators.

In our affective processing task, the rs1049353 CB1R gene SNP modulated the effects of stress on brain regions important for appropriate affective responding and emotion regulation during the presentation of negative compared to neutral pictures. During negative picture processing, carriers of the protective AA/AG genotype showed increased vmPFC activity after stress and

enhanced vlPFC-amygdala connectivity under no stress conditions. During affective processing, individuals carrying this genotype may be able to effectively regulate their emotions, possibly by downregulating the amygdala through inhibitory projections from the vlPFC and vmPFC (Ochsner et al., 2012). As part of the DMN, the vmPFC has also been shown to be activated by acute stress (van Oort et al., 2017). In AA/AG genotype carriers in particular, vmPFC activity may be enhanced to promote regulation of negative affect in response to emotional images under stress. Although one can only speculate as to whether and how the rs1049353 SNP may influence CB1R gene expression or functionality, the role of eCBs in vmPFC functioning (Lisboa et al., 2010) and the supposedly protective role of this SNP against stress-related psychopathologies (Hill and Patel, 2013) suggests that eCB signaling may be enhanced in these individuals. In addition to affective processing, the rs1049353 SNP modulated the recruitment of brain systems during emotional picture encoding under stress that contributed to the formation of memories. Only in stressed carriers of the AA/AG genotype did emotional memory positively correlate with activation of the amygdala, insula and hippocampus, as well as with coupling of the hippocampus with the BLA during negative picture encoding. This is in line with pharmacological experiments showing that enhanced eCB signaling via CB1Rs in the BLA is required for the enhancing effects of GCs on memory formation (Campolongo et al., 2009). In this way, AA/AG genotype carriers may be able to efficiently incorporate the learning experience into their autobiographical memory, so that should the same or a similar stressor reoccur the individual is prepared for optimal coping. Importantly, this contrasts with the excessive hippocampal engagement seen in ADRA2B deletion carriers, which may lead to overly strong consolidation of the stressful experience.

4.1.1 Receptor distribution and the acute stress response

It is highly important to unite our experimental findings with what we know about distributions of adrenergic, cannabinoid and mineralocorticoid receptors, as well as with what we know about stress-induced changes in stress hormones and their functionality. The effects that we observed were likely mediated by receptors located in the membrane of presynaptic neurons through which the release of neurotransmitters such as glutamate, GABA or NA is inhibited or facilitated. Whereas MR and α_{2B}-ARs can be expressed both pre- and postsynaptically, particularly important for the effects of stress are NA actions via presynaptic α_{2B}-adrenergic auto- and heteroreceptors that regulate the synthesis and release of NA and other neurotransmitters such as glutamate (Langer,

1980; Cousijn et al., 2010; Gilsbach and Hein, 2012), as well as rapid, non-genomic GC effects via membrane-bound MRs located at presynaptic sites (Karst et al., 2005; Olijslagers et al., 2008; Sandi, 2011). The CB1R is known to only exist as a presynaptic heteroreceptor and regulates mainly GABAergic but also glutamatergic neurotransmission (Piomelli, 2003). The fact that stress effects are mediated by presynaptic receptors is important for three reasons: first, presynaptic receptors rapidly and directly affect neurotransmission, without requiring genomic mechanisms; second, these receptors, in particular inhibitory α_2-adrenergic autoreceptors and cannabinoid heteroreceptors, are required for inhibitory feedback control (Raiteri et al., 1992; Schwartz, 1997; Marsicano et al., 2003), whereas presynaptic membrane-bound MRs are necessary for rapid excitatory effects of GCs (Groeneweg et al., 2011); third, presynaptic receptors are involved in pathophysiological processes and as such are often the target of various pharmacological interventions (Schlicker and Feuerstein, 2017).

Based on previous studies (de Quervain et al., 2007a; Rasch et al., 2009), the ADRA2B deletion variant supposedly has antagonistic effects, possibly mediated by decreased agonist-promoted phosphorylation and/or receptor desensitization, which would decrease the inhibitory influence of these α_{2B}-adrenergic autoreceptors, leading to enhanced NA availability. As was revealed by these studies (de Quervain et al., 2007a; Rasch et al., 2009), episodic memory dependent on the hippocampus and amygdala activity was increased in ADRA2B deletion carriers which is in line with high α_2-AR expression in the amygdala and hippocampus (Scheinin et al., 1994; see **Figure 2**). Despite this presumably enhanced noradrenergic activation, we did not observe a shift toward dorsal striatum-dependent memory in PCL under no stress conditions, adding to the evidence suggesting that GC actions via MRs mediate this shift under stress (Vogel et al., 2016). Although stress can lead to even further increases in NA in ADRA2B deletion carriers (Cousijn et al., 2010) and cortisol levels were enhanced, such as to suggest activation of non-genomic MRs, memory system engagement in ADRA2B deletion carriers did not change in response to stress. Strengthening of coupling between the amygdala and hippocampus might have prevented the stress-induced shift toward dorsal striatum-dependent memory, leading to overly strong engagement of the hippocampus even under stress.

In contrast, MR haplotype carriers showed a shift toward dorsal striatum memory under stress. It is not clear whether or how membrane MR-mediated changes in neurotransmission may translate into enhanced engagement of dorsal striatal memory, but it has been speculated that MR

activation in the ventral tegmental area may, via dopaminergic mechanisms, enhance amygdala function and its connectivity with the dorsal striatum (Vogel et al., 2016). However, considering that receptors of most stress mediators seem to be expressed in the dorsal striatum only very scarcely (see **Figure 2**), direct effects of stress hormones and neurotransmitters on receptors in the dorsal striatum seem less likely than their actions on amygdala and hippocampal neurons. Indeed, mineralocorticoid receptors are highly expressed in the hippocampus and amygdala but whereas glutamatergic transmission in the hippocampus is quickly enhanced, these changes are not as long-lasting as the rapid increases in excitability of neurons in the amygdala (Karst et al., 2010).

CB1Rs are strongly expressed in frontal cortex regions (see **Figure 2**), including the vlPFC and vmPFC. These regions are highly important for HPA axis regulation as well as emotion regulation, defined as the implementation of strategies that influence the intensity, duration and type of emotion experienced (Ochsner et al., 2012). In the mPFC, CB1Rs have been shown to inhibit GABAergic neurons, thereby increasing activity in this region (McLaughlin et al., 2014). In turn, this leads to enhanced negative feedback inhibition of the HPA axis (Hill et al., 2011) and suppression of the BLA, thereby reducing excessive responding to emotional stimuli (Morena and Campolongo, 2014). In rs1049353 AA/AG genotype carriers, more efficient CB1R functioning may lead to better emotion regulation and negative feedback inhibition mediated by disinhibition of mPFC regions. It has been suggested that stress impairs PFC functions through the reallocation of resources away from higher-order cognitive processes (Schwabe and Wolf, 2013; Hermans et al., 2014; Arnsten, 2015) which may not only promote vigilance and attention toward emotional material but may also enhance the habitual use of maladaptive emotion regulation strategies. Improved eCB signaling may prevent such maladaptive strategies and instead promote a more cognitive, vlPFC- and vmPFC-mediated pathway to downregulate the amygdala, thereby reducing negative affect. In addition, after stressor cessation engagement of the hippocampus during encoding may promote the appropriate integration of emotional information into hippocampus-dependent autobiographical memory, an effect likely mediated by CB1Rs located in the amygdala and hippocampus which may strengthen connectivity between these regions. This way, not only may acute emotion regulation be improved, but encoding and consolidation of the knowledge acquired during this encounter may serve to improve prospective coping behavior.

To sum up, whereas around 15 minutes after stress ADRA2B deletion non-carriers and MR haplotype carriers showed a stress-induced shift from cognitive, hippocampus-dependent toward habit, dorsal striatum-dependent memory, a shift which may be beneficial in response to acute stress, around 40 minutes after stress the rs1049353 AA/AG genotype seems to promote cognitive, vmPFC-mediated affect regulation and enhanced hippocampal engagement. Interestingly, although MR haplotype carriers showed lower cortisol levels in our second experiment, as would be expected from enhanced negative feedback control of the HPA axis and as is in line with pharmacological manipulations (Schwabe et al., 2010b; Schwabe et al., 2013b; Otte et al., 2015) and genetic studies (DeRijk et al., 2006; DeRijk, 2009), after controlling for these differences in cortisol concentration, the results remained largely unchanged. Also, the ADRA2B deletion and rs1049353 AA/AG genotype did not influence sympathetic arousal (blood pressure) or salivary cortisol levels. Together, this suggests that pure differences in sympathetic arousal or HPA axis activation may not be crucial in genotype-dependent modulations of stress effects on affective processing and memory. It remains to be seen how genotype-dependent structural and functional differences may be responsible for the effects that we observed. Importantly, since the genetic variants that we investigated have been associated with stress-related disorders (de Quervain et al., 2007a; Klok et al., 2011; Agrawal et al., 2012), how our findings may translate into resilience and vulnerability is discussed in the following paragraph.

4.1.2 Implications for stress-related psychopathologies

The rs1049353 AA/AG genotype and the MR haplotype (rs2070951 C and rs5522 A) have been proposed as protective genotypes against the detrimental effects of stress (Klok et al., 2011; Agrawal et al., 2012), whereas an ADRA2B deletion has been associated with enhanced PTSD risk (de Quervain et al., 2007a). Treatment options for PTSD that tackle the eCB and GC systems have been investigated and show promising results (Hill and Patel, 2013; de Quervain et al., 2017). Our findings add to this evidence by showing genotype-dependent differences in affective processing and memory, both of which are dysfunctional in stress-related psychopathologies (Joormann and Gotlib, 2010; Campbell-Sills et al., 2011; de Quervain et al., 2017). Beyond these considerations, it is tempting to speculate that enhanced engagement of hippocampus-dependent memory in PCL and reduced vmPFC recruitment under stress may represent endophenotypes for different stress-related psychopathologies.

The protective role of endocannabinoid signaling

The eCB system is essential for gating and buffering stress effects and for regulating mood by facilitating coping with fear and anxiety (Hillard et al., 2012). It determines the value of anxiety-provoking stimuli and regulates neural and behavioral processes that are essential for homeostasis and resilience against the detrimental effects of stress (Lutz et al., 2015). As such, eCB signaling is a key process in all brain regions that are relevant for the processing and regulation of anxiety, fear and stress, including the PFC, hippocampus, amygdala and hypothalamus (Lutz et al., 2015). Since CB1Rs are also located at cholinergic, serotonergic and noradrenergic sites, they are likely crucially involved in the release of these neurotransmitters as well (Nyiri et al., 2005; Kirilly et al., 2013; Häring et al., 2015). Its main function thus seems to be to control excessive activation, thereby causing anxiolytic effects, as has been demonstrated by FAAH inhibitor- or CB1R agonist-induced enhancements of eCB signaling in the PFC and amygdala (Hill and Patel, 2013). In these regions, eCB signaling seems to increase PFC and to suppress amygdala activation to dampen the expression of anxiety-like behaviors (Hill and Patel, 2013). Not only anxiety but also excessive stress responding seems to be prevented by eCB signaling. At rest, increased AEA levels provide an inhibitory tone on HPA axis-activating neurons (see **Figure 3A**) and stress-induced increases in 2-AG are important to reduce the magnitude of the stress response and aid the return to homeostasis (Hill and Patel, 2013; see **Figure 4A**).

The logical consequence is that suppression of eCB signaling causes anxiogenic effects and augments the response to stress. Indeed, administration of a CB1R inverse agonist into the BLA increased anxiety (Dono and Currie, 2012) and evidence suggests that CB1R antagonists lead to strong increases in anxiety- and depression-like symptoms, enhanced cortisol levels (only found in some individuals), attenuated reward processing and an augmented negative memory bias (for a review see Hill and Patel, 2013). Similarly, frontal CB1R knockout mice showed increased anxiety-like behaviors but only under highly aversive conditions (Jacob et al., 2009), as well as enhanced sensitivity to anhedonia after chronic stress exposure (Martin et al., 2002). After chronic unpredictable stress, an animal model of depression, animals developed downregulation of CB1Rs and 2-AG levels in the hippocampus (Hill and Gorzalka, 2005). Chronic treatment with the CB1R antagonist rimonabant led to depression-like symptoms, similar to those seen in a model of chronic stress, as well as to decreased frontal serotonin levels and severe structural and functional changes in the hippocampus (Beyer et al., 2010). In humans, after 500 days without any contact to

family and friends, subjects showed reduced positive emotions, high levels of catecholamines and reductions in 2-AG levels (Yi et al., 2016). Also, stress-related psychopathologies are characterized by a dysfunctional eCB system. Specifically, in patients with depression, basal and stress-induced eCB signaling seems to be reduced (Hill et al., 2008; Hill and Gorzalka, 2009; Hill et al., 2009a). Similarly, PTSD patients showed reduced 2-AG and AEA concentrations (Neumeister et al., 2013; Schaefer et al., 2014) and AEA concentrations negatively correlated with intrusive symptom severity (Hill et al., 2013). Increased CB1R availability in PTSD patients may likely have a compensatory function and in combination with reduced AEA and blunted cortisol concentration, (Neumeister et al., 2013) were able to correctly classify around 85% of PTSD patients.

The rs1049353 SNP investigated in our study provided us with clues as to how a CB1R gene variant may be involved in individual differences in affective processing and memory. The A allele has been shown to significantly reduce the risk of developing anhedonia and depression after early life stress (Agrawal et al., 2012). In contrast, the G allele of this SNP is a known risk factor for antidepressant treatment resistance and diminished social reward responsivity, indicated by blunted limbic and striatal activation in response to happy faces (Chakrabarti et al., 2006; Domschke et al., 2008). Given that in our study rs1049353 AA/AG genotype carriers showed increased vmPFC activity after stress and enhanced crosstalk of the vlPFC with the amygdala under no stress conditions during negative picture processing, which may indicate proper functioning of cognitive emotion regulation, our findings are in line with a protective role of the A allele, possibly by preventing excessive affective responding. Individuals suffering from psychopathologies such as depression show predominant use of avoidance, rumination or emotion suppression, maladaptive strategies that have been associated with enhanced sympathetic arousal, as well as with sustained activation of emotion generating brain regions such as the amygdala and insula (Goldin et al., 2008; Moore et al., 2008). Cognitive emotion regulation depends on intact functioning and engagement of PFC regions. Since stress is known to impair PFC-dependent processing (Arnsten, 2009) even healthy individuals seem to opt for more maladaptive strategies in highly threatening situations (Sheppes et al., 2014), likely because the use of more beneficial strategies dependent on the PFC are impaired under stress (Raio et al., 2013). Elevated cortisol levels are associated with increased amygdala and decreased vmPFC activation during negative affect regulation and deficiencies in effective emotion regulation due to HPA axis dysfunction may contribute to psychopathologies (Urry et al., 2006). Since effective

emotion regulation skills have been proposed as the key mechanism for resilience (Kalisch et al., 2015), it is not surprising that when emotion regulation is absent or applied poorly, affective responses may be excessive or inadequate, as is seen in several disorders (Aldao et al., 2010). In patients with late life depression, diminished vmPFC activity in response to negative stimuli is seen, an effect that positively correlated with symptom severity (Brassen et al., 2008). In PTSD patients, hyperarousal symptoms were associated with impaired downregulation of the amygdala by the mPFC during performance of an emotion-word Stroop task (Sadeh et al., 2014). Thus, in rs1049353 AA/AG genotype carriers, recruitment of the vmPFC despite stress may protect against maladaptive coping and excessive affective responding. Although so far none of the identified CB1R gene polymorphisms have been found to result in deletion or mutation of CB1R protein but other mechanisms such as enhanced mRNA stability are possible and could contribute to the protection against the detrimental effects of stress (Hillard et al., 2012). More direct evidence for genetic differences in eCB signaling comes from a study that investigated a SNP of the FAAH gene (rs324420) showing a C to A allele change which leads to destabilization of the FAAH enzyme, thereby augmenting AEA levels (Dincheva et al., 2015). In this study, homozygous A allele carriers showed decreased anxiety-like behavior and increased fear extinction learning, effects paralleled by stronger vmPFC-amygdala connectivity. It is tempting to speculate that the AA/AG genotype may, through enhanced or more efficient eCB signaling, attenuate the stress-induced reallocation of resources away from higher-order cognitive processes to prevent the habitual use of maladaptive emotion regulation, thereby allowing for more effective regulation of negative affect.

This, of course, has highly relevant implications for the pharmacological treatment of stress-related psychopathologies. In line with impaired vmPFC functioning and mPFC-amygdala coupling in response to emotional stimuli in patients with depression and PTSD, respectively (Brassen et al., 2008; Sadeh et al., 2014), and in line with enhanced vmPFC activity during negative stimulus processing in rs1049353 AA/AG genotype carriers after stress exposure, pharmacological enhancement of eCB signaling may enhance mPFC activity, thereby improving negative affect regulation and possibly reducing the risk for psychopathologies. Indeed, several studies now suggest that CB1R agonists may be a useful pharmacological treatment option for generalized anxiety disorder, depression and PTSD, likely by increasing activation of frontal brain regions (Hill and Patel, 2013). Evidence also suggests that FAAH inhibitors which increase AEA levels that regulate HPA axis activation (see **Figure 3A**), dampen the magnitude of the stress response and

amygdala activation (Bedse et al., 2014; Gray et al., 2015) and reduce anxiety under threatening conditions (Morena et al., 2016). During chronic stress exposure they were also shown to significantly reduce the risk of developing anhedonia (Bortolato et al., 2007; Rademacher and Hillard, 2007). In another experiment, 3 weeks of antidepressant treatment led to increased CB1R expression in the hippocampus and hypothalamus, regions in which eCBs may suppress CRH secretion, thereby reducing GC secretion, an effect that is prevented by CB1R antagonist administration (Hill et al., 2006). This clearly demonstrated the importance of the eCB system in mediating antidepressant effects on HPA axis activation. It is also in line with the finding that both CB1R agonists and FAAH inhibitors seem to exert their anxiolytic and antidepressant effects by eCB-mediated stimulation of serotonergic activation in the PFC, as was shown by injections of these agents directly into the vmPFC in mice exposed to forced swim stress (Bambico et al., 2007; McLaughlin et al., 2012). Interestingly, in response to stress, also selective inhibition of CB1Rs in glutamatergic neurons led to antidepressant-like effects in mice (Steiner et al., 2008; Häring et al., 2015). Importantly, this was not the case when CB1Rs in forebrain GABAergic neurons were inhibited. This is in accordance with a proposal suggesting that eCB actions on GABAergic interneurons disinhibit no-fear pathways and at the same time eCB actions on glutamatergic neurons decrease excitation of fear-pathways (Lafenetre et al., 2007). Due to the importance of the eCB system in appropriate and efficient release from fear (Lutz et al., 2015), it is not surprising that CB1Rs seem to be essential for fear extinction (Akirav, 2011). Indeed, several experiments using a rat model of PTSD showed that intra-BLA injections of cannabinoid receptor agonists led to diminished GC secretion, enhanced extinction, reduced avoidance – a maladaptive coping strategy – and attenuated anxiety (Ganon-Elazar and Akirav, 2009, 2012, 2013). Also FAAH inhibitors seem to potentiate memory extinction through CB1R activation and synaptic plasticity in the BLA (de Bitencourt et al., 2013; Gunduz-Cinar et al., 2013). These findings are in line with the memory-enhancing effects of increased eCB levels (Campolongo et al., 2009; see **Figure 7**). Treatment influencing the eCB system has been proposed as the optimal means to treat both the cognitive and the affective symptoms of PTSD by blocking the continuous retrieval of the traumatic experience as well as by enhancing extinction and diminishing symptoms of anxiety (Akirav, 2013). By using eCB degradation inhibitors such as FAAH inhibitors, psychotropic effects of compounds which bind to CB1Rs could be prevented (Moreira et al., 2009; Trezza and Campolongo, 2013). The effects of GCs as a means to treat fear-related disorders (de Quervain et

al., 2017) are possibly mediated by eCB actions on extinction and reconsolidation processes, a highly intriguing possibility when considering the benefits and side-effects of either GC or eCB treatment options. However, due to the pleiotropic nature of eCB signaling, developing more brain region- and neuron- (GABAergic or glutamatergic) specific pharmacological agents will be a great advancement.

To summarize, the findings of our study are in line with a protective role of eCB signaling against stress-related psychopathologies by means of enhanced PFC recruitment during affective processing under stress, allowing for improved cognitive regulation of negative affect. Enhanced recruitment of the vmPFC in rs1049353 AA/AG genotype carriers may also facilitate extinction, and the engagement of the hippocampus during memory encoding may aid (re)consolidation processes. These beneficial effects on emotion regulation, extinction and reconsolidation are important pathways through which these individuals may show resilience or enhanced treatment sensitivity after a traumatic experience.

The protective role of noradrenergic inhibition and mineralocorticoid receptor activation

Noradrenaline plays an important role in memory and emotion. For the enhancing effects of stress on encoding and consolidation processes and for increased emotional memory formation, noradrenergic activation in the BLA is required (Cahill and McGaugh, 1998; McGaugh, 2004; Roozendaal and McGaugh, 2011). These enhancing effects of NA, however, can become maladaptive and, through functional and structural changes in the amygdala and memory systems, can have important long-term consequences for affective processing and memory (Rauch et al., 2000; Roozendaal et al., 2009). In line with this, an ADRA2B deletion has been associated with enhanced memory for traumatic experiences and greater re-experiencing symptoms in Rwandan civil war survivors (de Quervain et al., 2007a). In accordance with stronger episodic memory formation in ADRA2B deletion carriers we observed enhanced crosstalk between the amygdala and hippocampus in these individuals. This strengthened connectivity may have prevented a stress-induced shift toward dorsal striatum-dependent memory, a process which is assumed to be adaptive, possibly rescuing performance and conserving available resources (Schwabe and Wolf, 2012; Schwabe et al., 2013b). Although this shift has also been proposed to lead to less flexible knowledge which is not easily applicable to new situations (Schwabe and Wolf,

2013), our results suggest that the continued use of hippocampus memory in response to stress may actually be a risk factor for developing a psychiatric disorder such as PTSD, possibly leading to overconsolidation of traumatic experiences (de Quervain et al., 2009) and maladaptive responding, which may come at the cost of impaired performance. In addition, the direct projections from NA-secreting neurons in the LC to PFC areas (Arnsten, 2009), may, when NA levels are excessive, impair PFC function (Arnsten, 2009, 2015). When chronically activated, increased noradrenergic signaling may promote the development of disorders such as PTSD which is characterized by impaired mPFC activity (Bremner, 2002) and heightened NA activity (Southwick et al., 1999). Enhanced noradrenergic activity in the amygdala may also explain hyperarousal symptoms in PTSD (Ronzoni et al., 2016) which is likely attributable to a reduced number or diminished binding affinity of α_2-ARs (Maes et al., 1999; Southwick et al., 1999).

Therefore, pharmacological agents that target noradrenergic hyperactivity might be useful in preventing or treating PTSD. Specifically, it has been suggested that well-timed noradrenergic treatment might be able to prevent overencoding or –consolidation of traumatic memories (Southwick et al., 1999). Also, α_2-AR agonists and α_1- or β-AR antagonists were shown to ameliorate symptoms of hyperarousal, hypervigilance, sleep disturbance, exaggerated startle response, nightmares, irritability and aggression in PTSD patients (Southwick et al., 1999; Pitman et al., 2002; Taylor et al., 2006; Raskind et al., 2007; Strawn and Geracioti, 2008). As an example, a study administering propranolol shortly after trauma exposure found that PTSD rates and symptom severity were significantly higher in individuals who had not taken propranolol for a week (Vaiva et al., 2003). However, since these clinical studies have not been replicated consistently (Pitman et al., 2012) and adrenergic agents are associated with several side effects (Strawn and Geracioti, 2008), selective serotonin reuptake inhibitors are still the current choice of treatment for PTSD. Also, unfortunately the differential contribution of α_2-AR subfamilies in the effects of stress is unknown and to date there are no pharmacological agents that are able to only influence these specific receptor subtypes, although some effort has been made to developing agents that at least have a higher or lower affinity for a specific subtype of α_2-ARs (Lalchandani et al., 2002). It remains to be seen whether α_{2B}-ARs play a special role in the NA-mediated effects of stress and whether more specialized agents may serve as better treatment options. Our data at least do suggest that a genetic variant in the gene coding for this receptor, leading to enhanced noradrenergic

availability in the amygdala, is importantly involved in stress effects on the engagement of multiple memory systems.

Our findings apply very well to the facilitation of a stress-induced shift toward dorsal striatum memory in carriers of an MR haplotype, which has been associated with enhanced MR expression and functionality that is directly linked to resilience against depression (Klok et al., 2011) and traumatic stress (ter Heegde et al., 2015; de Kloet et al., 2016). As stated before, this shift may actually be adaptive and the fact that several studies have shown health promoting effects of these MR gene variants provides additional strong evidence for this hypothesis. When faced with an aversive event, activation of MRs may promote adaptive coping and the shift away from hippocampus-dependent memory may prevent overconsolidation of fear memories (de Kloet et al., 2016). In line with a beneficial role of the MR, the MR agonist fludrocortisone has been shown to enhance antidepressant treatment efficacy, as well as to improve memory and executive functioning in depressed subjects. Specifically, fludrocortisone enhanced memory and executive function and reduced cortisol levels in healthy and depressed subjects (Otte et al., 2015). In contrast, the MR antagonist spironolactone significantly impaired selective attention, memory and mental flexibility and augmented cortisol secretion in healthy individuals (Otte et al., 2007). Directly related to these MR-mediated effects and in addition to evidence suggesting that GC actions via MRs impair memory retrieval (Dorey et al., 2011; Rimmele et al., 2013), it has been proposed that fear-related disorders such as phobias or PTSD can be treated with GCs (de Quervain et al., 2017). The work by Dominique de Quervain and his group has likely led to the most promising advancements concerning new treatment options for fear-related disorders in the last decade. The idea is that since GCs have been found to inhibit long-term memory retrieval (de Quervain et al., 1998; de Quervain et al., 2000), which may partly be MR-mediated, this could be used to inhibit retrieval of a traumatic experience, thereby reducing symptoms in patients with phobias and PTSD (de Quervain and Margraf, 2008). Specifically, it is assumed that ongoing retrieval and reconsolidation of the traumatic event may be what keeps these memories vivid. By inhibiting this process, cortisol may weaken the traumatic memory trace, ultimately and long-lastingly reducing symptoms. Several experiments provided first evidence for the effectiveness of this approach (de Quervain and Margraf, 2008). Treatment of low cortisol doses for one month had no adverse side effects and reduced traumatic memory symptoms, effects that seemed to be long-lasting. In patients with social phobia, cortisone administration 1 hour before exposure to a social

stressor diminished self-reported fear before, during and after stress exposure. Also, fear in reaction to a spider progressively lessened in patients with spider phobia when cortisone was repeatedly administered 1 hour before exposure. In another study, the addition of cortisol to exposure therapy significantly reduced fear and led to greater decreases during acute exposure to the phobic situation 3-5 days after the last session (de Quervain et al., 2011). Skin conductance levels as a measure of autonomic arousal upon exposure were significantly diminished even 1 month after the last session. Given that stress mediators and their effects on memory are highly time sensitive (see **Figures 3, 4** and **5**), optimal dosage, time point and duration of GC treatment seem crucial (de Quervain et al., 2017). A study investigating the neural underpinnings of these effects revealed that cortisol administration before extinction training reduced fear memory retrieval, indicated by diminished amygdala-hippocampus activity, and facilitated extinction memory consolidation, indicated by increased hippocampus activity and connectivity to the vmPFC during exposure to an extinguished stimulus one week later (Merz et al., 2018). A highly intriguing finding in patients receiving dexamethasone treatment revealed that the severe adverse side effects (e.g. psychosis and mood disturbances) could be ameliorated by co-administration of low doses of cortisol (Meijer and de Kloet, 2017). This apparently paradoxical finding was argued to be caused by dexamethasone-induced and GR-mediated suppression of cortisol secretion, leading to depletion of MRs. Additional cortisol administration is argued to replenish cortisol availability and increase binding to MRs. This led to the proposal that balancing MR- and GR-mediated actions may be important for the maintenance of homeostasis and to prevent side effects of pharmacological treatments which may lead to an imbalance of these receptor actions (de Kloet et al., 2018).

In sum, our findings provide further evidence that non-excessive NA-, as well as augmented MR- and eCB signaling may represent protective mechanisms against the development of stress-related psychopathologies. Genetic differences in these stress mediators play an important role in individual risk factors and provide a starting point for customized treatment options.

4.2 Methodological considerations and recommendations

Several methodological considerations have to be taken into account to critically contemplate the advantages and disadvantages of genetic research and to successfully design and conduct future studies, especially since these factors determine whether and which conclusions can or cannot be drawn from such studies.

4.2.1 Genetic differences and pharmacological agents

Important differences exist between pharmacological manipulations and genetic variability. Not only will genetic differences likely entail whole-system and tonic activation changes, but acute increases in endogenous stress mediators have a spatial and temporal specificity that pharmacological agents are largely lacking. Acute stress sets in motion fine-tuned actions of endogenous increases in NA, cortisol and eCBs in a region-specific manner (Ulrich-Lai and Herman, 2009; Morena et al., 2016). Neurotransmitter synthesis and release and receptor activation occurs in certain activated areas, whereas pharmacological agents lead to a release of neurotransmitters that likely over- or undershoot natural increases and to activation of all receptors in the brain regardless of their specific involvement in a particular process. With respect to our studies this means that although we observed enhanced recruitment of hippocampus memory in stressed ADRA2B deletion carriers, we cannot be certain that this is due to augmented noradrenergic signaling in the amygdala, strengthening its connectivity to the hippocampus so as to boost episodic memory formation. Although this is an intriguing proposal, strong evidence for such antagonistic effects on α_{2B}-ARs in ADRA2B deletion carriers is still lacking. Potentially, this variant has agonistic effects (Small et al., 2001), mediated by more efficient binding capability, receptor upregulation or inhibition of adenylcyclase, which may reduce NA availability, thereby attenuating the stress-induced shift toward dorsal striatum-dependent memory. Pharmacological studies investigating the effects of noradrenergic agonists and/or antagonists may provide additional information about whether NA, possibly in interaction with cortisol, is necessary for a shift toward dorsal striatum memory and whether excessive noradrenergic activation, as may be the case in ADRA2B deletion carriers, may actually prevent this shift due to strengthening of the hippocampal system. Additionally, not much is known yet about the potential regional and functional differences of the α_2-AR subtypes, although advances in the development of more specific pharmacological agents are underway. For example, it was shown that the α_2-AR

antagonist yohimbine can be improved to a more than 1,000-fold increased specificity for α_{2C}- compared to α_{2A}-AR subtypes (Lalchandani et al., 2002). In addition to the importance of MR-mediated GC effects on the engagement of multiple memory systems, studies are needed to investigate the involvement of eCB signaling, for example by means of CB1R agonist or FAAH inhibitor administration. Since GCs increase the synthesis and release of eCBs, enhanced eCB signaling may facilitate a stress-induced shift toward dorsal striatum memory. Considering that GC-induced eCB increases are mediated by GRs (Groeneweg et al., 2011), this may also point to an important role of membrane-bound GRs. Several studies have been conducted using corticosterone conjugated to bovine serum albumin, which cannot cross the membrane and therefore only binds to membrane receptors, and made great advances in differentiating the roles of membrane versus cytosolic receptor binding in memory consolidation (Roozendaal et al., 2010), retrieval (Dorey et al., 2011) and eCB-mediated memory enhancement (Atsak et al., 2015). The development of agents only binding to certain α_2-AR, MRs or GRs and to only specific CB1Rs on either GABAergic or glutamatergic neurons will be a great advancement. Since the ADRA2B deletion, the MR haplotype and possibly also the rs1049353 AA/AG genotype may have consequences for the synthesis and release of NA, GCs and eCBs, it will be highly important to test these assumptions and to investigate the role of such changes in the effects of genetic modulation. For this reason, it is indispensable to measure the circulating levels of these neurotransmitters. In humans, however, although salivary cortisol and alpha-amylase levels have been shown to relate to brain concentrations of cortisol (Kirschbaum and Hellhammer, 1989, 1994) and NA (Chatterton et al., 1996; Petrakova et al., 2017), respectively, circulating eCB levels measured in blood or hair samples do not do so in a straightforward manner and taking blood samples poses additional stress for participants (Hillard, 2018). Advancing the methods with which these neurotransmitters can be measured and gaining better understanding of the correlation between circulating levels in saliva or blood samples and active levels in the brain, will greatly improve future research.

4.2.2 Methods in genetics: costs and benefits

There are many different approaches to investigate genetic differences and they all come with certain advantages and disadvantages that mainly concern the issue of sample and effect sizes. Candidate gene studies in which only specific genetic variants are tested have the advantage of being completely hypothesis-driven, meaning that there is pre-existing information about the

biological mechanism and the relevance to the outcome. This is why we decided to investigate receptors of some of the main stress mediators for which we had specific hypotheses. Also highly relevant when it comes to EEG or fMRI studies is the advantage that candidate gene studies do not require as many participants as are needed for other approaches (Rasch et al., 2010). Another advantage is that this approach is much more precise than linkage studies and has the potential to identify specific genetic variants that are important for a certain phenotype rather than just a specific chromosomal region (Dick et al., 2015). This is, however, also directly linked to a disadvantage of this approach: behavioral traits are highly complex and effect sizes of single genetic polymorphisms are likely small, as is the ability to exactly predict which genes are most likely relevant. Indeed for many behavioral traits, the ability of correctly predicting the importance of a certain gene or SNP has been poor, likely leading to a high false discovery rate (Dick et al., 2015). What could also cause a problem is the fact that due to small numbers of homozygous carriers of certain SNPs' minor alleles, homo- and heterozygous carriers are often tested together against homozygous carriers of the major allele, which may undermine the actual complexity of the underlying genetic effects. The most severe problem with this approach is probably the high chance of inducing a bias toward certain established and accessible molecular pathways, which limits the potential of identifying novel genes and pathways (Papassotiropoulos and de Quervain, 2011).

Related to the problem of genetic complexity is the fact that most studies neglect the existence of epistatic interactions, likely because they are under-powered. Ignoring these interactions could partly be responsible for the phenomenon of 'missing heritability', because a large proportion of unexplained variance can probably be accounted for by these interactions (Papassotiropoulos and de Quervain, 2011). Epistatic interaction between two genes similar to the interaction between the ADRA2B deletion and MR haplotype investigated in an additional exploratory analyses can be seen as a proof of concept and as a starting point for more sophisticated approaches. Ignoring these interactions may be an important reason for why many effects cannot be replicated, probably due to two reasons: first, interactions may be non-linear and therefore remain undetected when using analyses only testing for linear and additive effect (Moore and Williams, 2009) and second, directly related to this point, a simulation study showed that the power to replicate a SNP's main effect can drop from more than 80 % to less than 20 % when the allele frequency of a second interacting SNP changes by less than 1% (Greene et al., 2009). For our

data, this has two implications. On the one hand, the fact that we were able to replicate our effects in a second and independent sample, showing that an ADRA2B deletion and MR haplotype modulate the effects of stress on the engagement of multiple memory systems, is highly convincing evidence that genetic variants in these genes are able to explain a large proportion of individual differences. On the other hand, our exploratory analysis for an ADRA2B deletion × MR haplotype × stress interaction could show that these genotypes may interact and that the effects of the ADRA2B deletion may depend on the presence or absence of the MR haplotype. Other examples of gene × gene interactions are two studies that investigated the interaction between the ADRA2B deletion and a G to A variant of the Catechol-O-methyltransferase (COMT) gene (also called met variant), which has been associated with enhanced episodic long-term memory (Scheggia et al., 2018). Whereas one study showed that the ADRA2B deletion prevented the emotional memory impairment seen in COMT val allele carriers (Gibbs et al., 2010), this finding was not replicated in another study (Naudts et al., 2012). This clearly also shows that investigating the interaction between only two genetic variants is not enough to explain the complexity of genetic effects and can only be considered as a starting point for more sophisticated methods and the investigation of multiple gene interactions.

Another approach to investigate the additive effects of specific genetic risk factors is to calculate multilocus genetic profile or risk scores. With the help of such scores researchers identified genetic clusters which were able to explain individual differences in episodic memory (de Quervain and Papassotiropoulos, 2006) and, in interaction with early life stress, variability in HPA axis function, threat-related amygdala reactivity, anxiety, as well as hippocampal and amygdala brain volume (Pagliaccio et al., 2014; Di Iorio et al., 2017). Probably the most substantial undertakings are genome wide association studies (GWAS) that screen the entire genome for associations with heritable traits. The greatest advantage compared to candidate gene studies is certainly the potential of identifying novel genes and molecular pathways related to certain phenotypes (Papassotiropoulos and de Quervain, 2011). However, DeRijk and colleagues (2011) pointed out several limitations of this approach, two of which are the requirement of an immense number of participants to increase their power, because of which they may lack data concerning the phenotype of interest, and the fact that even when such associations are found, it is unclear whether the tagged SNPs are actually capable of modulating the phenotype.

Since the phenotype of our interest is relatively well established with clear biological mechanisms, we investigated plausible genetic polymorphisms. With this approach allowing a smaller sample size, we were able to investigate the genetic modulation of stress effects on affective processing and memory by means of EEG and fMRI. To extent our analyses to more than a single SNP of interest, we also performed haplotype analyses and detected significantly high linkage disequilibrium between 6 SNPs of the MR gene, forming 5 different haplotypes. Other combinations of these SNP alleles than those detected are highly unlikely and the identified haplotypes capture much of the genetic variability across sizable regions of a gene (Gabriel et al., 2002).

Investigating endophenotypes and gene × environment interactions are valuable possibilities to improve candidate gene studies (Caspi and Moffitt, 2006). There are, however, additional challenges when it comes to gene × environment interactions and imaging genetics studies. Investigating the interaction between genetic differences and the effects of stress requires detailed knowledge of the mechanisms of stress and a reliable way to measure it. Since the effects of stress are well studied and the experimental induction of stress by means of the TSST reliably increase stress hormone and neurotransmitter levels (Kudielka et al., 2007), our experiments fulfill these requirements. Imaging genetics studies have a good chance of finding significant genotype-dependent differences in brain activity (Rasch et al., 2010), although a more recent review showed that experiments of 50-100 subjects per group will only have moderate power to reject the null hypothesis (Carter et al., 2017; **Figure 27**). An additional problem is that imaging genetics studies may be accompanied by non-significant behavioral results, because these require even larger sample sizes.

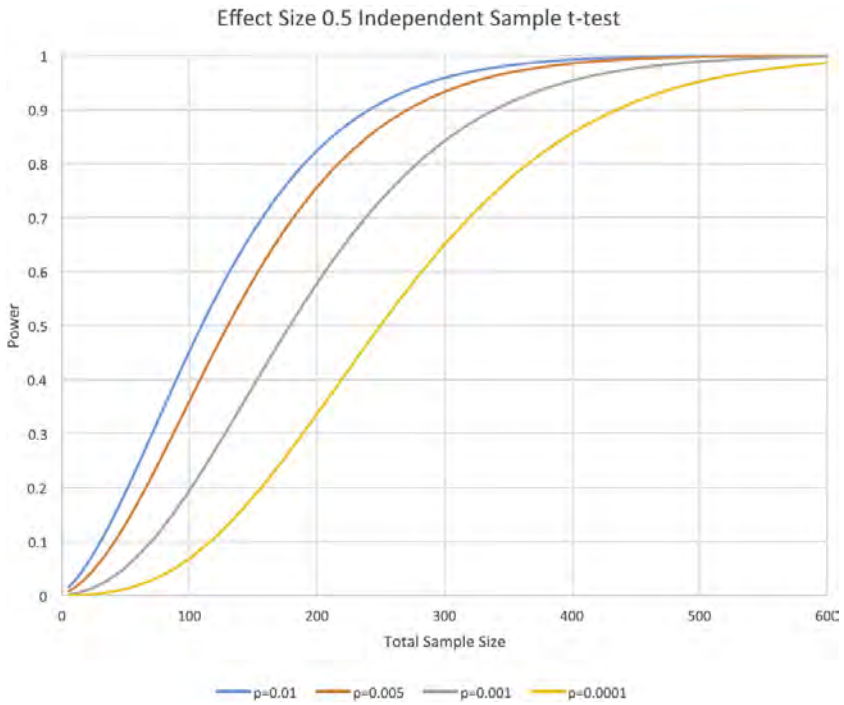


Figure 27. Power calculations in imaging genetics for independent samples t-tests for an effect size of 0.5 with different height thresholds used in imaging genetics studies. For typically used thresholds and this effect size 50-100 subjects per group will only have moderate power to reject the null hypothesis (from Carter et al., 2017).

To avoid reporting false positives and seemingly meaningful effects that may have occurred by chance, independent replication of the effects is indispensable. The fact that we tested 252 and 128 participants in our EEG and fMRI experiments, respectively, and that we were able to replicate our behavioral findings shows the strength of our approach and led us to detect medium effect sizes in our behavioral outcome. Specifically, effect sizes in our experiments, indicated by Cramer's V which gives a good norming independent of table size and that can be used for chi-square tables greater than 2 × 2 (Cramér and Hinkley, 1946; Liebetrau, 1983) are the following: ADRA2B = 0.218 (EEG) and 0.294 (fMRI), MR haplotype = 0.200 and 0.210 (fMRI). This could also be interpreted as an indication that the maximum possible variation between stress and strategy use in ADRA2B deletion non-carriers and MR haplotype carriers would be on average 25.6 % and 20.5 %, respectively. Importantly, although imaging genetics are correlational in nature (Papassotiropoulos and de Quervain, 2011) and it cannot be concluded that the brain regions in which differences between genotypes are detected are also the main sites where they exert their effects on a molecular or cellular level (Rasch et al., 2010), our findings show support for the involvement of ADRA2B, NR3C2 and CNR1 gene SNPs in stress effects on affective processing and memory.

4.2.3 Prenatal, early life and chronic stress: importance of epigenetics

Genetic selection of rodent inbreeding studies showed that individual differences in stress reactivity are not eliminated, indicating that susceptibility to the effects of stress is not solely determined by genetic factors (Ebner and Singewald, 2017). Instead in addition to genetic factors, epigenetic mechanisms set in motion by prenatal, early life, chronic and acute stress are highly relevant. Epigenetic mechanisms such as DNA methylation, histone modifications and micro RNA activity are crucial in explaining how environmental factors that have no effect on the DNA sequence can have dramatic and long-lasting consequences on cognition and behavior. Directly such epigenetic modifications can induce changes in chromatin structure and indirectly lead to alterations of gene expression, mechanisms which are particularly relevant for brain plasticity and memory (Levenson and Sweatt, 2005), as well as stress (Mifsud et al., 2011). Epigenetic modification of the GR gene (Nuclear receptor subfamily 3 group C member 1 (NR3C1)) promotor for example has been associated with individual and sex-dependent differences in memory and PTSD risk (Vukojevic et al., 2014). Epigenetic regulation of gene expression is particularly important for the long-term consequences of early life stress (Vaiserman, 2015). Specifically, early life stress has been shown to increase the number and function of excitatory synaptic connections, changes that are sufficient to affect gene expression via epigenetic mechanisms (Bolton et al., 2017). These changes have long-term consequences for stress system reactivity, coping behavior and vulnerability to future stressors (Maccari et al., 2003; Green et al., 2011; Chocyk et al., 2013). When stress occurs chronically, stress mediators induce processes that start out as protective adaptations but that have damaging effects when the regulatory imbalance in stress mediators remains unresolved (McEwen, 2001). Consequently, epigenetically-mediated structural and functional reorganizations take place, resulting in impairments of HPA axis feedback inhibition (Sousa, 2016). Chronic stress can influence epigenetic mechanisms of about 2,000 genes and the magnitude of epigenetic changes correlates with stressor intensity (Stankiewicz et al., 2013). Particularly interesting are the findings of chronic stress effects on eCB signaling. Specifically, whereas eCBs are important to limit the effects of chronic stress on structural changes in limbic brain regions by preventing excessive glutamatergic signaling, chronic stress-induced downregulation of CB1Rs and reductions in AEA levels mediated by increased FAAH availability disrupt these protective functions (McEwen et al., 2015). Interestingly, when repeated stress

exposure was followed by a 40-day period of recovery, changes in CB1R binding normalized and receptor density was even upregulated in the hippocampus (Lee and Hill, 2013). Being able to promote this process of rehabilitation after chronic stress exposure would greatly improve recovery from stress and may even prevent the development of stress-related psychopathologies in genetically predisposed individuals. The development of epigenetic drugs is underway (Weaver et al., 2005) and will have an immense impact on preventative measures and treatment options.

Several conclusions can be drawn from these methodological considerations (chapter 4.2.2) and the importance of epigenetics (chapter 4.2.3). Although highly important, pharmacological experiments are not able to replace genetic studies since these genetic studies capture endogenous processes initiated by stress that have effects with a unique spatial and temporal specificity. Also, it seems that GWAS should not obliterate candidate gene studies, because they can provide additional important insights into the mechanisms of how genes modulate the effects of stress. To improve the reputation of this approach in the genetic community, however, stringent requirements should be fulfilled, such as the provision of information on power calculations and effect sizes, independent replication and a well-defined presentation of the biological mechanisms. In addition, it is important that genetic studies – by default – also gather information about traumatic, prenatal, early life or chronic stress experiences. With advancements in the field of epigenetics, histone modifications and DNA methylation status need to be measured to take these mechanisms into account.

Based on these conclusions, I propose the following course of action for the purpose of investigating stress effects on cognition and behavior (**Figure 28**). First, behavioral studies in humans and animals will provide knowledge about the effects of stress on a behavior or cognitive process of interest. Subsequently, molecular studies in animals and brain imaging studies in humans will tell us which brain regions are involved in these effects. Lesion and pharmacological studies in animals, as well as patient, pharmacological and – if possible – brain stimulation studies in humans will identify causal mechanisms and the necessity for specific brain regions and neurotransmitters. Genome wide association and candidate gene studies will identify genetic markers that are associated with a certain (endo)phenotype and examine the role of specific genetic variants, respectively. Transgenic or knock-out mouse models, e.g. mutant mice lacking CB1Rs in glutamatergic neurons (Steiner et al., 2008) or forebrain MRs (ter Horst et al., 2013) as well as knock-in mouse models can show remarkable parallels with naturally occurring human

polymorphisms (Bogdan et al., 2016) and increase knowledge about the functional role of these polymorphisms. Importantly, in all of these approaches, additional modulatory factors need to be considered, such as traumatic, prenatal, early life and chronic stress experiences, but also highly positive experiences, epistatic and epigenetic mechanisms, as well as sex and age differences. In general, meta-analyses will provide information about the reliability of single study results and greater advancements will be achieved when data are being made available to other researchers.

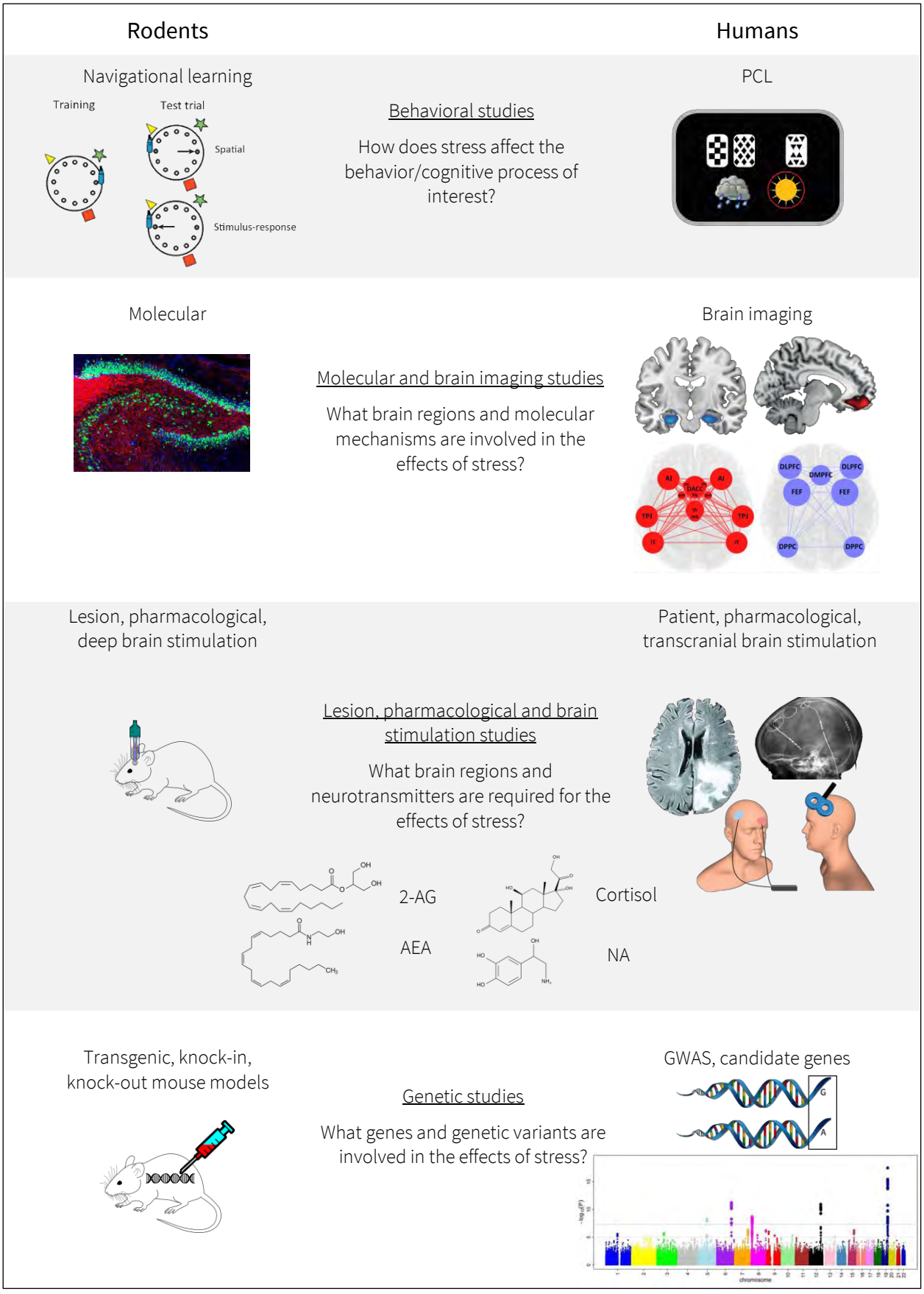


Figure 28. Course of action to investigate stress effects on cognition and behavior, exemplified by navigational and probabilistic classification learning (PCL). Insights from these different approaches will reciprocally stimulate new experiments (partly taken and modulated from Dayan et al., 2013; Schwabe and Wolf, 2013; Hermans et al., 2014). GWAS genome-wide association study

4.3 Stress × gene interactions: a model of vulnerability and resilience

Many behavioral, neuroimaging and genetic studies have investigated how individual differences in stress responsiveness and adaptation are translated into relative resilience or vulnerability to the pathogenic effects of stress (de Kloet et al., 2005; Lupien et al., 2009; McCrory et al., 2011; Hillard et al., 2012; Bogdan et al., 2013; McEwen and Morrison, 2013; Lucassen et al., 2014). Generally, in genetically predisposed individuals dysfunctional control mechanisms in response to stress can introduce a risk of developing a disorder after adverse experiences. In contrast, "resilience is the process of adapting well in the face of adversity, trauma, tragedy, threats or significant sources of stress" (American Psychological Association, 2010). Resilience is likely, at least to some extent, genetically determined, as is indicated by the importance of genetic differences in the ADRA2B, NR3C2 and CNR1 genes for stress-related psychopathologies discussed in chapter 4.1.2. Resilience can also be acquired to some extent and depends on our upbringing and life experiences (Franklin et al., 2012). Thus, it seems that behavioral, social and neurobiological factors of resilience can protect against and also develop in response to adverse experiences. Stress vulnerability models propose that biological, social and psychosocial factors determine resilience (Ingram and Luxton, 2005). It is also emphasized that the impact of stressful life events depends on the nature and intensity of the stressor, as well as on genetic and (endo)phenotypic risk factors. Among the behavioral and social factors, the most frequently listed are a strong social support system, social competence and agreeableness, beneficial self- and emotion regulation, optimism, cognitive flexibility, the use of active coping strategies, experiences made with controllable stressors – and mastering these challenges – as a preparation for future adverse encounters, the capacity to find some meaning in these adverse experience and to recover quickly from them (Yehuda et al., 2006; Feder et al., 2009; Franklin et al., 2012; Southwick and Charney, 2012). The most important neurobiological factors are rapid activation and efficient termination of the stress response axes, adaptive transcriptional mechanisms in response to adverse experiences, a stable dopaminergic reward system, a functional (v)mPFC and a well-modulated amygdala (Feder et al., 2009; Franklin et al., 2012; Southwick and Charney, 2012). Thus, risk and protective factors interact and determine the individual degree of resilience and vulnerability. Possessing many genetic, neurobiological, developmental and psychosocial risk factors increases stress vulnerability, whereas possessing

and augmenting protective factors increases the likelihood of stress resilience (Southwick and Charney, 2012).

Three observations and hypotheses need to be emphasized that are particularly relevant in the framework of resilience and vulnerability and that are directly related to the experimental findings discussed in this thesis. First, excessive noradrenergic activation promotes exaggerated affective responding and overconsolidation of fear memories (Rauch et al., 2000; Roozendaal et al., 2009; Ronzoni et al., 2016), which leads to the proposal that somewhat reduced noradrenergic signaling may promote resilience (Feder et al., 2009). Second, the vmPFC has been implicated in inhibitory control of stress pathways and has been associated with several important factors of resilience such as active coping, optimism and positive emotions, and the acquisition of stress resilience (Franklin et al., 2012). Importantly, the eCB system has been proposed to maintain vmPFC functioning and facilitate the transition of beneficial stress coping into lasting resilience (Worley et al., 2017). Third, the different stress response phases fulfill specific functions that contribute to stress resilience and that depend on balanced MR and GR functioning. Specifically, rapid non-genomic MR actions increase alertness, focused attention and appraisal, non-genomic GR actions facilitate stress recovery and appropriate encoding of the stressful experience, and genomic receptor actions promote consolidation of the stressful experience and adaptations to prepare the organism for future stressors (de Kloet, 2008). High MR functionality in particular has repeatedly been associated with stress resilience (Kanatsou et al., 2015; ter Heegde et al., 2015), whereas GR gene variants associated with increased sensitivity to GCs are known risk polymorphisms for depression and PTSD (Bachmann et al., 2005; van Rossum et al., 2006; Hauer et al., 2011). Thus, reduced noradrenergic activity, as well as enhanced eCB and MR signaling (in balance with GR signaling) seem to promote resilience. The results of our experimental studies can illustrate how genetic differences in affective processing and the engagement of multiple memory systems under stress may be integral factors of stress resilience and vulnerability. According to previous studies (de Quervain et al., 2007a; Hillard et al., 2012; ter Heegde et al., 2015), the ADRA2B deletion seems to be a stress vulnerability factor, whereas genetic variants associated with high MR functionality and the rs1049353 A allele seem to be stress resilience factors. In combination with the protective functions of noradrenergic inhibition and enhanced MR and eCB signaling, I propose the following model of stress resilience and vulnerability (**Figure 29**).

The genetic predisposition of enhanced stress resilience (ADRA2B deletion non-carriers, MR haplotype and rs1049343 AA/AG genotype carriers) has specific consequences depending on the stress response phase and the cognitive domain of interest (**Figure 29A**). In accordance with studies investigating the effects of stress on emotional responding (Hermans et al., 2014) and memory quantity (Roozendaal et al., 2009) and quality (Schwabe, 2017), the amygdala is at the center of our model. Actions of NA, cortisol and eCBs in the amygdala coordinate the engagement of sensory processing and memory regions, whereas prefrontal areas can exert regulatory top-down control over the amygdala to prevent excessive responding and overly strong hippocampus-dependent memory encoding. Specifically, under acute stress when NA levels rapidly rise, increased activation of the salience network promotes alertness and focused attention. In response to rising cortisol levels, enhanced membrane-bound MR and eCB signaling in the vmPFC and limbic brain regions has several important consequences. First, CB1R actions protect against excessive excitation of limbic regions and fear responding. Second, eCBs together with membrane MRs, appropriate affective processing, cognitive emotion regulation and adaptive coping are facilitated, partly by enhanced downregulation of the amygdala. Third, GC actions via membrane-bound MRs promote a shift toward the dorsal striatum, an adaptive mechanisms that saves cognitive resources, facilitates learning and rescues performance under stress. Fourth, despite this shift, enhanced CB1R functioning engages the hippocampus to store and incorporate important information, e.g. regarding the successful coping with the stressor, into autobiographical memory. Since the engagement of dorsal striatal memory has also been associated with rather rigid and inflexible memories that are difficult to generalize to novel situations and to link to existing knowledge structures (Plessow et al., 2011; Dandolo and Schwabe, 2016), I also propose that in the aftermath of stress, a highly relevant stress resilience factor is the ability to shift back to the hippocampus. This shift back to the hippocampus further promotes the CB1R-mediated consolidation of the stressful experience and initiates adaptive mechanisms which will prepare the organism for future stress encounters. Although not investigated here, NR3C1 gene polymorphisms that have been associated with GC sensitivity and stress-related psychopathologies (DeRijk and de Kloet, 2008; Ackermann et al., 2013) may be highly relevant in this respect. Earlier successful experiences with manageable stressors and associated epigenetic mechanisms may facilitate these processes and increase efficiency of stress coping behaviors.

In contrast, accumulation of genetic risk factors (ADRA2B deletion and rs1049353 GG genotype carriers, MR haplotype non-carriers) may increase vulnerability to stress-related psychopathologies (**Figure 29B**). Specifically, excessive noradrenergic activation of the amygdala under acute stress may lead to overly strong connections of the amygdala with brain regions important for visual processing (occipital cortex), attention (ACC) and episodic memory (hippocampus). As a consequence, vulnerable individuals are characterized by exaggerated affective responding, as well as overly strong encoding of the stressful encounter. Additionally, impaired MR functionality and excessive noradrenergic activation prevent the shift toward the dorsal striatum, which comes at the cost of impaired learning under stress and further strengthens memory formation of the stressful experience. Dysfunctional eCB signaling and reduced recruitment of the (v)mPFC may lead to the use of maladaptive coping strategies. Enhanced consolidation of the stressful experience, including strong feelings of anxiety caused by excessive affective processing, and the inability to effectively cope with the stressor may be able to explain the negative memory bias observed in patients with depression, the excessive fear responding and fear memory seen in anxiety disorders, and the overly strong and vivid traumatic memories displayed by PTSD patients.

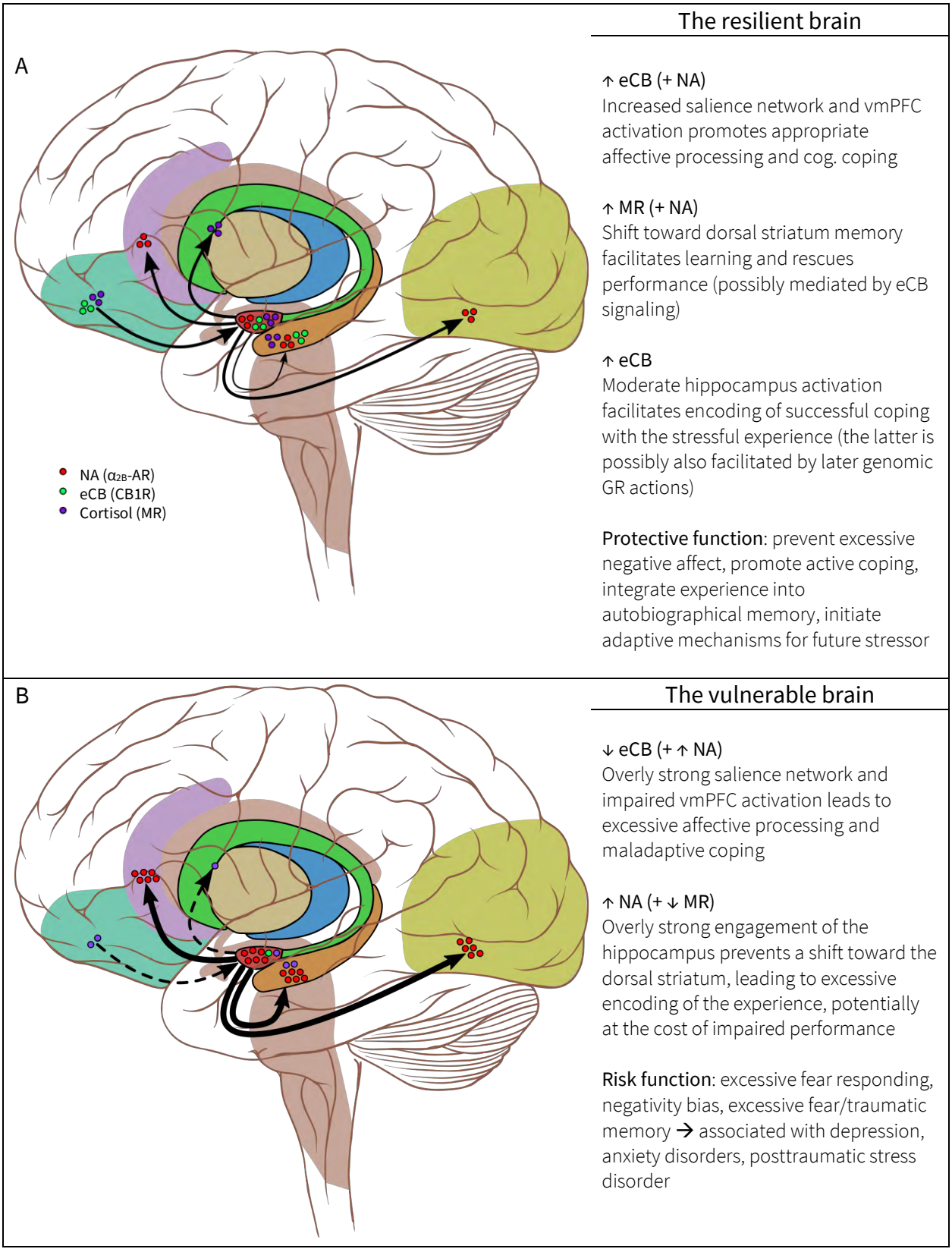


Figure 29. Proposed model of the resilient and vulnerable brain. (**A**) The resilient individual carrying the ADRA2B non-deletion, MR haplotype and rs1049353 AA/AG genotype may be characterized by appropriate affective responding (increased attention (ACC in purple), enhanced sensory processing (visual cortex in yellow) and cognitive coping (vmPFC in turquois)), and a shift toward the dorsal striatum to facilitate learning and rescue performance under stress. (**B**) Carrying the ADRA2 deletion but not the MR haplotype and rs1049353 AA/AG genotype may lead to enhanced stress vulnerability characterized by excessive affective responding, maladaptive coping and overly strong encoding of the stressful event.

This model illustrates that resilience is not simply the extent to which individuals are able to engage cognitive and flexible processes under stress. Rather, it is the adaptive distribution of the available resources and the optimal balance of the effects of different stress modulators in the different stress response phases. This model also has several implications that illustrate the complexity of the effects of stress and that need to be considered. The effects of stress strongly depend on the stress response phase that is being investigated and the relative contribution of different stress mediators may lead to such fine-tuned changes in brain activation and connectivity patterns that more sophisticated real-time, multivariate methods are required to capture them. Also, although the model depicts the genetic modulation of stress effects through differences in neurotransmitter quantity, this is not necessarily the mechanism causing these effects. In fact, since our results remained largely unchanged when cortisol differences between MR haplotype carriers and non-carriers in our fMRI experiment were accounted for points to other or at least additional mechanisms which may be at work.

4.4 Conclusion and outlook

To answer our general research question, it seems that genetic differences do have a large impact on individual variability in the effects of stress on affective processing and memory (**Box 1**). Our data provide further evidence for the involvement of important stress mediators and suggest that in response to acute stress, a functional vmPFC during affective processing and a shift toward the dorsal striatum during learning may be indicators of enhanced stress resilience (**Box 1**). Therefore, investigating genetic differences may benefit research in stress effects on other cognitive domains such as attention (Goldfarb et al., 2016) and decision-making (Doll et al., 2015) as well. Especially for the sake of more personalized and effective prevention and treatment options, further investigating genetic differences and understanding the mechanisms underlying the effects of stress is indispensable. Our experimental investigations of genetic differences in the ADRA2B, NR3C2 and CNR1 genes are a starting point for such future investigations. More sophisticated genetic and imaging approaches making use of multivariate analyses and genetic risk/profile scores will greatly advance the understanding of network changes and the contribution of several genetic polymorphisms in conjunction. Additionally, the analysis of epistatic interaction between the ADRA2B deletion and MR haplotype provided first evidence for interactive effects which needs to be further pursued. Advancements in the specificity of pharmacological agents may allow

investigation of the differential roles of the two eCBS AEA and 2-AG and of the AR subtypes, thereby improving pharmacological treatment efficacy and reducing side effects eventually. Although our results suggest improved emotion regulation in rs1049353 AA/AG genotype carriers, this should be examined more explicitly. Future studies hopefully will find out how eCBs modulate emotion regulation and whether certain CNR1 polymorphisms promote the use of cognitive strategies under stress, a highly relevant resilience factor. Since the roles of both MRs and CB1Rs in the mPFC are similar in their effects on negative feedback control of the HPA axis and adaptive coping under stress, interaction between these receptor actions need to be examined. This also suggests that eCBs may not only mediate GR effects on memory quantity (Campolongo et al., 2009), but that they may have a similar role to MRs in facilitating the stress-induced shift toward the dorsal striatum. Although the focus has so far been on the actions of membrane-bound MRs, it is likewise important to investigate the effects of membrane GRs for two reasons. First, non-genomic GR actions increase eCB levels (Groeneweg et al., 2011) and promote early memory consolidation under stress (Roozendaal et al., 2010). Second, the balance between MRs and GRs has received increasing attention and seems to be highly relevant for processes promoting coping, memory and resilience in response to stress (de Kloet et al., 2018). Another intriguing question concerns the role of NA in the relative engagement of multiple memory systems under stress. Our results indicate that excessive noradrenergic activation in the amygdala may strengthen the hippocampal system to such an extent that MR actions may not be able to induce the beneficial shift toward the dorsal striatum under stress. This still leaves the question whether NA is actually necessary for the stress-induced shift to occur. It may well be that NA is relevant for memory quantity, due to its hippocampus-enhancing effects but that it may not play such an important role for memory quality or may even be able to prevent the shift toward the dorsal striatum when levels are excessive. Pharmacological studies may shed further light on this issue and in accordance with our findings, an α_{2B}-AR antagonist may prevent the stress-induced shift toward the dorsal striatum, whereas this shift may still be observed when an α_{2B}-AR agonist is given. Generally, since stress does not only change activation and connectivity of single brain regions but has been shown to lead to large-scale network changes (Hermans et al., 2011) that have been related to noradrenergic arousal (Young et al., 2017), to further elucidate the role of genetic differences in stress effects, the investigation of real-time network changes as a function of cortisol, NA and eCB levels is required. Another goal of future research endeavors will be to test

the efficiency or flexibility of multiple memory systems in the sense that whereas a shift in response to an acute stressor likely is beneficial, a shift back toward the hippocampus may be necessary to consolidate the experience into autobiographical memory and to initiate adaptive mechanisms for future stressor encounters, effects which may already be facilitated by eCBs in the acute stress phase but that may require further facilitation by enhanced cognitive, hippocampus-dependent processes in the aftermath of stress. An overreliance on the hippocampus under stress may promote depression, anxiety disorders and possibly PTSD (see **Figure 29**), whereas an overreliance on habitual processes (under no stress conditions) may be a risk factor for drug addiction (Everitt and Robbins, 2005), alcohol dependency (Sjoerds et al., 2013), OCD (Gillan et al., 2011; Voon et al., 2015) and Tourette syndrome (Singer, 2016; **Box 1**). It is the fine balance mediated by different neuromodulators that is crucial and dysregulations in either direction will have extensive consequences.

It is highly relevant for the identification of factors contributing to resilience and vulnerability to consider genetic differences, the effects of sex, age, social and psychosocial factors, as well as prenatal, early and chronic stress experiences but also highly positive life experiences and their associated epigenetic mechanisms (**Box 1**). Although identifying the factors contributing to individual differences in stress effects on cognition and emotion will help us understand some of the mechanisms contributing to resilience and vulnerability, the highly complex phenotypes and the manifold interactions between genetic and environmental factors indeed emphasize that "every human being […] is in this world just once […] and no accident […] will throw together a second time into a unity such a curious and diffuse plurality" (Friedrich Nietzsche, 1874; translation taken from (Kaufmann, 1956).

Box 1 |	Take-home messages
1.	Genetic polymorphisms can have a large impact on stress effects on memory and emotion.
2.	Engagement of the vmPFC is crucial for appropriate emotional responding and stress coping.
3.	The shift toward the dorsal striatum facilitates learning under acute stress.
4.	Resilience is not measured as the extent to which cognitive and flexible processes are engaged under stress. Instead it is measured as the extent to which the available resources are distributed to ensure appropriate affective responding, learning and consolidation of the stressful experience. This may involve cognitive as well as habitual processes.
5.	The ADRA2B non-deletion, MR haplotype and rs1049353 AA/AG genotype are genetic resilience factors.
6.	Reducing NA and enhancing MR and CB1R signaling may promote resilience or treatment efficacy in genetically predisposed individuals.
7.	Research needs to assess and take into consideration the influence of genetic differences, the effects of sex, age, social support and psychosocial factors such as coping style, as well as prenatal/early life/chronic stress exposure but also highly positive life events, and epigenetic mechanisms.
8.	Overreliance on the hippocampus under stress may promote depression, anxiety disorders and possibly PTSD, whereas overreliance on habitual processes (under no stress conditions or in the aftermath of stress) may be a risk factor for drug addiction, alcohol dependency, obsessive-compulsive disorder and Tourette syndrome.

References

Ackermann S, Heck A, Rasch B, Papassotiropoulos A, de Quervain DJ (2013) The BclI polymorphism of the glucocorticoid receptor gene is associated with emotional memory performance in healthy individuals. *Psychoneuroendocrinology* 38:1203-1207.

Agrawal A, Nelson EC, Littlefield AK, Bucholz KK, Degenhardt L, Henders AK, Madden PA, Martin NG, Montgomery GW, Pergadia ML, Sher KJ, Heath AC, Lynskey MT (2012) Cannabinoid receptor genotype moderation of the effects of childhood physical abuse on anhedonia and depression. *Arch Gen Psychiatry* 69:732-740.

Akirav I (2011) The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus. *Front Behav Neurosci* 5:34.

Akirav I (2013) Targeting the endocannabinoid system to treat haunting traumatic memories. *Front Behav Neurosci* 7:1-3.

Aldao A, Nolen-Hoeksema S, Schweizer S (2010) Emotion-regulation strategies across psychopathology: A meta-analytic review. *Clin Psychol Rev* 30:217-237.

Alexander JK, Hillier A, Smith RM, Tivarus ME, Beversdorf DQ (2007) Beta-adrenergic modulation of cognitive flexibility during stress. *J Cogn Neurosci* 19:468-478.

American Psychological Association (2010) The road to resilience. In. Washington, DC: APA.

Arloth J et al. (2015) Genetic Differences in the Immediate Transcriptome Response to Stress Predict Risk-Related Brain Function and Psychiatric Disorders. *Neuron* 86:1189-1202.

Arnsten AF (2009) Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci* 10:410-422.

Arnsten AF (2015) Stress weakens prefrontal networks: molecular insults to higher cognition. *Nat Neurosci* 18:1376-1385.

Aron AR, Robbins TW, Poldrack RA (2004) Inhibition and the right inferior frontsal cortex. *Trends Cogn Sci* 8:170-177.

Atsak P, Hauer D, Campolongo P, Schelling G, McGaugh JL, Roozendaal B (2012) Glucocorticoids interact with the hippocampal endocannabinoid system in impairing retrieval of contextual fear memory. *Proc Natl Acad Sci U S A* 109:3504-3509.

Atsak P, Hauer D, Campolongo P, Schelling G, Fornari RV, Roozendaal B (2015) Endocannabinoid signaling within the basolateral amygdala integrates multiple stress hormone effects on memory consolidation. *Neuropsychopharmacology* 40:1485-1494.

Atsak P, Guenzel FM, Kantar-Gok D, Zalachoras I, Yargicoglu P, Meijer OC, Quirarte GL, Wolf OT, Schwabe L, Roozendaal B (2016) Glucocorticoids mediate stress-induced impairment of retrieval of stimulus-response memory. *Psychoneuroendocrinology* 67:207-215.

Bachmann AW, Sedgley TL, Jackson RV, Gibson JN, Young RM, Torpy DJ (2005) Glucocorticoid receptor polymorphisms and post-traumatic stress disorder. *Psychoneuroendocrinology* 30:297-306.

Baddeley A (1992) Working memory. *Science* 255:556-559.

Bambico FR, Katz N, Debonnel G, Gobbi G (2007) Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. *J Neurosci* 27:11700-11711.

Banks SJ, Eddy KT, Angstadt M, Nathan PJ, Phan KL (2007) Amygdala-frontal connectivity during emotion regulation. *Soc Cogn Affect Neurosci* 2:303-312.

Bartels M, Van den Berg M, Sluyter F, Boomsma DI, de Geus EJC (2003) Heritability of cortisol levels: review and simultaneous analysis of twin studies. *Psychoneuroendocrinology* 28:121-137.

Bedse G, Colangeli R, Lavecchia AM, Romano A, Altieri F, Cifani C, Cassano T, Gaetani S (2014) Role of the basolateral amygdala in mediating the effects of the fatty acid amide hydrolase inhibitor URB597 on HPA axis response to stress. *Eur Neuropsychopharmacol* 24:1511-1523.

Bellebaum C, Daum I (2008) Learning-related changes in reward expectancy are reflected in the feedback-related negativity. *Eur J Neurosci* 27:1823-1835.

Benoit JD, Rakic P, Frick KM (2015) Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. *Behav Brain Res* 281:1-8.

Beyer CE, Dwyer JM, Piesla MJ, Platt BJ, Shen R, Rahman Z, Chan K, Manners MT, Samad TA, Kennedy JD, Bingham B, Whiteside GT (2010) Depression-like phenotype following chronic CB1 receptor antagonism. *Neurobiol Dis* 39:148-155.

Bogdan R, Williamson DE, Hariri AR (2012) Mineralocorticoid receptor Iso/Val (rs5522) genotype moderates the association between previous childhood emotional neglect and amygdala reactivity. *Am J Psychiatry* 169:512-522.

Bogdan R, Hyde LW, Hariri AR (2013) A neurogenetics approach to understanding individual differences in brain, behavior, and risk for psychopathology. *Mol Psychiatry* 18:288-299.

Bogdan R, Perlis RH, Fagerness J, Pizzagalli DA (2010) The impact of mineralocorticoid receptor ISO/VAL genotype (rs5522) and stress on reward learning. *Genes Brain Behav* 9:658-667.

Bogdan R, Pagliaccio D, Baranger DA, Hariri AR (2016) Genetic Moderation of Stress Effects on Corticolimbic Circuitry. *Neuropsychopharmacology* 41:275-296.

Bogdanov M, Schwabe L (2016) Transcranial stimulation of the dorsolateral prefrontal cortex prevents stress-induced working memory deficits. *J Neurosci* 36:1429-1437.

Bolton JL, Molet J, Ivy A, Baram TZ (2017) New insights into early-life stress and behavioral outcomes. *Curr Opin Behav Sci* 14:133-139.

Bortolato M, Mangieri RA, Fu J, Kim JH, Arguello O, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D (2007) Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. *Biol Psychiatry* 62:1103-1110.

Bossong MG, van Hell HH, Jager G, Kahn RS, Ramsey NF, Jansma JM (2013) The endocannabinoid system and emotional processing: a pharmacological fMRI study with 9-tetrahydrocannabinol. *Eur Neuropsychopharmacol* 23:1687-1697.

Brassen S, Kalisch R, Weber-Fahr W, Braus DF, Büchel C (2008) Ventromedial prefrontal cortex processing during emotional evaluation in late-life depression: a longitudinal functional magnetic resonance imaging study. *Biol Psychiatry* 64:349-355.

Bremner JD (2002) Neuroimaging studies in post-traumatic stress disorder. *Curr Psychiatry Rep* 4:254-263.

Buchanan TW, Lovallo WR (2001) Enhanced memory for emotional material following stress-level cortisol treatment in humans. *Psychoneuroendocrinology* 26:307-317.

Buchanan TW, Tranel D, Adolphs R (2006) Impaired memory retrieval correlates with individual differences in cortisol response but not autonomic response. *Learn Mem* 13:282-287.

Budisavljevic S, Kawadler JM, Dell'Acqua F, Rijsdijk FV, Kane F, Picchioni M, McGuire P, Toulopoulou T, Georgiades A, Kalidindi S, Kravariti E, Murray RM, Murphy DG, Craig MC, Catani M (2016) Heritability of the limbic networks. *Soc Cogn Affect Neurosci* 11:746-757.

Cahill L, McGaugh JL (1998) Mechanisms of emotional arousal and lasting declarative memory. *Trends Neurosci* 21:294-299.

Cahill L, Gorski L, Le K (2003) Enhanced human memory consolidation with post-learning stress: interaction with the degree of arousal at encoding. *Learn Mem* 10:270-274.

Cahill L, Haier RJ, fallon J, Alkire MT, Tang C, Keator D, Wu J, McGaugh JL (1996) Amygdala activity at encoding correlated with long-term, free recall of emotional information. *Proc Natl Acad Sci U S A* 93:8016-8021.

Campbell-Sills L, Simmons AN, Lovero KL, Rochlin AA, Paulus MP, Stein MB (2011) Functioning of neural systems supporting emotion regulation in anxiety-prone individuals. *Neuroimage* 54:689-696.

Campolongo P, Roozendaal B, Trezza V, Hauer D, Schelling G, McGaugh JL, Cuomo V (2009) Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. *Proc Natl Acad Sci U S A* 106:4888-4893.

Carter CS, Bearden CE, Bullmore ET, Geschwind DH, Glahn DC, Gur RE, Meyer-Lindenberg A, Weinberger DR (2017) Enhancing the Informativeness and Replicability of Imaging Genomics Studies. *Biol Psychiatry* 82:157-164.

Carver CS, Connor-Smith J (2010) Personality and coping. *Annu Rev Psychol* 61:679-704.

Caspi A, Moffitt TE (2006) Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat Rev Neurosci* 7:583-590.

Chakrabarti B, Kent L, Suckling J, Bullmore E, Baron-Cohen S (2006) Variations in the human cannabinoid receptor (CNR1) gene modulate striatal responses to happy faces. *Eur J Neurosci* 23:1944-1948.

Champagne DL, Bagot RC, van Hasselt F, Ramakers G, Meaney MJ, de Kloet ER, Joels M, Krugers H (2008) Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. *J Neurosci* 28:6037-6045.

Chattarji S, Tomar A, Suvrathan A, Ghosh S, Rahman MM (2015) Neighborhood matters: divergent patterns of stress-induced plasticity across the brain. *Nat Neurosci* 18:1364-1375.

Chatterton RTJ, Vogelsong KM, Lu YC, Ellman AB, Hudgens GA (1996) Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin Physiol* 16:433-448.

Childs E, White TL, de Wit H (2014) Personality traits modulate emotional and physiological responses to stress. *Behav Pharmacol* 25:493-502.

Chiu CQ, Puente N, Grandes P, Castillo PE (2010) Dopaminergic modulation of endocannabinoid-mediated plasticity at GABAergic synapses in the prefrontal cortex. *J Neurosci* 30:7236-7248.

Chocyk A, Bobula B, Dudys D, Przyborowska A, Majcher-Maslanka I, Hess G, Wedzony K (2013) Early-life stress affects the structural and functional plasticity of the medial prefrontal cortex in adolescent rats. *Eur J Neurosci* 38:2089-2107.

Chrousos GP (2009) Stress and disorders of the stress system. *Nat Rev Endocrinol* 5:374-381.

Corkin S (2002) What's new with the amnesic patient H.M.? *Nat Rev Neurosci* 3:153-160.

Cornelisse S, Joels M, Smeets T (2011) A randomized trial on mineralocorticoid receptor blockade in men: effects on stress responses, selective attention, and memory. *Neuropsychopharmacology* 36:2720-2728.

Cousijn H, Rijpkema M, Qin S, van Marle HJ, Franke B, Hermans EJ, van Wingen G, Fernandez G (2010) Acute stress modulates genotype effects on amygdala processing in humans. *Proc Natl Acad Sci U S A* 107:9867-9872.

Cramér H, Hinkley D (1946) Theoretical statistics: Chapman & Hall.

D'Esposito M, Postle BR (2015) The cognitive neuroscience of working memory. *Annu Rev Psychol* 66:115-142.

Dandolo LC, Schwabe L (2016) Stress-induced cortisol hampers memory generalization. *Learn Mem* 23:679-683.

Datson NA, van der Perk J, de Kloet ER, Vreugdenhil E (2001) Identification of corticosteroid-responsive genes in rat hippocampus using serial analysis of gene expression. *Eur J Neurosci* 14:675-689.

Datson NA, Morsink MC, Meijer OC, de Kloet ER (2008) Central corticosteroid actions: Search for gene targets. *Eur J Pharmacol* 583:272-289.

Day HE, Campeau S, Watson SJJ, Akil H (1997) Distribution of alpha 1a-, alpha 1b- and alpha 1d-adrenergic receptor mRNA in the rat brain and spinal cord. *J Chem Neuroanat* 13:115-139.

Dayan E, Censor N, Buch ER, Sandrini M, Cohen LG (2013) Noninvasive brain stimulation: from physiology to network dynamics and back. *Nat Neurosci* 16:838-844.

de Bitencourt RM, Pamplona FA, Takahashi RN (2013) A current overview of cannabinoids and glucocorticoids in facilitating extinction of aversive memories: potential extinction enhancers. *Neuropharmacology* 64:389-395.

de Boer SF, van der Gugten J (1987) Daily variations in plasma noradrenaline, adrenaline and corticosterone concentrations in rats. *Physiol Behav* 40:323-328.

de Kloet ER (2008) About stress hormones and resilience to psychopathology. *J Neuroendocrinol* 20:885-892.

de Kloet ER, Reul JM (1987) Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems. *Psychoneuroendocrinology* 12:83-105.

de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 6:463-475.

de Kloet ER, Vreugdenhil E, Oitzl MS, Joels M (1998) Brain corticosteroid receptor balance in health and disease. *Endocr Rev* 19:269-301.

de Kloet ER, Meijer OC, de Nicola AF, de Rijk RH, Joels M (2018) Importance of the brain corticosteroid receptor balance in metaplasticity, cognitive performance and neuro-inflammation. *Front Neuroendocrinol*.

de Kloet ER, Otte C, Kumsta R, Kok L, Hillegers MH, Hasselmann H, Kliegel D, Joels M (2016) Stress and depression: a crucial role of the mineralocorticoid receptor. *J Neuroendocrinol* 28.

de Oliveira Alvares L, Engelke DS, Diehl F, Scheffer-Teixeira R, Haubrich J, de Freitas Cassini L, Molina VA, Quillfeldt JA (2010) Stress response recruits the hippocampal endocannabinoid system for the modulation of fear memory. *Learn Mem* 17:202-209.

de Quervain D, Schwabe L, Roozendaal B (2017) Stress, glucocorticoids and memory: implications for treating fear-related disorders. *Nat Rev Neurosci* 18:7-19.

de Quervain D, Kolassa IT, Ertl V, Onyut PL, Neuner F, Elbert T, Papassotiropoulos A (2007a) A deletion variant of the alpha2b-adrenoceptor is related to emotional memory in Europeans and Africans. *Nat Neurosci* 10:1137-1139.

de Quervain DF, Roozendaal B, Nitsch RM, McGaugh JL, Hock C (2000) Acute cortisone administration impairs retrieval of long-term declarative memory in humans. *Nat Neurosci* 3:313-314.

de Quervain DJ, Papassotiropoulos A (2006) Identification of a genetic cluster influencing memory performance and hippocampal activity in humans. *Proc Natl Acad Sci U S A* 103:4270-4274.

de Quervain DJ, Margraf J (2008) Glucocorticoids for the treatment of post-traumatic stress disorder and phobias: a novel therapeutic approach. *Eur J Pharmacol* 583:365-371.

de Quervain DJ, Roozendaal B, McGaugh JL (1998) Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature* 394:787-790.

de Quervain DJ, Aerni A, Roozendaal B (2007b) Preventive effect of beta-adrenoceptor blockade on glucocorticoid-induced memory retrieval deficits. *Am J Psychiatry* 164:967-969.

de Quervain DJ, Aerni A, Schelling G, Roozendaal B (2009) Glucocorticoids and the regulation of memory in health and disease. *Front Neuroendocrinol* 30:358-370.

de Quervain DJ, Bentz D, Michael T, Bolt OC, Wiederhold BK, Margraf J, Wilhelm FH (2011) Glucocorticoids enhance extinction-based psychotherapy. *Proc Natl Acad Sci U S A* 108:6621-6625.

de Quervain DJ, Henke K, Aerni A, Treyer V, McGaugh JL, Berthold T, Nitsch RM, Buck A, Roozendaal B, Hock C (2003) Glucocorticoid-induced impairment of declarative memory retrieval is associated with reduced blood flow in the medial temporal lobe. *Eur J Neurosci* 17:1296-1302.

DeRijk RH (2009) Single nucleotide polymorphisms related to HPA axis reactivity. *Neuroimmunomodulation* 16:340-352.

DeRijk RH, de Kloet ER (2008) Corticosteroid receptor polymorphisms: determinants of vulnerability and resilience. *Eur J Pharmacol* 583:303-311.

DeRijk RH, de Kloet ER, Zitman FG, van Leeuwen N (2011) Mineralocorticoid receptor gene variants as determinants of HPA axis regulation and behavior. *Endocr Dev* 20:137-148.

DeRijk RH, Wust S, Meijer OC, Zennaro MC, Federenko IS, Hellhammer DH, Giacchetti G, Vreugdenhil E, Zitman FG, de Kloet ER (2006) A common polymorphism in the mineralocorticoid receptor modulates stress responsiveness. *J Clin Endocrinol Metab* 91:5083-5089.

Di Iorio CR, Carey CE, Michalski LJ, Corral-Frias NS, Conley ED, Hariri AR, Bogdan R (2017) Hypothalamic-pituitary-adrenal axis genetic variation and early stress moderates amygdala function. *Psychoneuroendocrinology* 80:170-178.

Dick DM, Agrawal A, Keller MC, Adkins A, Aliev F, Monroe S, Hewitt JK, Kendler KS, Sher KJ (2015) Candidate gene-environment interaction research: reflections and recommendations. *Perspect Psychol Sci* 10:37-59.

Dickerson KC, Li J, Delgado MR (2011) Parallel contributions of distinct human memory systems during probabilistic learning. *Neuroimage* 55:266-276.

Dickerson SS, Kemeny ME (2004) Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull* 130:355-391.

Dincheva I, Drysdale AT, Hartley CA, Johnson DC, Jing D, King EC, Ra S, Gray JM, Yang R, DeGruccio AM, Huang C, Cravatt BF, Glatt CE, Hill MN, Casey BJ, Lee FS (2015) FAAH genetic variation enhances fronto-amygdala function in mouse and human. *Nat Commun* 6:6395.

Dolcos F, LaBar KS, Cabeza R (2004) Interaction between the amygdala and the medial temporal lobe memory system predicts better memory for emotional events. *Neuron* 42:855-863.

Doll BB, Shohamy D, Daw ND (2015) Multiple memory systems as substrates for multiple decision systems. *Neurobiol Learn Mem* 117:4-13.

Domschke K, Dannlowski U, Ohrmann P, Lawford B, Bauer J, Kugel H, Heindel W, Young R, Morris P, Arolt V, Deckert J, Suslow T, Baune BT (2008) Cannabinoid receptor 1 (CNR1) gene: impact on antidepressant treatment response and emotion processing in major depression. *Eur Neuropsychopharmacol* 18:751-759.

Dono LM, Currie PJ (2012) The cannabinoid receptor CB(1) inverse agonist AM251 potentiates the anxiogenic activity of urocortin I in the basolateral amygdala. *Neuropharmacology* 62:192-199.

Dorey R, Pierard C, Chauveau F, David V, Beracochea D (2012) Stress-induced memory retrieval impairments: different time-course involvement of corticosterone and glucocorticoid receptors in dorsal and ventral hippocampus. *Neuropsychopharmacology* 37:2870-2880.

Dorey R, Pierard C, Shinkaruk S, Tronche C, Chauveau F, Baudonnat M, Beracochea D (2011) Membrane mineralocorticoid but not glucocorticoid receptors of the dorsal hippocampus mediate the rapid effects of corticosterone on memory retrieval. *Neuropsychopharmacology* 36:2639-2649.

Dubreucq S, Matias I, Cardinal P, Haring M, Lutz B, Marsicano G, Chaouloff F (2012) Genetic dissection of the role of cannabinoid type-1 receptors in the emotional consequences of repeated social stress in mice. *Neuropsychopharmacology* 37:1885-1900.

Ebner K, Singewald N (2017) Individual differences in stress susceptibility and stress inhibitory mechanisms. *Current Opinion in Behavioral Sciences* 14:54-64.

Eichenbaum H (2000) A cortical-hippocampal system for declarative memory. *Nat Rev Neurosci* 1:41-50.

Elliott AE, Packard MG (2008) Intra-amygdala anxiogenic drug infusion prior to retrieval biases rats towards the use of habit memory. *Neurobiol Learn Mem* 90:616-623.

Ernst B, Steinhauser M (2012) Feedback-related brain activity predicts learning from feedback in multiple-choice testing. *Cogn Affect Behav Neurosci* 12:323-336.

Etkin A, Wager TD (2007) Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry* 164:1476-1488.

Etkin A, Egner T, Kalisch R (2011) Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci* 15:85-93.

Evanson NK, Tasker JG, Hill MN, Hillard CJ, Herman JP (2010) Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling. *Endocrinology* 151:4811-4819.

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* 8:1481-1489.

Fastenrath M, Coynel D, Spalek K, Milnik A, Gschwind L, Roozendaal B, Papassotiropoulos A, de Quervain DJ (2014) Dynamic modulation of amygdala-hippocampal connectivity by emotional arousal. *J Neurosci* 34:13935-13947.

Feder A, Nestler EJ, Charney DS (2009) Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci* 10:446-457.

Ferry B, Roozendaal B, McGaugh JL (1999) Basolateral amygdala noradrenergic influences on memory storage are mediated by an interaction between beta- and alpha1-adrenoceptors. *J Neurosci* 19:5119-5123.

Foerde K, Knowlton BJ, Poldrack RA (2006) Modulation of competing memory systems by distraction. *Proc Natl Acad Sci U S A* 103:11778-11783.

Foerde K, Poldrack RA, Knowlton BJ (2007) Secondary-task effects on classification learning. *Mem Cognit* 35:864-874.

Foerde K, Race E, Verfaellie M, Shohamy D (2013) A role for the medial temporal lobe in feedback-driven learning: evidence from amnesia. *J Neurosci* 33:5698-5704.

Franklin TB, Saab BJ, Mansuy IM (2012) Neural mechanisms of stress resilience and vulnerability. *Neuron* 75:747-761.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. *Science* 296:2225-2229.

Gaffan EA, Davies J (1981) The role of exploration in win-shift and win-stay performance on a radial maze. *Learn Motiv* 12:282-299.

Ganon-Elazar E, Akirav I (2009) Cannabinoid receptor activation in the basolateral amygdala blocks the effects of stress on the conditioning and extinction of inhibitory avoidance. *J Neurosci* 29:11078-11088.

Ganon-Elazar E, Akirav I (2012) Cannabinoids prevent the development of behavioral and endocrine alterations in a rat model of intense stress. *Neuropsychopharmacology* 37:456-466.

Ganon-Elazar E, Akirav I (2013) Cannabinoids and traumatic stress modulation of contextual fear extinction and GR expression in the amygdala-hippocampal-prefrontal circuit. *Psychoneuroendocrinology* 38:1675-1687.

Gibbs AA, Naudts KH, Azevedo RT, David AS (2010) Deletion variant of alpha2b-adrenergic receptor gene moderates the effect of COMT val(158)met polymorphism on episodic memory performance. *Eur Neuropsychopharmacol* 20:272-275.

Gillan CM, Papmeyer M, Morein-Zamir S, Sahakian BJ, Fineberg NA, Robbins TW, de Wit S (2011) Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. *Am J Psychiatry* 168:718-726.

Gilsbach R, Hein L (2012) Are the pharmacology and physiology of alpha(2) adrenoceptors determined by alpha(2)-heteroreceptors and autoreceptors respectively? *Br J Pharmacol* 165:90-102.

Glienke K, Wolf OT, Bellebaum C (2015) The impact of stress on feedback and error processing during behavioral adaptation. *Neuropsychologia* 71:181-190.

Gluck MA, Shohamy D, Myers C (2002) How do people solve the "weather prediction" task?: individual variability in strategies for probabilistic category learning. *Learn Mem* 9:408-418.

Goldfarb EV, Phelps EA (2017) Stress and the trade-off between hippocampal and striatal memory. *Current Opinion in Behavioral Sciences* 14:47-53.

Goldfarb EV, Chun MM, Phelps EA (2016) Memory-Guided Attention: Independent Contributions of the Hippocampus and Striatum. *Neuron* 89:317-324.

Goldfarb EV, Frobose MI, Cools R, Phelps EA (2017) Stress and cognitive flexibility: cortisol increases are associated with enhanced updating but impaired switching. *J Cogn Neurosci* 29:14-24.

Goldin PR, McRae K, Ramel W, Gross JJ (2008) The neural bases of emotion regulation: reappraisal and suppression of negative emotion. *Biol Psychiatry* 63:577-586.

Goodman J, Leong KC, Packard MG (2012) Emotional modulation of multiple memory systems: implications for the neurobiology of post-traumatic stress disorder. *Rev Neurosci* 23:627-643.

Gray JM, Vecchiarelli HA, Morena M, Lee TT, Hermanson DJ, Kim AB, McLaughlin RJ, Hassan KI, Kuhne C, Wotjak CT, Deussing JM, Patel S, Hill MN (2015) Corticotropin-releasing hormone drives anandamide hydrolysis in the amygdala to promote anxiety. *J Neurosci* 35:3879-3892.

Green MK, Rani CS, Joshi A, Soto-Pina AE, Martinez PA, Frazer A, Strong R, Morilak DA (2011) Prenatal stress induces long term stress vulnerability, compromising stress response systems in the brain and impairing extinction of conditioned fear after adult stress. *Neuroscience* 192:438-451.

Greene CS, Penrod NM, Williams SM, Moore JH (2009) Failure to replicate a genetic association may provide important clues about genetic architecture. *PLoS One* 4:e5639.

Groeneweg FL, Karst H, de Kloet ER, Joels M (2011) Rapid non-genomic effects of corticosteroids and their role in the central stress response. *J Endocrinol* 209:153-167.

Gunduz-Cinar O, Hill MN, McEwen BS, Holmes A (2013) Amygdala FAAH and anandamide: mediating protection and recovery from stress. *Trends Pharmacol Sci* 34:637-644.

Haller J, Barna I, Barsvari B, Gyimesi Pelczer K, Yasar S, Panlilio LV, Goldberg S (2009) Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats. *Psychopharmacology (Berl)* 204:607-616.

Hamann S (2001) Cognitive and neural mechanisms of emotional memory. *Trends Cogn Sci* 5:394-400.

Häring M, Enk V, Aparisi Rey A, Loch S, Ruiz de Azua I, Weber T, Bartsch D, Monory K, Lutz B (2015) Cannabinoid type-1 receptor signaling in central serotonergic neurons regulates anxiety-like behavior and sociability. *Front Behav Neurosci* 9:235.

Hartley T, Burgess N (2005) Complementary memory systems: competition, cooperation and compensation. *Trends Neurosci* 28:169-170.

Hauer D, Weis F, Papassotiropoulos A, Schmoeckel M, Beiras-Fernandez A, Lieke J, Kaufmann I, Kirchhoff F, Vogeser M, Roozendaal B, Briegel J, de Quervain D, Schelling G (2011) Relationship of a common polymorphism of the glucocorticoid receptor gene to traumatic memories and posttraumatic stress disorder in patients after intensive care therapy. *Crit Care Med* 39:643-650.

Hauser TU, Iannaccone R, Stampfli P, Drechsler R, Brandeis D, Walitza S, Brem S (2014) The feedback-related negativity (FRN) revisited: new insights into the localization, meaning and network organization. *Neuroimage* 84:159-168.

Hayes SM, Nadel L, Ryan L (2007) The effect of scene context on episodic object recognition: parahippocampal cortex mediates memory encoding and retrieval success. *Hippocampus* 17:873-889.

Henckens MJ, Hermans EJ, Pu Z, Joels M, Fernandez G (2009) Stressed memories: how acute stress affects memory formation in humans. *J Neurosci* 29:10111-10119.

Hermans EJ, Henckens MJ, Joels M, Fernandez G (2014) Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends Neurosci* 37:304-314.

Hermans EJ, van Marle HJ, Ossewaarde L, Henckens MJ, Qin S, T. vKM, Schoots VC, Cousijn H, Rijpkema M, Oostenvel R, Fernández G (2011) Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. *Science* 334:1151-1153.

Hill MN, Gorzalka BB (2005) Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test. *Eur Neuropsychopharmacol* 15:593-599.

Hill MN, Gorzalka BB (2009) Impairments in endocannabinoid signaling and depressive illness. *JAMA* 301:1165-1166.

Hill MN, McEwen BS (2010) Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. *Prog Neuropsychopharmacol Biol Psychiatry* 34:791-797.

Hill MN, Patel S (2013) Translational evidence for the involvement of the endocannabinoid system in stress-related psychiatric illnesses. *Biol Mood Anxiety Disord* 3.

Hill MN, Karatsoreos IN, Hillard CJ, McEwen BS (2010) Rapid elevations in limbic endocannabinoid content by glucocorticoid hormones in vivo. *Psychoneuroendocrinology* 35:1333-1338.

Hill MN, Miller GE, Ho WS, Gorzalka BB, Hillard CJ (2008) Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. *Pharmacopsychiatry* 41:48-53.

Hill MN, Miller GE, Carrier EJ, Gorzalka BB, Hillard CJ (2009a) Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. *Psychoneuroendocrinology* 34:1257-1262.

Hill MN, Ho WS, Sinopoli KJ, Viau V, Hillard CJ, Gorzalka BB (2006) Involvement of the endocannabinoid system in the ability of long-term tricyclic antidepressant treatment to suppress stress-induced activation of the hypothalamic-pituitary-adrenal axis. *Neuropsychopharmacology* 31:2591-2599.

Hill MN, McLaughlin RJ, Morrish AC, Viau V, Floresco SB, Hillard CJ, Gorzalka BB (2009b) Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. *Neuropsychopharmacology* 34:2733-2745.

Hill MN, Bierer LM, Makotkine I, Golier JA, Galea S, McEwen BS, Hillard CJ, Yehuda R (2013) Reductions in circulating endocannabinoid levels in individuals with post-traumatic stress disorder following exposure to the World Trade Center attacks. *Psychoneuroendocrinology* 38:2952-2961.

Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, Lee TT, Karatsoreos IN, Mackie K, Viau V, Pickel VM, McEwen BS, Liu QS, Gorzalka BB, Hillard CJ (2011) Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. *J Neurosci* 31:10506-10515.

Hillard CJ (2000) Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. *Prostaglandins Other Lipid Mediat* 61:3-18.

Hillard CJ (2018) Circulating Endocannabinoids: From Whence Do They Come and Where are They Going? *Neuropsychopharmacology* 43:155-172.

Hillard CJ, Weinlander KM, Stuhr KL (2012) Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. *Neuroscience* 204:207-229.

Horder J, Harmer CJ, Cowen PJ, McCabe C (2010) Reduced neural response to reward following 7 days treatment with the cannabinoid CB1 antagonist rimonabant in healthy volunteers. *Int J Neuropsychopharmacol* 13:1103-1113.

Horder J, Cowen PJ, Di Simplicio M, Browning M, Harmer CJ (2009) Acute administration of the cannabinoid CB1 antagonist rimonabant impairs positive affective memory in healthy volunteers. *Psychopharmacology (Berl)* 205:85-91.

Horder J, Browning M, Di Simplicio M, Cowen PJ, Harmer CJ (2012) Effects of 7 days of treatment with the cannabinoid type 1 receptor antagonist, rimonabant, on emotional processing. *J Psychopharmacol* 26:125-132.

Ingram RE, Luxton DD (2005) Vulnerability-stress models. In: Development of psychopathology: a vulnerability stress perspective (Hankin BL, Abela JRZ, eds), pp 32-46. Thousand Oaks, CA: Sage Publications.

Jacob W, Yassouridis A, Marsicano G, Monory K, Lutz B, Wotjak CT (2009) Endocannabinoids render exploratory behaviour largely independent of the test aversiveness: role of glutamatergic transmission. *Genes Brain Behav* 8:685-698.

Joëls M, Baram TZ (2009) The neuro-symphony of stress. *Nat Rev Neurosci* 10:459-466.

Joëls M, Karst H, DeRijk R, de Kloet ER (2008) The coming out of the brain mineralocorticoid receptor. *Trends Neurosci* 31:1-7.

Joëls M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ (2006) Learning under stress: how does it work? *Trends Cogn Sci* 10:152-158.

Joormann J, Gotlib IH (2010) Emotion regulation in depression: relation to cognitive inhibition. *Cogn Emot* 24:281-298.

Kalisch R, Muller MB, Tuscher O (2015) A conceptual framework for the neurobiological study of resilience. *Behav Brain Sci* 38:e92.

Kanatsou S, Kuil LE, Arp M, Oitzl MS, Harris AP, Seckl JR, Krugers HJ, Joels M (2015) Overexpression of mineralocorticoid receptors does not affect memory and anxiety-like behavior in female mice. *Front Behav Neurosci* 9:182.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M (2009) Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev* 89:309-380.

Karst H, Joels M (2016) Severe stress hormone conditions cause an extended window of excitability in the mouse basolateral amygdala. *Neuropharmacology* 110:175-180.

Karst H, Berger S, Erdmann G, Schutz G, Joels M (2010) Metaplasticity of amygdalar responses to the stress hormone corticosterone. *Proc Natl Acad Sci U S A* 107:14449-14454.

Karst H, Berger S, Turiault M, Tronche F, Schutz G, Joels M (2005) Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proc Natl Acad Sci U S A* 102:19204-19207.

Katona I, Rancz EA, Acsady L, Ledent C, Mackie K, Hajos N, Freund TF (2001) Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J Neurosci* 21:9506-9518.

Kaufmann W (1956) Existentialism from Dostoevsky to Sartre: World Publishing Co.

Kim JJ, Baxter MG (2001) Multiple brain-memory systems: the whole does not equal the sum of its parts. *Trends Neurosci* 24:324-330.

Kim JJ, Lee HJ, Han JS, Packard MG (2001) Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. *J Neurosci* 21:5222-5228.

Kirilly E, Hunyady L, Bagdy G (2013) Opposing local effects of endocannabinoids on the activity of noradrenergic neurons and release of noradrenaline: relevance for their role in depression and in the actions of CB(1) receptor antagonists. *J Neural Transm (Vienna)* 120:177-186.

Kirschbaum C, Hellhammer DH (1989) Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 22:150-169.

Kirschbaum C, Hellhammer DH (1994) Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology* 19:313-333.

Kirschbaum C, Wüst S-, Hellhammer D (1992) Consistent sex differences in cortisol responses to psychological stress. *Psychosom Med* 54:648-657.

Kirschbaum C, Pirke KM, Hellhammer D (1993) The 'Trier Social Stress Test' - a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28:76-81.

Klok MD, Giltay EJ, Van der Does AJ, Geleijnse JM, Antypa N, Penninx BW, de Geus EJ, Willemsen G, Boomsma DI, van Leeuwen N, Zitman FG, de Kloet ER, DeRijk RH (2011) A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. *Transl Psychiatry* 1:e62.

Knowlton BJ, Mangels JA, Squire LR (1996a) A neostriatal habit learning system in humans. *Science* 273:1399-1402.

Knowlton BJ, Squire LR, Paulsen JS, Swerdlow N, Swenson M, Butters N (1996b) Dissociations within nondeclarative memory in Huntington's disease. *Neuropsychology* 10:538-548.

Korem N, Zer-Aviv TM, Ganon-Elazar E, Abush H, Akirav I (2016) Targeting the endocannabinoid system to treat anxiety-related disorders. *J Basic Clin Physiol Pharmacol* 27:193-202.

Kreek MJ, Nielsen DA, Butelman ER, LaForge KS (2005) Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. *Nat Neurosci* 8:1450-1457.

Krugers HJ, Karst H, Joels M (2012) Interactions between noradrenaline and corticosteroids in the brain: from electrical activity to cognitive performance. *Front Cell Neurosci* 6:15.

Kudielka BM, Hellhammer D, Kirschbaum C (2007) Ten years of research with the Trier Social Stress Test (TSST) - revisited. In: Social neuroscience: integrating biological and psychological explanations of social behavior (Harmon-Jones E, Winkielman P, eds). New York: Guilford Press.

Kuhlmann S, Piel M, Wolf OT (2005) Impaired memory retrieval after psychosocial stress in healthy young men. *J Neurosci* 25:2977-2982.

Kukolja J, Klingmuller D, Maier W, Fink GR, Hurlemann R (2011) Noradrenergic-glucocorticoid modulation of emotional memory encoding in the human hippocampus. *Psychol Med* 41:2167-2176.

LaBar KS, Cabeza R (2006) Cognitive neuroscience of emotional memory. *Nat Rev Neurosci* 7:54-64.

Lafenetre P, Chaouloff F, Marsicano G (2007) The endocannabinoid system in the processing of anxiety and fear and how CB1 receptors may modulate fear extinction. *Pharmacol Res* 56:367-381.

Lalchandani SG, Lei L, Zheng W, Suni MM, Moore BM, Liggett SB, Miller DD, Feller DR (2002) Yohimbine dimers exhibiting selectivity for the human alpha 2C-adrenoceptor subtype. *J Pharmacol Exp Ther* 303:979-984.

Langer SZ (1980) Presynaptic regulation of the release of catecholamines. *Pharmacol Rev* 32:337-362.

Lazarus RS (1966) Psychological Stress and the Coping Process. New York: McGraw-Hill.

Lazarus RS, Folkman S (1984) Stress, appraisal and coping. NewYork: Springer.

LeDoux JE (2000) Emotion circuits in the brain. *Annu Rev Neurosci* 23.

Lee AS, Duman RS, Pittenger C (2008) A double dissociation revealing bidirectional competition between striatum and hippocampus during learning. *Proc Natl Acad Sci U S A* 105:17163-17168.

Lee TT, Hill MN (2013) Age of stress exposure modulates the immediate and sustained effects of repeated stress on corticolimbic cannabinoid CB1 receptor binding in male rats. *Neuroscience* 249:106-114.

Levenson JM, Sweatt JD (2005) Epigenetic mechanisms in memory formation. *Nat Rev Neurosci* 6:108-118.

Liberzon I, Taylor SF, Amdur R, Jung TD, Chamberlain KR, Minoshima S, Koeppe RA, Fig LM (1999) Brain activation in PTSD in response to trauma-related stimuli. *Biol Psychiatry* 45:817-826.

Liberzon I, King AP, Ressler KJ, Almli LM, Zhang P, Ma ST, Cohen GH, Tamburrino MB, Calabrese JR, Galea S (2014) Interaction of the ADRB2 gene polymorphism with childhood trauma in predicting adult symptoms of posttraumatic stress disorder. *JAMA Psychiatry* 71:1174-1182.

Liebetrau A (1983) Measures of association: Sage Publications, Inc.

Lisboa SF, Reis DG, da Silva AL, Correa FM, Guimaraes FS, Resstel LB (2010) Cannabinoid CB1 receptors in the medial prefrontal cortex modulate the expression of contextual fear conditioning. *Int J Neuropsychopharmacol* 13:1163-1173.

Liston C, McEwen BS, Casey BJ (2009) Psychosocial stress reversibly disrupts prefrontal processing and attentional control. *Proc Natl Acad Sci U S A* 106:912-917.

Lucassen PJ, Pruessner J, Sousa N, Almeida OF, Van Dam AM, Rajkowska G, Swaab DF, Czeh B (2014) Neuropathology of stress. *Acta Neuropathol* 127:109-135.

Lupien SJ, McEwen BS, Gunnar MR, Heim C (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* 10:434-445.

Lutz B (2009) Endocannabinoid signals in the control of emotion. *Curr Opin Pharmacol* 9:46-52.

Lutz B, Marsicano G, Maldonado R, Hillard CJ (2015) The endocannabinoid system in guarding against fear, anxiety and stress. *Nat Rev Neurosci* 16:705-718.
Maccari S, Darnaudery M, Morley-Fletcher S, Zuena AR, Cinque C, Van Reeth O (2003) Prenatal stress and long-term consequences: implications of glucocorticoid hormones. *Neurosci Biobehav Rev* 27:119-127.
Maes M, Lin AH, Verkerk R, Delmeire L, Van Gastel A, Van der Planken M, Scharpé S (1999) Serotonergic and noradrenergic markers of post-traumatic stress disorder with and without major depression. *Neuropsychopharmacology* 20:188-197.
Marsicano G, Lutz B (1999) Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain *Eur J Neurosci* 11:4213-4225.
Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutiérrez SO, van der Stelt M, López-Rodríguez ML, Casanova E, Schütz G, Zieglgänsberger W, di Marzo V, Behl C, Lutz B (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302:84-88.
Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O (2002) Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl)* 159:379-387.
McCrory E, De Brito SA, Viding E (2011) The impact of childhood maltreatment: a review of neurobiological and genetic factors. *Front Psychiatry* 2:48.
McCune SK, Voigt MM, Hill JM (1993) Expression of multiple alpha adrenergic receptor subtype messenger RNAs in the adult rat brain. *Neuroscience* 57:143-151.
McDonald RJ, White NM (1993) A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. *Behav Neurosci* 107:3-22.
McDonald RJ, White NM (1994) Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. *Behav Neural Biol* 61:260-270.
McEwen BS (1998) Stress, adaptation, and disease. Allostasis and allostatic load. *Ann N Y Acad Sci* 1:33-44.
McEwen BS (2001) Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. *Ann N Y Acad Sci* 933.
McEwen BS (2004) Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci* 1032:1-7.
McEwen BS, Morrison JH (2013) The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course. *Neuron* 79:16-29.
McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN, Nasca C (2015) Mechanisms of stress in the brain. *Nat Neurosci* 18:1353-1363.
McGaugh JL (2000) Memory - a century of consolidation. *Science* 287:248-251.
McGaugh JL (2004) The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu Rev Neurosci* 27:1-28.
McGaugh JL, Cahill L (1997) Interaction of neuromodulatory systems in modulating memory storage. *Behav Brain Res* 83:31-38.
McGaugh JL, Cahill L, Roozendaal B (1996) Involvement of the amygdala in memory storage: interaction with other brain systems. *Proc Natl Acad Sci U S A* 93:13508-13514.
McGaugh JL, Introini-Collison IB, Cahill LF, Castellano C, Dalmaz C, Parent MB, Williams CL (1993) Neuromodulatory systems and memory storage: role of the amygdala. *Behav Brain Res* 58:81-90.

McIntyre CK, Miyashita T, Setlow B, Marjon KD, Steward O, Guzowski JF, McGaugh JL (2005) Memory-influencing intra-basolateral amygdala drug infusions modulate expression of Arc protein in the hippocampus. *Proc Natl Acad Sci U S A* 102:10718-10723.

McLaughlin RJ, Hill MN, Gorzalka BB (2014) A critical role for prefrontocortical endocannabinoid signaling in the regulation of stress and emotional behavior. *Neurosci Biobehav Rev* 42:116-131.

McLaughlin RJ, Hill MN, Bambico FR, Stuhr KL, Gobbi G, Hillard CJ, Gorzalka BB (2012) Prefrontal cortical anandamide signaling coordinates coping responses to stress through a serotonergic pathway. *Eur Neuropsychopharmacol* 22:664-671.

McPartland JM, Glass M, Pertwee RG (2007) Meta-analysis of cannabinoid ligand binding affinity and receptor distribution: interspecies differences. *Br J Pharmacol* 152:583-593.

McReynolds JR, Donowho K, Abdi A, McGaugh JL, Roozendaal B, McIntyre CK (2010) Memory-enhancing corticosterone treatment increases amygdala norepinephrine and Arc protein expression in hippocampal synaptic fractions. *Neurobiol Learn Mem* 93:312-321.

Medina AC, Charles JR, Espinoza-Gonzalez V, Sanchez-Resendis O, Prado-Alcala RA, Roozendaal B, Quirarte GL (2007) Glucocorticoid administration into the dorsal striatum facilitates memory consolidation of inhibitory avoidance training but not of the context or footshock components. *Learn Mem* 14:673-677.

Meijer OC, de Kloet ER (2017) A Refill for the Brain Mineralocorticoid Receptor: The Benefit of Cortisol Add-On to Dexamethasone Therapy. *Endocrinology* 158:448-454.

Merz CJ, Hamacher-Dang TC, Stark R, Wolf OT, Hermann A (2018) Neural Underpinnings of Cortisol Effects on Fear Extinction. *Neuropsychopharmacology* 43:384-392.

Mifsud KR, Gutierrez-Mecinas M, Trollope AF, Collins A, Saunderson EA, Reul JM (2011) Epigenetic mechanisms in stress and adaptation. *Brain Behav Immun* 25:1305-1315.

Mitchell PB, Morris MJ (2007) Depression and anxiety with rimonabant. *The Lancet* 370:1671-1672.

Mitra R, Ferguson D, Sapolsky RM (2009) Mineralocorticoid receptor overexpression in basolateral amygdala reduces corticosterone secretion and anxiety. *Biol Psychiatry* 66:686-690.

Moore JH, Williams SM (2009) Epistasis and its implications for personal genetics. *Am J Hum Genet* 85:309-320.

Moore SA, Zoellner LA, Mollenholt N (2008) Are expressive suppression and cognitive reappraisal associated with stress-related symptoms? *Behav Res Ther* 46:993-1000.

Moreira FA, Grieb M, Lutz B (2009) Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. *Best Pract Res Clin Endocrinol Metab* 23:133-144.

Morena M, Campolongo P (2014) The endocannabinoid system: an emotional buffer in the modulation of memory function. *Neurobiol Learn Mem* 112:30-43.

Morena M, Patel S, Bains JS, Hill MN (2016) Neurobiological interactions between stress and the endocannabinoid system. *Neuropsychopharmacology* 41:80-102.

Morena M, De Castro V, Gray JM, Palmery M, Trezza V, Roozendaal B, Hill MN, Campolongo P (2015) Training-associated emotional arousal shapes endocannabinoid modulation of spatial memory retrieval in rats. *J Neurosci* 35:13962-13974.

Motzkin JC, Philippi CL, Wolf RC, Baskaya MK, Koenigs M (2015) Ventromedial prefrontal cortex is critical for the regulation of amygdala activity in humans. *Biol Psychiatry* 77:276-284.

Naudts KH, Azevedo RT, David AS, van Heeringen C, Gibbs AA (2012) Influence of COMT val158met and ADRA2B deletion polymorphisms on recollection and familiarity components of human emotional memory. *J Psychopharmacol* 26:819-829.

Neumeister A, Normandin MD, Pietrzak RH, Piomelli D, Zheng MQ, Gujarro-Anton A, Potenza MN, Bailey CR, Lin SF, Najafzadeh S, Ropchan J, Henry S, Corsi-Travali S, Carson RE, Huang Y (2013) Elevated brain cannabinoid CB1 receptor availability in post-traumatic stress disorder: a positron emission tomography study. *Mol Psychiatry* 18:1034-1040.

New AS, Fan J, Murrough JW, Liu X, Liebman RE, Guise KG, Tang CY, Charney DS (2009) A functional magnetic resonance imaging study of deliberate emotion regulation in resilience and posttraumatic stress disorder. *Biol Psychiatry* 66:656-664.

Nieuwenhuis S, Slagter HA, von Geusau NJ, Heslenfeld DJ, Holroyd CB (2005) Knowing good from bad: differential activation of human cortical areas by positive and negative outcomes. *Eur J Neurosci* 21:3161-3168.

Nyiri G, Szabadits E, Cserep C, Mackie K, Shigemoto R, Freund TF (2005) GABAB and CB1 cannabinoid receptor expression identifies two types of septal cholinergic neurons. *Eur J Neurosci* 21:3034-3042.

O'Dell TJ, Connor SA, Guglietta R, Nguyen PV (2015) β-Adrenergic receptor signaling and modulation of long-term potentiation in the mammalian hippocampus. *Learn Mem* 22:461-471.

Ochsner KN, Silvers JA, Buhle JT (2012) Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. *Ann N Y Acad Sci* 1251:E1-24.

Ochsner KN, Ray RD, Cooper JC, Robertson ER, Chopra S, Gabrieli JD, Gross JJ (2004) For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion. *Neuroimage* 23:483-499.

Oitzl MS, Champagne DL, van der Veen R, de Kloet ER (2010) Brain development under stress: hypotheses of glucocorticoid actions revisited. *Neurosci Biobehav Rev* 34:853-866.

Olijslagers JE, de Kloet ER, Elgersma Y, van Woerden GM, Joels M, Karst H (2008) Rapid changes in hippocampal CA1 pyramidal cell function via pre- as well as postsynaptic membrane mineralocorticoid receptors. *Eur J Neurosci* 27:2542-2550.

Olofson JK, Nordin S, Sequeira H, Polich J (2008) Affective picture processing: an integrative review of ERP findings. *Biol Psychol* 77:247-265.

Oomen CA, Soeters H, Audureau N, Vermunt L, van Hasselt FN, Manders EM, Joels M, Lucassen PJ, Krugers H (2010) Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood. *J Neurosci* 30:6635-6645.

Otte C, Moritz S, Yassouridis A, Koop M, Madrischewski AM, Wiedemann K, Kellner M (2007) Blockade of the mineralocorticoid receptor in healthy men: effects on experimentally induced panic symptoms, stress hormones, and cognition. *Neuropsychopharmacology* 32:232-238.

Otte C, Wingenfeld K, Kuehl LK, Kaczmarczyk M, Richter S, Quante A, Regen F, Bajbouj M, Zimmermann-Viehoff F, Wiedemann K, Hinkelmann K (2015) Mineralocorticoid receptor stimulation improves cognitive function and decreases cortisol secretion in depressed patients and healthy individuals. *Neuropsychopharmacology* 40:386-393.

Packard MG (1999) Glutamate infused posttraining into the hippocampus or caudate-putamen differentially strengthens place and response learning. *Proc Natl Acad Sci U S A* 96:12881-12886.

Packard MG, McGaugh JL (1992) Double dissociation of fornix and caudate nucleus lesions on acquisition of two water maze tasks: further evidence for multiple memory systems. *Behav Neurosci* 106:439-446.

Packard MG, McGaugh JL (1996) Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol Learn Mem* 65:65-72.

Packard MG, Teather LA (1998) Amygdala modulation of multiple memory systems: hippocampus and caudate-putamen. *Neurobiol Learn Mem* 69:163-203.

Packard MG, Wingard JC (2004) Amygdala and "emotional" modulation of the relative use of multiple memory systems. *Neurobiol Learn Mem* 82:243-252.

Packard MG, Gabriele A (2009) Peripheral anxiogenic drug injections differentially affect cognitive and habit memory: role of basolateral amygdala. *Neuroscience* 164:457-462.

Packard MG, Hirsh R, White NM (1989) Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems. *J Neurosci* 9:1465-1472.

Packard MG, Goodman J, Ressler RL (2018) Emotional modulation of habit memory: neural mechanisms and implications for psychopathology. *Current Opinion in Behavioral Sciences* 20:25-32.

Pagliaccio D, Luby JL, Bogdan R, Agrawal A, Gaffrey MS, Belden AC, Botteron KN, Harms MP, Barch DM (2014) Stress-system genes and life stress predict cortisol levels and amygdala and hippocampal volumes in children. *Neuropsychopharmacology* 39:1245-1253.

Papassotiropoulos A, de Quervain DJ (2011) Genetics of human episodic memory: dealing with complexity. *Trends Cogn Sci* 15:381-387.

Patel S, Roelke CT, Rademacher DJ, Hillard CJ (2005) Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling. *European Journal of Neuroscience* 21:1057-1069.

Payne JD, Jackson ED, Hoscheidt S, Ryan L, Jacobs WJ, Nadel L (2007) Stress administered prior to encoding impairs neutral but enhances emotional long-term episodic memories. *Learn Mem* 14:861-868.

Pechtel P, Pizzagalli DA (2011) Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology (Berl)* 214:55-70.

Pessoa L, Adolphs R (2010) Emotion processing and the amygdala: from a 'low road' to 'many roads' of evaluating biological significance. *Nat Rev Neurosci* 11:773-783.

Petrakova L, Boy K, Mittmann L, Möller L, Engler H, Schedlowski M (2017) Salivary alpha-amylase and noradrenaline responses to corticotropin-releasing hormone administration in humans. *Biol Psychol* 127:34-39.

Phan KL, Wager T, Taylor SF, Liberzon I (2002) Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage* 16:331-348.

Phan KL, Angstadt M, Golden J, Onyewuenyi I, Popovska A, de Wit H (2008) Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. *J Neurosci* 28:2313-2319.

Piomelli D (2003) The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* 4:873-884.

Pitman RK, Sanders KM, Zusman RM, Healy AR, Cheema F, Laski NB, Cahill L, Orr SP (2002) Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biol Psychiatry* 51:189-192.

Pitman RK, Rasmusson AM, Koenen KC, Shin LM, Orr SP, Gilbertson MW, Milad MR, Liberzon I (2012) Biological studies of post-traumatic stress disorder. *Nat Rev Neurosci* 13:769-787.

Plessow F, Kiesel A, Kirschbaum C (2012) The stressed prefrontal cortex and goal-directed behaviour: acute psychosocial stress impairs the flexible implementation of task goals. *Exp Brain Res* 216:397-408.

Plessow F, Fischer R, Kirschbaum C, Goschke T (2011) Inflexibly focused under stress: acute psychosocial stress increases shielding of action goals at the expense of reduced cognitive flexibility with increasing time lag to the stressor. *J Cogn Neurosci* 23:3218-3227.

Plomin R (1990) The role of inheritance in behavior. *Science* 248:183-188.

Plomin R, Owen MJ, McGuffin P (1994) The genetic basis of complex human behaviors. *Science* 264:1733-1739.

Poldrack RA, Packard MG (2003) Competition among multiple memory systems: converging evidence from animal and human brain studies. *Neuropsychologia* 41:245-251.

Poldrack RA, Foerde K (2008) Category learning and the memory systems debate. *Neurosci Biobehav Rev* 32:197-205.

Poldrack RA, Prabhakaran V, Seger CA, Gabrieli JD (1999) Striatal activation during acquisition of a cognitive skill. *Neuropsychology* 13:564-574.

Poldrack RA, Clark J, Paré-Blagoev EJ, Shohamy D, Creso M, J., Myers C, Gluck MA (2001) Interactive memory systems in the human brain. *Nature* 414:546-550.

Polich J (2007) Updating P300: an integrative theory of P3a and P3b. *Clin Neurophysiol* 118:2128-2148.

Prager EM, Brielmaier J, Bergstrom HC, McGuire J, Johnson LR (2010) Localization of mineralocorticoid receptors at mammalian synapses. *PLoS One* 5:e14344.

Prüssner JC, Dedovic K, Khalili-Mahani N, Engert V, Prüssner M, Buss C, Renwick R, Dagher A, Meaney MJ, Lupien S (2008) Deactivation of the limbic system during acute psychosocial stress: evidence from positron emission tomography and functional magnetic resonance imaging studies. *Biol Psychiatry* 63:234-240.

Quirarte GL, de la Teja IS, Casillas M, Serafin N, Prado-Alcala RA, Roozendaal B (2009) Corticosterone infused into the dorsal striatum selectively enhances memory consolidation of cued water-maze training. *Learn Mem* 16:586-589.

Rademacher DJ, Hillard CJ (2007) Interactions between endocannabinoids and stress-induced decreased sensitivity to natural reward. *Prog Neuropsychopharmacol Biol Psychiatry* 31:633-641.

Raio CM, Orederu TA, Palazzolo L, Shurick AA, Phelps EA (2013) Cognitive emotion regulation fails the stress test. *Proc Natl Acad Sci U S A* 110:15139-15144.

Raiteri M, Bonanno G, Maura G, Pende M, Andrioli GC, Ruelle A (1992) Subclassification of release-regulating alpha 2-autoreceptors in human brain cortex. *Br J Pharmacol* 107:1146-1151.

Rasch B, Papassotiropoulos A, de Quervain DF (2010) Imaging genetics of cognitive functions: Focus on episodic memory. *Neuroimage* 53:870-877.

Rasch B, Spalek K, Buholzer S, Luechinger R, Boesiger P, Papassotiropoulos A, de Quervain DJ (2009) A genetic variation of the noradrenergic system is related to differential amygdala activation during encoding of emotional memories. *Proc Natl Acad Sci U S A* 106:19191-19196.

Raskind MA, Peskind ER, Hoff DJ, Hart KL, Holmes HA, Warren D, Shofer J, O'Connell J, Taylor F, Gross C, Rohde K, McFall ME (2007) A parallel group placebo controlled study of prazosin for trauma nightmares and sleep disturbance in combat veterans with post-traumatic stress disorder. *Biol Psychiatry* 61:928-934.

Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, Orr SP, Pitman RK (2000) Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biol Psychiatry* 47:769-776.

Reber PJ, Knowlton BJ, Squire LR (1996) Dissociable properties of memory systems: differences in the flexibility of declarative and nondeclarative knowledge. *Behav Neurosci* 110:861-871.

Reul JM, de Kloet ER (1985) Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology* 117:2505-2511.

Reznikoff GA, Manaker S, Rhodes CH, Winokur A, Rainbow TC (1986) Localization and quantification of beta-adrenergic receptors in human brain. *Neurology* 36:1067-1073.

Rimmele U, Besedovsky L, Lange T, Born J (2013) Blocking mineralocorticoid receptors impairs, blocking glucocorticoid receptors enhances memory retrieval in humans. *Neuropsychopharmacology* 38:884-894.

Ritchey M, Dolcos F, Cabeza R (2008) Role of amygdala connectivity in the persistence of emotional memories over time: an event-related FMRI investigation. *Cereb Cortex* 18:2494-2504.

Ronzoni G, Del Arco A, Mora F, Segovia G (2016) Enhanced noradrenergic activity in the amygdala contributes to hyperarousal in an animal model of PTSD. *Psychoneuroendocrinology* 70:1-9.

Roozendaal B (2002) Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. *Neurobiology of Learning and Memory* 78:578-595.

Roozendaal B, McGaugh JL (1996) Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task. *Neurobiol Learn Mem* 65:1-8.

Roozendaal B, McGaugh JL (2011) Memory modulation. *Behav Neurosci* 125:797-824.

Roozendaal B, Hermans EJ (2017) Norepinephrine effects on the encoding and consolidation of emotional memory: improving synergy between animal and human studies. *Current Opinion in Behavioral Sciences* 14:115-122.

Roozendaal B, Carmi O, McGaugh JL (1996a) Adrenocortical suppression blocks the memory-enhancing effects of amphetamine and epinephrine. *Proc Natl Acad Sci U S A* 1996:4.

Roozendaal B, Bohus B, McGaugh JL (1996b) Dose-dependent suppression of adrenocortical activity with metyrapone: effects on emotion and memory. *Psychoneuroendocrinology* 21:681-693.

Roozendaal B, McEwen BS, Chattarji S (2009) Stress, memory and the amygdala. *Nat Rev Neurosci* 10:423-433.

Roozendaal B, Okuda S, Van der Zee EA, McGaugh JL (2006) Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proc Natl Acad Sci U S A* 103:6741-6746.

Roozendaal B, Hernandez A, Cabrera SM, Hagewoud R, Malvaez M, Stefanko DP, Haettig J, Wood MA (2010) Membrane-associated glucocorticoid activity is necessary for modulation of long-term memory via chromatin modification. *J Neurosci* 30:5037-5046.

Rozeboom AM, Akil H, Seasholtz AF (2007) Mineralocorticoid receptor overexpression in forebrain decreases anxiety-like behavior and alters the stress response in mice. *Proc Natl Acad Sci U S A* 104:4688-4693.

Rubino T, Realini N, Castiglioni C, Guidali C, Vigano D, Marras E, Petrosino S, Perletti G, Maccarrone M, Di Marzo V, Parolaro D (2008) Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. *Cereb Cortex* 18:1292-1301.

Russo SJ, Murrough JW, Han MH, Charney DS, Nestler EJ (2012) Neurobiology of resilience. *Nat Neurosci* 15:1475-1484.

Rustemeier M, Schwabe L, Bellebaum C (2013) On the relationship between learning strategy and feedback processing in the weather prediction task--Evidence from event-related potentials. *Neuropsychologia* 51:695-703.

Rypma B, Berger JS, Prabhakaran V, Bly BM, Kimberg DY, Biswal BB, D'Esposito M (2006) Neural correlates of cognitive efficiency. *Neuroimage* 33:969-979.

Sadeh N, Spielberg JM, Warren SL, Miller GA, Heller W (2014) Aberrant neural connectivity during emotional processing associated with posttraumatic stress. *Clin Psychol Sci* 2:748-755.
Sandi C (2011) Glucocorticoids act on glutamatergic pathways to affect memory processes. *Trends Neurosci* 34:165-176.
Sänger J, Bechtold L, Schoofs D, Blaszkewicz M, Wascher E (2014) The influence of acute stress on attention mechanisms and its electrophysiological correlates. *Front Behav Neurosci* 8:353.
Schaefer C, Enning F, Mueller JK, Bumb JM, Rohleder C, Odorfer TM, Klosterkotter J, Hellmich M, Koethe D, Schmahl C, Bohus M, Leweke FM (2014) Fatty acid ethanolamide levels are altered in borderline personality and complex posttraumatic stress disorders. *Eur Arch Psychiatry Clin Neurosci* 264:459-463.
Scheggia D, Zamberletti E, Realini N, Mereu M, Contarini G, Ferretti V, Manago F, Margiani G, Brunoro R, Rubino T, De Luca MA, Piomelli D, Parolaro D, Papaleo F (2018) Remote memories are enhanced by COMT activity through dysregulation of the endocannabinoid system in the prefrontal cortex. *Mol Psychiatry* 23:1040-1050.
Scheinin M, Lomasney JW, Hayden-Hixson DM, Schambra UB, Caron MG, Lefkowitz RJ, Fremeau RTJ (1994) Distribution of a2-adrenergic receptor subtype gene expression in rat brain. *Brain Res Mol Brain Res* 21:133-149.
Schlicker E, Feuerstein T (2017) Human presynaptic receptors. *Pharmacol Ther* 172:1-21.
Schroeder JP, Wingard JC, Packard MG (2002) Post-training reversible inactivation of hippocampus reveals interference between memory systems. *Hippocampus* 12:280-284.
Schulz P, Schlotz W (1999) Das Trierer Inventar zur Erfassung von chronischem Streß (TICS): Skalenkonstruktion, teststatistische Überprüfung und Validierung der Skala Arbeitsüberlastung. *Diagnostica* 45:8-19.
Schulz P, Schlotz W, Becker P (2004) Das Trier Inventar zum Chronischen Stress. In. Göttingen: Hogrefe Verlag.
Schümann D, Sommer T (2018) Dissociation of immediate and delayed effects of emotional arousal on episodic memory. *Neurobiol Learn Mem* 148.
Schwabe L (2017) Memory under stress: from single systems to network changes. *Eur J Neurosci* 45:478-489.
Schwabe L, Wolf OT (2012) Stress modulates the engagement of multiple memory systems in classification learning. *J Neurosci* 32:11042-11049.
Schwabe L, Wolf OT (2013) Stress and multiple memory systems: from 'thinking' to 'doing'. *Trends Cogn Sci* 17:60-68.
Schwabe L, Wolf OT (2014) Timing matters: temporal dynamics of stress effects on memory retrieval. *Cogn Affect Behav Neurosci* 14:1041-1048.
Schwabe L, Schachinger H (2018) Ten years of research with the socially evaluated cold pressor test: data from the past and guidelines for the future. *Psychoneuroendocrinology*.
Schwabe L, Wolf OT, Oitzl MS (2010a) Memory formation under stress: quantity and quality. *Neurosci Biobehav Rev* 34:584-591.
Schwabe L, Dalm S, Schachinger H, Oitzl MS (2008) Chronic stress modulates the use of spatial and stimulus-response learning strategies in mice and man. *Neurobiol Learn Mem* 90:495-503.
Schwabe L, Schächinger H, de Kloet R, Oitzl MS (2010b) Corticosteroids operate as a switch between memory systems. *J Cogn Neurosci* 22:1362-1372.
Schwabe L, Hoffken O, Tegenthoff M, Wolf OT (2013a) Stress-induced enhancement of response inhibition depends on mineralocorticoid receptor activation. *Psychoneuroendocrinology* 38:2319-2326.

Schwabe L, Tegenthoff M, Hoffken O, Wolf OT (2013b) Mineralocorticoid receptor blockade prevents stress-induced modulation of multiple memory systems in the human brain. *Biol Psychiatry* 74:801-808.

Schwabe L, Joels M, Roozendaal B, Wolf OT, Oitzl MS (2012) Stress effects on memory: an update and integration. *Neurosci Biobehav Rev* 36:1740-1749.

Schwabe L, Oitzl MS, Philippsen C, Richter S, Bohringer A, Wippich W, Schachinger H (2007) Stress modulates the use of spatial versus stimulus-response learning strategies in humans. *Learn Mem* 14:109-116.

Schwartz DD (1997) Activation of alpha-2 adrenergic receptors inhibits norepinephrine release by a pertussis toxin-insensitive pathway independent of changes in cytosolic calcium in cultured rat sympathetic neurons. *J Pharmacol Exp Ther* 282:248-255.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry* 20:11-21.

Scult MA, Hariri AR (2018) A brief introduction to the neurogenetics of cognition-emotion interactions. *Curr Opin Behav Sci* 19:50-54.

Selye H (1950) Stress and the general adaptation syndrome. *Br Med J* 1:1383-1392.

Sergerie K, Chochol C, Armony JL (2008) The role of the amygdala in emotional processing: a quantitative meta-analysis of functional neuroimaging studies. *Neurosci Biobehav Rev* 32:811-830.

Sharot T, Phelps EA (2004) How arousal modulates memory: disentangling the effects of attention and retention. *Cogn Affect Behav Neurosci* 4:294-306.

Sheppes G, Scheibe S, Suri G, Radu P, Blechert J, Gross JJ (2014) Emotion regulation choice: a conceptual framework and supporting evidence. *J Exp Psychol Gen* 143:163-181.

Shields GS, Sazma MA, Yonelinas AP (2016) The effects of acute stress on core executive functions: a meta-analysis and comparison with cortisol. *Neurosci Biobehav Rev* 68:651-668.

Shields GS, Sazma MA, McCullough AM, Yonelinas AP (2017) The effects of acute stress on episodic memory: a meta-analysis and integrative review. *Psychol Bull* 143:636-675.

Shohamy D, Myers C, Grossman S, Sage J, Gluck MA, Poldrack RA (2004) Cortico-striatal contributions to feedback-based learning: converging data from neuroimaging and neuropsychology. *Brain* 127:851-859.

Siegle GJ, Thompson W, Carter CS, Steinhauer SR, Thase ME (2007) Increased amygdala and decreased dorsolateral prefrontal BOLD responses in unipolar depression: related and independent features. *Biol Psychiatry* 61:198-209.

Singer HS (2016) Habitual and goal-directed behaviours and Tourette syndrome. *Brain* 139:312-316.

Sjoerds Z, de Wit S, van den Brink W, Robbins TW, Beekman AT, Penninx BW, Veltman DJ (2013) Behavioral and neuroimaging evidence for overreliance on habit learning in alcohol-dependent patients. *Transl Psychiatry* 3:e337.

Small KM, Brown KM, Forbes SL, Liggett SB (2001) Polymorphic deletion of three intracellular acidic residues of the alpha 2B-adrenergic receptor decreases G protein-coupled receptor kinase-mediated phosphorylation and desensitization. *J Biol Chem* 276:4917-4922.

Smeets T, Otgaar H, Candel I, Wolf OT (2008) True or false? Memory is differentially affected by stress-induced cortisol elevations and sympathetic activity at consolidation and retrieval. *Psychoneuroendocrinology* 33:1378-1386.

Smoller JW (2016) The genetics of stress-related disorders: PTSD, depression, and anxiety disorders. *Neuropsychopharmacology* 41:297-319.

Sousa N (2016) The dynamics of the stress neuromatrix. *Mol Psychiatry* 21:302-312.

Southwick SM, Charney DS (2012) The science of resilience: implications for the prevention and treatment of depression. *Science* 338:79-82.
Southwick SM, Bremner JD, Rasmusson A, Morgan CAr, Arnsten A, Charney DS (1999) Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol Psychiatry* 46:1192-1204.
Squire LR (1992a) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev* 99:195-231.
Squire LR (1992b) Declarative and nondeclarative memory: multiple brain systems supporting learning and memory. *J Cogn Neurosci* 4:232-243.
Squire LR (2004) Memory systems of the brain: a brief history and current perspective. *Neurobiol Learn Mem* 82:171-177.
Squire LR, Zola M (1996) Structure and function of declarative and nondeclarative memory systems. *Proc Natl Acad Sci U S A* 93:13515-13522.
Stankiewicz AM, Swiergiel AH, Lisowski P (2013) Epigenetics of stress adaptations in the brain. *Brain Res Bull* 98:76-92.
Steiner MA, Marsicano G, Wotjak CT, Lutz B (2008) Conditional cannabinoid receptor type 1 mutants reveal neuron subpopulation-specific effects on behavioral and neuroendocrine stress responses. *Psychoneuroendocrinology* 33:1165-1170.
Strange BA, Dolan RJ (2004) Beta-adrenergic modulation of emotional memory-evoked human amygdala and hippocampal responses. *Proc Natl Acad Sci U S A* 101:11454-11458.
Strawn JR, Geracioti TD, Jr. (2008) Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder. *Depress Anxiety* 25:260-271.
Sutherland RJ, McDonald RJ, Savage DD (2000) Prenatal exposure to moderate levels of ethanol can have long-lasting effects on learning and memory in adult offspring. *Psychobiology* 28:532-539.
Taylor FB, Lowe K, Thompson C, McFall MM, Peskind ER, Kanter ED, Allison N, Williams J, Martin P, Raskind MA (2006) Daytime prazosin reduces psychological distress to trauma specific cues in civilian trauma posttraumatic stress disorder. *Biol Psychiatry* 59:577-581.
ter Heegde F, DeRijk RH, Vinkers CH (2015) The brain mineralocorticoid receptor and stress resilience. *Psychoneuroendocrinology* 52:92-110.
ter Horst JP, van der Mark MH, Arp M, Berger S, de Kloet ER, Oitzl MS (2012) Stress or no stress: mineralocorticoid receptors in the forebrain regulate behavioral adaptation. *Neurobiol Learn Mem* 98:33-40.
ter Horst JP, Kentrop J, Arp M, Hubens CJ, de Kloet ER, Oitzl MS (2013) Spatial learning of female mice: a role of the mineralocorticoid receptor during stress and the estrous cycle. *Front Behav Neurosci* 7:56.
Thompson PW, Cannon TD, Narr KL, van Erp T, Poutanen VP, Huttunen M, Lönngvist J, Standertskjöld-Nordenstam CG, Kaprio J, Khaledy M, Dail R, Zoumalan CI, Toga AW (2001) Genetic influences on brain structure. *Nat Neurosci* 4:1253-1258.
Todd RM, Palombo DJ, Levine B, Anderson AK (2011) Genetic differences in emotionally enhanced memory. *Neuropsychologia* 49:734-744.
Tolman EC (1938) The determiners of behavior at a choice point. *Psychological Review* 45:1-41.
Tolman EC (1948) Cognitive maps in rats and men. *Psychological Review* 55:189-208.
Trezza V, Campolongo P (2013) The endocannabinoid system as a possible target to treat both the cognitive and emotional features of post-traumatic stress disorder (PTSD). *Front Behav Neurosci* 7:100.
Tulving E (2002) Episodic memory: from mind to brain. *Annu Rev Psychol* 53:1-25.

Ulrich-Lai YM, Herman JP (2009) Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci* 10:397-409.

Urner M, van Wingen G, Franke B, Rijpkema M, Fernandez G, Tendolkar I (2011) Genetic variation of the alpha2b-adrenoceptor affects neural correlates of successful emotional memory formation. *Hum Brain Mapp* 32:2096-2103.

Urry HL, van Reekum CM, Johnstone T, Kalin NH, Thurow ME, Schaefer HS, Jackson CA, Frye CJ, Greischar LL, Alexander AL, Davidson RJ (2006) Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *J Neurosci* 26:4415-4425.

Vaiserman AM (2015) Epigenetic programming by early-life stress: evidence from human populations. *Dev Dyn* 244:254-265.

Vaiva G, Ducrocq F, Jezequel K, Averland B, Lestavel P, Brunet A, Marmar CR (2003) Immediate treatment with propranolol decreases posttraumatic stress disorder two months after trauma. *Biological Psychiatry* 54:947-949.

van Leeuwen N, Kumsta R, Entringer S, de Kloet ER, Zitman FG, DeRijk RH, Wust S (2010) Functional mineralocorticoid receptor (MR) gene variation influences the cortisol awakening response after dexamethasone. *Psychoneuroendocrinology* 35:339-349.

van Marle HJ, Hermans EJ, Qin S, Fernandez G (2009) From specificity to sensitivity: how acute stress affects amygdala processing of biologically salient stimuli. *Biol Psychiatry* 66:649-655.

van Marle HJ, Hermans EJ, Qin S, Fernandez G (2010) Enhanced resting-state connectivity of amygdala in the immediate aftermath of acute psychological stress. *Neuroimage* 53:348-354.

van Oort J, Tendolkar I, Hermans EJ, Mulders PC, Beckmann CF, Schene AH, Fernandez G, van Eijndhoven PF (2017) How the brain connects in response to acute stress: A review at the human brain systems level. *Neurosci Biobehav Rev* 83:281-297.

van Rossum EF, Binder EB, Majer M, Koper JW, Ising M, Modell S, Salyakina D, Lamberts SW, Holsboer F (2006) Polymorphisms of the glucocorticoid receptor gene and major depression. *Biol Psychiatry* 59:681-688.

van Stegeren AH, Wolf OT, Everaerd W, Rombouts SA (2008) Interaction of endogenous cortisol and noradrenaline in the human amygdala. *Prog Brain Res* 167:263-268.

van Stegeren AH, Roozendaal B, Kindt M, Wolf OT, Joels M (2010) Interacting noradrenergic and corticosteroid systems shift human brain activation patterns during encoding. *Neurobiol Learn Mem* 93:56-65.

van Stegeren AH, Wolf OT, Everaerd W, Scheltens P, Barkhof F, Rombouts SA (2007) Endogenous cortisol level interacts with noradrenergic activation in the human amygdala. *Neurobiol Learn Mem* 87:57-66.

van Stegeren AH, Goekoop R, Everaerd W, Scheltens P, Barkhof F, Kuijer JP, Rombouts SA (2005) Noradrenaline mediates amygdala activation in men and women during encoding of emotional material. *Neuroimage* 24:898-909.

Veer IM, Oei NY, Spinhoven P, van Buchem MA, Elzinga BM, Rombouts SA (2011) Beyond acute social stress: increased functional connectivity between amygdala and cortical midline structures. *Neuroimage* 57:1534-1541.

Voermans NC, Petersson KM, Daudey L, Weber B, Van Spaendonck KP, Kremer HP, Fernandez G (2004) Interaction between the human hippocampus and the caudate nucleus during route recognition. *Neuron* 43:427-435.

Vogel S, Fernandez G, Joels M, Schwabe L (2016) Cognitive adaptation under stress: a case for the mineralocorticoid receptor. *Trends Cogn Sci* 20:192-203.

Vogel S, Klumpers F, Krugers HJ, Fang Z, Oplaat KT, Oitzl MS, Joels M, Fernandez G (2015) Blocking the mineralocorticoid receptor in humans prevents the stress-induced enhancement of centromedial amygdala connectivity with the dorsal striatum. *Neuropsychopharmacology* 40:947-956.

Vogel S, Klumpers F, Schroder TN, Oplaat KT, Krugers HJ, Oitzl MS, Joels M, Doeller CF, Fernandez G (2017) Stress induces a shift towards striatum-dependent stimulus-response learning via the mineralocorticoid receptor. *Neuropsychopharmacology* 42:1262-1271.

Voon V, Derbyshire K, Ruck C, Irvine MA, Worbe Y, Enander J, Schreiber LR, Gillan C, Fineberg NA, Sahakian BJ, Robbins TW, Harrison NA, Wood J, Daw ND, Dayan P, Grant JE, Bullmore ET (2015) Disorders of compulsivity: a common bias towards learning habits. *Mol Psychiatry* 20:345-352.

Vuilleumier P (2005) How brains beware: neural mechanisms of emotional attention. *Trends Cogn Sci* 9:585-594.

Vuilleumier P, Richardson MP, Armony JL, Driver J, Dolan RJ (2004) Distant influences of amygdala lesion on visual cortical activation during emotional face processing. *Nat Neurosci* 7:1271-1278.

Vukojevic V, Kolassa IT, Fastenrath M, Gschwind L, Spalek K, Milnik A, Heck A, Vogler C, Wilker S, Demougin P, Peter F, Atucha E, Stetak A, Roozendaal B, Elbert T, Papassotiropoulos A, de Quervain D (2014) Epigenetic modification of the glucocorticoid receptor gene is linked to traumatic memory and post-traumatic stress disorder risk in genocide survivors. *J Neurosci* 34:10274-10284.

Wager TD, Davidson ML, Hughes BL, Lindquist MA, Ochsner KN (2008) Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron* 59:1037-1050.

Weaver IC, Champagne FA, Brown SE, Dymov S, Sharma S, Meaney MJ, Szyf M (2005) Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *J Neurosci* 25:11045-11054.

Weymar M, Schwabe L, Löw A, Hamm AO (2012) Stress sensitizes the brain: increased processing of unpleasant pictures after exposure to acute stress. *J Cogn Neurosci* 24:1511-1518.

White NM, McDonald RJ (2002) Multiple parallel memory systems in the brain of the rat. *Neurobiol Learn Mem* 77:125-184.

Wingard JC, Packard MG (2008) The amygdala and emotional modulation of competition between cognitive and habit memory. *Behav Brain Res* 193:126-131.

Wirz L, Bogdanov M, Schwabe L (2018) Habits under stress: mechanistic insights across different types of learning. *Current Opinion in Behavioral Sciences* 20:9-16.

Wirz L, Reuter M, Felten A, Schwabe L (under revision) An endocannabinoid receptor polymorphism modulates affective processing under stress. *Soc Cogn Affect Neurosci*.

Wirz L, Wacker J, Felten A, Reuter M, Schwabe L (2017a) A deletion variant of the alpha2b-adrenoceptor modulates the stress-induced shift from "cognitive" to "habit" memory. *J Neurosci* 37:2149-2160.

Wirz L, Reuter M, Wacker J, Felten A, Schwabe L (2017b) A haplotype associated with enhanced mineralocorticoid receptor expression facilitates the stress-induced shift from "cognitive" to "habit" learning. *eNeuro* 4.

Worley NB, Hill MN, Christianson JP (2017) Prefrontal endocannabinoids, stress controllability and resilience: A hypothesis. *Prog Neuropsychopharmacol Biol Psychiatry*.

Wüst S, Federenko I, Hellhammer DH, Kirschbaum C (2000) Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology* 25:707-720.

Yehuda R, Flory JD, Southwick S, Charney DS (2006) Developing an agenda for translational studies of resilience and vulnerability following trauma exposure. *Ann N Y Acad Sci* 1071:379-396.

Yi B, Nichiporuk I, Nicolas M, Schneider S, Feuerecker M, Vassilieva G, Thieme D, Schelling G, Chouker A (2016) Reductions in circulating endocannabinoid 2-arachidonoylglycerol levels in healthy human subjects exposed to chronic stressors. *Prog Neuropsychopharmacol Biol Psychiatry* 67:92-97.

Young CB, Raz G, Everaerd D, Beckmann CF, Tendolkar I, Hendler T, Fernandez G, Hermans EJ (2017) Dynamic shifts in large-scale brain network balance as a function of arousal. *J Neurosci* 37:281-290.

Zarzer CA, Puchinger MG, Köhler G, Kügler P (2013) Differentiation between genomic and non-genomic feedback controls yields an HPA axis model featuring hypercortisolism as an irreversible bistable switch. *Theor Biol Med Model* 10.

Zoladz PR, Dailey AM, Nagle HE, Fiely MK, Mosley BE, Brown CM, Duffy TJ, Scharf AR, Earley MB, Rorabaugh BR (2017) ADRA2B deletion variant influences time-dependent effects of pre-learning stress on long-term memory. *Neurobiol Learn Mem* 140:71-81.

Appendix A

Wirz L, Wacker J, Felten A, Reuter M, Schwabe L (2017) A deletion variant of the alpha2b-adrenoceptor modulates the stress-induced shift from “cognitive” to “habit” memory. *J Neurosci* 37:2149-2160.

Behavioral/Cognitive

A Deletion Variant of the α2b-Adrenoceptor Modulates the Stress-Induced Shift from "Cognitive" to "Habit" Memory

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Stress induces a shift from hippocampus-based "cognitive" toward dorsal striatum-based "habitual" learning and memory. This shift is thought to have important implications for stress-related psychopathologies, including post-traumatic stress disorder (PTSD). However, there is large individual variability in the stress-induced bias toward habit memory, and the factors underlying this variability are completely unknown. Here we hypothesized that a functional deletion variant of the gene encoding the α2b-adrenoceptor (*ADRA2B*), which has been linked to emotional memory processes and increased PTSD risk, modulates the stress-induced shift from cognitive toward habit memory. In two independent experimental studies, healthy humans were genotyped for the *ADRA2B* deletion variant. After a stress or control manipulation, participants completed a dual-solution learning task while electroencephalographic (Study I) or fMRI measurements (Study II) were taken. Carriers compared with noncarriers of the *ADRA2B* deletion variant exhibited a significantly reduced bias toward habit memory after stress. fMRI results indicated that, whereas noncarriers of the *ADRA2B* deletion variant showed increased functional connectivity between amygdala and putamen after stress, this increase in connectivity was absent in carriers of the deletion variant, who instead showed overall enhanced connectivity between amygdala and entorhinal cortex. Our results indicate that a common genetic variation of the noradrenergic system modulates the impact of stress on the balance between cognitive and habitual memory systems, most likely via altered amygdala orchestration of these systems.

Key words: amygdala; dorsal striatum; hippocampus; memory; noradrenaline; stress

Significance Statement

Stressful events have a powerful effect on human learning and memory. Specifically, accumulating evidence suggests that stress favors more rigid dorsal striatum-dependent habit memory, at the expense of flexible hippocampus-dependent cognitive memory. Although this shift may have important implications for understanding mental disorders, such as post-traumatic stress disorder, little is known about the source of individual differences in the sensitivity for the stress-induced bias toward habit memory. We report here that a common genetic variation of the noradrenergic system, a known risk factor for post-traumatic stress disorder, modulates the stress-induced shift from cognitive to habit memory, most likely through altered crosstalk between the hippocampus and dorsal striatum with the amygdala, a key structure in emotional memory.

Introduction

Stress has a major impact on health and well-being (McEwen, 1998; de Kloet et al., 2005). These stress effects may be partly due to stress-induced changes in cognition, including altered learning and memory processes (Diamond et al., 2007; Lupien et al., 2009; Schwabe et al., 2010; Roozendaal and McGaugh, 2011; Sandi and Haller, 2015). Stress enhances memory consolidation and impairs memory retrieval. These modulatory effects are critically mediated by noradrenaline, in interaction with glucocorticoids (McGaugh et al., 1996; Roozendaal et al., 2004; de Quervain et al., 2007a; Schwabe et al., 2009; Roozendaal and McGaugh, 2011). Beyond its effects on consolidation and retrieval, stress alters the contribution of multiple, anatomically and functionally distinct memory systems to learning and behavior (Schwabe et al., 2010; Schwabe and Wolf, 2013). Specifically, stress promotes a shift from hippocampus-dependent cognitive toward dorsal striatum-dependent habit memory (Kim et al., 2001; Schwabe and Wolf, 2012, 2013; Schwabe et al., 2007). Neuroimaging data indicate that the amygdala orchestrates this stress-induced shift in the

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balance between flexible and more rigid memory processes (Packard and Wingard, 2004; Schwabe et al., 2013; Vogel et al., 2016).

However, not all individuals show the bias toward dorsal striatal habit memory after stress (Kim et al., 2001; Schwabe et al., 2007; Schwabe and Wolf, 2012). Given the critical clinical implications associated with the stress-induced modulation of the balance between cognitive and habit memory (Schwabe et al., 2010; Goodman et al., 2012; de Quervain et al., 2017), identifying the source of this variability is crucial. Genetic differences explain part of the variance in human memory (de Quervain and Papassotiropoulos, 2006; de Quervain et al., 2012; Papassotiropoulos et al., 2006, 2013). With respect to the emotional modulation of memory, a deletion variant of the gene encoding the α2b-adrenoceptor (*ADRA2B*), which is present in ~30% of Caucasians (Small et al., 2001), is of particular interest. Corroborating studies showing that pharmacological manipulations of α2-adrenoceptors affect emotional memory (O'Carroll et al., 1999; Southwick et al., 2002), healthy *ADRA2B* deletion carriers exhibited enhanced episodic (i.e., hippocampus-dependent) memory for emotional material (de Quervain et al., 2007b; Rasch et al., 2009). This emotional memory enhancement was also observed in Rwandan civil war survivors carrying the deletion variant (de Quervain et al., 2007b) and directly linked to increased PTSD risk (Liberzon et al., 2014). At the neural level, the *ADRA2B* deletion variant was associated with increased amygdala activation during encoding of emotional stimuli (Rasch et al., 2009) and following stress (Cousijn et al., 2010) as well as increased connectivity between the amygdala and the insular cortex (Rasch et al., 2009), a region closely linked to the medial temporal lobe network relevant for episodic memory (Miranda and Bermúdez-Rattoni, 2007). Based on this evidence, suggesting that the *ADRA2B* deletion variant is associated with stronger amygdala activation and enhanced hippocampus-dependent memory for emotional material, we hypothesized that the deletion variant would be linked to enhanced crosstalk between the amygdala and the hippocampus, thereby reducing the stress-induced shift from hippocampal to dorsal striatal memory.

To test the modulatory role of the *ADRA2B* deletion, we exposed healthy individuals, genotyped for the *ADRA2B* polymorphism, to a stressor (or control manipulation) before they completed a probabilistic classification learning (PCL) task that can be solved by the hippocampus and the dorsal striatum (Fig. 1) (Knowlton et al., 1996; Poldrack et al., 2001). The engagement of these memory systems can be assessed by the analysis of distinct learning strategies known to rely on the hippocampus and the dorsal striatum, respectively (Shohamy et al., 2004; Schwabe and Wolf, 2012). Additionally, in our first experiment, we used EEG measurements to assess the feedback-related negativity (FRN), which likely reflects striatal processing (Nieuwenhuis et al., 2005; Hauser et al., 2014). In a second experiment, we used the same experimental setup, but fMRI to elucidate the neural correlates of the modulatory effect of the *ADRA2B* polymorphism on the stress-induced shift toward habit memory.

Materials and Methods

Study I: role of an ADRA2B deletion variant in the stress-induced modulation of multiple memory systems

Participants and experimental design. A total of 252 healthy volunteers without medication intake, including adrenergic agonists and antagonists, or any current or previous neurological or psychiatric disorders participated in this experiment (127 women; mean ± SD age, 25.1 ± 3.5 years). To control for factors known to influence the responsiveness of the hypothalamus-pituitary-adrenal axis to stress, smokers as well as women taking hormonal contraceptives were excluded and women were only tested outside their menstrual cycle phase (Kirschbaum et al., 1999; Rohleder and Kirschbaum, 2006). The study was approved by the ethical review board of the German Psychological Society (reference: LS072014). Participants gave written informed consent and received a moderate monetary compensation of 25€ for their participation.

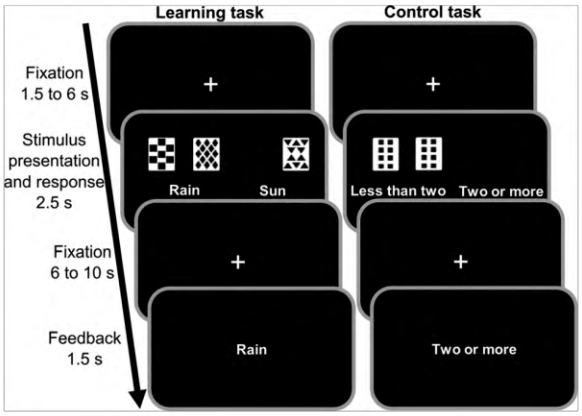

Figure 1. Probabilistic classification learning task. Participants were presented with 1–3 of 4 possible cues per trial and asked to predict the weather outcome ("rain" or "sun"). Each cue has a certain probability for the 2 outcomes. Feedback about the correct outcome was presented after each trial. The task can be solved by using single-cue strategies based on the hippocampus and multi-cue strategies based on the dorsal striatum. In a visual-motor control task, participants were required to indicate whether ≤2 or >2 cards were presented. The shown task setup refers to Study II. In Study I, there was no visual-motor control task and the timing was slightly different.

A 2 × 2 between-subjects design with the factors treatment (stress vs control manipulation) and *ADRA2B* deletion variant (carriers vs noncarriers) was used to examine modulatory effects of the *ADRA2B* genotype on stress-induced changes in the preferential engagement of multiple memory systems. Participants were randomly assigned to the stress or control condition. To control for the diurnal rhythm of cortisol, all testing took place in the afternoon.

Genotyping. A common variant (rs29000568) of the *ADRA2B* gene consists of an inframe deletion of three acidic residues. Specifically, glutamic acid residues 301–303 in the third intracellular loop of the receptor, which are part of a large glutamic acid stretch (glu12; amino acids 297–309), are absent in ~30% of Caucasians (Small et al., 2001; de Quervain et al., 2007b). This deletion variant is associated with increased extracellular noradrenaline availability (Small et al., 2001). For genetic analysis, DNA was extracted from buccal cells. Automated purification of genomic DNA was conducted by means of the MagNA Pure LC system using a commercial extraction kit (MagNA Pure LC DNA isolation kit; Roche Diagnostics). Genotyping of the polymorphism was performed by real-time PCR using fluorescence melting curve detection analysis by means of the Light Cycler System (Roche Diagnostics) and a LightSNiP Assay (TIB MOLBIOL).

Stress manipulation. Participants in the stress condition underwent the Trier Social Stress Test (TSST), the gold standard in experimental stress research that reliably induces autonomic nervous system and hypothalamus-pituitary-adrenal axis activation (Kirschbaum et al., 1993). After a short preparation time, each participant was asked to give a 5 min free speech about why he or she is the ideal candidate for a job tailored to his or her interests and subsequently had to solve a difficult mental-arithmetic task for another 5 min (counting backwards from 2043 in steps of 17). Throughout the TSST, participants were videotaped and evaluated by a reserved and nonreinforcing panel. In the control condition, participants were asked to talk about a self-chosen topic and had to count forwards from zero in steps of 15, while being alone in the room and without video recordings.

To assess the effectiveness of the stress induction, subjective and physiological measures were taken at several time points across the experiment. Changes in subjective mood were evaluated using a German mood questionnaire (subscales: depressed vs elevated, restless vs calm, sleepy vs awake; high scores indicate elevated mood, calmness, and wakefulness) (Steyer et al., 1994). In addition, participants rated how difficult, unpleasant, and stressful they had experienced the manipulation on a scale from 0 (not at all) to 100 (very much). Blood pressure was measured using a Dinamap system (Critikon) before (−25 min), during (10 min), and after (20, 60, 80 min) the stress manipulation. Furthermore, saliva samples were collected before (−25 min) and after (20, 30, 40, 80 min) the experimental treatment using Salivette collection devices (Sarstedt). Samples were stored at −18°C; and at the end of the study, the free fraction of cortisol was analyzed from saliva using a commercially available chemiluminescence immunoassay (IBL).

Probabilistic classification learning (PCL) task. Fifteen minutes after the stress or control manipulation, when peak cortisol levels were expected, participants completed a modified version of the weather prediction task (Fig. 1) (Knowlton et al., 1994; Gluck et al., 2002) while EEG was recorded. In this PCL task, participants had to learn how to classify stimuli into two given categories ("rain" vs "sun") based on trial-by-trial feedback. Between 1 and 3 (of 4) cards appeared on each trial, yielding 14 different cue patterns. These cue patterns were associated with two possible outcomes (sun and rain) in a probabilistic manner, such that a particular cue was associated with the outcome "sun" with a probability of 75.6%, 57.5%, 42.5%, or 24.4% across 100 trials; these probabilities were the same as in previous studies using this task (Gluck et al., 2002; Lagnado et al., 2006; Schwabe and Wolf, 2012; Schwabe et al., 2013). A response was counted as correct if it matched the outcome with the highest probability for that cue pattern. Participants completed 100 PCL trials (duration ~25 min). On each trial, they saw 1 of the 14 cue patterns and had 5 s to respond by pressing one of the buttons that corresponded with either "sun" or "rain." The chosen outcome was highlighted with a red circle for 500 ms. Following a black screen that was shown for another 500 ms, a feedback stimulus in the form of a happy or sad face was presented for 1 s. The intertrial interval varied between 1 and 2.5 s.

Assessment of learning strategies. The PCL task can be solved by using different learning strategies that rely on distinct brain systems. In particular, patient and neuroimaging studies showed that participants may acquire the task using single-cue strategies supported by a hippocampus-dependent system or by using multi-cue strategies that are based on the dorsal striatum (Knowlton et al., 1996; Shohamy et al., 2004; Foerde et al., 2006; Schwabe et al., 2012). To assess the learning strategy that participants used during the PCL task, participants' actual responses were compared with ideal response patterns for each strategy (Gluck et al., 2002; Lagnado et al., 2006). A least mean squares measure resulted in a fit-value ranging from 0 to 1 (0 indicating a perfect fit). The strategy with the lowest score was defined as the best fit for that participant. If the best fit score was >0.15, participants' strategies were classified as "nonidentifiable" (Gluck et al., 2002); and because the multi-match strategy contains a "random" element (Lagnado et al., 2006), when single-cue and multi-match scores were very close, the single-cue strategy was chosen. In Study I, there were 20 participants for which no strategy could be identified; experimental groups did not differ in the number of participants for which no strategy could be identified ($\chi^2_{(1)} = 0.220$, $p = 0.639$). For the sake of simplicity and in line with previous studies (Schwabe and Wolf, 2012; Schwabe et al., 2013), we classified the strategies that participants may use to solve the PCL task into hippocampus-dependent single-cue (comprising one cue and singleton) and dorsal striatum-dependent multi-cue strategies.

Behavioral and physiological data analyses. Subjective and physiological measurements were analyzed using mixed-design ANOVAs with time as within-subject factor and treatment (TSST vs control) as well as *ADRA2B* deletion variant (carriers vs noncarriers) as between-subjects factors. A mixed-design ANOVA with blocks of 10 trials as within-subject factor was used to assess learning performance on the PCL task. Group differences in learning strategy were analyzed by means of χ^2 tests. Statistical analyses were performed using SPSS Statistics 22 (RRID: SCR_002865, IBM). All reported p values are two-tailed. In case of violation of the sphericity assumption, Greenhouse-Geisser corrections were applied. Significant main and interaction effects were followed by the appropriate *post hoc* tests.

EEG recording and analyses. During the PCL task, EEG was recorded from 64 active electrodes arranged according to the international 10–20 system. In addition, horizontal electro-oculograms were measured, and the most frontal electrodes served as recording sites for vertical eye movements. A Biosemi Active-Two amplifier system was used with a sampling rate of 2048 Hz (Biosemi). Common mode sense and driven right leg electrodes served as recording reference and ground, respectively.

EEG data were analyzed offline using the Brain Vision Analyzer software (Brain Products) and a costume-written MATLAB script (The MathWorks). After the EEG signal was downsampled to 512 Hz, the data were high-pass filtered at 0.01 Hz. To remove artifacts from electrical lines, a 50 Hz notch filter was applied. EEG data were then visually inspected to discard any extreme artifacts. Additionally, eye-blink and eye-movement artifacts were removed using an independent component-based approach. Bad channels were replaced by means of topographic interpolation, and the data were rereferenced to the average of all electrodes. To analyze ERPs reflecting feedback processing, data were segmented into epochs from −200 to 800 ms with respect to feedback stimulus onset and subsequently baseline corrected relative to the 200 ms preceding the feedback stimulus. Before averaging, trials were rejected if there was a voltage step >50 μV /ms, or a difference of >100 μV as well as a signal <0.1 μV was detected in any of the intervals. Because of technical difficulties or excessive noise, 24 participants were excluded from further EEG data analyses. For the remaining 228 participants (stress group: 68 carriers, 46 noncarriers; control group: 64 carriers, 50 noncarriers), on average 37.5 trials (SD 13.8 trials) in the negative feedback condition were available. The FRN, which likely reflects striatal feedback processing (Nieuwenhuis et al., 2005; Hauser et al., 2014), was calculated as the most negative peak amplitude in the time window between 200 and 350 ms following feedback presentation relative to the preceding positive peak amplitude between 150 ms and the latency of that negative peak (Frank et al., 2005; Eppinger et al., 2008; Rustemeier et al., 2013). A mixed-design ANOVA with electrode site and feedback (positive vs negative) as within-subject factors and treatment as well as genotype as between-subjects factors was used to investigate stress- or genotype-related differences in feedback processing. Frontal electrodes (FC1, Fz, FCz, FC2), where the FRN was most pronounced, were included in the analyses.

Study II: neural underpinnings of the *ADRA2B* modulation of memory system engagement under stress

Participants and experimental design. A total of 128 volunteers of the Bonn Gene Brain Behavior Project participated in this experiment (62 women; mean ± SD age, 23.0 ± 3.6 years). Participants were young and healthy nonsmokers without medication intake, including adrenergic agonists and antagonists, or lifetime history of neurological or psychiatric disorders. Furthermore, any contraindications for fMRI measurements served as exclusion criteria. The study was approved by the ethical review board of the German Psychological Society (reference: LS072014) as well as by a local committee at the University of Bonn. Participants gave written informed consent and received a moderate monetary compensation of 35€.

In line with the first experiment, we used a 2 × 2 between-subjects design with the factors treatment (TSST vs control manipulation) and *ADRA2B* genotype (deletion vs nondeletion carriers). In this experiment, however, participants were prescreened and groups were stratified by the *ADRA2B* genotype. We aimed for 30 participants in each of the four experimental groups ($N = 120$). However, because of technical difficulties and excessive head motion in the MRI scanner, 8 participants had to be excluded from the fMRI analyses (stress: 2 deletion, 3 nondeletion; control: 1 deletion, 2 nondeletion) and 8 additional participants were recruited, thus leading to a sample of 120 participants ($N = 30$/group) for the fMRI analyses. For the behavioral analyses, data from all 128 tested participants were used (stress: 32 deletion, 33 nondeletion; control: 31 deletion, 32 nondeletion).

Experimental procedure. The experimental procedure, including the stress manipulation, the parameters measured, and the PCL task, was the same as in the first study, with two exceptions. First, fMRI

instead of EEG measurements were taken while participants performed the PCL task. Second, the task was slightly modified to accommodate fMRI requirements. More specifically, in addition to 100 PCL trials, participants completed 100 visuo-motor control trials in which they were asked to indicate whether <2 or ≥2 cards appeared on the screen (trial type was randomly alternated; task duration ~45 min). Moreover, the timing of the events was adjusted to the slow BOLD response. In Study II, there were 31 participants for whom no strategy could be identified; experimental groups did not differ in that number ($p > 0.05$). The behavioral and statistical analyses were in line with those of the first experiment.

MRI acquisition and analyses. MRI measurements were acquired using a 3T Trio Scanner (Siemens) equipped with a 32-channel head coil. BOLD T2-weighted echoplanar functional images were acquired parallel to the anterior commissure-posterior commissure plane (37 transversal slices; TR = 2000 ms; TE = 30 ms; ascending acquisition; effective voxel size = 3 × 3 × 3 mm). Additionally, a high-resolution T1-weighted anatomical image was acquired (208 sagittal slices, TR = 1660 ms, TE = 2.54 ms, voxel size = 0.8 × 0.8 × 0.8 mm).

Preprocessing and analyses of the fMRI data using GLMs were performed with the SPM12 MATLAB toolbox (RRID:SCR_007037, Wellcome Trust Centre for Neuroimaging, London). Functional data were slice-time and head-motion corrected as well as coregistered to the structural image using rigid-body transformations. The T1-weighted image was segmented into gray and white matter, cerebrospinal fluid, bone, soft tissue, and air. Forward deformation fields were then used to spatially normalize the functional and structural scans to the MNI standard brain. Finally, normalized functional images were smoothed using an 8 mm FWHM Gaussian kernel.

Correct and incorrect PCL trials as well as visuo-motor control trials were modeled using canonical hemodynamic response functions. Additionally, fixation, button presses and the six movement parameters were included into the model. Data were filtered in the temporal domain using a nonlinear high-pass filter with a 128 s cutoff. Contrast images were generated for PCL minus control trials and for correct minus incorrect PCL trials. These difference contrasts were then entered into a second-level (group) analyses, using a full-factorial model with the factors treatment (control vs stress) and genotype (deletion vs nondeletion carriers). Psycho-Physiological-Interaction (PPI) analyses were performed to assess whether the coupling of the amygdala with the hippocampus and the dorsal striatum (regions of interest [ROIs]) was altered by stress and/or *ADRA2B* genotype. For this purpose, the first eigenvariate of the time course of each ROI in the contrast PCL correct minus PCL incorrect was extracted from the appropriate brain atlases and used as seed. The PPI was then computed as the element-by-element product of the BOLD signal time course of this seed and a vector coding for successful classification learning. Next, each time course was added separately as a covariate of interest in addition to the first-level regressors. The individual PPI contrasts were then entered in a second-level random-effects analysis. Results of these analyses give insight into brain regions that show a similar and task-dependent pattern of activation. These regions are therefore supposed to be functionally connected during correct classification learning.

Explorative whole-brain analyses as well as ROI analyses were used. A priori ROIs were memory system structures (hippocampus, caudate nucleus, and putamen) that are consistently activated in the PCL task (Poldrack et al., 2001; Foerde et al., 2006; Schwabe and Wolf, 2012; Schwabe et al., 2013) as well as the amygdala because this area has been shown to be affected by the *ADRA2B* deletion variant (Cousijn et al., 2010) and is known to modulate multiple memory systems (Elliott and Packard, 2008; Schwabe et al., 2013; Vogel et al., 2015). Anatomical masks of the caudate nucleus, the putamen, and the amygdala were taken from the Harvard-Oxford subcortical atlas, whereas masks of the hippocampal subregions were taken from the Anatomy Toolbox for SPM (Institute of Neuroscience and Medicine, Jülich, Germany). For the explorative whole-brain analysis, the significance threshold was set to $p < 0.05$ at cluster level and corrected for multiple testing (family-wise error [FWE] correction). ROI analyses were performed using small-volume correction with an initial threshold of $p < 0.05$ uncorrected, followed by FWE correction ($p < 0.05$). Thresholds at 50% were used to include only voxels with a probability of at least 50% to belong to each subregion. To assess stress- or genotype-dependent alterations in the connectivity between the ROIs, PPI analyses were performed. After voxels of interest of the time courses of the ROIs were extracted from the appropriate brain atlases, they were added separately in the model in addition to the first-level regressors. Correlations between these time series and the activity of the rest of the brain are indicative of similar activation patterns and thus brain regions that are supposedly functionally connected. Again, for ROI analyses, small-volume correction with an initial threshold of $p < 0.05$ uncorrected, followed by FWE correction ($p < 0.05$) was performed.

Results

Study I: role of an *ADRA2B* deletion variant in the stress-induced modulation of multiple memory systems

Genotyping

Our first study included 114 heterozygous and 29 homozygous carriers of the *ADRA2B* deletion variant. In line with previous studies (de Quervain et al., 2007b; Rasch et al., 2009) and due to the relatively small number of homozygous carriers of the deletion variant, heterozygous and homozygous carriers were statistically treated as one group ($N = 143$). A total of 109 participants did not carry the deletion variant. Genotype frequencies were in Hardy-Weinberg equilibrium ($\chi^2_{(1)} = 0.780$, $p = 0.377$) and in line with the genotype and allele frequencies typically observed in Caucasians (Small et al., 2001). Genotype was not significantly associated with sex or age (both $p > 0.279$) and the distribution of carriers and noncarriers of the *ADRA2B* deletion variant was comparable in the stress (74 carriers, 52 noncarriers) and control group (69 carriers, 57 noncarriers; $\chi^2_{(1)} = 0.40$, $p = 0.525$).

Subjective, autonomic, and endocrine stress response

Changes in subjective feeling, blood pressure, and salivary cortisol verified the successful stress induction by the TSST (Table 1). Compared with the control procedure, exposure to the TSST was rated as significantly more difficult, unpleasant, and stressful (all $F_{(1,248)} \geq 223.55$, all $p < 0.001$). Moreover, the TSST, but not the control manipulation, resulted in increases of depressed mood and restlessness (time × treatment: both $F_{(2,2494)} \geq 53.76$, both $p < 0.001$). Additionally, exposure to the TSST led to significant autonomic activation, reflected by increases in systolic (Fig. 2*A*) and diastolic blood pressure (time × treatment: both $F \geq 62.91$, both $p < 0.001$). Finally, we obtained a significant increase in cortisol concentrations following the stress but not the control manipulation (time × treatment: $F_{(2,493.2)} = 71.15$, $p < 0.001$). As shown in Figure 2*B*, peak cortisol levels were reached ~15 min following the stressor, when behavioral testing started. Importantly, carriers and noncarriers of the *ADRA2B* deletion variant did not differ in measures of blood pressure, cortisol, or mood (all $p > 0.299$).

ADRA2B *deletion variant is associated with a reduced stress-induced shift toward multi-cue strategies*

In the PCL task, participants' performance gradually improved from 59% to 74% correct responses ($F_{(7.8,\ 1921.1)} = 21.52$, $p < 0.001$; Fig. 2*C*), thus indicating successful learning. In line with previous studies showing that different memory systems may contribute equally well to learning performance (Schwabe and Wolf, 2009, 2012), stress had no effect on performance ($F_{(3.1,\ 762.2)} = 0.80$, $p = 0.597$). Moreover, there was no difference between carriers and noncarriers of the *ADRA2B* deletion variant in learning performance ($F_{(3.1,\ 762.2)} = 1.22$, $p = 0.287$).

The engagement of multiple memory systems is reflected in the use of single-cue strategies, which are based on the hippocampus; and multi-cue strategies, which are based on the dorsal stria-

Table 1. Subjective, autonomic, and endocrine stress response in Study I[a]

	Control		Stress	
	Deletion	No deletion	Deletion	No deletion
Subjective assessment				
Stressful	26.67 ± 2.63	29.30 ± 2.90	71.76 ± 2.64***	68.08 ± 2.03***
Difficult	25.51 ± 2.58	32.63 ± 2.84	77.70 ± 2.49***	71.73 ± 2.97***
Unpleasant	27.97 ± 2.78	30.53 ± 3.06	76.89 ± 2.68***	69.42 ± 3.20***
Subjective mood				
Good versus bad mood				
Before treatment	32.58 ± 0.52	32.66 ± 0.58	33.70 ± 0.51	33.56 ± 0.60
1 min after treatment	32.30 ± 0.72	32.04 ± 0.80	27.28 ± 0.69***	25.75 ± 0.83***
65 min after treatment	31.23 ± 0.68	31.00 ± 0.76	31.07 ± 0.66	29.48 ± 0.79
Calm versus restless				
Before treatment	30.58 ± 0.66	30.80 ± 0.73	31.51 ± 0.63	31.15 ± 0.76
1 min after treatment	30.06 ± 0.72	30.09 ± 0.80	23.82 ± 0.70***	22.94 ± 0.83***
65 min after treatment	31.19 ± 0.71	31.02 ± 0.78	30.82 ± 0.68	29.56 ± 0.81
Tired versus awake				
Before treatment	29.06 ± 0.74	29.41 ± 0.83	30.68 ± 0.72	30.14 ± 0.86
1 min after treatment	28.33 ± 0.75	29.23 ± 0.84	30.18 ± 0.73	28.65 ± 0.87
65 min after treatment	22.70 ± 0.83	23.34 ± 0.93	23.28 ± 0.81	23.00 ± 0.96
Systolic blood pressure (bpm)				
Before treatment	134.22 ± 2.20	132.75 ± 2.42	131.80 ± 2.16	132.42 ± 2.54
During treatment	136.45 ± 2.21	135.76 ± 2.43	158.22 ± 2.16***	158.64 ± 2.54***
5 min after treatment	132.91 ± 2.05	130.50 ± 2.25	138.17 ± 2.00	138.35 ± 2.36*
45 min after treatment	130.34 ± 2.03	126.24 ± 2.23	130.13 ± 1.98	127.49 ± 2.33
65 min after treatment	129.70 ± 1.92	129.79 ± 2.11	132.01 ± 1.88	128.56 ± 2.21
Diastolic blood pressure (bpm)				
Before treatment	76.90 ± 1.04	75.83 ± 1.14	76.28 ± 1.02	75.85 ± 1.19
During treatment	81.59 ± 1.32	80.25 ± 1.45	95.22 ± 1.29***	94.10 ± 1.52***
5 min after treatment	78.37 ± 1.01	76.97 ± 1.11	81.98 ± 0.99*	80.89 ± 1.16
45 min after treatment	76.27 ± 0.94	74.25 ± 1.04	77.52 ± 0.92	76.50 ± 1.09
65 min after treatment	77.54 ± 0.94	75.93 ± 1.04	77.88 ± 0.92	75.77 ± 1.08
Salivary cortisol (nmol/l)				
Before treatment	4.90 ± 0.51	5.66 ± 0.56	5.04 ± 0.49	6.30 ± 0.59
5 min after treatment	5.04 ± 0.68	4.72 ± 0.75	9.21 ± 0.65***	11.34 ± 0.78***
15 min after treatment	4.31 ± 0.81	3.98 ± 0.89	11.86 ± 0.78***	13.95 ± 0.93***
25 min after treatment	3.73 ± 0.64	3.46 ± 0.71	9.32 ± 0.62***	10.65 ± 0.74***
65 min after treatment	3.06 ± 0.28	2.85 ± 0.31	4.66 ± 0.27***	5.12 ± 0.32***

[a]Data are mean ± SEM.

*$p < 0.05$; ***$p < 0.001$.

tum. Corroborating earlier results from our laboratory (Schwabe and Wolf, 2012; Schwabe et al., 2013), stressed participants showed a shift toward more multi-cue and fewer single-cue strategies compared with controls ($\chi^2_{(1)} = 4.25$, $p = 0.039$). Most importantly, however, this stress-induced shift was modulated by *ADRA2B* genotype (Fig. 2*D*). In noncarriers of the deletion variant, stress increased the use of multi-cue strategies from 70% to 88% ($\chi^2_{(1)} = 4.85$, $p = 0.028$). This, however, was not the case in *ADRA2B* deletion carriers who did not show any changes in the used strategy after stress ($\chi^2_{(1)} = 0.67$, $p = 0.412$).

Stress increases the amplitude of the feedback-related negativity during probabilistic classification learning

Our EEG data showed a larger FRN, thought to reflect dorsal striatal processing (Nieuwenhuis et al., 2005; Hajcak et al., 2007; Hauser et al., 2014), with a typical frontocentral distribution in stressed participants compared with controls following negative feedback ($F_{(1,224)} = 6.62$, $p = 0.011$; Fig. 2*E*). As the FRN is particularly important for learning from negative feedback (van der Helden et al., 2010), this difference was not observed in response to positive feedback ($F_{(1,223)} = 0.33$, $p = 0.564$; feedback × condition: $F_{(1,9.8)} = 4.99$, $p = 0.026$). Carriers and noncarriers of the *ADRA2B* deletion variant, however, neither differed in FRN amplitude, nor was the stress effect on the FRN modulated by *ADRA2B* genotype (all $F < 0.59$, all $p > 0.321$). We did not obtain significant correlations between task performance and FRN amplitude (all $p > 0.05$).

Study II: neural underpinnings of the *ADRA2B* modulation of memory system engagement under stress

Subjective, autonomic, and endocrine stress responses

Exposure to the TSST was rated as significantly more difficult, unpleasant, and stressful compared with the control manipulation (all $F_{(1,124)} > 58.68$, all $p < 0.001$) and participants' mood decreased only following the TSST (all $F_{(2,246)} > 21.48$, all $p < 0.001$; Table 2). Moreover, systolic and diastolic blood pressure (Fig. 3*A*) as well as salivary cortisol increased following the TSST, but not the control manipulation (all $F > 20.28$, all $p < 0.001$). As shown in Figure 3*B*, cortisol concentrations peaked ~15 min following the stressor, when the scanning session began. *ADRA2B* deletion and nondeletion carriers did not differ in their physiological or subjective responses to stress (all $p > 0.136$).

ADRA2B *deletion variant is associated with a reduced stress-induced shift toward multi-cue strategies*

Participants gradually learned to correctly classify the card stimuli and correct responses increased from 37% to 63% across trials ($F_{(6.7,\ 826.5)} = 28.64$, $p < 0.001$; Fig. 3*C*). Stress and the *ADRA2B* deletion variant had no effect on task performance (both $F_{(6.7,\ 826.5)} < 1.01$, both $p > 0.421$). Analysis of the used learning

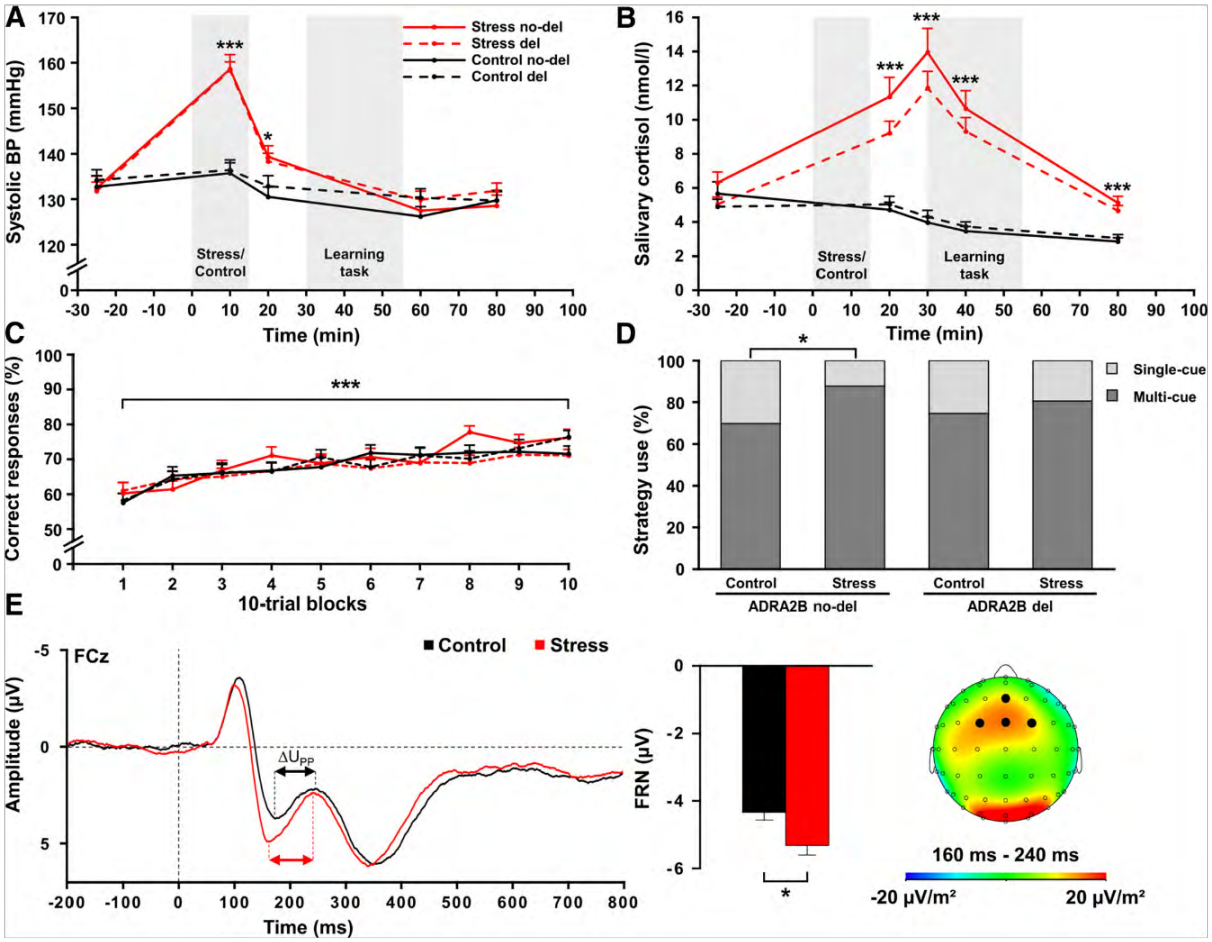

Figure 2. Physiological, behavioral, and EEG data of Study I. Compared with a nonstressful control manipulation, exposure to the TSST led to significant increases in (***A***) systolic blood pressure (BP) and (***B***) salivary cortisol concentrations, regardless of the allelic variant. ***C***, Classification learning performance increased across trials, without any effects of stress or α2b-adrenoceptor gene (*ADRA2B*) variant. ***D***, Stress increased the bias toward more multi-cue strategies in noncarriers (*ADRA2B* no-del) but not in carriers of the deletion variant (*ADRA2B* del). ***E***, EEG data revealed a significant increase in the FRN at FCz electrode, without differences between carriers and noncarriers of the *ADRA2B* deletion variant. The FRN was calculated as the most negative peak amplitude in the time window between 200 and 350 ms following feedback presentation relative to the preceding positive peak amplitude between 150 ms and the latency of that negative peak. ΔU_{PP}, Voltage difference between the positive and the negative peak amplitude after negative feedback. *$p < 0.05$. ***$p < 0.001$. Error bars indicate SEM.

strategies, however, revealed that carriers of the ADRA2B deletion variant used overall more single-cue strategies compared with noncarriers of the deletion variant ($\chi^2_{(1)} = 5.49$, $p = 0.019$). Also, replicating the findings of our first study, stressed participants used single-cue strategies significantly less often and multi-cue strategies significantly more often compared with participants in the control group ($\chi^2_{(1)} = 4.54$, $p = 0.033$). Most importantly, as shown in Figure 3*D*, this stress effect was again mainly due to noncarriers of the ADRA2B deletion variant who showed a significant stress-induced shift toward multi-cue strategies ($\chi^2_{(1)} = 4.41$, $p = 0.036$), whereas there was no such effect in carriers of the deletion variant ($\chi^2_{(1)} = 1.7$, $p = 0.193$).

Neural mechanisms of stress-induced modulation of learning

Corroborating previous studies (Poldrack et al., 2001; Foerde et al., 2006; Schwabe and Wolf, 2012; Schwabe et al., 2013), our fMRI data showed that PCL (vs visuo-motor control trials) was associated with bilateral activation of the caudate nucleus, the putamen, and the hippocampus (all $p_{FWE} \leq 0.031$). In addition, we observed activation in other regions known to be involved in learning processes, including the cingulate and paracingulate cortex, the orbitofrontal cortex, the insular cortex, and the precuneus (all $p < 0.001$; Table 3). There were no significant correlations between activity in those regions and performance (all $p > 0.05$). In line with the idea that stress biases multiple memory systems toward the dorsal striatum, we obtained significantly increased activity of the caudate nucleus in stressed participants compared with controls ($F_{(1,116)} = 12.57$, $p_{FWE} = 0.028$, $k = 40$; Fig. 4*A*). This stress-induced increase in dorsal striatal activity, however, was not modulated by the ADRA2B deletion variant. In medial temporal lobe regions, we did not find any differences (all $p_{FWE} > 0.05$).

ADRA2B *deletion variant modulates stress-induced changes in amygdala connectivity with the dorsal striatum*

In a next step, we performed functional connectivity analyses to assess how stress altered the crosstalk of multiple memory systems and whether this crosstalk was modulated by the *ADRA2B* polymorphism. Because the amygdala is known to play a key role in the modulation of memory (Packard and Goodman, 2012;

Table 2. Subjective, autonomic, and endocrine stress response in Study II[a]

	Control		Stress	
	Deletion	No deletion	Deletion	No deletion
Subjective assessment				
Stressful	30.65 ± 3.67	28.44 ± 3.61	68.13 ± 3.61***	63.64 ± 3.55***
Difficult	26.45 ± 3.85	26.25 ± 3.79	71.88 ± 3.79***	69.70 ± 3.73***
Unpleasant	34.84 ± 4.41	35.94 ± 4.34	70.94 ± 4.34***	66.36 ± 4.28***
Subjective mood				
Good versus bad mood				
Before treatment	35.10 ± 0.73	33.88 ± 0.72	34.53 ± 0.72	34.59 ± 0.72
1 min after treatment	34.87 ± 0.98	33.00 ± 0.96	28.38 ± 0.97***	28.34 ± 0.97***
75 min after treatment	33.36 ± 0.93	32.06 ± 0.91	29.87 ± 0.91	32.56 ± 0.91
Calm versus restless				
Before treatment	33.03 ± 0.92	31.31 ± 0.91	31.59 ± 0.91	31.84 ± 0.91
1 min after treatment	30.58 ± 1.11	30.53 ± 1.09	24.47 ± 1.09***	24.25 ± 1.09***
75 min after treatment	32.36 ± 0.91	31.34 ± 0.89	31.56 ± 0.89	32.28 ± 0.89
Tired versus awake				
Before treatment	30.87 ± 0.96	29.81 ± 0.95	30.31 ± 0.95	30.81 ± 0.95
1 min after treatment	30.45 ± 1.03	28.94 ± 1.02	29.00 ± 1.02	28.44 ± 1.02
75 min after treatment	23.61 ± 1.06	23.31 ± 1.04	22.22 ± 1.04	22.34 ± 1.04
Systolic blood pressure (bpm)				
Before treatment	123.52 ± 2.40	125.19 ± 2.40	122.33 ± 2.36	122.80 ± 2.32
During treatment	118.63 ± 2.64	119.97 ± 2.64	128.64 ± 2.60**	128.03 ± 2.60**
5 min after treatment	120.55 ± 2.53	120.73 ± 2.53	129.55 ± 2.49**	128.76 ± 2.45**
75 min after treatment	121.74 ± 2.24	120.10 ± 2.24	121.31 ± 2.21	121.32 ± 2.17
Diastolic blood pressure (bpm)				
Before treatment	83.15 ± 1.57	82.92 ± 1.57	80.83 ± 1.55	83.26 ± 1.52
During treatment	83.37 ± 1.82	82.66 ± 1.82	90.36 ± 1.79***	91.32 ± 1.76***
5 min after treatment	84.68 ± 1.63	83.13 ± 1.63	87.69 ± 1.61**	89.50 ± 1.58**
75 min after treatment	83.97 ± 1.59	84.10 ± 1.59	83.84 ± 1.57	83.77 ± 1.54
Salivary cortisol (nM)				
Before treatment	3.78 ± 0.88	3.10 ± 0.86	3.93 ± 0.86	4.82 ± 0.85
5 min after treatment	3.90 ± 1.19	3.43 ± 1.17	7.30 ± 1.17***	9.22 ± 1.15***
15 min after treatment	3.57 ± 1.24	3.22 ± 1.22	8.78 ± 1.22***	11.26 ± 1.20***
75 min after treatment	2.41 ± 0.79	2.69 ± 0.77	3.88 ± 0.77**	5.92 ± 0.76**

[a]Data are mean ± SEM.
**$p < 0.01$; ***$p < 0.001$.

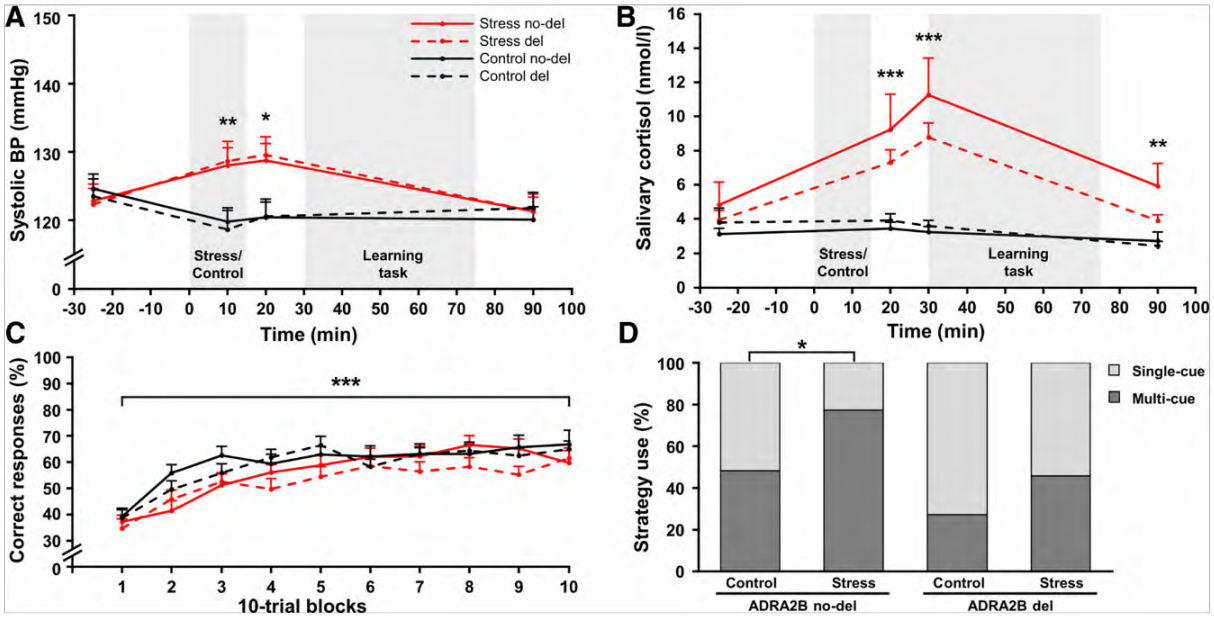

Figure 3. Physiological and behavioral data of Study II. Compared with a nonstressful control manipulation, exposure to the TSST led to significant increases in (***A***) systolic blood pressure (BP) and (***B***) salivary cortisol concentrations, regardless of the α2b-adrenoceptor gene (*ADRA2B*) variant. ***C***, Classification learning performance increased across trials, without any effects of stress or *ADRA2B* variant. ***D***, Stress led to a bias toward more multi-cue strategies only in noncarriers (*ADRA2B* no-del) but not in carriers of the deletion variant (*ADRA2B* del). *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. Error bars indicate SEM.

Table 3. Peak voxels and *t* values of significantly activated clusters during the PCL task[a]

PCL > control	Cluster size	MNI coordinates (mm)			t_{max}	p_{corr}
		x	*y*	*z*		
L supplementary motor area	866	0	23	47	19.51	<0.001
L insula left; L caudate; R caudate	3.083	−30	20	−4	18.32	<0.001
R insula; R inferior frontal gyrus triangular; R precentral gyrus	900	33	20	−4	17.11	<0.001
L inferior parietal sulcus; R angular gyrus; L precuneus	2.337	−33	−58	44	15.02	<0.001
R middle occipital gyrus; R cuneus; R fusiform gyrus	879	33	−88	−1	12.61	<0.001
L middle occipital gyrus left; inferior temporal gyrus	512	−15	−103	2	9.03	<0.001
Middle cingulate cortex	101	−3	−28	29	8.54	<0.001
L middle frontal gyrus	58	−30	5	56	6.68	<0.001
R anterior orbitofrontal cortex	20	27	38	−22	6.29	<0.001
Cerebellar crus	11	−36	−61	−28	5.83	0.001
Calcarine cortex	9	3	−88	−7	5.69	0.001
R anterior orbitofrontal cortex	5	48	47	−16	5.66	0.001
R middle frontal gyrus; superior frontal gyrus	42	33	53	2	5.22	0.008
L anterior cingulate cortex	5	−6	−1	29	5.18	0.010
L hippocampus CA	7	−18	−37	5	3.33	0.031[b]
R hippocampus CA	23	18	−34	2	6.89	<0.001[b]
L hippocampus DG	5	−21	−37	2	3.97	0.001[b]
L hippocampus DG	5	21	−34	2	6.89	<0.001[b]
L hippocampus	5	−21	−28	−10	3.91	0.005[b]
L caudate nucleus	106	−9	11	−1	15.55	<0.001[b]
R caudate nucleus	107	9	11	2	14.87	<0.001[b]
L putamen	38	−15	8	−4	10.96	<0.001[b]
R putamen	36	18	11	−1	7.32	<0.001[b]

[a]Data indicate local maxima. All labels are taken from the Automatic Anatomical Labeling atlas. The significance threshold was set to $p < 0.05$ (FWE-corrected).

[b]Small-volume corrected (ROI); all other activations are significant at the whole-brain level.

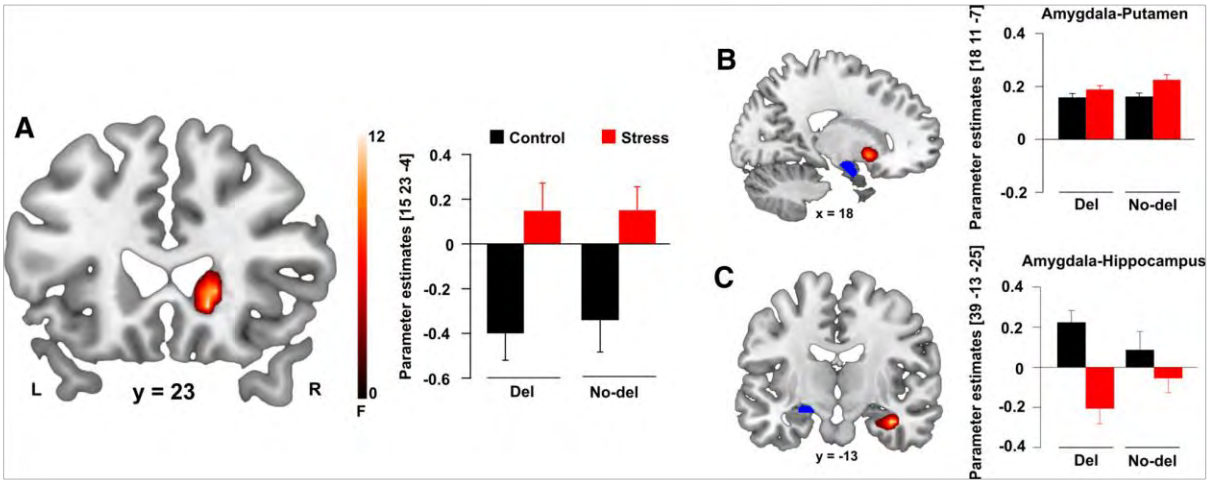

Figure 4. Stress effects on brain activity and connectivity. ***A***, The right caudate nucleus was significantly more activated in the stress compared with the control group ($p_{FWE} < 0.05$). ***B***, ***C***, Functional connectivity between the left amygdala and the hippocampus was decreased after stress ($p_{FWE} < 0.05$), whereas functional connectivity between amygdala and putamen tended to increase after stress ($p_{FWE} = 0.063$). Activations are superimposed on coronal and sagittal axial sections of a T1-weighted template image (red). Blue represents anatomical masks. L, Left side of the brain; R, right side of the brain. Error bars indicate SEM.

Schwabe, 2013) and previous studies showed altered amygdala coupling with the hippocampus and the dorsal striatum after stress (Schwabe et al., 2013; Vogel et al., 2015), we focused on the connectivity of the amygdala. Overall, stress had opposite effects on amygdala connectivity with the dorsal striatum ($F_{(1,116)} = 11.09$, $p_{FWE} = 0.063$, $k = 31$; Fig. 4*B*) and the hippocampus ($F_{(1,116)} = 17.41$, $p_{FWE} = 0.005$, $k = 33$; Fig. 4*C*). More specifically, stress increased amygdala connectivity with the putamen ($t_{(1,118)} = 4.15$, $p_{FWE} = 0.003$, *k*: 38) but decreased amygdala coupling with the hippocampus, in particular with the cornu ammonis subregion ($t_{(1,118)} = 4.12$, $p_{FWE} = 0.003$, $k = 44$).

Critically, coupling of the amygdala with the hippocampus and dorsal striatum was significantly affected by *ADRA2B* genotype ($F_{(1,116)} = 13.77$, $p_{FWE} = 0.017$, $k = 32$). Regardless of stress, carriers compared with noncarriers of the deletion variant showed increased task-related connectivity between the amygdala and the entorhinal cortex (EC; $t_{(1,118)} = 3.72$, $p_{FWE} = 0.008$, $k = 40$; Fig. 5*A*), the main input structure to the hippocampus that is highly important for hippocampus-dependent declarative memory processes (Squire and Zola, 1996; Eichenbaum, 2000; Hargreaves et al., 2005). Even more interestingly, we obtained a significant stress × *ADRA2B* interaction for the connectivity between the amygdala and the putamen ($F_{(1,116)} = 9.73$, $p_{FWE} < 0.05$, $k = 31$). As is shown in Figure 5*B*, noncarriers compared with carriers of the deletion variant showed significantly increased amygdala-putamen connectivity after stress

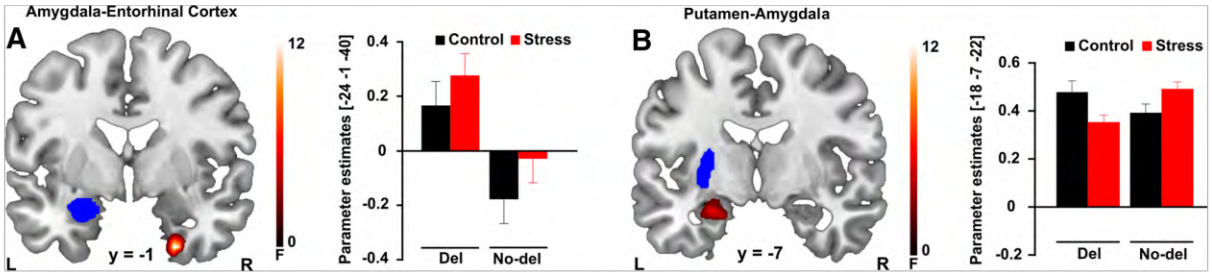

Figure 5. Alpha2b-adrenoceptor gene (*ADRA2B*) effects on brain connectivity. ***A***, Regardless of stress, carriers of the *ADRA2B* deletion variant showed stronger functional connectivity between the left amygdala and the right entorhinal cortex ($p_{FWE} < 0.05$). ***B***, *ADRA2B* genotype additionally modulates stress-induced changes in amygdala-putamen connectivity. Under stress, noncarriers of the deletion variant showed significantly increased amygdala-putamen connectivity than carriers of the *ADRA2B* deletion variant ($p_{FWE} = 0.006$), whereas genotype groups did not differ under control conditions ($p_{FWE} = 0.352$). Activations are superimposed on coronal axial sections of a T1-weighted template image (red). Blue represents anatomical masks. L, Left side of the brain; R, right side of the brain. Error bars indicate SEM.

($t_{(1,58)} = 3.72$, $p_{FWE} = 0.006$, $k = 53$), whereas genotype groups did not differ under control conditions ($p_{FWE} > 0.05$).

Discussion

Stress biases memory toward rigid dorsal striatum-based processes, and this bias is thought to play a crucial role in stress-related psychopathology (Packard and Goodman, 2012; Schwabe and Wolf, 2013; Schwabe, 2013). We show here, in two independent studies, that a genetic polymorphism on the gene coding the α2b-adrenoceptor modulates the stress-induced bias toward habit memory in healthy individuals. Specifically, the shift toward habit memory was diminished in carriers of an *ADRA2B* deletion variant compared with noncarriers of this variant. Our neuroimaging data suggest that this modulatory effect of the *ADRA2B* polymorphism is mediated by altered connectivity of the amygdala with the hippocampus and dorsal striatum.

In line with previous studies (Schwabe and Wolf, 2012; Schwabe et al., 2013), we showed that stress leads to an increase in multi-cue strategies known to rely on the dorsal striatum and a decrease in single-cue strategies supported by the hippocampus (Shohamy et al., 2004; Schwabe and Wolf, 2012). This shift in strategy use may be due to impaired hippocampal or enhanced dorsal striatal memory. Previous studies found evidence for both alternatives (Schwabe and Wolf, 2012; Schwabe et al., 2013; Vogel et al., 2015), which are not mutually exclusive. Our EEG data showed a stress-induced increase in the FRN, which might be taken as evidence for increased striatal processing (Nieuwenhuis et al., 2005; Foti et al., 2011). In line with this interpretation, our fMRI data revealed increased dorsal striatal activation after stress. Moreover, our data support earlier findings suggesting that the amygdala, a region critically involved in emotional memory modulation (McGaugh, 2000; Packard and Wingard, 2004), orchestrates the shift from hippocampal to dorsal striatal memory after stress (Schwabe et al., 2013; Vogel et al., 2016). Specifically, we observed that stress led to increased amygdala-dorsal striatum connectivity but decreased amygdala-hippocampus connectivity.

Most importantly, however, the stress-induced shift in the preferential engagement of hippocampal and striatal memory systems was modulated by the *ADRA2B* deletion variant. The relative bias toward multi-cue strategies after stress was only observed in noncarriers of the *ADRA2B* deletion variant. This genotype effect appeared not to be mediated by changes in the activity of the hippocampus or the dorsal striatum because activity of these systems remained unaffected by genotype and the stress-induced increases in FRN amplitude and striatal activity were independent of *ADRA2B* genotype. Instead, the *ADRA2B* deletion variant had a significant impact on functional amygdala-EC and amygdala-dorsal striatum connectivity. Regardless of stress, the *ADRA2B* deletion variant was linked to increased amygdala-EC coupling. This finding suggests that the amygdala-EC crosstalk is particularly strong in carriers of the *ADRA2B* deletion variant, which may explain their superior episodic memory for emotional stimuli reported earlier (de Quervain et al., 2007b; Rasch et al., 2009). Although the increased amygdala-EC coupling in carriers of the deletion variant remained largely unchanged by stress, we obtained genotype-specific stress effects on the connectivity between amygdala and putamen. Specifically, whereas stress increased amygdala-putamen coupling in noncarriers of the *ADRA2B* deletion variant, this was not the case in carriers of the deletion variant. Together, these data suggest that the *ADRA2B* deletion variant, known to affect amygdala processing (Rasch et al., 2009; Cousijn et al., 2010), primarily modulates the crosstalk between amygdala and EC (regardless of stress) as well as between amygdala and putamen (under stress) and that, in line with recent pharmacological data (Schwabe et al., 2013), it is this modulation in connectivity patterns that is critical for the changes in learning strategies under stress in carriers of the *ADRA2B* deletion variant.

Previous studies on the stress-induced modulation of multiple memory systems suggested a critical role of glucocorticoids in the shift toward habit memory (Vogel et al., 2016). There is, however, also evidence that noradrenaline is critically involved in the shift from cognitive to habit memory. For instance, it has been shown that intra-amygdala injections of an α2-adrenoceptor antagonist, leading to increased noradrenergic stimulation, biased memory toward the dorsal striatum-dependent system in rats (Packard and Wingard, 2004; Wingard and Packard, 2008). At first glance, these findings, suggesting increased habit memory following noradrenergic activation, might seem to be in conflict with the present finding that a deletion variant of the *ADRA2B* gene, which is also linked to increased noradrenergic activation (Small et al., 2001), reduces the stress-induced shift toward habit memory. However, the α2-adrenoceptor antagonist used in pharmacological studies is not specific for any of the adrenergic receptor subtypes (Hieble and Ruffolo, 1995; Hieble et al., 1996), whereas the effects of the *ADRA2B* deletion variant are specific for the α2b-adrenoceptor subtype. Although specific adrenergic receptor subtypes may exert different effects on learning under stress, direct evidence supporting this view is still missing. Moreover and perhaps even more importantly, the administration of the α2-adrenoceptor antagonist leads to an acute, tran-

sient increase in noradrenergic stimulation, whereas the *ADRA2B* deletion variant is most likely associated with more constant changes. Specifically, the deletion variant is assumed to be associated with inherent and constant differences in noradrenaline availability (Small et al., 2001), although direct evidence from humans for this claim is still lacking. It is tempting to speculate that the *ADRA2B* deletion variant is associated with some sort of homeostatic compensations, an important mechanism of neuronal functioning (Marder and Goaillard, 2006). Indeed, our neuroimaging data suggest that carriers of the *ADRA2B* deletion variant show, compared with noncarriers of this variant, increased amygdala-EC coupling, which may be such a compensatory mechanism. Thus, acute pharmacological manipulations of a neurotransmitter system can hardly be compared with genetic differences in such a system. However, both the available pharmacological data and our behavioral genetics findings argue for an important role of the noradrenergic system in the balance between cognitive and habit memory.

We tested the role of the *ADRA2B* genotype in the stress-induced modulation of cognitive and habit memory in two experiments. In both experiments, task performance started ~30 min after stress, when cortisol levels were significantly elevated, and lasted for 25 min (Experiment I) and 45 min (Experiment II), respectively. Thus, stress-induced sympathetic activity should have mainly vanished before behavioral testing and genomic cortisol actions should not have fully developed yet, suggesting that the present effects were mainly the result of nongenomic cortisol actions. Nevertheless, accumulating evidence indicates that there might be substantial variation in stress effects on cognition within relatively short time windows (Bendahan et al., 2016; Vogel and Schwabe, 2016) and the conclusion that the stress effect has been stable across the PCL task in this study might be premature. Such (possible) time-dependent changes in task performance or brain activity due to temporal dynamics of the stress response, however, can hardly be dissociated from changes resulting from learning processes.

The experimental setup used in the two experiments was virtually the same, but different methods were used to measure brain activity (EEG vs fMRI). Because of differences in the temporal resolution of the measured signals, feedback timing varied between the two studies. Feedback timing has considerable implications for the predominance of cognitive versus habit memory. Specifically, the striatum is very important for immediate feedback processing, whereas a delay in feedback presentation leads to increased engagement of the hippocampus (Foerde and Shohamy, 2011). Indeed, in the first experiment, in which feedback followed shortly after the response, larger hippocampal engagement possibly led to overall more multi-cue learning than in the second experiment, in which feedback was delayed by a few seconds and learning has a generally larger striatal contribution. Critically, however, regardless of feedback timing and the general distribution of single-cue versus multi-cue strategies, stress increased multi-cue learning and the *ADRA2B* deletion variant modulated this effect. This underlines the robustness of the stress-induced bias toward dorsal striatal memory and its modulation by the *ADRA2B* genotype.

In conclusion, we showed, in two independent experiments, that a deletion variant of the *ADRA2B* gene encoding the α2b-adrenoceptor reduces the stress-induced shift from hippocampal cognitive toward dorsal striatal habit memory, most likely via overall increased amygdala-EC connectivity and altered amygdala-putamen connectivity under stress. Although the stress-induced bias toward habit memory may hamper memory flexibility (Seehagen et al., 2015; Dandolo and Schwabe, 2016), it is thought to be a generally adaptive mechanism that aids coping with stressful events (Vogel et al., 2016). The reduced ability to shift toward a more suitable memory system under stress, together with aberrant emotional memory formation (de Quervain et al., 2007b; Rasch et al., 2009), may thus be an important factor that renders carriers of the *ADRA2B* deletion variant particularly vulnerable for developing PTSD (Liberzon et al., 2014).

References

Bendahan S, Goette L, Thoresen J, Loued-Khenissi L, Hollis F, Sandi C (2016) Acute stress alters individual risk taking in a time-dependent manner and leads to anti-social risk. Eur J Neurosci. Advance online publication. Retrieved Sep. 8, 2016. CrossRef Medline

Cousijn H, Rijpkema M, Qin S, van Marle HJ, Franke B, Hermans EJ, van Wingen G, Fernández G (2010) Acute stress modulates genotype effects on amygdala processing in humans. Proc Natl Acad Sci U S A 107:9867–9872. CrossRef Medline

Dandolo LC, Schwabe L (2016) Stress-induced cortisol hampers memory generalization. Learn Mem 23:679–683. CrossRef Medline

de Kloet ER, Joëls M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6:463–475. CrossRef Medline

de Quervain DJ, Papassotiropoulos A (2006) Identification of a genetic cluster influencing memory performance and hippocampal activity in humans. Proc Natl Acad Sci U S A 103:4270–4274. CrossRef Medline

de Quervain DJ, Aerni A, Roozendaal B (2007a) Preventive effect of beta-adrenoceptor blockade on glucocorticoid-induced memory retrieval deficits. Am J Psychiatry 164:967–969. CrossRef Medline

de Quervain DJ, Kolassa IT, Ertl V, Onyut PL, Neuner F, Elbert T, Papassotiropoulos A (2007b) A deletion variant of the alpha2b-adrenoceptor is related to emotional memory in Europeans and Africans. Nat Neurosci 10:1137–1139. CrossRef Medline

de Quervain DJ, Kolassa IT, Ackermann S, Aerni A, Boesiger P, Demougin P, Elbert T, Ertl V, Gschwind L, Hadziselimovic N, Hanser E, Heck A, Hieber P, Huynh KD, Klarhöfer M, Luechinger R, Rasch B, Scheffler K, Spalek K, Stippich C, et al. (2012) PKCα is genetically linked to memory capacity in healthy subjects and to risk for posttraumatic stress disorder in genocide survivors. Proc Natl Acad Sci U S A 109:8746–8751. CrossRef Medline

de Quervain D, Schwabe L, Roozendaal B (2017) Stress, glucocorticoids and memory: implications for treating fear-related disorders. Nat Rev Neurosci 18:7–19. CrossRef Medline

Diamond DM, Campbell AM, Park CR, Halonen J, Zoladz PR (2007) The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. Neural Plast 2007:60803. CrossRef Medline

Eichenbaum H (2000) A cortical-hippocampal system for declarative memory. Nat Rev Neurosci 1:41–50. CrossRef Medline

Elliott AE, Packard MG (2008) Intra-amygdala anxiogenic drug infusion prior to retrieval biases rats towards the use of habit memory. Neurobiol Learn Mem 90:616–623. CrossRef Medline

Eppinger B, Kray J, Mock B, Mecklinger A (2008) Better or worse than expected? Aging, learning, and the ERN. Neuropsychologia 46:521–539. CrossRef Medline

Foerde K, Shohamy D (2011) Feedback timing modulates brain systems for learning in humans. J Neurosci 31:13157–13167. CrossRef Medline

Foerde K, Knowlton BJ, Poldrack RA (2006) Modulation of competing memory systems by distraction. Proc Natl Acad Sci U S A 103:11778–11783. CrossRef Medline

Foti D, Weinberg A, Dien J, Hajcak G (2011) Event-related potential activity in the basal ganglia differentiates rewards from nonrewards: response to commentary. Hum Brain Mapp 32:2267–2269. CrossRef Medline

Frank MJ, Woroch BS, Curran T (2005) Error-related negativity predicts reinforcement learning and conflict biases. Neuron 47:495–501. CrossRef Medline

Gluck MA, Shohamy D, Myers C (2002) How do people solve the "weather prediction" task? Individual variability in strategies for probabilistic category learning. Learn Mem 9:408–418. CrossRef Medline

Goodman J, Leong KC, Packard MG (2012) Emotional modulation of multiple memory systems: implications for the neurobiology of post-traumatic stress disorder. Rev Neurosci 23:627–643. CrossRef Medline

Hajcak G, Moser JS, Holroyd CB, Simons RF (2007) It's worse than you

thought: the feedback negativity and violations of reward prediction in gambling tasks. Psychophysiology 44:905–912. CrossRef Medline

Hargreaves EL, Rao G, Lee I, Knierim JJ (2005) Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. Science 308: 1792–1794. CrossRef Medline

Hauser TU, Iannaccone R, Stämpfli P, Drechsler R, Brandeis D, Walitza S, Brem S (2014) The feedback-related negativity (FRN) revisited: new insights into the localization, meaning and network organization. Neuroimage 84:159–168. CrossRef Medline

Hieble JP, Ruffolo RR Jr (1996) Subclassification and nomenclature of alpha 1- and alpha 2-adrenoceptors. Prog Drug Res 47:81–130. Medline

Hieble JP, Bondinell WE, Ruffolo RR Jr (1995) Alpha- and beta-adrenoceptors: from the gene to the clinic: I. Molecular biology and adrenoceptor subclassification. J Med Chem 38:3415–3444. CrossRef Medline

Kim JJ, Lee HJ, Han JS, Packard MG (2001) Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. J Neurosci 21:5222–5228. Medline

Kirschbaum C, Pirke KM, Hellhammer DH (1993) The 'Trier Social Stress Test': a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28:76–81. Medline

Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH (1999) Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom Med 61:154–162. CrossRef Medline

Knowlton BJ, Squire LR, Gluck MA (1994) Probabilistic classification learning in amnesia. Learn Mem 1:106–120. CrossRef Medline

Knowlton BJ, Mangels JA, Squire LR (1996) A neostriatal habit learning system in humans. Science 273:1399–1402. CrossRef Medline

Lagnado DA, Newell BR, Kahan S, Shanks DR (2006) Insight and strategy in multiple-cue learning. J Exp Psychol Gen 135:162–183. CrossRef Medline

Liberzon I, King AP, Ressler KJ, Almli LM, Zhang P, Ma ST, Cohen GH, Tamburrino MB, Calabrese JR, Galea S (2014) Interaction of the ADRB2 gene polymorphism with childhood trauma in predicting adult symptoms of posttraumatic stress disorder. JAMA Psychiatry 71:1174–1182. CrossRef Medline

Lupien SJ, McEwen BS, Gunnar MR, Heim C (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 10:434–445. CrossRef Medline

Marder E, Goaillard JM (2006) Variability, compensation and homeostasis in neuron and network function. Nat Rev Neurosci 7:563–574. CrossRef Medline

McEwen BS (1998) Stress, adaptation, and disease: allostasis and allostatic load. Ann N Y Acad Sci 840:33–44. CrossRef Medline

McGaugh JL (2000) Memory: a century of consolidation. Science 287:248–251. CrossRef Medline

McGaugh JL, Cahill L, Roozendaal B (1996) Involvement of the amygdala in memory storage: interaction with other brain systems. Proc Natl Acad Sci U S A 93:13508–13514. CrossRef Medline

Miranda MI, Bermúdez-Rattoni F (2007) Cholinergic activity in the insular cortex is necessary for acquisition and consolidation of contextual memory. Neurobiol Learn Mem 87:343–351. CrossRef Medline

Nieuwenhuis S, Slagter HA, von Geusau NJ, Heslenfeld DJ, Holroyd CB (2005) Knowing good from bad: differential activation of human cortical areas by positive and negative outcomes. Eur J Neurosci 21:3161–3168. CrossRef Medline

O'Carroll RE, Drysdale E, Cahill L, Shajahan P, Ebmeier KP (1999) Stimulation of the noradrenergic system enhances and blockade reduces memory for emotional material in man. Psychol Med 29:1083–1088. CrossRef Medline

Packard MG, Goodman J (2012) Emotional arousal and multiple memory systems in the mammalian brain. Front Behav Neurosci 6:14. CrossRef Medline

Packard MG, Wingard JC (2004) Amygdala and "emotional" modulation of the relative use of multiple memory systems. Neurobiol Learn Mem 82: 243–252. CrossRef Medline

Papassotiropoulos A, Stephan DA, Huentelman MJ, Hoerndli FJ, Craig DW, Pearson JV, Huynh KD, Brunner F, Corneveaux J, Osborne D, Wollmer MA, Aerni A, Coluccia D, Hänggi J, Mondadori CR, Buchmann A, Reiman EM, Caselli RJ, Henke K, de Quervain DJ (2006) Common Kibra alleles are associated with human memory performance. Science 314: 475–478. CrossRef Medline

Papassotiropoulos A, Stefanova E, Vogler C, Gschwind L, Ackermann S, Spalek K, Rasch B, Heck A, Aerni A, Hanser E, Demougin P, Huynh KD, Luechinger R, Klarhöfer M, Novakovic I, Kostic V, Boesiger P, Scheffler K, de Quervain DJ (2013) A genome-wide survey and functional brain imaging study identify CTNNBL1 as a memory-related gene. Mol Psychiatry 18:255–263. CrossRef Medline

Poldrack RA, Clark J, Paré-Blagoev EJ, Shohamy D, Creso Moyano J, Myers C, Gluck MA (2001) Interactive memory systems in the human brain. Nature 414:546–550. CrossRef Medline

Rasch B, Spalek K, Buholzer S, Luechinger R, Boesiger P, Papassotiropoulos A, de Quervain DJ (2009) A genetic variation of the noradrenergic system is related to differential amygdala activation during encoding of emotional memories. Proc Natl Acad Sci U S A 106:19191–19196. CrossRef Medline

Rohleder N, Kirschbaum C (2006) The hypothalamic-pituitary-adrenal (HPA) axis in habitual smokers. Int J Psychophysiol 59:236–243. CrossRef Medline

Roozendaal B, McGaugh JL (2011) Memory modulation. Behav Neurosci 125:797–824. CrossRef Medline

Roozendaal B, Hahn EL, Nathan SV, de Quervain DJ, McGaugh JL (2004) Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala. J Neurosci 24:8161–8169. CrossRef Medline

Rustemeier M, Schwabe L, Bellebaum C (2013) On the relationship between learning strategy and feedback processing in the weather prediction task: evidence from event-related potentials. Neuropsychologia 51:695–703. CrossRef Medline

Sandi C, Haller J (2015) Stress and the social brain: behavioural effects and neurobiological mechanisms. Nat Rev Neurosci 16:290–304. CrossRef Medline

Schwabe L (2013) Stress and the engagement of multiple memory systems: integration of animal and human studies. Hippocampus 23:1035–1043. CrossRef Medline

Schwabe L, Wolf OT (2009) Stress prompts habit behavior in humans. J Neurosci 29:7191–7198. CrossRef Medline

Schwabe L, Wolf OT (2012) Stress modulates the engagement of multiple memory systems in classification learning. J Neurosci 32:11042–11049. CrossRef Medline

Schwabe L, Wolf OT (2013) Stress and multiple memory systems: from 'thinking' to 'doing.' Trends Cogn Sci 17:60–68. CrossRef Medline

Schwabe L, Oitzl MS, Philippsen C, Richter S, Bohringer A, Wippich W, Schachinger H (2007) Stress modulates the use of spatial versus stimulus-response learning strategies in humans. Learn Mem 14:109–116. CrossRef Medline

Schwabe L, Römer S, Richter S, Dockendorf S, Bilak B, Schächinger H (2009) Stress effects on declarative memory retrieval are blocked by a beta-adrenoceptor antagonist in humans. Psychoneuroendocrinology 34:446–454. CrossRef Medline

Schwabe L, Wolf OT, Oitzl MS (2010) Memory formation under stress: quantity and quality. Neurosci Biobehav Rev 34:584–591. CrossRef Medline

Schwabe L, Joëls M, Roozendaal B, Wolf OT, Oitzl MS (2012) Stress effects on memory: an update and integration. Neurosci Biobehav Rev 36:1740–1749. CrossRef Medline

Schwabe L, Tegenthoff M, Höffken O, Wolf OT (2013) Mineralocorticoid receptor blockade prevents stress-induced modulation of multiple memory systems in the human brain. Biol Psychiatry 74:801–808. CrossRef Medline

Seehagen S, Schneider S, Rudolph J, Ernst S, Zmyj N (2015) Stress impairs cognitive flexibility in infants. Proc Natl Acad Sci U S A 112:12882–12886. CrossRef Medline

Shohamy D, Myers CE, Grossman S, Sage J, Gluck MA, Poldrack RA (2004) Cortico-striatal contributions to feedback-based learning: converging data from neuroimaging and neuropsychology. Brain 127:851–859. CrossRef Medline

Small KM, Brown KM, Forbes SL, Liggett SB (2001) Polymorphic deletion of three intracellular acidic residues of the alpha 2B-adrenergic receptor decreases G protein-coupled receptor kinase-mediated phosphorylation and desensitization. J Biol Chem 276:4917–4922. CrossRef Medline

Southwick SM, Davis M, Horner B, Cahill L, Morgan CA 3rd, Gold PE,

Bremner JD, Charney DC (2002) Relationship of enhanced norepinephrine activity during memory consolidation to enhanced long-term memory in humans. Am J Psychiatry 159:1420–1422. CrossRef Medline

Squire LR, Zola SM (1996) Structure and function of declarative and nondeclarative memory systems. Proc Natl Acad Sci U S A 93:13515–13522. CrossRef Medline

Steyer R, Schwenkmezger P, Notz P, Eid M (1994) Theoretical analysis of a multidimensional mood questionnaire (MDBF). Diagnostica 40:9.

van der Helden J, Boksem MA, Blom JH (2010) The importance of failure: feedback-related negativity predicts motor learning efficiency. Cereb Cortex 20:1596–1603. CrossRef Medline

Vogel S, Schwabe L (2016) Stress in the zoo: tracking the impact of stress on memory formation over time. Psychoneuroendocrinology 71:64–72. CrossRef Medline

Vogel S, Klumpers F, Krugers HJ, Fang Z, Oplaat KT, Oitzl MS, Joëls M, Fernández G (2015) Blocking the mineralocorticoid receptor in humans prevents the stress-induced enhancement of centromedial amygdala connectivity with the dorsal striatum. Neuropsychopharmacology 40:947–956. CrossRef Medline

Vogel S, Fernández G, Joëls M, Schwabe L (2016) Cognitive adaptation under stress: a case for the mineralocorticoid receptor. Trends Cogn Sci 20:192–203. CrossRef Medline

Wingard JC, Packard MG (2008) The amygdala and emotional modulation of competition between cognitive and habit memory. Behav Brain Res 193:126–131. CrossRef Medline

Appendix B

Wirz L, Reuter M, Wacker J, Felten A, Schwabe L (2017b) A haplotype associated with enhanced mineralocorticoid receptor expression facilitates the stress-induced shift from "cognitive" to "habit" learning. *eNeuro* 5:e0359-17.2017.

Cognition and Behavior

A Haplotype Associated with Enhanced Mineralocorticoid Receptor Expression Facilitates the Stress-Induced Shift from "Cognitive" to "Habit" Learning

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Abstract

Stress induces a shift from hippocampus-dependent "cognitive" toward dorsal striatum-dependent "habit" memory. However, not all individuals are susceptible to this shift under stress. Based on pharmacological studies indicating a critical role of the mineralocorticoid receptor (MR) in the stress-induced bias toward dorsal striatal learning, we hypothesized that *MR* gene variants contribute to these individual differences. In two experiments, healthy participants were genotyped, exposed to a stressor or control manipulation and performed a learning task that can be solved using hippocampal or dorsal striatal systems, while electroencephalography (EEG; Experiment I) or functional magnetic resonance imaging (fMRI; Experiment II) measurements were taken. Stress led to a shift from hippocampal to dorsal striatal learning which was more pronounced in homo- and heterozygous carriers of a six single nucleotide polymorphisms (SNPs)-comprising haplotype containing the alleles of two *MR SNPs* associated with increased MR expression and transactivational activity (*MR*-2G/C **C** [rs2070951], *MR*-I180V **A** [rs5522]). This stress-induced shift toward habit memory was paralleled by an increased feedback-related negativity (FRN), which may reflect striatal processing, and increased caudate activation. Carriers of the *MR* haplotype showed a reduced P3a, an event-related potential thought to indicate cognitive processing, and reduced hippocampal activity after stress. Moreover, stress resulted in reduced amygdala-hippocampus connectivity and the decrease in amygdala connectivity to the parahippocampal cortex was particularly pronounced in *MR* haplotype carriers. Our findings indicate that genetic variants associated with enhanced MR expression facilitate a stress-induced shift from hippocampal toward dorsal striatal learning, most likely via impaired hippocampal processing and reduced amygdala-hippocampus cross talk, allowing the dorsal striatum to guide behavior under stress.

Key words: glucocorticoids; hippocampus; mineralocorticoid receptor; stress

Significance Statement

Stressful events may trigger a shift from hippocampus-dependent, "cognitive" toward dorsal striatum-dependent, "habitual" control of learning. While being generally adaptive for performance under stress, this shift may contribute to stress-related psychopathology. However, there are substantial individual differences in the stress-induced bias toward habit learning, the source of which is not fully understood. In line with pharmacological studies pointing to a critical role of the mineralocorticoid receptor (MR) in the stress-induced learning bias, we report here that a *MR* haplotype associated with enhanced MR expression facilitates habit learning under stress. Using electroencephalography (EEG) and functional magnetic resonance imaging (fMRI), we show that this genetic modulation is most likely mediated by altered hippocampus activity and amygdala-hippocampus crosstalk.

Introduction

Stressful events may modulate the engagement of multiple, anatomically and functionally distinct memory systems (Packard and Wingard, 2004; Schwabe, 2013; Schwabe and Wolf, 2013). Specifically, stress has been shown to favor simple but rigid "habit" learning supported by the dorsal striatum over more complex "cognitive" learning dependent on the hippocampus or prefrontal cortex (Kim et al., 2001; Schwabe et al., 2007; Schwabe and Wolf, 2009, 2012). This stress-induced bias toward habit learning is thought to contribute to stress-related psychopathologies, including posttraumatic stress disorder (PTSD) and addiction (Schwabe et al., 2010a, 2011; Packard and Goodman, 2012).

The shift from cognitive to habit learning under stress may be accompanied by reduced hippocampal and increased dorsal striatal activity and evidence suggests that the amygdala orchestrates the engagement of these memory systems (Packard and Wingard, 2004; Schwabe and Wolf, 2012; Schwabe et al., 2013; Vogel et al., 2015). These stress-induced changes in the preferential engagement of multiple memory systems are critically driven by glucocorticoids (mainly cortisol in humans) binding to membrane-bound mineralocorticoid receptors (MRs; Vogel et al., 2016). In particular, pharmacological studies in rodents and humans showed that blockade or absence of MRs prevented the stress-induced bias toward dorsal striatum-dependent memory (Schwabe et al., 2010b; ter Horst et al., 2012; Schwabe et al., 2013).

However, not all individuals show the bias toward dorsal striatal habit learning under stress. Given the potential clinical relevance of the stress-induced memory bias, it is important to identify factors that contribute to this individual variance. If the stress-induced shift toward habit memory is mediated by MRs, genetic differences in the *MR* gene (*NR3C2*) are a likely source contributing to individual differences in the engagement of multiple memory systems under stress. Two common variants in the *MR* gene, the *MR*-2G/C **C** (rs2070951) and *MR*-I180V **A** (rs5522) alleles, are associated with increased expression and transactivation capacity of the MR *in vitro* (DeRijk and De Kloet, 2008; van Leeuwen et al., 2010) and altered hypothalamic-pituitary-adrenal (HPA) axis reactivity (DeRijk, 2009). Similarly to pharmacological blockade of the MR, *MR*-I180V **G** and *MR*-2G/C **G** allele carriers showed increased levels of cortisol in response to psychosocial stress (DeRijk et al., 2006). In addition, these *MR* single nucleotide polymorphisms (SNPs) together result in four haplotypes (GA, CA, CG, GG).The common and functional CA haplotype, which results in higher transcriptional, translational and transactivational *MR* activity, has been associated with enhanced resilience to depression (Klok et al., 2011) as well as traumatic stress (ter Heegde et al., 2015; de Kloet et al., 2016).

The present study aimed to test whether *MR* haplotypes with known differences in *MR* transactivation and expression contribute to individual variance in stress effects on multiple memory systems. For this purpose we conducted two independent experiments in which healthy participants, genotyped for several *MR* haplotypes, were exposed to a stressor (or control manipulation) before completing a probabilistic classification learning (PCL) task that can be solved using hippocampus-dependent single-cue or dorsal striatum-dependent multi-cue strategies (Gluck et al., 2002; Shohamy et al., 2004; Schwabe and Wolf, 2012). In Experiment I, we used electroencephalography (EEG) to assess the feedback-related negativity (FRN) and the P3, event-related brain potentials (ERPs) reflecting, at least partly, dorsal striatal (Nieuwenhuis et al., 2005; Hauser et al., 2014) and hippocampal processes (Knight, 1996; Polich, 2007), respectively. In Experiment II, we employed functional magnetic resonance imaging (fMRI) to elucidate the neural underpinnings of modulatory effects of the *MR* haplotype on the stress-induced shift toward habit memory. We hypothesized that the *MR* haplotype associated with increased MR functionality (*MR*-2G/C **C** and *MR*-I180V **A**) enhances the stress-induced shift from hippocampal toward dorsal striatal memory processes. At the neural level, we expected this shift to be mediated by changes in P3 and FRN magnitude, as well as by alterations in activation of the dorsal striatum and hippocampus and in connectivity of these memory systems with the amygdala.

Materials and Methods

Experiment I: *MR* haplotype, stress, and the engagement of multiple memory systems

Participants and experimental design

Healthy volunteers ($N = 252$) without current or previous neurologic or psychiatric disorders or present medication intake participated in this experiment (127 women; mean age: 25.1 years, SD 3.5 years). Factors influencing the reactivity of the HPA axis were controlled for by excluding smokers and women taking hormonal contraceptives and by testing women outside their menstrual cycle phase (Kirschbaum et al., 1999; Rohleder and Kirschbaum, 2006). To control for the diurnal rhythm of cortisol, all testing took place in the afternoon. The experiment was approved by the ethical review board of the German Psychological Society (reference: LS072014). Participants gave written informed consent and received a moderate monetary compensation of 25€ for their participation. This sample is part of a larger project on indi-

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vidual differences in stress effects on multiple memory systems (Wirz et al., 2017).

To examine modulatory effects of an *MR* haplotype on stress-induced changes in the preferential engagement of multiple memory systems, we used a 2 × 2 between-subjects design with the factors treatment (stress vs control manipulation) and *MR* haplotype (homo- and heterozygous carriers vs noncarriers) in which participants were randomly assigned to the stress or control condition. Due to technical difficulties and excessive artifacts in the EEG, 24 participants were excluded from the ERP analyses, leading to a sample of 228 participants (stress: 81 carriers, 33 noncarriers; control: 91 carriers, 23 noncarriers), whereas for the behavioral analyses data from all 252 tested participants were used (stress: 90 carriers, 36 noncarriers; control: 101 carriers, 25 noncarriers).

Genetic analyses

Participants were genotyped for seven SNPs of the gene coding for the *MR* (*NR3C2*; rs1512344, rs2070950, **rs2070951 [*MR*-2G/C]**, rs4835519, **rs5522 [*MR*-I180V]**, rs5534, rs7658048). Two of them are functional SNPs located on exon 2 of the *MR* gene (*MR*-2G/C, allele frequency 50% and *MR*-I180V, allele frequency 12%) that may alter HPA axis responsiveness, thereby affecting individual stress responsivity and vulnerability to stress-related disorders (DeRijk, 2009; Klok et al., 2011; van Leeuwen et al., 2011; Medina et al., 2013). For genetic analysis, DNA was extracted from buccal cells. Automated purification of genomic DNA was conducted by means of the MagNA Pure LC system using a commercial extraction kit (MagNA Pure LC DNA isolation kit; Roche Diagnostics). Genotyping of the *MR* polymorphisms was performed by MALDI-TOF mass spectrometry using the iPLEX assay and the Sequenom MassARRAY platform.

Linkage analyses between SNPs and construction of haplotype blocks were conducted by means of Haploview 4.2 (https://www.broadinstitute.org/haploview/haploview). Haplotype blocks were defined by the method suggested by (Gabriel et al., 2002). Individual haplotypes were calculated with PHASE, version 2.1. PHASE implements a Bayesian statistical method for reconstructing haplotypes from population genotype data. To test for deviations from Hardy-Weinberg Equilibrium, allele frequencies were analyzed using χ^2 tests. Homo- and heterozygous carriers of the *MR*-2G/C **C** and *MR*-I180V **A** haplotype (= *MR* haplotype carriers), showing higher *MR* transactivation and expression compared to the other haplotypes (GA, CG, GG), were treated as one group and tested against all other haplotypes (= *MR* haplotype noncarriers) in further analyses.

Stress and control manipulation

Participants in the stress condition underwent the Trier Social Stress Test (TSST), which is known to reliably increase activity of the autonomic nervous system (ANS) and the HPA axis (Kirschbaum et al., 1993). After 3 min of preparation, each participant was asked to give a 5-min free speech about why he or she is the ideal candidate for a job tailored to his or her interests and subsequently had to solve a difficult mental-arithmetic task for another 5 min (counting backwards from 2043 in steps of 17). Throughout the TSST, participants were videotaped and evaluated by a reserved and nonreinforcing panel. In the control condition, participants talked about a self-chosen topic and performed an easy calculation task (counting forward in steps of 15) without panel and video recordings.

To assess the effectiveness of the stress induction, subjective and physiologic measures were taken at several time points across the experiment. Changes in subjective mood were evaluated using a German mood questionnaire (MDBF; subscales: depressed vs elevated, restless vs calm, sleepy vs awake; high scores indicate elevated mood, calmness, and wakefulness; Steyer et al., 1994). Additionally, participants rated the difficulty, unpleasantness and stressfulness of the stress or control manipulation on a scale from 0 (“not at all”) to 100 (“very much”). Blood pressure was measured using a Dinamap system (Critikon) before (−25 min), during (+10 min), and after (+20 min, +60 min, +80 min) the experimental manipulation. Furthermore, saliva samples were collected before (-25 min) and after (+20 min, +30 min, +40 min, +80 min) the experimental treatment using Salivette collection devices (Sarstedt). Saliva samples were stored at −18°C until the free fraction of cortisol was determined using commercially available chemiluminescence immunoassays (IBL).

PCL task

To assess the engagement of multiple memory systems, participants completed a modified version of the weather prediction task (Knowlton et al., 1994; Knowlton et al., 1996), while EEG was recorded and ~15 min after the treatment, when peak cortisol levels after stress were expected. In this PCL task, participants learned to classify stimuli into the categories “rain” and “sun” based on trial-by-trial feedback. One, two or three (out of four) cards appeared on each trial, yielding 14 different cue patterns. These cue patterns were associated with the outcomes sun and rain in a probabilistic manner, such that a particular cue was associated with the outcome sun with a probability of 75.6, 57.5, 42.5, or 24.4 percentage across 100 trials; these probabilities are in line with previous studies using this task (Gluck et al., 2002; Lagnado et al., 2006; Schwabe and Wolf, 2012; Schwabe et al., 2013). A response was counted as correct if it matched the outcome with the highest probability for that cue pattern. Participants completed 100 PCL trials (duration: ~25 min). On each trial, 1 of the 14 cue patterns appeared and participants had 5 s to respond by pressing one of two buttons that corresponded with the outcomes sun and rain. Responses were highlighted with a red circle (500 ms) before a black screen appeared (500 ms), which was followed by a feedback stimulus in the form of a happy or sad face (1.000 ms). The intertrial interval varied between 1 and 2.5 s.

Assessment of learning strategies

The PCL task can be solved by using different learning strategies that rely on distinct brain systems. Patient and neuroimaging studies showed that participants may ac-

quire the task using single-cue strategies supported by a hippocampus-dependent system or by using multi-cue strategies that are based on the dorsal striatum (Knowlton et al., 1996; Shohamy et al., 2004; Foerde et al., 2006; Schwabe and Wolf, 2012). In order to assess participants' learning strategies during PCL, participants' actual responses were compared with ideal response patterns for each strategy (Gluck et al., 2002; Lagnado et al., 2006). A least mean squares measure resulted in a fit value ranging from 0 to 1 (0 indicating a perfect fit). Participants were assigned the strategy with the best fit score. If none of the scores for all possible strategies was <0.16, participants' strategies were classified as "nonidentifiable" (Gluck et al., 2002; Wirz et al., 2017). Independent of the experimental group, no strategy was identifiable in 20 participants ($\chi^2_{(1)} = 0.220$, $p = 0.639$). In line with previous studies (Schwabe and Wolf, 2012; Schwabe et al., 2013), strategies were classified into hippocampus-dependent single-cue and dorsal striatum-dependent multi-cue strategies. Although this dichotomization may reduce some of the variation, the classification into single- and multi-cue strategies (above the actual fit score and the substrategies) is useful to analyze differences in the predominant engagement of either one of these systems, and it promotes the comparison to previous studies using this task (Shohamy et al., 2004; Schwabe and Wolf, 2012; Schwabe et al., 2013).

Behavioral and physiologic data analyses

Subjective and physiologic measurements were analyzed using mixed-design ANOVAs with time as within-subject factor and treatment (TSST vs control) as well as *MR* haplotype (carriers vs noncarriers) as between-subjects factors. A mixed-design ANOVA with blocks of 10 trials as within-subject factor was used to assess learning performance on the PCL task. Group differences in learning strategy were analyzed by means of χ^2 tests. Statistical analyses were performed using SPSS Statistics 22 (IBM). All reported p values are two-tailed. In case of violation of the sphericity assumption, Greenhouse-Geisser corrections were applied. Significant main and interaction effects were followed by the appropriate *post hoc* tests.

EEG recording and analyses

During the PCL task, EEG was recorded from 64 active electrodes arranged according to the international 10–20 system. Horizontal electro-oculograms were measured and the most frontal electrodes Fp1 and Fp2 served as recording sites for vertical eye movements. A Biosemi Active-Two amplifier system was used with a sampling rate of 2048 Hz (Biosemi). Common mode sense and driven right leg electrodes served as recording reference and ground.

EEG data were analyzed offline using the Brain Vision Analyzer software (Brain Products). After the EEG signal was downsampled to 512 Hz, the data were high-pass filtered at 0.01 Hz. To remove artifacts from electrical lines, a 50 Hz notch filter was applied. EEG data were then visually inspected to discard any extreme artifacts. Additionally, artifacts originating from eye-blinks or -movements were removed using an independent component based approach. Bad channels were replaced by means of topographic interpolation and the data were re-referenced to the average of all electrodes. To analyze ERPs reflecting feedback processing, data were segmented into epochs from -200 to 800 ms with respect to feedback stimulus onset and subsequently baseline corrected relative to the 200 ms preceding the feedback stimulus. Before averaging, trials were rejected if there was a voltage step higher than 50 μV/ms, or a difference of >100 μV as well as a signal lower than 0.1 μV was detected in any of the intervals.

The FRN, and event-related potential which likely reflects striatal feedback processing (Nieuwenhuis et al., 2005; Hauser et al., 2014), was calculated as the most negative peak amplitude in the time window between 200 and 350 ms following feedback presentation relative to the preceding positive peak amplitude between 150 ms and the latency of that negative peak (Eppinger et al., 2008; Rustemeier et al., 2013). A mixed-design ANOVA with electrode site and feedback (positive vs negative) as within-subject factors and treatment as well as *MR* haplotype as between-subjects factors was used to investigate stress- or genotype-related differences in the FRN. Feedback was added as a factor, since the FRN is particularly important for learning from negative feedback (van der Helden et al., 2010). For each participant, on average 37.5 (SD = 13.8 trials) negative feedback trials were available. Frontal electrodes (FC1, Fz, FCz, FC2), where the FRN was most pronounced, were included in the analyses.

The P3a and P3b components are proposed to reflect cognitive mechanisms facilitating attention and promoting memory processes and to involve frontal areas and the hippocampus (Knight, 1996; Polich, 2007). The P3a was calculated as the mean activity in a time window between 235 and 425 ms at central electrodes (C1, Cz, C2). The P3b was defined as the mean activity in a time window between 270 and 420 ms at parietal electrodes P1, Pz, P2. Since no differences between negative and positive feedback in P3a or P3b were expected, analyses included all feedback trials (mean number of trials = 77, SD = 17 trials). Repeated measures ANOVA with electrode site as within-subject factor and treatment as well as *MR* haplotype as between-subjects factors were used to investigate stress- or genotype-dependent differences in the P3 components.

Experiment II: Neural signature of *MR* haplotype modulation of stress-induced changes in multiple memory systems

Participants and experimental design

A total of 128 volunteers of the Bonn Gene Brain Behavior Project participated in this experiment (62 women; mean age = 23.0 years, SD = 3.6 years). Participants were healthy, young nonsmokers without medication intake, or lifetime history of any neurologic or psychiatric disorders. Furthermore, any contraindications for fMRI measurements served as exclusion criteria. The experiment was approved by the ethical review board of the

German Psychological Society (DGPs; reference: LS072014) as well as by the local committee at the University of Bonn. Participants gave written informed consent and received a moderate monetary compensation of 35 €. This sample is part of a larger project on individual differences in stress effects on multiple memory systems (Wirz et al., 2017).

In line with the first experiment, we used a 2 × 2 between-subjects design with the factors treatment (TSST vs control manipulation) and *MR* haplotype (carriers vs noncarriers). Participants were randomly assigned to the stress or control condition. Due to technical difficulties and excessive head motion in the MRI scanner, 8 participants were excluded from the fMRI analyses, leading to a sample of 120 participants (stress: 47 carriers, 13 noncarriers; control: 45 carriers, 15 noncarriers) for the fMRI analyses. For the behavioral analyses, data from all 128 tested participants were used (stress: 50 carriers, 15 noncarriers; control: 48 carriers, 15 noncarriers).

Experimental procedure

The experimental procedure, including the stress manipulation, the parameters measured and the PCL task, was identical to the first experiment, except that fMRI instead of EEG measurements were taken and that the PCL task was slightly modified to accommodate fMRI requirements. More specifically, in addition to 100 PCL trials, participants completed 100 visuomotor control trials in which they were asked to indicate whether <2 or ≥ 2 cards appeared on the screen (trial type was randomly alternated; task duration: ~45 min). Additionally, the timing of the events was adjusted to the slow blood oxygenation level-dependent (BOLD) response. In Experiment II, there were 31 participants for whom no strategy could be identified; experimental groups did not differ in that number ($p = 0.316$). The behavioral analyses were in line with those of the first experiment.

MRI acquisition and analyses

MRI measurements were acquired using a 3T Trio Scanner (Siemens) equipped with a 32-channel head coil. BOLD T2-weighted echoplanar functional images were acquired parallel to the anterior commissure-posterior commissure plane (37 transversal slices; TR = 2000 ms; TE = 30 ms; ascending acquisition; effective voxel size = 3 × 3 × 3 mm). Additionally, a high-resolution T1-weighted anatomic image was acquired (208 sagittal slices, TR = 1660 ms, TE = 2.54 ms, voxel size = 0.8 × 0.8 × 0.8 mm).

Preprocessing and analyses of the fMRI data using general linear modeling were performed with the SPM12 Matlab toolbox (Wellcome Trust Center for Neuroimaging). Functional data were slice-time and head-motion corrected as well as coregistered to the structural image using rigid-body transformations. The T1-weighted image was segmented into gray and white matter, cerebrospinal fluid, bone, soft tissue, and air. Forward deformation fields were then used to spatially normalize the functional and structural scans to the Montreal Neurologic Institute standard brain. Finally, normalized functional images were smoothed using an 8-mm full-width half-maximum Gaussian kernel.

Correct and incorrect PCL trials as well as visuomotor control trials were modeled using canonical hemodynamic response functions. Additionally, fixation, button presses and the six movement parameters were included into the model. Data were filtered in the temporal domain using a nonlinear high-pass filter with a 128-s cutoff. Contrast images were generated for PCL minus control trials and for correct minus incorrect PCL trials. These difference contrasts were then entered into second-level (group) analyses, using a full-factorial model with the factors treatment (control vs stress) and *MR* haplotype (carriers vs noncarriers). Psycho-physiologic interaction (PPI) analyses were performed to assess whether the coupling of the amygdala with the hippocampus, the dorsal striatum and the putamen was altered by stress and/or *MR* haplotype. For this purpose, the first eigenvariate of the time course of each ROI in the contrast PCL correct minus PCL incorrect was extracted from the appropriate brain atlases and used as seed. The PPI was then computed as the element-by-element product of the BOLD signal time course of this seed and a vector coding for successful classification learning. Next, each time course was added separately as a covariate of interest in addition to the first-level regressors. The individual PPI contrasts were then entered in a second-level random-effects analysis. Results of these analyses give insight into brain regions that show a similar and task-dependent pattern of activation. These regions are therefore supposed to be functionally connected during correct classification learning.

Explorative whole brain analyses as well as ROI analyses were used. A priori ROIs were the memory system structures of interest (hippocampus, caudate nucleus and putamen) as well as the amygdala because this area is assumed to modulate multiple memory systems (Elliott and Packard, 2008; Schwabe et al., 2013; Vogel et al., 2015) and is affected by MR activation (Karst et al., 2010). Anatomic masks of the caudate nucleus, the putamen and the amygdala were taken from the Harvard-Oxford subcortical atlas, whereas masks of the hippocampal subregions were taken from the Anatomy Toolbox for SPM (Institute of Neuroscience and Medicine). For the explorative whole-brain analysis, the significance threshold was set to $p < 0.05$ at cluster level and corrected for multiple testing [familywise error (FEW) correction]. ROI analyses were performed using small-volume correction with an initial threshold of $p < 0.05$ uncorrected, followed by FEW correction ($p < 0.05$). Thresholds at 50 percentage were used to include only voxels with a probability of at least 50 percentage to belong to each subregion.

Results

Experiment I: *MR* haplotype, stress, and the engagement of multiple memory systems

MR haplotype analyses

Haplotype analyses revealed significantly strong linkage between six *MR* SNPs (rs1512344, rs2070950, **rs2070951**, rs4835519, **rs5522**, rs7658048) building a

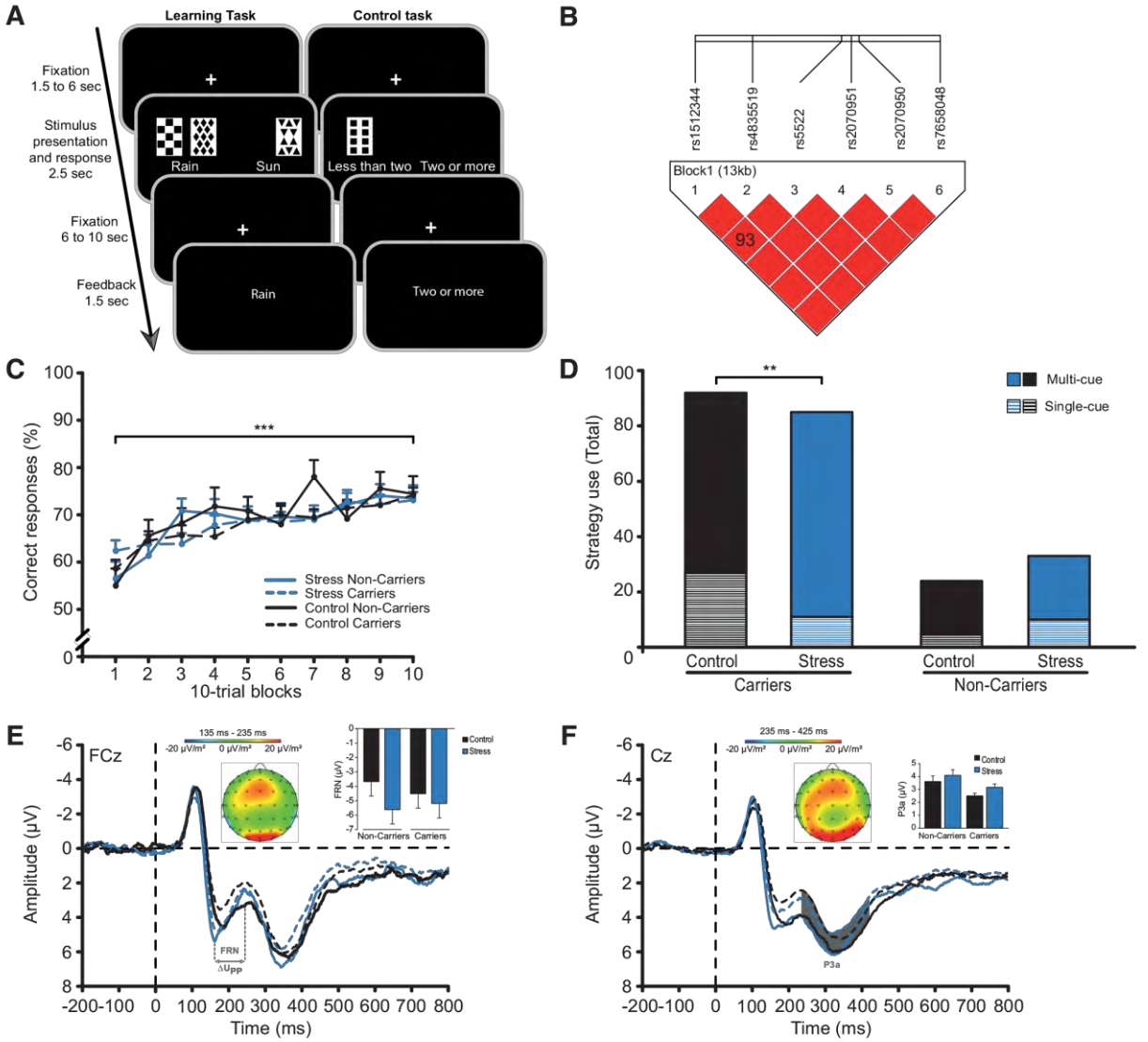

Figure 1. PCL task, haplotype analysis, and behavioral and EEG data of Experiment I. ***A***, Participants were required to learn how to predict the weather (rain or sun) from presentation of three to four out of four possible cues based on trial-by-trial feedback. In Experiment II, participants also completed a visual-motor control task in which they had to indicate whether ≤2 or >2 cards were presented. ***B***, Haplotype block covering 13 kb on the *MR* gene. Homo- and heterozygous *MR* haplotype carriers (*MR*-2G/C **C** [rs2070951], *MR*-I180V **A** [rs5522]) were tested against all other haplotypes. ***C***, Classification learning performance increased across trials but was unaffected by stress or the *MR* haplotype. ***D***, Stress, however, increased the use of multi-cue strategies, thought to rely on the dorsal striatum, and decreased the use of single-cue strategies, assumed to be supported by the hippocampus. This bias toward enhanced dorsal striatal processing was only observed in stressed *MR* haplotype carriers. ***E***, EEG data revealed a significant stress-induced increase in the FRN at FCz electrode, which was calculated as the most negative peak amplitude in the time window irrespective between 200 and 350 ms following feedback presentation relative to the preceding positive peak amplitude between 150 ms and the latency of that negative peak. ***F***, *MR* haplotype carriers showed, irrespective of stress, an enhanced P3a at central electrodes (C1, Cz, C2), which was calculated as the mean activity in the time window between 235 and 425 ms following feedback presentation. ΔU_{PP} represents the voltage difference between the positive and the negative peak amplitude after negative feedback. $^{***}p < 0.001$, $^{**}p < 0.01$, error bars represent SEM.

haplotype block covering 13 kb on the *NR3C2* gene (Fig. 1*B*). *MR* haplotype details and allele frequencies can be found in Tables 1 and 2. The *MR* haplotype, including the *MR*-2 G/C **C** and *MR*-I180V **A** alleles previously associated with increased MR expression and transactivational capacity (DeRijk and De Kloet, 2008; van Leeuwen et al., 2010), was of particular interest (alleles in order of the SNPs: CC**CT**A**G**). Experiment I included 132 participants

Table 1. Haplotypes in Experiments I and II

		MR-2G/C		*MR*-I180V	
rs1512344	rs2070950	**rs2070951**	rs4835519	**rs5522**	rs17658048
C	**C**	**C**	**T**	**A**	**G**
T	**G**	**G**	**C**	**A**	**A**
T	**G**	**G**	**T**	**G**	**G**
T	**G**	**G**	**T**	**A**	**G**
C	**G**	**G**	**T**	**A**	**G**

Combinations of 6 *MR* SNP alleles as detected by the haplotype analyses in Experiments I and II.

carrying one and 59 participants carrying two alleles of this haplotype. Another four haplotypes were identified and one participant carried an unknown haplotype (Tables 1 and 2). Importantly, however, that participant did not carry the **C** and **A** alleles of the *MR* SNPs of interest (**rs2070951** and **rs5522** respectively). Homo- and heterozygous carriers of the CC**C**T**A**G haplotype (= *MR* haplotype carriers), which shows higher *MR* transactivation and expression compared to the other haplotypes, were tested against all other haplotypes (= *MR* haplotype noncarriers), leading to 90 carriers and 36 noncarriers in the stress and 101 carriers and 25 noncarriers in the control condition. Frequencies of the *MR* SNPs were in accordance with those documented in the database of the National Center for Biotechnology Information (NCBI) for Europeans. Frequencies were in Hardy-Weinberg equilibrium (all $p \geq 0.23$) and the distribution of carriers and noncarriers of the *MR* haplotype did not differ in the stress (90 carriers, 36 noncarriers) and control group (101 carriers, 25 noncarriers; $\chi^2_{(1)} = 2.617$, $p = 0.106$). The *MR* haplotype was not significantly associated with sex or age (both $p \geq 0.827$).

Successful stress induction by the TSST

Significant changes in subjective mood, blood pressure and concentrations of the glucocorticoid stress hormone cortisol verified the successful stress induction by the TSST (Table 3). Compared to the control procedure, exposure to the TSST was rated as significantly more difficult, unpleasant and stressful (all $F_{(1248)} \geq 165.821$, all $p < 0.001$). Moreover, the TSST but not the control manipulation, resulted in increases of depressed mood and restlessness (time × treatment: both $F_{(2246)} \geq 37.536$, both $p < 0.001$); all participants, irrespective of experimental group, became increasingly tired throughout the experiment (time × treatment: $F_{(1.8440.4)} = 129.645$, $p < 0.001$). In addition, exposure to the TSST led to significant autonomic activation, reflected by increases in systolic (Fig. 2*A*) and diastolic blood pressure (time × treatment: both $F \geq 51.146$, both $p < 0.001$). Finally, we obtained a significant increase in cortisol concentrations following the stress but not the control manipulation (time × treatment: $F_{(2493.1)} = 50.777$, $p < 0.001$). As shown in Figure 2*B*, peak cortisol levels were reached ~15 min following the stressor, when behavioral testing started. The *MR* haplotype did not influence measures of blood pressure, cortisol or mood (all $F \leq 1.548$, all $p \geq 0.215$).

Table 2. Haplotype distribution in Experiments I and II

	Experiment I		Experiment II	
Haplotype	Allele frequency	%	Allele frequency	%
CCCTAG	250	49.6	125	48.2
TGGCAA	173	34.3	89	34.8
TGGTGG	58	11.5	29	11.3
TGGTAG	18	3.6	10	3.9
CGGTAG	4	0.8	-	-
Unknown	1	0.2	3	1.2

Allele frequencies and percentage of the haplotypes detected in Experiments I and II. **Unknown** represents participants not carrying the **CCCTAG** haplotype but who could not be assigned to any of the other haplotypes.

Carriers of the MR haplotype show enhanced stress-induced shift toward multi-cue strategies

Participants successfully learned the cue-outcome associations, as reflected in a gradual performance improvement from 58 to 74 percent correct responses across PCL trials ($F_{(7.7,\ 1918.2)} = 17.730$, $p < 0.001$; Fig. 1*C*). In line with previous studies showing that different memory systems may contribute equally well to learning performance (Schwabe and Wolf, 2009, 2012), stress and *MR* haplotype did not influence performance (all $F \leq 1.413$, $p \geq 0.240$). However, stress tended to change the learning strategies that were used to solve the PCL task: compared to controls, stressed participants tended to engage multi-cue strategies that supposedly depend on the dorsal striatum more often and single-cue strategies that are assumed to depend on the hippocampus less often ($\chi^2_{(1)} = 3.200$, trend: $p = 0.074$). Most importantly, as shown in Figure 1*D*, stress effects on strategy use differed between *MR* haplotype carriers versus noncarriers. In carriers of this haplotype, stress led to a significant increase in the use of multi-cue strategies from 71-87 percentage and a decrease in the use of single-cue strategies from 29-13 percentage ($\chi^2_{(1)} = 7.054$, $p = 0.008$, *Cramer's V* = 0.200). This stress-induced shift in strategy use, was absent in noncarriers of the *MR* haplotype ($\chi^2_{(1)} = 0.643$, $p = 0.423$, *Cramer's V* = 0.106). Since previous animal studies suggested that *MR* genotype effects may be sex-dependent (Klok et al., 2011; Vinkers et al., 2015; Hamstra et al., 2017), we performed explorative analyses on our behavioral data, adding sex as another variable in the χ^2 test. Results indicate that in males, *MR* haplotype carriers use more multi-cue strategies after stress compared to the no-stress control condition ($\chi^2_{(1)} = 5.792$, $p = 0.016$, *Cramer's V* = 0.255), whereas in females, there was no significant modulation of strategy use under stress by the *MR* haplotype ($\chi^2_{(1)} = 2.091$, $p = 0.148$, *Cramer's V* = 0.154). Similarly, when directly comparing male and female *MR* haplotype carriers in the stress condition, a trend toward a similar effect, namely increased multi-cue

Table 3. Subjective, autonomic, and endocrine stress response in Experiment I

	Control		Stress	
	Carriers	Noncarriers	Carriers	Noncarriers
Subjective assessment				
Stressful	27.62 ± 2.25	28.80 ± 4.01	69.89 ± 2.08	71.11 ± 4.36***
Difficult	28.51 ± 2.31	29.60 ± 4.34	76.00 ± 2.13	73.33 ± 3.40***
Unpleasant	29.50 ± 2.45	27.60 ± 4.25	74.78 ± 2.17	71.39 ± 4.39***
Subjective mood				
Good vs bad mood				
Before treatment	32.65 ± 0.46	32.48 ± 0.77	33.44 ± 0.44	34.14 ± 0.71
1 min after treatment	32.35 ± 0.51	31.76 ± 0.98	26.80 ± 0.69	26.28 ± 1.22***
65 min after treatment	31.19 ± 0.55	31.12 ± 1.02	29.93 ± 0.65	31.61 ± 0.98
Calm vs restless				
Before treatment	30.68 ± 0.59	30.68 ± 0.92	31.23 ± 0.52	31.69 ± 0.96
1 min after treatment	30.19 ± 0.56	29.72 ± 1.10	23.22 ± 0.63	24.06 ± 1.23***
65 min after treatment	31.16 ± 0.57	31.12 ± 1.02	29.60 ± 0.65	32.06 ± 0.95
Tired vs awake				
Before treatment	29.52 ± 0.59	28.00 ± 1.21	30.21 ± 0.68	31.06 ± 1.02
1 min after treatment	29.07 ± 0.65	27.48 ± 1.28	29.36 ± 0.64	30.03 ± 0.99
65 min after treatment	22.85 ± 0.68	23.88 ± 1.39	22.88 ± 0.68	23.89 ± 1.36
Systolic blood pressure (bpm)				
Before treatment	133.02 ± 1.85	135.68 ± 3.50	132.87 ± 1.93	129.99 ± 2.84
During treatment	135.68 ± 1.74	137.98 ± 3.60	157.73 ± 2.05	160.36 ± 3.02***
5 min after treatment	131.05 ± 1.69	134.90 ± 4.20	139.22 ± 1.76	137.59 ± 2.41*
45 min after treatment	127.35 ± 1.59	133.06 ± 3.45	129.72 ± 1.73	126.76 ± 3.23
65 min after treatment	128.95 ± 1.62	132-94 ± 3.23	130.49 ± 1.66	130.56 ± 2.53
Diastolic blood pressure (bpm)				
Before treatment	76.30 ± 0.82	76.88 ± 1.75	76.21 ± 0.92	75.79 ± 1.51
During treatment	80.93 ± 0.79	81.22 ± 2.16	94.62 ± 1.42	94.72 ± 1.92***
5 min after treatment	77.87 ± 0.73	77.20 ± 1.80	81.02 ± 0.95	82.77 ± 1.52**
45 min after treatment	75.23 ± 0.68	75.84 ± 1.65	76.56 ± 0.90	78.11 ± 1.42
65 min after treatment	76.70 ± 0.69	77.26 ± 1.69	76.89 ± 0.91	76.82 ± 1.33
Salivary cortisol (nmol/l)				
Before treatment	5.16 ± 0.46	5.60 ± 0.67	5.32 ± 0.42	6.16 ± 0.70
5 min after treatment	4.69 ± 0.35	5.71 ± 0.85	9.62 ± 0.72	11.25 ± 1.27***
15 min after treatment	4.06 ± 0.27	4.57 ± 0.64	12.33 ± 0.97	13.71 ± 1.50***
25 min after treatment	3.51 ± 0.22	4.00 ± 0.49	9.67 ± 0.79	10.36 ± 1.09***
65 min after treatment	2.93 ± 0.16	3.13 ± 0.39	4.74 ± 0.30	5.11 ± 0.43***

Data represent means ± SEM. bpm, beats per minute. Stress versus control ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

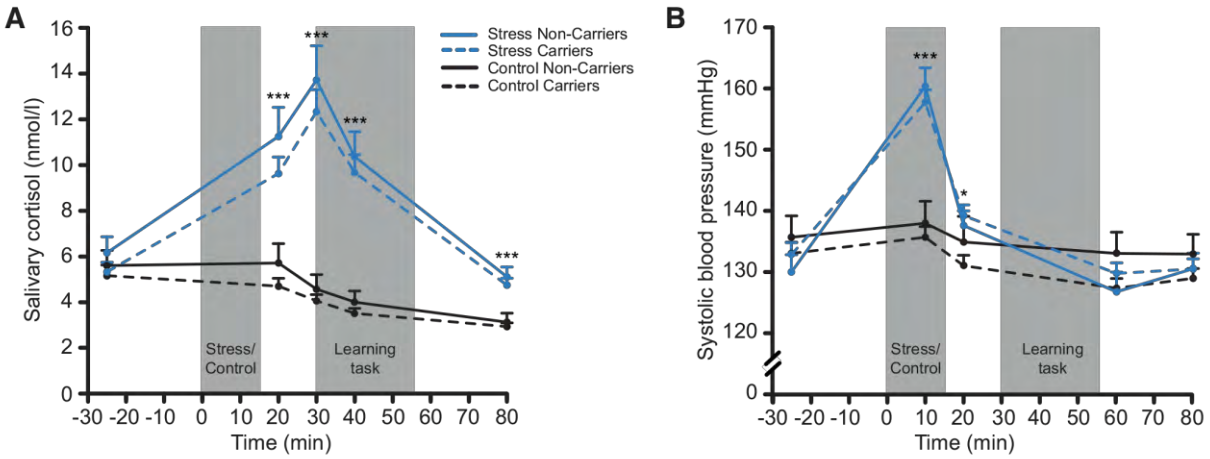

Figure 2. Physiological data of Experiment I. Independent of the *MR* haplotype and compared to a nonstressful control manipulation, exposure to the TSST led to significant increases in (***A***) salivary cortisol concentrations and (***B***) systolic blood pressure. ***$p < 0.001$, *$p < 0.05$, error bars represent SEM.

strategy use in males, is observed ($\chi^2_{(1)} = 2.748$, $p = 0.097$, *Cramer's V* = 0.180), whereas in stressed noncarriers of the *MR* haplotype, we do not detect any gender differences ($\chi^2_{(1)} = 0.013$, $p = 0.909$, *Cramer's V* = 0.020). This is in line with evidence showing that female mice with a genetic deletion of forebrain MR continued to use hippocampus-dependent spatial strategies in a maze task despite stress (Ter Horst et al., 2013).

Stress and MR haplotype alter electrocortical activity during learning

Our EEG data show that the FRN followed the typical frontocentral distribution and was increased in stressed compared to control participants following negative feedback ($F_{(1223)} = 9.956$, $p = 0.037$; Fig. 1*E*). Since the FRN is particularly important for learning in response to negative feedback (van der Helden et al., 2010), this difference was not observed in response to positive feedback ($F_{(1223)} = 0.587$, $p = 0.444$; feedback × treatment: $F_{(1223)} = 6.404$, $p = 0.012$). Carriers and noncarriers of the *MR* haplotype, neither differed in FRN amplitude ($F_{(1223)} = 0.254$, $p = 0.615$), nor was the stress effect on the FRN modulated by *MR* haplotype ($F_{(1223)} = 0.646$, $p = 0.422$).

In contrast to the FRN, the P3a was reduced in carriers compared to noncarriers of the *MR* haplotype ($F_{(1224)} = 5.331$, $p = 0.022$; Fig. 1*F*), but was not affected by stress (both $F \leq 0.043$, $p \geq 0.837$), whereas the P3b was neither influenced by the stress manipulation nor by the *MR* haplotype (all $F \leq 0.044$, $p \geq 0.834$). Visual inspection of the EEG time course suggested that group differences already developed earlier. Indeed, explorative analyses showed that at frontocentral electrodes the P2 (mean activity 135-235 ms) and the N2 (mean activity 185-285 ms), two early attentional components, were reduced in *MR* haplotype carriers (both $F \geq 5.328$, $p \leq 0.022$). Additionally, the P2 tended to be reduced in stressed participants ($F_{(1224)} = 3.837$, $p = 0.051$).

Experiment II: Neural signature of *MR* haplotype modulation of stress-induced changes in multiple memory systems

Our first experiment showed that the *MR* haplotype modulates the influence of stress on the engagement of different learning strategies in a PCL task. We found evidence that stress led to an increase in FRN amplitude, presumably indicative of increased striatal feedback processing, and that the *MR* haplotype was associated with an increase in early P3a amplitude, which likely reflects cognitive mechanisms that facilitate attention. However, how exactly the *MR* haplotype modulated the stress effect on strategy use on a neural level, remained unclear. Therefore, we ran a second experiment, in which we used fMRI to unravel the neural underpinnings of the modulatory effect of the *MR* haplotype on stress-induced changes in multiple memory systems.

MR haplotype analysis

In line with Experiment I, haplotype analyses revealed significantly strong linkage between six *MR* SNPs (rs1512344, rs2070950, **rs2070951**, rs4835519, **rs5522**, rs7658048; Fig. 3*A*). *MR* haplotype details and allele frequencies can be found in Tables 1, 2. This second experiment included 98 carriers of the CC**C**T**A**G haplotype (= *MR* haplotype) that had also been identified in Experiment I (71 participants with one allele and 27 participants with two alleles). Again, the same four other haplotypes were identified and three participants carried unknown haplotypes (Tables 1, 2). In total, 30 participants did not carry the *MR* haplotype. Frequencies of the *MR* SNPs were in accordance with frequencies for Europeans as reported by the NCBI. Frequencies were in Hardy-Weinberg equilibrium (all $p \geq 0.11$) and genotype was not significantly associated with sex or age (both $F \leq 0.376$, $p \geq 0.540$). Carriers and noncarriers of the *MR* haplotype were equally distributed in the stress (50 carriers, 15 noncarriers) and control group (48 carriers, 15 noncarriers; $\chi^2_{(1)} = 0.010$, $p = 0.922$).

Successful stress induction by the TSST

As in Experiment I, exposure to the TSST was rated as significantly more difficult, unpleasant and stressful compared to the control manipulation (all $F_{(1124)} \geq 46.367$, all $p < 0.001$*)* and participants' mood decreased only following the TSST (all $F_{(2, 246)} \geq 12.258$, all $p < 0.001$; Table 4). Again, participants became overall increasingly tired throughout the experiment ($F_{(2246)} = 101.880$, $p < 0.001$). Moreover, systolic and diastolic blood pressure as well as salivary cortisol increased following the TSST but not after the control manipulation (all $F \geq 10.472$, all $p < 0.001$; Fig. 4), with cortisol reaching peak levels shortly before PCL in the MRI scanner.

Although the subjective response to the stressor was not affected by the *MR* haplotype (all $F_{(1123)} \leq 1.814$, $p \geq 0.165$), carriers of the *MR* haplotype felt more awake throughout the experiment ($F_{(1123)} = 14.359$, $p < 0.001$). Moreover, the *MR* haplotype affected blood pressure and cortisol levels. Approximately 75 min after the TSST, the *MR* haplotype was associated with decreased systolic blood pressure levels (time × treatment × *MR* haplotype: $F_{(2121)} = 4.079$, $p < 0.001$; *post hoc* comparison of time point of measurement: $p = 0.044$). Diastolic blood pressure remained unaffected by the *MR* haplotype (time × *MR* haplotype: $F_{(2.5311)} = 0.330$, $p = 0.769$; main effect *MR* haplotype: $F_{(1123)} = 0.777$, $p = 0.380$). Salivary cortisol was reduced in carriers compared to noncarriers of the *MR* haplotype irrespective of the experimental condition ($F_{(1124)} = 4.224$, $p = 0.042$; Fig. 4*A*). Furthermore, although not statistically significant, carriers of the *MR* haplotype tended to show an attenuated cortisol response to the TSST ($p = 0.077$).

MR haplotype is associated with enhanced stress-induced bias toward multi-cue strategies

Participants gradually learned to correctly classify the cues and correct responses increased from 37 to 62% across PCL trials ($F_{(6.7,828.5)} = 20.901$, $p < 0.001$; Fig. 3*B*). Stress and *MR* haplotype had no effect on task performance (all $F \leq 2.916$, all $p \geq 0.90$). Corroborating the behavioral findings of Experiment I, stress led, compared to the control manipulation, to more multi-cue and less single-cue learning ($\chi^2_{(1)} = 4.173$, $p = 0.041$). This stress-

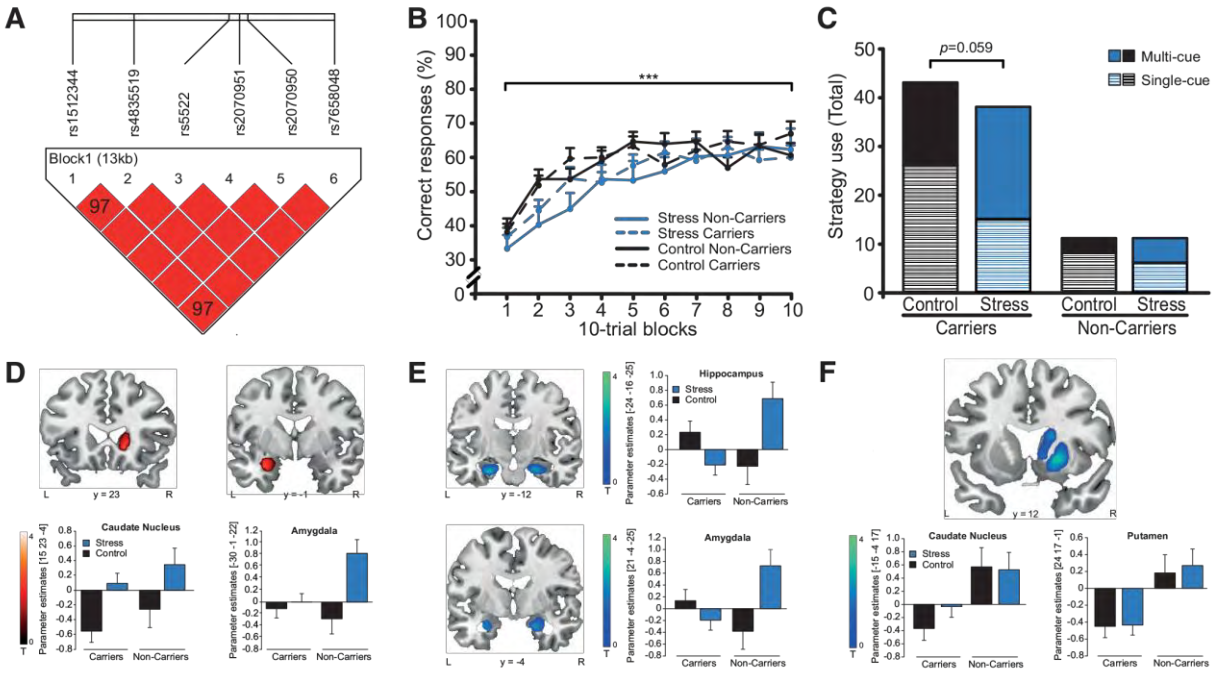

Figure 3. Haplotype analysis, behavioral data and stress and *MR* haplotype effects on brain activity in Experiment II. ***A***, Haplotype block covering 13 kb on the *MR* gene. Homo- and heterozygous *MR* haplotype carriers (*MR*-2G/C **C** [rs2070951], *MR*-I180V **A** [rs5522]) were tested against all other haplotypes. ***B***, Classification learning performance increased across trials but was unaffected by stress or the *MR* haplotype. ***C***, However, stress increased the use of multi-cue strategies, thought to rely on the dorsal striatum, and decreased the use of single-cue strategies, assumed to be supported by the hippocampus. This bias toward enhanced dorsal striatal processing was only observed in stressed *MR* haplotype carriers. Stress increased activation of the caudate nucleus (p_{FWE} = 0.035) and the amygdala (p_{FWE} = 0.030; ***D***), whereas *MR* haplotype carriers showed enhanced bilateral amygdala activation under stress (both $p_{FWE} \leq 0.067$) and reduced bilateral hippocampus (both $p_{FWE} \leq 0.047$; ***E***) and overall reduced activation of the caudate nucleus (p_{FWE} = 0.032) and putamen (p_{FWE} = 0.006; ***F***). Activations are superimposed on coronal sections of a T1-weighted template image and represented in red. Activation that is reduced in *MR* haplotype carriers is shown in blue. L corresponds to the left, R to the right side of the brain, and error bars represent SEM. ***$p < 0.001$.

induced shift in learning strategy was modulated by the *MR* haplotype. As shown in Figure 3*C*, only *MR* haplotype carriers tended to shift toward multi-cue strategies after stress ($\chi^2_{(1)}$ = 3.556, p = 0.059, *Cramer's V* = 0.210), whereas there was no such effect in noncarriers of this haplotype ($\chi^2_{(1)}$ = 0.786, p = 0.375, *Cramer's V* = 0.189). The effect sizes for the modulatory effect of the MR haplotype on the stress-induced bias toward multi-cue strategies were comparable between Experiment I (*Cramer's V* = 0.2) and Experiment II (*Cramer's V* = 0.21), indicating that the trend-level significance of the MR modulation in this fMRI experiment was most likely due to lower statistical power. Although in our first experiment explorative analyses revealed that the modulatory effects of the *MR* haplotype on strategy use under stress may be sex dependent, the smaller sample size of this fMRI experiment did not allow for such analyses.

Neural underpinnings of the MR haplotype-dependent modulation of multiple memory systems after stress

Corroborating previous studies (Poldrack et al., 2001; Foerde et al., 2006; Schwabe and Wolf, 2012; Schwabe et al., 2013), PCL (vs visuomotor control trials) led to bilateral activation of the caudate nucleus, putamen and hippocampus (all $p_{FWE} \leq 0.031$). In addition, PCL activated regions such as the cingulate and paracingulate cortex, orbitofrontal cortex, insular cortex and precuneus (all $p_{FWE} < 0.001$; Table 5). Stress led to a significant increase in caudate activation during learning (t =3.21, p_{FWE} = 0.035, k: 34; Fig. 3*D*). Whereas this stress-induced increase in dorsal striatal activity was not modulated by the *MR* haplotype, the *MR* haplotype affected activation in the hippocampus under stress, in that stressed *MR* haplotype carriers showed significantly reduced bilateral hippocampal activation (right: t = 3.15, p_{FWE} = 0.047, k: 32; left: t = 3.48, p_{FWE} = 0.019, k: 38; Fig. 3*E*). In addition, stress increased amygdala activity (t = 3.01, p_{FWE} = 0.030, k: 20) and this stress-induced increase was modulated by the *MR* haplotype, with stressed participants not carrying the *MR* haplotype showing greater bilateral amygdala activation (right: t = 2.98, p_{FWE} = 0.038, k: 20; left: t = 2.67, p_{FWE} = 0.067, k: 21; Fig. 3*E*). Irrespective of stress manipulation, the *MR* haplotype was associated with reduced activation of the caudate nucleus (t = 3.25, p_{FWE} = 0.032, k: 69) and the putamen (t = 3.93, p_{FWE} = 0.006, k: 56; Fig. 3*F*).

Table 4. Subjective, autonomic, and endocrine stress response in Experiment II

	Control		Stress	
	Carriers	Noncarriers	Carriers	Noncarriers
Subjective assessment				
Stressful	29.17 ± 2.96	30.67 ± 6.28	65.80 ± 2.73	66.00 ± 5.24***
Difficult	26.67 ± 3.01	25.33 ± 6.61	71.80 ± 2.63	67.33 ± 6.93***
Unpleasant	34.38 ± 3.55	38.67 ± 7.61	66.40 ± 3.33	76.00 ± 5.42***
Subjective mood				
Good vs bad mood				
Before treatment	34.65 ± 0.62	33.93 ± 1.09	34.84 ± 0.49	33.07 ± 1.37
1 min after treatment	34.13 ± 0.59	33.27 ± 1.24	28.31 ± 0.92	28.53 ± 1.73***
75 min after treatment	33.21 ± 0.68	31.07 ± 1.44	31.80 ± 0.70	29.27 ± 1.74
Calm vs restless				
Before treatment	31.83 ± 0.79	33.20 ± 0.91	32.26 ± 0.72	29.87 ± 1.33
1 min after treatment	30.63 ± 0.81	30.33 ± 1.67	24.61 ± 0.93	23.53 ± 1.60***
75 min after treatment	31.63 ± 0.77	32.53 ± 1.33	32.38 ± 0.72	30.73 ± 0.91
Tired vs awake				
Before treatment	31.17 ± 0.79	27.67 ± 1.56	31.14 ± 0.65	28.13 ± 1.37
1 min after treatment	30.46 ± 0.85	27.20 ± 1.58	29.61 ± 0.69	25.80 ± 1.62
75 min after treatment	24.65 ± 0.78	19.67 ± 1.41	22.88 ± 0.86	20.47 ± 1.17
Overall tired vs awake	31.36 ± 0.65	32.02 ± 1.15	29.73 ± 0.64	28.04 ± 1.15##
Systolic blood pressure (bpm)				
Before treatment	124.09 ± 1.97	124.00 ± 2.84	122.19 ± 1.90	123.83 ± 3.67
During treatment	119.05 ± 1.89	119.73 ± 3.92	128.68 ± 2.14	127.17 ± 4.42**
5 min after treatment	120.04 ± 1.83	121.83 ± 3.99	129.39 ± 2.06	128.33 ± 3.81**
75 min after treatment	121.52 ± 1.77	118.97 ± 2.76	119.55 ± 1.88	127.20 ± 2.61+
Diastolic blood pressure (bpm)				
Before treatment	83.25 ± 1.34	82.07 ± 2.53	81.21 ± 0.97	84.90 ± 2.79
During treatment	83.24 ± 1.17	82.50 ± 2.26	89.59 ± 1.49	95.03 ± 3.58***
5 min after treatment	84.07 ± 1.26	83.53 ± 1.83	88.05 ± 1.26	90.47 ± 3.08**
75 min after treatment	84.35 ± 1.42	83.03 ± 1.78	82.90 ± 1.13	86.83 ± 2.51
Salivary cortisol (nmol/l)				
Before treatment	3.37 ± 0.69	3.66 ± 1.24	3.68 ± 0.68	6.71 ± 1.242
5 min after treatment	3.62 ± 0.93	3.78 ± 1.67	7.03 ± 0.91	12.43 ± 1.67***
15 min after treatment	3.31 ± 0.98	3.66 ± 1.75	8.91 ± 0.96	13.80 ± 1.75***
75 min after treatment	2.46 ± 0.62	2.85 ± 1.12	4.16 ± 0.61	7.44 ± 1.12***
Overall salivary cortisol (nmol/l)	3.19 ± 0.75	3.48 ± 1.34	5.94 ± 0.73	10.10 ± 1.34#

Data represent means ± SEM. bpm, beats per minute. Time × treatment (stress vs control) interaction ***$p < 0.001$ and **$p < 0.01$. *MR* haplotype (carriers vs noncarriers) main effect ##$p < 0.01$ and #$p < 0.05$. Time × treatment × *MR* haplotype interaction +$p < 0.05$.

MR haplotype modulates stress-induced changes in amygdala connectivity with the dorsal striatum and the hippocampus

Because previous evidence suggested that the amygdala may orchestrate the engagement of multiple memory systems under stress (Vogel et al., 2016), we analyzed functional connectivity of the amygdala with the hippocampus, caudate nucleus and putamen. In line with a stress-induced modulation of multiple memory systems at the expense of the hippocampus-dependent system, stressed participants showed decreased amygdala connectivity with the cornu ammonis subregion of the hippocampus ($t = 3.23$, $p_{FWE} = 0.043$, k: 318) as well as a trend toward decreased amygdala-entorhinal cortex coupling ($t = 2.95$, $p_{FWE} = 0.061$, k: 10; Fig. 5*A*). Critically, the *MR* haplotype modulated the connectivity of the amygdala with structures of the cognitive and habitual systems under stress (treatment × *MR* haplotype interactions all $t \geq 2.83$, $p_{FWE} \leq 0.065$, all $k \geq 24$). In the stress condition, amygdala connectivity with the anterior parahippocampal region was reduced in carriers of the *MR* haplotype (right: $t = 3.01$, $p_{FWE} = 0.032$, k: 24; left: $t = 2.91$, $p_{FWE} = 0.063$, k: 29; Fig. 5*B*). Conversely, in the control condition, amygdala connectivity with the caudate nucleus was increased in *MR* haplotype carriers relative to noncarriers ($t = 3.58$, $p_{FWE} = 0.018$, k: 44; Fig. 5*B*).

Role of altered cortisol levels in the MR haplotype-dependent modulation of multiple memory systems under stress

Because carriers of the *MR* haplotype showed overall lower cortisol concentrations and tended to have a reduced cortisol response to the TSST, we tested whether the behavioral and neuronal effects of the *MR* haplotype were mediated by altered cortisol levels or whether these effects occurred independently of changes in cortisol responses. Results of mediation and moderation analyses using the PROCESS plugin for SPSS (Hayes, 2013) showed that cortisol (expressed as area under the curve with respect to ground) neither moderated nor mediated the stress-induced increase in dorsal striatum-dependent, multi-cue strategies in *MR* haplotype carriers (moderation $p = 0.930$, mediation $p = 0.562$). Similarly, our imaging data remained largely unchanged after including cortisol as a covariate. Activation of the hippocampus and the amygdala was still reduced in stressed *MR* haplotype

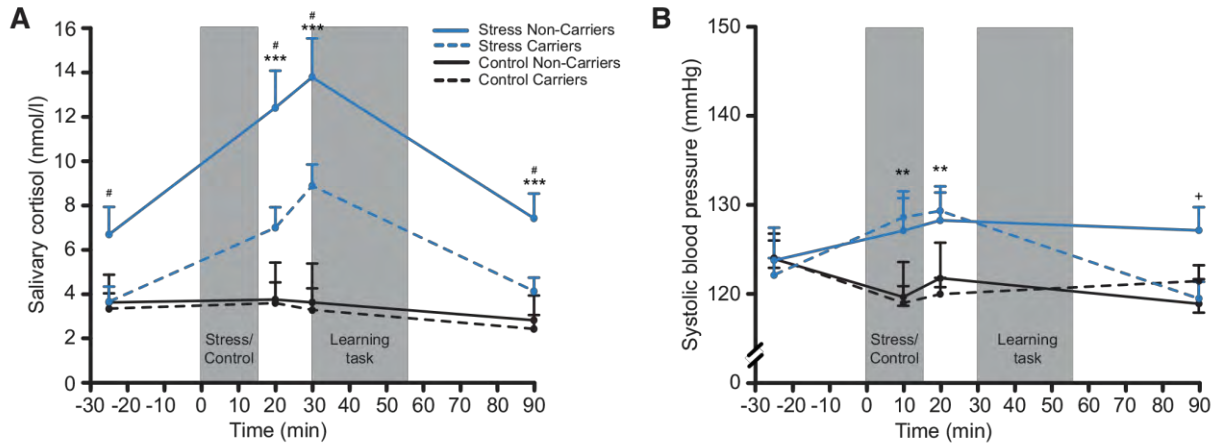

Figure 4. Physiological data of Experiment II. ***A***, Salivary cortisol concentrations were increased in response to the TSST but were generally diminished in *MR* haplotype carriers. ***B***, Similarly, stress exposure led to significant increases in systolic blood pressure and carriers of the *MR* haplotype show increased levels ~75 min following the stressor. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ indicate significant differences between stress and control group; #$p < 0.05$ indicates significant differences between *MR* haplotype carriers and no-carriers; +$p < 0.05$ indicates differences between *MR* haplotype carriers and no-carriers under stress, error bars represent SEM.

carriers (hippocampus: right: $t = 3.16$, $p_{FWE} = 0.046$, k: 30; left: $t = 3.46$, $p_{FWE} = 0.020$, k: 39) and ireespective of the stress manipulation, putamen activity was still reduced in *MR* haplotype carriers ($t = 3.44$, $p_{FWE} = 0.025$, k: 38), whereas the significant caudate nucleus activation became a trend ($t = 2.91$, $p_{FWE} = 0.075$, k: 21). The reduced amygdala-anterior parahippocampus connectivity in stressed *MR* haplotype carriers dropped to trend level (right: $t = 2.66$, $p_{FWE} = 0.095$, k: 9; left: $t = 2.55$, $p_{FWE} = 0.109$, k: 6). However, in the control condition, increased amygdala-caudate nucleus connectivity in carriers of the *MR* haplotype remained significant ($t = 3.13$, $p_{FWE} = 0.040$, k: 28).

Discussion

It is increasingly acknowledged that stress, whether acute or chronic, promotes a shift from more complex, cognitive toward rather simple but rigid forms of learning

Table 5. Significantly activated cluster peak voxels and *T* values during PCL

		MNI coordinates (mm)				
PCL > control	Cluster size	*x*	*y*	*z*	T_{max}	p_{corr}
L supplementary motor area	866	0	23	47	19.51	<0.001
L insula left; L caudate; R caudate	3.083	-30	20	-4	18.32	<0.001
R insula; R inferior frontal gyrus triangular; R precentral gyrus	900	33	20	-4	17.11	<0.001
L inferior parietal sulcus; R angular gyrus; L precuneus	2.337	-33	-58	44	15.02	<0.001
R middle occipital gyrus; R cuneus; R fusiform gyrus	879	33	-88	-1	12.61	<0.001
L middle occipital gyrus left; inferior temporal gyrus	512	-15	-103	2	9.03	<0.001
Middle cingulate cortex	101	-3	-28	29	8.54	<0.001
L middle frontal gyrus	58	-30	5	56	6.68	<0.001
R anterior orbitofrontal cortex	20	27	38	-22	6.29	<0.001
Cerebellar crus	11	-36	-61	-28	5.83	0.001
Calcarine cortex	9	3	-88	-7	5.69	0.001
R anterior orbitofrontal cortex	5	48	47	-16	5.66	0.001
R middle frontal gyrus; superior frontal gyrus	42	33	53	2	5.22	0.008
L anterior cingulate cortex	5	-6	-1	29	5.18	0.010
L hippocampus CA	7	-18	-37	5	3.33	0.031*
R hippocampus CA	23	18	-34	2	6.89	<0.001*
L hippocampus DG	5	-21	-37	2	3.97	0.001*
L hippocampus DG	5	21	-34	2	6.89	<0.001*
L hippocampus	5	-21	-28	-10	3.91	0.005*
L caudate nucleus	106	-9	11	-1	15.55	<0.001*
R caudate nucleus	107	9	11	2	14.87	<0.001*
L putamen	38	-15	8	-4	10.96	<0.001*
R putamen	36	18	11	-1	7.32	<0.001*

Table shows local maxima of functional voxels (normalized voxel size = 3 × 3 × 3 mm^3). MNI, Montreal Neurologic Institute; corr, corrected. All labels are taken from the Automatic Anatomic Labeling (ALL) atlas. The significance threshold was set to $p < 0.05$ (FWE corrected). *, small volume corrected; all other activations are sig. at the whole-brain level.

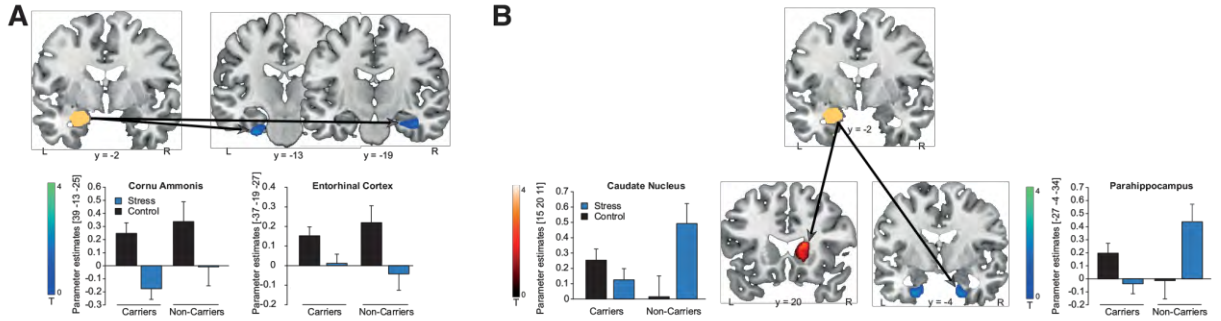

Figure 5. Stress and *MR* haplotype effects on brain connectivity in Experiment II. ***A***, Stress resulted in decreased amygdala-hippocampus connectivity (cornu ammonis: $p_{FWE} = 0.043$, enthorhinal cortex: $p_{FWE} = 0.061$). *MR* haplotype carriers showed reduced amygdala-anterior parahippocampus connectivity under stress (both $p_{FWE} \leq 0.063$; ***B***), whereas under control conditions, *MR* haplotype carriers showed increased amygdala-caudate nucleus coupling ($p_{FWE} = 0.018$; ***C***). Activations are superimposed on coronal sections of a T1-weighted template image and represented in red when greater blue when reduced in *MR* haplotype carriers, the anatomic mask is indicated in yellow. L corresponds to the left, R to the right side of the brain, and error bars represent SEM.

and memory (Schwabe et al., 2008; Dias-Ferreira et al., 2009; Packard and Goodman, 2012; Soares et al., 2012; Schwabe and Wolf, 2012; Schwabe and Wolf, 2013; Vogel et al., 2016; Wirz et al., 2018). Although this shift might contribute to stress-related psychopathology (Packard, 2009; Schwabe et al., 2011; de Quervain et al., 2017), not all individuals are equally susceptible to this stress-induced bias. Here, we showed in two independent experiments that a haplotype containing one or two copies of the alleles of two functional *MR* SNPs previously associated with enhanced MR expression (*MR*-2G/C **C**, *MR*-I180V **A**; DeRijk and De Kloet, 2008) facilitates the stress-induced shift from hippocampus-dependent toward dorsal striatum-dependent memory. In fact, the stress-induced shift toward dorsal striatal processing was solely observed in carriers of this haplotype. This modulation of the stress-induced shift toward habit memory by the *MR* haplotype was accompanied by specific changes in memory networks, indicating that the influence of this haplotype was mainly linked to impaired hippocampal processing and reduced amygdala-hippocampus cross talk under stress.

Since the discovery of membrane-bound MRs (Karst et al., 2005), several studies have demonstrated a role of rapid, nongenomic MR signaling in cognition. For instance, MR antagonists have been shown to impair selective attention and working memory performance and to enhance long-term memory (Otte et al., 2007; Cornelisse et al., 2011), whereas MR agonists have been shown to improve verbal memory and executive function in depressed patients (Otte et al., 2015) and to be associated with risky decision making (Deuter et al., 2017). Moreover, converging lines of evidence from rodent and human experiments pointed to a critical role of the MR in the engagement of multiple memory systems under stress. Specifically, pharmacological blockade of the MR prevented the shift from hippocampal toward dorsal striatal learning strategies as well as stress-induced alterations in amygdala connectivity with the hippocampus and dorsal striatum, respectively (Schwabe and Wolf, 2012; Schwabe et al., 2013; Vogel et al., 2015). Here we show that individual differences in the *MR* gene, involving two SNPs with known functionality, modulate the shift in the engagement of multiple memory systems under stress and may therefore explain at least part of the individual variability in this shift. Our finding that an *MR* haplotype associated with increased MR expression and transactivational activity is associated with increased probability of engaging the dorsal striatum-dependent memory system under stress is in line with the previous pharmacological data (Schwabe et al., 2010b, 2013) and underlines the critical involvement of the MR in stress effects on the engagement of multiple memory systems. Interestingly and further in line with previous evidence for the stress-induced shift in the engagement of multiple memory systems (Schwabe and Wolf, 2009, 2012), neither stress nor the MR haplotype affected actual task performance. This underlines that both systems can support performance. The impact of the engaged system, however, may be seen when the learning environment changes and the flexibility of learned is probed (Schwabe and Wolf, 2013; Quaedflieg and Schwabe, 2017).

Using EEG and fMRI, we investigated the neural underpinnings of the role of the *MR* haplotype in the stress-induced modulation of multiple memory systems. Our EEG data showed that stress was overall associated with a larger FRN, suggesting increased striatal processing (Nieuwenhuis et al., 2005; Foti et al., 2011), in line with the assumed bias toward dorsal striatal learning after stress. The *MR* haplotype was, irrespective of stress, associated with a reduced P3a, an ERP component related to attentional and memory processes as well as hippocampal functioning (Knight, 1996; Polich, 2007). Additionally, already earlier components (P2, N2) were reduced in *MR* haplotype carriers, suggesting that early attentional processes are affected by differences in MR functionality. Corroborating an influence of the *MR* haplotype on hippocampal processing, our fMRI data revealed that stress reduced hippocampal activity, particularly in carriers of the *MR* haplotype. Together these data suggest that the *MR* haplotype is generally linked to reduced processing in cognitive areas such as the hippocampus, which may

render these areas in *MR* haplotype carriers particularly vulnerable to the impact of stress. Reduced hippocampal involvement in learning may allow the dorsal striatum to dominate learning under stress. Indeed, in contrast to the hippocampus and in line with our EEG findings, stress led to increased striatal activity during learning, irrespective of genotype. Whereas these *MR* haplotype- and stress-related changes in hippocampal and dorsal striatal activity fit very well with the existing literature, the finding that the *MR* haplotype was associated with attenuated amygdala activation after stress and, under no-stress conditions, with reduced caudate and putamen activity was less expected. Although these latter results clearly require further investigation, the reduced activations might reflect more efficient processing in *MR* haplotype carriers (Rypma et al., 2006), enabling them to shift more easily to the dorsal striatal system after stress.

In addition to these changes in single brain areas, the *MR* haplotype modulated stress-induced alterations in connectivity of the amygdala with multiple memory systems. Previous findings showed that stress increases amygdala connectivity with the dorsal striatum but reduces amygdala connectivity with the hippocampus and that these opposite changes in amygdala connectivity are abolished by an MR antagonist (Schwabe et al., 2013). In line with these findings, we obtained reduced amygdala-hippocampus connectivity under stress and the stress-induced decrease in amygdala cross talk with medial temporal cortices adjacent to the hippocampus (in particular, the parahippocampal cortex) was present only in carriers of the *MR* haplotype.

Beyond its role in cognition, the MR has been associated with negative feedback control of the HPA axis. Accordingly, pharmacological manipulations of MR functioning typically result in altered cortisol levels (Schwabe et al., 2010b; Cornelisse et al., 2011; Schwabe et al., 2013; Otte et al., 2015) and two *MR* polymorphisms have been linked to altered cortisol responses to stress (DeRijk et al., 2006; DeRijk, 2009). Since we obtained reduced cortisol levels in carriers of the MR haplotype, as one would expect in carriers of MR variants associated with enhanced functioning, only in Experiment II but not in Experiment I, our present data remain inconclusive with respect to the role of the MR haplotype in the modulation of the HPA axis. Even more important, however, is the question whether effects of the *MR* haplotype on the engagement of multiple memory systems are mainly related to altered cortisol responses. The fact that we observed an influence of the *MR* haplotype on the engagement of multiple memory systems under stress in both experiments, while its impact on cortisol concentrations was only present in one of the experiments, renders a mere dependency on different cortisol levels unlikely. Moreover, we did not find any effects of cortisol when directly testing for mediation or moderation effects and our neuroimaging results remained largely unchanged when cortisol was added as a covariate. Thus, we argue that the behavioral and neural effects of the *MR* haplotype are not owing to an altered cortisol response to a stressor, but most likely to increased efficiency in how cortisol binding to MRs induces a shift toward the dorsal striatum, i.e. in how cortisol acting through the MR can translate into behavioral changes. Determining how exactly a genetic variation in the *MR* gene translates into a more pronounced bias toward habit learning under stress remains a challenge for future molecular studies. Importantly, future studies will need to investigate additive gene dose-dependent effects of the *MR* haplotype (Hamstra et al., 2017) as well as sex-dependent effects (Vinkers et al., 2015). In particular, explorative analyses of the behavioral data of our first experiment lent some support for sex-dependent differences in the interactive influence of stress and *MR* genotype on the engagement of multiple learning strategies. Specifically, only male *MR* haplotype carriers showed enhanced use of multi-cue strategies after stress, which is in line with previous evidence in rodents (Ter Horst et al., 2013). As sex differences were not the focus of this study, the present analyses of potential sex effects are rather preliminary. Given the potential relevance of such effects in the face of different prevalences of stress-related mental disorders in men and women (Bangasser and Valentino, 2014), determining whether there are significant differences in stress $\times$ *MR* genotype interactions on the use of multiple memory systems between men and women is an important challenge for future studies. Similarly, and in line with the finding that also genetic differences in the noradrenergic system modulate stress effects on multiple memory systems (Wirz et al., 2017), it will be important for future research to investigate interactive effects of several genes, which will allow pooling of the relative small effects of individual polymorphisms.

Because of differences in the temporal resolution of the EEG and fMRI measurements, feedback timing varied between the two experiments. Importantly, whereas the striatum is highly important for immediate feedback processing, the engagement of the hippocampus increases when feedback is delayed (Foerde and Shohamy, 2011). In line with this idea, there was overall a higher percentage of single-cue strategies in our EEG experiment, in which feedback followed shortly after the response, whereas in our fMRI study, due to the slow BOLD response, feedback was delayed, leading to a generally stronger engagement of the dorsal striatal memory system. Critically, however, stress increased multi-cue learning and the *MR* haplotype modulated this effect, irrespective of these timing differences and the general differences in the distribution of the strategies between the experiments.

To conclude, we showed in two independent experiments that genetic variations in several *MR* SNPs synergistically modulate the stress-induced bias toward dorsal striatal memory and thus explain at least part of the individual variance in this bias. Although the stress-induced shift from hippocampus-dependent cognitive toward dorsal striatum-dependent habit memory may impair memory flexibility (Seehagen et al., 2015), it is thought to be generally beneficial for coping with a stressor (Vogel et al., 2016). The ability to shift flexibly between these systems may have important implications for stress-related mental disorders such as PTSD, for which

glucocorticoid-based therapeutic approaches have been proposed (de Quervain et al., 2017). In order for such interventions to be successful, personalized treatment strategies taking individual vulnerability to stress-induced changes in cognition into account are crucial. Our data suggest that, in addition to genetic variations of glucocorticoid and adrenergic receptors (de Quervain et al., 2017; Wirz et al., 2017), genetic variations of the MR may be very important in this respect.

References

Bangasser DA, Valentino RJ (2014) Sex differences in stress-related psychiatric disorders: neurobiological perspectives. Front Neuroendocrinol 35:303–319. CrossRef Medline

Cornelisse S, Joëls M, Smeets T (2011) A randomized trial on mineralocorticoid receptor blockade in men: effects on stress responses, selective attention, and memory. Neuropsychopharmacology 36: 2720–2728. CrossRef Medline

de Kloet ER, Otte C, Kumsta R, Kok L, Hillegers MH, Hasselmann H, Kliegel D, Joëls M (2016) Stress and depression: a crucial role of the mineralocorticoid receptor. J Neuroendocrinol 28:CrossRef

de Quervain D, Schwabe L, Roozendaal B (2017) Stress, glucocorticoids and memory: implications for treating fear-related disorders. Nat Rev Neurosci 18:7–19. CrossRef Medline

DeRijk RH (2009) Single nucleotide polymorphisms related to HPA axis reactivity. Neuroimmunomodulation 16:340–352. CrossRef Medline

DeRijk RH, De Kloet ER (2008) Corticosteroid receptor polymorphisms: determinants of vulnerability and resilience. Eur J Pharmacol 583:303–311. CrossRef Medline

DeRijk RH, Wüst S, Meijer OC, Zennaro MC, Federenko IS, Hellhammer DH, Giacchetti G, Vreugdenhil E, Zitman FG, de Kloet ER (2006) A common polymorphism in the mineralocorticoid receptor modulates stress responsiveness. J Clin Endocrinol Metab 91: 5083–5089. CrossRef Medline

Deuter CE, Wingenfeld K, Schultebraucks K, Hellmann-Regen J, Piber D, Otte C (2017) Effects of mineralocorticoid-receptor stimulation on risk taking behavior in young healthy men and women. Psychoneuroendocrinology 75:132–140. CrossRef Medline

Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ, Costa RM, Sousa N (2009) Chronic stress causes frontostriatal reorganization and affects decision-making. Science 325:621–625. CrossRef Medline

Elliott AE, Packard MG (2008) Intra-amygdala anxiogenic drug infusion prior to retrieval biases rats towards the use of habit memory. Neurobiol Learn Mem 90:616–623. CrossRef

Eppinger B, Kray J, Mock B, Mecklinger A (2008) Better or worse than expected? Aging, learning, and the ERN. Neuropsychologia 46:521–539. CrossRef Medline

Foerde K, Shohamy D (2011) Feedback timing modulates brain systems for learning in humans. J Neurosci 31:13157–13167. CrossRef Medline

Foerde K, Knowlton BJ, Poldrack RA (2006) Modulation of competing memory systems by distraction. Proc Natl Acad Sci USA 103:11778–11783. CrossRef Medline

Foti D, Weinberg A, Dien J, Hajcak G (2011) Event-related potential activity in the basal ganglia differentiates rewards from nonrewards: temporospatial principal components analysis and source localization of the feedback negativity. Hum Brain Mapp 32:2207–2216. CrossRef Medline

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. Science 296:2225–2229. CrossRef Medline

Gluck MA, Shohamy D, Myers C (2002) How do people solve the "weather prediction" task?: individual variability in strategies for probabilistic category learning. Learn Mem 9:408–418.

Hamstra DA, de Kloet ER, Quataert I, Jansen M, Van der Does W (2017) Mineralocorticoid receptor haplotype, estradiol, progesterone and emotional information processing. Psychoneuroendocrinology 76:162–173. CrossRef Medline

Hauser TU, Iannaccone R, Stämpfli P, Drechsler R, Brandeis D, Walitza S, Brem S (2014) The feedback-related negativity (FRN) revisited: new insights into the localization, meaning and network organization. Neuroimage 84:159–168. CrossRef Medline

Hayes AF (2013) Introduction to mediation, moderation, and conditional process analysis: a regression-based approach, Ed 1. New York: The Guilford Press.

Karst H, Berger S, Turiault M, Tronche F, Schütz G, Joëls M (2005) Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. Proc Natl Acad Sci USA 102:19204–19207. CrossRef Medline

Karst H, Berger S, Erdmann G, Schütz G, Joëls M (2010) Metaplasticity of amygdalar responses to the stress hormone corticosterone. Proc Natl Acad Sci USA 107:14449–14454. CrossRef Medline

Kim JJ, Lee HJ, Han J-S, Packard MG (2001) Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. J Neurosci 21:5222–5228. Medline

Kirschbaum C, Pirke KM, Hellhammer DH (1993) The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28:76–81. CrossRef

Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH (1999) Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamic-pituitary-adrenal axis. Psychosom Med 61:154–162. CrossRef

Klok MD, Giltay EJ, Van der Does AJ, Geleijnse JM, Antypa N, Penninx BW, de Geus EJ, Willemsen G, Boomsma DI, van Leeuwen N, Zitman FG, de Kloet ER, DeRijk RH (2011) A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. Transl Psychiatry 1:1–8. CrossRef

Knight RT (1996) Contribution of human hippocampal region to novelty detection. Nature 383:256–259. CrossRef Medline

Knowlton BJ, Squire LR, Gluck MA (1994) Probabilistic classification learning in amnesia. Learn Mem 1:106–120. Medline

Knowlton BJ, Mangels JA, Squire LR (1996) A neostriatal habit learning system in humans. Science 273:1399–1402. Medline

Lagnado DA, Newell BR, Kahan S, Shanks DR (2006) Insight and strategy in multiple-cue learning. J Exp Psychol Gen 135:162–183. CrossRef Medline

Medina A, Seasholtz AF, Sharma V, Burke S, Bunney W Jr, Myers RM, Schatzberg A, Akil H, Watson SJ (2013) Glucocorticoid and mineralocorticoid receptor expression in the human hippocampus in major depressive disorder. J Psychiatr Res 47:307–314. CrossRef

Nieuwenhuis S, Slagter HA, von Geusau NJ, Heslenfeld DJ, Holroyd CB (2005) Knowing good from bad: differential activation of human cortical areas by positive and negative outcomes. Eur J Neurosci 21:3161–3168. CrossRef Medline

Otte C, Moritz S, Yassouridis A, Koop M, Madrischewski AM, Wiedemann K, Kellner M (2007) Blockade of the mineralocorticoid receptor in healthy men: effects on experimentally induced panic symptoms, stress hormones, and cognition. Neuropsychopharmacology 32:232–238. CrossRef Medline

Otte C, Wingenfeld K, Kuehl LK, Kaczmarczyk M, Richter S, Quante A, Regen F, Bajbouj M, Zimmermann-Viehoff F, Wiedemann K, Hinkelmann K (2015) Mineralocorticoid receptor stimulation improves cognitive function and decreases cortisol secretion in depressed patients and healthy individuals. Neuropsychopharmacology 40:386–393. CrossRef Medline

Packard MG (2009) Anxiety, cognition, and habit: a multiple memory systems perspective. Brain Res 1293:121–128. CrossRef Medline

Packard MG, Wingard JC (2004) Amygdala and "emotional" modulation of the relative use of multiple memory systems. Neurobiol Learn Mem 82:243–252. CrossRef Medline

Packard MG, Goodman J (2012) Emotional arousal and multiple memory systems in the mammalian brain. Front Behav Neurosci 6:14. CrossRef Medline

Poldrack RA, Clark J, Paré-Blagoev EJ, Shohamy D, Moyano JC, Myers C, Gluck MA (2001) Interactive memory systems in the human brain. Nature 414:546–550. CrossRef Medline

Polich J (2007) Updating P300: an integrative theory of P3a and P3b. Clin Neurophysiol 118:2128–2148. CrossRef Medline

Quaedflieg CWEM, Schwabe L (2017) Memory dynamics under stress. Memory 1–13.

Rohleder N, Kirschbaum C (2006) The hypothalamic-pituitary-adrenal (HPA) axis in habitual smokers. Int J Psychophysiol 59: 236–243. CrossRef Medline

Rustemeier M, Schwabe L, Bellebaum C (2013) On the relationship between learning strategy and feedback processing in the weather prediction task–evidence from event-related potentials. Neuropsychologia 51:695–703. CrossRef Medline

Rypma B, Berger JS, Prabhakaran V, Bly BM, Kimberg DY, Biswal BB, D'Esposito M (2006) Neural correlates of cognitive efficiency. Neuroimage 33:969–979. CrossRef Medline

Schwabe L (2013) Stress and the engagement of multiple memory systems: integration of animal and human studies. Hippocampus 23:1035–1043. CrossRef Medline

Schwabe L, Wolf OT (2009) Stress prompts habit behavior in humans. J Neurosci 29:7191–7198. CrossRef Medline

Schwabe L, Wolf OT (2012) Stress modulates the engagement of multiple memory systems in classification learning. J Neurosci 32:11042–11049. CrossRef Medline

Schwabe L, Wolf OT (2013) Stress and multiple memory systems: from 'thinking' to 'doing.' Trends Cogn Sci 17:60–68. CrossRef Medline

Schwabe L, Oitzl MS, Philippsen C, Richter S, Bohringer A, Wippich W, Schachinger H (2007) Stress modulates the use of spatial versus stimulus-response learning strategies in humans. Learn Mem 14:109–116. CrossRef

Schwabe L, Dalm S, Schächinger E, Oitzl MS (2008) Chronic stress modulates the use of spatial stimulus-response learning strategies in mice and man. Neurobiol Learn Mem 90:495–503. CrossRef

Schwabe L, Wolf OT, Oitzl MS (2010a) Memory formation under stress: quantity and quality. Neurosci Biobehav Rev 34:584–591.

Schwabe L, Schächinger E, de Kloet ER, Oitzl MS (2010b) Corticosteroids operate as a switch between memory systems. J Cogn Neurosci 22:1362–1372. CrossRef Medline

Schwabe L, Dickinson A, Wolf OT (2011) Stress, habits, and drug addiction: a psychoneuroendocrinological perspective. Exp Clin Psychopharmacol 19:53–63. CrossRef

Schwabe L, Tegenthoff M, Höffken O, Wolf OT (2013) Mineralocorticoid receptor blockade prevents stress-induced modulation of multiple memory systems in the human brain. Biol Psychiatry 74:801–808. CrossRef Medline

Seehagen S, Schneider S, Rudolph J, Ernst S, Zmyj N (2015) Stress impairs cognitive flexibility in infants. Proc Natl Acad Sci USA 112:12882–12886. CrossRef Medline

Shohamy D, Myers CE, Grossman S, Sage J, Gluck MA, Poldrack RA (2004) Cortico-striatal contributions to feedback-based learning: converging data from neuroimaging and neuropsychology. Brain 127:851–859. CrossRef Medline

Soares JM, Sampaio A, Ferreira LM, Santos NC, Marques F, Palha JA, Cerqueira JJ, Sousa N (2012) Stress-induced changes in human decision-making are reversible. Transl Psychiatry 2:e131. CrossRef

Steyer R, Schwenkmezger P, Notz P, Eid M (1994) Theoretical analysis of a multidimensional mood questionnaire (MDBF). Diagnostica 40:320–328.

ter Heegde F, De Rijk RH, Vinkers CH (2015) The brain mineralocorticoid receptor and stress resilience. Psychoneuroendocrinology 52:92–110. CrossRef Medline

ter Horst JP, van der Mark MH, Arp M, Berger S, de Kloet ER, Oitzl MS (2012) Stress or no stress: mineralocorticoid receptors in the forebrain regulate behavioral adaptation. Neurobiol Learn Mem 98:33–40. CrossRef Medline

Ter Horst JP, Kentrop J, Arp M, Hubens CJ, de Kloet ER, Oitzl MS (2013) Spatial learning of female mice: a role of the mineralocorticoid receptor during stress and the estrous cycle. Front Behav Neurosci 7:56. CrossRef Medline

van der Helden J, Boksem MA, Blom JH (2010) The importance of failure: feedback-related negativity predicts motor learning efficiency. Cereb Cortex 20:1596–1603. CrossRef Medline

van Leeuwen N, Kumsta R, Entringer S, de Kloet ER, Zitman FG, DeRijk RH, Wüst S (2010) Functional mineralocorticoid receptor (MR) gene variation influences the cortisol awakening response after dexamethasone. Psychoneuroendocrinology 35:339–349. CrossRef Medline

van Leeuwen N, Bellingrath S, de Kloet ER, Zitman FG, DeRijk RH, Kudielka BM, Wüst S (2011) Human mineralocorticoid receptor (MR) gene haplotypes modulate MR expression and transactivation: implication for the stress response. Psychoneuroendocrinology 36:699–709. CrossRef Medline

Vinkers CH, Joëls M, Milaneschi Y, Gerritsen L, Kahn RS, Penninx BW, Boks MP (2015) Mineralocorticoid receptor haplotypes sex-dependently moderate depression susceptibility following childhood maltreatment. Psychoneuroendocrinology 54:90–102. CrossRef Medline

Vogel S, Klumpers F, Krugers HJ, Fang Z, Oplaat KT, Oitzl MS, Joëls M, Fernández G (2015) Blocking the mineralocorticoid receptor in humans prevents the stress-induced enhancement of centromedial amygdala connectivity with the dorsal striatum. Neuropsychopharmacology 40:947–956. CrossRef Medline

Vogel S, Fernández G, Joëls M, Schwabe L (2016) Cognitive adaptation under stress: a case for the mineralocorticoid receptor. Trends Cogn Sci 20:192–203. CrossRef Medline

Wirz L, Bogdanov M, Schwabe L (2018) Habits under stress: mechanistic insights across different types of learning. Curr Opin Bevah Sci 20:9–16. CrossRef

Wirz L, Wacker J, Felten A, Reuter M, Schwabe L (2017) A deletion variant of the α2b-adrenoceptor modulates the stress-induced shift from "cognitive" to "habit" memory. J Neurosci 37:2149–2160. CrossRef

Appendix C

Wirz L, Reuter M, Felten A, Schwabe L (*under revision*) An endocannabinoid receptor polymorphism modulates affective processing under stress. *Soc Cogn Affect Neurosci*.

An endocannabinoid receptor polymorphism modulates affective processing under stress

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Abstract

Stress has a critical impact on affective and cognitive processing. Based on rodent data suggesting that endocannabinoid signaling via CB1 receptors serves as an emotional buffer, we hypothesized that a common variant of the gene coding for the CB1 receptor modulates affective processing under stress (CNR1; rs1049353 A vs. G allele). Therefore, 139 participants, genotyped for this polymorphism, underwent a stress or control manipulation before they viewed emotionally neutral and negative pictures in an MRI scanner. The ventromedial prefrontal cortex, known for its crucial role in emotion regulation, was significantly more activated in A vs. homozygous G allele carriers when viewing negative pictures after stress. Already at rest, AA/AG genotype carriers showed enhanced crosstalk between the ventrolateral prefrontal cortex and the amygdala. We further assessed participants' 24hours-delayed memory for the presented pictures and found that memory performance correlated with amygdala and hippocampus activity and connectivity in stressed carriers of the AA/AG but not the GG genotype. These findings underline the modulatory role of the endocannabinoid system in stress effects on emotion and cognition and provide insights into the neural mechanisms that may contribute to the suggested protective effect of the AA/AG genotype of the CB1 receptor polymorphism against stress-related psychopathologies.

Keywords: endocannabinoids, stress, medial prefrontal cortex, affective processing, emotional memory

Introduction

Stressful events have a major impact on mental health and may contribute to psychopathologies such as addiction, posttraumatic stress disorder (PTSD) or major depression (McEwen, 2004; de Kloet et al., 2005; Chrousos, 2009). These psychopathologies are at least partly driven by stress-induced changes in affective processing (Karl et al., 2006; Leppänen, 2006). Indeed, there is strong evidence that stress alters our responses to emotional stimuli (Ellenbogen et al., 2002; van Stegeren et al., 2005; Fox et al., 2010; Weymar et al., 2012). These changes in affective processing have mainly been attributed to the effects of stress-induced increases in catecholamines and glucocorticoids on the amygdala, prefrontal cortex (PFC) and hippocampus (de Kloet et al., 2005; Arnsten, 2009; Joels and Baram, 2009; Schwabe et al., 2013), brain regions crucial for affective processing (Sergerie et al., 2008; Kalisch, 2009; Etkin et al., 2011). Recent findings, however, point to another important player in the effects of stress on affective processing: the endocannabinoid (eCB) system (Campolongo and Trezza, 2012; Morena et al., 2016).

The eCB system is a lipid signaling system in the brain that modulates neurotransmitter release (Kogan and Mechoulam, 2006). The system is composed of the eCB ligands anandamide and 2-arachidonoylglycerol (2-AG), the cannabinoid receptors CB1 and CB2, and the enzymes involved in endocannabinoid synthesis and metabolism (FAAH for anandamide and MAGL for 2-AG; Mechoulam and Parker, 2013). ECBs and CB1 receptors are abundantly present in the amygdala, hippocampus and PFC (CB2 receptors are located mainly in the periphery; McPartland et al., 2007; Morena and Campolongo, 2014). In addition, eCBs are rapidly synthesized on demand and retrogradely activate CB1 receptors in these brain regions, thus putting the eCB system and in particular CB1 receptors in a prime position to modulate stress effects on affective processing. In line with this idea, rodent studies showed that eCB signaling via the CB1 receptor can regulate activation of the hypothalamus-pituitary-adrenal (HPA) axis and modulate affective processing under stress (Lutz, 2009; McLaughlin et al., 2012; Wang et al., 2012; Bedse et al., 2014; Gray et al., 2015). For instance, injection of a CB1 receptor agonist into the basolateral part of the amygdala (BLA) prevented the stress-induced glucocorticoid increase in rats (Ganon-Elazar and Akirav, 2009) and anandamide was reported to have anxiolytic effects (Lutz et al., 2015). Furthermore, administration of a CB1 receptor antagonist into the medial PFC prolonged the corticosterone response to a stressor, suggesting that termination of HPA axis activation by glucocorticoids within the medial PFC critically depends on eCB signaling via the CB1 receptor (Hill et al., 2011). Thus, the

eCB system has been suggested to act as an emotional buffer system that is crucial for appropriate affective responding (Lutz, 2009; Campolongo and Trezza, 2012; McLaughlin et al., 2014; Lutz et al., 2015).

So far, experimental evidence for a role of the eCB system in affective processing under stress comes almost exclusively from animal studies. There is, however, first evidence that at least some of the animal findings can be translated to humans. In particular, clinical studies tested the effects of the CB1 receptor antagonist rimonabant and showed that it led to decreased activity of brain reward regions in response to pleasant stimuli (Horder et al., 2010) and to a negative bias in memory recall (Horder et al., 2009; Horder et al., 2012). However, because rimonabant administration also led to significant increases in anxiety and depressive mood (Mitchell and Morris, 2007; Hill and Gorzalka, 2009; Goodwin et al., 2012), which further underlines the relevance of CB1 receptors in affective processing, it had to be taken off the market and pharmacological manipulations of the eCB system are thus not feasible in humans anymore. Furthermore, studies on the effects of cannabis and CB1 receptor agonists are less informative in this context, because the effects of exogenous cannabinoids may be substantially different since they lack the spatial and temporal specificity of endogenous eCBs (Steiner and Wotjak, 2008; Akirav, 2011, 2013). An alternative strategy to target the function of eCBs in humans, however, is a behavioral genetics approach, employing the individual genetic variance in eCB activity. Single nucleotide polymorphisms (SNPs) in the CB1 receptor gene (CNR1) have been linked to mood and anxiety disorders, such as PTSD and major depression (Hillard et al., 2012). In particular, the minor A allele of the exonic rs1049353 polymorphism has been proposed as a protective factor that reduces the risk of depression after stressful events (Agrawal et al., 2012), whereas carriers of the major G allele were found to be at higher risk for antidepressant treatment resistance (Domschke et al., 2008). While these findings suggest that a genetic variant of the CB1 receptor may be linked to stress-related psychopathologies, how eCBs and in particular this CB1 receptor polymorphism (rs1049353) may alter affective processing in humans under stress, is completely unknown.

The primary aim of the present study was therefore to determine if and how a genetic variant of the CB1 receptor gene (rs1049353) modulates the neural processing of affective information after stress. For this purpose, healthy participants were genotyped for the rs1049353 polymorphism and randomly assigned to a stress (Trier Social Stress Test [TSST]; Kirschbaum et al., 1993) or control manipulation. Following the experimental manipulation, participants were presented with

emotionally negative and neutral pictures, while their brain activity was measured using functional magnetic resonance imaging (fMRI). We hypothesized that the CB1 receptor polymorphism (rs1049353) would modulate the stress effects on activity in brain regions that are crucial for affective processing, such as the amygdala and the medial PFC. In particular, we predicted that in response to the stress manipulation, carriers of the proposed protective A allele, compared to G allele carriers, would show reduced amygdala activity and increased activity in prefrontal areas that are implicated in emotion regulation (Urry et al., 2006; Banks et al., 2007). Although this study focused mainly on the modulatory role of the rs1049353 genotype in affective processing after stress, we were also interested in potential effects of this polymorphism on subsequent memory for the neutral and emotional stimuli, because eCBs are also thought to play a crucial role in the emotional modulation of memory (Campolongo et al., 2009; Atsak et al., 2015) and emotional memory processes are highly relevant in stress-related psychopathologies such as PTSD (Pitman et al., 2012; de Quervain et al., 2017). Therefore, participants additionally completed free recall and recognition tests for the presented pictures 24 hours after encoding. In terms of the modulation of emotional memory processes under stress, we expected that the CB1 receptor polymorphism might modulate the activity and interplay of the amygdala and hippocampus, the two key regions in emotional memory formation (Cahill and McGaugh, 1998; McGaugh, 2004; Roozendaal et al., 2009).

Materials and Methods

Participants and experimental design

In total, 139 young, healthy, normal-weight volunteers (67 women; mean age = 23.4 ± SD: 3.5 years; mean body-mass index [BMI] = 22.51 kg/m^2, SD = 2.27) participated in this experiment. Exclusion criteria, assessed by means of a standardized interview, included medication or drug intake (including cannabis consumption), any past or current neurological or psychiatric disorders, smoking, a BMI < 18 kg/m^2 or > 26 kg/m^2, as well as any contraindications to fMRI measurements. In addition, women were not tested during their menses. The experiment was approved by the ethical review board of the German Psychological Society (reference: LS072014) and the local ethics committee at the University of Bonn and is in accordance with the Declaration of Helsinki. This experiment is part of a larger study on genotype-dependent differences in cognitive processes under stress (Wirz et al., 2017).

A 2 x 2 x 2 mixed design with the between-subjects factors treatment (stress vs. control manipulation) and CNR1 genotype (homo- and heterozygous A allele carriers vs. homozygous G allele carriers) and the within-subject factor emotionality (negative vs. neutral) was used to investigate CNR1 genotype-dependent differences in stress effects on neural processing of affective information. Participants were randomly assigned to a stress or control condition. Technical difficulties and excessive head motion in the MRI scanner led to the exclusion of two participants. Additionally, three participants had to be excluded due to missing picture ratings or recall data, leading to a final sample of 134 participants (stress: 34 males, 33 females, control: 36 males, 31 females). For the genetic analyses, another three participants had to be excluded due to missing data for the CNR1 single nucleotide polymorphism (SNP) of interest (131 participants; stress-AA/AG genotype: 16 males, 17 females, stress-GG genotype: 18 males, 15 females; control-AA/AG genotype: 19 males, 8 females, control-GG genotype: 17 males, 21 females).

Genetic analyses

Participants were genotyped for the rs1049353 SNP of the gene coding for the CB1 receptor on chromosome 6q14-q15 (CNR1). This SNP is located on the coding exon of CNR1 and has been associated with depression in response to stress exposure as well as with post-traumatic stress disorder (Hill and Patel, 2013; Mota et al., 2015). For genetic analysis, DNA was extracted from buccal cells. Automated purification of genomic DNA was conducted by means of the MagNA Pure® LC system using a commercial extraction kit (MagNA Pure LC DNA isolation kit; Roche Diagnostics, Mannheim, Germany). Genotyping of the MR polymorphisms was performed by MALDI-TOF mass spectrometry using the iPLEX assay and the Sequenom MassARRAY platform. For all further analyses, homo- and heterozygous carriers of the rs1049353 minor allele (A), which seems to be protective against the effects of stress (Hill and Patel, 2013), were treated as one group and tested against homozygous carriers of the major allele (G).

Stress and control manipulation

In the stress condition, participants underwent the TSST, a commonly used laboratory stressor that has been shown to induce a reliable increase in autonomic nervous system and HPA axis activity (Kirschbaum et al., 1993). In a mock-job interview, participants were introduced to a reserved and non-reinforcing panel that evaluated participants' performance on two tasks. The first task consisted of a 5 min free speech about why he or she is the ideal candidate for a job

tailored to his or her interests, whereas in the second task the participant was asked to count backwards from 2043 in steps of 17 for another 5 minutes. During both tasks, participants were videotaped. In the control condition, the participant was alone in the room, without video recordings, talked about a self-chosen topic and performed an easy calculation task (counting forward in steps of 15).

The effectiveness of the stress induction was assessed by means of questionnaires, blood pressure measurements and saliva samples. Changes in subjective mood were evaluated with a German mood questionnaire (MDBF; subscales: depressed vs. elevated, restless vs. calm, sleepy vs. awake; high scores indicate elevated mood, calmness and wakefulness; Steyer et al., 1994) and an additional questionnaire in which participants rated on a scale from 0 ('not at all') to 100 ('very much') how difficult, unpleasant and stressful they had experienced the stress or control manipulation. Blood pressure was measured using a Dinamap system (Critikon, Tampa, USA) before (-25 min), during (+10 min) and at several time points after the experimental treatment (+20 min, +90 min). To assess the HPA axis response to the TSST and control manipulation, saliva samples were collected before (-25 min) and after (+20 min, +30 min, +90 min) the experimental treatment using Salivette® collection devices (Sarstedt, Nümbrecht, Germany). Saliva samples were stored at -18 °C until the end of the experiment, when the free fraction of cortisol was determined using chemiluminescence immunoassays (IBL, Hamburg, Germany).

Affective picture task

In order to investigate CNR1 genotype-dependent differences in the neural processing of emotional information under stress, participants viewed negative and neutral pictures while fMRI was recorded. Pictures were taken from the International Affective Picture System (IAPS; Lang et al., 2008) and an in-house database which includes pictures depicting scenes with a more contemporary relevance (e.g. pictures of refugees). On the basis of previous valence ratings on a scale from 0 ('negative') over 50 ('neutral') to 100 ('positive'), the pictures were categorized as emotionally negative (24.19 ± 7.38) and emotionally neutral (55 ± 7.42), with 25 pictures in each emotionality category. These previous ratings (0 = 'not arousing', 100 = 'very arousing') showed that mean arousal levels were significantly larger for negative (50.32 ± 8.93) compared to neutral pictures (11.35 ± 2.84; $t(18) = 18.78$, $p < 0.001$). In the current study, the pictures were presented for 2.5 sec in the middle of the screen in a quasi-randomized order, ensuring that no more than two pictures of the same emotionality were seen one after another. Participants were asked to rate the

pictures on a four-point scale ('negative' [1], 'rather negative' [2], 'rather neutral' [3], 'neutral' [4]), but they were not explicitly instructed to memorize the pictures for a subsequent memory test. Between pictures, there were fixation periods of 6-10 sec (mean = 7 sec), resulting in a total task duration of 8 minutes.

Experimental procedure

All testing took place in the afternoon to control for the diurnal rhythm of cortisol. After participants had given written informed consent, buccal cells were collected for later genetic analyses. Participants then underwent the stress or control manipulation before they were placed inside the MRI scanner. Approximately 50 minutes after the onset of the stress or control manipulation, participants performed the affective picture task. At the end of the experiment, participants received a moderate monetary compensation (35 €). Although this experiment focused mainly on the modulatory effect of a CB1 receptor polymorphism (rs1049353) on the influence of stress on the neural processing of affective material, we aimed also to assess potential effects on emotional memory formation. To this end, participants were called 24 hours after the affective picture task and asked to describe as many pictures as possible in as much detail as possible, so that the experimenter knew for sure to which picture the participant was referring to. When more than 60 sec had elapsed after the last picture was recalled, a link to a forced choice recognition test was sent to the participants which they completed immediately after the free recall test. In the recognition test, participants saw all pictures they had seen the day before, as well as 25 negative and 25 neutral pictures that were not presented before in a randomized order. Participants indicated by button press whether they thought it was an old or a new picture and additionally specified whether they were 'very sure', 'rather sure', 'rather unsure', or 'sure' in their decision.

Behavioral and physiological data analyses

Physiological, subjective, and behavioral parameters were analyzed using mixed-design analyses of variance (ANOVAs) with time as within-subject factor and treatment (stress vs. control manipulation) as well as CNR1 genotype (AA/AG vs. GG genotype carriers) as between-subjects factors. For the analyses of picture ratings, certainty ratings, free recall and recognition performance we added emotionality (negative vs. neutral) as within-subject factor. For our memory analyses, we focused on the number of correctly recalled pictures in the free recall test

as well as hits and false alarms in the recognition test. In addition, the sensitivity index d-prime (d') was calculated for negative and neutral pictures, using hits and false alarms according to signal detection theory (Wickens, 2002), because this measure corrects for individual response biases. Statistical analyses were performed using SPSS Statistics 22 (IBM, Armonk, USA). All reported P-values are two-tailed and in case of violation of the sphericity assumption, Greenhouse-Geisser correction was applied.

MRI acquisition and analyses

Functional MRI measurements were acquired using a 3 T Trio Scanner (Siemens, München, Germany) with a 32-channel head coil. BOLD T2-weighted echoplanar functional images parallel to the anterior commissure-posterior commissure plane (37 transversal slices; TR = 2000 ms; TE = 30 ms; ascending acquisition; effective voxel size = 3x3x3 mm) and a high-resolution T1-weighted anatomical image (208 sagittal slices, TR = 1660 ms, TE = 2.54 ms, voxel size = 0.8 x 0.8 x 0.8 mm) were acquired.

FMRI preprocessing and data analyses using general linear modeling were performed using the SPM12 Matlab toolbox (Wellcome Trust Centre for Neuroimaging, London, UK). Functional data were slice-time and head-motion corrected as well as coregistered to the structural image using rigid-body transformations. The T1-weighted image was segmented into gray and white matter, cerebro-spinal fluid, bone, soft tissue and air. Using forward deformation fields, the functional and structural scans were spatially normalized to the Montreal Neurological Institute standard brain. Finally, an 8 mm full-width half-maximum Gaussian kernel was used to smooth the normalized functional images.

Negative and neutral picture trials were modeled using canonical hemodynamic response functions. Fixation, button press and the six movement parameters were included as regressors of no interest. A temporal high-pass filter with a 128 s cutoff was used and contrast images were generated for negative minus neutral picture trials. These difference contrasts were then entered into second-level (group) analyses using a full-factorial model with the factors treatment (stress vs. control manipulation) and CNR1 genotype (AA/AG vs. GG genotype carriers). Exploratory whole brain analyses as well as region of interest (ROI) analyses were used. A priori ROIs were cortico-limbic structures known to be involved in affective processing and memory formation (i.e. the amygdala, insula and hippocampus; McGaugh, 2000; Phan et al., 2002), as well as PFC areas

(medial PFC [mPFC], ventromedial PFC [vmPFC}, ventrolateral PFC [vlPFC]) that play a pivotal role in emotion regulation (Wager et al., 2008; Motzkin et al., 2015). Anatomical masks of subcortical brain regions (amygdala, insula, hippocampus) and the mPFC were taken from the Harvard-Oxford Atlas with a probability threshold of 50 %, so that only voxels with a probability of at least 50 % to belong to each brain region were included. Anatomical masks of the vmPFC and vlPFC were created using MARINA software (http://www.bion.de/eng/MARINA.php). For the exploratory whole-brain analyses, the significance threshold was set to $p < 0.05$ at cluster level (in a minimum of five adjacent voxels) and corrected for multiple testing (family-wise error [FWE] correction). ROI analyses using small-volume correction (SVC) with an initial threshold of $p < 0.05$ uncorrected were followed by FWE correction ($p < 0.05$). Within a ROI, only clusters of at least 5 significant voxels are reported.

To assess group differences in the connectivity between our ROIs, we performed Psycho-Physiological Interaction (PPI) analyses. Accordingly, the first eigenvariate of the time course of our ROIs in the contrast negative minus neutral was extracted from the appropriate brain atlases and used as seed region. A general linear model with a physiological regressor (time course response in the seed region), a psychological regressor (negative minus neutral pictures) and a PPI regressor, which was calculated as the cross-product of the previous two regressors, was computed. The individual PPI contrasts were then entered into second-level random-effects analyses. As these analyses reveal brain regions with a similar and task-dependent activation pattern, these regions are supposed to be functionally connected during the processing of negative versus neutral pictures.

To investigate whether brain activation and connectivity during picture processing were directly associated with subsequent memory performance, we correlated brain activity and functional connectivity of our ROIs to participants' individual memory performances. For this purpose, we ran a second full-factorial model and PPI models with memory performance entered as a covariate. We then extracted the contrast values of the significant clusters of voxels with Marsbar (http://marsbar.sourceforge.net/) and correlated these with the participants' memory performance. To compare correlations between our experimental groups, we additionally ran separate models for A and G allele carriers in the stress and control group, including memory performance as a covariate. Subsequently, contrast estimates were correlated with participants'

memory performance. Correlation coefficients were then transformed using the Fisher's r-to-z transformation and the resulting z-scores were statistically compared.

Results

Genetic analyses

Genotyping participants for the rs1049353 SNP of the CNR1 gene coding for the CB1 receptor revealed 74 (53.2 %) homozygous G allele, 4 (2.9 %) homozygous A allele and 58 (41.7 %) heterozygous G/A allele carriers. In line with previous studies (Agrawal et al., 2012; Mota et al., 2015) and due to the small number of homozygous A allele carriers, homo- and heterozygous carriers were treated as one group (62 AA/AG genotype carriers [44.6 %]) and tested against homozygous G allele (GG genotype) carriers. Allele frequencies (minor allele frequency = 24.26 %, major allele frequency = 75.74 %) were in accordance with those documented by the National Center for Biotechnology Information (NCBI) for Europeans and in Hardy-Weinberg equilibrium ($\chi^2(1) = 3.50$, $P = 0.061$). GG and AA/AG genotype carriers were equally distributed in the stress (33 AA/AG genotype carriers, 38 GG genotype carriers) and control group (28 AA/AG genotype carriers, 33 GG genotype carriers; $\chi^2(1) = 0.77$, $P = 0.381$).

Successful stress induction by the TSST

Subjective mood, as well as blood pressure and cortisol concentrations significantly changed in response to the TSST and verified the successful stress induction. Independent of CNR1 genotype, exposure to the TSST was rated as significantly more difficult, unpleasant and stressful than the control manipulation (all $F(1,132) \geq 71.62$, all $P < 0.001$; Table 1). In addition, GG genotype carriers of the rs1049353 SNP were overall more restless than AA/AG genotype carriers ($F(1,130) = 4.92$, $P = 0.028$). More importantly, however, independent of CNR1 genotype, participants' mood decreased and they became increasingly restless in response to the TSST compared to the control condition (time×treatment: both $F(2,129) \geq 21.45$, both $P < 0.001$; Table 1). Independent of CNR1 genotype and treatment condition, participants became increasingly tired during the course of the experiment (time: $F(2,129) = 96.06$, $P < 0.001$).

Table 1. Subjective stress response

	Control		Stress	
	AA/AG genotype	GG genotype	AA/AG genotype	GG genotype
Subjective assessment				
Stressful	24.64 ± 4.07	31.03 ± 3.36	64.12 ± 3.56	67.43 ± 3.49***
Difficult	25.71 ± 4.32	26.41 ± 3.30	70.88 ± 4.01	71.14 ± 3.40***
Unpleasant	28.93 ± 4.78	37.69 ± 3.93	66.18 ± 4.35	70.57 ± 3.50***
Subjective mood				
Good vs. bad mood				
Before treatment	33.86 ± 0.71	34.45 ± 0.82	34.38 ± 0.73	34.43 ± 0.65
1 min after treatment	32.64 ± 0.82	34.15 ± 0.78	28.15 ± 1.13	27.91 ± 1.16***
75 min after treatment	32.14 ± 0.96	32.85 ± 0.81	30.35 ± 1.09	31.31 ± 0.82
Calm vs. restless				
Before treatment	29.93 ± 0.96	33.26 ± 0.84	30.97 ± 0.98	32.60 ± 0.71
1 min after treatment	29.11 ± 1.03	31.38 ± 0.91	23.76 ± 1.15	24.79 ± 1.07***
75 min after treatment	30.68 ± 0.88	32.00 ± 0.98	31.59 ± 0.98	31.80 ± 0.67
Overall calm vs. restless	29.90 ± 0.78	32.13 ± 0.79	28.77 ± 0.86	29.82 ± 0.58#
Tired vs. awake				
Before treatment	29.93 ± 1.07	30.76 ± 0.98	30.03 ± 0.78	30.43 ± 0.85
1 min after treatment	28.82 ± 1.13	30.10 ± 0.99	28.82 ± 0.95	28.24 ± 0.88
75 min after treatment	22.93 ± 0.97	24.26 ± 1.00	21.38 ± 1.01	23.51 ± 0.97

Data represent means ± SEM. bpm – beats per minute.
Time × Treatment (stress vs. control) *** $P < 0.001$ ** $P < 0.01$ * $P < 0.05$
CNR1 genotype (AA/AG vs. GG) # $P < 0.05$

Exposure to the TSST, compared to the control condition, further led to a significant increase in cortisol concentrations (time×treatment: $F(3,130) = 17.73$, $P < 0.001$; Figure 1A), which provides evidence for a stress-induced activation of the HPA axis. Stress-induced increases in salivary cortisol were not affected by CNR1 genotype (all main and interaction effects: all $F \leq 0.82$, all $P \geq 0.368$). Finally, activity of the autonomic nervous system significantly increased in response to the TSST, but not after the control manipulation, as was shown by significant increases in systolic and diastolic blood pressure (time×treatment: both $F \geq 17.51$, both $P \leq 0.001$). Although systolic blood pressure was not influenced by CNR1 genotype (time×genotype: $F(3,129) = 1.43$, $P = 0.236$; genotype: $F(1,131) = 0.55$, $P = 0.460$; Figure 1B), a time×treatment×genotype interaction for diastolic blood pressure ($F(3,129) \geq 3.49$, $P = 0.018$) showed that in rs1049353 GG genotype carriers, diastolic blood pressure increased during and immediately following the stress induction (both

F(1,72) ≥ 7.57, both P ≤ 0.008), whereas in AA/AG genotype carriers no such effect was observed (both F(1,59) ≤ 1.15, both P ≥ 0.287; Figure 1C).

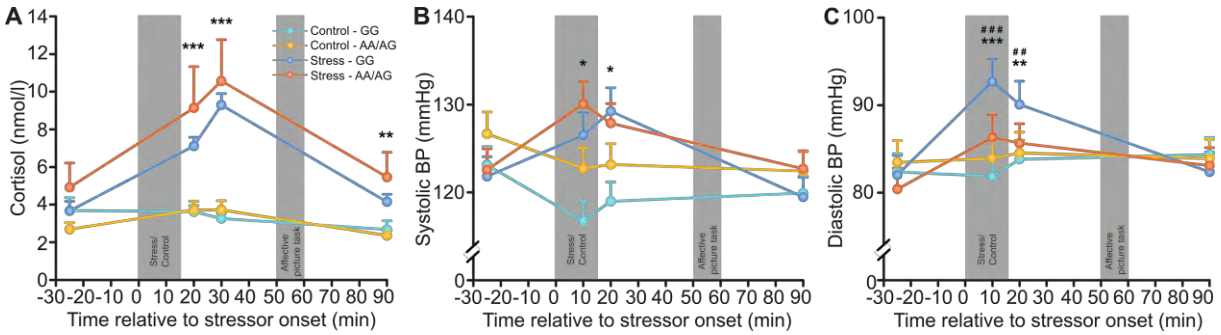

Figure 1. Physiological changes in the experimental groups. Independent of *CNR1* genotype and compared to a non-stressful control manipulation, exposure to the Trier Social Stress Test led to significant increases in (A) salivary cortisol concentrations and (B) systolic blood pressure. Whereas stress also increased (C) diastolic blood pressure, this increase was only observed in rs1049353 GG genotype carriers. Stress vs. Control *** $P < 0.001$ * $P < 0.05$, Stress/GG genotype vs. all other groups ### $P < 0.001$ # $P < 0.01$, error bars represent SEM.

Neural correlates of affective picture processing

As expected, pictures that were a priori classified as negative were rated as significantly more negative (mean = 1.6, SD = 0.39) than those that had been classified as neutral (mean = 3.57, SD = 0.42; F(1,130) = 1033.02, P < 0.001; Table 2). The experimental manipulation (stress vs. control) and the CNR1 genotype had no influence on these emotionality ratings (all F ≤ 0.77, all P ≥ 0.381). With respect to reaction times, participants were faster to respond to neutral than negative pictures (F(1,129) = 5.28, P = 0.023), which is in line with previous studies showing that emotional stimuli automatically capture our attention and are, for example, viewed longer than neutral pictures (Hajcak et al., 2010), which might indicate more in-depth processing of negative material as is supported by EEG studies with a high temporal sensitivity (Palomba et al., 1997; Hajcak et al., 2010). In addition, G allele carriers rated the pictures, irrespective of picture emotionality, faster than AA/AG genotype carriers (F(1,129) = 4.04, P = 0.047).

Table 2. Picture ratings and memory performance

	Control		Stress	
	AA/AG genotype	GG genotype	AA/AG genotype	GG genotype
Picture ratings				
Negative pictures	1.62 ± 0.07	1.56 ± 0.06	1.67 ± 0.08	1.58 ± 0.06***
Neutral pictures	3.55 ± 0.09	3.56 ± 0.06	3.55 ± 0.09	3.60 ± 0.07
Free recall				
Negative pictures	6.61 ± 0.53	5.95 ± 0.35	6.52 ± 0.34	6.74 ± 0.53***
Neutral pictures	3.07 ± 0.38	3.41 ± 0.41	3.39 ± 0.26	3.76 ± 0.38
Recognition				
Negative pictures hit rate	92.67 ± 1.00	91.18 ± 1.26	88.85 ± 1.54	86.94 ± 2.52***
Neutral pictures hit rate	85.26 ± 2.20	86.15 ± 1.89	84.85 ± 2.22	83.82 ± 2.32
Negative pictures false alarm rate	9.19 ± 1.68	9.44 ± 1.34	9.45 ± 2.49	9.88 ± 1.36***
Neutral pictures false alarm rate	17.56 ± 1.76	21.95 ± 2.66	19.64 ± 2.31	21.41 ± 2.75
Certainty ratings				
Negative pictures	3.71 ± 0.04	3.62 ± 0.04	3.62 ± 0.05	3.60 ± 0.05***
Neutral pictures	3.43 ± 0.06	3.40 ± 0.07	3.41 ± 0.06	3.47 ± 0.06
D-prime				
Negative pictures	3.04 ± 0.12	2.98 ± 0.12	2.88 ± 0.15	2.76 ± 0.14***
Neutral pictures	2.18 ± 0.13	2.09 ± 0.12	2.13 ± 0.13	2.04 ± 0.12

Table shows picture ratings as well as free recall and recognition performance in dependence of experimental manipulation (TSST vs. control condition) and *CNR1* genotype (AA/AG vs. GG). Data represent means ± SEM. Emotionality (negative vs. neutral) *** $P < 0.001$

In line with previous findings (van Stegeren, 2009; van Stegeren et al., 2010; Etkin et al., 2011), negative (vs. neutral) picture processing led overall to significant increases in activation in brain regions associated with affective processing and emotion regulation. Specifically, irrespective of stress and CNR1 variant, the presentation of negative pictures increased bilateral activation of the amygdala (right: t = 10.81, PFWE < 0.001, k = 84; left: t = 10.68, PFWE < 0.001, k = 64), vlPFC (right: t = 6.01, PFWE < 0.001, k = 36, left: t = 4.74, PFWE < 0.001, k = 46), vmPFC (right: t = 6.07, PFWE < 0.001, k = 114; left: t = 6.31, PFWE < 0.001, k = 138) and insula (right: t = 8.78, PFWE < 0.001, k = 122), with the left insula even surviving FWE-correction at whole-brain level (left: t = 11.59, PFWE < 0.001, k = 2,053). In addition, the occipital inferior gyrus (left: t = 12.33, PFWE < 0.001, k = 1,242) and anterior parietal regions (postcentral gyrus [right: t = 17.74, PFWE < 0.001, k = 1,226], supramarginal gyrus [left: t = 6.15, PFWE < 0.001, k = 20]), which have been associated with emotional arousal and the regulation of an individual's internal state ('as-if-body-loop'; (Damasio et al., 2000; Anders et al.,

2004), were more strongly activated when negative pictures were presented (see Table 3). No brain regions were stronger activated during neutral compared to negative picture presentation.

Table 3. Significantly activated cluster peak voxels during negative picture processing

	MNI coordinates (mm)					
Negative > Neutral	Cluster size	x	y	z	t_{max}	$P_{FWE\text{-}corr}$
R postcentral gyrus	1,226	51	-19	59	17.74	<0.001
R temporal inferior gyrus	917	45	-64	-7	17.51	<0.001
L medial superior frontal gyrus	1,069	-6	50	20	12.56	<0.001
L occipital inferior gyrus	1,242	-42	-76	-4	12.33	<0.001
L insula	2,053	-30	17	-16	11.59	<0.001
L fusiform gyrus	19	-30	-7	-34	6.95	<0.001
L posterior cingulate	25	0	-49	26	6.50	<0.001
L medial OFC	51	-3	44	-19	6.31	<0.001
L supramarginal gyrus	20	-66	-28	35	6.15	<0.001
R fusiform gyrus	12	30	-7	-34	5.47	<0.001
R amygdala	84	24	-1	-16	10.81	<0.001*
L amygdala	64	-21	-4	-16	10.68	<0.001*
L ventromedial PFC	20	-6	50	11	9.24	<0.001*
R insula	122	33	14	-16	8.78	<0.001*
R ventromedial PFC	19	0	50	-19	6.07	<0.001*
R ventrolateral PFC	12	54	32	8	6.01	<0.001*

Table shows local maxima of functional voxels (normalized voxel size = 3 x 3 x 3 mm^3). MNI Montreal Neurological Institute, corr corrected, PFC prefrontal cortex, OFC orbitofrontal cortex, FWE family-wise error. All labels are taken from the Automatic Anatomical Labeling (ALL) atlas. The significance threshold was set to p<0.05 (FWE corrected). * small volume corrected; all other activations are sig. at whole brain level.

CNR1 genotype modulates prefrontal activity and connectivity with the amygdala during affective picture processing

Corroborating earlier studies that emphasized an essential role of endocannabinoids in the maintenance of emotional homeostasis in the face of a stressor (Lutz, 2009; McLaughlin et al., 2014), the rs1049353 SNP modulated brain activation in response to negative (vs. neutral) pictures under stress. Specifically, we observed an interaction between treatment (stress vs. control) and CNR1 genotype (rs1049353 A vs. G allele) on vmPFC activity for negative vs. neutral pictures (left: t = 3.55, PFWE = 0.034, k = 24; Figure 2A). Post-hoc tests revealed that under stress, AA/AG compared to GG genotype carriers showed enhanced activity of the vmPFC (left: t = 3.64, PFWE = 0.035, k = 22), whereas there were no genotype-dependent effects in the control condition. No main effects

of CNR1 genotype or treatment were observed, nor was any other brain area significantly modulated by CNR1 genotype or treatment (no suprathreshold clusters).

In order to gain insight into the network structure underlying the modulatory effect of the CNR1 genotype on stress-induced changes in neural affective processing, we performed, in a next step, functional connectivity analyses. These analyses revealed a significant CNR1 genotype × treatment interaction for the coupling of the vlPFC and the amygdala (t = 3.43, PFWE = 0.013, k = 27). As displayed in Figure 2B, AA/AG compared to GG genotype carriers showed significantly increased vlPFC-amygdala connectivity under no-stress control conditions during negative picture processing (t = 3.76, PFWE = 0.007, k = 38), whereas genotype groups did not differ after stress (no suprathreshold clusters).

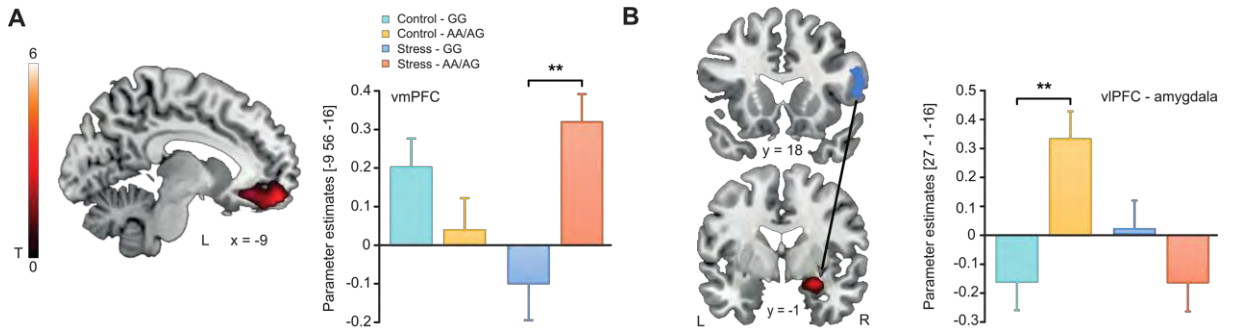

Figure 2. Stress and *CNR1* genotype effects on brain activity during affective picture processing. (A) Activity in the ventromedial prefrontal cortex was increased in rs1049353 AA/AG compared to GG genotype carriers for negative (vs. neutral) picture processing under stress. (B) Already under no-stress control conditions, the venrolateral prefrontal cortex showed enhanced functional connectivity with the amygdala when participants viewed negative (vs. neutral) pictures. Activations are superimposed on coronal sections of a T1-weighted template image and represented in red. vmPFC ventromedial prefrontal cortex, vlPFC ventrolateral prefrontal cortex, L corresponds to the left, R to the right side of the brain and error bars represent SEM. ** $p < 0.01$

Memory performance in AA/AG genotype carriers correlates with activation of and connectivity between limbic areas after stress

Performance in the surprise free recall test 24 hours after picture presentation was overall rather moderate (participants recalled on average 20 ± 8 percent of all pictures). As expected, memory was significantly better for negative (mean = 6, SD = 2) than for neutral pictures (mean = 3, SD = 2; F(1,130) = 203.98, P < 0.001; Table 2). Performance in the recognition task was overall very high, with an average hit rate of 88 percent and a false alarm rate of only 15 percent. In line with the free recall data, we observed superior recognition memory for negative items (increased hit rate, reduced false alarm rate; all F(1,129) ≥ 25.46, all P < 0.001; Table 2). An increased sensitivity index d' (F(1,129) = 140.53, P < 0.001) and higher confidence ratings (F(1,129) = 99.68, P < 0.001) for

negative relative to neutral pictures lent further support for the emotional memory enhancement (Table 2). Importantly, recall and recognition performance for negative and neutral pictures as well as confidence ratings were unaffected by stress and CNR1 genotype (all $F \leq 2.49$, all $P \geq 0.117$).

In order to investigate whether the neural underpinnings of emotional memory formation were affected by stress and the CNR1 polymorphism, we correlated brain activity for negative compared to neutral items during encoding with the 24 hours delayed memory performance for negative pictures. Since free recall performance was rather moderate and variance was small, we used the sensitivity index d' in our correlation analyses. In line with a crucial role of limbic brain regions in the processing of and memory formation for emotional material (McGaugh, 2004; LaBar and Cabeza, 2006), our analyses revealed overall significant clusters in the amygdala (left: $t = 2.85$, $P_{FWE} = 0.046$, $k = 10$), insula (right: $t = 4.24$, $P_{FWE} = 0.001$, $k = 113$; left: $t = 4.52$, $P_{FWE} = 0.001$, $k = 90$) and hippocampus (left: $t = 3.76$, $P_{FWE} = 0.008$, $k = 36$) during negative compared to neutral picture presentation. These clusters were positively correlated with participants' memory performance for negative pictures (amygdala: left: $r = 0.310$, $P < 0.001$; insula: right: $r = 0.310$, $P = 0.001$, left: $r = 0.275$, $P = 0.001$; hippocampus: $r = 0.209$, $P = 0.016$). Importantly, the neural correlates of emotional memory enhancement were modulated by stress and CNR1 genotype. Specifically, we observed that in stressed AA/AG genotype carriers emotional memory performance positively correlated with clusters in the amygdala (left: $t = 4.24$, $P_{FWE} = 0.003$, $k = 28$; $r = 0.571$, $P = 0.001$), insula (left: $t = 4.13$, $P_{FWE} = 0.012$, $k = 62$; $r = 0.555$, $P = 0.001$, right: $t = 3.61$, $P_{FWE} = 0.039$, $k = 26$; $r = 0.571$, $P = 0.001$) and hippocampus (left: $t = 4.16$, $P_{FWE} = 0.009$, $k = 56$; $r = 0.468$, $P = 0.006$; Figure 3A). In contrast, no such correlations were found in stressed GG genotype carriers (no suprathreshold clusters, all $r \leq 0.289$, $P \geq 0.108$). In the control condition, however, there were no significant clusters that were activated during negative picture encoding and that correlated with emotional memory performance in AA/AG genotype carriers (no suprathreshold clusters, all $r \leq 0.247$, $P \geq 0.215$), whereas in GG genotype carriers emotional memory performance was positively correlated with activation of the insula (right: $t = 4.22$, $P_{FWE} = 0.008$, $k = 14$; $r = 0.455$, $P = 0.004$; Figure 3B). In support of CNR1 genotype-dependent differences in the neural basis of emotional memory formation, the correlations between emotional memory performance and activation of limbic brain regions during negative compared to neutral picture encoding significantly differed between AA/AG and GG genotype carriers in the stress (amygdala and insula: both z between -2.06 and -1.61, $P \leq 0.054$) and control condition (insula: $z = 2.72$, $P = 0.003$).

In addition to the activation of single brain regions, we analyzed how functional connectivity patterns during negative picture encoding may relate to later memory performance. Interestingly, functional connectivity of the hippocampus and the BLA was associated with enhanced emotional memory only in stressed AA/AG genotype carriers ($t = 3.78$, $P_{FWE} = 0.004$, $k = 31$; $r = 0.449$, $P = 0.009$; Figure 3C), whereas no such correlations were observed in stressed GG genotype carriers or AA/AG and GG genotype carriers in the control condition (no suprathreshold clusters, all r between -0.112 and 0.213, all $P > 0.102$; no differences between these groups: all $z \geq -1.26$, all $P \geq 0.103$).

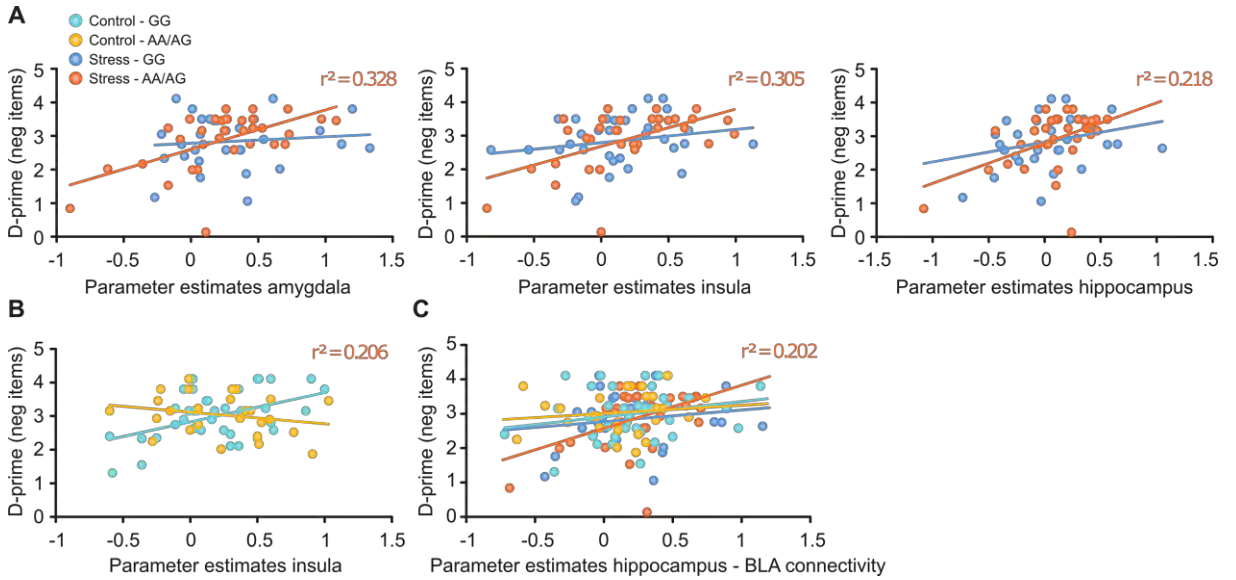

Figure 3. Correlations between brain activity during encoding of negative (vs. neutral) pictures and emotional memory performance, expressed as sensitivity index d' for negative pictures. (A) Following the stress manipulation, activity of the amygdala, insula and hippocampus positively correlated with memory performance in stressed AA/AG genotype carriers. (B) In the no-stress control condition, insula activity positively correlated with memory performance in GG genotype allle carriers. (C) Enhanced functional connecitivity of the hippocampus with the basolateral amygdala (BLA) during negative (vs. neutral) picture encoding was associated with enhanced memory performance for negative pictures only in stressed AA/AG genotype carriers.

Discussion

The eCB system has been suggested to act as an emotional buffer under stress (Morena and Campolongo, 2014). However, how eCBs may modulate affective responding in humans remained unclear. Here we combined a behavioral genetics approach with fMRI to investigate how a variant of the gene coding for the CB1 receptor may alter the neural processing of affective information under stress. Our results show, in line with a crucial role of eCB signaling in affective responding, significant changes of affective processing under stress depending on the CB1 receptor gene variant.

More specifically, we obtained stronger activity of the vmPFC in AA/AG compared to GG genotype carriers when processing emotionally negative (vs. neutral) information after stress. The mPFC coordinates cognitive, emotional and behavioral responses to stressful stimuli and regulates glucocorticoid-mediated negative feedback inhibition of the HPA axis (Diorio et al., 1993; McLaughlin et al., 2014). The vmPFC specifically is known to play a crucial role in emotion regulation and extinction processes (Ochsner and Gross, 2005; Kalisch et al., 2006; Goldin et al., 2008) and reduced activation of the vmPFC during negative picture processing has been associated with depression (Brassen et al., 2008), which suggests that the stronger recruitment of the vmPFC may allow more efficient affective processing and emotion regulation under stress. Indeed, the vmPFC has been shown to regulate limbic brain regions that are involved in the generation of emotional responses (Etkin et al., 2011) and enhanced activation of this brain region is associated with reduced negative affect (Urry et al., 2006). Endocannabinoid signaling appears to be crucial for effective mPFC functioning (Morena et al., 2016). Indeed, eCBs have been shown to regulate GABAergic inhibition of the mPFC (McLaughlin et al., 2014) and increased eCB signaling in the mPFC and amygdala were able to suppress anxiety (Rubino et al., 2008). The eCB-induced increase in dopamine and the reduced GABAergic inhibition of the mPFC (Chiu et al., 2010) in concert with enhanced serotonergic activation (McLaughlin et al., 2012) may promote self-focused emotion regulation and active stress coping strategies dependent on the mPFC (Ochsner et al., 2004; McLaughlin et al., 2014), which is in line with anxiety-reducing and antidepressant-like effects of CB1 receptor agonists (Bambico et al., 2007; Akirav, 2011). These mechanism may contribute to the proposed protective effect of the rs1049353 A allele against stress-related psychopathologies.

Interestingly, we obtained already under no-stress control conditions differential prefrontal engagement during affective processing in AA/AG compared to GG geotype carriers. In particular, the vlPFC, another critical area for emotion regulation (Ochsner and Gross, 2005), showed in the control condition stronger connectivity with the amygdala during affective processing in AA/AG compared to GG genotype carriers. Although functional connectivity data do not allow conclusions regarding the direction of the interaction, this finding is generally in line with previous studies suggesting that the vlPFC inhibits activation of the amygdala, thereby diminishing the influence of the amygdala during affective processing, which is crucial for successful emotion regulation in the face of threatening stimuli (Banks et al., 2007; Wager et al., 2008; Etkin et al., 2011). Thus, this increased vlPFC-amygdala connectivity may represent another mechanism

contributing to beneficial effects of the A allele in coping with adverse events. While these neural data provide evidence for differences between AA/AG and GG genotype carriers in affective processing under stress and control conditions, it is important to note that these differences at the neural level were not accompanied by behavioral changes (i.e., changes in emotionality ratings), which may be, at least in part, due to the reduced sensitivity of the four-point rating scale. Interestingly, however, GG genotype carriers were generally faster in their emotionality ratings, which might be indicative of more automatic, reflexive affective responding.

Beyond a mere modulation of affective processing under stress, the eCB system has been implicated in memory formation for emotional events, most likely through its influence on rapid glucocorticoid signaling (Campolongo et al., 2009; Atsak et al., 2012a; Atsak et al., 2012b; Atsak et al., 2015). In line with a number of previous studies (for a review see Hamann, 2001), our results showed better memory for negative compared to neutral information. This emotional memory enhancement is commonly assumed to rely on the actions of catecholamines and glucocorticoids in the amygdala, which then modulate memory processes in areas such as the hippocampus (McGaugh, 2000; LaBar and Cabeza, 2006; Roozendaal et al., 2009). In line with these ideas, we obtained, across genotype- and treatment-groups, significant correlations between memory performance for emotionally arousing stimuli and activity in the amygdala, insula and hippocampus. The neural underpinnings of the emotional memory enhancement, however, were distinct in carriers of the AA/AG and GG genotype of the CNR1 gene polymorphism. When analyzing the experimental groups separately, the correlations between hippocampal, amygdala and insula activity and emotional memory performance were observed in stressed AA/AG but not GG genotype carriers. Moreover, we obtained a significant correlation between the functional connectivity of BLA and hippocampus after stress, as predicted by prominent models of emotional memory formation (Roozendaal et al., 2004; Roozendaal and McGaugh, 2011; Roozendaal and Hermans, 2017), in stressed AA/AG but not GG genotype carriers. This result is generally in line with the finding that injection of a CB1 receptor agonist into the BLA improved emotional memory in rats, whereas the corticosterone-induced emotional memory enhancement was blocked already by injection of a very low dose of a CB1 receptor antagonist into the BLA (Campolongo et al., 2009). These findings support the view that a stress-induced increase in glucocorticoids stimulates eCB signaling, leading to increased (noradrenergic) activity in the BLA, most likely by inhibiting GABAergic influences (Duvarci and Pare, 2007; Hill and McEwen, 2009), which then enhances

memory consolidation through changes in hippocampal synaptic plasticity. Similarly, to the data on affective processing, however, it is important to note that the neural differences in memory formation did not translate into performance differences in the present study. The absence of behavioral differences may be owing to the overall rather moderate performance level in the surprise memory tests. Alternatively, differential memory performance might be revealed in more sophisticated memory tests that assess the actual level of elaboration of the encoded material (Schwabe and Wolf, 2013). CB1 genotype related changes in the neural signature of emotional memory formation may result in encoding and consolidation changes that are highly relevant in the context of PTSD and anxiety disorders and are further related to the protective effects of the A allele of the rs1049353 polymorphism on mental health.

The fact that we see a (potentially) beneficial influence of the rs1049353 AA/AG genotype on the neural correlates of affective processing and emotional memory enhancement under stress, but not under control conditions, is in line with previous results showing that a certain degree of emotional arousal is needed for a modulation by eCB signaling (Campolongo et al., 2012). Although it is unknown whether the rs1049353 polymorphism is functional or not, it has been postulated to affect mRNA stability (Chakrabarti et al., 2006; Domschke et al., 2008; Hill and Patel, 2013). In addition, clinical studies showed some level of protection against stress and the development of depression in AA/AG genotype carriers (Domschke et al., 2008; Agrawal et al., 2012) and a neuroimaging study in healthy participants revealed diminished activation of the striatum in response to happy faces in GG genotype carriers, which may be indicative of reduced social reward responsivity (Chakrabarti et al., 2006). Based on these results and the proposal that the rs1049353 AA/AG genotype may results in a more stable mRNA (Hill and Patel, 2013), it is tempting to speculate that the AA genotype is associated with enhanced CB1 receptor functioning in vivo. Whereas future molecular studies are needed to explicitly test this prediction, we propose that enhanced CB1 receptor functioning in A allele carriers improves emotion regulation strategies in the face of stress and perhaps the incorporation of emotional events into autobiographical memory, for which the hippocampus is essential (Cabeza and St. Jacques, 2007). In contrast to previous studies that suggested a role of eCBs and the CB1 receptor in the regulation of the HPA axis (McLaughlin et al., 2014), we obtained no influence of the CB1 receptor polymorphism on the cortisol response to stress. The absence of such an effect suggests that the effects of the rs1049353 polymorphism on the neural underpinnings of affective processing and emotional memory

formation are not simply driven by changes in the cortisol response to stress. It is very likely that the influence of eCB signaling on other neurotransmitter systems is crucial, such as the influence of eCBs on GABAergic neurons in the mPFC (McLaughlin 2014) and BLA (Duvarci 2007), as well as on serotonergic pathways to other limbic brain regions (McLaughlin 2012).

In sum, our results show that a common variant of the gene coding for the CB1 receptor (rs10493453 AA/AG genotype) is associated with increased recruitment of prefrontal regions that are important for emotion regulation during affective processing under stress and with enhanced connectivity of the hippocampus with the BLA during emotional memory formation. These data may point to improved emotion regulation abilities and more appropriate consolidation of emotional events into autobiographical memory in individuals with one or more copies of the minor A allele of this polymorphism. This modulation of affective and cognitive processing under stress may contribute to a certain degree of protection against stress-related disorders such as PTSD. Indeed, first evidence suggests that the eCB system may be an effective target for treating the cognitive and affective characteristics of PTSD (Hill et al., 2006; Ganon-Elazar and Akirav, 2012; Akirav, 2013; Trezza and Campolongo, 2013; Korem et al., 2016). Specifically, in rats it was shown that injections of a CB1/CB2 receptor agonist into the BLA and hippocampus prevented the stress-induced glucocorticoid receptor upregulation in these brain regions as well as in the PFC and prevented an impairment in fear extinction (Ganon-Elazar and Akirav, 2013). In combination with evidence suggesting glucocorticoid-based therapies for the attenuation of aversive memories (de Quervain et al., 2017), these findings may hopefully advance the development of new treatment options for PTSD and other stress-related disorders that are characterized by aberrant affective processing.

References

Agrawal, A., Nelson, E. C., Littlefield, A. K., Bucholz, K. K., Degenhardt, L., Henders, A. K., Madden, P. A., Martin, N. G., Montgomery, G. W., Pergadia, M. L., Sher, K. J., Heath, A. C. & Lynskey, M. T. (2012) 'Cannabinoid receptor genotype moderation of the effects of childhood physical abuse on anhedonia and depression', Arch Gen Psychiatry, 69(7), pp. 732-740.

Akirav, I. (2011) 'The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus', Front Behav Neurosci, 5, p. 34.

Akirav, I. (2013) 'Targeting the endocannabinoid system to treat haunting traumatic memories', Front Behav Neurosci, 7(124), pp. 1-3.

Anders, S., Lotze, M., Erb, M., Grodd, W. & Birbaumer, N. (2004) 'Brain activity underlying emotional valence and arousal: a response-related fMRI study', Hum Brain Mapp, 23(4), pp. 200-209.

Arnsten, A. F. (2009) 'Stress signalling pathways that impair prefrontal cortex structure and function', Nat Rev Neurosci, 10(6), pp. 410-422.

Atsak, P., Hauer, D., Campolongo, P., Schelling, G., Fornari, R. V. & Roozendaal, B. (2015) 'Endocannabinoid signaling within the basolateral amygdala integrates multiple stress hormone effects on memory consolidation', Neuropsychopharmacology, 40(6), pp. 1485-1494.

Atsak, P., Hauer, D., Campolongo, P., Schelling, G., McGaugh, J. L. & Roozendaal, B. (2012a) 'Glucocorticoids interact with the hippocampal endocannabinoid system in impairing retrieval of contextual fear memory', Proc Natl Acad Sci U S A, 109(9), pp. 3504-3509.

Atsak, P., Roozendaal, B. & Campolongo, P. (2012b) 'Role of the endocannabinoid system in regulating glucocorticoid effects on memory for emotional experiences', Neuroscience, 204, pp. 104-116.

Bambico, F. R., Katz, N., Debonnel, G. & Gobbi, G. (2007) 'Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex', J Neurosci, 27(43), pp. 11700-11711.

Banks, S. J., Eddy, K. T., Angstadt, M., Nathan, P. J. & Phan, K. L. (2007) 'Amygdala-frontal connectivity during emotion regulation', Soc Cogn Affect Neurosci, 2(4), pp. 303-312.

Bedse, G., Colangeli, R., Lavecchia, A. M., Romano, A., Altieri, F., Cifani, C., Cassano, T. & Gaetani, S. (2014) 'Role of the basolateral amygdala in mediating the effects of the fatty acid amide hydrolase inhibitor URB597 on HPA axis response to stress', Eur Neuropsychopharmacol, 24(9), pp. 1511-1523.

Brassen, S., Kalisch, R., Weber-Fahr, W., Braus, D. F. & Büchel, C. (2008) 'Ventromedial predrontal cortex processing during emotional evaluation in late-life depression: a longitudinal functional magnetic resonance imaging study', Biol Psychiatry, 64, pp. 349-355.

Cabeza, R. & St. Jacques, P. (2007) 'Functional neuroimaging of autobiographical memory', Trends Cogn Sci, 11(5), pp. 219-227.

Cahill, L. & McGaugh, J. L. (1998) 'Mechanisms of emotional arousal and lasting declarative memory', Trends Neurosci, 21(7), pp. 294-299.

Campolongo, P., Ratano, P., Manduca, A., Scattoni, M. L., Palmery, M., Trezza, V. & Cuomo, V. (2012) 'The endocannabinoid transport inhibitor AM404 differentially modulates recognition memory in rats depending on environmental aversiveness', Front Behav Neurosci, 6, p. 11.

Campolongo, P., Roozendaal, B., Trezza, V., Hauer, D., Schelling, G., McGaugh, J. L. & Cuomo, V. (2009) 'Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory', Proc Natl Acad Sci U S A, 106(12), pp. 4888-4893.

Campolongo, P. & Trezza, V. (2012) 'The endocannabinoid system: a key modulator of emotions and cognition', Front Behav Neurosci, 6(73), pp. 1-2.

Chakrabarti, B., Kent, L., Suckling, J., Bullmore, E. & Baron-Cohen, S. (2006) 'Variations in the human cannabinoid receptor (CNR1) gene modulate striatal responses to happy faces', Eur J Neurosci, 23(7), pp. 1944-1948.

Chiu, C. Q., Puente, N., Grandes, P. & Castillo, P. E. (2010) 'Dopaminergic modulation of endocannabinoid-mediated plasticity at GABAergic synapses in the prefrontal cortex', J Neurosci, 30(21), pp. 7236-7248.

Chrousos, G. P. (2009) 'Stress and disorders of the stress system', Nat Rev Endocrinol, 5(7), pp. 374-381.

Damasio, A. R., Grabowski, T. J., Bechara, A., Damasio, H., Ponto, L. L. B., Parvizi, J. & Hichwa, R. D. (2000) 'Subcortical and cortical brain activity during the feeling of self-generated emotions', Nat Neurosci, 3(10), pp. 1049-1056.

de Kloet, E. R., Joels, M. & Holsboer, F. (2005) 'Stress and the brain: from adaptation to disease', Nat Rev Neurosci, 6(6), pp. 463-475.

de Quervain, D., Schwabe, L. & Roozendaal, B. (2017) 'Stress, glucocorticoids and memory: implications for treating fear-related disorders', Nat Rev Neurosci, 18(1), pp. 7-19.

Diorio, D., Viau, V. & Meaney, M. J. (1993) 'The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress', J Neurosci, 13(9), pp. 3839-3847.

Domschke, K., Dannlowski, U., Ohrmann, P., Lawford, B., Bauer, J., Kugel, H., Heindel, W., Young, R., Morris, P., Arolt, V., Deckert, J., Suslow, T. & Baune, B. T. (2008) 'Cannabinoid receptor 1 (CNR1) gene: impact on antidepressant treatment response and emotion processing in major depression', Eur Neuropsychopharmacol, 18(10), pp. 751-759.

Duvarci, S. & Pare, D. (2007) 'Glucocorticoids enhance the excitability of principal basolateral amygdala neurons', J Neurosci, 27(16), pp. 4482-4491.

Ellenbogen, M. A., Schwartzman, A. E., Stewart, J. & Walker, C. D. (2002) 'Stress and selective attention: the interplay of mood, cortisol levels, and emotional information processing', Psychophysiology, 39(6), pp. 723-732.

Etkin, A., Egner, T. & Kalisch, R. (2011) 'Emotional processing in anterior cingulate and medial prefrontal cortex', Trends Cogn Sci, 15(2), pp. 85-93.

Fox, E., Cahill, S. & Zougkou, K. (2010) 'Preconscious processing biases predict emotional reactivity to stress', Biol Psychiatry, 67(4), pp. 371-377.

Ganon-Elazar, E. & Akirav, I. (2009) 'Cannabinoid receptor activation in the basolateral amygdala blocks the effects of stress on the conditioning and extinction of inhibitory avoidance', J Neurosci, 29(36), pp. 11078-11088.

Ganon-Elazar, E. & Akirav, I. (2012) 'Cannabinoids prevent the development of behavioral and endocrine alterations in a rat model of intense stress', Neuropsychopharmacology, 37(2), pp. 456-466.

Ganon-Elazar, E. & Akirav, I. (2013) 'Cannabinoids and traumatic stress modulation of contextual fear extinction and GR expression in the amygdala-hippocampal-prefrontal circuit', Psychoneuroendocrinology, 38(9), pp. 1675-1687.

Goldin, P. R., McRae, K., Ramel, W. & Gross, J. J. (2008) 'The neural bases of emotion regulation: reappraisal and suppression of negative emotion', Biol Psychiatry, 63(6), pp. 577-586.

Goodwin, R. S., Baumann, M. H., Gorelick, D. A., Schwilke, E., Schwope, D. M., Darwin, W. D., Kelly, D. L., Schroeder, J. R., Ortemann-Renon, C., Bonnet, D. & Huestis, M. A. (2012) 'CB1 - cannabinoid receptor antagonist effects on cortisol in cannabis-dependent men', Am J Drug Alcohol Abuse, 38(1), pp. 114-119.

Gray, J. M., Vecchiarelli, H. A., Morena, M., Lee, T. T., Hermanson, D. J., Kim, A. B., McLaughlin, R. J., Hassan, K. I., Kuhne, C., Wotjak, C. T., Deussing, J. M., Patel, S. & Hill, M. N. (2015) 'Corticotropin-releasing hormone drives anandamide hydrolysis in the amygdala to promote anxiety', J Neurosci, 35(9), pp. 3879-3892.

Hajcak, G., MacNamara, A. & Olvet, D. M. (2010) 'Event-related potentials, emotion, and emotion regulation: an integrative review', Dev Neuropsychol, 35(2), pp. 129-155.

Hamann, S. (2001) 'Cognitive and neural mechanisms of emotional memory', Trends Cogn Sci, 5(9), pp. 394-400.

Hill, M. N. & Gorzalka, B. B. (2009) 'Impairments in endocannabinoid signaling and depressive illness', JAMA, 301(11), pp. 1165-1166.

Hill, M. N., Ho, W.-S. V., Sinopoli, K. J., Viau, V., Hillard, C. J. & Gorzalka, B. B. (2006) 'Involvement of the endocannabinoid system in the ability of long-term tricyclic antidepressant treatment to suppress stress-induced activation of the hypothalamic-pituitary-adrenal axis', Neuropsychopharmacology, 31, pp. 2591-2599.

Hill, M. N. & McEwen, B. S. (2009) 'Endocannabinoids: The silent partner of glucocorticoids in the synapse', Proc Natl Acad Sci U S A, 106(12), pp. 4579-4580.

Hill, M. N., McLaughlin, R. J., Pan, B., Fitzgerald, M. L., Roberts, C. J., Lee, T. T., Karatsoreos, I. N., Mackie, K., Viau, V., Pickel, V. M., McEwen, B. S., Liu, Q. S., Gorzalka, B. B. & Hillard, C. J. (2011) 'Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response', J Neurosci, 31(29), pp. 10506-10515.

Hill, M. N. & Patel, S. (2013) 'Translational evidence for the involvement of the endocannabinoid system in stress-related psychiatric illnesses', Biol Mood Anxiety Disord, 3(19).

Hillard, C. J., Weinlander, K. M. & Stuhr, K. L. (2012) 'Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence', Neuroscience, 204, pp. 207-229.

Horder, J., Browning, M., Di Simplicio, M., Cowen, P. J. & Harmer, C. J. (2012) 'Effects of 7 days of treatment with the cannabinoid type 1 receptor antagonist, rimonabant, on emotional processing', J Psychopharmacol, 26(1), pp. 125-132.

Horder, J., Cowen, P. J., Di Simplicio, M., Browning, M. & Harmer, C. J. (2009) 'Acute administration of the cannabinoid CB1 antagonist rimonabant impairs positive affective memory in healthy volunteers', Psychopharmacology (Berl), 205(1), pp. 85-91.

Horder, J., Harmer, C. J., Cowen, P. J. & McCabe, C. (2010) 'Reduced neural response to reward following 7 days treatment with the cannabinoid CB1 antagonist rimonabant in healthy volunteers', Int J Neuropsychopharmacol, 13(8), pp. 1103-1113.

Joels, M. & Baram, T. Z. (2009) 'The neuro-symphony of stress', Nat Rev Neurosci, 10, pp. 459-466.

Kalisch, R. (2009) 'The functional neuroanatomy of reappraisal: time matters', Neurosci Biobehav Rev, 33, pp. 1215-1226.

Kalisch, R., Korenfeld, E., Stephan, K. E., Weiskopf, N., Seymour, B. & Dolan, R. J. (2006) 'Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network', J Neurosci, 26(37), pp. 9503-9511.

Karl, A., Malta, L. S. & Maercker, A. (2006) 'Meta-analytic review of event-related potential studies in post-traumatic stress disorder', Biol Psychol, 71(2), pp. 123-147.

Kirschbaum, C., Pirke, K. M. & Hellhammer, D. H. (1993) 'The 'Trier Social Stress Test': a tool for investigating psychobiological stress responses in the laboratory setting', Neuropsychobiology, 28, pp. 76-81.

Kogan, N. M. & Mechoulam, R. (2006) 'The chemistry of endocannabinoids', J Endocrinol Invest, 29(3), pp. 3-14.

Korem, N., Zer-Aviv, T. M., Ganon-Elazar, E., Abush, H. & Akirav, I. (2016) 'Targeting the endocannabinoid system to treat anxiety-related disorders', J Basic Clin Physiol Pharmacol, 27(3), pp. 193-202.

LaBar, K. S. & Cabeza, R. (2006) 'Cognitive neuroscience of emotional memory', Nat Rev Neurosci, 7(1), pp. 54-64.

Lang, P. J., Bradley, M. M. & Cuthbert, B. N. (2008) 'International affective picture system (IAPS): Affective ratings of pictures and instruction manual', Technical Report A-8, University of Florida, Gainesville, FL.

Leppänen, J. M. (2006) 'Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings', Curr Opin Psychiatry, 19(1), pp. 34-39.

Lutz, B. (2009) 'Endocannabinoid signals in the control of emotion', Curr Opin Pharmacol, 9(1), pp. 46-52.

Lutz, B., Marsicano, G., Maldonado, R. & Hillard, C. J. (2015) 'The endocannabinoid system in guarding against fear, anxiety and stress', Nat Rev Neurosci, 16(12), pp. 705-718.

McEwen, B. S. (2004) 'Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders', Ann N Y Acad Sci, 1032, pp. 1-7.

McGaugh, J. L. (2000) 'Memory - a century of consolidation', Science, 287(5451), pp. 248-251.

McGaugh, J. L. (2004) 'The amygdala modulates the consolidation of memories of emotionally arousing experiences', Annu Rev Neurosci, 27, pp. 1-28.

McLaughlin, R. J., Hill, M. N., Bambico, F. R., Stuhr, K. L., Gobbi, G., Hillard, C. J. & Gorzalka, B. B. (2012) 'Prefrontal cortical anandamide signaling coordinates coping responses to stress through a serotonergic pathway', Eur Neuropsychopharmacol, 22(9), pp. 664-671.

McLaughlin, R. J., Hill, M. N. & Gorzalka, B. B. (2014) 'A critical role for prefrontocortical endocannabinoid signaling in the regulation of stress and emotional behavior', Neurosci Biobehav Rev, 42, pp. 116-131.

McPartland, J. M., M., G. & Pertwee, R. G. (2007) 'Meta-analysis of cannabinoid ligand binding affinity and receptor distribution: interspecies differences', Br J Pharmacol, 152(5), pp. 583-593.

Mechoulam, R. & Parker, L. A. (2013) 'The endocannabinoid system and the brain', Ann Rev Psychol, 64, pp. 21-47.

Mitchell, P. B. & Morris, M. J. (2007) 'Depression and anxiety with rimonabant', The Lancet, 370(9600), pp. 1671-1672.

Morena, M. & Campolongo, P. (2014) 'The endocannabinoid system: an emotional buffer in the modulation of memory function', Neurobiol Learn Mem, 112, pp. 30-43.

Morena, M., Patel, S., Bains, J. S. & Hill, M. N. (2016) 'Neurobiological Interactions Between Stress and the Endocannabinoid System', Neuropsychopharmacology, 41(1), pp. 80-102.

Mota, N., Sumner, J. A., Lowe, S. R., Neumeister, A., Uddin, M., Aiello, A. E., Wildman, D. E., Galea, S., Koenen, K. C. & Pietrzak, R. H. (2015) 'The rs1049353 polymorphism in the CNR1 gene interacts with childhood abuse to predict posttraumatic threat symptoms', J Clin Psychiatry, 76(12), pp. e1622-1623.

Motzkin, J. C., Philippi, C. L., Wolf, R. C., Baskaya, M. K. & Koenigs, M. (2015) 'Ventromedial prefrontal cortex is critical for the regulation of amygdala activity in humans', Biol Psychiatry, 77(3), pp. 276-284.

Ochsner, K. N. & Gross, J. J. (2005) 'The cognitive control of emotion', Trends Cogn Sci, 9(5), pp. 242-249.

Ochsner, K. N., Ray, R. D., Cooper, J. C., Robertson, E. R., Chopra, S., Gabrieli, J. D. & Gross, J. J. (2004) 'For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion', Neuroimage, 23(2), pp. 483-499.

Palomba, D., Angrilli, A. & Mini, A. (1997) 'Visual evoked potentials, heart rate responses and memory to emotional pictorial stimuli', Int J Psychophysiol, 27, pp. 55-67.

Phan, K. L., Wager, T., Taylor, S. F. & Liberzon, I. (2002) 'Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI', Neuroimage, 16(2), pp. 331-348.

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., Milad, M. R. & Liberzon, I. (2012) 'Biological studies of post-traumatic stress disorder', Nat Rev Neurosci, 13(11), pp. 769-787.

Roozendaal, B., Hahn, E. L., Nathan, S. V., de Quervain, D. J. & McGaugh, J. L. (2004) 'Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala', J Neurosci, 24(37), pp. 8161-8169.

Roozendaal, B. & Hermans, E. J. (2017) 'Norepinephrine effects on the encoding and consolidation of emotional memory: improving synergy between animal and human studies', Curr Opin Behav Sci, 14, pp. 115-122.

Roozendaal, B., McEwen, B. S. & Chattarji, S. (2009) 'Stress, memory and the amygdala', Nat Rev Neurosci, 10, pp. 423-433.

Roozendaal, B. & McGaugh, J. L. (2011) 'Memory modulation', Behav Neurosci, 125(6), pp. 797-824.

Rubino, T., Realini, N., Castiglioni, C., Guidali, C., Vigano, D., Marras, E., Petrosino, S., Perletti, G., Maccarrone, M., Di Marzo, V. & Parolaro, D. (2008) 'Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex', Cereb Cortex, 18(6), pp. 1292-1301.

Schwabe, L., Hoffken, O., Tegenthoff, M. & Wolf, O. T. (2013) 'Opposite effects of noradrenergic arousal on amygdala processing of fearful faces in men and women', Neuroimage, 73, pp. 1-7.

Schwabe, L. & Wolf, O. T. (2013) 'Stress and multiple memory systems: feom 'thinking' to 'doing'', Trends Cogn Sci, 17(2), pp. 60-68.

Sergerie, K., Chochol, C. & Armony, J. L. (2008) 'The role of the amygdala in emotional processing: a quantitative meta-analysis of functional neuroimaging studies', Neurosci Biobehav Rev, 32, pp. 811-830.

Steiner, M. & Wotjak, C. (2008) 'Role of the endocannabinoid system in regulation of the hypothalamic-pituitary-adrenocortical axis', Prog Brain Res, 170, pp. 397-432.

Steyer, R., P., S., Notz, P. & Eid, M. (1994) 'Theoretical analysis of a multidimensional mood questionnaire (MDBF)', Diagnostica, 40(4), pp. 320-328.

Trezza, V. & Campolongo, P. (2013) 'The endocannabinoid system as a possible target to treat both the cognitive and emotional features of post-traumatic stress disorder (PTSD)', Front Behav Neurosci, 7(100), pp. 1-5.

Urry, H. L., van Reekum, C. M., Johnstone, T., Kalin, N. H., Thurow, M. E., Schaefer, H. S., Jackson, C. A., Frye, C. J., Greischar, L. L., Alexander, A. L. & Davidson, R. J. (2006) 'Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults', J Neurosci, 26(16), pp. 4415-4425.

van Stegeren, A. H. (2009) 'Imaging stress effects on memory: a review of neuroimaging studies', Can J Psychiatry, 54(1), pp. 16-27.

van Stegeren, A. H., Goekoop, R., Everaerd, W., Scheltens, P., Barkhof, F., Kuijer, J. P. & Rombouts, S. A. (2005) 'Noradrenaline mediates amygdala activation in men and women during encoding of emotional material', Neuroimage, 24(3), pp. 898-909.

van Stegeren, A. H., Roozendaal, B., Kindt, M., Wolf, O. T. & Joels, M. (2010) 'Interacting noradrenergic and corticosteroid systems shift human brain activation patterns during encoding', Neurobiol Learn Mem, 93(1), pp. 56-65.

Wager, T. D., Davidson, M. L., Hughes, B. L., Lindquist, M. A. & Ochsner, K. N. (2008) 'Prefrontal-subcortical pathways mediating successful emotion regulation', Neuron, 59(6), pp. 1037-1050.

Wang, M., Hill, M. N., Zhang, L., Gorzalka, B. B., Hillard, C. J. & Alger, B. E. (2012) 'Acute restraint stress enhances hippocampal endocannabinoid function via glucocorticoid receptor activation', J Psychopharmacol, 26(1), pp. 56-70.

Weymar, M., Schwabe, L., Löw, A. & Hamm, A. O. (2012) 'Stress sensitizes the brain: increased processing of unpleasant pictures after exposure to acute stress', J Cogn Neurosci, 24(7), pp. 1511-1518.

Wickens, T. D. (2002) 'Elementary signal detection theory', New York, Oxford University Press.

Wirz, L., Wacker, J., Felten, A., Reuter, M. & Schwabe, L. (2017) 'A Deletion Variant of the alpha2b-Adrenoceptor Modulates the Stress-Induced Shift from "Cognitive" to "Habit" Memory', J Neurosci, 37(8), pp. 2149-2160.