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(Not) building on what you know:

Understanding the impact of stress and major stress mediators on our ability to successfully use prior knowledge

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Summary

Learning and memory constitute critical processes that enable us to behave adaptively and respond adequately to our surroundings. The latter is dependent on our ability to utilize prior knowledge, to form schemas, i.e. specific knowledge structures that can act as scaffolds and aid learning of schema-related information, or for the purpose of generalizing across new but related situations. Both, schema-based learning and memory generalization have been shown to rely on specific brain structures, such as the ventromedial prefrontal cortex as well as the hippocampus. These structures are highly sensitive to stress and major stress mediators such as cortisol and noradrenaline. However, how our ability to utilize prior knowledge is impacted by stress and these stress mediators is not yet clear. To this end, we conducted three studies to investigate the impact of acute stress, as well as of the stress mediators cortisol and noradrenaline on the performance and neural underpinnings of the use of prior knowledge in schema-based learning and memory generalization. Results showed that stress or increased cortisol levels led to an inability to use prior knowledge to aid learning and hampered with the neural ensemble involved in schema-detection and learning, while increased noradrenergic arousal led to impaired memory generalization specifically in women. Hence, the current work is the first to show an impact of stress and specific stress mediators on our ability to use prior knowledge in different contexts and settings. Results have important implications for educational purposes and are also of clinical relevance as many stress-related mental disorders prominently feature impaired memory processes.

Index

TABLE OF FIGURES

ABBREVIATIONS

adrenocorticotropic releasing hormone (**ACTH**)

central nervous system (**CNS**)

cornu ammonis (**CA1**)

corticotrophin releasing hormone (**CRH**)

cyclic adenosine monophosphate (**cAMP**)

cyclic-adenosine3'5'-monophosphate (cAMP)-dependent protein kinase A (**PKA**)

dorsolateral prefrontal cortex (**dlPFC**)

glucocorticoid receptor (**GR**)

hypothalamic-pituitary-adrenal (**HPA**)

long-term potentiation (**LTP**)

medial prefrontal cortex (**mPFC**)

medial temporal lobes (**MTL**)

mineralocorticoid receptor (**MR**)

paraventricular nucleus (**PVN**)

prefrontal cortex (**PFC**)

repetitive transcranial magnetic stimulation (**rTMS**)

serotonin (**5-HT**)

socially evaluated cold pressure test (**SECPT**)

stimulus response learning (**S-R**)

subtypes of the 5-HT receptors (**5-HT1A, 5-HT4**)

sympathetic nervous system (**SNS**)

temporo-parietal junction (**TPJ**)

transcranial direct current stimulation (**tDCS**),

trier social stress test (**TSST**)

ventromedial prefrontal cortex (**vmPFC**)

ventrolateral part of the prefrontal cortex (**vlPFC**)

Chapter I

Learning and Memory

"Yesterday is but today's memory and tomorrow is today's dream" – Khalil Gibran

Imagine you cannot remember your next doctors' appointment or the birthday of your best friend. While this is not out of the ordinary and happens frequently, when the inability to remember is extended to crucial personal facts, such as your place of birth, who your parents are or how you spend your childhood, it becomes apparent that memory is absolutely crucial in daily life and to whom you are. Besides our ability to mentally travel back into the past, our memory also enables us to imagine the future (Schacter, Benoit, & Szpunar, 2017). Hence, memory is not a static construct, but rather flexible and allows us to benefit from previous experiences and to tackle future encounters successfully (Schacter & Addis, 2007; Shohamy & Wagner, 2008; Tulving, 2002; van Kesteren, Ruiter, Fernandez, & Henson, 2012). However, memory is nothing without learning and learning has been designated as the acquisition of information that is then stored in our memory (Okano, Hirano, & Balaban, 2000). Learning therefore constitutes a critical process, as it allows us to benefit from our mistakes, respond adequately to changes in our surroundings and behave adaptively (Jing, Madore, & Schacter, 2017; Schacter, Addis, & Buckner, 2007; Wimmer & Shohamy, 2012). The latter is dependent on our ability to use previous knowledge to build networks or scaffolds that we can get back to and utilize to aid learning of related content (van Kesteren et al., 2012). Furthermore, we can also utilize memories of previous experiences and apply these to similar situations, allowing us to react appropriately (Shohamy & Wagner, 2008). These different concepts of learning and memory are termed schema-based learning and memory generalization, respectively. Hence, when considering learning and memory, it is also important to not only describe the single processes but also to take into account that we benefit from previous knowledge.

There are different theories and concepts of learning and memory that were developed over the last centuries. These theories cover conceptual aspects of learning and memory, such as the presence of different systems and forms of learning and memory, but also the physiological aspects

underlying the process of learning and storage of information. In the next paragraphs I will consider the above mentioned conceptual, systemic and physiological aspects of learning and memory in general but also and most importantly with respect to the beneficial aspects of previous knowledge in the form of a schema or when used during memory generalization.

Short-term and Long-Term Memory

When we consider memory, we discriminate between short-term and long-term memory, a distinction that has been made originally by Donald Hebb (1949), although it was suggested already by William James (1890). The distinction between the concepts of short-term and long-term memory was made based on the different electrical and neurochemical activities in the brain (Baddeley, 2001). Early research to distinguish these two types of memory was based on the type of forgetting that could be observed (Brown, 1958, Petterson & Peterson, 1959, Melton, 1963), while later work considered amnesic patients and their impaired long term memory (Aggleton & Pearce, 2001; Milner, 1966). Additional work was done that led to the distinction of episodic and semantic memory as separate forms of long-term memory (Tulving, 1972), while the distinction between short-term memory and long-term memory remained unchanged (Baddeley, 2001). The current work is predominantly concerned with processes in long-term memory. The following sections will therefore only consider aspects of long-term memory, starting with a distinction between different memory systems, as well as a detailed description of each system, its functionality and interactions with and between structures that support processes within long-term memory.

First of all, it is important to define the crucial memory processes that will be referred to frequently. Encoding is the initial storage of memories that remain labile for a certain amount of time, while consolidation describes the process by which an encoded memory that was labile, is transformed into a stable memory (Genzel et al., 2017; Lechner, Squire, & Byrne, 1999; Misanin, Miller, & Lewis, 1968; Müller & Pilzecker, 1900; Ribot, 1882; Squire, Genzel, Wixted, & Morris, 2015). Retrieval on the other hand is what we would describe as ‘remembering’ in lay terms (Dimsdale-Zucker, Ritchey, Ekstrom, Yonelinas, & Ranganath, 2018; Ebbinghaus, 1885). An additional mechanism, termed reconsolidation turns already consolidated, stable memories into labile representations again, that can be modified by new information or interferences (Alberini, 2011; Dudai, 2006; Hupbach, Gomez, Hardt, & Nadel, 2007; Inda, Muravieva, & Alberini, 2011). This mechanism therefore further

contributes to the dynamic nature of memories, as even a consolidated memory can still be modulated (Alberini, 2011; Lee, 2010; McKenzie & Eichenbaum, 2011).

Multiple Learning and Memory Systems

Extensive evidence indicates that there is no single system supporting long-term memory, but that there are multiple memory systems that function by different mechanisms and are supported by different structures, such as the hippocampus, prefrontal cortex, amygdala as well as striatum and primary sensory cortex (Foerde & Shohamy, 2011; Ji & Wilson, 2007; McDonald & White, 1993; Squire & Zola, 1996). When memory is discussed in daily settings, the medial temporal lobe-dependent declarative memory is mostly referred to. Declarative memory is a complex construct that is based on an amalgamation of memories or representations of our very own personal events, i.e. our episodic memory and with respect to our basic knowledge of facts, i.e. our semantic memory (Tulving, 1972, 2002). An additional memory system is suggested to support a simpler, more rigid, non-declarative form of learning, often called habit learning that is dependent on the basal ganglia, specifically the dorsal striatum. As early as in the 1920s, William James reported a distinction between declarative and habit systems and was already able to characterize habits as primitive, reflex-like behaviors. He attributed an important, preparative function to these behaviors (James, 1918), but was not yet able to trace his observations back to anatomical structures in the brain (Eichenbaum, 2001; Zola-Morgan, Squire, Amaral, & Suzuki, 1989).

Hence, it is critical to make further qualitative distinctions between different brain systems that support memory. As mentioned above, declarative memory is suggested to be based on the medial temporal lobe (MTL) and specifically the hippocampus. The memory system mostly supporting habit or stimulus response (S-R) learning on the other hand is dependent on the basal ganglia, specifically the dorsal striatum (Faure, Haberland, Conde, & El Massioui, 2005; Featherstone & McDonald, 2004). It is suggested that while these systems support different functions, they are not completely independent, but interact to support memory functioning (Knowlton, Mangels, & Squire, 1996; McDonald & White, 1993; Mishkin, Malamut, & Bachevalier, 1984; Packard & Knowlton, 2002; Squire, 2004).

Medial Temporal Lobe Dependent Learning and Memory

As mentioned above, declarative memory is supported by the MTL and particularly the hippocampus and supports memory encoding, consolidation, retrieval and reconsolidation (Alvarez & Squire, 1994; Dimsdale-Zucker et al., 2018; Dolan & Fletcher, 1997; Ekstrom & Bookheimer, 2007; McGaugh, 2000; McKenzie & Eichenbaum, 2011; Schiller & Phelps, 2011; Shohamy & Wagner, 2008). Early research that has promoted the understanding of the anatomy of the MTL region and the functionality have come from lesion studies of memory impairments, such as what was seen in patient H. M. (Squire, Stark, & Clark, 2004; Squire & Zola-Morgan, 1991). H.M. suffered from severe epilepsy and received surgery to alleviate symptoms. During the surgery, parts of the medial temporal lobes (MTL) were removed bilaterally. As a result, H.M. was not able to form new declarative memories. However, H.M. and other patients with similar lesions were able to gradually develop new habits – an ability that is thought to be specifically supported by the basal ganglia (Foerde & Shohamy, 2011; Scoville & Milner, 1957). Cohen and Squire (1980), who have performed research in amnesic patients, were among the first to report that declarative memory functions belonged majorly to the hippocampus, as part of the MTL (Cohen & Squire, 1980). Later studies stated similar findings of selective impairments of explicit memory expression and retrieval after damage to the hippocampus or cell loss specifically within this structure (Corkin, 1984; Zola-Morgan, Squire, & Amaral, 1986).

Studies using animal models, especially in monkeys and rats, have further described the anatomy of the MTL structures and their connectivity with other brain regions in great detail (Burwell & Amaral, 1998; Lavenex & Amaral, 2000; Suzuki & Amaral, 1994a, 1994b; van Strien, Cappaert, & Witter, 2009). The medial temporal lobes comprise the hippocampus with the CA1 (cornu ammonis) and CA3 regions, the dentate gyrus and subicular complex as well as the distinct structures of the perirhinal, entorhinal and parahippocampal cortices that constitute the majority of the parahippocampal gyrus (Squire et al., 2004). These structures are extensively connected within the MTL but also with structures in different brain regions, such as the parietal and prefrontal cortex. This connectivity is critical as it enables memory processing (Carmichael & Price, 1995; Simons & Spiers, 2003; Wagner, Shannon, Kahn, & Buckner, 2005). The perirhinal and lateral entorhinal cortex that receive input from areas within the frontal, temporal and parietal cortices, are important for object-specific stimuli and for identifying whether a stimulus is familiar or not (Miller & Desimone, 1991; Preston & Eichenbaum, 2013; Squire et al., 2004). The parahippocampal gyrus

has been suggested to be concerned with spatial memory and navigational features (Ekstrom & Bookheimer, 2007; Squire & Zola-Morgan, 1991). More recently however, research suggests, that the parahippocampal and medial entorhinal cortex are critical concerning the spatial context of the incoming information, rather than spatial information per se (Bar, 2004; Bar & Aminoff, 2003; Mullally & Maguire, 2011). Within the hippocampus, the information regarding the stimulus identity and the spatial context converges. This in turn allows the hippocampus to build complex memories out of these incoming stimuli (Burgess & Maguire, 2002; Diana, Yonelinas, & Ranganath, 2007; Ekstrom & Bookheimer, 2007; Preston & Eichenbaum, 2013). Retrieval of information is supported by specific feedback streams that run from the hippocampus, via the perirhinal and lateral entorhinal cortices, as well as the parahippocampal and medial entorhinal cortex, to the cortical areas, where the specific information is then processed (Hayes, Ryan, Schnyer, & Nadel, 2004; Preston & Eichenbaum, 2013).

The hippocampus is therefore critical for memory formation, i.e. encoding and consolidation, as well as memory retrieval and is concerned with a specific time-window, after initial memory formation and final long-term storage which may occur in the neocortex (Alvarez & Squire, 1994; Eichenbaum, 2000; Genzel et al., 2017; van Strien et al., 2009). The connection between the hippocampus and neocortex is important as it is suggested, that the hippocampus stores novel memories as representations that comprise elaborate details, while long term storage as a more gist-like, even schematic representations is supported by the neocortex (Alvarez & Squire, 1994; Nadel, Samnonovich, Ryan, & Moscovitch, 2000). This transformation is based upon processing along the long-axis of the hippocampus, as well as its tight connections with neocortical areas, especially the ventromedial prefrontal cortex (vmPFC) and neocortex. However, there is still ongoing research to determine how this transformation takes place and how the individual structures are involved (Dandolo & Schwabe, 2018; Robin & Moscovitch, 2017).

Another important characteristic of hippocampal memory is that it is flexible and supports the encoding of facts and personal experiences in relation to one another. It can therefore be described as inferential memory (Zeithamova, Schlichting, & Preston, 2012). This flexibility of the hippocampus-based memory has also been shown to be critical for our ability to form memory representations and benefit from these when we are dependent on the use of our previous knowledge (Shohamy & Wagner, 2008). Hippocampal memory does therefore support critical process that are underlying our ability to learn and are thus crucial for survival.

Striatum Dependent Learning and Memory

Another memory system that is also critical for learning, but lacks the flexibility of the hippocampus-based memory system is the striatum-based memory system. Early work described this system to be responsible for habit learning and S-R learning, which supports a form of long-term memory that differs from what is observed in hippocampal-based memory (Battig, Rosvold, & Mishkin, 1960; Knowlton et al., 1996; Packard & Knowlton, 2002; Yin, Knowlton, & Balleine, 2004). Starting in the 1960s, early work investigated the role of the basal ganglia in memory processes in mammals through specific lesion studies and dissociation methodologies revealing the crucial role of the dorsal striatum in S-R learning (Battig et al., 1960; Gross, Chorover, & Cohen, 1965; Packard & Knowlton, 2002). However, later research further specified that the striatum is not unanimously involved with S-R learning or habit learning, but shows a functional heterogeneity within its structure. More precisely, the lateral part of the dorsal striatum is important for S-R learning, while the medial part of the dorsal striatum supports memory processes that resemble hippocampus-based memory (Devan & White, 1999; Featherstone & McDonald, 2004; Packard & Knowlton, 2002). Hence, both the MTL as well as the dorsal striatum constitute a specific memory system that comprise specific and important functions in learning and memory (McDonald & White, 1993). Additionally, critical functions are also taken over by structures within the prefrontal cortex (PFC).

Prefrontal Structures – Learning and Memory

Prefrontal brain structures have also been shown to serve important functions in learning and memory. More precisely, the prefrontal cortex interacts with the hippocampus-based declarative memory system (Preston & Eichenbaum, 2013; van Kesteren, Fernandez, Norris, & Hermans, 2010). Furthermore, research into the mechanisms of prefrontal functioning have revealed that these are specifically implicated in learning, memory formation, retrieval, as well as supporting the use of prior knowledge during learning (Blumenfeld & Ranganath, 2007; Depue, Burgess, Willcutt, Ruzic, & Banich, 2010; van Kesteren, Fernandez, et al., 2010; van Kesteren, Rijpkema, Ruiter, & Fernandez, 2010). The prefrontal cortex has long been associated with cognitive control processes (Braver, Reynolds, & Donaldson, 2003; Cole & Schneider, 2007; Depue et al., 2010) as well as working memory (D'Esposito et al., 1995; Fregni et al., 2005). However, recent research has now shown that these control processes exerted by the prefrontal cortex may enhance or suppress specific

memories (Anderson et al., 2004; Depue et al., 2010; Javadi & Walsh, 2012). While the latter seems to be primarily carried out by the ventrolateral part of the prefrontal cortex (vlPFC), the dorsolateral part (dlPFC) has been associated with an organizing role, that may be apparent during memory retrieval processes (Blumenfeld & Ranganath, 2007). However, beyond these superordinate organizing functions, findings showed that specifically the dlPFC seems to hold a critical role not only in retrieval but also memory encoding. A study by Rossi et al (2001) showed that a disruptive form of repetitive transcranial magnetic stimulation (rTMS), resulted in a distinct impairment in either memory retrieval or encoding processes depending on whether the left or right dlPFC was targeted. More specifically, disruption of the left dlPFC led to deficits in memory encoding, while rTMS over the right dlPFC impaired memory retrieval (Rossi et al., 2001). Additional research by Javadi and Walsh (2012), investigating the role of the dlPFC in memory processing showed that using anodal transcranial direct current stimulation (tDCS), i.e. an excitatory stimulation of the left dlPFC during encoding lead to an improved subsequent memory performance, while cathodal, i.e. inhibitory stimulation lead to an impairment during a subsequent recognition test. A similar pattern was observed when tDCS stimulation was applied during a recognition test. Explicitly, cathodal stimulation disrupted memory performance, while anodal stimulation led to a trend for a significant improvement during recognition performance (Javadi & Walsh, 2012). Hence, both the left and right dlPFC may prove important during specific memory processes, with functions that go beyond the role of an organizing structure (Javadi & Walsh, 2012; Rossi et al., 2001; Sandrini et al., 2014; Sandrini, Censor, Mishoe, & Cohen, 2013). The vlPFC, on the other hand, may rather exert selection or classification functions that aid during recall or recognition procedures or are important when directing attention toward relevant information or inhibiting irrelevant incoming information (Blumenfeld & Ranganath, 2007). Importantly, the vmPFC is considered when investigating the use of prior knowledge during learning of related information (van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012). Furthermore, the vmPFC has been related to integrate relevant and suppress irrelevant information coming from the hippocampus. Over time, specifically representations in the vmPFC become more important as the hippocampus representations fade (Nieuwenhuis & Takashima, 2011). Additionally, the vmPFC signals congruency of incoming information with prior knowledge, even when the relevance may not be high (Brod & Shing, 2018). The vmPFC is also concerned with concept learning, relying on the generalization across abstract category representations (Bowman & Zeithamova, 2018). Even though critically important, the vmPFC has only recently been considered in terms of its role in memory processing. While, memory functions

supported by the prefrontal cortex are important in itself, a number of studies have reported strong interactions with the hippocampus that have proven crucial during learning and memory (Benchenane et al., 2010; Dobbins, Foley, Schacter, & Wagner, 2002; Preston & Eichenbaum, 2013; Siapas, Lubenov, & Wilson, 2005).

Interaction between Prefrontal Areas and Hippocampus in Learning and Memory

The above mentioned functions of the prefrontal cortex in learning and memory are crucial, but may not only depend on prefrontal cortex structures, but critically on bidirectional interactions with the hippocampus (Preston & Eichenbaum, 2013). What has been suggested, is an important interplay during which the hippocampus is concerned with the establishment and reiteration of memories while the prefrontal cortex adds representations of related memories together with the contexts in which they occurred. During retrieval, the prefrontal cortex enables a detection of how these related memories interact (Preston & Eichenbaum, 2013). Damage to the prefrontal cortex has been shown to impair the ability to flexibly shift between representations as the specific context cannot be accessed (Birrell & Brown, 2000; Preston & Eichenbaum, 2013; Rich & Shapiro, 2007). In lay terms, there is a division of labor between the hippocampus and prefrontal cortex in the consolidation and retrieval of memories, whereby the hippocampus is concerned with forming new memories and the prefrontal cortex with flexibly switching between these new memories (Miller & Cohen, 2001; Preston & Eichenbaum, 2013). This has been shown in different experiments in animals and humans that showed an impairment in the ability to switch between different strategies or memories (Dias, Robbins, & Roberts, 1996; Ragozzino & Kesner, 1999; Rich & Shapiro, 2007).

Evidence that the hippocampus - prefrontal cortex interaction is also critical during memory retrieval has been investigated in patients with damage to the prefrontal cortex (Shimamura, Jurica, Mangels, Gershberg, & Knight, 1995). Impairments are not obvious in simple memory retrieval, but during situations with increased cognitive load or interference. Furthermore, patients were not able to successfully complete a paired association task that required the retrieval of learned associations and then use this prior knowledge to form new associations with the known items (Preston & Eichenbaum, 2013; Shimamura et al., 1995). Another important aspect of hippocampal-prefrontal coordination is an inhibitory control function, as the prefrontal cortex can exert inhibitory control

over retrieval mechanisms (Depue, 2012). This function is tested with different tasks, namely, the 'white bear experiment', directed forgetting paradigms and think/no-think tests (Anderson & Green, 2001; Bjork & Woodward Jr, 1973; Reitman, Malin, Bjork, & Higman, 1973; Wegner, Schneider, Carter III, & White, 1987). These tasks all require participants to exert control over retrieving specific items and to suppress memory retrieval (Depue, 2012). The neural underpinnings of these control mechanisms exerted by the prefrontal cortex have been revealed in an animal model (Navawongse & Eichenbaum, 2013). In a study by Navawongse and Eichenbaum (2013), rats learned two contradictory associations in two distinct spatial contexts. The associations are selectively encoded by the hippocampus in their corresponding spatial context. Later, being placed in a specific context may aid the retrieval of the corresponding association. Critically, the selection of which item or association is retrieved depends on the functional integrity of the mPFC, as lesions to this structure resulted in a retrieval of both associations, independent of the spatial context (Navawongse & Eichenbaum, 2013). It is therefore suggested that prefrontal interactions with the hippocampus via the entorhinal cortex support these control mechanisms (Komorowski, Manns, & Eichenbaum, 2009; Navawongse & Eichenbaum, 2013).

These results therefore highlight the importance of the interaction between the prefrontal cortex and the hippocampus in the formation but also in the retrieval of memories. However, in our daily life, encoding, consolidation and retrieval of memories rarely happen in isolation. Instead, we constantly need to integrate newly encoded information into our existing knowledge and retrieve older information that might be useful in our current situation. Forming connections between related memories the (medial) prefrontal cortex serves a crucial function that allows us to profit from memories and experiences we have made (Dias et al., 1996; Preston & Eichenbaum, 2013; Rich & Shapiro, 2007; van Kesteren, Fernandez, et al., 2010). The question how exactly we make use of previous experiences has been investigated in several lines of research. More specifically, research on schema-based learning implies that we can use our knowledge as a framework which aids our learning of new items that are congruent with the established framework (van Kesteren et al., 2012). When confronted with a new situation, on the other hand, we may also benefit from previous knowledge of analogous problems that share common characteristics with the current situation but are not completely identical, as is the case in memory generalization (Shohamy & Wagner, 2008). These two learning and memory mechanisms are the focus of the current work and are described and discussed in detail below.

Schema-Based Learning and Memory

The concept of memory schemas has been introduced to psychology very early, whereby a schema essentially describes the existence of previous knowledge or knowledge structures that facilitate the integration of new but schema-related knowledge, which in turn aids learning (van Kesteren et al., 2012). Early in the 20th century, Barlett (Barlett, 1932) as well as Piaget (Piaget, 1926) have referred to a schema as a mental representation of knowledge, comprising a certain structure. In other contexts, the schema construct has been referred to by different names, such as scripts, story grammars and frames (Minsky, 1975; Rumelhart, 1980; Schank & Abelson, 1977), though all serving the same goal, to provide a structure of pre-existing knowledge that facilitates learning of congruent information. The idea of a mental schema is quite intriguing as it allows us to gain insight into how acquired knowledge can facilitate new learning and accelerate consolidation (van Kesteren et al., 2012). More precisely, a mental schema aids the integration of new information while utilizing existing information that matches the context and acts as a scaffold (van Kesteren et al., 2012) and this can be applied to semantic as well as episodic memories (McKenzie & Eichenbaum, 2011).

A prominent example of an experiment assessing schema related memory stems from Brewer and Treyens (1981), who examined the role of schemas in memory specific for places. Participants were put into a room that was made to look like a graduate students' office. The room contained items that would be expected to exist in such a type of office, in addition to items that were out of place and did not belong in such a setting. Subjects were left in the room for a brief period of time and where then asked what they remembered about the room and specifically about the items. Participants remembered items that were present in the office particularly well when these typically belonged into an office. In addition to the items actually in the room, subjects also remembered things that were not there, but that one would expect to be in an office. Moreover participants remembered items that were clearly out of place and were not associated with the specific setting (Brewer & Treyens, 1981). This experiment impressively showed the effect of a pre-existing schema on our memory, specifically, a schema that is acquired gradually over the course of our life. Brewer and Treyens (1981) had to assume that all their participants had a similar mental image of an office, so that participants would not only remember the items that were there and belonged into an office, but also falsely remembered items that were not actually in the experimental office. Hence, the existence of a memory schema does not only facilitate learning of

schema-related information but also the identification of information that is schema-inconsistent and in addition, augments the false recognition of items that are not there but are compatible with a certain schema (Brewer & Treyens, 1981; van Kesteren et al., 2012). Furthermore, a schema may be activated to compensate for a declining memory, however, this could trigger wrong memories (Kleider, Pezdek, Goldinger, & Kirk, 2008).

Investigating schema-based learning and memory in the lab is a challenging task, as schemas are suggested to be acquired over a very long time. However, there are established tasks that can be used successfully to investigate the behavioral, but also neural underpinnings of schema-based learning (Gilboa & Marlatte, 2017), such as transitive inference paradigms, paired associates tasks, as was used in animals or a movie-based recognition tasks (Bethus, Tse, & Morris, 2010; Kleider et al., 2008; Kumaran, 2013; Tse et al., 2007; Tse et al., 2011; van Kesteren, Fernandez, et al., 2010). Using these tasks, schema-based learning was reported to activate the medial prefrontal cortex (mPFC), specifically the vmPFC, as well as the angular gyrus and precuneus, while novel information that was not schema-related activated the hippocampus (Tse et al., 2007; van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012; Wagner et al., 2015). However, if a schema is extensively trained, even over-trained, then retrieval of this schema after a period of three months triggered activity in different structures. Precisely, the vlPFC showed activity, together with the anterior temporal lobe, the angular gyrus and temporo-parietal junction (TPJ; Sommer, 2017). Although over-training of a schema is associated with activity in structures different to the vmPFC, the vmPFC was shown to be crucial for the reinstatement of a schema, even after over-training (Gilboa & Marlatte, 2017). Hence, the mPFC is a critical structure in schema-based learning and may detect the fit of incoming information with a pre-existing schema (Richards et al., 2014). Furthermore, its interaction with the hippocampus is critical during initial schema formation (Gilboa & Marlatte, 2017; Preston & Eichenbaum, 2013). Van Kesteren et al (2012) have proposed a model that describes the mPFC as a critical structure detecting the congruency of incoming information and comparing this incoming information with pre-existing information in the neocortex. This detection functions through a sort of resonance, as information matching prior knowledge triggers a synchronous oscillation within the mPFC (Engel, Fries, & Singer, 2001; van Kesteren et al., 2012). The higher the congruency of the incoming information with the pre-existing information, the greater the resonance and the greater the activity within the mPFC (van Kesteren et al., 2012).

Hence, the mPFC is critical as it detects whether incoming information is schema-congruent and therefore facilitates the use of a pre-existing schema to aid learning while the hippocampus is

concerned with novel information that may not match a pre-existing schema, as well as the establishment with a new schema before the information is transferred to the neocortex. (Gilboa & Marlatte, 2017; Packard & Wingard, 2004; Richards et al., 2014; van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012).

Memory Generalization

Another memory process that makes use of pre-existing knowledge is referred to as memory generalization. Memory generalization is distinct from schema-based learning in its functioning as well as the neural mechanisms and is suggested to depend majorly on the flexible hippocampus-based memory (Myers et al., 2003; Shohamy & Wagner, 2008).

Memory generalization enables us to utilize what we have learned previously and apply this knowledge to new situations that bear some similarity to what we have experienced before instead of adjusting completely anew (Shohamy & Wagner, 2008). It is suggested that our memories are stored as separate entities in the hippocampus and can be linked together according to their similarity in context or content, as an integrated representation, that can also be also retrieved together by the hippocampus (Shohamy & Wagner, 2008; Zeithamova, Dominick, & Preston, 2012). Based on this specific ability to utilize our experiences, we can deduce outcomes or situations from our memory and are therefore able to adapt more rapidly to changing environments and aid our learning and decision-making (Shohamy & Wagner, 2008). In contrast to schema-related memory, memory generalization was found to be crucially dependent on the flexible, declarative memory of the hippocampus. Work in rodents has shown that when synaptic plasticity is blocked in the hippocampus, the animals' ability to generalize across the experienced and new situation is disrupted (Iordanova, Good, & Honey, 2011). Work in humans indicated that the MTL area, but specifically the hippocampus, is distinctively activated when overlapping events are encoded, for example during an acquired equivalence paradigm (Shohamy & Wagner, 2008; Zeithamova, Dominick, et al., 2012). Further evidence has been obtained from an experiment with patients suffering from hippocampal damage as well as patients with Parkinson's disease who suffer from damage to the basal ganglia. Patients were asked to complete a generalization task. More specifically, participants were required to transfer previously learned information to a novel recombination. While Parkinson's patients were slow in the initial learning, their ability to

generalize was not impaired. In patients suffering from hippocampal damage however, initial learning was not compromised, but these patients were not able to generalize previously learned information to novel situations, therefore confirming assumptions that specifically the hippocampus is concerned with associative learning and generalization (Eichenbaum, 2000; Myers et al., 2003; Squire & Zola-Morgan, 1991).

Shohamy and Wagner (2008) proposed a mechanism by which the hippocampus supports generalization quickly without relying on making inferences. They suggested, that the hippocampus stores memories as discreet entities and when a new memory is acquired that bears similarities with an already existing memory, both memories are linked together and stored as integrated representations. Following input from dopaminergic midbrain, these representations might later be retrieved together allowing for memory generalization. Hence the hippocampus is particularly suited to support generalization as it supports a highly flexible memory and allows dynamic switches between memory formation and retrieval modes (Shohamy & Wagner, 2008). While this proposed mechanism sounds plausible and may actually support memory generalization it is to note that the results reported by Myers et al (2003) point to no impaired generalization in Parkinson's patients, a disease that is associated with decreased dopaminergic signaling in the midbrain, specifically the substantia nigra (Braak, Ghebremedhin, Rub, Bratzke, & Del Tredici, 2004). Hence, these two suggestions may not seem to be in accord. However, Myers et al (2003) described their patient populations as suffering from 'mild' Parkinson's disease and 'mild to moderate' hippocampal atrophy (Myers et al., 2003), respectively. It may therefore be the case that the dopamine signaling even in a slightly downregulated form, as can be expected in mild Parkinson's disease, is still sufficient to support the switch between the formation and retrieval of integrated representations in the hippocampus, as is suggested by Shohamy and Wagner (2008), while mild to moderate hippocampal atrophy may already impair the formation and retrieval of these integrated representations.

The hippocampus and prefrontal cortices, implicated in memory generalization (hippocampus; Myers et al., 2003; Shohamy & Wagner, 2008) and schema-based learning (hippocampus and vmPFC; van Kesteren, Fernandez, et al., 2010; van Kesteren, Rijpkema, et al., 2010), therefore support complex and critical memory processes that are important in daily life, i.e. for our ability to benefit from prior knowledge. However, it is not yet clear how prone these processes are to the modulation by external and intrinsic mechanisms, such as stress and the stress mediators cortisol and noradrenaline. Especially the experience of acute stress and major stress mediators cortisol and

noradrenaline have been shown to strongly affect learning and memory processes (Atsak et al., 2016; Buchanan & Tranel, 2008; Buchanan, Tranel, & Adolphs, 2006; Cahill, Gorski, & Le, 2003; Diamond et al., 2006; Kukolja, Klingmuller, Maier, Fink, & Hurlemann, 2011; Maroun & Akirav, 2008; Payne et al., 2007). So far, however there is no insight on how stress impacts the use of previous knowledge to aid learning (schema-based learning) and our ability to use our memories to generalize across experiences (memory generalization).

Chapter II

Stress and Cognition

The above mentioned processes, schema-based learning and memory generalization, are highly relevant in our daily life, as they facilitate learning and enable us to adjust to an ever changing environment and thus support our survival due our ability to utilize previous knowledge. However, in everyday life we are often confronted with stressful situations that may impact our behavior, specifically learning and decision-making processes (Buckert, Schwieren, Kudielka, & Fiebach, 2014; Cahill et al., 2003; Gathmann et al., 2014; Joels, Pu, Wiegert, Oitzl, & Krugers, 2006). Yet, we lack insight into how stress affects our ability to use and benefit from prior knowledge during learning.

Stress is omnipresent in our daily life. We may be exposed to a stressor in different contexts or situations, such as the stress we experience during an important exam or during a presentation, or the psychosocial stress that we may be subjected to during a job interview. What is common to these situations is the fact that we notice changes in our cognitive abilities while under stress, such as forgetting important facts, which we are asked about in the exam, important points we were going to mention during a presentation or not being able to recall where we worked before our current job. Later, we may however explicitly remember the stressful situation very detailed, sitting the exam, standing in front of a large group of people during the presentation or sitting in that job interview, during which we could not remember anything. These examples highlight two things quite strongly: the disruptive effect stress has on our abilities to retrieve information and the enhancing effect stress can have on memory formation (Wolf, 2017; although there is also the suggestion, that learning under stress actually disrupts formation of new memory, see: Schwabe & Wolf, 2010a). While we often experience situations as stressful, in the current work stress is considered as our physiological reaction to a potential stressor, which may be a situation or threat.

Stress actually functions as a protective mechanism that enables us to adequately react to a threat. Thus, even this short-lived impairment of memory retrieval due to stress prevents distraction and facilitates crucial memory formation to enable us to remember this potential threat we were exposed to (Schwabe, Joels, Roozendaal, Wolf, & Oitzl, 2012; Vogel, Fernandez, Joels, & Schwabe, 2016).The impact of stress is therefore certainly adaptive as it aids us to focus on the current situation and to form memories that allow us to remember this stressful experience, but certainly also disruptive to other, possibly unrelated memory processes (Vogel et al., 2016). The next

sections will therefore provide a general overview of previous research and important findings of stress on memory processes, followed by the physiology of the fast and slow stress response and how this specifically affects structures in the brain that lead to the observed impact on memory processes.

Stress Effects on Learning and Memory

Due to the prevalence of stress in our daily life, there is already an extensive body of research and an interest to examine how stress effects on memory are mediated and precisely which learning and memory processes are affected and how. Up to this point however, we lack insight into how stress impacts the use of prior knowledge, through schema-based learning and memory generalization.

When considering long-term memory, a main interest has been the flexible hippocampus-based memory and the associated functions, such as declarative memory encoding, consolidation, retrieval and reconsolidation. Previous results have indicated, that stress may have a variable impact on these processes that may lead to an enhancement or impairment, depending on the specific process under investigation (Cahill et al., 2003; Cornelisse, van Stegeren, & Joels, 2011; De Quervain, Roozendaal, & McGaugh, 1998; Diamond et al., 2006; Guez, Saar-Ashkenazy, Keha, & Tiferet-Dweck, 2016; Joels et al., 2006; Roozendaal, 2002; Schwabe, 2013). For example, stress has been shown to boost encoding of stressor related material, while it may impair encoding for stressor unrelated events (Schwabe, Bohringer, Chatterjee, & Schachinger, 2008; Smeets, Giesbrecht, Jelicic, & Merckelbach, 2007; Vogel & Schwabe, 2016a). Furthermore, stress enhances consolidation (de Quervain, Schwabe, & Roozendaal, 2017; McGaugh, 2018; Roozendaal & Hermans, 2017; Roozendaal et al., 2009) but impairs memory retrieval (De Quervain et al., 1998; Schilling et al., 2013; Smeets, 2011; Wolf, 2017). The impact of stress on reconsolidation processes is heterogeneous, as results reported point to an enhancing, but also impairing effect (Bos, Schuijer, Lodestijn, Beckers, & Kindt, 2014; Maroun & Akirav, 2008; Schwabe & Wolf, 2010c; Wang, Zhao, Ghitza, Li, & Lu, 2008), though it may be critically dependent on how reconsolidation was tested. Furthermore, the results crucially depend on the timing of the stressor (Joels, Fernandez, & Roozendaal, 2011; Schwabe, Joels, et al., 2012; Schwabe & Wolf, 2013). Stress before learning may impact encoding and thus successive memory, as stated above. Whether subsequent memory processes are enhanced or impaired however, depends on different factors, such as emotionality of

the learned stimuli, how long stress was applied before learning and when memory is tested (Diamond, Campbell, Park, Halonen, & Zoladz, 2007; Diamond et al., 2006; Elzinga, Bakker, & Bremner, 2005; Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Maroun & Akirav, 2008; Payne et al., 2007; Schwabe, Joels, et al., 2012; Smeets et al., 2007; Zoladz et al., 2011).

Effects of Stress and Sex

Considering the variable effects of stress on memory processes, it may also be important to take into account possible effects of sex. A multitude of studies has reported diverse effects of stress in men and women, such as impaired S-R learning in men, but not in women, while spatial memory was impaired in women and not in men after exposure to a stressor (Guenzel, Wolf, & Schwabe, 2014). Furthermore, while exposure to a stressor results in similar increased levels of salivary cortisol, only men showed enhanced memory (Cahill et al., 2003). Besides differential effects of stress on memory performance in men and women, findings also showed increased risk taking in men after intake of hydrocortisone or exposure to an acute stressor (Kluen, Agorastos, Wiedemann, & Schwabe, 2017; Lighthall, Mather, & Gorlick, 2009). These differences observed between the sexes may come about as sex hormones, menstrual cycle phase, but also oral contraceptives impact stress reactivity (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999).

To appreciate these results and also to develop an understanding as to how stress could impact schema-based learning and memory generalization, it is crucial to appreciate the mechanisms that may be active during a stress response and how these may impact specific brain regions and in turn behavior.

Stress Mechanisms and Modulators

Stress in our daily life indicates a potential threat to an individual and its intrinsic homeostasis, which triggers changes in current and even future behavior. Stress may be signaled by a stressor, which can be a physical or psychological threat. Physical stressors include injuries to the body or unpleasant or painful bodily experiences, such as ice cold water on the skin for an extended period of time and lead to the activation of hypothalamic and brain stem neurons (de Kloet, Joels, &

Holsboer, 2005; Ulrich-Lai & Herman, 2009) . Psychological stressors describe embarrassment, as well as exposure to unpleasant situations without bodily pain. It is to note however, that physical and psychological stressors also act in combination (Joels & Baram, 2009). When we are exposed to a stressor, a stress response is elicited that consists of the activation of discrete but also interacting systems, a fast catecholaminergic stress response and a slow glucocorticoid response, as well as the activity of fast acting neuropeptides (Joels & Baram, 2009; Pu, Krugers, & Joels, 2007; Quirarte, Roozendaal, & McGaugh, 1997).

Fast Stress Response

Immediately after the exposure to a stressor, a fast reaction is initiated that involves the central and peripheral parts of the nervous system and quickly (within a few minutes) leads to the liberation of catecholamines such as adrenaline and noradrenaline as well as peptides such as corticotrophin releasing hormone (CRH) and rarely lasts longer than exposure to the stressor (Joels & Baram, 2009). The incoming stressful stimuli are processed immediately after exposure and the information is relayed by the parasympathetic nervous system to parts of the central nervous system (CNS). Within the CNS, information is then transferred via the hypothalamus and sensory regions of the brain to the prefrontal, but in particular limbic structures (Romero & Butler, 2007; Smith & Vale, 2006). Activation of limbic nuclei is initiated that project to brain stem nuclei, such as the locus coeruleus, raphe nucleus and, outside of the brainstem, the hypothalamus, which constitutes an important relay center. Hypothalamic activation then triggers the release of CRH from the paraventricular nucleus (PVN) of the hypothalamus (Smith & Vale, 2006; Valentino, Foote, & Aston-Jones, 1983; Whitnall, 1993). CRH release is a crucial step in the tight regulation of the stress response as it is involved in the regulation of the glucose metabolism as well as the regulation of the cardiovascular and respiratory system, but also in the slower part of the stress response by initiating the secretion of adrenocorticotropic releasing hormone (ACTH; Smith & Vale, 2006). In addition to CRH, the fast stress response is also characterized by activation of the sympathetic nervous system (SNS) through the hypothalamus and a rapid release of adrenaline and noradrenaline from the adrenal medulla (Joels & Baram, 2009; Joels et al., 2006). However, noradrenaline is also directly released within the brain. This release is mediated by the vagal nerve and the solitary tract nucleus, which is part of the medulla oblongata in the brain stem (McGaugh & Roozendaal, 2002). Subsequently, it has also been shown that there is an increased firing of

noradrenergic neurons from the locus coeruleus, which leads to an increased exposure of the basolateral amygdala to noradrenaline (McIntyre, Hatfield, & McGaugh, 2002). Noradrenaline actions are mediated by fast G-Protein coupled receptors that allow for the rapid action of noradrenaline (Joels & Baram, 2009). More specifically, in the basolateral amygdala adenylate cyclase binds to beta-adrenoceptors that lead to cyclic adenosine monophosphate (cAMP) formation. cAMP is a second messenger, which in turn activates the cyclic-adenosine3'5'-monophosphate (cAMP)-dependent protein kinase A (PKA). Through phosphorylation, PKA activates enzymes that promote the formation of glucose from glycogen, as well as enzymes that endorse muscle contraction in the heart, allowing the animal to react quickly to the stressor, by either fight or flight (McGaugh & Roozendaal, 2002; Schwabe, Joels, et al., 2012). The locus coeruleus exclusively releases noradrenaline that is transported to and affects structures within the cortex and the hippocampus (Valentino & Van Bockstaele, 2008). Both CRH and noradrenaline have been found to strengthen synaptic connectivity within the hippocampus and facilitate synaptic plasticity, therefore leading to an increase in functionality and better learning of the events associated with the stressor (de Kloet et al., 2005)

Slow Stress Response

After the initiation of the fast stress response, the slower part of the stress response is activated as well. This part is constituted by the Hypothalamic-Pituitary-Adrenal (HPA) axis. As mentioned above, CRH is released from the PVN of the hypothalamus and transported in hypophysial portal vessels to the pituitary gland. Within the pituitary, CRH binds to specific receptors, so-called pituitary corticotropes that cause the liberation of ACTH into the circulation. ACTH then acts on the adrenal cortex to initiate synthesis and release of glucocorticoids. Glucocorticoids, such as cortisol in humans, are released from the zona fasciculate in the adrenal cortex (Smith & Vale, 2006). Glucocorticoid levels increase at about five to ten minutes after stress onset and reach their peak levels at about 20 to 30 minutes past stressor onset (Dickerson & Kemeny, 2004). Glucocorticoids also function as a restoration system after stress. Glucocorticoids act on the pituitary gland and PVN to inhibit the HPA axis and thus aid the return to homeostasis after the stress response. Additionally, glucocorticoids are also involved in reinstating energy resources after these have been depleted after stress (Joels et al., 2011). Generally, the feedback and restoration mechanisms of

this stress response operate reliably to ensure a reinstatement of homeostasis. However, pathologies may develop as excessive HPA activation cannot be buffered, or when there is decreased HPA activation (Burke, Fernald, Gertler, & Adler, 2005; Jansen et al., 1998; Lovallo, Dickensheets, Myers, Thomas, & Nixon, 2000; Smith & Vale, 2006; Stokes, 1995; Stratakis & Chrousos, 1995).

Glucocorticoid effects are mediated via two different receptor types: glucocorticoid (GR) and mineralocorticoid (MR) receptors that differ in their affinity for glucocorticoids and their location within the brain. More specifically, MRs are located in the cerebral cortex, the limbic regions, notably the hippocampus and amygdala, the periventricular region as well as outside of the brain, in the heart (de Kloet, Karst, & Joëls, 2008; de Kloet et al., 2000; Gomez-Sanchez & Gomez-Sanchez, 2014; Groeneweg, Karst, de Kloet, & Joels, 2012; Harris, Holmes, de Kloet, Chapman, & Seckl, 2013). MRs have further been shown to be involved in synaptic plasticity, however only when aldosterone instead of cortisol/corticosterone is active (Maggio & Segal, 2012). GRs have been reported to co-exist with the MR, although the GR is much more widespread throughout the brain, but specifically a high concentration is located in areas that are involved in orchestrating the stress response (Han, Ozawa, Matsuda, Nishi, & Kawata, 2005; Joels & De Kloet, 1994, 2017; Oitzl & De Kloet, 1992; Wang et al., 2013). GRs are involved in the reinstatement of homeostasis and the recovery from stress and therefore constitute the negative feedback mechanism. GRs have a low affinity for glucocorticoids, hence these receptor types are only active when plasma glucocorticoid levels are heightened. This may be the case after a stress response or a high concentration during the day due to the circadian rhythm (de Kloet & Reul, 1987; Groeneweg et al., 2012; Smith & Vale, 2006). The MR on the other hand has a high glucocorticoid affinity, which means that even at baseline concentrations, this receptor type may be occupied (Groeneweg et al., 2012). Due to this almost general occupancy of the receptor, it was suggested that it may be involved in determining an individuals' threshold for stress sensitivity (Joels, Karst, DeRijk, & de Kloet, 2008), while both GRs and MRs are proposed to set the stress responsiveness of an individual (Groeneweg et al., 2012). Initially it was assumed that both receptor types only mediate slow genomic actions of glucocorticoids, as both receptors are ligand-driven transcription factors that translocate into the nucleus of the cell to alter gene transcription (Joels & de Kloet, 2017; Joels, Sarabdjitsingh, & Karst, 2012). However, recent evidence also points to rapid cortisol effects that act via membrane bound G-protein coupled mineralocorticoid receptors and mediate the fast, non-genomic glucocorticoid effects (Joels et al., 2008; Joels, Pasricha, & Karst, 2013; Vogel et al., 2016). Rapid, non-genomic

effects were suggested to exert impairing effects on specific cognitive functions, while slow genomic effects were thought to exert the opposite effects. Evidence comes from studies in rodents and humans, showing that glucocorticoids (corticosterone in rodents and cortisol in humans) can rapidly eradicate irrelevant behaviors, affect hippocampus-based behavioral flexibility and reactions toward new, unknown objects. Further evidence then pinpointed the rapid non-genomic effects in the hippocampus, as well as in the amygdala (Bohus & De Kloet, 1981; Joels et al., 2008; Karst, Berger, Erdmann, Schutz, & Joels, 2010; Karst et al., 2005; Oitzl & De Kloet, 1992; Oitzl, Fluttert, & De Kloet, 1994). However, impairing effects of cortisol on memory were also reported to be present at 25 minutes after stress onset but remained at 90 minutes, although cortisol levels had already decreased to baseline (Schwabe & Wolf, 2014). The fact that memory retrieval was still impaired, even when cortisol levels returned to baseline after an extended period of time, may point to a disruptive effect of slow genomic cortisol (Dorey, Pierard, Chauveau, David, & Beracochea, 2012; Schwabe & Wolf, 2014; Wolf, 2017).

Glucocorticoids also impact the noradrenergic system, as they act on brainstem noradrenergic cell groups that project to the basolateral amygdala (Roozendaal, Okuda, de Quervain, & McGaugh, 2006). These projections couple with alpha-adrenoceptors and postsynaptically interact with the beta-adrenergic receptors in the basolateral amygdala. Furthermore, action of glucocorticoids and catecholamines in the basolateral amygdala affect other brain regions as well, such as the hippocampus and prefrontal regions (Roozendaal, Okuda, de Quervain, et al., 2006). Hence, the stress response encompasses different systems that comprise different components and act at different timings throughout the tightly regulated stress response. It is also important to note that brain regions are differentially impacted, which is especially critical when regarding the divergent effects on memory observed after stress.

Impact of Stress and Stress Mediators on Brain Structures and Memory Functions

Stress effects on memory have been described in various studies (Kim, Lee, Han & Packard, 2001; Kim & Diamond, 2002; Meir Drexler & Wolf, 2017; Payne et al., 2007; Schwabe, Joels, et al., 2012). In addition to our knowledge about which effects stress has on the distinct memory functions, the mechanisms behind the impairing, but also enhancing effects are of great interest. Especially, when it is known that a memory process is impacted by stress, it is crucial to understand whether all

components of the stress response are necessary for the impact stress has on a memory function or if specific parts of this stress response are sufficient to lead to a disruption or an enhancement.

Effects of Stress and major Stress Mediators on Hippocampus-Based Memory Formation

As mentioned before, there is a great difference between the stress effects exerted on different memory functions. Researchers have considered the enhancing effects on memory formation processes (encoding and consolidation) already in the late 1960s (Bohus & Lissak, 1968). In an experiment by Bohus and Lissak (1968), rats were given either a cortisol treatment or an adrenalectomy and were conditioned in a fear-response paradigm. Increased cortisol led to a facilitation of the extinction of an avoidance response, while a delay in the extinction was reported when adrenocortical hormones were removed after an adrenalectomy (Bohus & Lissak, 1968). Similar findings were already obtained even earlier by de Wied in 1966. He investigated the effects of ACTH and glucocorticoids on avoidance behavior. De Wied (1966) showed that administration of corticosterone or the synthetic compound dexamethasone led to a facilitated extinction, while ACTH did not have such an effect (De Wied, 1966). Additional and later work showed impressively that stress effects on behavior mediated by glucocorticoids seemed to be receptor specific. Oitzl et al (1992) showed that the use of MR and GR antagonists in rats led to an impairment of different characteristics of spatial learning in a water maze. More specifically, injection of a GR antagonist into the hippocampus disrupted consolidation of spatial information after learning. MR antagonists however led to a change in behavior of the animal in the use of different escape strategies. It was therefore concluded, that MRs are specifically involved in evaluating and responding to certain situations (Oitzl & De Kloet, 1992).

Opposing Effects of Cortisol on Hippocampus-Based Memory Encoding and Consolidation

Research further showed opposing effects of glucocoticoids on memory depending on context and location of action. More specifically, circulating corticosteroids that could be associated with a current learning task lead to an increase in performance (Roozendaal & McGaugh, 1997), while the opposite was true for circulating glucocorticoids that could not be associated with a current task, showing that stress effects, or the effect of the stress mediator cortisol may be context dependent (Oitzl, Fluttert, & De Kloet, 1998). Furthermore, it is suggested that modulation of stress or the

stress hormones on hippocampus-based memory may be mediated through the amygdala (Fastenrath et al., 2014; Kim et al., 2001; McGaugh, 2002). However, only infusions of glucocorticoids directly into the basolateral part of the amygdala led to an enhancing effect on memory and no effect was found for infusions into the central part of the amygdala, specifically indicating that the basolateral amygdala is critical for the observed effects (Roozendaal & McGaugh, 1997). Oitzl et al (1998) were able to show later that a blockage of the GR receptors within the hippocampus, either uni- or bilaterally, resulted in increased spatial learning, in a dose-dependent matter. Thus, blocking this receptor enabled enhanced consolidation, specifically for spatial information (Oitzl et al., 1998). While the effects observed are opposing to previous findings of the same group, the authors explain that the findings are specific to the site of blockade. More specifically, intraventricular injection of a GR antagonist led to an impairment of memory consolidation (Oitzl et al., 1998), while injections of GR antagonists in the dorsal part of the hippocampus exerted a facilitation of spatial memory consolidation (Oitzl & De Kloet, 1992) therefore preventing either general action of corticosterone and other receptor agonists or a localized action within the hippocampus (Oitzl et al., 1998). Hence, stress effects, or the action of the individual stress mediators on memory processes are highly specific, depending on the timing of the stressor, but also the location of action.

Noradrenergic Effects on Memory Formation in the Hippocampus

While the above effects are primarily dependent on increased glucocorticoid levels, it is also necessary to specifically consider the effect of noradrenaline on memory processes. Already in the 1970s, Kety (1972) and Gold and Buskirk (1978) provided evidence for catecholaminergic action on memory processing (Gold & van Buskirk, 1978; Kety, 1972; Roozendaal & Hermans, 2017). Later research showed that noradrenaline administration or injection of a beta-adrenoceptor agonist directly into the amygdala led to a memory enhancement, although only if administered immediately after an emotionally arousing training experience. Injection of noradrenaline into the hippocampus or entorhinal cortex facilitated inhibitory avoidance training and later consolidation of the experience (Barsegyan, McGaugh, & Roozendaal, 2014; Hatfield & McGaugh, 1999; Liang, McGaugh, & Yao, 1990; Yang & Liang, 2014). Further research in humans indicated that administration of an alpha2-adrenoceptor antagonist (yohimbine) led to a memory enhancement of emotional material, if given before initial learning (O'Carroll, Drysdale, Cahill, Shajahan, & Ebmeier,

1999). However, if the action of noradrenaline was blocked, memory enhancement of particularly arousing stimuli was impaired (Cahill, Prins, Weber, & McGaugh, 1994). The impact of noradrenaline, released by the locus coeruleus is also apparent during memory encoding, as it regulates attentional processes that facilitate input of information that will be encoded later on (Roozendaal & Hermans, 2017), most efficiently at moderate concentrations as its mode of action is dependent on an inverted U-shape, with too high or too little concentration not being beneficial (Aston-Jones & Cohen, 2004). Furthermore, noradrenaline is also implicated in the activation of the salience network, including the dorsal anterior cingulate cortex, anterior insula and amygdala and functions to provide adequate homeostatic regulation of attentional and affective processing, such as what is necessary after exposure to a stressful stimulus (Hermans et al., 2011; Seeley et al., 2007). Additional research has indicated that increased noradrenergic activity together with increased amygdala activity lead to an enhanced memory formation, however specifically for emotional items or information (Roozendaal & Hermans, 2017)

Stress Effects on Hippocampus-Based Memory Retrieval

In contrast to the usually enhancing effects of stress on memory encoding and consolidation, memory retrieval is often found to be impaired. In an early experiment by De Quervain and colleagues (1998), rats were trained in a spatial learning task (water maze) and subjected to footshocks, two-, 30 and 240 minutes before retention testing in the water maze. Rats that received footshocks at 30 minutes before retrieval testing were impaired in comparison to controls and could not recall the positon of the target area within the maze very well. Recall testing at two or 240 minutes revealed no impairment. Administration of exogenous corticosterone caused similar, but dose-dependent effects. When the synthesis of corticosterone was inhibited by a drug, these impairing effects on retrieval were not observed. Hence, stress, through the activation of the HPA axis, has strong effects on the retrieval of hippocampus dependent memory in a time- but also dose-dependent manner (De Quervain et al., 1998). Glucocorticoids specifically also exert direct effects on central memory structures, such as the hippocampus. These effects may come about via the MR and GR receptors (Oitzl et al., 1998).

In addition to specific effects of stress mediators on memory functions and the resulting behavioral effects, it is also important to consider where in the brain these mediators exert their action. The amygdala has been shown to be a crucial structure for mediating stress effects on hippocampus-

based retrieval (Kim, Lee, Han, & Packard, 2001). Kim and colleagues (2001) examined the involvement of the amygdala in the effects of stress on hippocampus-based memory retrieval and showed in an experiment that behavioral and physiological stress effects in the hippocampus could be abolished when the amygdala was lesioned. Physiologically, hippocampal slices from normal animals showed aberrant synaptic plasticity after stress, in comparison to normal controls, while animals with amygdala lesions presented with normal synaptic plasticity after stress. Behaviorally it was also shown that stress led to an impairment of the retrieval of spatial memory and that this impairment was also abolished when animals had lesions in the amygdala (Kim et al., 2001). It was shown further that activity in the amygdala is only detrimental for memory processes during stress and not shortly after stress (Kim, Koo, Lee, & Han, 2005).

Direct effects of stress and glucocorticoids on memory retrieval were investigated in an experiment by Roozendaal et al (2004). Roozendaal and colleagues assessed hippocampus-based memory retrieval mechanisms and could show that, in rats, the retrieval of long-term spatial, i.e. hippocampus dependent memory is impaired by glucocorticoids, as these facilitate noradrenergic activity within the hippocampus. A noradrenergic mechanism acting in the basolateral amygdala, on the other hand, facilitates the impairing effects of glucocorticoids in the hippocampus and thus on memory retrieval (Roozendaal, Hahn, Nathan, de Quervain, & McGaugh, 2004).

Stress Effects on Prefrontal Cortex Functionality

In addition to the effects of stress on learning and memory that are crucially supported by the hippocampus, there are two additional structures that are important to consider in the current context. Firstly, stress effects on the prefrontal cortex are critical, as this region is crucially important for memory processes (Javadi & Walsh, 2012; Rossi et al., 2001; Sandrini et al., 2014), such as schema-based learning (van Kesteren et al., 2012) and has been shown also to be highly sensitive to stress (Arnsten, 1998, 2009). Early observations showed that pilots, even though highly skilled, made many errors during times when they were stressed. These errors were traced back to the detrimental effects stress has on prefrontal functioning, as flying complex maneuvers during battles required activity of the prefrontal cortex to control behavior and flexibly apply what was learned (Arnsten, 1998, 2009). Later evidence indicated that stress led to impaired working memory, cognitive control mechanisms and goal-directed behavior, all mechanisms that are crucially dependent on the prefrontal cortex (Barsegyan, Mackenzie, Kurose, McGaugh, &

Roozendaal, 2010; Bogdanov & Schwabe, 2016; Ramos & Arnsten, 2007; Schwabe, Hoffken, Tegenthoff, & Wolf, 2011). Stress effects on the prefrontal cortex are mediated by GR and MRs that lead to a downregulation of this structure and therefore a decrease in activation (Izquierdo, Wellman, & Holmes, 2006; McKlveen et al., 2013). Noradrenaline also exerts a powerful modulatory function on the prefrontal cortex. More specifically, the impact of noradrenaline in combination with dopamine is detrimental when concentrations are too high or too low, which then leads to an impairment of prefrontal functioning (Arnsten, 2000; McCormick, Pape, & Williamson, 1991). The concentration of noradrenaline that reaches the PFC is mediated also by the amygdala, as studies have shown that lesions in the amygdala can in turn modulate noradrenergic activity on the prefrontal cortex (Arnsten, 2009; Ferry, Roozendaal, & McGaugh, 1999; Roozendaal, Quirarte, & McGaugh, 2002).

Stress Effects on Striatum-Based Memory Processes

In addition to the effects of stress, catecholamines and glucocorticoids on the hippocampus and prefrontal cortex, findings suggest that stress also mediates a switch in memory systems. More specifically, stress increases the use of the striatum-based memory system that supports habit or stimulus-response learning and memory (Schwabe et al., 2007; Schwabe, Schachinger, de Kloet, & Oitzl, 2010). However, recent research points out that there may not just be a switch from one memory system to another but that there may be more than one mechanism, leading to enhancement or impairment of one or both of the two systems. More specifically, classically it is assumed that stress leads to a downregulation of hippocampal memory and enhanced striatal memory. This has been shown impressively in rodents through the presence of c-fos that constitutes an indirect marker of neuronal activity (action potentials). Increased c-fos expression in the striatum and decreased c-fos expression in the hippocampus signal that there is increased and decreased activity in the striatum and hippocampus, respectively (Vanelzakker et al., 2011). Furthermore, this switch from the impaired hippocampal to the striatal memory is triggered through stress before learning (Goldfarb & Phelps, 2017; Kim et al., 2001; Packard & Wingard, 2004; Schwabe et al., 2007; Schwabe & Wolf, 2012). It is further suggested that both memory systems may be impaired, though the striatal system to a lesser degree. This assumption was made after observing animal and human behavior after pre-retrieval stress. More specifically, in humans, it was shown that both recognition memory, depending on the hippocampus and S-R memory were

impaired after exposure to an acute stressor (Goldfarb & Phelps, 2017; Guenzel, Wolf, & Schwabe, 2013; Schwabe & Wolf, 2014). Stress before retrieval may therefore initiate a memory formation mode that rather supports the formation of new memories that adhere to the context of the experienced stressor and inhibits memory retrieval of items not associated with the current situations (Goldfarb & Phelps, 2017; Schwabe, Joels, et al., 2012). While there need to be additional research concerning the impact of stress and the timing of the stressor on the individual memory systems, it is already evident, that not only the hippocampal memory is modulated by stress effects, but also critical prefrontal functionality as well as the striatum dependent memory and that these effects are also mediated through amygdala activity.

Hence, stress effects have been reported concerning different processes, such as hippocampus-based consolidation and retrieval processes as well as prefrontal and striatum dependent memory processes. What is to note however, is a general lack of information regarding the modulating effect of stress or the major stress mediators on the use of prior knowledge in general, but specifically on schema-based learning and memory generalization. These processes have been shown to serve crucial functions in everyday life, as they facilitate learning of schema-based information and allow us to behave adaptively in new situations and to generalize across experiences. It is therefore essential to shed light on the sensitivity of schema-based learning and memory generalization to the effect of stress to develop a better understanding not only of the general processes involved in these learning and memory functions but also how they are modulated.

Scope and Aim

The learning and memory processes, schema-based learning and memory generalization, described above, are crucial in our everyday life. They allow us to flexibly learn, make decisions and behave adaptively (Shohamy & Wagner, 2008; van Kesteren et al., 2012). In the case of schema-based learning, there is enhanced encoding, consolidation and retrieval of schema-related material (Preston & Eichenbaum, 2013; Richards et al., 2014; van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012). During memory generalization, similar events are encoded together to allow for a combined retrieval of these items, thus avoiding rather elaborate inference processes (Shohamy & Wagner, 2008). Hence, both processes are examples of how we use and benefit from prior knowledge and show that prior knowledge is not only beneficial in terms of our behavior, it is also an advantage on the level of processing and storing incoming information (Shohamy & Wagner, 2008; van Kesteren, Fernandez, et al., 2010; van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012). Thus these processes go beyond isolated encoding, consolidation and retrieval processes and represent more holistic memory functions akin to how we use memory in our daily lives. Furthermore, both memory generalization and schema-based learning rely on different, but overlapping brain structures. The integration of new information into prior knowledge representations has been associated specifically with the mPFC, after initial schema-formation by the hippocampus (Gilboa & Marlatte, 2017; van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012). In memory generalization, utilization of prior knowledge has been specifically associated with the flexible hippocampus-based memory (Myers et al., 2003; Shohamy & Wagner, 2008). Our ability to utilize prior knowledge is therefore majorly associated with two brain structures that take over different but related functions.

However, we are also almost constantly exposed to changes in our environment and some of these changes might be experienced as stressful. Stress and the physiological mechanisms underlying the stress response have been described above. What is to note, is the fact that precisely schema-based learning and memory generalization are supported by brain structures that are highly sensitive to stress and specifically the stress hormones cortisol and noradrenaline (Arnsten, 2009; Barsegyan et al., 2010; Kim et al., 2001; Kim & Diamond, 2002). Up to this date, there has been immense research examining memory processes such as encoding, consolidation and retrieval under stress (Cahill et al., 2003; Cornelisse et al., 2011; De Quervain et al., 1998; Diamond et al., 2006; Guez et

al., 2016; Joels et al., 2006; Roozendaal, 2002; Schwabe, 2013), how stress impacts our ability to utilize prior knowledge has however received little attention, even though it extends previous research as it incorporates more than one single process alone. Furthermore, we do not have blank slates when it comes to learning and when we learn in our everyday life we may always rely on similar experiences or previous knowledge or build new schemas that we can rely on for future learning. Hence, the overarching question of the current work is whether and how stress and major stress mediators, such as cortisol and noradrenaline impact our ability to use prior knowledge. Furthermore, as described above, the use of prior knowledge may incorporate intricate functions going above simple encoding, consolidation and retrieval processes. Hence, the impact of stress on the use of prior knowledge may represent a more complete picture of how stress and major stress mediators exert an impact on our memory and learning functions.

To this end, three studies have been conducted considering schema-based learning, using an established transitive inference paradigm (Kumaran, 2013) and memory generalization using an acquired equivalence paradigm (Myers et al., 2003). Both tasks have been chosen, as they serve our aim to induce the acquisition of knowledge as well as its later use. More specifically, in the transitive inference paradigm, participants learn an age-related hierarchy of different galaxies (from oldest to youngest), building a schema-like representation that can later be used to facilitate the integration of new galaxies into the existing knowledge structure. (Kumaran, 2013; Kumaran, Melo, & Duzel, 2012). In terms of memory generalization, the task provides participants with associations in the form of pairs between fish and several persons. These associations can then be used to generalize to new associations between a known person and a new fish. More precisely, the knowledge participants acquired at the beginning of the task enables them to make generalizations to new, but similar items (Myers et al., 2003). Hence, both tasks used in the current work allow us to show the possible benefit of using prior knowledge and to investigate the possible impairing effects of stress and stress mediators.

Chapter III

In the following I will provide short summaries of the three studies that were conducted with the aim to shed light how stress and major stress mediators manipulate our ability to benefit from prior knowledge. These summaries comprise a brief background, description of the task and general procedure, the main results without statistical analyses as well as a brief discussion of the main finding. The full publication for each study can be found in the appendix.

Study I: Impact of Stress and Glucocorticoids on Schema-Based Learning

Kluen LM, Nixon P, Agorastos A, Wiedemann K and Schwabe L (2016) *Neuropsychopharmacology* (The full publication can be found in appendix A).

Background

In this first study we wanted to investigate the effects of stress and major stress mediators cortisol and noradrenaline on the use of prior knowledge using a schema-based learning paradigm (Kumaran, 2013). As stated above, a schema aids learning of schema-related information and has been associated specifically with the mPFC (Gilboa & Marlatte, 2017; Sommer, 2017; Tse et al., 2007; Tse et al., 2011; van Kesteren, Fernandez, et al., 2010; van Kesteren, Rijpkema, et al., 2010). This structure has been shown to be especially important for the integration of schema-congruent information into a pre-existing knowledge structure, while the hippocampus was appointed crucial for the encoding of new, but not schema-related information (Tse et al., 2007; Tse et al., 2011; van Kesteren, Fernandez, et al., 2010; van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012). However, while previous studies have reported also that specifically the mPFC is sensitive to stress and major stress mediators cortisol and noradrenaline (Arnsten, 2000, 2009; Oei et al., 2007; Roozendaal et al., 2009), how the latter impacts our ability to utilize prior knowledge is not known. Hence, the aim of the current study was to elucidate the effect of stress, as well as the major stress mediators cortisol and noradrenaline on schema-based learning. We hypothesized that stress would lead to an impairment in the use of prior knowledge, as previous studies have shown a

detrimental effects of stress on prefrontal functioning (Barsegyan et al., 2010; Elzinga & Roelofs, 2005; Schwabe & Wolf, 2009). Furthermore, we hypothesized that cortisol and noradrenaline might interact to impact schema-based learning.

Methods

Procedure

In two experiments, participants completed a schema-based learning task (Experiment I n = 96, 48 female, five participants were excluded due to sickness or inadequate knowledge of German, leaving a sample of 91 participants (45 females); Experiment II n = 96, 48 female, six participants had to be excluded due to low initial schema-acquisition, leaving a sample of 90 participants (44 females)). Participants acquired a schema on day 1. On day 2, about 24 h after day 1, participants learned new schema-related and schema-unrelated items. In experiment I, participants were exposed to a stressor or a control condition either immediately before learning, taking advantage of the still increased noradrenergic arousal and the not yet augmented cortisol levels, or about 25 minutes before learning, taking advantage of the peak cortisol levels and the decreased noradrenergic arousal at the time of testing. More specifically, participants were exposed to the socially evaluated cold pressure test (SECPT; Schwabe, Haddad, & Schachinger, 2008). During this test, participants were instructed to place their hand into a bucket of ice-cold water and keep it there for as long as possible (for a maximum of 3 minutes), while being evaluated by a cold, non-responsive experimenter. This test has been extensively validated and is expected to raise noradrenergic arousal as well as cortisol release in response to the psycho-physical stress (Schwabe, Haddad, et al., 2008; Schwabe & Schachinger, 2018). In experiment II, participants received orally a placebo, hydrocortisone (20 mg), the alpha-2-adrenoceptor-antagonist yohimbine (20 mg), stimulating noradrenergic arousal, or both drugs about 45 minutes before learning. The timing and dosage were chosen according to previous studies (Buchanan & Lovallo, 2001; Schwabe, Tegenthoff, Hoffken, & Wolf, 2010).

Task

Participants completed a transitive inference paradigm (Kumaran, 2013; Kumaran et al., 2012). This task comprised two phases, an acquisition phase (day 1) and a schema-based learning phase (day 2). During the acquisition phase, participants learned the relative age of six galaxies in the form of a

hierarchy. During this phase, participants completed three different trial types, namely learning trials, inference trials and baseline trials. During each trial, participants saw two galaxies, presented next to each other on the screen. During the baseline trials, a cross was presented below one of the images and participants had to indicate, by button press, under which image the cross was placed and received feedback immediately. Baseline trials were used as control trials, as they probed attention throughout the task. During the learning trials, two galaxies immediately related in age were presented and participants were asked to indicate which galaxy was older. After participants made their choice, they received feedback immediately. During the inference trials, participants saw two galaxies that were not neighboring in age, i.e. separated by two other galaxies (short inference trials) or three to four (long-inference trials) and were again asked to indicate which of the galaxies was older. This time however, participants did not receive feedback but were asked to indicate on a scale comprising four items, how certain they were about their answer. About 24 hours later, participants completed the schema-based learning phase. During this phase, participants learned two new hierarchies, one that comprised four completely new items and four items that were already part of the galaxy participants learned during the acquisition phase (related hierarchy) and a second hierarchy that comprised only new items (novel hierarchy). Participants were instructed that the information concerning the order of the galaxies still applied and again completed baseline, learning and inference trials. For the related hierarchy, learning trials consisted of neighboring items and always contained a galaxy from the acquisition phase and a new item. For the novel hierarchy, learning trials again consisted of neighboring items, however this time only new items were presented. Inference trials on the other hand, only showed new items for the novel and related hierarchy and again for short inference trials and long inference trials (see figure 1).

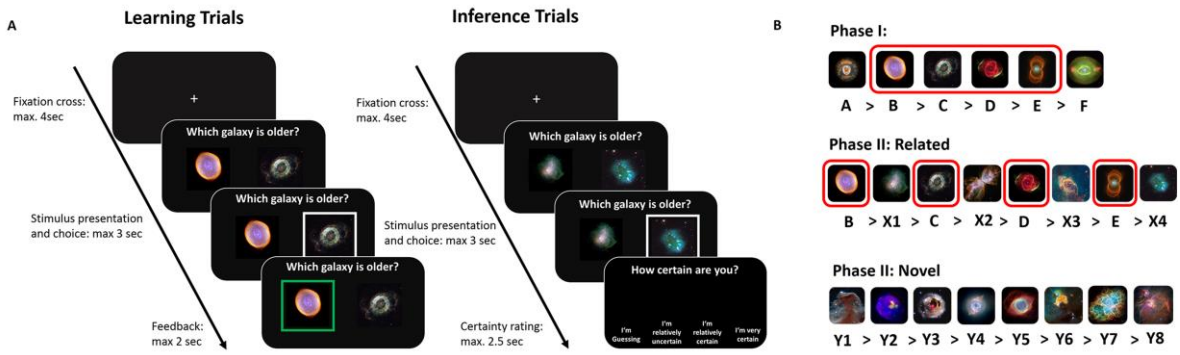

Figure 1 – Schema-Based Learning Task

Schematic representation of the learning and inference trials during the schema-based learning phase (A) as well as the individual hierarchies for the acquisition phase (phase I) and the schema-based learning phase (phase II). During the learning trials (A), participants always saw two items immediately related in age from the hierarchy that was learned during phase I and one of the four new galaxies that was added during phase II (B). When participants chose which galaxy was older, a frame was placed around the choice. Participants received feedback immediately in the form of a green frame around the older galaxy (A). During inference trials, only new items were presented, i.e. the items that were added to the related galaxy or the items from the novel hierarchy, however, items where never immediately related in age (B). When participants chose which galaxy was older, a frame was placed around that item and participants were asked how certain they were about their choice on a scale from 'guessing' to 'very certain' (A).

Results - Experiment I

Phase I

Results showed that participants learned the schema very well on day 1, with about 81 percent correct answers. There was also no difference in performance between groups. As schema-based learning is the main interest of the current work, inference trials were disregarded.

Manipulation check

Stress induction led to a significant increase in sympathetic nervous system activity as well as in salivary cortisol levels, compared to the control group.

Phase II

On day 2, participants showed the expected schema-effect, with a strong trend for a better performance in the learning trials that utilized the schema-related galaxies compared to the new galaxies. Stress however led to an impairment in the ability to utilize prior knowledge. More precisely, control participants showed a significantly better performance in the related learning trials, i.e. when schema-related information was tested, compared to when novel information was

tested. In stressed participants, there was no such effect. Stressed participants were not able to benefit from a previously established schema, independent of the timing of the stressor and showed a similar performance in novel and related learning trials (see figure 2).

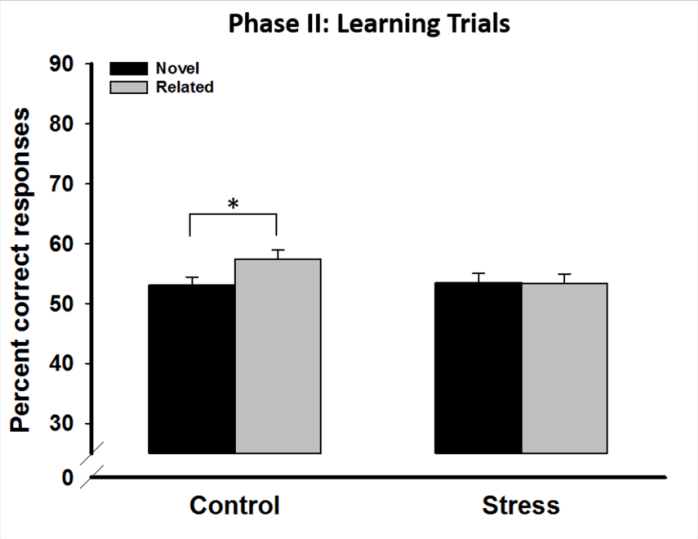

Figure 2 – Day 2 Performance in Learning (Experiment I)

During the learning trials of phase II, control participants were able to profit from the previously acquired schema and showed a significantly increased performance in the related hierarchy, compared to the novel hierarchy. Stressed participants, irrespective of the timing of the stressor, did not show an improved performance in any of the hierarchies. Error bars represent standard error of the mean. * $p < .05$.

Results - Experiment II

Phase I

On day 1, participants performed again very well in learning trials, with about 80 percent correct answers. Treatment groups did not differ in learning performance.

Manipulation check

Intake of hydrocortisone and yohimbine led to a significant increase in noradrenergic arousal as well as salivary cortisol in the respective groups and in comparison to the participants receiving a placebo treatment.

Phase II

Results showed again the expected schema-effect, as participants performed better in the schema-related compared to the novel trials. More importantly however, results indicated that the use of a schema was impacted by hydrocortisone intake. Participants taking hydrocortisone either alone or in combination with yohimbine showed a similar performance in related compared to novel trials indicating no beneficial effect of prior knowledge. In contrast, participants taking a placebo or yohimbine alone showed a significantly better performance in related compared to novel trials

(figure 3), hence the ability to use and benefit from prior knowledge was still intact. We did however not obtain a significant interaction effect of hydrocortisone and yohimbine.

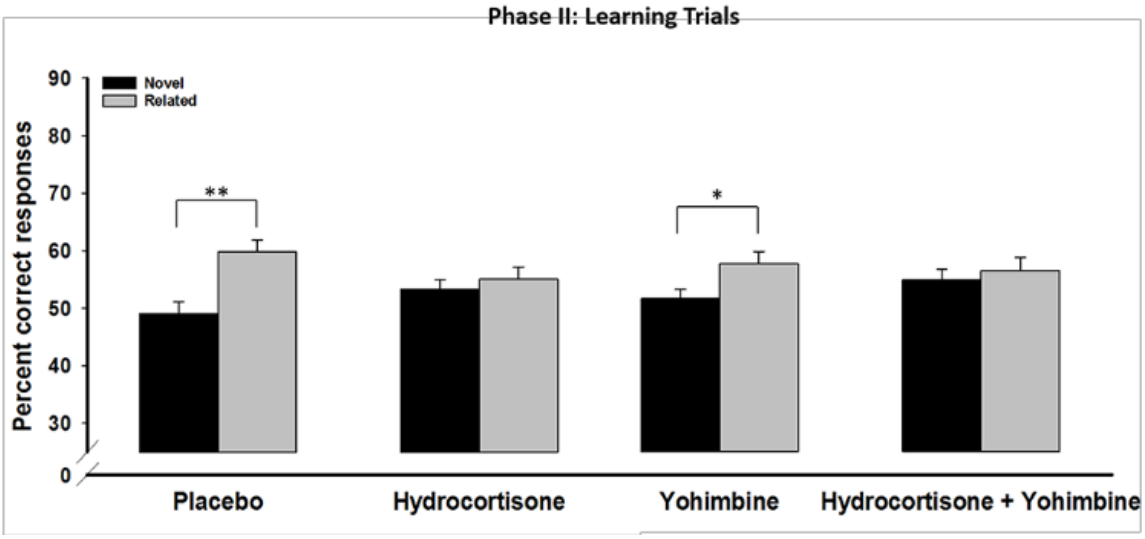

Figure 3 – Day 2 Performance in Learning Trials (Experiment II)

Participants in the placebo and yohimbine only groups showed a significantly increased performance in the related learning trials compared to the novel learning trials, indicating that they did indeed benefit from the previously acquired schema. Participants that received hydrocortisone alone or in combination showed a similar performance in the novel and related learning trials. Error bars indicate standard error of the mean. * $p < .05$, ** $p < .001$.

Discussion

The aim of the current study was to investigate the impact of stress and major stress mediators on our ability to use prior knowledge. We tested this using a schema-based learning task that allowed us to control for the acquisition of knowledge, in the form of a schema that can later be applied to new information that is either schema-related or unrelated. Numerous studies have shown that the presence of a schema aids learning of schema-related information (Alba & Hasher, 1983; Ghosh & Gilboa, 2014; Gilboa & Marlatte, 2017; Tse et al., 2007), up to this point however, it was not clear whether this schema-effect could be modulated by stress or major stress mediators cortisol or noradrenaline. The current findings indicate that the positive effect of a schema does not persist after stress exposure or elevated levels of cortisol. Results showed that stressed participants could not benefit from pre-existing knowledge, irrespective of the timing of the stressor and the same was true for participants that received hydrocortisone, either alone or in combination with yohimbine. Yohimbine alone, did not impact schema-based learning.

It is suggested that schema-based learning depends on the mPFC (Izquierdo et al., 2006; Tse et al., 2007; Tse et al., 2011; van Kesteren et al., 2013), a structure that is highly sensitive to stress (Arnsten, 2009), due to a high density of glucocorticoid receptors (McEwen, De Kloet, & Rostene, 1986). Thus, the observed impairment of schema-based learning in stressed participants and participants receiving hydrocortisone may arise because stress and glucocorticoids led to a downregulation of activity in the mPFC, disabling the detection of whether incoming information was schema-related or not. While it was initially suggested that the immediate stress group was predominantly characterized by increased noradrenaline levels and cortisol levels that were not elevated at the time of testing, it may be possible that cortisol levels may have already been rising shortly after the stressor. In the delayed group however, noradrenaline levels were likely to be decreased about 25 minutes past stressor onset, while cortisol levels may have reached its peak concentration. These assumptions allow the hypothesis that the observed effects, i.e. a decrease in the ability to utilize a pre-existing schema in both the immediate and delayed stress groups, were due to the increased cortisol levels. This proposition is also supported by results observed in experiment II. Specifically, results have clearly shown impaired schema-related learning in participants receiving hydrocortisone alone or in combination. Hence, the current study is the first to shed light on the modulatory role of stress and the major stress mediator cortisol on our ability to utilize and benefit from prior knowledge tested during schema-based learning. How stress or the major stress mediator cortisol impacts the neural underpinnings of schema-based learning is however not known and the following study aims to fill this gap.

Study II: Stress Affects the Neural Ensemble For Integrating New Information And Prior Knowledge

Vogel S, **Kluen LM**, Fernández G and Schwabe L (2018) Neuroimage

(The full publication can be found in appendix B).

Background

In the previous study, we could show that stress and the major stress mediator cortisol have a negative impact on the ability to benefit from prior knowledge during schema-based learning. As stated above, the mPFC has been appointed as a crucial structure facilitating schema-based learning, also through the interaction with the angular gyrus and precuneus (Gilboa & Marlatte, 2017; Spalding, Jones, Duff, Tranel, & Warren, 2015; Tse et al., 2007; Tse et al., 2011; van Kesteren, Fernandez, et al., 2010; van Kesteren, Rijpkema, et al., 2010; Wagner et al., 2015). The mPFC specifically acts as a detector, indicating whether new information is related to prior knowledge and integrates this information in the already existing schema structure (Ghosh, Moscovitch, Melo Colella, & Gilboa, 2014; Richards et al., 2014). The hippocampus on the other hand encodes novel information that is not congruent with a pre-existing schema or schema-relevant (Eichenbaum, 1999; Scoville & Milner, 1957; van Kesteren et al., 2012). The hippocampus is also critical for initial acquisition of the schema before it gets transferred to the mPFC and transformed into an abstract representation (Gilboa & Marlatte, 2017; Sommer, 2017). Previous studies have shown that specifically the mPFC and hippocampus are sensitive to stress (Joels et al., 2006; Schwabe, Joels, et al., 2012) but the underlying mechanism by which stress and cortisol impact schema-based learning has not yet been elucidated. Hence, the aim of the current experiment was to investigate the impact of stress on schema-based learning and specifically the impact of stress on the underlying neural structures. Based on the results from study I, we hypothesized that stress would downregulate activity in the mPFC in response to schema-related material resulting in an impairment of congruency detection and thus schema-based learning (Arnsten, 2009; Barsegyan et al., 2010; Schwabe, Tegenthoff, Hoffken, & Wolf, 2012; van Kesteren, Fernandez, et al., 2010; van Kesteren et al., 2012)

Methods

Procedure

Participants (n=50, 25 females) completed the transitive inference paradigm (Kumaran, 2013; Kumaran et al., 2012) described above (Study I). Learning on day 1 was done outside of the scanner. On day 2, participants underwent a stress induction protocol before schema-based learning in the MRI scanner.

More specifically, participants were exposed to the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993), a stress induction paradigm that has been successfully validated (Bogdanov & Schwabe, 2016; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Schwabe et al., 2007). As part of the procedure, participants were led into a room with a committee of two people in white lab coats sitting at a table. Participants were asked to prepare a short speech about their personal attributes qualifying them for their dream job. During the speech, the committee took notes while keeping a neutral expression and refraining from any encouraging or friendly gestures. Additionally, participants were videotaped and saw themselves on a large video screen. After the free speech, participants were asked to count backwards from 2043 in steps of 17, again while again being evaluated by the committee members. This task has been shown to increase subjective stress levels, as well as cortisol and noradrenergic arousal robustly (Kirschbaum et al., 1993).

Results

Phase I

Schema acquisition was successful on day 1, with participants reaching a final performance of about 85 percent correct answers in learning trials. Importantly however, there was no significant difference in behavioral performance between stress and control participants. Although inference trials were tested and recorded throughout the experiment, neuroimaging results have shown that processes active in these trials are different to schema-based learning trials. Hence, for the current experiment, only schema-based learning trials are considered in detail.

Manipulation check

Exposure to the TSST led to significantly increased blood pressure as well as salivary cortisol levels in the stress- compared to the control group.

Phase II

Behavioral Results

In phase II, performance in schema related learning trials was better compared to schema-unrelated trials, providing evidence again for the positive effect of a present schema. There was however no significant difference in performance between schema-related and novel trials between the stress and control group (figure 4).

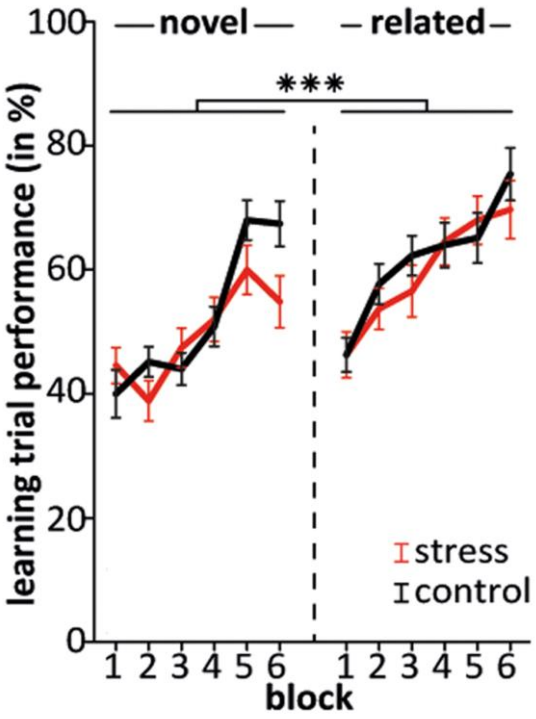

Figure 4 – Task Performance on Day 2

Learning performance on day 2 was significantly increased for the related compared to novel hierarchy. The presence of a schema thus aided learning of schema-related items. There was however no significant difference in performance between stressed and control participants. Error bars represent standard error of the mean. *** $p < .001$

Neuroimaging Results

Considering the neural mechanisms active, results showed that learning of schema-related information led to the activation of the angular gyri, the precuneus and the mPFC. These brain structures have been shown previously to be active during schema-based learning and memory

processes (van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012; Wagner et al., 2015). Due to its specific role in schema-based learning, the focus was centered on activation of the mPFC. mPFC activity during schema-based learning was shown, although only marginally significant in the control group, while there was no significant activation in the stress group. Additionally, activity in the mPFC during the presentation of schema-based learning trials was negatively correlated with the individual cortisol response and participants with higher levels of cortisol release showed less schema-related mPFC activation. This result was however only present across all participants and is thus to be considered with caution (figure 5).

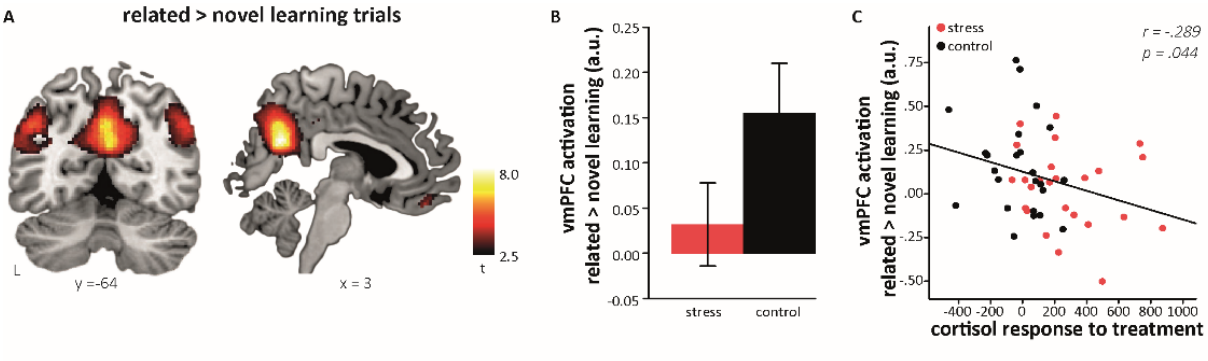

Figure 5 – Individual Differences in Cortisol Response Impact Brain Regions Supporting Schema-Based Learning

There was a significantly increased activity in the mPFC, the precuneus and both angular gyri when presenting related compared to novel stimuli (A). This activation pattern was shown across groups. However, this schema activation was less prominent in the stress, compared to the control group (B; parameter estimates were extracted using an anatomical mask). Across all participants there was a negative correlation of the mPFC activation and cortisol response (C). Images are displayed at $p < .005$, uncorrected for illustration purposes.

Furthermore, as it is suggested that congruency detection is done majorly by the mPFC, there should be less activation in the hippocampus when schema-related information is present. Results showed that in stressed participants, hippocampal activity during schema-related trials was negatively associated with schema-related learning performance. This particular association was only detected in the stress group and was not found in control participants or across both groups (figure 6).

Additionally, a connectivity analysis showed that in the control group, there was no significant connectivity between the hippocampus and angular gyrus and hippocampus and mPFC during schema-related learning trials. In the stress group however, there was an enhanced connectivity

between the hippocampus and angular gyrus and hippocampus and medial prefrontal cortices. Hence, the negative association of the hippocampus with schema-based learning performance, as well as the connectivity analysis would suggest that stressed participants were not able to detect the schema-congruency and thus treated schema-related, as novel information.

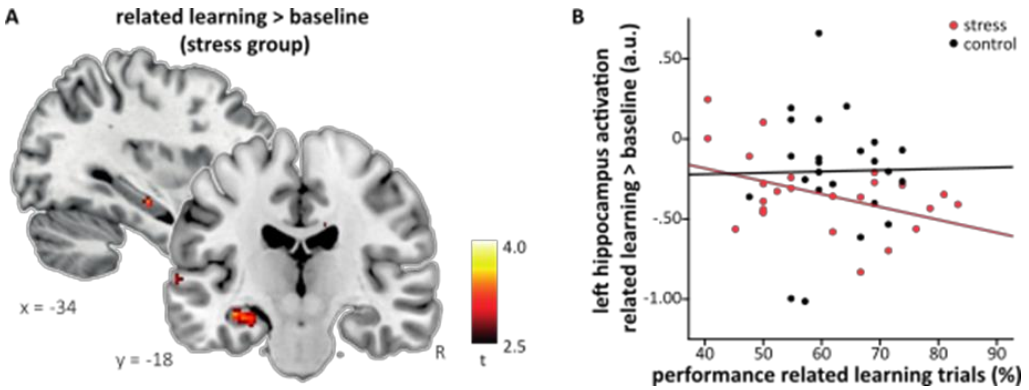

Figure 6 – Negative Impact of Hippocampal Activity during Schema-Related Learning in the Stress Group

In stressed participants, there was a negative association between performance in schema-related learning trials and hippocampus activation in related compared to baseline trials (A). Hence, activity of the hippocampus during schema-related learning trials may actually be damaging to performance. (B) Correlation between the left hippocampus for related compared to baseline trials is shown for the stress group, as well as control group. Images are displayed at $p < .005$, uncorrected, for illustration purposes.

Discussion

In the previous study, we could show that stress and pharmacologically elevated levels of cortisol led to an inability to benefit from prior knowledge during a schema-based learning task. Results of the current study now shed light on the impact of stress on the neural structures involved in schema-based learning and the possible mechanism by which the impairment that we have observed in the previous study has come about. It is to note that behavioral results in the current study did not lead to significant findings. While we could show that this task may actually detect differences in the ability to benefit from a previously acquired schema, the current study may have lacked power to detect even subtle behavioral differences. This may all be more apparent as the previous study did actually test almost double the number of participants. Neuroimaging results however reflect the impact of stress during schema-based learning.

More precisely, current results show the activation of brain structures, such as the mPFC, precuneus and angular gyrus in schema-related learning, in comparison to novel learning.

Specifically the mPFC occupies a major role in schema-based learning and previous human studies showed that lesions in the mPFC led to a diminished ability to access prior knowledge, thus impairing the use of prior knowledge during learning (Ghosh et al., 2014; Gilboa, Alain, He, Stuss, & Moscovitch, 2009). The hippocampus however, is suggested to be active when information is learned that does not correspond to a pre-existing schema (van Kesteren et al., 2012) and should therefore show decreased activity during schema-based learning (Tse et al., 2011; van Kesteren, Rijpkema, Ruiter, Morris, & Fernández, 2014). In control participants this pattern could be shown, while stressed participants presented with decreased mPFC activity when schema-related items where shown, that may also be fostered by an increased cortisol release. Additionally stressed participants also presented with increased hippocampal activity for schema-related items. Specifically the interaction between increased cortisol and a decrease in mPFC activity may also be in accord with the previous study. When control participants learned schema-related information, there was less involvement of the hippocampus, as the information may be integrated into neocortical schema-networks by the mPFC. In stressed participants however, schema-related information led to strong connectivity between the hippocampus and angular gyrus and mPFC. This connectivity might be interrupting the schema-based learning since information is treated as new episodic information. Hence, it may be the case that stressed participants classed the schema-related information as novel information. It is therefore possible to suggest that stressed participants were unable to utilize the pre-existing schema to detect the congruency of the schema-related information and could therefore not benefit from the existing prior knowledge.

[*LS and GF conceptualized the study. SV and LMK performed data acquisition. SV analyzed the data. LMK contributed to preprocessing. SV and LS drafted initial manuscript. LMK and GF provided critical revisions. All authors contributed to writing the manuscript*]

Study III: Noradrenergic Stimulation Impairs Memory Generalization in Women

Kluen LM, Agorastos A, Wiedemann K and Schwabe L (2017) Journal of Cognitive Neuroscience

(The full publication can be found in appendix C).

Background

The third study has also focused on the impact of cortisol and noradrenaline on the use of prior knowledge, however this time targeting a different memory process that utilized prior knowledge. More specifically, we aimed at investigating the modulatory role of noradrenaline and cortisol on memory generalization, a process during which one benefits from previous experiences that are similar to new experiences (Myers et al., 2003; Shohamy & Wagner, 2008). Memory generalization is critically dependent on flexible hippocampus-based memory as previous research has shown that the hippocampus stores events or experiences as discreet representations that can be accessed at a later date (Kirwan & Stark, 2007; Leutgeb, Leutgeb, Moser, & Moser, 2007; Shohamy & Wagner, 2008). Furthermore, although items are stored as separate representations, similar items might show a certain overlap between these representations. Detecting this overlap is critical as similar representations may be integrated into a cohesive representation comprising the former discreet events (Eichenbaum, 2000; Gluck & Myers, 1993; Shohamy & Wagner, 2008). However, the hippocampus is a structure that has been shown to be highly sensitive to stress and various studies have reported severe impairments of hippocampal memory after a stressful encounter (Dorey et al., 2011; Kim & Diamond, 2002; Kim, Song, & Kosten, 2006; Qin, Hermans, van Marle, & Fernandez, 2012; Roozendaal, Griffith, Buranday, De Quervain, & McGaugh, 2003). Furthermore, it has been shown that stress mediates the switch from the cognitive, hippocampus-based memory system, to the dorsal striatum-based memory system, supporting procedural or habit memory (Schwabe, Tegenthoff, et al., 2010; Schwabe & Wolf, 2009, 2012). A previous study from our lab has indicated, that acute stress leads to an impaired memory generalization, which may be traced back to impaired hippocampal activity (Dandolo & Schwabe, 2016). It is however not yet clear what the driving force behind this impairment is. Hence, the current study employs a pharmacological manipulation to test the impact of increased glucocorticoid and noradrenergic activity on memory generalization in both men and women. We hypothesized that both hydrocortisone and yohimbine would be sufficient to alter the ability to generalize across experiences. As women are more

sensitive to stress effects specifically when considering hippocampal memory (Guenzel et al., 2014; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001), it is tempting to speculate that effects observed will be stronger in women.

Methods

Procedure

We tested 103 healthy participants (52 female) in a double blind, fully-crossed, placebo-controlled between-subjects design. Depending on the group, participants received either a placebo, the alpha-2-adrenoceptor antagonist yohimbine (20 mg), hydrocortisone (20 mg) or both drugs. About 70 minutes after pill intake, participants completed a memory generalization task.

Task

More specifically, participants completed the acquired equivalence task by Myers et al (2003). The task comprised two phases, namely the acquisition phase and the generalization phase. Participants first completed the acquisition phase during which pictures of specific individuals were paired with differentially colored fish (eight fish and eight individuals in total). Individuals differed in age, gender and hair color. Participants were asked to learn the specific associations between fish and individual by trial and error. In the first of three stages of the acquisition phase (shaping), participants learned four pairings between individual and fish. To do so participants saw a picture of an individual on a computer screen and the images of two fish below the individual. Participants were then asked to indicate by button press, to which fish the individual belonged. Participants received feedback about the correctness of their choice. The equivalence stage immediately followed the shaping stage and comprised four additional individuals that were also paired with the already shown fish. Hence, participants were required to form equivalences between the individuals that were associated with the same fish and comprehend that these individuals always shared the same features (gender and hair color). In the third stage, new consequents, the eight individuals from the previous two stages were shown again, however, the four individuals from the shaping stage were now associated with an additional, completely new and differently colored fish. Participants were required to learn that each individual can be associated with two fish. Then the generalization phase followed. During the generalization phase, all trials from the acquisition phase were shown as

well as new trials during which the individuals from the equivalence training stage were paired with new fish. In the generalization phase however, participants were not given feedback. Hence, the generalization phase can be used well to test whether participants were able to generalize the associations they had learned in the previous phase to the new trials (Collie, Myers, Schnirman, Wood, & Maruff, 2002; Myers et al., 2003; figure 7). To assess participants' generalization ability, a generalization score was calculated. This score was calculated by subtracting the percentage correct of the trials during the new consequents stage from the percentage correct of the new trials from the generalization phase with an added constant of 100, to avoid negative scores. This score specifically allows to separate the ability to generalize across associations and initial memory (Dandolo & Schwabe, 2016).

Results

Manipulation Check

Intake of yohimbine alone or in combination led to a significant increase in blood pressure. Hydrocortisone intake, alone or in combination caused a significant increase in salivary cortisol, in comparison to participants receiving a placebo.

Generalization Performance

Results showed that participants learned very well and that the intake of yohimbine and/or hydrocortisone did not hamper initial learning in the acquisition phase, i.e. performance was comparable between the treatment groups as well as between men and women. The ability to generalize across new pairs in the acquired equivalence task however, was impacted by the intake of yohimbine and this impact was different in men and women. More specifically, yohimbine alone or in combination with hydrocortisone led to a decrease in generalization performance in women, while the effect was almost opposite in men (although this did not reach statistical significance). Yohimbine intake also had a significant impact on the generalization score in women. Cortisol on the other hand did not affect the ability to generalize in the current task (figure 8).

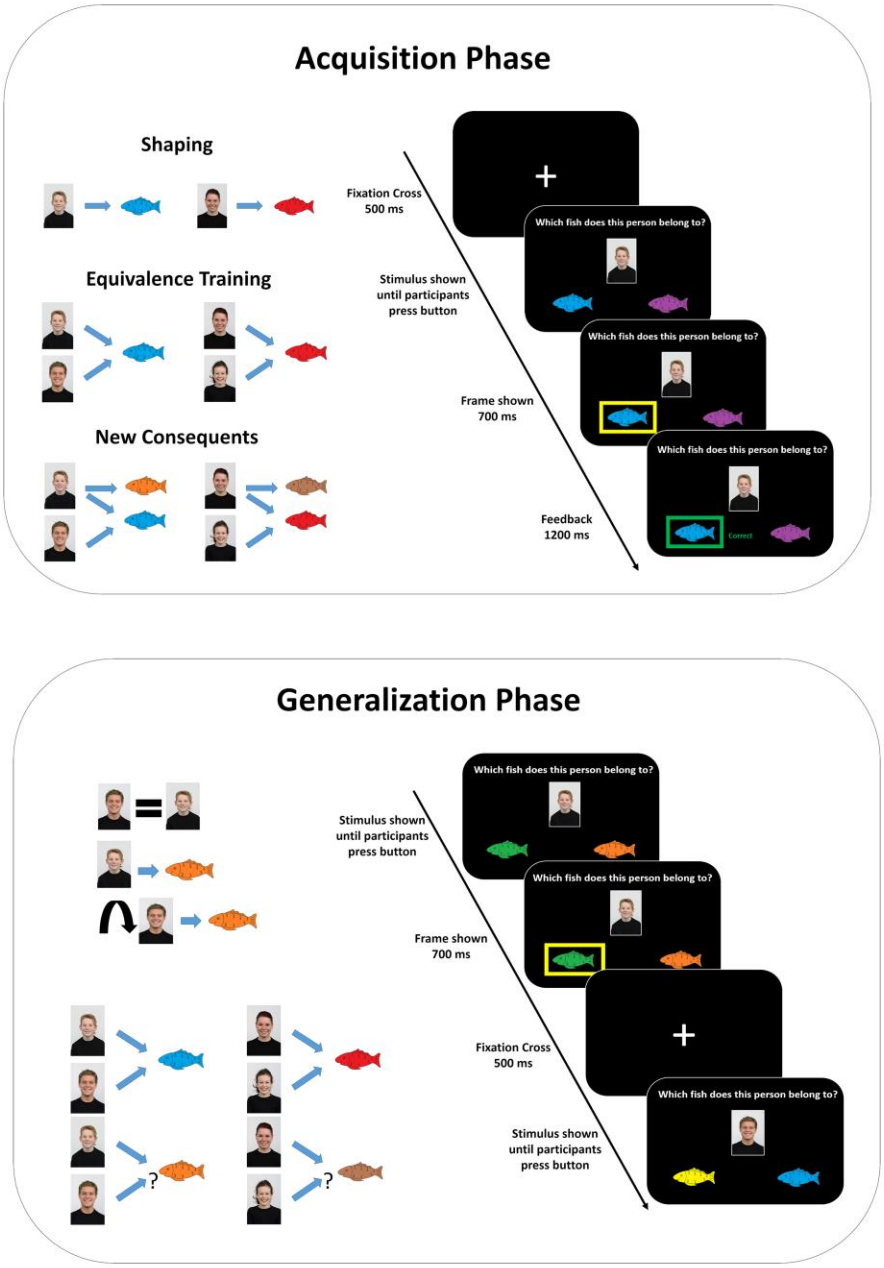

Figure 7 – Acquired Equivalence Task

The acquired equivalence task has been adapted from Myers et al (2003). The task comprises two phases, the acquisition phase consisting of three stages and the generalization phase. During the acquisition phase, the participant learns the associations between an individual and a specifically colored fish. In the first stage (shaping), participants learn four pairings between individual and fish. In the second stage (equivalence training) four additional individuals are associated with the four fish from the shaping stage, so that each fish is associated with two individuals, based on a specific characteristic (gender and hair color). During the third stage (new consequents), the individuals from the shaping stage are associated with an additional fish each. During the acquisition stage, participants always received immediate feedback. During the generalization phase, participants were required to indicate to which of the newly introduced fish during the new consequents stage, the individuals from the equivalence training stage belonged to. This time participants did not receive feedback, but were required to generalize across new items.

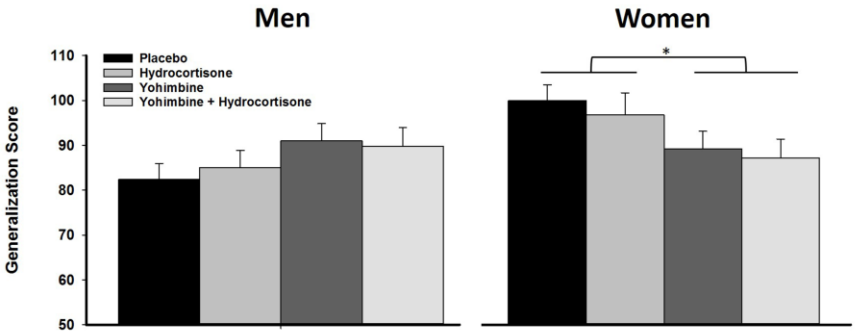

Figure 8 – Noradrenaline is Detrimental to Generalization Performance in Women

The generalization score indicates participants' generalization performance with respect to initial learning. A score of 100 represents an equal performance between old and new trials in the generalization phase, while a score below 100 indicates a generalization impairment as it reflects a better performance in old compared to new trials. There was a significant decrease in generalization performance in women that received yohimbine alone or in combination with hydrocortisone, while women that received a placebo or hydrocortisone performed well and were able to generalize across discreet experiences. In men, there was no significant difference between generalization performance in the individual treatment groups. Women receiving a placebo showed a significantly better performance compared to men that received a placebo. Error bars represent standard error of the mean. * $p < .05$.

Discussion

The aim of the current study was to elucidate the specific effects of the major stress mediators on our ability to utilize previous knowledge and generalize across experiences, an ability crucial for our everyday life, decision-making and survival (Shohamy & Wagner, 2008). Recent evidence indicates that stress has an impact on generalization ability (Dandolo & Schwabe, 2016) and our results show that elevated noradrenergic stimulation, triggered by the alpha-2-adrenoceptor-antagonist yohimbine disrupts memory generalization in women exclusively, while this result could not be found in men. Cortisol on the other hand, did not affect performance, neither in men nor in women.

Current results seem to pinpoint a modulatory effect of specifically noradrenaline on generalization performance. Previous reports have also indicated the importance of noradrenaline in mediating the effect of stress on memory, showing direct effects of noradrenaline on memory processes as well as an absence of effects when noradrenergic activity is blocked (Packard & Wingard, 2004; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006; Schwabe, Hoffken, Tegenthoff, & Wolf, 2013; Schwabe et al., 2009; Williams, Men, Clayton, & Gold, 1998). While this can only be speculated, it is possible that noradrenaline acts on the hippocampus via the amygdala, impacting generalization performance (McGaugh, Cahill, & Roozendaal, 1996). This mode of action may also explain the

observed gender effects, as previous studies have reported structural and functional differences in the amygdala of men and women (Cahill, 2006). Hence noradrenergic arousal plays an important role when considering the impact of stress on memory processes and seemingly also on memory generalization. While the current data showed a strong impact of noradrenaline on memory generalization, there was no impact of increased cortisol levels. However, Dandolo and Schwabe (2016) showed a negative association between cortisol and generalization performance. It can be speculated that the effect of cortisol requires concurrent noradrenergic activity, despite the fact that current results did not indicate any interaction effects. It may however still be possible that, while not sufficient, effects of cortisol are necessary to induce generalization impairments, as the concurrent activity of noradrenaline and cortisol is frequently reported and emphasized (Roozendaal et al., 2004; Roozendaal, Okuda, de Quervain, et al., 2006; Roozendaal, Okuda, Van der Zee, et al., 2006). Hence, the current study provides direct evidence for a specific impact of noradrenaline on memory generalization in women and that noradrenaline may be sufficient to exert this disruptive effect.

Furthermore, current results also extend our knowledge on how stress, but specifically stress mediators impact the use of prior knowledge during learning, beyond its influence on schema-based learning described above. Additionally, we may present a different mechanism by which our ability to use prior knowledge may be impacted by stress. In the following I will discuss and integrate the present findings to allow for a better understanding of the underlying mechanisms that may impact our ability to use and benefit from prior knowledge.

Chapter IV

General Discussion

Learning and memory have fascinated psychological and neuroscientific research for a very long time, as the two concepts or abilities enable us to mentally travel back in time, remember and relive important personal events, adapt to ever changing surroundings and thus support survival (Schacter & Addis, 2007; Shohamy & Daw, 2015; Tulving & Markowitsch, 1998). However, in our environment we are also almost constantly exposed to stressful occurrences and a multitude of studies has already provided evidence for the diverse effects stress has on learning and memory processes, such as the mostly detrimental effects of stress on specifically memory retrieval (Schwabe, Joels, et al., 2012; Schwabe & Wolf, 2010a; Schwabe, Wolf, & Oitzl, 2010; Shors, 2001; Smeets, 2011). This research has grossly contributed to our understanding of how our behavior and capabilities change under stress and has additionally provided relevant insight into various stress-related pathologies (Gotlib & Joormann, 2010; Shohamy et al., 2010). Yet, while research on the impact of stress on memory encoding, consolidation and retrieval has been extensive (Andreano & Cahill, 2006; Barsegyan et al., 2010; Barsegyan et al., 2014; Buchanan & Tranel, 2008; Buchanan et al., 2006; De Quervain et al., 2003; De Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Kukolja et al., 2011; Payne et al., 2007; Roozendaal & Hermans, 2017; van Stegeren et al., 2005), we lack an understanding about the impact of stress on our ability to use previous knowledge. This is especially important since prior knowledge has been shown to aid learning and allow us to benefit from previous experiences in new but similar situations (Shohamy & Wagner, 2008; van Kesteren et al., 2012). Furthermore, the use of prior knowledge, in different contexts, may also represent a more complete and realistic picture of combined memory processes, going beyond simple, isolated testing of individual mechanisms, such as retrieval or consolidation. The current work therefore aims to provide relevant insight into how stress and the major stress mediators exert a modulating function on schema-based learning as well as memory generalization, as a way to reflect the use of prior knowledge and how this can be modulated by stress. Additionally, we provide further insight into how the associated brain structures are involved in the use of prior knowledge and how they may be impacted by stress. Hence, present results will also add to the bigger picture on how external and internal factors modulate our behavior and the underlying neural structures.

In study I, Kluen et al (2016) performed two experiments to investigate the impact of stress and major stress mediators cortisol and noradrenaline on schema-based learning. Participants acquired a schema on day 1 and learned schema-related and unrelated information on the next day. Before participants completed the task on day 2, they were exposed to a stress manipulation, either immediately or with a short delay before schema-based learning (experiment I) or received a pharmacological manipulation (hydrocortisone, yohimbine, both drugs or a placebo) before testing (experiment II). Results showed that participants who were exposed to stress, regardless of the timing of the stressor, as well as participants that received hydrocortisone alone or in combination were not able to use previously acquired knowledge in the form of a schema, to aid learning of schema-related information. In control participants however, we did observe a stable schema-effect, showing that performance in schema-related trials was significantly better compared to novel trials. The same was observed in participants that received a placebo treatment or yohimbine alone as they were very well able to make use of their previous knowledge and showed increased performance in schema-related compared to novel trials. Hence, stress, immediately before as well as with a short delay and the intake of hydrocortisone, alone or in combination with yohimbine, exerted a severely disruptive effect on the ability to utilize previous knowledge to aid learning.

The second study (study II) by Vogel et al (2018) has aimed to fill the gap in our understanding about the modulating effect of stress on the underlying neural structures of schema-based learning. We utilized the same design as before, this time however, participants completed the second part of the task in the MRI scanner after being exposed to the TSST. While behavioral performance was not significantly different between stressed and control participants, neuroimaging results showed specific differences in the activity of the neural structures supporting schema-related learning. More precisely, the fact that behavioral performance was not significantly different between the stress and control group may be explained by a lack of power, as indicated by a post-hoc power analysis and the fact that the first study tested a larger number of participants. However, the impact of stress on the neural underpinnings of schema-based learning may also shed light on the mechanisms involved. While schema-based learning and detection of a schema has been specifically associated with activation of the mPFC, learning of novel, schema-unrelated information has been linked to increased hippocampal activity (Tse et al., 2007; van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012). mPFC activity was observed during the presentation of schema-related compared to novel trials in control participants, although only marginally significant, while this was not the case in stressed participants. Furthermore, irrespective of the treatment group,

there was a trend for a negative correlation between the individual cortisol response, hence the higher the cortisol response, the lower the activation of the mPFC. Hippocampal activity in the context of schema-based learning is typically associated with the processing of novel, schema-unrelated information (Tse et al., 2007; van Kesteren, Rijpkema, et al., 2010; van Kesteren et al., 2012). Control participants showed precisely this pattern, i.e. increased activity during the processing of novel, compared to baseline trials, while increased hippocampal activity in stressed participants was associated with schema-related trials compared to baseline trials. In stressed participants, activity in the hippocampus was further associated with impaired performance in schema-based learning. Hence, results indicate that stressed participants were not able to identify schema-related information as such, reflected in the aberrant pattern of brain regions activated during schema-related trials and could therefore not benefit from previous knowledge. Results further suggest that cortisol may also play an important role, as already found in the first study, although there needs to be further investigation to confirm this trend-level result.

In the third study (Kluen et al, 2017), the aim was to investigate the impact of the stress mediators cortisol and noradrenaline on a different form of learning that also requires the use of previous knowledge: memory generalization. To this end, participants received a placebo, hydrocortisone, yohimbine or both drugs before performing the acquired equivalence paradigm by Myers et al (2003). Results showed that all participants, regardless of treatment, performed well in the acquisition phase and learned the associations between person and fish very well. In the generalization phase however, women that received yohimbine, alone or in combination with hydrocortisone, showed a decrease in generalization performance when acquisition performance was controlled for. In men, although not reaching significance, performance tended to be increased after yohimbine intake (result did however not reach statistical significance), while there was no effect of hydrocortisone on performance in men or women. When considering the generalization score, i.e. a behavioral index of generalization ability (Dandolo & Schwabe, 2016), results again pointed to a disruptive effect of increased noradrenaline levels after yohimbine (alone or in combination) intake on memory generalization in women. In men, results again showed a tendency of increased performance after yohimbine intake without reaching statistical significance. Results therefore showed that increased levels of noradrenaline had a detrimental effect on the ability to generalize across discreet experiences in women, while this could not be observed in men.

Effects of Stress and Stress Mediators on the Use of Prior Knowledge

Results in all three studies therefore indicate a detrimental effect of stress or major stress mediators cortisol and noradrenaline on the ability to use prior knowledge. Furthermore, results showed that stress and increased cortisol had a disruptive effect on the ability to utilize their prior knowledge to aid learning of schema-related information. Results showed further that in stressed participants, the underlying neural structures that have been associated with schema-based learning were activated less during the presentation of schema-related trials and more when information that was not congruent with a pre-existing schema was presented, confirming the assumption that a pre-existing schema and the incoming information could not be identified as congruent as participants may not have been able to use their previously acquired knowledge to make these inferences. Regarding memory generalization, increased levels of noradrenaline hampered with the ability to use previous knowledge for generalization (in women), while learning of equivalences, i.e. the initial acquisition of information that participants were required to use later on, was not impacted by the intake of hydrocortisone, yohimbine or both, neither in men nor in women. Hence, stress or the major stress mediators cortisol and noradrenaline led to an impairment in the ability to utilize and most importantly benefit from prior knowledge in two different contexts. These findings therefore extend results obtained previously, as they show that stress and specific compounds involved in the stress response also impact complex memory and learning processes that go beyond simple consolidation and retrieval processes, but may actually incorporate these. While results observed after the stress manipulation are in agreement with what was expected, i.e. a disruption of the use of previous knowledge and aberrant activation in relevant brain structures, results obtained after drug administration may have not shown the effects that were predicted initially. More precisely, previous literature has emphasized an impact of the combined activity of the glucocorticoid and catecholaminergic systems during stress and studies investigating the individual components of the stress response have also pointed out that both stress mediators are required in interaction to elicit effects on learning and memory processes (de Quervain, Aerni, & Roozendaal, 2007; Elzinga & Roelofs, 2005; Roozendaal et al., 2004; Schwabe et al., 2011; Schwabe et al., 2009; Schwabe, Tegenthoff, et al., 2010, 2012; van Stegeren, Roozendaal, Kindt, Wolf, & Joels, 2010).

Specific Impact of Cortisol and Noradrenaline

Although the interaction of both noradrenaline and cortisol is often emphasized, there are also accounts reporting that specifically increased levels of glucocorticoids (cortisol in humans and corticosterone in rodents) are required for observable effects on memory, not necessitating increased noradrenergic stimulation, as may be the case in the current work. A study by De Quervain, Roozendaal and McGaugh (1998) also investigated the impact of stress mediators on memory recall and reported that corticosterones impaired the ability of rats to accurately recall memories 30 minutes after acute stress (footshocks), or after a dose of systemic corticosterone administration. Furthermore, to rule out that the combined activity of catecholamines and glucocorticoids was required, rats received a corticosterone synthesis blocker. Blocking corticosterone action also blocked the stress induced memory impairment (De Quervain et al., 1998), showing that corticosterone may indeed be sufficient to lead to a disruptive effect on memory recall. However, it may be the case that effects observed are not triggered by cortisol action alone, but that the activation of the sympathetic nervous system and with that, a release of catecholamines is beneficial for corticosterone action on memory processes. Even after the administration of systemic corticosterone, it may be the case that catecholamines are released, possibly in a lower dose than after an acute stress administration. Our findings may point in the same direction, indicating a cortisol effect. It is however not yet clear whether cortisol is actually sufficient to yield these results alone or whether noradrenaline is also necessary, since we did not block the action of noradrenaline. However, the lack of an interaction effect may indicate that cortisol is actually sufficient. To further shed light on these assumptions, future studies should inhibit the action of cortisol, possibly using cortisol synthesis inhibitor metyrapone (Maheu, Joober, Beaulieu, & Lupien, 2004).

Another study by De Quervain et al (2000) reported a retrieval impairment of non-arousing words in humans after the intake of cortisone before task completion, compared to a control group. This retrieval deficit was only present in a delayed free recall test, while no effects were found in a recognition test, or in experimental groups receiving cortisone at different time points (De Quervain et al., 2000). Although the authors discussed the fact that their experiment did not lead to an autonomic arousal due to the use of non-arousing stimuli, it was not tested whether there was an increased stimulation of the noradrenergic system. This study therefore points in the same direction as was shown above, a sole action of cortisol, but additional research is needed to confirm

this assumption. Further results highlighting the impact of cortisone alone showed that hydrocortisone administration, without concurrent yohimbine intake, led to an impaired memory retrieval which may be due to a decrease in cerebral blood flow in the parahippocampal gyrus, a structure crucial for the retrieval of spatial information (De Quervain et al., 2003). Another study in rats reported that direct infusions of a glucocorticoid receptor agonist into the hippocampus impaired memory retrieval (Roozendaal et al., 2003). In both studies, a possible rise in sympathetic activation was not tested. Nonetheless SNS activation may have been a possible co-occurrence of the circumstances that were apparent in the study, such as being in a PET scanner, for example (De Quervain et al., 2003). Both studies also did not elicit a stress response but administered cortisone or activated glucocorticoid receptors with a specific agonist. This again, points to the fact that cortisol has an impairing effect of memory functions by itself, without necessitating concurrent noradrenergic activity, as may be the case in the current study, when investigating the use of prior knowledge.

Specifically considering effects of noradrenergic activity, a study by Van Stegeren et al (2005) reported aberrant amygdala activation in participants when viewing emotionally arousing pictures in the MRI scanner after taking the beta-adrenoceptor blocker propranolol (van Stegeren et al., 2005). The results only addressed noradrenergic activity, although the fact that participants were in an MRI scanner could have triggered the release of cortisol. In our third study, we also observed a strong effect of noradrenaline on generalization ability, hypothesizing that the effect observed may have been mediated by amygdala action on hippocampal functioning. Roozendaal et al (2006) also point in the same direction, however indicating that glucocorticoid action may necessitate noradrenergic activity. More specifically Roozendaal et al (2006) reported that beneficial effects of glucocorticoids on conditioning experiments in rats were only observed when noradrenaline in the amygdala was not blocked (Roozendaal, Hui, et al., 2006), providing evidence for a concerted action of corticosterone and noradrenaline but also the critical role of noradrenaline on the amygdala. Another study by Schwabe et al (2013) reported that noradrenaline led to an increased amygdala activity in women when viewing fearful faces, while this was not observed in men (Schwabe, Hoffken, et al., 2013). Hence, while concurrent activation of the sympathetic nervous system and the HPA axis cannot be ruled out in the experiments described above, their findings also point to the conclusion that, as found in our studies, each individual stress mediator plays a major role in the effects observed.

An important distinction to earlier studies is that both paradigms used in the current work (memory generalization and schema-based learning) did not test for a stress effect on an isolated retrieval or consolidation mechanism, but a more complex process that requires the recognition of the congruency of previous knowledge with an existing schema or to generalize across a new event that bears similar features to what has been experienced before (Gilboa & Marlatte, 2017; Shohamy & Wagner, 2008; van Kesteren et al., 2013; van Kesteren, Rijpkema, et al., 2010). Furthermore, the activation of underlying brain structures during schema-based learning is rather complex, compared to mere consolidation and retrieval, activating a network of structures, such as the mPFC, angular gyrus and precuneus (Gilboa & Marlatte, 2017; Spalding et al., 2015; Tse et al., 2007; Tse et al., 2011; van Kesteren et al., 2013; van Kesteren, Rijpkema, et al., 2010; Wagner et al., 2015). It may therefore be the case that due to the anatomical structures involved, i.e. the mPFC and its sensitivity to specifically cortisol (Barsegyan et al., 2010; Butts, Weinberg, Young, & Phillips, 2011; McEown & Treit, 2011), schema-detection may be hindered by the presence of a heightened level of cortisol and does not necessarily require an increased stimulation of the noradrenergic system. This may also be reflected in the results obtained from the first experiment. In both groups, the immediate as well as delayed stress groups, data indicated an impairment in schema-based learning. In the immediate group that started the task immediately after stress exposure, there was an increase in nor-adrenergic activity, while cortisol was already rising during schema-based learning. In the delayed group, nor-adrenergic activity may have decreased while there were still increased cortisol levels during task completion. In the second experiment utilizing a pharmacological manipulation, effects obtained were also dependent on the increase of cortisol, either alone or in combination with yohimbine. Hence, the underlying mechanism of the observed deficit may indeed be due to the decrease in mPFC activity as a results of an increase in cortisol after stress and the inability to detect schema-congruent information as such. Furthermore, structures such as the angular gyrus and precuneus, also need to be considered in their reactivity to stress. While there is a lack of literature considering the precise action of the stress mediators on these structures, the current study indicates that stress leads to an increased connectivity between these structures when schema-unrelated items were presented. Hence, the current results highlight the complexity of the process in question, especially when considering the modulating effect of stress and the major stress mediators on schema-based learning. The same may be true for generalization. Although critically dependent on the hippocampus, the mechanism governing

generalization may be rather multifaceted, compared to isolated retrieval and consolidation processes.

In our third study, results did not reflect a specific impact of cortisol, but of increased noradrenergic arousal on memory generalization in women. It is possible to rule out direct effects of noradrenaline on the hippocampus to impair memory generalization, as noradrenaline has been shown to lead to increased synaptic plasticity in the hippocampus (Gray & Johnston, 1987), which would aid learning. Instead, it is suggested that the results observed are caused by increased amygdala activity, modulating the functionality of the hippocampus in turn (McGaugh et al., 1996) and therefore leading to a decreased generalization ability in women, while almost the opposite effect was observed in men. This sex difference fits with the broader literature, as noradrenergic action on the functionality of the amygdala may also be impacted by the presence of estrogen as the combined activity of these compounds may lead to an increased amygdala functioning (Matsumoto, 1991; McEwen & Alves, 1999), while testosterone in men may lead to a decrease in amygdala functioning (Flügge, Kramer, & Fuchs, 2001). Thus, in this case, a concurrent effect of cortisol may not be necessary as noradrenaline may be sufficient to disrupt the complex process of memory generalization. Due to the fact that testing took place in the afternoon, the baseline level of cortisol, though present, might have been too low to exert strong effects on its own. However, Dandolo and Schwabe (2016) showed an association between increased cortisol levels and impaired generalization performance (Dandolo & Schwabe, 2016). Hence, while cortisol may not be sufficient to impair memory generalization, it may still be required, even in low doses to facilitate the effect of noradrenaline, leading to impaired generalization performance in women.

Hence, effects of stress and glucocorticoids as well as noradrenaline may not be directly translatable from previous accounts investigating isolated processes, to the current work. However, previous studies already reported that the action of a single stress mediator was sufficient to show modulations on the memory processes of interest. The current work thus extends our knowledge and again shows that rather complex learning and memory processes, such as schema-based learning and memory generalization are actually impaired by the action of a single stress mediator, in this case cortisol or noradrenaline. Although we cannot rule out that a concurrent action of both systems is required, our results did not indicate any interaction effects. In the sections below, I will further elaborate and provide more detail on the mechanisms that may be involved in the modulation of stress or the major stress mediators on schema-based learning and memory generalization.

Mechanism Underlying the Impaired Use of Prior Knowledge in a Schema-Based Learning Task

Regarding the effect of stress and the major stress mediators on the use of prior knowledge in schema-based learning, results showed that stress as well as an increase in cortisol lead to the inability to utilize a pre-existing schema to aid schema-based learning. As pointed out above, schema-based learning is primarily supported by the mPFC (Damoiseaux & Greicius, 2009; Hare, Hakimi, & Rangel, 2014; Tse et al., 2007; Tse et al., 2011; van Kesteren, Fernandez, et al., 2010; van Kesteren, Rijpkema, et al., 2010), which is highly sensitive to stress and expresses a high density of glucocorticoid receptors (Arnsten, 2009; McEwen et al., 1986; Schwabe & Wolf, 2009). Hence, the observed behavioral deficits during schema-based learning after acute stress or hydrocortisone administration may be due to a downregulation of mPFC activity, which then hinders the detection of schema-congruent information. Results from our neuroimaging study also showed that in the control group, there was a marginally significant effect for an increased activity in the mPFC during schema-related compared to novel (schema-unrelated) trials, while this was not the case in stressed participants. Furthermore, across all participants, there was a negative correlation between mPFC activity and individual cortisol response that allowed the interpretation that a higher cortisol response led to a decreased activity in the mPFC and may therefore hamper schema-detection. Hence, cortisol seems to be exerting a strong influence on the ability to detect schema-related information, i.e. the congruency with a pre-existing schema, by leading to a downregulation of the mPFC. This cortisol action may involve the activation of GRs and MRs. While these have traditionally been thought to only mediate slow genomic effects, recent evidence indicates that membrane bound MRs and GRs actually support rapid action of cortisol (Barsegyan et al., 2010; Joels et al., 2012). Regarding the timing of the effects reported, this mode of action, via membrane-bound receptors, may be highly likely (Henckens, van Wingen, Joels, & Fernandez, 2010; Tasker, Di, & Malcher-Lopes, 2006). However, this is only speculation, as the current work cannot confirm these assumptions. Future studies should therefore employ specific blockers of these receptors to be able to make more precise claims.

The importance of the vmPFC for congruency detection has also been pointed out by a recent study investigating whether the vmPFC is activated during the presentation of items that may be perceived as congruent with pre-existing knowledge or relevant to pre-existing knowledge. Results showed that the vmPFC shows increased activity and contributes more strongly to memory formation when the congruency that is perceived of incoming information is high, rather than the

relevance (Brod & Shing, 2018), confirming the importance of this structure to schema-based learning and memory. Further evidence of the relevance of the vmPFC to schema-based learning and the detrimental effect that a downregulation or lesion can have was shown in a study by Gosh et al (2014). The group investigated the ability of patients with vmPFC damage and confabulation, to complete a schema-based learning task. These patients were presented with words that belonged to an 'everyday schema', such as the word 'chalkboard' to 'school' and were asked to indicate whether the words belonged together in one schema or not. Results showed an impairment in the ability to identify these associations even though there was no sign of memory impairment (Ghosh et al., 2014). Gosh et al (2014) suggested several explanations for the impairment in schema processing, highlighting specifically the assumption that damage to the vmPFC causes a 'nebulous' schema-structure, that impairs the identification of schema-related and unrelated events, expressed in longer reaction times for both items in patients compared to controls (Ghosh et al., 2014). Additional analyses of our own data showed that there was no difference in reaction times between participants in either treatment group, nor any interaction effects with treatment (no main or interaction effects, all $F \leq 1.88$, all $p \geq .17$). Although the assumption of a nebulous schema structure may still hold true for the current data, the fact that we did not observe a treatment related difference in reaction times may be reasoned by the differences in the participant sample tested. Gosh et al (2014) utilized a specific sample of confabulating patients with vmPFC damage that showed these effects, while our participants were exposed to a mild laboratory stressor or a pharmacological manipulation that is suggested to lead to a downregulation of vmPFC activity but not to a severe loss of function or confabulation. Furthermore, patients tested by Gosh et al (2014) were older than the samples discussed in the current work, as these comprise only participants from 18 to 32. Patients tested by Gosh et al ranged from 43 to 67 years of age, which may also explain the differences in reaction times recorded.

Early accounts by Minsky (1975) and Neisser (1976) have also suggested that perception is guided by a schema (Minsky, 1975; Neisser, 1976) and that this assumption could be extended to our attention that may be influenced by pre-existing and activated schemas. Hence, it may be the case that detection of whether incoming information is actually congruent to a schema is essentially influenced by our attention that may in turn be modulated by an activated schema. In the current study, stressed participants may have not been able to activate the current schema and may thus not have been able to detect congruency and benefit from the schema per se, as attention was not

specifically guided. The precise mechanism underlying the results observed however would need further investigation.

Furthermore, our results showed that activity in the hippocampus during schema-related learning trials in stressed participants was negatively associated with schema-related learning. Hence there is not only the difference in activation in the mPFC but also in the hippocampus, i.e. the structure that is concerned with information not related to a present schema. It may be the case, that incoming information may not be detected as schema-related, which may be reasoned with the decreased activation in the mPFC as well as the increased activity in the hippocampus in stressed participants.

Additionally, it is important to consider effects of stress or cortisol on initial acquisition of a schema, or new information, as has been the case when participants were presented with the novel hierarchy. As suggested above, initial acquisition may be hippocampus dependent. However, there are accounts that suggest that stress or cortisol may actually lead to a switch in memory systems, from the hippocampus-based system to the striatum, which has been shown to spare performance in several learning tasks (Schwabe et al., 2007; Schwabe, Schachinger, et al., 2010; Schwabe & Wolf, 2012, 2013). It may therefore be the case that learning of novel information was actually mediated by the striatum in stressed participants instead of the hippocampus, which would also explain the lack of a performance deficit. However, pre-learning stress effects have not led to clear results, indicating, positive, negative and null effects (Kirschbaum et al., 1996; Sandi & Pinelo-Nava, 2007; Schwabe, Bohringer, et al., 2008; Smeets et al., 2007; Zoladz et al., 2011). Hence, unimpaired initial learning may have simply been the result of a lack of any stress effects. This may be confirmed as stressed participants showed hippocampus activity when schema-related trials were presented, while they seemed to have classed this information as novel. Whole brain analyses also revealed activation of the striatum, although the ventral striatum, when learning trials were presented. It is however important to note, that specifically the dorsolateral striatum is implicated in the rather rigid memory that is enhanced after stress (Featherstone & McDonald, 2004; Packard & Knowlton, 2002). Hence, there need to be further investigations targeting specifically a possible switch in memory systems that may be active after stress and the consequences of such a switch for the use of prior knowledge.

While the vmPFC has been strongly associated with schema-based learning, this association seems to depend, at least partly, on how well a schema is established. A study by Sommer (2017) tested

the neural underpinnings of an overlearned schema and showed that initial acquisition led to an activation of the hippocampus, while on the next day the schema was transferred to the vmPFC. Three month later, retrieval of the schema led to activity in the vlPFC, as well as connectivity with the anterior temporal lobe, angular gyrus and TPJ (Sommer, 2017). Reinstatement of a schema however, was shown to then require vmPFC activity again (Gilboa & Marlatte, 2017). However, since the current experiments only involved a delay of 24 hours between initial schema acquisition and schema-related learning, it is likely that effects observed are due to the impact of stress or cortisol on the vmPFC, rather than other structures involved. Though it is to keep in mind that highly established schemas, as we may have due to natural occurrences and a lifelong acquisition and maintenance, involve additional structures to the vmPFC, which also need to be investigated in future experiments.

Generally, it is not yet clear how stress impacts the use of already long established schemas. Although a recent study by Vogel et al. (2018) provided some insight, there need to be additional studies that investigate this topic. More specifically, Vogel et al. (2018) used a task during which participants were presented with words that either matched or did not match a specific category that was shown previously. Participants were asked to indicate whether the word shown actually belonged to the category or not, thereby relying on already established schemas, such as bathroom, or school. Before completing the task, participants underwent the TSST or a control condition. Three to five days later, participants performed a free recall and recognition test of the words presented and showed a better recall and recognition performance for schema-related items, compared to control items that did not belong to an activated schema (Vogel, Kluen, Fernandez, & Schwabe, 2018). Furthermore, stress led to an engagement of the hippocampus, during the presentation of schema-related items. Hence, even when activating well established schemas, such as the general schema of a bathroom or school, stress has an impact on the general processing of information that belongs to a schema and also affects subsequent memory performance, depending on hippocampal activity (Vogel et al., 2018). While these results already provide some insight, there need to be additional investigations into the impact stress has on our ability to profit from schemas established long ago and are maintained during a lifetime, to add to our understanding of this complex memory function.

Additional Compounds Involved in the Stress Response

However, while the results obtained are already allowing us to further our comprehension of the effects of stress and the major stress mediators on schema-based learning, it is to keep in mind that the stress response is a rather complex construct that comprises more discreet compounds than cortisol and noradrenaline alone. There are additional mediators that are released in response to stress, such as serotonin, dopamine, CRH, nitric oxide and vasopressin (Joels & Baram, 2009; Joels et al., 2006), to name a few. The release of these specific stress mediators are mainly dependent on the specific type and timing of the stressor, but also vary in concentration depending on the characteristics of the individual, such as gender and age. Up to the current stage of research, not all substances that are released during a stress response have been associated with a specific function (Joels & Baram, 2009). It is however highly likely that substances such as nitric oxide, dopamine or serotonin bear important functions during the stress response that may also have an impact on learning and memory processes. Nitric oxide for example, has been found to increase the likelihood of the induction of a specific form of synaptic plasticity (long-term potentiation, LTP) and is involved in memory formation (Gülpinar & Yegen, 2004). Release of dopamine, noradrenaline as well as serotonin have been described in the hippocampus, striatum, nucleus accumbens, amygdala and prefrontal cortices, i.e. regions that have been associated with learning and memory processes (Abercrombie, Keefe, DiFrischia, & Zigmond, 1989; Joels & Baram, 2009). Considering serotonin (5-HT), a study in rats showed that it is likely to occupy an important role in the recovery after stress and may be beneficial to prevent dendritic spine loss in the hippocampus as a result of stress (Luine, Villegas, Martinez, & McEwen, 1994). There are additional accounts that report a protective effect of decreased levels of 5-HT on memory processes after stress and that specific subtypes of the 5-HT receptors (5-HT1A, 5-HT4) also play important roles in memory impairment and cognitive enhancement (Gülpinar & Yegen, 2004). It has also been stated that specifically females show an increased release of serotonin in reaction to acute stress, which may also be sensitive to the current menstrual cycle position (Andreano & Cahill, 2009; Mitsushima, Yamada, Takase, Funabashi, & Kimura, 2006; Moses et al., 2000). Although we did not find any gender specific effects relating to the effect of stress and cortisol on schema-based learning, it may still prove important to consider this compound, as it may shed light on the underlying mechanisms that act to modulate this memory process. Although discussed further below, serotonin may also be important with respect to memory generalization and the specific effect we obtained in women.

Regarding the impact of dopamine, results indicate that activation of dopamine D1 receptors as a response to acute stress impairs emotional learning and memory in the rat (Wang, Wu, Zhu, Li, & Cai, 2012). Dopamine is part of the fast stress response and is released immediately after stress exposure together with noradrenaline and serotonin. The action of these compounds is mediated via G-protein coupled receptors (Joels & Baram, 2009). Results regarding the mechanism underlying the dopamine response to stress indicated that in prefrontal regions, dopamine is released in response to increased glucocorticoid levels and is reduced in response to a blockage of glucocorticoid receptors (Butts et al., 2011). To understand the impact of stress on specific learning and memory functions, especially complex processes such as schema-based learning that involve pre-existing knowledge, it is important to consider all aspects of the stress response and future research may be extended to also investigate compounds such as 5-HT, dopamine and many more specifically on schema-based learning processes, but also memory generalization.

Impact of Stress Mediators on Memory Generalization

In the third study, we observed strong impairing effects of increased levels of noradrenaline on generalization ability in an acquired equivalence paradigm, however solely in women. The observed effects may reflect an impact on hippocampal functioning as memory generalization is proposed to mainly depend on the hippocampus, especially when generalization is considered a form of integrative encoding (Shohamy & Wagner, 2008). More specifically, it is suggested that the confrontation with an event that bears similarities with what has already been experienced before may initiate retrieval processes of that particular event, which allows the formation of a so-called integrated representation that comprises the two memories as discreet events. This form of encoding and retrieval mechanisms also facilitate generalization as the integrated representation will be retrieved together as opposed to forming inferences (Shohamy & Wagner, 2008). The proposed mechanism is based on accounts that report a shifting between memory encoding and retrieval mechanisms within the hippocampus, which is mediated by dopamine signaling from the midbrain (Hasselmo & McClelland, 1999; Hasselmo, Schnell, & Barkai, 1995). The observed deficits in the ability to generalize may however not be the results of a direct action of noradrenaline on hippocampal functioning, but act via a different mechanism.

Previous literature indicates similar gender-specific findings in terms of the impact of increased noradrenergic activity, though in a different paradigm. A study by Schwabe et al (2013) reported

that yohimbine administration led to an augmented rating of fearfulness in images of faces, however solely by women, while this was not found in men. Furthermore, neuroimaging data indicated that yohimbine intake increased amygdala reactivity to fearful faces in women, while this could not be shown in men. Increased cortisol levels, as a results of hydrocortisone intake, did not have any effect on performance or neural activity (Schwabe, Hoffken, et al., 2013). These results point towards the possibility that observed effects of noradrenaline on memory generalization may be due to an increase in activity of the amygdala which in turn impacts hippocampal functioning (McGaugh et al., 1996; Rasch et al., 2009; Vogel & Schwabe, 2016b). Further evidence for the effect of increased noradrenergic stimulation on cognitive functions via the amygdala comes from studies utilizing pharmacological manipulations of the action of noradrenaline on the amygdala. Specifically, blockage of beta-adrenergic receptors lead to a reduction in activity as a reaction to emotional stimuli, while a reuptake inhibitor lead to increased activity in the amygdala (Hurlemann et al., 2010; Onur et al., 2009; van Stegeren et al., 2005). Another account reports that glucocorticoid effects require noradrenergic activity in the basolateral amygdala (Roozendaal, Hui, et al., 2006; Roozendaal, Okuda, de Quervain, et al., 2006; Roozendaal, Okuda, Van der Zee, et al., 2006). Hence, it seems that noradrenergic effects are specifically prevalent at the basolateral amygdala and the driving force to then impact cognitive functions, such as memory processes, as it may be the case in the current work.

The amygdala is part of a salience network which shows increased activity in response to stress and primarily noradrenaline (Hermans, Henckens, Joels, & Fernandez, 2014). This may also be the reason we observed effects of sex. Functionality of the amygdala is different between the sexes and there are accounts that also report developmental differences (Newman, 1999). Furthermore, in addition to the functionality and developmental differences, reports state that amygdala functioning is impacted by male and female sex hormones, albeit differently. While female sex hormones estrogen and progesterone lead to an increased amygdala activity, also through the interaction with catecholamines, testosterone may have the opposite effect (Flügge et al., 2001; Matsumoto, 1991; Schiess, Joels, & Shinnick-Gallagher, 1988; Schwabe, Hoffken, et al., 2013). Hence, the current effects may be observed in females, since there is increased amygdala activity due to the assumed interaction between noradrenaline and estrogen, which may then lead to a strong modulation on hippocampal functioning. This in turn, strongly impacts the retrieval of integrated representations that would facilitate generalization, but may have also already impacted the initial formation of integrated representations. This may be the case as integrated

representations are formed when the likeness between an incoming event that bears similarities with what has already been experienced before may initiate retrieval processes of that particular event (Shohamy & Wagner; 2008). On the other hand, it may be possible that the simple retrieval is not impacted. However, it is necessary to further investigate these assumptions and to also increase and decrease levels of estrogen and progesterone in females to test whether effects become more pronounced or may even disappear. Furthermore, while results in men were only at trend level, there was still an indication that performance tended to be improved in men compared to women during increased noradrenergic stimulation. This may be due to the fact that testosterone has been reported to rather decrease amygdala activity (Flügge et al., 2001). Thus, there may not be such a strong modulating impact from the amygdala to the hippocampus, therefore not hindering generalization. Even though this is highly speculative, one could assume that less amygdala activity would lead to an enhanced ability to generalize across experiences, as was found in men.

The concerted action of increased noradrenaline and the sex hormone estrogen may therefore lead to an increased activity in the amygdala, which then negatively impacts hippocampal functioning and leads to an impairment in memory generalization in women. When hippocampal activity is impacted by the amygdala, the complex mechanism underlying memory generalization may in turn be impaired. The neurochemical mechanism that is suggested to facilitate the dynamic switch between the encoding and retrieval mechanism in the hippocampus is supported by a midbrain dopaminergic system (Shohamy & Wagner, 2008). This system is assumed to aid the encoding of individual memory episodes or events but research has also suggested that hippocampal plasticity, a prerequisite for the role the hippocampus plays in memory formation, is also modulated by dopamine (Lisman & Grace, 2005; Morris et al., 2003; Otmakhova & Lisman, 1996; Schultz, Dayan, & Montague, 1997). This dopaminergic input that also projects to the striatum, suggests an involvement of the latter in addition to the hippocampus in memory generalization (Adcock, Thangavel, Whitfield-Gabrieli, Knutson, & Gabrieli, 2006; Lisman & Grace, 2005; Shohamy & Wagner, 2008). Regarding the effects observed, it can be speculated that the release of dopamine in the midbrain is modulated by acute stress and the main stress-mediators cortisol and noradrenaline. While there are not many accounts that provide insight into the mechanism, there are few results that indicate an increase of dopamine release in response to stress but also a modulation through the sex hormones testosterone and estrogen (Sinclair, Purves-Tyson, Allen, & Weickert, 2014). While Sinclair et al (2014) mainly review studies indicating this interaction with

cortisol, there is also the suggestion that noradrenaline interacts with dopamine (Antelman & Caggiula, 1977). It seems however necessary to consider the interaction of all three compounds (estrogen, noradrenaline and dopamine) together as it is suggested, at least in the prefrontal cortex, that this interaction may lead to an impairment (Shansky, Bender, & Arnsten, 2009). More specifically, under stress there is an increased projection of dopamine signaling from the midbrain to the prefrontal cortex. This dopamine signaling adheres to an inverted U-shape indicating that a concentration of dopamine that is too low or too high, which may be the case after stress, may be detrimental to signaling within the prefrontal cortex. Estrogen does not adhere to such a dose-response regulation (Shansky et al., 2009). However, in higher concentrations, i.e. in females with high estrogen release, estrogen interacts with dopamine and might hamper prefrontal functioning already at otherwise optimal dopamine levels. There is however a mechanism present that exerts regulatory functions on this dopamine and estrogen interaction which is mediated by noradrenaline. Noradrenaline may interact with alpha-2a receptors that modulate cAMP functioning which in turn changes the gating of specific ion channels and may serve to recover PFC functionality after increased dopamine release. Estrogen however may lead to a disengagement of the alpha-2a agonist and may thus hamper with the functioning of this receptor and its regulatory role. Thus, the increased dopamine signaling cannot be buffered (Ansonoff & Etgen, 2001; Kritzer & Creutz, 2008; Shansky et al., 2009; Shansky & Lipps, 2013; Xiao & Becker, 1994).

While this is highly speculative, a similar mechanism may be active in the hippocampus, which might receive dopaminergic input from the midbrain area that mediates the dynamic switch between encoding and retrieval states of the integrated representations. It is possible that increased dopaminergic signaling, facilitated through increased noradrenergic signaling under stress (Antelman & Caggiula, 1977) and the interaction between noradrenaline and estrogen may hinder the switch between these two modes of operation in the hippocampus, or even facilitate the switch from the hippocampus to the striatum under stress (Schwabe, Schächinger, de Kloet, & Oitzl, 2010), hence possibly hindering the retrieval of the integrated representations, especially in women.

While the explanations above mainly target the likely mechanism, that hippocampal activity is downregulated through increased amygdala functioning in women and the effect this has on the mechanisms that facilitate memory generalization, it is important to also elaborate on another mechanism that is likely to be active after exposure to an acute stressor. Previous studies have already reported that stress can lead to a shift from the declarative hippocampus-based memory system to the striatum dependent memory system (Schwabe, Schächinger, et al., 2010; Schwabe &

Wolf, 2010b). It is further suggested that this switch is mediated by the amygdala (Packard & Wingard, 2004; Vogel et al., 2016). This system is rather rigid and does not support flexible learning, as what would be required in memory generalization, it does however still support learning and memory (Myers et al., 2003). What would speak against the idea that stressed participants in our study relied on the striatal system instead of the hippocampus is the fact that results did not indicate a performance difference in treatment groups in the acquisition phase that comprised learning of the critical associations. However, it may be the case that this task was too easy, which would also be suggested by the fact that we did not find any significant increase between the individual stages of the acquisition phase. Hence, stressed participants might have still relied on the striatum, which may have been sufficient to support initial acquisition learning (Knowlton & Squire, 1993). During the generalization phase, the impairment in the ability to generalize in women that received yohimbine alone or in combination may however point to the possibility that the striatal memory systems was not able to support memory generalization (Dandolo & Schwabe, 2016). It may also be the case that women who showed the impairment relied on the striatum and did not form integrated representations in the first place but remained dependent on distinct representations of learned stimulus pairs in the acquisition phase.

Results therefore indicate that there may not only be a disruptive effect of noradrenaline, through increased activity of the amygdala in women, which in turn impaired hippocampal functioning, but also a disruptive effect of noradrenaline and estrogen on the dopamine signaling from the midbrain to the hippocampus. As these are highly speculative assumptions, there need to be additional investigations, probing the mechanisms underlying the crucial dynamic switch in the hippocampus and its sensitivity to modulatory action of noradrenaline and estrogen, but also dopamine signaling from the midbrain area and the impact of stress on this structure.

Timing and Cortisol Action

While we observed a clear effect of noradrenaline on memory generalization, cortisol did not exert an impact, neither in women nor in men. However, previous results showed a negative association between generalization performance and cortisol, though this interaction was independent of gender (Dandolo & Schwabe, 2016). While this association clearly suggests an impact of cortisol on generalization performance in the previous study, it may be the case that this disruption is actually mediated by an interaction between cortisol and noradrenaline. It may also be the case that cortisol

may require the presence of noradrenaline to exert a function, while noradrenaline is mediating the disruptive effects (Roozendaal, Hui, et al., 2006). This is however still to be confirmed and there need to be additional studies that employ further manipulations, such as blocking the action of one mediator or the other to obtain more insight into the functioning.

Another important point to take into account concerning any glucocorticoid effects, is the timing of the experiment. Participants had completed the task about 85 minutes after the intake of hydrocortisone and yohimbine. Cortisol is known to act via intracellular GRs and MRs that mediate slow genomic stress effects (Joels et al., 2012). As of recently however, research suggests that there are also membrane associated glucocorticoid and mineralocorticoid receptors that facilitate the rapid cortisol effects (Joels et al., 2013; Joels et al., 2012; Vogel et al., 2016). At this point there is no clear definition for how long rapid effects persist and when slow effects come about (Joels et al., 2012). Manipulation checks confirmed that blood pressure (as an index of noradrenergic arousal) and salivary cortisol were still significantly elevated in the respective groups compared to the groups that did not receive that particular treatment at the time of task completion. Since participants finished the task at about 85 minutes past drug intake, rapid cortisol effects may have started to decay, while slow effects have begun to develop, hence there may have been an overlap of both modes of action present (Joels et al., 2012). Rapid glucocorticoid action has been shown to exert rather impairing effects on memory retrieval, while slow effects were reported to exert almost opposite effects (Henckens et al., 2012; Henckens et al., 2010; van Ast, Cornelisse, Meeter, Joels, & Kindt, 2013). It may however even be the case that there is an intermediate mode, at which the rapid non-genomic effect transitions into the slow genomic effect (Joels et al., 2012). These assumption may explain the lack of behavioral effects observed that can be associated with increased cortisol levels, as impairing and facilitating effects may have canceled each other out.

Furthermore, it may be the case, that slow genomic glucocorticoid effects are actually context dependent, meaning that impairing effects of cortisol on memory retrieval, may only be apparent when these are tested in a distinctly new context. This context-dependency has been pointed out in a recent study by Vogel and Schwabe (2016), with respect to enhanced memory formation after stress. While it is suggested that slow genomic glucocorticoid actions exert almost opposite effects, results observed still indicated an enhanced memory formation at 2 h after stress induction (Vogel & Schwabe, 2016b). However, Schwabe and Wolf (2014) pointed to a persistent, even worsened impairment on memory retrieval 90 minutes after stress exposure, even though there was no severe change of context. Though there needs to be additional investigation into the mechanisms

and resulting effects of genomic cortisol actions, it may also be the case that since we did not test a simple retrieval, encoding or consolidation mechanism, we cannot translate effects from previous studies directly to the current experiment.

However, another point to make regarding glucocorticoid effects, may be that there is a lack of power. More specifically, while context dependency may be an explanation for the lack of slow genomic glucocorticoid effects, it may also be the case that particularly these slow effects are more subtle than the rapid glucocorticoid effects and may therefore require a larger sample size (Vogel & Schwabe, 2016b). While the overall sample size with 103 participants was rather large, due to eight experimental groups (12-13 males and 12-14 females per group) the size of each individual group was relatively small. Future studies should bear that in mind and increase sample sizes as well as manipulate the timing of the study to further investigate any possible effects of cortisol on memory generalization.

Gender Effects in Response to Stress

There are several accounts that have emphasized the importance to take into account gender effects in the neurosciences (Andreano & Cahill, 2006, 2009; Apicella, Carré, & Dreber, 2015; Apicella et al., 2011; Cahill, 2006; Cazzell, Li, Lin, Patel, & Liu, 2012; McLean, Asnaani, Litz, & Hofmann, 2011; Olff, Langeland, Draijer, & Gersons, 2007; Schipper, 2015a, 2015b), which may also well be true when considering the modulating effects of stress on cognition (Andreano & Cahill, 2006; Daughters, Gorka, Matusiewicz, & Anderson, 2013; Kirschbaum et al., 1999; Kluen et al., 2017; Kudielka et al., 2004; Lighthall et al., 2012; Shors, Chua, & Falduto, 2001; Stephens, Mahon, McCaul, & Wand, 2016). Stress is based on the release of many different compounds that interact with one another, also including sex hormones such as estrogen, progesterone and testosterone (Ansonoff & Etgen, 2001; Cahill, 2006; Shansky et al., 2009; van den Bos, Harteveld, & Stoop, 2009; Welker, Zilioli, Carre, & Mehta, 2016). As already described above, the effect of noradrenaline on memory generalization in women, may be due to differences in amygdala structure, including anatomy and functionality (Cahill, 2006) between the sexes, but may also involve differential interaction between the sex hormones and stress mediators (Flügge et al., 2001; Matsumoto, 1991; McEwen & Alves, 1999). To rule out that effects observed were due to the differential menstrual phases of women participating, an additional analysis confirmed that the number of women in the follicular and luteal phase were not different between groups ($\chi^2(6)=6.26$, $p=.395$). In the

experiments regarding the effects of stress and stress-mediators on schema-based learning we did not observe a difference in performance in men and women in interaction with treatment, as stated by Vogel et al (2018) and as additional analyses of our data revealed (no significant main or interaction effects, all $F \leq 2.12$, all $p \geq .149$) in Kluen et al (2016). Hence it seems that the effects of stress and the major stress-mediators on schema-based learning are independent of gender, i.e. that both sexes show a similar impairment to utilize a pre-existing schema after stress or cortisol treatment. While there seem to be certain cognitive functions that are more sensitive to stress and sex hormone interactions than others, it is important to consider sex as a variable to increase our understanding of the mechanisms behind the modulating function of stress.

Linking Schema-Based Learning and Memory Generalization

While the above points targeting the individual mechanisms of stress and stress mediators on schema-based learning and memory generalization are critical to further our understanding of the underlying processes, it is also important to take one step back and again consider how our findings might be integrated to relate to the overarching question of how stress and individual stress mediators affect our ability to utilize prior knowledge. As stated above, we chose the tasks, probing schema-based learning in a transitive inference paradigm (Kumaran, 2013) and memory generalization in an acquired equivalence task (Myers et al., 2003), to investigate the use of prior knowledge, but also the different mechanisms that may underlie the latter. In schema-based learning, the vmPFC plays a critical role as it acts as a detector, indicating the congruency of incoming information with that of a pre-existing schema but also when this new information is integrated into that schema (Richards et al., 2014; van Kesteren, Fernandez, et al., 2010). Furthermore, the hippocampus is critical for initial schema formation and also for processing information that is novel and does not fit in an activated schema (van Kesteren, Fernandez, et al., 2010; van Kesteren et al., 2012). Memory generalization relies majorly on the hippocampus. More precisely, the hippocampus detects similarities between incoming information as well as already stored items and combines them to form integrated representations that are then stored and also retrieved as such. Hence there is a constant switch between retrieval and storage mode (Shohamy & Wagner, 2008). Although featuring different underlying mechanisms, both processes enable the use of prior knowledge that aids our learning and behavior (figure 9 A). This is especially critical, as in everyday life we do not have a blank slate when we learn. Most of the time we are confronted

with information or situations that relate to previous and similar information or situations. We can therefore rely on our experiences and our knowledge to provide a framework to incorporate this new information and to guide our behavior. Hence, we rely on our ability to make use of this knowledge and if we are stressed this ability is disrupted (figure 9 B).

The current work therefore goes beyond research that has been done in the context of stress effects on memory and learning behavior on isolated mechanisms, such as consolidation and retrieval. Although we can show an impairment after stress, which is not unfamiliar when regarding stress effects on memory retrieval, for example, the underlying processes may be different. First of all, in both processes the detector that signals whether incoming information is related to prior knowledge is disrupted by stress. Hence, without this detector, the need to provide access to prior knowledge is not signaled. In the case of memory generalization, the hippocampus detects congruency between incoming information and stored information (Shohamy & Wagner, 2008). It may be the case that this critical process is disrupted, thus prior knowledge cannot be accessed as retrieval is impaired. On the other hand, the retrieval of integrated representations may be hampered with, which would again hinder the use of prior knowledge, although possibly not the detector function. During schema-based learning, the mPFC is critical and results showed a stress induced impairment of this structure when schema-related information was shown (figure 9 B). In schema-based learning, the mPFC acts as a detector (Richards et al., 2014; van Kesteren et al., 2013), as stated above and when mPFC activity is decreased this function may be impaired and a schema cannot be accessed. Furthermore, the mPFC has not been targeted primarily in (long-term) memory research and has originally been considered in terms of working memory and executive functioning (Fregni et al., 2005; Miller, 2000; Miller & Cohen, 2001). However, investigations targeting schema-based learning have considered the mPFC a crucial structure, together with the hippocampus (Schlichting & Preston, 2015; Spalding et al., 2018; van Kesteren, Rijpkema, et al., 2010). The current results are now the first to explore the impact of stress and stress mediators also on mPFC activity and its effects on schema-based learning and thus on the ability to use prior knowledge. Recent evidence also indicates a role of the PFC in a form of memory generalization (Bowman & Zeithamova, 2018).

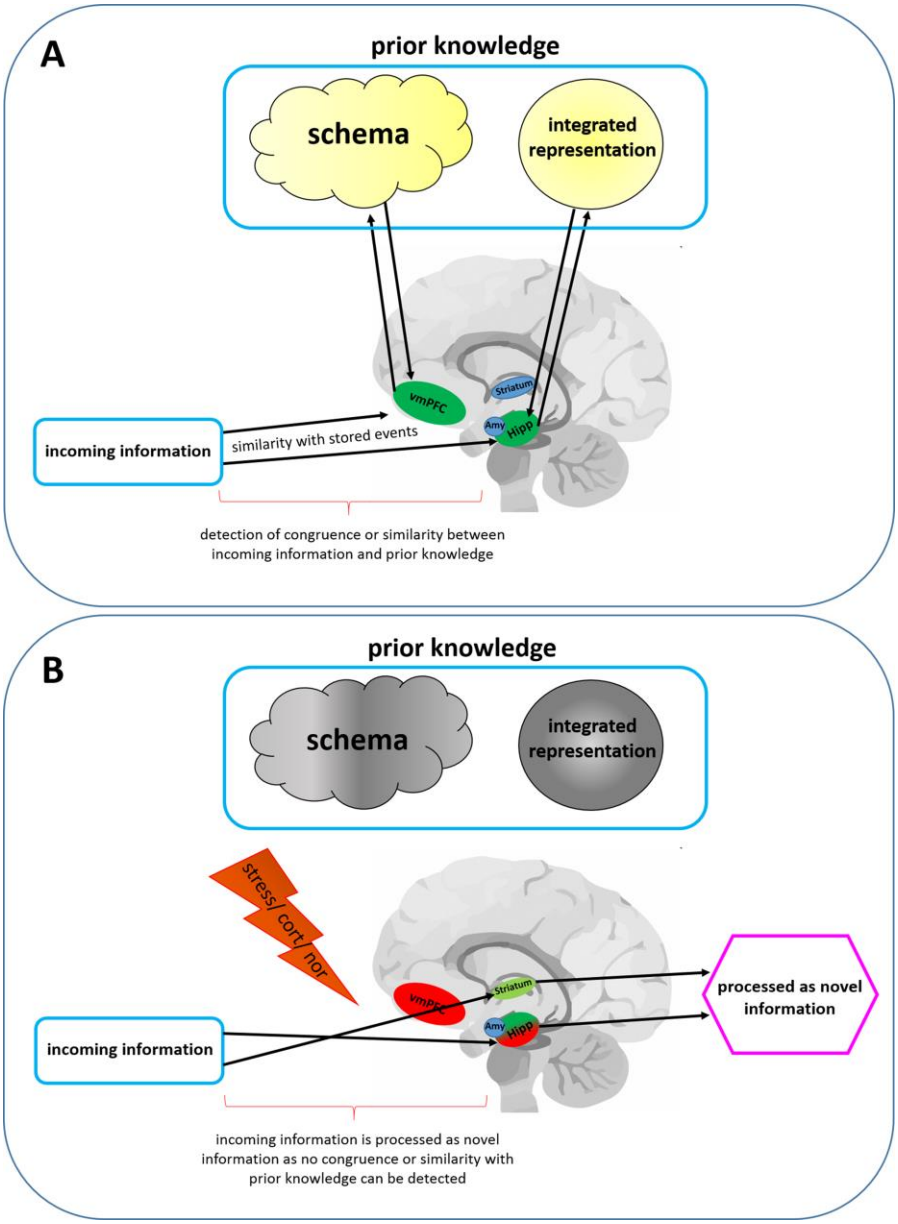

Figure 9 – Schematic Representation of the Impact of Stress on our Ability to Utilize Prior Knowledge

(A) Under normal conditions, incoming information is assessed of whether it is congruent with pre-existing knowledge. If congruency is detected (in this case a blue frame), this may aid learning of that information as well as our ability to benefit from that knowledge that we can apply to new but similar situations. Depending on the congruency, or fit of the information, it is processed by the vmPFC (in case of a congruency with a schema) or the hippocampus where similarity with a similar event/item is detected (generalization) and an integrated representation is formed. These two mechanisms constitute examples of how previous knowledge can be operationalized. Under stress or increased levels of stress mediators cortisol (cort) or noradrenaline (nor, B), activity in relevant structures for congruency detection is downregulated (shown in red) or a switch in structures that process the information takes place (green). Congruency of incoming information with prior knowledge cannot be detected anymore and information is treated as novel (shown in pink) and processed by the hippocampus (in case of schema-incongruent information) or possibly the striatum. Schema-based learning and memory generalization are two different mechanisms that both illustrate the use of prior knowledge and the different mechanisms by which our ability to use this prior knowledge is hampered with through the impact of stress, noradrenaline and cortisol. Hipp – hippocampus, Amy – amygdala, vmPFC – ventromedial prefrontal cortex. The image of the brain is downloaded from: https://upload.wikimedia.org/wikipedia/commons/1/13/Brain_logo.svg. The image is denoted as open access and was accessed last on the 16th of April 2018.

Concept generalization is also described as memory generalization that relies on abstract category representations and has been associated with hippocampal as well as vmPFC activity (Bowman & Zeithamova, 2018). More specifically, the vmPFC represents abstract category information and tracks these as model predictors. In a similar task, the vmPFC has been associated with tracking overall generalization success (Bowman & Zeithamova, 2018; Zeithamova, Maddox, & Schnyer, 2008). Although the task we employed in the current experiment was different to the tasks employed by Bowman and Zeithamova (2018) and Zeithamova et al. (2008), it may be necessary to consider a role of the vmPFC in memory generalization tested in an acquired equivalence task, as well. In the current task, we assessed generalizations that relied on associations that were acquired immediately before generalization testing and may have therefore relied on the hippocampus (Shohamy & Wagner, 2008). However, if generalizations are made based on associations that have been acquired some time ago, there may have been a transfer of these representations to neocortical structures, possibly including the vmPFC. During stress, there may again be a downregulation of this structure (Arnsten, 2009), similar to what we showed during schema-based learning, which would also disable memory generalization. Although there need to be further investigations concerning the assumptions made, the fact that there is a critical involvement of the hippocampus, but also and specifically the vmPFC allows us to further extend previous findings in memory research concerning isolated functions and implicate additional structures such as the mPFC in long-term memory. Hence, the effect of stress and stress mediators on the use of prior knowledge is critical and allows us to develop a better understanding of the impact of stress and stress mediators on the use of prior knowledge. Current research also extends findings not only in terms of constituting a complex process but also in terms of the underlying neural structures, specifically the effect of stress/stress mediators on the mPFC in the context of schema-based learning. Furthermore it shows opportunities for further investigation, specifically targeting the vmPFC in an additional process that utilized prior knowledge, such as memory generalization.

Limitations and Future Directions

The current work describes three studies that shed light on the modulating role of stress and the major stress mediators on schema-based learning, the neural underpinnings of this modulation as well as memory generalization. To confirm as well as to extend current results there should be future studies investigating these topics further by taking on certain advancements.

First of all, while the current experiments report the action of a single stress mediator as crucial, future studies should conduct additional experiments that allow the blockage of the glucocorticoid/mineralocorticoid receptors as well as the adrenergic system. Only when one system is completely inactive, it is possible to confirm the assumptions made in the current experiments. An additional point to consider is that in our current pharmacological experiments, we only investigated a single dose of hydrocortisone and yohimbine that led to the effects observed. However, while administering these drugs is an adequate way to investigate the action of these specific stress mediators and there are previous studies that have used the same or very similar dosage (Buchanan & Lovallo, 2001; Henckens, van Wingen, Joels, & Fernandez, 2011; Margittai et al., 2016; Schwabe, Tegenthoff, et al., 2010, 2012), it is important to also test whether effects remain stable or change when doses are reduced or increased and not lead to reversed or enhanced/weakened effects. What is also important to bear in mind is a possible interaction of the stress hormones cortisol and noradrenaline with sex hormones, as these may have buffering effects (Flügge et al., 2001; Shansky et al., 2009), which may in lower doses not lead to observable behavioral effects.

Additionally and as mentioned above, the sample size of the second and third study were relatively small. While effects in the second study may show this lack of power in only marginally significant results, results with respect to the impact of noradrenaline on memory generalization in the third study were significant regardless of the smaller experimental groups. It is generally necessary to confirm results through replication of the experiment as well as by using larger samples, as effects may become stronger and a cortisol effect that may have been absent may be observed when power is further increased in study III. In study III, the effect may also be tested at a different timing, to specifically target rapid glucocorticoid effects or slow genomic effects. This may also clarify results and the underlying mechanism.

Furthermore, while we aimed at providing insight into the effect of stress and stress mediators on our ability to use prior knowledge, we can still not provide a complete picture, despite the fact that we employed two different tasks, assessing different underlying functions. Future studies should utilize additional and different tasks, testing further aspects of the use of prior knowledge in learning to contribute to the big picture. It is also important to consider even more aspects of the stress response in isolation to further shed light on specific mechanisms that may lead to a disruption or enhancement. Hence, while we can provide evidence for a modulating role of stress and stress mediators on the use of prior knowledge and the possible underlying neural mechanisms, there is still room for further investigations.

Conclusion

The current work provides insight into the impact of stress and major stress mediators cortisol and noradrenaline on our ability to use prior knowledge during learning in two highly complex learning and memory functions, memory generalization and schema-based learning. While the precise mechanisms tested are very different, results show that stress as well as the stress mediators cortisol and noradrenaline have a disruptive effect on the use of prior knowledge. Schema-based learning may be disrupted as stress as well as cortisol lead to a downregulation of the mPFC, which serves as a detector of how congruent incoming information is associated with an activated schema (Richards et al., 2014; van Kesteren, Fernandez, et al., 2010). Stressed participants as well as participants presenting with pharmacologically increased levels of cortisol were not able to identify schema-related information as such and may therefore treat it as novel information. This assumption may be confirmed by results from study II, as stressed participants also show a strong involvement of the hippocampus during schema-based learning, even though the hippocampus is suggested to be concerned with novel incoming information. Memory generalization that relies on the concept of encoding integrated representations of similar experiences and the retrieval of these newly combined memories (Shohamy & Wagner, 2008), is impaired by increased noradrenergic arousal in women. This impairment may be due to a downregulation of the hippocampus, through increased amygdala activity or a possible switch in memory systems, from the hippocampus-based memory to the striatum-based memory. Hence, although the processes active are very dissimilar as well as the mechanisms that are assumed be responsible for the disruptions observed, stress and the stress mediators cortisol and noradrenaline hamper with our ability to benefit from prior

knowledge. These findings therefore extend our understanding regarding stress effects on specific memory functions as the processes tested here are complex and combine a multitude of mechanisms that go beyond simple consolidation and retrieval. Furthermore, we show the effect of stress on the neural ensemble involved in schema-based learning and results specifically indicate an effect of stress on the mPFC. The use of prior knowledge facilitates learning and is thus critical for our behavior, but results have shown how sensitive this ability is to the impact of stress and specific stress mediators. Our findings therefore match with previous results, but also offer even more room for additional research, that could employ variations in the timing when stress is applied and how to manipulate prior knowledge.

As stress effects have an adaptive as well as protective role, one question remaining may be whether this impairment of the use of prior knowledge might be beneficial under certain conditions. It can be speculated that under stress the use of prior knowledge is not as necessary as the focus lies on the formation of new memories that are associated with the stressful situation (Schwabe, Wolf, et al., 2010). Hence, mainly consolidation mechanisms may be active while retrieval of similar events or integrated representations for memory generalization and the detection of congruency with active schemas is impaired. Furthermore, schema-related information may be treated as novel as prefrontal activity is downregulated, while current results also showed increased hippocampal activity. Furthermore, previous studies have reported a shift from hippocampal to striatum dependent memory, underlining the assumption, that under stress S-R learning, compared to rather complex processes is supported (Schwabe et al., 2011).

To conclude, learning is a crucial process in our everyday life that enables us to adapt to our environment and supports our survival (Jing et al., 2017; Schacter & Addis, 2007). Schema-based learning and memory generalization are two complex and critical processes that aid learning and behavior through the use of prior knowledge (Shohamy & Wagner, 2008; van Kesteren et al., 2012). However, as critical as these processes are, they are also sensitive to modulatory effects by stress and the stress mediators cortisol and noradrenaline. The current work is the first to provide crucial insight into the underlying processes and may therefore prove important not only for our general understanding of the impact of stress on learning and memory processes, but also into the underlying mechanisms. Precisely the latter is important when we consider schema-based learning and memory generalization in everyday life, such as in educational or clinical settings. Specifically schema-based learning is important to education and it may be critical to take into account that this process may be hampered with in stressful environments (Vogel & Schwabe, 2016a). Furthermore,

several stress related mental disorders, such as depression and schizophrenia have been associated with an impaired memory performance (Aleman, Hijman, de Haan, & Kahn, 1999; Kaouane et al., 2012; Saykin, Gur, & Gur, 1991). Interestingly, these disorders are also more prevalent in women than in men (Gotlib & Joormann, 2010; Shohamy et al., 2010). Hence, when considering learning and memory, it is also important to take into account the use of previous knowledge that aids us to tackle new situations or learn new, related content, but also the modulatory impact of stress and stress mediators.

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APPENDIX

Appendix A

Impact of Stress and Glucocorticoids on Schema-Based Learning

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Impact of Stress and Glucocorticoids on Schema-Based Learning

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Pre-existing knowledge, a 'schema', facilitates the encoding, consolidation, and retrieval of schema-relevant information. Such schema-based memory is key to every form of education and provides intriguing insights into the integration of new information and prior knowledge. Stress is known to have a critical impact on memory processes, mainly through the action of glucocorticoids and catecholamines. However, whether stress and these major stress mediators affect schema-based learning is completely unknown. To address this question, we performed two experiments, in which participants acquired a schema on day 1 and learned schema-related as well as schema-unrelated information on day 2. In the first experiment, participants underwent a stress or control manipulation either immediately or about 25 min before schema-based memory testing. The second experiment tested whether glucocorticoid and/or noradrenergic activation is sufficient to modulate schema-based memory. To this end, participants received orally a placebo, hydrocortisone, the α2-adrenoceptor-antagonist yohimbine, leading to increased noradrenergic stimulation, or both drugs, before completing the schema-based memory test. Our data indicate that stress, irrespective of the exact timing of the stress exposure, impaired schema-based learning, while leaving learning of schema-unrelated information intact. A very similar effect was obtained after hydrocortisone, but not yohimbine, administration. These data show that stress disrupts participants' ability to benefit from prior knowledge during learning and that glucocorticoid activation is sufficient to produce this effect. Our findings provide novel insights into the impact of stress and stress hormones on the dynamics of human memory and have important practical implications, specifically for educational contexts.
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INTRODUCTION

Prior knowledge, referred to as a schema, provides a framework for the organization and efficient incorporation of new information. Although the powerful impact of pre-existing schemas on learning and memory has long been observed (Alba and Hasher, 1983; Anderson, 1984; Barlett, 1932), the neural underpinnings of schema-based memory have been elucidated only over the past decade. Rodent studies and neuroimaging studies in humans identified the medial prefrontal cortex (mPFC) as a key region in schema-based memory (Ghosh *et al*, 2014b; Tse *et al*, 2007; Tse *et al*, 2011; van Kesteren *et al*, 2010). In particular, the mPFC is thought to detect the schema-congruence of information and to facilitate the integration of schema-relevant information into the neocortical schema representation (van Kesteren *et al*, 2012). The hippocampus, in turn, although being critical for learning novel information, is thought to be less relevant for schema-based memory (Tse *et al*, 2007; van Kesteren *et al*, 2012).

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Stress is well-known to have a critical influence on learning and memory (Diamond *et al*, 2007; Roozendaal *et al*, 2009; Schwabe *et al*, 2012a) and the PFC is among the brain areas that are most sensitive to stress. For instance, stress may affect the goal-directed control of instrumental learning (Schwabe and Wolf, 2009), known to rely on the mPFC (Valentin *et al*, 2007), as well as dendritic morphology in the mPFC (Izquierdo *et al*, 2006; Moench *et al*, 2016). These effects of stress on prefrontal functions are critically mediated by glucocorticoids and noradrenaline (Barsegyan *et al*, 2010; Schwabe *et al*, 2012b). Glucocorticoid and noradrenaline effects are not necessarily independent of one another but there is compelling evidence that glucocorticoids and noradrenaline interact to decrease PFC-dependent functions (Barsegyan *et al*, 2010; Schwabe *et al*, 2011; Schwabe *et al*, 2010a). Although the effects of stress, glucocorticoids and noradrenaline on memory are well-established, the fundamental idea that learning often occurs against the background of existing prior knowledge has been barely considered and whether stress and major stress mediators affect schema-based memory processes is unknown.

Here, we examined the impact of stress, glucocorticoids, and noradrenaline on the use of prior knowledge during learning. We performed two experiments, in which participants acquired a schema on day 1 and learned schema-related and -unrelated material on day 2. The first experiment

assessed the impact of stress on schema-based memory. To address potential differences in the role of noradrenaline and glucocorticoids in the putative stress effect, we employed the different temporal profiles of action of the two stress response systems. Specifically, participants performed the test of schema-based memory either immediately after the stress (or control) manipulation when noradrenergic arousal was high and cortisol levels were hypothesized to not be elevated yet, or 25 min thereafter when cortisol levels peaked and noradrenergic activation had returned to baseline. In the second experiment, we used a pharmacological manipulation of the glucocorticoid and noradrenergic systems to test whether glucocorticoid and/or noradrenergic stimulation is sufficient to affect schema-based learning. Therefore, participants received a placebo, hydrocortisone, the α2-adrenoceptor antagonist yohimbine that leads to increased noradrenergic stimulation, or both drugs before the test session on day 2. In both experiments, participants performed schema-based-learning as well as inference trials after the stress or pharmacological manipulation. On the basis of findings indicating that stress interferes with prefrontal functions (Barsegyan *et al*, 2010; Elzinga and Roelofs, 2005; Schwabe and Wolf, 2009), we hypothesized that stress would impair the use of prior knowledge during learning (ie, schema-based learning). With respect to the role of glucocorticoids and noradrenaline, it was tempting to predict that both systems might act synergistically to disrupt schema-based learning.

MATERIALS AND METHODS

Experiment I: Impact of Stress on Schema-Based Learning

Participants. Ninety-six healthy individuals (48 female; age (M ± SEM): 25.24 ± 0.36 years), without life-time history of any neurological or mental disorders, current pharmacological treatment or medication intake within the four weeks prior to participation, tobacco- or drug-use, over- or underweight, or intake of hormonal contraceptives in females participated in this experiment. Women were not invited for an appointment during their menses. Participants gave written informed consent before taking part in the study and received a compensation of 20€. The local ethics committee approved the study protocol. Five participants had to be excluded from the analyses because of sickness on experimental day 2 or inadequate knowledge of German, leaving a sample of 91 participants (45 females; Supplementary Material).

Experimental design and procedure. We used a two-day between-subjects design with the factors treatment (control *vs* stress) and interval (immediate *vs* delayed learning), resulting in four experimental groups to which participants were randomly assigned ($n = 22$–24/group). All testing took place between 1300 and 1800 hours. On day 1, participants learned a hierarchy of six galaxies. On day 2, ~24 h after day 1, participants underwent a stress or control manipulation and learned both a novel hierarchy of galaxies and a hierarchy containing both elements of the hierarchy learned on day 1 and new elements (related hierarchy) either immediately or 25 min after the stress or control manipulation.

Stress manipulation. Participants in the stress condition were exposed to the Socially Evaluated Cold Pressor Test (SECPT), as described in detail elsewhere (Schwabe *et al*, 2008). In short, participants were instructed to submerge their left hand into ice water (0–2 °C) for 3 min. A cold and non-reinforcing experimenter took notes to prompt a feeling of being evaluated. Furthermore, participants were videotaped for pretended analysis of facial expression. In the control condition, participants were instructed to immerse their hand into warm water (35–37 °C) for 3 min. There was no camera nor were they being evaluated by the experimenter.

To assess the successful stress induction by the SECPT, we took subjective and physiological measurements repeatedly across the experiment. Immediately after the SECPT or control manipulation participants rated on a scale from 0 ('not at all') to 100 ('very much') how stressful, difficult, painful, and unpleasant they experienced the treatment. Blood pressure was measured using a Dinamap system (Critikon, FL, USA) at the beginning of the experiment, during the SECPT/control manipulation, immediately after the SECPT/control manipulation, before and after the task. For salivary cortisol measurements, saliva samples were collected using Salivette collection devices (Sarstedt, Germany) at baseline, immediately after the SECPT/control manipulation, as well as 10 and 30 min after the treatment; in the delayed learning groups also 40 and 60 min after the treatment. Saliva samples were stored at −18 °C. Free concentrations of cortisol were analyzed subsequently by means of a luminescence assay (IBL, Germany; intra- and inter-assay coefficients of variance below 13 percent).

Learning task. To assess schema-based learning processes, we used a modified version of a task previously introduced by Kumaran and colleagues (Kumaran, 2013; Kumaran *et al*, 2012) that consisted of two stages: a schema-acquisition phase (phase I) and a schema-based learning phase (phase II).

Schema-acquisition phase (phase I). Phase I testing was completed on day 1. In this phase, six galaxies were presented with a pre-determined age-hierarchy (A > B > C > D > E > F, with A being the 'oldest' galaxy; Figure 1). Participants were presented with three different types of trials: baseline-, learning- and inference trials. On each trial, participants saw two different galaxies next to each other. During baseline trials, a cross was presented below one of the images and participants were required to indicate under which image the cross was located by button press within 2 s. A blank screen was shown for 0.5 s, followed by feedback (green frame around the correct image) presented for 2 s. Baseline trials served as control trials, probing for attention across the task. During learning trials, participants were presented with two neighboring galaxies (eg, A–B) and asked to indicate which of the galaxies was older. Feedback and timings were exactly the same as during baseline trials. During inference trials, participants were asked to indicate which of two non-neighboring galaxies, separated by an age distance of two (ie, short: eg, A–D), three or four (ie, long: eg, A–E, A–F), was older. Furthermore, participants did not receive corrective feedback in inference trials but had to indicate their certainty on a 4-item scale from 'I'm guessing'

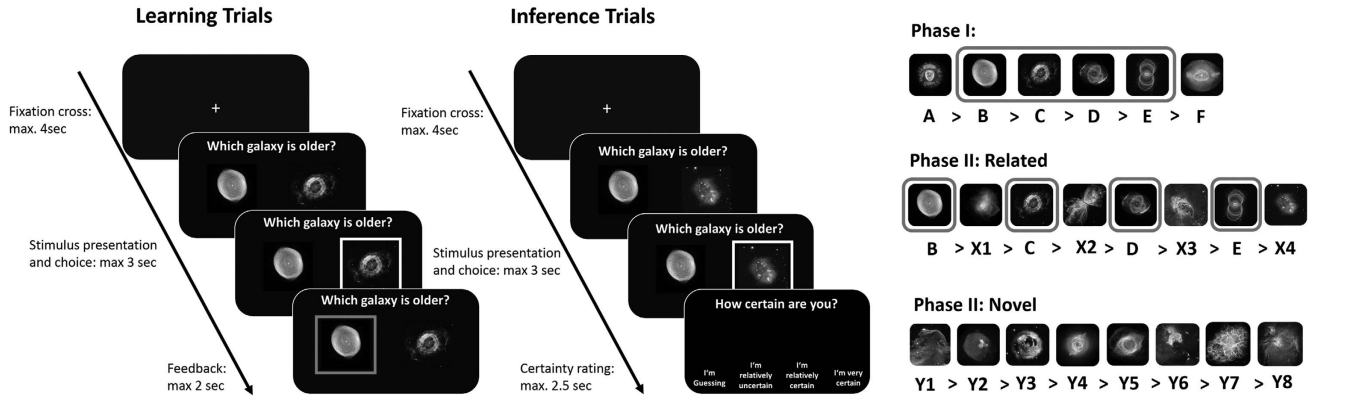

Figure 1 Learning task. In phase I, participants learned an age-hierarchy of six galaxies based on trial-by-trial feedback (learning trials, only neighboring galaxies were presented). In *inference* trials, participants were required to decide which of two non-neighboring items was older, without receiving feedback about the correct answer. In phase II, ~ 24 h after phase I, participants were presented with two hierarchies comprising 8 galaxies each. One of the hierarchies (related) contained four galaxies already learned in phase I, and four completely new galaxies. The other hierarchy (novel) contained only new galaxies. Participants learned the positions of the new items again through trial and error in learning trials. In inference trials, the ability to put new items into the already existing hierarchy was tested, as only non-neighboring items were presented. Schema-based memory is reflected in better learning performance for the related compared with the novel hierarchy in phase II.

to 'I'm very certain' within 2 s. After each trial, participants saw a fixation cross for a duration of 2–4 s.

During phase I, which took about 35 min, participants completed 10 blocks, each comprising 4 baseline-, 10 training and 10 test trials (ie, 240 trials in total). The galaxies presented in each block were pre-determined, the order of galaxies presented was randomized.

Schema-based learning (phase II). Phase II testing was completed on day 2, ~ 24 h after day 1 and either immediately or 25 min after the SECPT or control procedure. In phase II, participants learned two hierarchies, each comprising 8 galaxies. One of the hierarchies contained 4 galaxies from the hierarchy learned during phase I and 4 completely new items (B > X1 > C > X2 > D > X3 > E > X4-related hierarchy; see Figure 1). Participants were explicitly instructed that the hierarchy they learned in phase I was still true in phase II. The second hierarchy contained only new galaxies (Y1 > Y2 > Y3 > Y4 > Y5 > Y6 > Y7 > Y8-novel, Figure 1). Participants again completed baseline, learning, and inference trials for both hierarchies in the same way as in phase I. Importantly for the related hierarchy, learning trials always consisted of one galaxy from phase I and one new item. Inference trials, however, involved only galaxies that had not been shown on day 1.

For each hierarchy, related and novel, participants completed 6 blocks, comprising 2 baseline, 7 learning and 6 inference trials (180 trials in total). Related and novel blocks alternated and a change between them was not specifically indicated. Phase II testing lasted ~ 23 min. The galaxy images used in novel and related hierarchies as well as the hierarchy participants began with were counterbalanced.

Experiment II: Impact of Cortisol and Noradrenaline on Schema-Based Learning

Participants and experimental design. Ninety-six healthy participants were recruited for this experiment (48 female; age (M ± SEM): 24.78 ± 0.39 years, body-mass-index (M ± SEM): 22.79 ± 0.2 kg/m^2). Exclusion criteria for participation were identical to experiment I. In addition, participants were screened for hydrocortisone intolerance, cardiovascular disorders, including low and high blood pressure and diabetes as well as related disorders. Participants gave written informed consent before taking part in the study and received a compensation of 35€ for participation. The ethics committee of the Hamburg Medical Association approved the study protocol. Six participants had to be excluded from the analyses due to insufficient schema acquisition on day 1 (learning trial performance below 40% and/or combined performance of learning and inference trials below chance level), most likely reflecting difficulties in understanding the task instructions, leaving a sample of 90 participants (44 females, Supplementary Material).

Experimental design and procedure. We used a two-day, double-blind, fully-crossed, placebo-controlled, between-subjects design with the factors noradrenaline (placebo *vs* yohimbine) and cortisol (placebo *vs* hydrocortisone), resulting in four experimental groups to which participants were randomly assigned ($n = 22$ or 23 per group). All testing took place between 1300 and 1900 hours. On day 1, participants completed the first phase of the learning task (ie, schema acquisition). On day 2, ~ 24 h after day 1, participants were administered the drugs 45 min before they completed phase II of the task (ie, schema-based learning).

Pharmacological manipulation. Participants received orally either a placebo (12 male, 10 female), 20 mg hydrocortisone (12 male, 11 female), 20 mg yohimbine (10 male, 12 female), a α2-adrenoceptor-antagonist leading to increased noradrenergic stimulation, or both drugs (12 male, 11 female). Timing and dosages of drug administration were chosen in accordance with previous studies (Buchanan and Lovallo, 2001; Schwabe *et al*, 2010a). To validate the action of the drugs, blood pressure and salivary cortisol were measured before medication intake, 45 min after medication intake and after phase II of the learning task (about 70 min after drug intake).

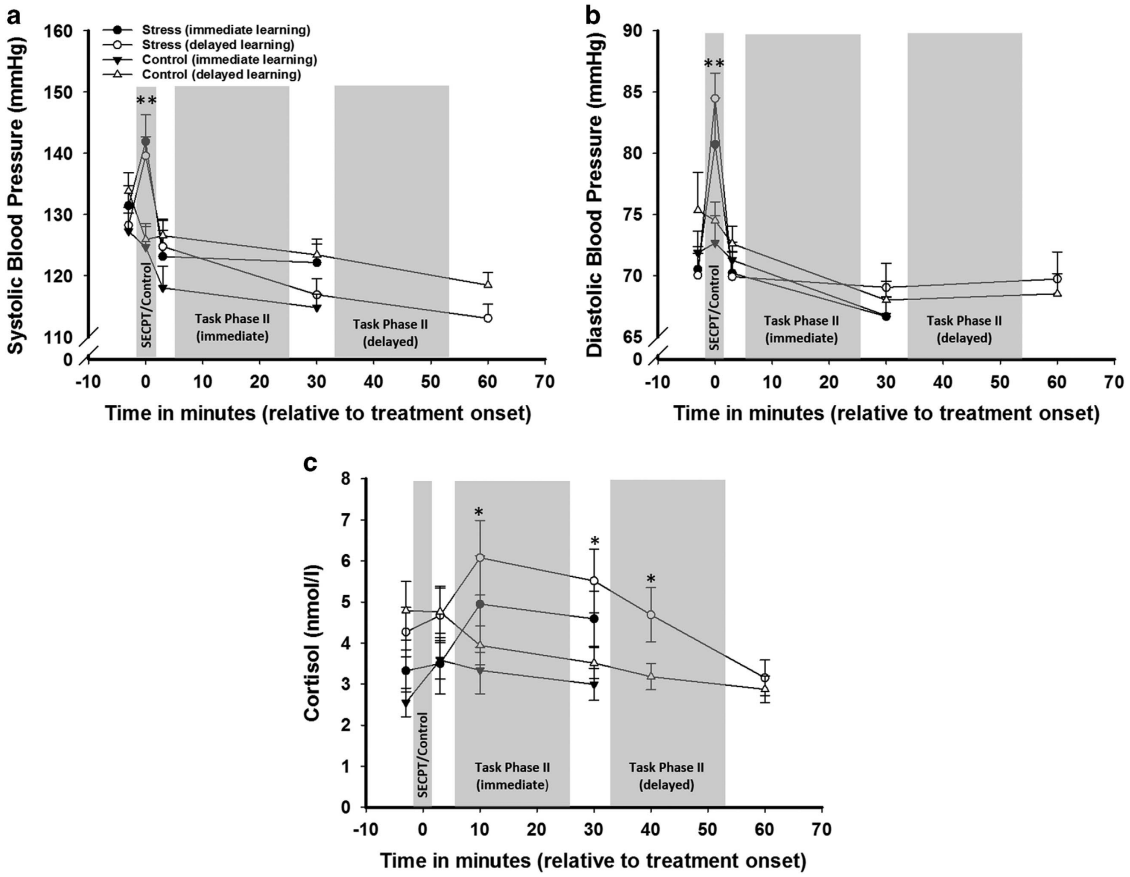

Figure 2 Successful stress induction in experiment I. The exposure to the Socially Evaluated Cold Pressor Test (SECPT) led to a significant increase in (a) systolic blood pressure and (b) diastolic blood pressure, whereas the exposure to the control manipulation did not. (c) Salivary cortisol levels were significantly increased in participants undergoing the SECPT, compared with participants that were subjected to the control manipulation. Data represent means ± SEM $^{**}p<0.001$, $^{*}p<0.05$.

Learning task. The learning task was identical as in experiment I, with two exceptions. First, the number of blocks of phase I was reduced to 15 blocks comprising 180 trials because participants already showed robust learning after 180 trials in experiment I. Second, we presented an explicit hierarchy recall test for the hierarchy learned on day 1 about 45 min after pill intake on day 2 (before phase II started), in order to rule out that potential drug effects are due to a simple memory retrieval deficit.

An overview of the statistical analyses is given in the Supplementary Material.

RESULTS

Experiment I: Stress Impairs Schema-Based Learning

Significant increases in subjective stress level, blood pressure and cortisol confirmed the successful stress induction by the SECPT. There were no significant differences between the immediate and delayed groups (Figure 2 and Supplementary Results).

Phase I: Successful schema acquisition. Participants showed a significant increase in performance in both learning and inference trials (both $p<0.001$, $\eta_p=0.279$; Supplementary Figure S1), with a performance of 81 percent in the learning trials and 77 percent correct responses in the inference trials the end of the acquisition phase, indicating that participants acquired a stable schema. Baseline trial performance was close to perfect (on average 92 percent correct, Supplementary Table S5). Most importantly, experimental groups did not differ in their performance on day 1, ie, they acquired the schema equally well (all effects including the factor experimental group: all $F<2.02$, all $p>0.12$).

Phase II: Schema-based learning under stress. Overall, participants showed increasing performance independent of hierarchy types ($F_{(3.97,\ 344.98)}=30.180$, $p<0.001$, $\eta_p=0.258$, Supplementary Figure S2A). Performance tended to be better in learning trials for the related compared to the novel hierarchy ($F_{(1,\ 87)}=3.519$, $p=0.064$, $\eta_p=0.039$), reflecting the expected schema-effect. Most importantly, however, stress affected the extent to which prior knowledge facilitated performance. Participants in the control groups performed significantly better in related compared with novel trials (71 *vs* 64 percent in the last block; $F_{(1,\ 43)}=7.976$, $p=0.007$, $\eta_p=0.156$) which demonstrates a schema-based memory effect. Stressed participants, however, did not perform better in related compared to novel trials (62 and 60 percent, respectively; $F_{(1,\ 44)}=0.003$, $p=0.959$, $\eta_p<0.001$; marginal hierarchy type × treatment interaction: $F_{(1,\ 87)}=3.806$,

$p = 0.054$, $\eta_p = 0.042$, Figure 3). Importantly, this effect of stress was comparable in the immediate learning and delayed learning groups (hierarchy type × treatment × interval and hierarchy type × block × treatment × interval interactions, both $F \leqslant 1.192$, both $p \geqslant 0.313$). Reaction times did not differ between groups (all $F \leqslant 1.569$, all $p \geqslant 0.184$). In baseline trials, participants reached an average performance of 98 percent. Because inference trials are less dependent on the medial PFC (Heckers *et al*, 2004) and reflect cognitive processes that are beyond the scope of the present experiment, data from these trials are presented in the Supplementary Material.

Experiment II: Hydrocortisone, but not Yohimbine, Disrupts Schema-Based Learning

As expected, hydrocortisone led to an increase in salivary cortisol but not to a change in blood pressure. Conversely, yohimbine increased blood pressure but not salivary cortisol (Figure 4 and Supplementary Results).

Phase I: Successful schema acquisition. Participants showed a significant increase in performance in both learning and inference trials across phase I of the task (both $F > 16.65$, both $p < 0.001$, both $\eta_p > 0.16$, Supplementary Figure S4). At the end of the acquisition phase, participants were correct on about 80 percent of the learning and inference trials, indicating that participants acquired a robust schema. Performance in baseline trials reached 92 percent on average (Supplementary Table S7). Notably, the experimental groups did not differ in schema acquisition (all $F < 1.28$, all $p > 0.14$).

Phase II: Schema-based learning after hydrocortisone and yohimbine intake. The explicit recall of the hierarchy learned on day 1 remained unaffected by yohimbine and hydrocortisone (all $F \leqslant 2.720$, all $p \geqslant 0.103$), thus showing that the drugs did not affect the simple retrieval of the hierarchy learned on day 1. Furthermore, participants showed almost perfect performance on baseline trials and reached 98 percent on average.

Performance in the learning trials increased over time ($F_{(5,\ 430)} = 39.929$, $p < .001$, $\eta_p = 0.317$) and was significantly better for related compared to novel trials, demonstrating the expected schema-effect ($F_{(1,\ 86)} = 18.459$, $p < 0.001$, $\eta_p = 0.177$, Supplementary Figure S5A). Most importantly, however, we obtained a significant interaction of hierarchy type (ie, novel *vs* related) and cortisol ($F_{(1,\ 86)} = 8.335$, $p = 0.005$, $\eta_p = 0.088$, Figure 5), indicating that hydrocortisone modulated participants' ability to utilize previous knowledge during learning. Specifically, both participants in the placebo group and those in the yohimbine only group showed significantly better performance in related than in novel trials (both $t \leqslant 2.616$, both $p \leqslant 0.016$). Participants that had received hydrocortisone, however, whether alone or in combination with yohimbine, did not benefit from the prior knowledge and performed comparably in novel and related trials (both $t \leqslant 0.673$, both $p \geqslant 0.465$). While hydrocortisone modulated schema-based learning, there was no main effect of yohimbine and no hydrocortisone × yohimbine interaction on schema-based learning (all $F \leqslant 1.595$, all $p \geqslant 0.160$). Reaction times did not differ between groups ($F \leqslant 1.221$, $p \geqslant 0.301$). The data from the inference trials are presented and discussed in the Supplementary Material.

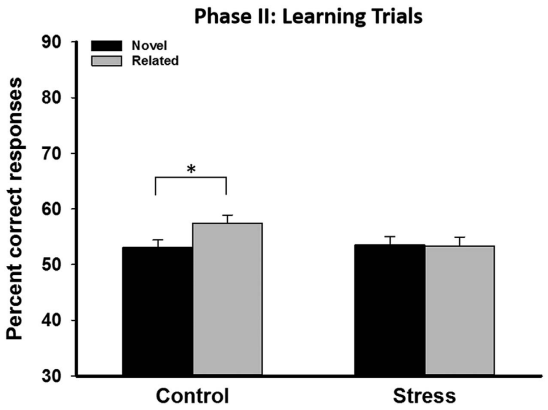

Figure 3 Performance in learning trials in phase II experiment I. Learning trial performance was improved for the related compared to novel hierarchy in control participants, indicating the successful utilization of a pre-existing schema. Stressed participants, however, did not perform better in related *vs* novel trials, indicating a reduced ability to utilize prior knowledge during learning, ie, impaired schema-based memory. Data represent means ± SEM. *$p < 0.05$.

DISCUSSION

Prior knowledge, a 'schema', is known to facilitate the encoding, consolidation, and retrieval of schema-related information (Alba and Hasher, 1983; Ghosh and Gilboa, 2014a; Morris, 2006; Tse *et al*, 2007; van Kesteren *et al*, 2012). In the present experiments, we examined the impact of stress and major stress mediators, ie, cortisol and noradrenaline, on schema-based learning. Our findings show that stress interferes with the ability to utilize a pre-existing schema to aid learning of new information. A similar impairment of schema-based learning was observed after hydrocortisone administration, but not after administration of the α2-adrenoceptor antagonist yohimbine, suggesting that glucocorticoid, but not noradrenergic activity is sufficient to hinder the use of prior knowledge during learning.

Converging lines of evidence from rodent and human neuroimaging studies identified the medial PFC as a key locus for schema-based memory processes (Tse *et al*, 2007; Tse *et al*, 2011; van Kesteren *et al*, 2013). The PFC expresses receptors for glucocorticoids at a particularly high density (McEwen *et al*, 1986) and stressful events may impair prefrontal functions (Arnsten, 2009; Izquierdo *et al*, 2006; Schwabe and Wolf, 2009). Accordingly, it appears reasonable to assume that the observed stress-induced deficit in schema-based learning was due to an impairing effect on the medial PFC, reducing its ability to detect the congruency of new information with an existing schema. Interestingly, the impairing effect of stress on schema-based learning was observed both when learning took place immediately after the stressful event and when it took place about 25 min later. Given that noradrenaline levels were most likely still increased in the immediate learning condition but not in the delayed learning condition, whereas cortisol levels were already rising during learning in the immediate learning condition and reached peak levels shortly before or during

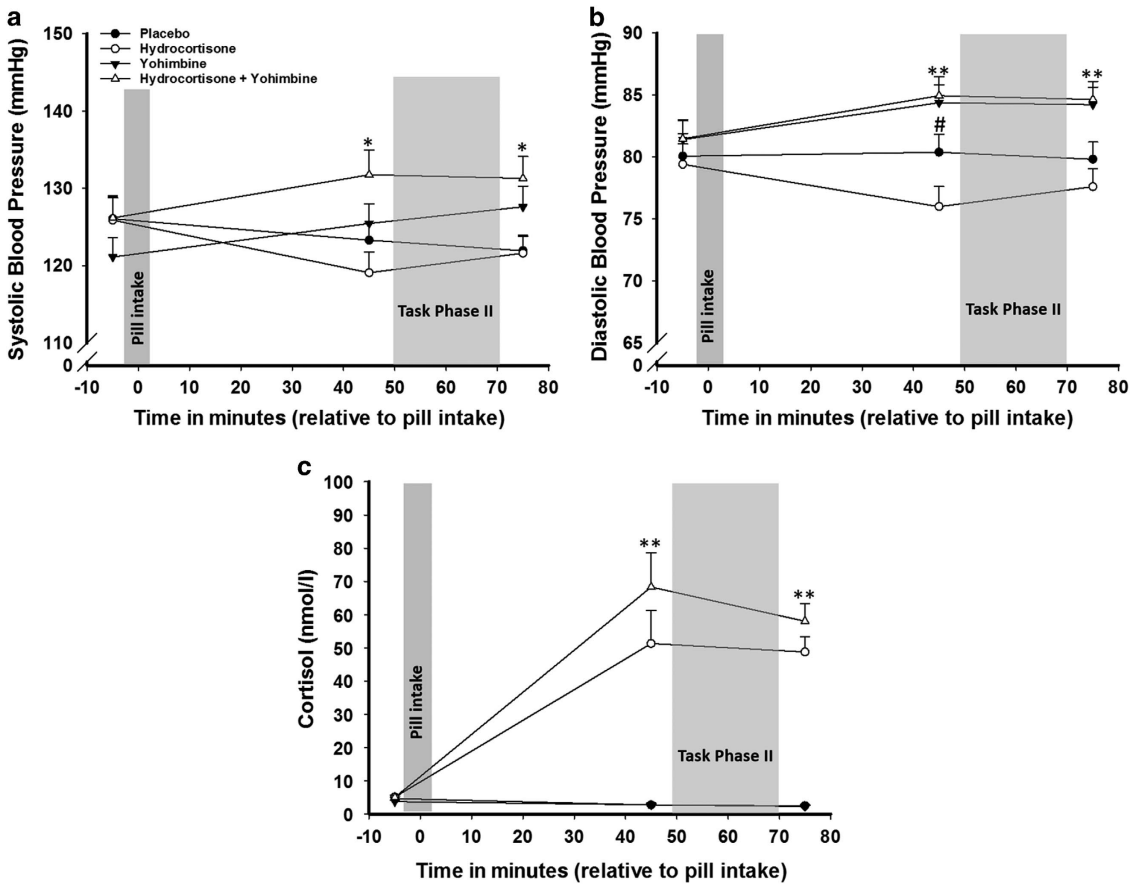

Figure 4 Effectiveness of the pharmacological manipulation in experiment II. Participants that received solely yohimbine or yohimbine in combination with hydrocortisone showed a significant increase in (a) systolic and (b) diastolic blood pressure, compared to participants that received a placebo or hydrocortisone only. 45 min after pill intake the placebo group showed a strong trend for a higher diastolic blood pressure, compared to the hydrocortisone only group. (c) Salivary cortisol levels were significantly increased in participants that received hydrocortisone only or in combination with yohimbine, compared with participants that received yohimbine only or a placebo. Data represent means ± SEM $^{**}p<0.001$, $^{*}p<0.05$, $^{\#}p<0.10$.

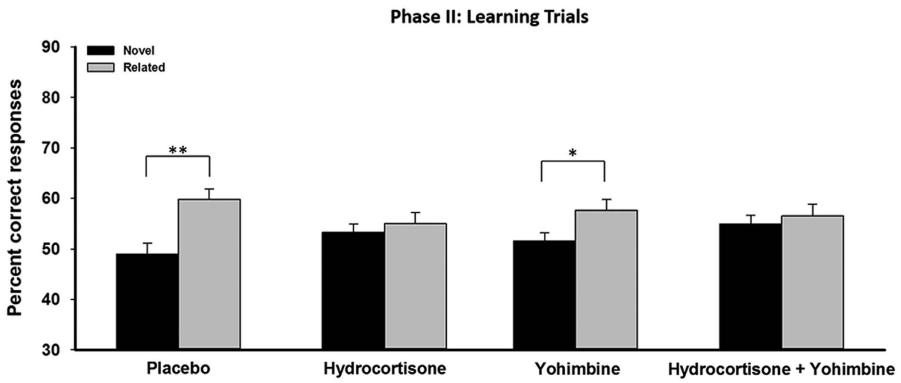

Figure 5 Performance in learning trials in phase II in experiment II. Placebo and yohimbine only groups showed a significant schema-based memory effect, indicated by better performance for related compared to novel hierarchies. This schema-based memory effect was absent in participants that received hydrocortisone alone or in combination. Data represent means ± SEM. $^{*}p<0.05$.

learning in the delayed condition, this finding suggests that the stress-induced deficit in schema-based learning was rather owing to the action of glucocorticoids than to noradrenaline.

The idea that glucocorticoids have a major role in the modulation of schema-based learning is supported by our pharmacological experiment. This experiment showed that a moderate dose of hydrocortisone led to an impairment in the use of prior knowledge that resembled the deficit observed after stress, suggesting that a rise in glucocorticoid levels is sufficient to impair schema-based learning processes. Previous studies indicated that prefrontal structures that are critically involved in schema-based learning are a major target of glucocorticoids (Liu and Aghajanian, 2008; Meaney *et al*, 1985). Thus, glucocorticoid-induced changes in prefrontal areas are a likely mechanism underlying the

impairment of schema-based learning after hydrocortisone intake. The classical mode of action of glucocorticoids involves intracellular glucocorticoid and mineralocorticoid receptors (GR and MR, respectively) that mediate relatively slow genomic changes by transactivation and transrepression of responsive genes (De Kloet *et al*, 1986; Joels *et al*, 2012). More recent research, however, indicates that there are also membrane-bound MR and GR that initiate rapid, non-genomic changes in neural excitability, cognition and behavior (Barsegyan *et al*, 2010; Joels *et al*, 2012; Roozendaal *et al*, 2010). Given the time-scale of the observed glucocorticoid effects on schema-based memory, we argue that these effects were most likely mediated by membrane-bound receptors. Future studies using selective MR and GR antagonists, possibly in combination with glucocorticoids conjugated to a membrane-impermeable bovine serum albumin molecule, are required to elucidate the specific role of these receptor types in the impact of glucocorticoids on the use of prior knowledge during learning.

Although our data show that glucocorticoid elevations are sufficient to impair schema-based learning, no such effect was found after increased noradrenergic stimulation by yohimbine. Previous studies showed that noradrenaline may affect PFC-dependent cognitive functions (Ramos and Arnsten, 2007) and that noradrenaline may interact with glucocorticoids in influencing prefrontal functions (Barsegyan *et al*, 2010; Schwabe and Wolf, 2010b, 2012c). However, we also obtained no evidence for an interaction of glucocorticoids and noradrenaline, neither in our pharmacological experiment nor in the stress experiment, in which a more pronounced effect in the immediate compared to the delayed condition may have been indicative for such an interaction. One potential explanation for the absence of an effect of noradrenaline may be seen in the specific cognitive function tested. Schema-based memory is centered on the mPFC interacting with a large network of other regions (van Kesteren *et al*, 2012) and whether noradrenaline may affect schema-based memory processes has not been tested before. Moreover, while noradrenergic activation appears not to be sufficient for the modulation of schema-based memory, it may well be necessary for stress and glucocorticoids to exert their detrimental effects on the use of prior knowledge during learning. In the stress experiment, noradrenaline levels were initially raised in all stressed participants and the pill intake and task performance was most likely associated with a certain level of arousal in the pharmacological experiment. Future studies may combine a stress or glucocorticoid manipulation with β-adrenergic receptor blockade to examine whether noradrenaline is necessary in enabling the impairing effects of stress and glucocorticoids on schema-based memory.

Finally, it is important to note that the task we used here was rather difficult and, in line with previous findings (Kumaran, 2013), performance was far from perfect in this task, even after initial learning on day 1. Thus, one might question, whether participants did acquire a robust schema. However, the fact that, in controls, performance was significantly better in related compared with novel trials suggests that they could benefit from their prior knowledge. Moreover, inference trial performance depended significantly on the distance between the hierarchy elements (Supplementary Material), which strongly suggests that participants had indeed constructed an associative structure (ie, a schema).

To conclude, whereas stress effects on memory formation or retrieval are very well documented (Diamond *et al*, 2007; Joels *et al*, 2011; Roozendaal *et al*, 2009; Schwabe *et al*, 2012a), we examined here the integration of new information and prior knowledge, ie, schema-based memory. Our findings go beyond the known effects of stress on memory and show that stress reduces the ability to benefit from prior knowledge during learning. Importantly, the present effects of stress and major stress mediators could be separated from the classical effects on memory formation and memory retrieval. Specifically, both the encoding of the novel hierarchy and (in experiment 2) the recall of the hierarchy learned on day 1 remained unaffected by our experimental manipulation on day 2 (Supplementary Discussion), thus making simple stress or glucocorticoid effects on encoding or retrieval unlikely. Instead, we argue that stress and glucocorticoids affected primarily the schema-congruency detection by the medial PFC, that is thought to facilitate the integration of schema-related information into neocortical memory representations (van Kesteren *et al*, 2012). On the basis of our finding that the administration of glucocorticoids was sufficient to produce a deficit in the ability to use prior knowledge during learning that strongly resembled the effect observed after stress, it is tempting to speculate that glucocorticoids play a key role in the stress-induced impairment of schema-based memory. The present results may have important implications for educational settings, in which the ability to utilize prior knowledge during learning is essential and in which stressful events are very common.

FUNDING AND DISCLOSURE

The authors declare no conflict of interest.

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Impact of stress and glucocorticoids on schema-based learning

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Supplemental Methods and Materials

Supplemental Results

Supplemental Discussion

Supplemental References

Supplemental Tables S1 to S7

Supplemental Figures S1 to S6

Supplemental Methods and Materials

Experiment I: Impact of stress on schema-based learning and inference

The final gender distributions in the groups were as follows: control immediate learning: 10 female, 12 male; control delayed learning: 12 female, 11 male; stress immediate learning: 11 females, 11 males; stress delayed learning: 12 females, 12 males.

Schema-based learning and inference

After completing the task on day 1 and day 2, participants were asked to complete an explicit hierarchy test. They were given a printout of the all galaxies belonging to the respective hierarchy and were asked to explicitly indicate the position of each galaxy in the hierarchy. On day 2, participants received different printouts for the related and novel hierarchies.

Control variables

In order to control for influences of chronic stress, participants completed the Trier Inventory for Chronic Stress after task completion on day 1 (TICS; (Schulz & Schlotz, 1999).

Experiment II: Impact of cortisol and noradrenaline on schema-based learning

The final gender distributions in the groups were as follows: placebo: 12 male, 10 female; hydrocortisone: 12 male, 11 female; yohimbine: 10 male, 12 female, and hydrocortisone+yohimbine: 12 male, 11 female.

Schema-based learning and inference

After completing the task on day 1, and on day 2, 45 min after drug intake and after task completion, participants were asked to complete an explicit hierarchy test. On day 2, after drug intake, the explicit hierarchy test of day 1 was repeated. After task completion, participants received different printouts for the related and novel hierarchies, which was the same as described for experiment I.

Control variables

In order to control for influences of depressive mood, chronic stress or state and trait anxiety, participants completed the Beck depression inventory (BDI; (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), the Trier Inventory for Chronic Stress (TICS; (Schulz & Schlotz, 1999) and the State-Trait Anxiety Inventory (STAI; (Spielberger, Sydeman, & Maruish, 1994).

Statistical analyses

Physiological and subjective parameters were analyzed using mixed-design ANOVAs with time-point of measurement as within-subject factor and treatment (stress vs. control) and interval (immediate learning vs. delayed learning; exp. I) or noradrenaline (placebo vs. noradrenaline) and cortisol (placebo vs cortisol; exp. II) as between-subjects factors. Phase I task performance was analyzed using mixed design ANOVAs with the within-subject factor block (10 or 15 levels) and the between-subject factor experimental treatment. Phase II task performance for training trials was analyzed using mixed design ANOVAs with the within-subjects factors hierarchy type (novel vs. related) and block (6 levels) and the between-subjects factors treatment and interval (exp. I) or cortisol and noradrenaline (exp. II). In the analyses of test trial performance, we added the factor difficulty (i.e. distance between the items in the hierarchy: short vs. long). Significant main or interaction effects were pursued by the appropriate post-hoc tests. All indicated p-values are two-tailed. All analyses were performed with the software SPSS (version 22, IBM).

Supplemental Results

Experiment I

Experimental groups did not differ in their chronic stress level or in their cortisol concentration before schema acquisition on day 1 (both $F \leq 1.87$, and both $p \geq .14$; supplemental Table S1).

Indicators of successful stress induction

Subjective and physiological parameters on day 2 confirmed the successful stress induction by the SECPT, both in the immediate and delayed learning groups. Participants who underwent the SECPT experienced the treatment as significantly more unpleasant, stressful, difficult and painful compared to participants who underwent the control manipulation (all $p < .001$; supplemental Table S2). At the physiological level, participants exposed to the SECPT showed significant increases in systolic and diastolic blood pressure, while participants in the control groups did not (both time×treatment interactions: $F > 12$, $p < .001$, $\eta_p > .14$). Whereas groups did not differ in blood pressure at baseline, before or after behavioral testing (all $p > .08$), during the treatment both systolic and diastolic blood pressure were significantly higher in participants exposed to the SECPT than in those exposed to the control manipulation (all $t \geq 3.803$, all

p<.001, Figure 2A and B). Finally, exposure to the SECPT also led to an increase in salivary cortisol (time×treatment: $F_{(1.98, 158.58)}$=7.387, *p*=.001, η_p=.085). While groups did not differ in their cortisol concentrations at baseline (t_{83}=.202, *p*=.840), cortisol levels were significantly higher in the stress compared to the control groups 10 and 30 minutes after the treatment (both t>2.33, both *p*<.05). In the delayed learning groups, cortisol levels were still significantly higher in stressed compared to control participants 40 minutes after the treatment ($t_{31.47}$=2.054, *p*=.048; at 60min post-treatment: t_{42}=.505, *p*=.616, Figure 2C). Importantly, the subjective, blood pressure and cortisol responses to the stressor were comparable in the immediate and delayed learning groups (all F<1.6, all *p*>.190).

Schema-based learning (learning trials)

Participants made on average only two mistakes in the explicit hierarchy test after the acquisition phase, hence participants acquired a stable schema (see supplemental table S4).

Schema-based inference (inference trials)

Overall, performance increased in inference trials across task phase II ($F_{(1.85, 160.62)}$=42.598, *p*< .001, η_p=.329). Although there was no overall difference in performance in novel vs. related inference trials, there was a trend for better inference performance in related trials in controls compared to stressed participants (t_{89} =-1.760, p=.082; hierarchy type × treatment $F_{(1, 87)}$=2.346, *p*=.129, η_p=.026). The performance level reached, however, was dependent on the distance of the items presented, i.e. the difficulty of the inference trials, with performance in the long distance trials being overall better than in the short distance trials ($F_{(1, 87)}$=43.629, *p*<.001, η_p=.334, supplemental figure S2B). Short distance trials were considered to be more difficult, as it was more challenging to identify the exact distance between items rather than to locate items at the opposite ends of a hierarchy. Furthermore, stress had a significant effect on how the difficulty of the required inference affected performance (difficulty × treatment: $F_{(1, 87)}$=6.747, *p*=.011, η_p=.072). Participants in the control condition showed better performance in the long-distance trials compared to stressed participants (75 vs 68 percent respectively, t_{89} =-1.981, *p*=.051, supplemental figure S3), independent of hierarchy type. No such effect was observed for short distance trials (t_{89} =.357, *p*=.722). The questionnaire probing participants' explicit knowledge at the end of day 2, did not reveal any effect of hierarchy type or treatment (all F < 2.7, and all p ≥ .127; see supplemental table S4).

Experiment II

Manipulation check

On day 1, there were no group differences in blood pressure or cortisol before schema acquisition (all F<.990 and p≥.404, supplemental table S3). Similarly, groups did not differ in these parameters before pill intake on day 2 (all F<1.999 and all p≥.161, figure 4). However, as expected, there was a significant increase in systolic and diastolic blood pressure over time in participants receiving yohimbine (time×noradrenaline: both F>8.44, both p<.001, both η_p>.085). Participants in the yohimbine groups showed increased systolic and diastolic blood pressure 45 minutes after medication intake and after completion of the task (all p≤.007). Hydrocortisone intake had no impact on autonomic arousal (both F≤.854, both p≥.428). Salivary cortisol concentrations, however, increased markedly in participants after hydrocortisone intake (time×cortisol: $F_{(1.39, 119.64)}$=52.589, p<.001, η_p=.379) but not after yohimbine intake ($F_{(1.39, 119.64)}$=1.122, p=.312, η_p=.013, figure 4). Importantly, there were no interaction effects of yohimbine and hydrocortisone on any subjective or physiological measures (all F<2.035, all p>.134).

Schema-based learning (learning trials)

In the explicit test of hierarchy knowledge given at the end of day 1, participants made on average only two mistakes, hence participants acquired the schema well (see supplemental table S5).

Schema-based inference (inference trials)

The explicit recall of the hierarchy learned on day 1 remained unaffected by yohimbine and hydrocortisone (all F ≤ 2.720, all p ≥ .103), thus showing that the drugs did not affect the simple retrieval of the hierarchy learned on day 1 (supplemental table S5). Overall, inference performance increased across phase II of the task ($F_{(1.77, 152.14)}$=37.488, p<.001, η_p=.304). Performance was enhanced in related compared to novel trials (69 vs. 64 percent correct; $F_{(1, 86)}$=5.570, p=.021, η_p=.061). Moreover, performance was better in long distance compared to short-distance trials (70 vs. 62 percent correct: $F_{(1, 86)}$=45.683, p<.001, η_p=.347, supplemental figure S5B). Interestingly, a schema-based inference effect was observed in participants that had received yohimbine (t_{44}=-2.247, p=.030) but not in those that were not administered yohimbine (t_{44}=-1.064, p=.293). Specifically, in long-distance trials for the related hierarchy, participants in the yohimbine groups tended to perform better than participants that had not been administered yohimbine ($t_{81.09}$=-1.683, p=.096; hierarchy type × difficulty × noradrenaline:

$F_{(1, 86)}$=4.069, p=.047, η_p=.045). Hydrocortisone, tended to impair inference performance specifically for long-distance related trails at the end of the inference session (hierarchy type × difficulty × block × cortisol: $F_{(2, 172)}$ = 3.666, p=.028, η_p=.041), the respective post-hoc tests, however, did not reach statistical significance (all p>.075, supplemental figure S6). After phase II, participants completed a questionnaire to test for explicit knowledge in the novel as well as related hierarchy. There was a significant main effect for hierarchy type ($F_{(1, 86)}$ = 10.416, p = .002, η_p = .108), indicating that performance across all groups was better in the related compared to the novel hierarchy questionnaire. Performance in this explicit knowledge questionnaire, however, was not influenced by hydrocortisone or yohimbine (both F < 2.35, both p ≥ .130, supplemental table S5).

Control variables

Groups did not differ in chronic stress levels, depressive symptoms or state anxiety (all F≤.547, all p≥.651, supplemental table S3). Trait anxiety, however, was slightly higher in the hydrocortisone group, compared to the other three groups (Bonferroni corrected post-hoc tests, all p<.05). In order to rule out that differences in trait anxiety could account for our findings, we ran all relevant analyses again, including trait anxiety as a covariate. Including trait anxiety as a covariate, however, left our pattern of results largely unchanged. Most importantly, the critical interaction between hierarchy type and hydrocortisone remained significant ($F_{(1, 84)}$=7.706, p=.007, η_p=.084).

Supplemental Discussion

Schema-dependent learning and schema-detection have been linked to medial prefrontal cortex activity (Morris, 2006; van Kesteren et al., 2012), while it was shown that the hippocampus is less involved in these processes (Tse et al., 2007; Tse et al., 2011; van Kesteren et al., 2012). However, initial learning during which a schema is built as well as learning of novel items may be hippocampus-dependent, and may thus be sensitive to stress effects. Pre-learning stress effects have led to unclear results, with studies showing positive as well as negative effects, or no effects at all (Kirschbaum et al., 1996; Sandi & Pinelo-Nava, 2007; Schwabe, Bohringer, et al., 2008; Smeets et al., 2007; Zoladz et al., 2011). The findings that stress did not affect the acquisition of the novel hierarchy items may however also be explained by a switch in the predominating memory system after stress. Previous studies have shown that stress induces a shift from hippocampus-based ‘cognitive’ memory towards rather rigid striatal memory (Kim et al., 2001; Schwabe et al., 2007; Schwabe, Schächinger, et al.,

2010; Schwabe & Wolf, 2012, 2013). This stress-induced shift, however, appeared to rescue performance after stress, so that learning performance remained intact despite stress (Schwabe, Schächinger, et al., 2010; Schwabe, Tegenthoff, Hoffken, & Wolf, 2013).

In addition to schema-based learning, which was the major focus of the present study, we also assessed schema-based inference processes. Inference processes differ from schema-based learning processes both at the functional and neural level. For instance, compared to schema-based learning processes, inference processes rely on a stronger activation of the hippocampus and lateral prefrontal areas (Heckers, Zalesak, Weiss, Ditman, & Titone, 2004; Kumaran et al., 2012; Preston & Eichenbaum, 2013). While these areas may be modulated by stress and stress hormones as well (Arnsten, 2009; Kim & Diamond, 2002; Kim et al., 2006), the influence of stress, glucocorticoids, and noradrenaline on schema-based inference was less clear than the effect on schema-based learning. In our first experiment, we obtained a (non-significant) trend indicating that stress may reduce schema-based inference processes. Our pharmacological study suggested that glucocorticoids might impair schema-based inference, whereas noradrenaline may even facilitate schema-based inference processes. Although there is some evidence that noradrenaline facilitates some cognitive functions (Sara, 2009) and that glucocorticoid and noradrenaline effects on cognition may differ (Margittai et al., 2016; Schonfeld, Ackermann, & Schwabe, 2014) whether there are indeed opposite effects of noradrenaline and glucocorticoids on inference processes remains to be further tested by studies directed specifically at this cognitive process.

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Supplemental Tables

Supplemental Table S1: Measures of cortisol and chronic stress levels on day 1**.**

Day 1

Variable	Stress	Control
Cortisol (nmol/l)	4.14 (0.55)	4.34 (0.55)
TICS Screening Score[1]	17.68 (1.48)	18.47 (1.94)

Data represent mean (standard error).
[1] A total of 69 participants completed the TICS Questionnaire.

Supplemental Table S2: Measures of subjective mood in both groups on day 2

Day 2

Variable	Stress	Control
Ratings of stress/control procedure		
Difficult	**56.30 (4.7)****	1.78 (0.86)
Unpleasant	**59.35 (3.78)****	6.22 (2.72)
Stressful	**47.17 (4.44)****	1.33 (0.68)
Painful	**57.83 (4.08)****	0.00 (0.00)

Data represent mean (standard error).
** $p < .001$, * $p < .05$ compared to control group.

Supplemental Table S3: Measures of control variables and physiological parameters on day 1

Variable	Placebo	Hydrocortisone	Yohimbine	Hydrocortisone + Yohimbine
Systolic blood pressure (mmHg)	121.59 (2.5)	123.2 (2.43)	122.64 (2.72)	125.22 (2.93)
Diastolic blood pressure (mmHg)	80.57 (1.72)	80.2 (1.95)	82.41 (1.55)	82.13 (1.85)
Cortisol (nmol/l)	4.86 (0.51)	5.27 (1.12)	3.89 (0.43)	5.92 (1.08)
Control Variables				
TICS Screening Score	13.55 (1.88)	14.87 (1.51)	14.91 (2.15)	14.78 (1.41)
BDI	4.64 (0.84)	5.83 (0.72)	5.27 (0.88)	5.04 (0.73)
STAI (State)	34.10 (1.55)	35.67 (1.06)	34.57 (1.27)	33.43 (1.20)
STAI (Trait)	35.45 (1.8)	**38.78 (1.66)***	35.14 (1.78)	32.43 (1.07)

Data represent mean (standard error). * $p < .05$

Supplemental Table S4: Mean performance in Galaxy Questionnaires on day1 and day2

Variable	Stress (immediate learning)	Stress (delayed earning)	Control (immediate learning)	Control (delayed learning)
Day 1				
Galaxy Questionnaire	2.50 (.46)	2.83 (.47)	1.41 (.43)	1.78 (.47)
Day 2				
Galaxy Questionnaire (Novel)	4.23 (.65)	4.75 (.54)	4.18 (.65)	4.91 (.60)
Galaxy Questionnaire (Related)	4.64 (.62)	5.33 (.55)	4.00 (.71)	5.13 (.55)

Data represent mean (standard error).

Supplemental Table S5: Mean performance in baseline trials on day 1 and day 2

Variable	Stress (immediate learning)	Stress (delayed learning)	Control (immediate learning)	Control (delayed learning)
Day 1				
Baseline Trials (%)	89.89 (3.08)	93.44 (1.15)	94.09 (1.48)	90.65 (2.16)
Day 2				
Baseline Trials (%)	95.27 (2.38)	97.74 (.83)	98.48 (.584)	99.28 (.43)

Supplemental Table S6: Mean performance in Galaxy Questionnaires on day1 and day2

Variable	Placebo	Hydrocortisone	Yohimbine	Hydrocortisone + Yohimbine
Day 1				
Galaxy Questionnaire	2.68 (.42)	2.43 (.46)	1.68 (.39)	2.27 (.44)
Day 2				
Galaxy Questionnaire (same as day 1)	2.73 (.42)	2.13 (.41)	2.55 (.78)	2.17 (.47)
Galaxy Questionnaire (Novel)	5.91 (.51)	4.70 (.55)	5.50 (.35)	5.65 (.46)
Galaxy Questionnaire (Related)	4.27 (.68)	4.83 (.49)	4.45 (.54)	4.57 (.57)

Data represent mean (standard error).

Supplemental Table S7: Mean performance in baseline trials on day 1 and day 2

Variable	Placebo	Hydrocortisone	Yohimbine	Hydrocortisone + Yohimbine
Day 1				
Baseline Trials (%)	90.30 (1.75)	92.61 (1.68)	95.00 (1.24)	91.88 (1.66)
Day 2				
Baseline Trials (%)	98.86 (.62)	97.64 (.78)	99.81 (.19)	97.46 (.63)

Data represent mean (standard error).

Supplemental Figure 1

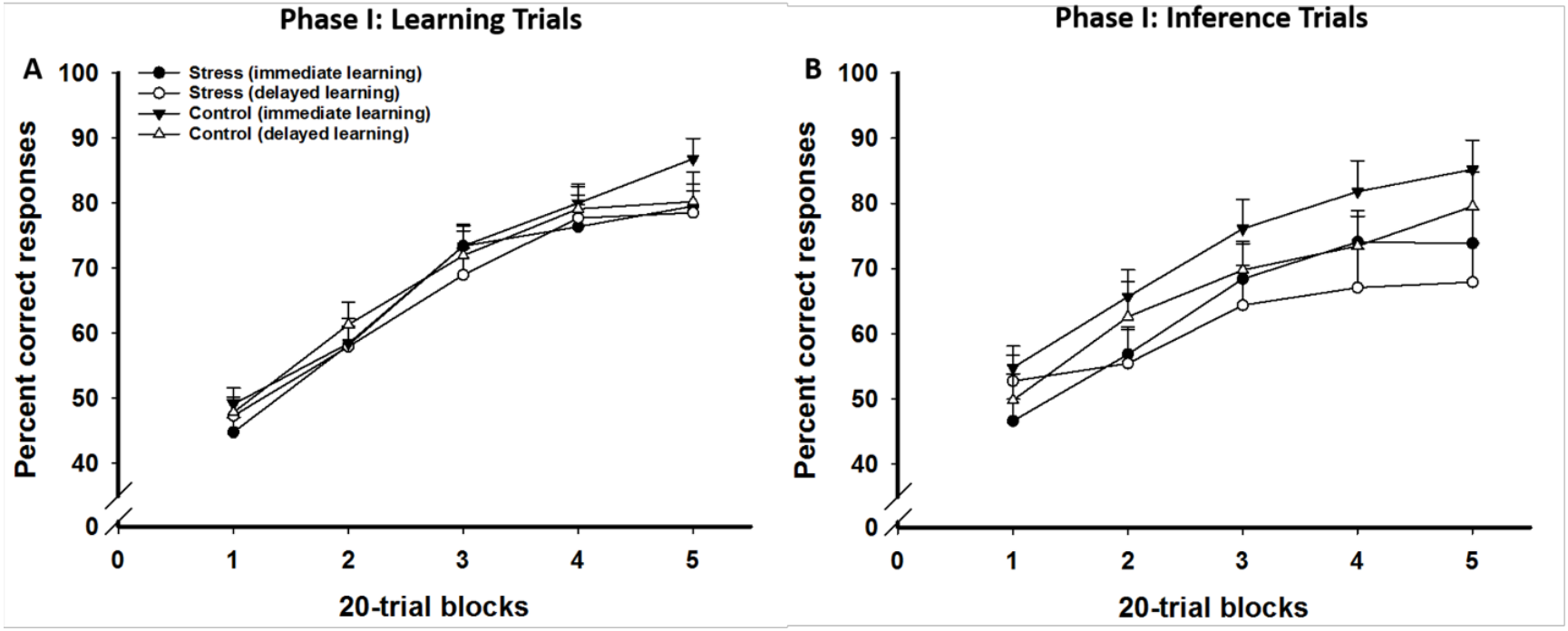

Figure S1. Learning and Inference trial performance in phase I of experiment I. Participants showed increasing performance in **A.** learning and **B.** inference trials across phase I, without any differences between the four experimental groups. Data represent means ± s.e.m.

Supplemental Figure 2

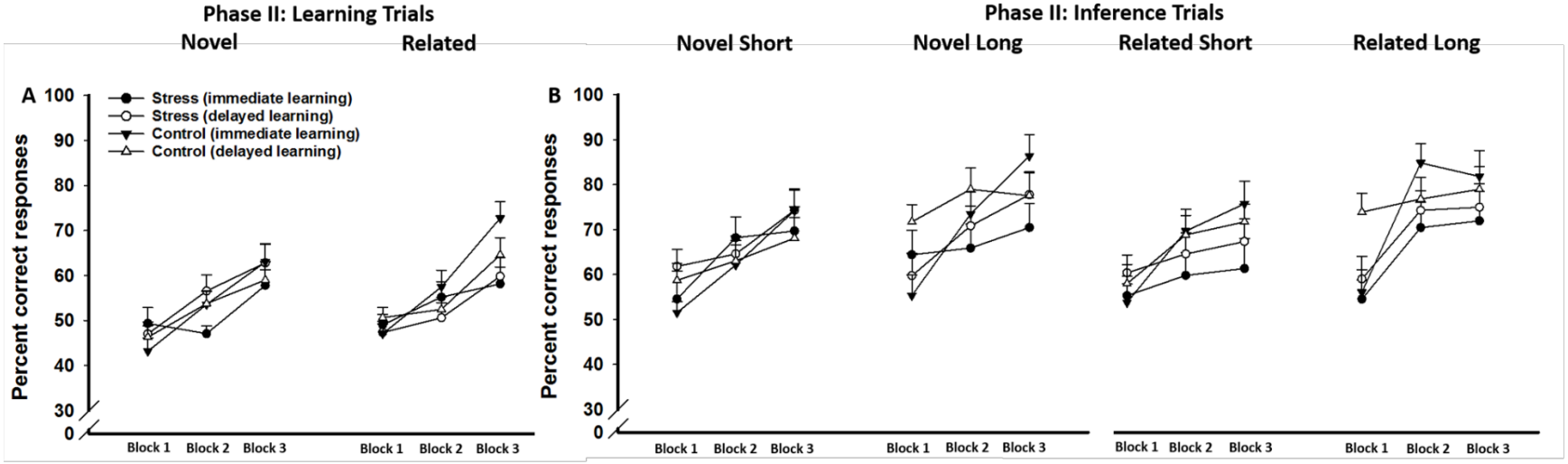

Figure S2. Learning and inference trial performance in phase II in experiment I.**A**. Performance in novel and related learning trials increased over the course of the task, with the expected schema-effect in control participants but not in stressed participants. Figure 3 in the main text shows the overall performance, indicating that participants in the control group showed improved performance in related hierarchies compared to novel hierarchies, while this result is not obtained in stressed participants. **B**. In inference trials, performance increased across the task. Difficulty, i.e. the distance in the age hierarchy between the galaxies presented, had an impact on performance in control as well as stressed participants. Data represent means ± s.e.m.

Supplemental Figure 3

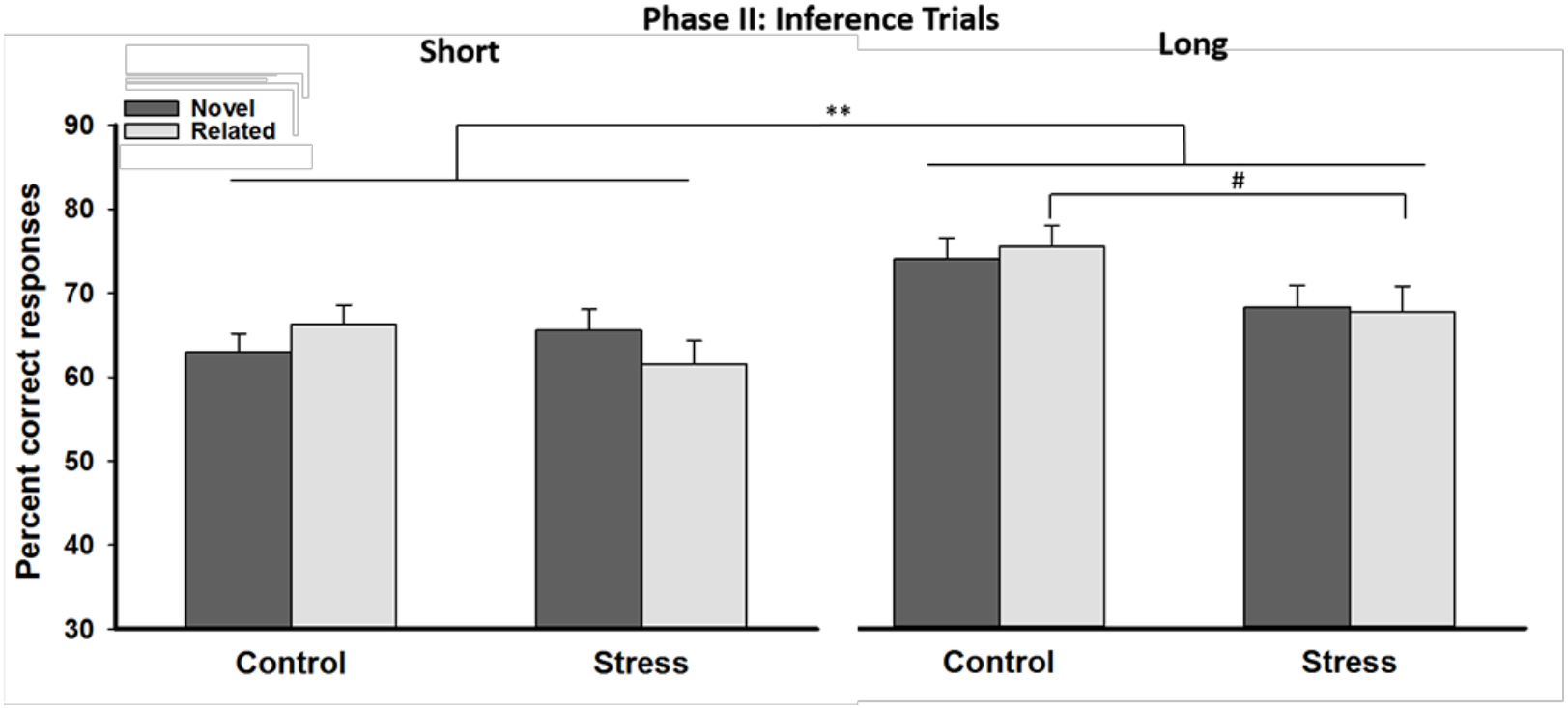

Figure S3. Inference trial performance in phase II in experiment I. Performance in inference trials was dependent on the distance between galaxies in the age hierarchy. Control as well as stressed participants showed improved performance in long distance trials compared to short distance trials. Control participants, but not stressed participants, showed a trend for better performance in related compared to novel trials. Data represent means ± s.e.m. ** $p < .001$, * $p < .05$, # < .10

Supplemental Figure 4

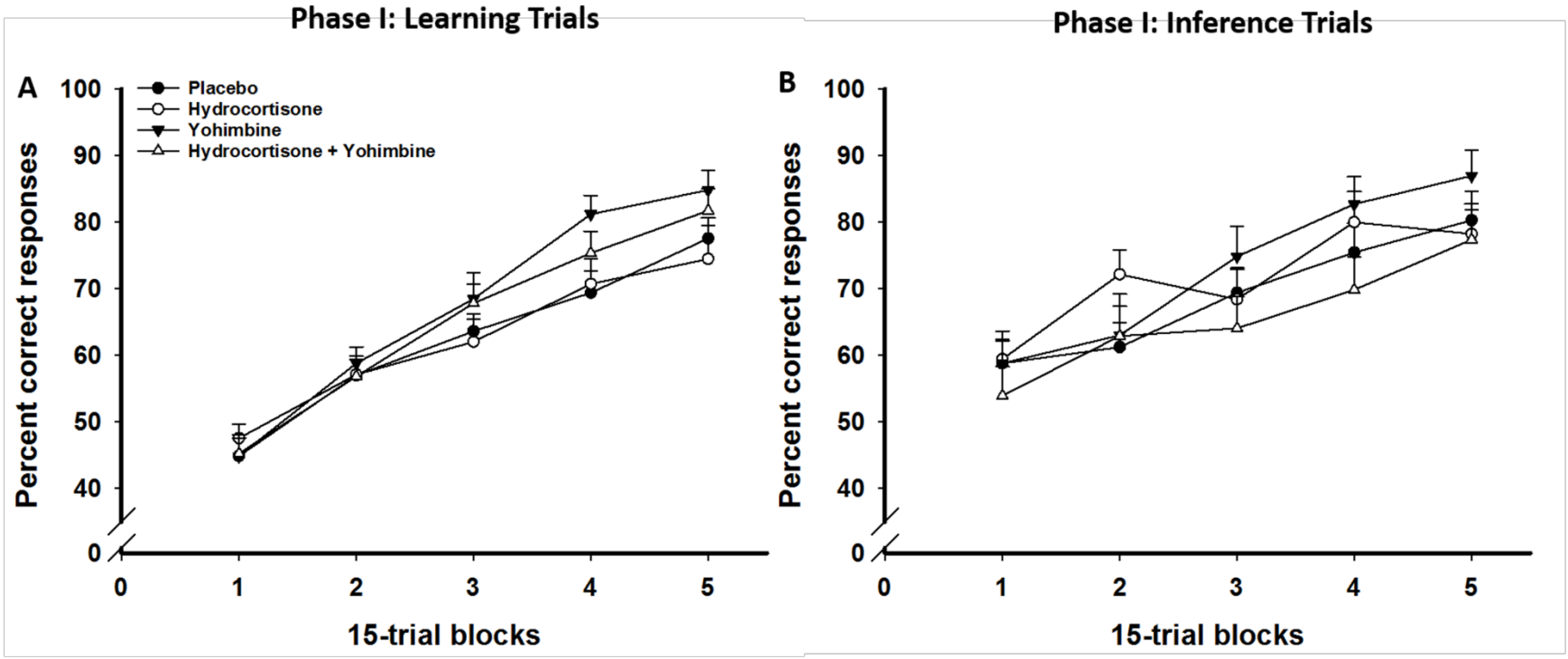

Figure S4. Learning and inference trial performance in phase I in experiment II. Participants showed increasing performance in **A.** learning and **B.** inference trials across phase I, without any differences between the four experimental groups. Data represent means ± s.e.m.

Supplemental Figure 5

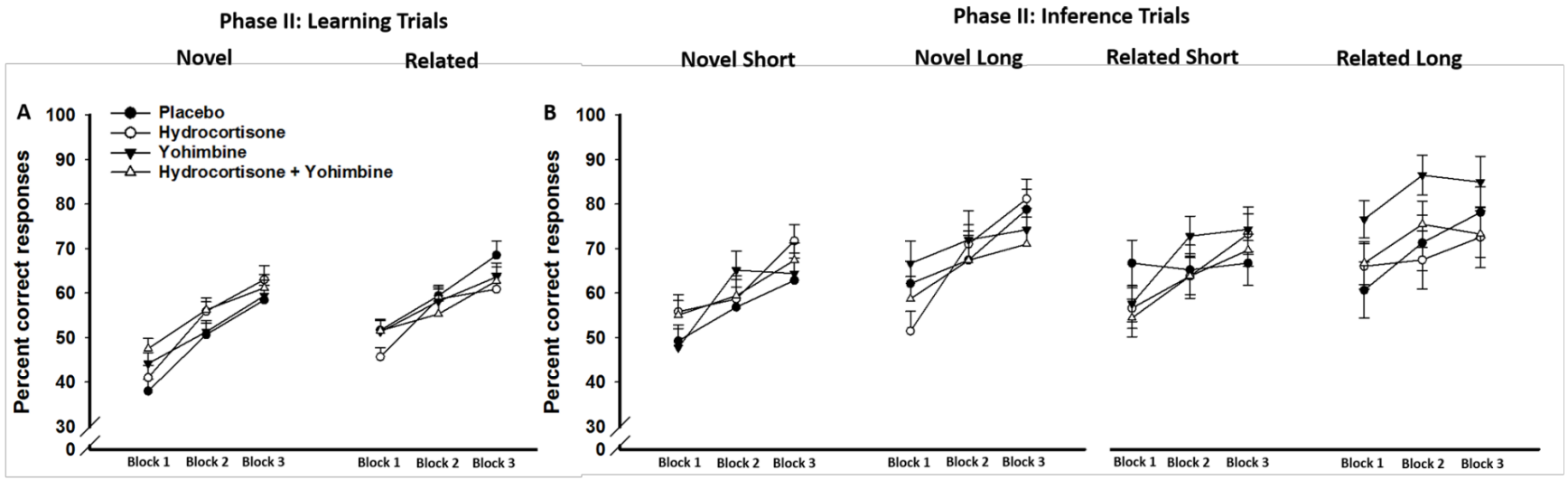

Figure S4. Learning and inference trial performance in phase II in experiment II. **A.** Performance in novel and related learning trials increased over the course of the task, with higher performance in related compared to novel hierarchies in participants that received a placebo or yohimbine only. This finding is shown for the overall performance in all groups in Figure 5 in the main text, highlighting the improved performance in related, compared to novel trials in the yohimbine and placebo groups. **B.** In inference trials, performance also increased across the task, although differently in groups. Participants that received yohimbine showed a trend for a schema-effect in the long distance trials. Data represent means ± s.e.m.

Supplemental Figure 6

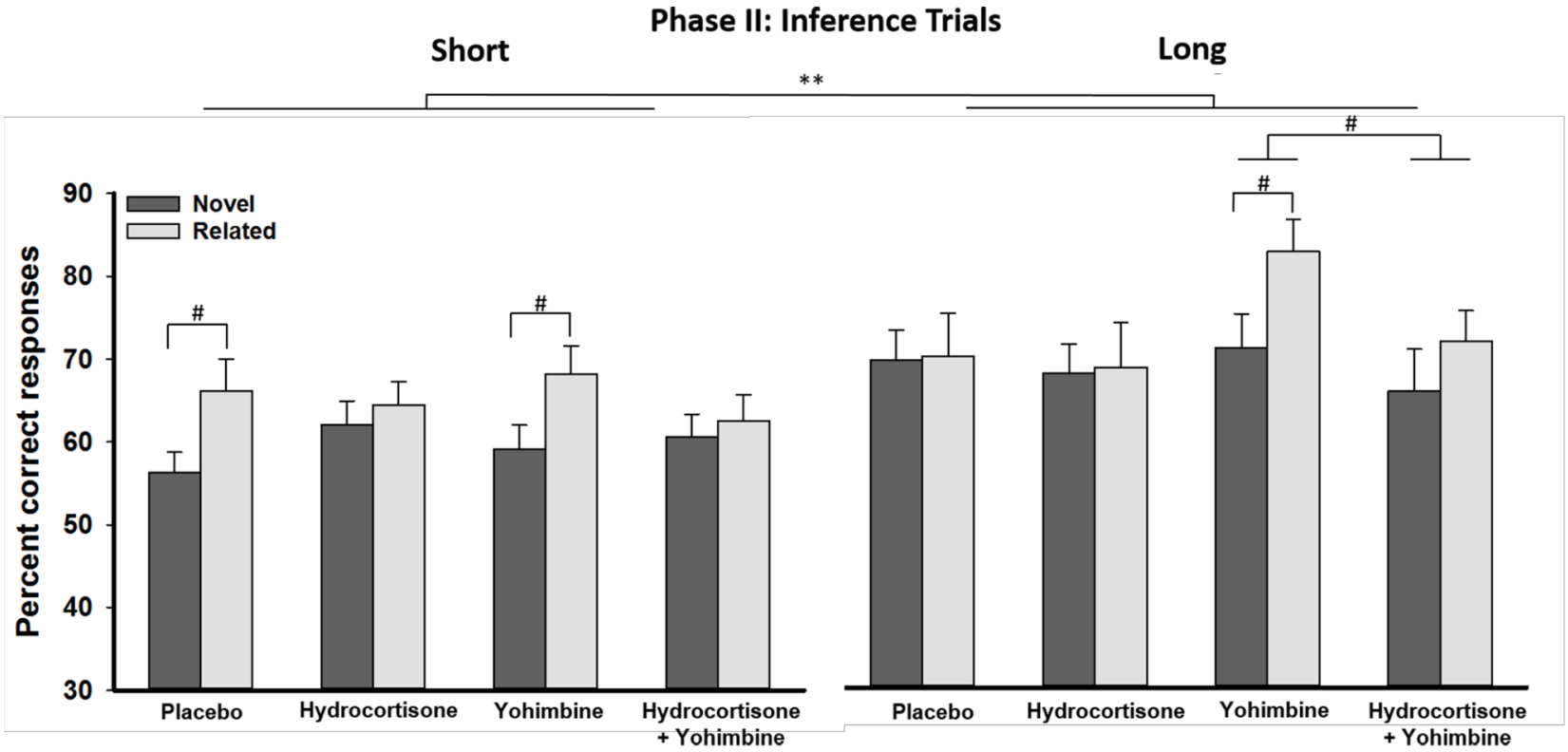

Figure S6. Inference trial performance in phase II in experiment II**.** Overall, performance was better in long- compared to short-distance trials. For short-distance trials, participants that received hydrocortisone or both hydrocortisone and yohimbine showed similar performance in novel and related trials, whereas the placebo group showed a trend for improved performance in related trials. In long-distance trials, the yohimbine groups displayed a trend for a schema effect with increased performance in related compared to novel structures (p=.096). Data represent means ± s.e.m. ** $p < .001$, * $p < .05$, # $p < .10$

Appendix B

Stress Affects the Neural Ensemble for Integrating New Information and Prior Knowledge

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Stress affects the neural ensemble for integrating new information and prior knowledge

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ABSTRACT

Prior knowledge, represented as a schema, facilitates memory encoding. This schema-related learning is assumed to rely on the medial prefrontal cortex (mPFC) that rapidly integrates new information into the schema, whereas schema-incongruent or novel information is encoded by the hippocampus. Stress is a powerful modulator of prefrontal and hippocampal functioning and first studies suggest a stress-induced deficit of schema-related learning. However, the underlying neural mechanism is currently unknown. To investigate the neural basis of a stress-induced schema-related learning impairment, participants first acquired a schema. One day later, they underwent a stress induction or a control procedure before learning schema-related and novel information in the MRI scanner. In line with previous studies, learning schema-related compared to novel information activated the mPFC, angular gyrus, and precuneus. Stress, however, affected the neural ensemble activated during learning. Whereas the control group distinguished between sets of brain regions for related and novel information, stressed individuals engaged the hippocampus even when a relevant schema was present. Additionally, stressed participants displayed aberrant functional connectivity between brain regions involved in schema processing when encoding novel information. The failure to segregate functional connectivity patterns depending on the presence of prior knowledge was linked to impaired performance after stress. Our results show that stress affects the neural ensemble underlying the efficient use of schemas during learning. These findings may have relevant implications for clinical and educational settings.

Introduction

Schemas are associative network structures, which often lack unit detail and are adaptable in the face of new, schema-congruent information (Ghosh and Gilboa, 2014; Gilboa and Marlatte, 2017). Such schemas facilitate memory formation by providing a framework to guide learning (Bartlett, 1932; Kumaran, 2013; van Kesteren et al., 2014). The enhancing effect of schemas on new learning is at the heart of our educational system and has a long standing research tradition in psychology (Bartlett, 1932). Only recently, however, neuroscientists unraveled the neural mechanisms underlying schema-related learning. These studies demonstrated that encoding, consolidating, and retrieving schema-related information critically relies on the medial prefrontal cortex (mPFC), interacting with the angular gyrus and the precuneus (Gilboa and Marlatte, 2017; Spalding et al., 2015; Tse et al., 2007, 2011; van Kesteren et al., 2010a,b; Wagner et al., 2015). Critically, the mPFC is assumed to detect whether new information is congruent with prior knowledge. If there is relevant prior knowledge, the mPFC integrates this information into the schema (Ghosh et al., 2014; Richards et al., 2014; van Kesteren et al., 2012). In contrast, schema-incongruent or unrelated information is encoded by the hippocampus as new episodic memory (Eichenbaum, 1999; Scoville and Milner, 1957; van Kesteren et al., 2012).

Stressful events are well-known to alter both hippocampal and PFC functioning (Joëls et al., 2006; Schwabe et al., 2012a). For instance, stress and stress mediators, such as corticosteroids and catecholamines, often enhance episodic memory formation (Barsegyan et al., 2010; Cahill et al., 2003; Luethi et al., 2008; Roozendaal et al., 2006; Sandi and Rose, 1994), but impair memory retrieval and prefrontal functions including working memory and goal-directed behavior (Barsegyan et al., 2010;

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Buchanan et al., 2006; de Quervain et al., 1998; de Quervain et al., 2000; Diamond et al., 2006; Roozendaal et al., 2003; Schwabe et al., 2012b; Schwabe and Wolf, 2014; Zoladz et al., 2012). In line with these findings, major stress mediators affect plasticity both in the hippocampus and the PFC (Arnsten, 2009; Diamond et al., 2006; Zoladz et al., 2012). Many previous studies on stress and memory, however, have not considered what an individual brings to a learning situation in terms of prior knowledge. Recent evidence from our lab indicates that stress and the administration of glucocorticoids impair schema-related learning (Kluen et al., 2017). The neural mechanisms underlying the impact of stress on the integration of new information and existing knowledge, however, are unknown. The present experiment therefore aimed at examining the neural mechanisms underlying a stress-induced impairment in schema-related learning.

To this end, participants first learned a hierarchy of six galaxies (Kumaran, 2013, Fig. 1A, 'phase 1'). One day later, participants underwent a stress induction or a control manipulation before they learned two new hierarchies ('phase 2') while neural activity was assessed using functional magnetic resonance imaging (fMRI). Importantly, one of these hierarchies ('related') included four galaxies from the original schema, which thus served as a scaffold to learn the new galaxies' positions in the hierarchy. In contrast, the other ('novel') hierarchy consisted of eight completely new galaxies such that there was no schema that could aid new learning (Kumaran, 2013). Based on evidence suggesting that stress may impair mPFC functioning (Arnsten, 2009; Barsegyan et al., 2010; Schwabe et al., 2012b), we hypothesized that stress would reduce schema-related mPFC activity, leading to impaired detection of schema-congruency (van Kesteren et al., 2010a; van Kesteren et al., 2012) and thus to impaired schema-related learning.

Materials and methods

Participants and experimental design

Fifty healthy individuals (25 males, 25 females) completed this experiment (mean age = 25.0 years, SEM = 0.48 years). Individuals with current medication intake, lifetime history of neurological or psychiatric disorders, or current non-admissibility to the MRI scanner were excluded from participation. Moreover, we excluded smokers and women taking hormonal contraceptives as both can affect the stress response (Kirschbaum et al., 1999; Rohleder and Kirschbaum, 2006). All participants had normal or corrected to normal vision and were screened by an MD prior to MRI scanning for possible MRI contraindications. The protocol was approved by the review board of the German Psychological Society (LS 062014_B), all participants provided written informed consent and received a moderate monetary compensation for participation.

We used a mixed design with the between-subjects factor treatment (stress vs. control manipulation) and the within-subjects factor schema (schema-related vs. novel information) to investigate the effects of stress on the neural mechanisms underlying schema-related learning. Participants were randomly assigned to the experimental groups (n = 25 per group).

Experimental procedure

All testing took place in the afternoon and early evening (12:00–20:00).

Day 1. Upon their arrival at the laboratory, participants provided baseline measures of blood pressure (assessed using an Omron blood pressure monitor with arm cuff) and salivary cortisol. To assess the concentrations of cortisol in saliva over time, each participant provided six samples using Salivette® collection devices (Sarstedt). Samples were stored at −18 °C. When data acquisition was finished, all samples were thawed and the fraction of free cortisol was measured using a chemiluminescence immunoassay (IBL, Tecan) with a lower detection limit of 0.33 nmol/l. All intra- and inter-assay coefficients of variance were <10%. In addition to these physiological measures, participants completed a German questionnaire assessing subjective mood, wakefulness, and calmness (MDBF; Steyer et al., 1994). Finally, participants performed phase 1 of the learning task during which they acquired a schema (Kumaran, 2013) followed by an explicit memory test (see below and Fig. 1A).

Day 2. One day later, participants came to the MRI scanning facility at the University Medical Center Hamburg-Eppendorf. They completed the

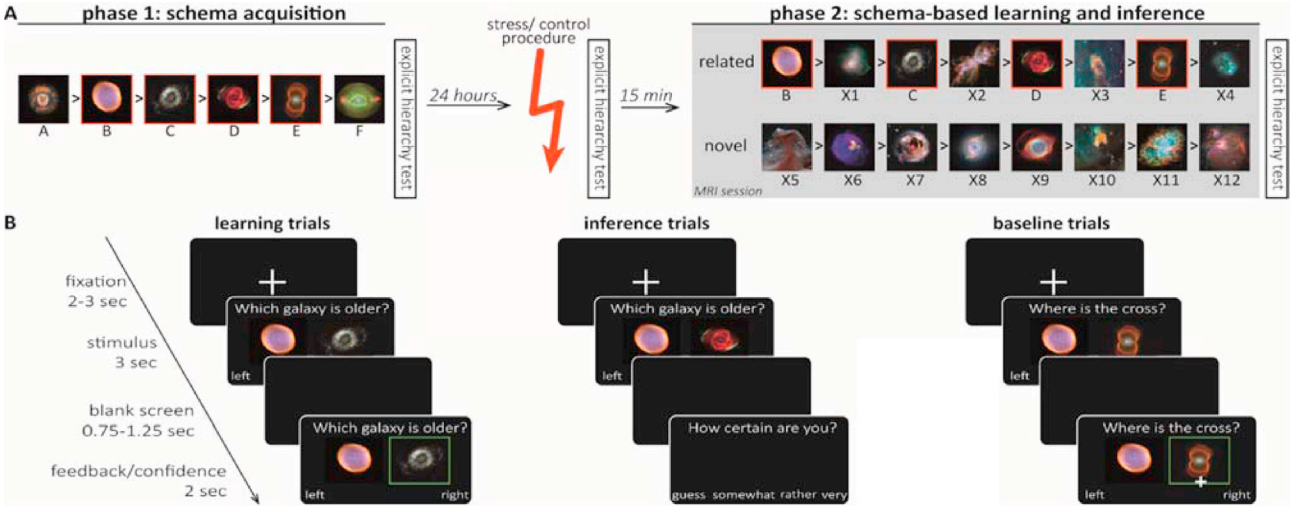

Fig. 1. Experimental setup and learning task. **A** Participants acquired an age hierarchy of six different galaxies by trial and error on day 1 ('phase 1', (Kumaran, 2013)), followed by an explicit hierarchy test. A day later, they came to the MRI scanning facility and underwent either a stress or a control manipulation. After another explicit hierarchy recall test, participants learned two new hierarchies of galaxies ('phase 2') while their brain activity was measured using functional magnetic resonance imaging (fMRI). Importantly, to acquire one of these hierarchies ('related') they could use their knowledge from phase 1 as a schema, as this hierarchy contained items from phase 1. In contrast, for the 'novel' hierarchy, the knowledge from phase 1 could not serve as a scaffold guiding new learning as the items were all new. **B** Three trial types were used during both phases. In learning trials, participants saw two neighboring galaxies for 3 s and had to indicate which one is older by button press. They were provided with the correct feedback (2 s) which they could use to learn about the age relationships between the galaxies. In inference trials, participants were presented with non-neighboring galaxies and had to infer the age relationship between these galaxies. Finally, baseline trials served as visuo-motor control trials for the fMRI analyses.

Pittsburgh Sleep Quality Index (German version; Backhaus and Riemann, 1996) and the MDBF, and provided a saliva sample and vital signs assessment. Next, they were brought to a separate room where they underwent either the Trier Social Stress Test (Kirschbaum et al., 1993) or a non-stressful control procedure, depending on group assignment. The TSST is a stress protocol well-known to activate both the autonomic nervous system and the hypothalamus-pituitary-adrenal axis (Kirschbaum et al., 1993). It simulates a 15-min job interview including a public speech about the participant's eligibility for his/her dream job and a mental arithmetic task while being videotaped and evaluated by two serious, non-reinforcing committee members. Participants in the control condition spoke about a topic of their choice followed by a simple arithmetic task (counting forwards in steps of 15), without committee or camera. Directly after the stressor/control manipulation, participants' vital signs were assessed again, followed by a saliva sample, the MDBF, and a rating of the difficulty, stressfulness, and unpleasantness of the experimental treatment. Participants then completed an explicit memory recall test and were brought to the MRI room and prepared for scanning. Approximately 15 min after stressor/control manipulation offset, participants learned schema-related and novel information (phase 2 of the learning task), followed by an anatomical scan and an explicit memory test. Participants left the scanning facility after providing a last saliva sample, vital signs and mood assessment.

Learning task

To investigate schema-related learning, we used a learning task that was modified from Kumaran (Kluen et al., 2017; Kumaran, 2013).

Phase 1: Schema acquisition. In phase 1, participants acquired a (fictive) age hierarchy of six galaxies, A > B > C > D > E > F (Fig. 1A). Three different trial types were presented in 15 blocks: learning, inference and baseline trials (Fig. 1B). In learning trials (5 per block), participants were presented with two neighboring galaxies for 3 s (e.g., B and C) and asked to indicate which one was older by pressing one of two buttons. After a jittered blank screen, feedback was provided for 2 s, highlighting the older galaxy with a green frame. In inference trials (5 per block), two non-neighboring galaxies were presented for 3 s (e.g., B and E) and participants had to infer the older galaxy based on what they had learned during the learning trials. In these inference trials, no feedback was provided, but participants were asked to rate their confidence from 1 ('guess') to 4 ('very sure') after a jittered blank screen. Finally, baseline trials (2 per block) were used as visuo-motor control trials and contained two randomly chosen galaxies for 3 s, one of which was marked with a white cross. Participants had to choose the galaxy with the cross and were provided with the correct feedback after a jittered blank screen. Each block contained two baseline trials and five learning trials that were randomly intermixed, followed by five inference trials.

Phase 2: *Schema-related and novel learning.* In phase 2, participants learned two new age hierarchies of eight galaxies each in the MRI scanner (Fig. 1A). Importantly, one hierarchy (termed 'related', B > X1>C > X2>D > X3>E > X4) included four galaxies from the schema acquired during phase 1, which could thus serve as a scaffold to learn the position of the new galaxies more rapidly. In contrast, the other hierarchy ('novel', X5>X6>X7>X8>X9>X10 > X11 > X12) included eight completely new galaxies for which the schema could not serve as a scaffold aiding learning during phase 2. Participants were presented with six blocks per hierarchy (12 in total), each comprising two baseline trials randomly intermixed with seven learning trials, followed by six inference trials, which contained only new galaxies and no galaxies from phase 1. Trial timing and setup was identical to phase 1. The assignments of galaxies to hierarchy position and the related vs. novel hierarchy, and whether phase 2 started with a related or novel block, were counterbalanced. Of special interest for the current study were the learning trials as they indexed schema-related learning during phase 2, whereas inference trials mainly targeted inferential reasoning.

Explicit hierarchy test. After phase 1, the stress/control manipulation, and phase 2, participants were asked to explicitly recall the hierarchical order of the presented galaxies. All galaxies presented up to that time point were shown and participants were asked to indicate the correct order (separately for 'novel' and 'related' after phase 2). In line with previous studies (Kumaran, 2013; Kumaran et al., 2012), we evaluated performance by the summed deviation of the true position from the position indicated by the participant per galaxy (hereafter referred to as 'errors'). Higher values thus represent more errors in explicit hierarchy knowledge.

Statistical analysis of behavioral and physiological data

To test whether the TSST successfully induced stress, data on mood, vital signs, and salivary cortisol were analyzed using mixed-design ANOVAs with the between-subjects factor treatment and the within-subjects factor time after stress/control manipulation onset. T-tests were used to investigate post-hoc group differences in these measures, to test for group differences in stress measures and explicit knowledge on day 1, and to analyze group differences in the ratings of the stress/control manipulation.

Task performance during learning and inference trials was averaged per block and subjected to mixed-design ANOVAs per phase with the between-subjects factor treatment and the within-subjects factors schema (novel vs. related) and block. For explicit memory after phase 2, a similar ANOVA was used with the factors treatment and schema. All analyses were performed in SPSS Statistics 22 (IBM). All P-values are two-tailed and Greenhouse-Geisser correction was applied when necessary.

MRI acquisition and analyses

MRI measurements were acquired using a 3T Skyra scanner (Siemens) equipped with a 32-channel head coil. A sequence sensitive to the blood-oxygenation level dependent (BOLD) response with the following parameters was used to measure brain activity during task performance: 27 transversal slices, slice thickness = 3 mm, distance factor 20%, repetition time (TR) = 2.00s, echo time (TE) = 30 ms, effective voxel size = 3.0 × 3.0 × 3.0 mm. Additionally, we acquired magnetic (B0) field maps to unwarp the functional images and a high-resolution T1-weighted anatomical image (TR = 2.5s, TE = 2.12 ms, 256 slices, voxel size = 0.8 × 0.8 × 0.9 mm). All fMRI data were preprocessed and analyzed in SPM12 (Wellcome Trust Center for Neuroimaging, London) using general linear modeling (GLM). One participant (male, control group) was excluded due to excessive head motion (>4 mm). The first three functional images were discarded to allow for T1 equilibration. Remaining functional images were spatially realigned and unwarped, coregistered to the structural image, and normalized to MNI space. Finally, the functional images were spatially smoothed using the default 8 mm FWHM Gaussian kernel.

To assess task-related activity and the effects of treatment, we used a model including separate regressors for stimulus and feedback/confidence presentation during baseline, learning, and inference trials, respectively. To dissociate activity for novel and related trials, the regressors for learning and inference were split, resulting in 10 regressors in total, all events modeled as boxcars with a duration of the events' presentation on screen (baseline, baseline feedback, novel learning, related learning, novel inference, related inference, feedback novel learning, feedback related learning, confidence novel inference, confidence related inference). Additionally, we added a spike regressor for button presses. All regressors were convolved with the canonical hemodynamic response function. Six realignment parameters were added to account for residual motion. Full-factorial designs were used to test for activation differences depending on schema and treatment. Behavioral covariates (average performance in learning and inference trials) were added to the second-level GLMs where indicated (see 3.4) to assess the correlation between neural activation and behavioral performance. Moreover, to correlate brain activity to the cortisol response to treatment,

we calculated the area under the curve with respect to the increase during day 2 (AUCi; Pruessner et al., 2003) and extracted mean parameter estimates for the contrast of interest from the anatomically defined mPFC using MarsBaR (see 3.4). To investigate the effects of treatment on hippocampal and mPFC connectivity during schema-related learning, the 'Psycho-Physiologic Interaction' tool was used as implemented in SPM12 to test for enhanced connectivity during related and novel learning trials as compared to baseline trials, respectively (see 3.5, 3.6). The models contained all task regressors, the interaction term and the time course of the ROI which was anatomically defined using the Harvard-Oxford atlas at a probability of 50%. Again, full-factorial designs were used to test for group differences.

For whole-brain analyses, we used a cluster-defining threshold of $P < .001$ with a cluster-probability of $P < .05$ family-wise error (FWE) corrected for multiple comparisons as suggested by previous research (Eklund et al., 2016). For our regions of interest (ROIs, hippocampus, mPFC, angular gyri, and precuneus), we implemented small volume correction (SVC) using an initial threshold of $P < .005$, uncorrected, which was followed by voxel-wise FWE-correction ($P < .05$) for multiple comparisons within ROIs. The more liberal initial threshold was chosen to enhance sensitivity considering that voxel-wise inference has been shown to be overly conservative (Eklund et al., 2016). The results obtained by SVC are indicated by 'P_{SVC}', all other results are based on whole-brain cluster-inference. Anatomical masks were taken from the Harvard-Oxford atlas using a probability threshold of 50%. For the mPFC, we used the masks for frontal medial cortex and the paracingulate cortex. All images are displayed at $P < .005$, uncorrected, for illustrative purposes.

Results

Successful schema acquisition

On day 1, participants successfully acquired the schema (Fig. 2A). Both during learning trials, in which neighboring galaxies were presented and corrective feedback was provided, and during inference trials, presenting non-neighboring galaxies without feedback (Fig. 2B), task performance increased significantly over blocks (both $F > 20$, $P < .001$, Fig. 2), reaching an average learning and inference performance of 85% in the last task block. Successful schema acquisition was further supported by the explicit hierarchy test in which participants made on average only 3 mistakes (range 0–18) and 58% of participants made no error at all (Fig. 2C). Furthermore, participants performed significantly better in inference trials in which the hierarchical distance between galaxies was long than in those in which the distance was short ($F(1,48) = 26.32$, $P < 0.001$), indicating that participants had indeed created an associative structure characteristic for a schema. Most importantly, stress and control groups did not differ in schema acquisition on day 1, neither in learning or inference trial performance, nor in the explicit hierarchy test (all main effects and interactions: $P > .25$). Moreover, groups did not differ in any measure of stress on day 1 (all $P > .15$, Table 1) or in self-reported sleep duration and quality in the night after the learning session (both $P > .50$).

Successful stress induction prior to schema-related vs. novel learning

As expected, the TSST induced pronounced subjective, autonomic, and endocrine stress responses. The TSST was rated as more difficult, stressful, and unpleasant (all $t > 4$, $P < .001$, Table 2) and decreased positive mood and calmness (time × treatment: both $F > 7$, $P \leq .001$) compared to the control manipulation. Whereas groups did not differ before the treatment (all $P > .15$, Table 2), the stress group felt less positive and calm prior to the learning task (both $P \leq .01$). Moreover, the TSST activated the autonomic nervous system as indicated by a pronounced increase in diastolic and systolic blood pressure (time × treatment: both $F > 8$, $P = .001$, Fig. 3A and B). Groups did not differ prior to treatment (all $P > .60$), but the stress group displayed higher blood pressure after treatment, i.e., before the learning session (both $P < .05$). Finally, the stressor also markedly increased salivary cortisol levels (time × treatment: $F(2.6,125.5) = 10.75$, $P < .001$, Fig. 3C): Whereas groups did not differ before treatment ($P = .507$), salivary cortisol levels were elevated from stressor offset onwards (stress vs. control directly after treatment: $P = .074$; immediately before learning, after learning, and at the end of day 2: all $P < .001$).

Pre-existing schema enhances learning of related learning material

The stress induced by the TSST did not affect explicit recall of the schema learned on day 1 ($t(48) = 0.00$, $P = 1.000$, Fig. 4C) and recall performance for this previously learned schema was overall very good

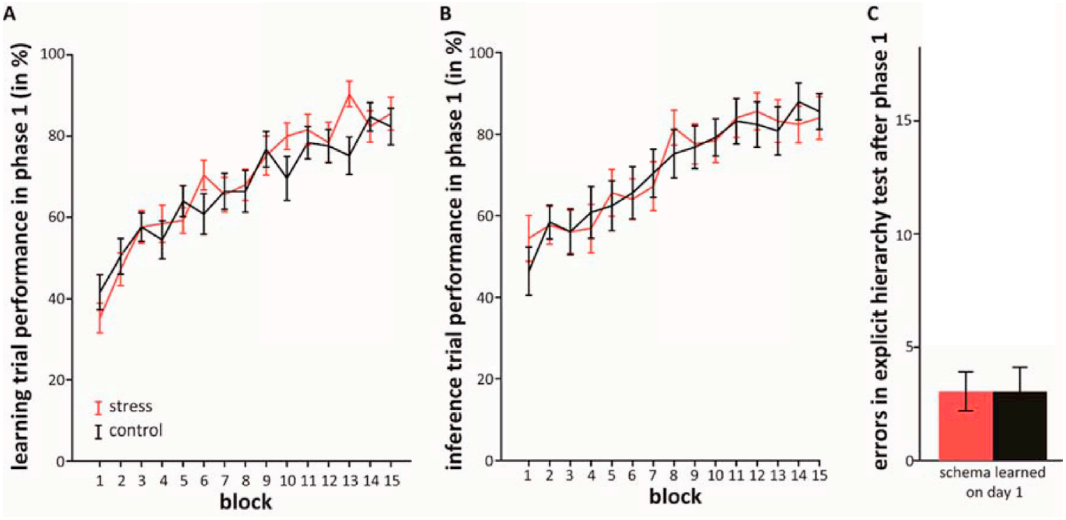

Fig. 2. Task performance and explicit knowledge on day 1. **A** Learning trial performance increased over blocks during phase 1 and was not affected by stress. **B** Similarly, inference trial performance increased during phase 1, but was unaffected by stress. **C** After phase 1, participants were able to explicitly recall the hierarchy learned during phase 1 as indicated by few errors (defined as the sum of all deviations of the hierarchy position indicated by the participant and its true position for all galaxies). The maximum possible amount of errors was 18. Color coding of the groups is the same for all panels, there was no effect of treatment. Data represent means ± s.e.m.

Table 1
Physiological, endocrine, and subjective measures of stress in both groups on day 1.

Variable	Stress group		Control group		Statistics	
	M	SEM	M	SEM	*t*	*P*
Systolic blood pressure (mmHg)	118.36	3.05	119.14	2.93	-.18	.854
Diastolic blood pressure (mmHg)	81.92	2.04	82.34	1.55	-.16	.870
Heart rate (bpm)	81.16	2.84	80.92	2.29	.07	.948
Salivary cortisol (nmol/l)	5.51	0.96	4.51	0.56	.89	.379
Subjective mood	35.56	0.69	34.88	0.74	.67	.506
Wakefulness	30.64	0.93	31.24	1.25	-.39	.702
Restlessness	33.96	0.84	31.92	1.14	1.44	.156

(only 3 mistakes on average, range 0–18). Thus, possible treatment effects on schema-congruent learning on day 2 cannot be explained by a simple stress-induced retrieval deficit.

As expected, the presence of a relevant schema boosted performance in learning trials, indicated by better acquisition of the related hierarchy compared to the novel hierarchy (schema: $F(1,48) = 41.20$, $P < .001$; block: $F(3.8, 181.2) = 25.23$, $P < .001$, schema × block: $F(4.2,202.6) = 2.83$, $P = .023$; Fig. 4A). Inference performance, in contrast, was comparable for both hierarchies (all $P > .15$, Fig. 4B), suggesting that inference was not modulated by the presence of a schema. The idea that learning and inference trials tracked different processes is supported by our fMRI results (Fig. 5) showing increased activation in memory-related areas during learning trials as compared to inference trials (hippocampus, mPFC, and angular gyrus), but no enhanced activation in the hippocampus, mPFC, angular gyrus, or precuneus in inference trials as compared to learning trials (no significant voxel even at $P < .005$, uncorrected). As we were mainly interested in learning and less in inference processes, we focused our further analyses mainly on learning trials. In line with the schema-related learning enhancement, participants made fewer errors in the explicit memory test at the end of day 2 for the congruent hierarchy than for the incongruent hierarchy ($F(1,48) = 5.05$, $P = .029$, Fig. 4C). However, at the group level we found no effect of treatment (all main effects or interactions: $P > .20$), also not when excluding the three participants of the stress group that were classified as cortisol-nonresponders based on a cortisol response to the stressor of less than 1.5 nmol/l (Miller et al., 2013). We also explored whether gender interacted with treatment or schema to affect performance. Although men outperformed women in the explicit knowledge test ($F(1,46) = 9.14$, $p = .004$) and inference trials ($F(1,46) = 6.93$,

Table 2
Subjective measures of stress in both groups over the course of day 2.

Variable	Stress group			Control group		
	Before stress induction	After stress induction	End of day 2	Before control procedure	After control procedure	End of day 2
Mood questionnaire						
Subjective mood	35.16 (0.77)	29.60* (1.31)	32.44 (1.00)	34.60 (1.04)	34.16 (1.09)	33.80 (0.80)
Wakefulness	30.04 (0.98)	29.88 (0.94)	26.08 (1.06)	31.92 (1.23)	31.44 (1.11)	25.68 (1.16)
Calmness	33.84 (0.81)	27.52** (1.17)	32.08 (0.98)	32.12 (1.21)	32.36 (1.22)	33.84 (0.78)
Ratings of stressor/control procedure						
Difficult	–	69.20*** (4.00)	–	–	32.80 (5.49)	–
Unpleasant	–	64.00*** (4.08)	–	–	30.00 (5.80)	–
Stressful	–	66.40*** (4.51)	–	–	26.40 (4.47)	–

Note: Data represent mean (standard error). Higher values in subjective mood represent elevated mood. ***$P < .001$ compared to control group, **$P < .01$ compared to control group, *$P < .05$ compared to control group.

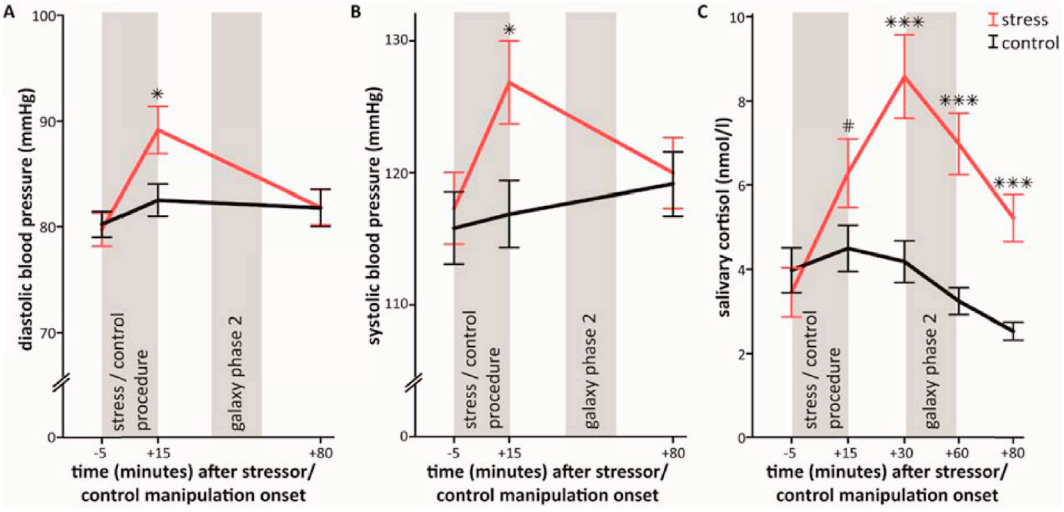

Fig. 3. Successful stress induction indicated by physiological parameters. **A** Participants in the stress group showed increased diastolic and **B** systolic blood pressure after treatment compared to participants in the control group. **C** The stress procedure increased salivary cortisol levels compared to the control procedure. Data represent means ± s.e.m.* $P < .001$, *$P < 0.05$.

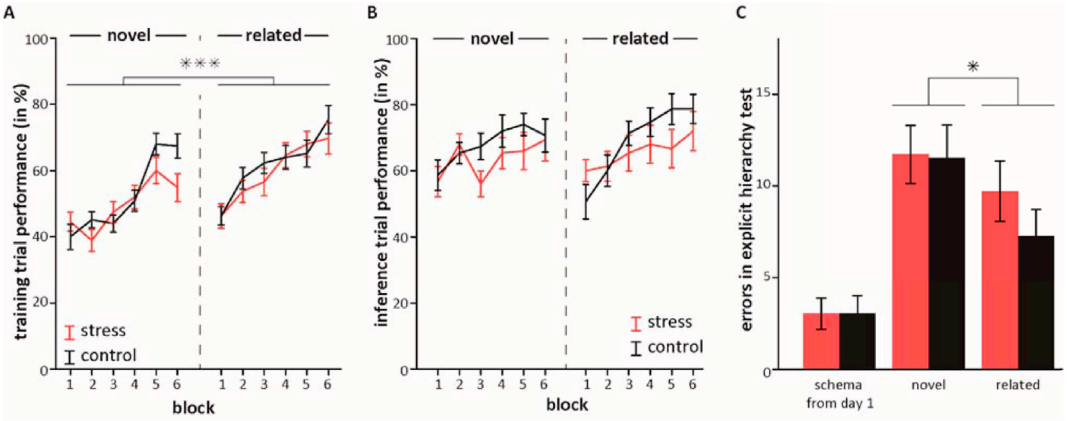

Fig. 4. Task performance and explicit hierarchy knowledge on day 2. **A** Learning trial performance was better for the related compared to the novel structure, indicating that the presence of a schema facilitated learning of schema-related information. **B** Inference trial performance increased across blocks but was not facilitated by the presence of prior knowledge. **C** Stress did not affect explicit retrieval of the hierarchy learned on day 1 (schema) as assessed directly after the stress/control manipulation. Supporting enhanced learning if a relevant schema is available, participants made fewer errors in the explicit hierarchy test for the related hierarchy compared to the novel hierarchy. Data represent means ± s.e.m. ***$P < .001$, *$P < .05$.

$p = .011$; learning trials: $p > .10$), these effects were independent of treatment or schema (all $p > .10$).

Stress affects neural activity underlying schema-related learning

Across groups, learning of schema-related information compared to novel information activated a set of brain regions known to be implicated in schema processing in humans and rodents (Gilboa and Marlatte, 2017; Spalding et al., 2015; Tse et al., 2011; van Buuren et al., 2014; van Kesteren et al., 2010b; van Kesteren et al., 2012; Wagner et al., 2015). Specifically, we found activation in the angular gyri, the precuneus (all $P_{FWE} < .05$, Fig. 6A), and the mPFC ($P_{SVC} = .017$, k = 44, T = 3.98). On the group level, schema-related neural activity was not affected by treatment (all $P > .10$). However, given its important role in learning new schema-related information (Gilboa and Marlatte, 2017; Sommer, 2017; Wagner et al., 2015), the mPFC activation was of special interest to us. This structure is assumed to detect the congruency between new information and recently learned (i.e., 24h ago) prior knowledge and to integrate the new information into the schema (van Kesteren et al., 2012). Interestingly, this mPFC activation to schema-related information was only present (as a trend) in the control group ($P_{SVC} = .051$, k = 20, T = 3.53), whereas we found no significantly activated voxel for related > novel learning in the mPFC in the stress group (even not at a lenient threshold of $P < .005$, uncorrected; extracted parameters from the underlying anatomical region, frontal medial cortex, shown in Fig. 6B). Although these group differences were only trend-level significant ($t(47) = -1.726$, $p = .091$), the activation for related > novel learning in the mPFC was negatively correlated with the individual cortisol response

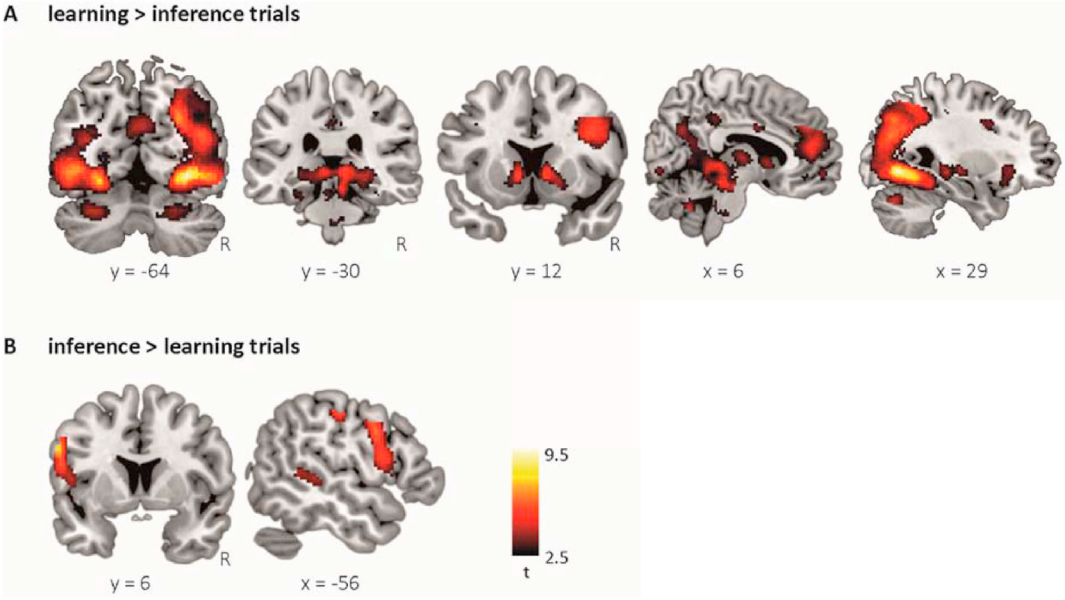

Fig. 5. Learning trials and inference trials resulted in different patterns of neural activation. **A** Brain regions more responsive to learning as compared to inference trials included widespread occipital brain regions, ventral striatum, right inferior frontal cortex (all $P_{FWE} < 0.05$), the hippocampus (right: $P_{SVC} = 0.006$, k = 78, T = 4.23; left: $P_{SVC} = 0.055$, k = 25, T = 3.38), the medial prefrontal cortex (mPFC, $P_{SVC} = 0.048$, k = 30, T = 3.42) and, at trend level, the right angular gyrus ($P_{SVC} = 0.063$, k = 41, T = 3.32). **B** In contrast, brain regions more activated by inference trials included the left inferior frontal cortex, the left superior temporal gyrus, and the left pre- and postcentral gyri (all $P_{FWE} < 0.05$). Importantly, there was no activated voxel in the hippocampus, mPFC, angular gyri, or precuneus in this contrast at $P < 0.005$, uncorrected. This supports that learning trials tracked the learning of schema-related and novel information whereas inference trials rather targeted inferential reasoning. Images are displayed at $P < 0.005$, uncorrected, for illustration purposes.

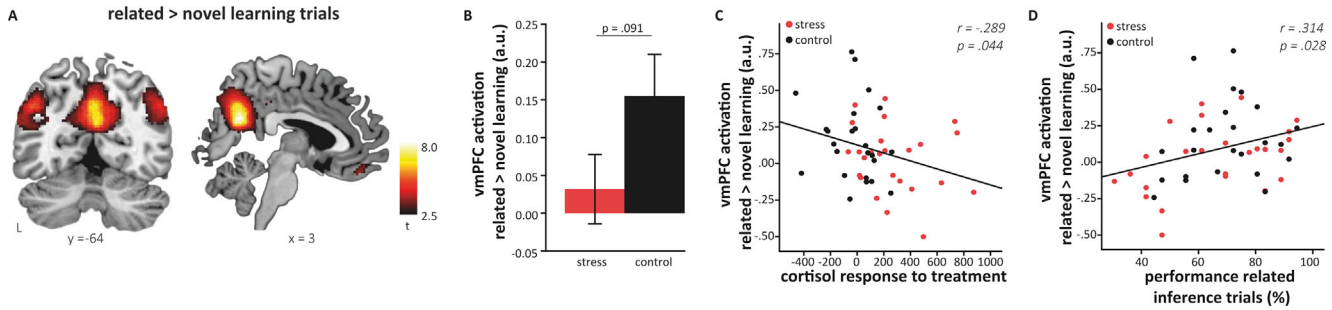

Fig. 6. Brain regions supporting schema-related learning are affected by individual differences in stress levels. **A** Across groups, brain regions responding more to related than novel learning trials were the medial prefrontal cortex (mPFC, $P_{SVC} = .017$, k = 44, T = 3.98), the precuneus, and both angular gyri (all $P_{FWE} < .05$). **B** Extracting the parameter estimates for this contrast from the mPFC using an anatomical mask showed that this schema-related mPFC activation tended to be less pronounced in the stress group than in the control group ($t(47) = -1.726$, $p = .091$). **C** Moreover, across groups this mPFC activation during learning of schema-related information was negatively correlated with the cortisol response to treatment as assessed using the area under the curve with respect to the increase. **D** Finally, mPFC activation during schema-related learning was positively correlated with performance in related inference trials, supporting the crucial role of the mPFC in integrating new information into an existing schema. Images are displayed at $P < 0.005$, uncorrected, for illustration purposes.

to the treatment ($r = -.289$, $P = .044$, anatomical mask, Fig. 6C). Although this finding should be interpreted with caution considering that the correlation was not significant in the stress group alone ($r = -.203$, $P = .332$, control group alone: $r = -.192$, $P = .368$), it suggests that individuals with a higher cortisol response to treatment might show less schema-related mPFC activation, possibly indicating reduced congruency detection and impaired integration of related information into the schema. In support of the hypothesis that mPFC activation is associated with successful schema-congruent learning, this mPFC activation was also positively correlated with performance in inference trials of the related hierarchy ($r = .314$, $P = .028$, Fig. 6D). According to a model of schema-related neural processing, the detection of congruency by the mPFC should lead to a suppression of memory encoding and neural activity in medial temporal lobe regions such as the hippocampus (van Kesteren et al., 2012). This model would suggest an enhanced activity in the hippocampus for related information if stress would affect mPFC functioning, as the hippocampus would be less inhibited, which would in turn be associated with less schema-related learning. Indeed, within the stress group, we found that hippocampal activity during related learning trials as compared to baseline trials was negatively associated with performance in related trials ($P_{SVC} = .028$, k = 48, T = 3.68, Fig. 7), suggesting impaired schema-related learning when stressed participants engaged the hippocampus. The association between hippocampal activity (anatomically defined) and related learning performance was not significant across groups ($r = -.174$, $p = .233$) or in the control group ($r = .016$, $p = .939$). It is to be noted, however, that the correlations did not significantly differ between groups ($p = .110$).

Stressed individuals show enhanced hippocampal connectivity with brain regions involved in schema-related learning

In order to investigate how this stronger involvement of the hippocampus during related learning trials may be detrimental to learning in stressed individuals, we assessed functional connectivity of the left hippocampus (anatomically defined) during related learning trials as compared to baseline trials, providing an index that is independent of any changes in novel trials, using a psychophysiological interaction (PPI) analysis. Across groups, we found enhanced schema-related hippocampal connectivity with the left angular gyrus, precuneus, left inferior temporal gyrus (all $P_{FWE} < .05$, Fig. 8A), mPFC ($P_{SVC} = .027$, k = 93, T = 3.49), right angular gyrus ($P_{SVC} = .031$, k = 111, T = 3.64), and right hippocampus ($P_{SVC} = .014$, k = 69, T = 3.95). In contrast, we found no enhanced hippocampal connectivity during baseline trials (no significant voxel even at $P < .005$, uncorrected). More importantly, stress enhanced connectivity between the hippocampus and the set of brain regions involved in schema processing during related learning trials, i.e., the angular gyri (left: $P_{FWE} < .05$, right: $P_{SVC} = .048$, k = 68, T = 3.46, Fig. 9), and the mPFC ($P_{SVC} = .044$, k = 86, T = 3.89), as compared to the control group. When examining both groups separately, there was no significantly enhanced connectivity during related learning trials between the

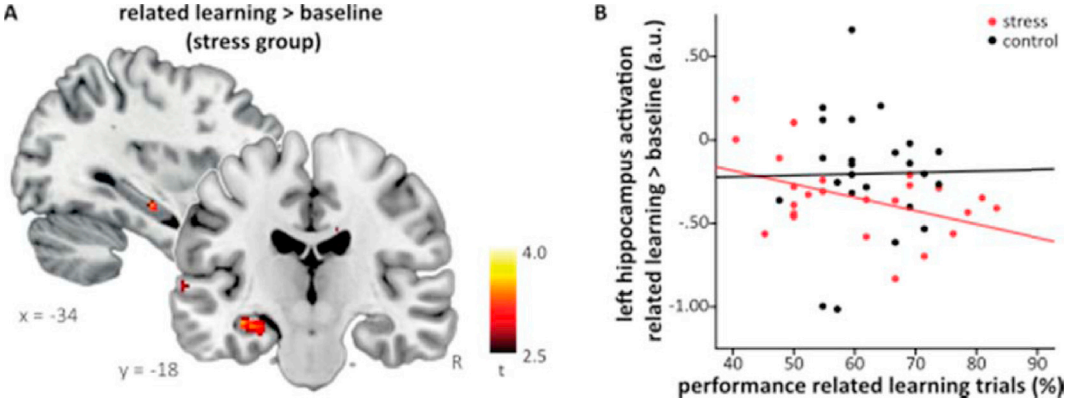

Fig. 7. Hippocampal activity during schema-related learning is detrimental to performance in the stress group. **A** In the stress group, we found a negative association between hippocampal activation during schema-related learning (as compared to baseline trials) and performance in related learning trials, suggesting that an enhanced hippocampal involvement during the related condition is disadvantageous for performance. **B** Scatterplot showing the correlation between activation of the anatomical left hippocampus (related learning > baseline) and related learning trial performance in the stress group but not the control group. Images are displayed at $P < .005$, uncorrected, for illustration purposes.

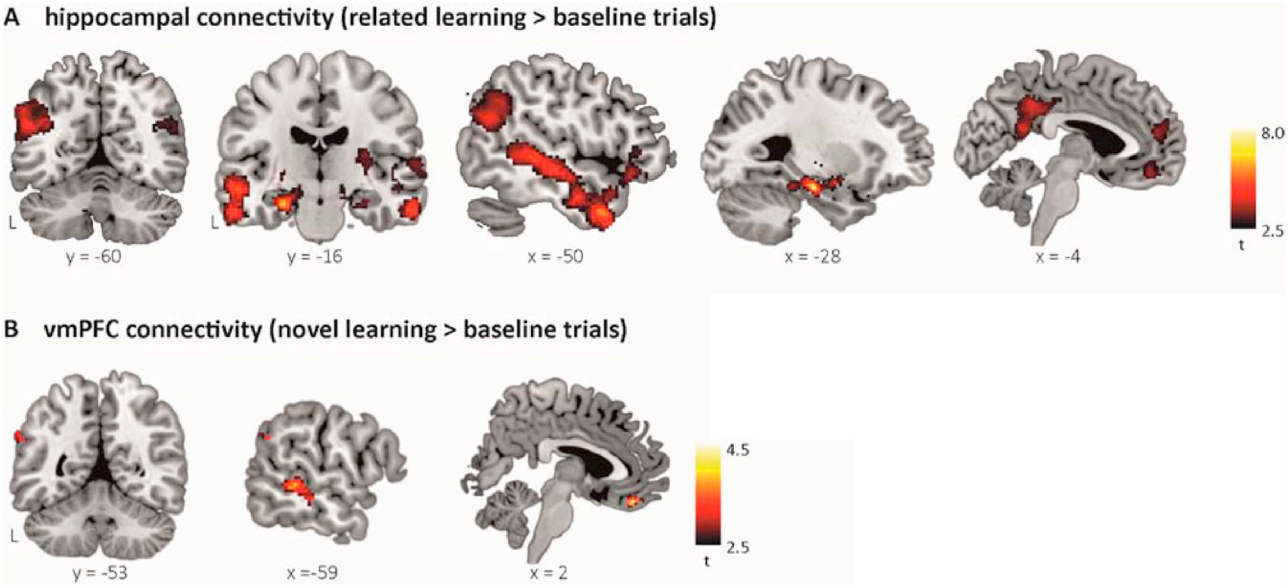

Fig. 8. Functional connectivity of the hippocampus and medial prefrontal cortex (mPFC) during schema-related and novel learning across groups. **A** Using a psychophysiological interaction (PPI) analysis, we found enhanced schema-related hippocampal connectivity with the left angular gyrus, precuneus, left inferior temporal gyrus (all $P_{FWE} < .05$, Fig. 8A), mPFC ($P_{SVC} = .027$, k = 93, T = 3.49), right angular gyrus ($P_{SVC} = .031$, k = 111, T = 3.64), and right hippocampus ($P_{SVC} = .014$, k = 69, T = 3.95). **B** Using another PPI analysis focusing on novel (versus baseline) trials, we found increased coupling of the mPFC with the left angular gyrus ($P_{SVC} = .047$, k = 8, T = 3.24, Fig. 8B) and the mPFC itself ($P_{SVC} = .003$, k = 68, T = 4.53). Images are displayed at $P < 0.005$, uncorrected, for illustration purposes.

hippocampus and these regions in the control group (no significant voxel even at $P < .005$, uncorrected), suggesting that the control group successfully isolated the hippocampus during schema-related learning from these structures. In the stress group, however, we found pronounced connectivity between the hippocampus and the angular gyri ($P_{FWE} < .05$), the mPFC ($P_{SVC} = .006$, k = 220, T = 4.24), and middle temporal cortices ($P_{FWE} < .05$) during related learning trials, suggesting that the hippocampus was inserted into this group of brain regions involved in schema-related learning. This might imply a lack of separation between memory networks for schema-related information and novel information, which may be associated with impaired congruency detection and less integration of related information into an existing schema.

Stressed individuals recruit brain regions involved in schema-related learning when learning novel information

As we observed a lack of segregation between functional connectivity patterns in the stress group during related learning trials, we reasoned that stressed individuals may have difficulties in separating brain regions suitable for learning of information for which prior knowledge exists (related) vs. learning of novel information. If this is the case, stress may also affect the brain regions recruited during the processing of novel information. To test this idea, we used a similar PPI model, now seeding on the mPFC during novel learning trials as compared to baseline trials. In general, novel learning increased mPFC coupling with the left angular gyrus ($P_{SVC} = .047$, k = 8, T = 3.24, Fig. 8B) and the mPFC itself ($P_{SVC} = .003$, k = 68, T = 4.53) whereas no brain region showed increased mPFC coupling during baseline trials. More importantly, however, we found in the stress group during novel learning trials enhanced mPFC connectivity with brain regions involved in schema-related learning, i.e. the mPFC itself ($P_{SVC} = .002$, k = 132, T = 4.68) and, at trend level, the angular gyri (left: $P_{SVC} = .051$, k = 31, T = 3.21; right: $P_{SVC} = .081$, k = 11, T = 3.28, Fig. 10A). In contrast, we found no enhanced connectivity of the mPFC with any region involved in schema processing during novel learning in the control group (no significant voxel at $P < .005$, uncorrected). Although the group differences did not reach statistical significance (all $P_{SVC} > .15$), this might suggest that stressed individuals recruited the cluster of brain regions involved in schema processing when faced with novel information for which no prior knowledge existed. Interestingly, a stronger connectivity between the mPFC and the angular gyrus during novel learning trials was associated with impaired performance in these trials across both groups ($P_{SVC} = .007$, k = 77, T = 4.27, Fig. 10B) and in the stress group alone ($P_{SVC} = .017$, k = 130, T = 3.95). This indicates that stronger connectivity between brain regions involved in schema-related learning during novel learning trials in the stress group (and a potential lack of segregation) was detrimental to performance.

Discussion

The integration of new information into pre-existing knowledge structures is key to efficient learning. Indeed it is well known that information is learned more easily if it can be linked to prior knowledge (Bartlett, 1932). Despite the crucial relevance of this so-called schema-congruent memory for educational settings, factors modulating this fundamental process of learning are largely unexplored. Recently, we reported that acute stress and the administration of glucocorticoids reduce schema-based learning (Kluen et al., 2017). Here, we investigated the underlying neural mechanisms and show that the exposure to acute stress impairs the separation of brain regions supporting the acquisition of schema-related and novel information. We found no stress effects on memory performance on group level, most likely owing to a lack of statistical power as a post-hoc power analysis using the software G*Power (Faul et al., 2009) indicated that a sample size of 94 participants would have been required to detect the previously reported behavioral effect (Kluen et al., 2017) with a power of 95 percent. Nonetheless, the stress-induced changes in neural processing reported here could explain how stress may hamper the use of prior knowledge to support memory performance.

In line with previous studies implicating the mPFC, precuneus, and angular gyrus in schema-congruent learning (Sommer, 2017; Tse et al., 2011; van Buuren et al., 2014; van Kesteren et al., 2010b; Wagner et al., 2015), we found enhanced activity in these regions when comparing the acquisition of schema-related information with learning of novel information. Earlier studies in rodents and humans suggested a key role for

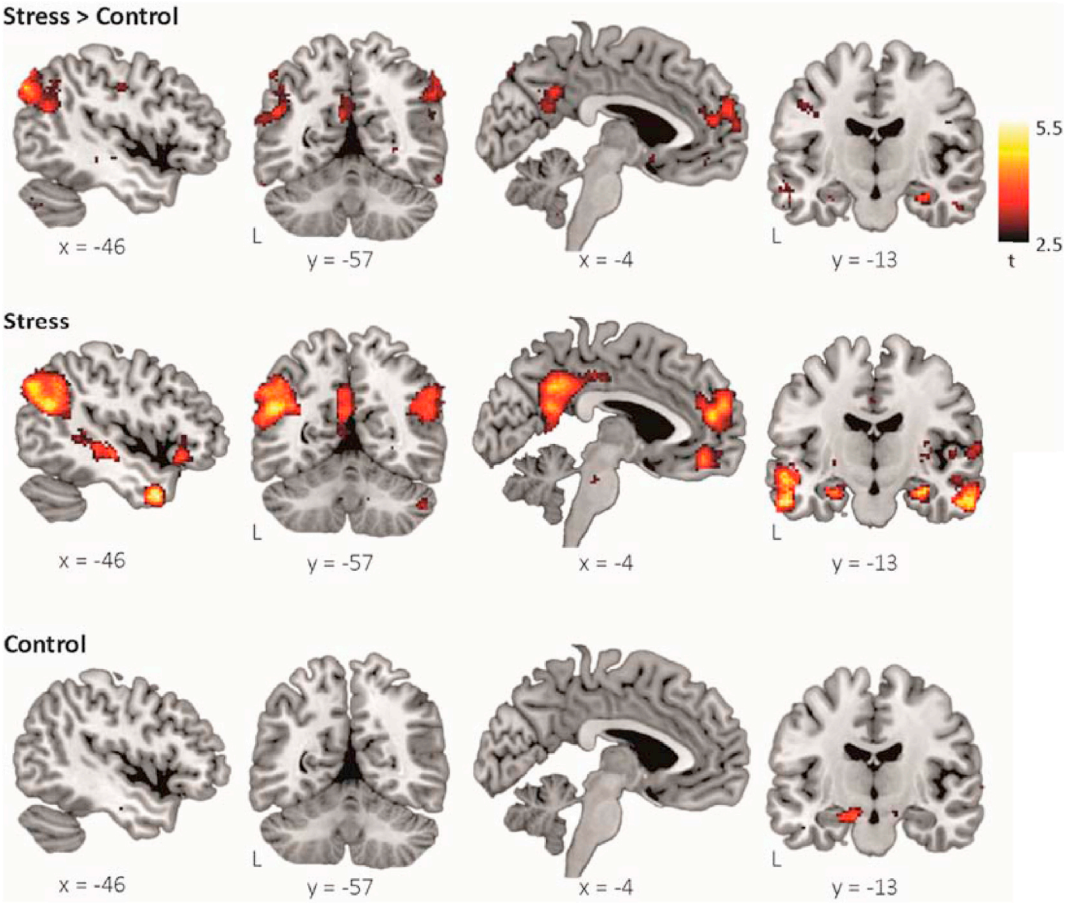

Fig. 9. Stress-induced incorporation of the hippocampus into the cluster of brain regions involved in schema-related learning. **Top:** Main effect of stress on functional connectivity of the left anatomical hippocampus in related learning trials as compared to baseline trials, modeled as psychophysiological interaction (PPI). Stress enhanced connectivity between the hippocampus and the angular gyri (left: $P_{FWE} < .05$, right: $P_{SVC} = .048$, k = 68, T = 3.46) and the medial prefrontal cortex (mPFC; $P_{SVC} = .044$, k = 86, T = 3.89). **Middle:** When examining the stress group separately, we found enhanced hippocampal connectivity during related learning trials to both angular gyri ($P_{FWE} < .05$), the mPFC ($P_{SVC} = .006$, k = 220, T = 4.24), and middle temporal cortices ($P_{FWE} < .05$), suggesting that the stress group inserted the hippocampus into the cluster involved in schema processing. **Bottom:** In contrast, there was no schema-related enhancement of connectivity between the hippocampus and these regions in the control group (no significant voxel at $P < .005$, uncorrected), suggesting that the control group successfully segregated the hippocampus during schema-related learning. Images are displayed at $P < 0.005$, uncorrected, for illustration purposes.

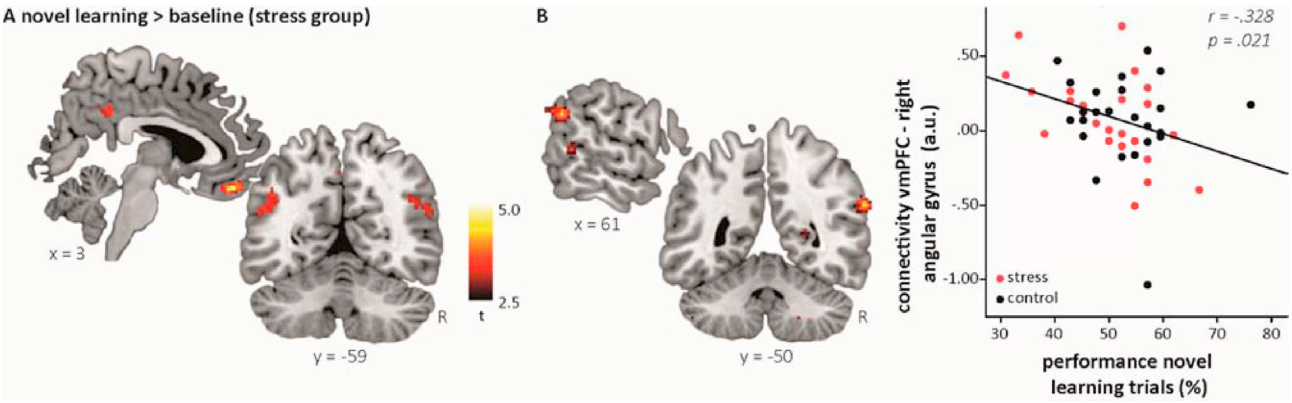

Fig. 10. Stress induced connectivity between regions involved in schema-related processing during learning of novel items. **A** Functional connectivity of the anatomical medial prefrontal cortex (mPFC) during novel learning as compared to baseline, modeled as psychophysiological interaction (PPI) in the stress group. We found enhanced mPFC connectivity to both angular gyri (left: $P_{SVC} = .051$, k = 31, T = 3.21; right: $P_{SVC} = .081$, k = 11, T = 3.28), and the mPFC itself ($P_{SVC} = .002$, k = 132, T = 4.68) during novel learning. **B** The enhanced connectivity with the angular gyrus during novel learning was negatively correlated with performance in novel learning trials across groups ($P_{SVC} = .007$, k = 77, T = 4.27) and in the stress group alone ($P_{SVC} = .017$, k = 130, T = 3.95, data not shown). The scatter plot illustrates the correlation of mPFC-angular gyrus connectivity (anatomically defined) and performance during novel learning trials. Images are displayed at $P < 0.005$, uncorrected, for illustration purposes.

the mPFC in the integration of related information into existing neocortical representation networks (Ghosh et al., 2014; Spalding et al., 2015; Tse et al., 2011; van Buuren et al., 2014; van Kesteren et al., 2010a). For instance, rodent data showed rapid activation in the mPFC when schema-related information was successfully learned (Tse et al., 2011), and patients with mPFC lesions showed less access to prior knowledge, resulting in difficulties to benefit from prior knowledge during learning (Ghosh et al., 2014; Gilboa et al., 2009; Kan et al., 2008). In line with this crucial role of the mPFC in schema-related learning, we found that the mPFC responded more to schema-related than novel items and schema-related activity in the mPFC predicted later performance. In contrast to the mPFC, the hippocampus is particularly important to learn detailed episodic information that is incongruent with prior knowledge or novel (van Kesteren et al., 2012) and should be less involved during learning of schema-related information (Tse et al., 2011; van Kesteren et al., 2014). Accordingly, when investigating brain regions underlying schema-related learning (as compared to baseline trials) in non-stressed controls, we did not find any significant activation in the hippocampus.

However, stress tended to impair schema-related mPFC activity and, within the stress group, increased hippocampal activity for the related hierarchy was associated with impaired schema-related learning. The hippocampus has previously been shown to respond to associative novelty (Köhler et al., 2005), supporting the idea that those individuals who engaged the hippocampus when presented with schema-related galaxy pairs might not have been able to make use of their schema and rather treated the information as if it was novel. Together, these findings suggest that stress might hinder successful schema-related learning by impairing mPFC functioning and aberrant hippocampal processing. Our results further suggest that the stress hormone cortisol may be mediating this stress effect, in line with recent findings showing that hydrocortisone administration is sufficient to hamper schema-related learning (Kluen et al., 2017).

In line with the hypothesis of reduced hippocampal involvement in learning schema-related information (van Kesteren et al., 2010a; van Kesteren et al., 2012), our results suggest that the hippocampus is less functionally connected to brain regions involved in schema processing when control participants are presented with information that relates to their prior knowledge. Supposedly, this information is rapidly integrated into the neocortical network by the mPFC without the need for strong hippocampal involvement (Tse et al., 2007, 2011; van Kesteren et al., 2012). Importantly, our results show that stress led to a strong coupling between the hippocampus, angular gyrus, and mPFC when stressed individuals were presented with schema-related information. As the hippocampus is crucial to encode detailed episodic memories (Eichenbaum, 1999), this episodic encoding might be hindering memory encoding when sufficient prior knowledge is present to encode the information more rapidly in the neocortex (van Kesteren et al., 2012). However, other studies reported enhanced coupling between mPFC and hippocampus to be beneficial when acquiring conceptual knowledge (Kumaran et al., 2012), suggesting that the exact relationship between mPFC-hippocampal interaction and learning may differ depending on, for instance, schema richness or the precise definition of schema-unrelated, novel trials (Gilboa and Marlatte, 2017).

Interestingly, we found stress-induced alterations in brain connectivity not only when participants encoded schema-related information, but also when presented with information for which they had no prior knowledge. In particular, connectivity between the mPFC and the angular gyrus was enhanced when stressed participants encoded novel information (compared to baseline trials) and this enhanced connectivity was detrimental to the successful acquisition of novel items. In contrast, there was no significantly enhanced mPFC connectivity with the angular gyrus during novel learning trials compared to baseline trials in the control group. Together, these findings indicate that stressed participants were less able to select the relevant set of brain regions depending on the presence or absence of relevant prior knowledge, which in turn deteriorated performance. Moreover, our findings show that an activation of brain regions involved in schema-related learning is not beneficial per se but can even be detrimental to learning if activated in the absence of relevant prior knowledge. However, with more practice or a longer period of consolidation, a new schema for the novel items may be built, which could be accompanied by changes in mPFC and angular gyrus activity and connectivity that are beneficial for performance (Bontempi et al., 1999; Takashima et al., 2006; Wagner et al., 2015).

Another interesting aspect of our results is that, while we often found bilateral activations and connectivity patterns, we sometimes obtained activation in or connectivity with structures in one hemisphere only. Particularly, we found that schema-related activity in the left hippocampus was associated with impaired schema-related learning in the stress group which is in line with previous reports showing that memory related activity in the left hippocampus was negatively related to congruency (or schema-relatedness), and congruency in turn was associated with better memory (van Kesteren et al., 2013). Additionally, schema-related connectivity of the hippocampus was somewhat stronger in the left hemisphere (note, however, that also the seed was in the left hemisphere), which might be related to previous findings suggesting that the left angular gyrus recombines consolidated schema components into one memory representation when learning new, schema-related information (Wagner et al., 2015). However, it is important to note that we did not assess laterality specifically, i.e., whether activation in or connectivity with a given structure was stronger than activity in or connectivity with the corresponding structure in the other hemisphere. Moreover, laterality is known to depend strongly on task characteristics and the baseline used (Harrington et al., 2006) and is therefore usually assessed using multiple tasks (Seghier, 2008). More research is thus needed to clarify potential lateralization processes in schema-related learning.

In contrast to previous findings (Kumaran, 2013), we did not find beneficial effects of a schema on inference performance. One possible explanation for this discrepancy is the difference in the extent of training on day 1. In the study by Kumaran (2013), participants were more extensively trained (120 learning trials and 120 inference trials) whereas the current project encompassed only 75 trials each due to practical limitations. In line with that reasoning, another recent study (Kluen et al., 2017, experiment 1) was not able to replicate the schema-effect on inference performance. Interestingly, the second experiment of this study that tested a larger sample did find the schema-effect on inference performance (Kluen et al., 2017, experiment 2). Thus, it might be that the ameliorating effect of a schema on inference performance depends on very thorough schema training and may need more statistical power to be detected.

Conclusion

Successful integration of new information into prior knowledge depends on the functional integrity of the mPFC, closely interacting with the angular gyrus and the precuneus to encode schema-related information. The present study suggests that individuals with strong cortisol responses to stress display less mPFC activity during encoding of schema-related information and that their performance is deteriorated if they rely on the hippocampus instead. Moreover, we show that stress reduces the separation between brain connectivity patterns for learning related and novel information, respectively, which was associated with impaired memory performance. The present findings go significantly beyond prior studies showing effects of stress on memory encoding, consolidation or retrieval, which did not take into account the individual background of established prior knowledge. The present study may thus be relevant with respect to distorted memory processes in patients with stress-related mental disorders (Ehlers and Clark, 2000) which often involve strong negative schemas (Beck, 2008; Beck and Clark, 1997). Moreover, a better understanding of how stress might impair learning in general (Vogel and Schwabe, 2016) and schema-related learning in particular may have critical implications for educational contexts, in which stress is common

(Valizadeh et al., 2012) and the use of prior knowledge during learning is a key factor for successful performance.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix C

Noradrenergic Stimulation Impairs Memory Generalization in Women

Kluen LM, Agorastos A, Wiedemann K and Schwabe L (2017) Journal of Cognitive Neuroscience

Noradrenergic Stimulation Impairs Memory Generalization in Women

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Abstract

■ Memory generalization is essential for adaptive decision-making and action. Our ability to generalize across past experiences relies on medial-temporal lobe structures, known to be highly sensitive to stress. Recent evidence suggests that stressful events may indeed interfere with memory generalization. Yet, the mechanisms involved in this generalization impairment are unknown. We tested here whether a pharmacological elevation of major stress mediators—noradrenaline and glucocorticoids—is sufficient to disrupt memory generalization. In a double-blind, placebo-controlled design, healthy men and women received orally a placebo, hydrocortisone, the α2-adrenoceptor antagonist yohimbine that leads to increased noradrenergic stimulation, or both drugs, before they completed an associative learning task probing memory generalization. Drugs left learning performance intact. Yohimbine, however, led to a striking generalization impairment in women, but not in men. Hydrocortisone, in turn, had no effect on memory generalization, neither in men nor in women. The present findings indicate that increased noradrenergic activity, but not cortisol, is sufficient to disrupt memory generalization in a sex-specific manner, with relevant implications for stress-related mental disorders characterized by generalization deficits. ■

INTRODUCTION

Networks of semantic knowledge are built from specific episodic experiences (Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila, 1999). These experiences are encoded by the hippocampus as separate representations (Kirwan & Stark, 2007; Leutgeb, Leutgeb, Moser, & Moser, 2007), thus allowing details of a single event to be remembered. However, the content of experiences is often similar, and such overlap provides a basis for generalizing across discrete episodic experiences to create more abstract, semantic representations. The hippocampus is thought to enable memory generalization by integrative encoding of episodes into a linked network of mnemonic nodes (Shohamy & Wagner, 2008) and by creating flexible memory representations that allow associative inference processes (Heckers, Zalesak, Weiss, Ditman, & Titone, 2004; Preston, Shrager, Dudukovic, & Gabrieli, 2004; Eichenbaum, 2000). The capacity to generalize across experiences is critically dependent on this flexibility of hippocampal memory. Other brain areas, such as the dorsal striatum, may support memory performance after hippocampal damage (Packard & McGaugh, 1996; McDonald & White, 1994), yet nonhippocampal memory lacks the flexibility that is required to generalize across discrete events (Myers et al., 2003; Collie, Myers, Schnirman, Wood, & Maruff, 2002).

Stress has a major impact on hippocampal functioning (Kim & Diamond, 2002; Lupien & Lepage, 2001). Although the effects of stress on hippocampal neuroplasticity and encoding are complex (Schwabe, Wolf, & Oitzl, 2010; Joels & Krugers, 2007; Diamond, Park, & Woodson, 2004), several studies reported impaired hippocampal memory processes after stress (Schwabe, Bohringer, & Wolf, 2009; Diamond et al., 2006). Moreover, stress has been shown to promote a shift from hippocampal to dorsal striatal control of memory (Schwabe & Wolf, 2012; Schwabe et al., 2007; Kim, Lee, Han, & Packard, 2001). Both of these effects are mediated by hormones and neurotransmitters that are released in response to stressful encounters, in particular, glucocorticoids (mainly cortisol in humans) and noradrenaline (Schwabe, Tegenthoff, Hoffken, & Wolf, 2010, 2013; Kukolja, Klingmuller, Maier, Fink, & Hurlemann, 2011; Roozendaal, McReynolds, et al., 2009; Roozendaal, Hui, et al., 2006; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006; Packard & Wingard, 2004). These stress mediators may alter hippocampal functioning directly or indirectly via increased amygdala activation (Diamond et al., 2006; Roozendaal, Okuda, Van der Zee, et al., 2006; Kim & Diamond, 2002; McGaugh, Cahill, & Roozendaal, 1996; Joels & De Kloet, 1989). If memory generalization relies on the hippocampus and this area is (directly or indirectly) affected by stress, one may predict that stress can interfere with memory generalization processes. Indeed, very recent evidence from our lab suggests that stress hinders memory generalization (Dandolo & Schwabe, 2016). However, the mechanisms underlying the stress-induced generalization deficit are still largely unknown. In particular, it is unclear whether the concerted activity of glucocorticoids and noradrenaline is required to impair memory

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generalization or whether the action of one of these stress mediators is sufficient to produce this effect.

Moreover, there is accumulating evidence showing that the influence of stress and major stress mediators such as glucocorticoids and noradrenaline on learning and memory may differ in men and women (Felmingham et al., 2010; Andreano & Cahill, 2006, 2009; Buchanan & Tranel, 2008). For instance, acute stress enhanced hippocampal spine density in male rats but reduced hippocampal spine density in female rats (Shors, Falduto, & Leuner, 2004; Shors, Chua, & Falduto, 2001). Similarly, stress shortly before the acquisition of a hippocampus-dependent spatial task impaired memory in women but not in men (Guenzel, Wolf, & Schwabe, 2014). Given that men and women differ in their prevalence of stress-related mental disorders, in which distorted memory is prominent (McLean, Asnaani, Litz, & Hofmann, 2011; Olff, Langeland, Draijer, & Gersons, 2007; Altemus, 2006), such sex differences in the impact of stress and stress hormones on memory are highly relevant. Whether stress hormones have a differential impact on memory generalization in men and women is unknown.

In this study, we employed pharmacological manipulations to examine the impact of elevated glucocorticoid and noradrenergic activity on memory generalization in men and women. Healthy participants received either a placebo, hydrocortisone, the α2-adrenoceptor antagonist yohimbine leading to increased noradrenergic stimulation, or both drugs before they completed an associative learning task probing memory generalization. In this task, participants first learned associations between antecedent and consequent stimuli and were then required to generalize across the learned associations to respond correctly to new items (Myers et al., 2003). The task design and experimental setup was comparable to our previous study (Dandolo & Schwabe, 2016), except for differences in the treatment testing interval due to the different experimental manipulations. On the basis of evidence showing that both glucocorticoids and noradrenaline may affect hippocampal functioning (Marzo, Bai, & Otani, 2009; Joels, Pu, Wiegert, Oitzl, & Krugers, 2006; Almaguer-Melian et al., 2005; Kim & Diamond, 2002; Katsuki, Izumi, & Zorumski, 1997) and that stress-induced elevations in both autonomic arousal and cortisol were correlated with impaired generalization (Dandolo & Schwabe, 2016), we predicted that both hydrocortisone and yohimbine would be sufficient to disrupt memory generalization. Because stress effects on hippocampal memory appear to be stronger in women than in men (Guenzel et al., 2014; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001), we further hypothesized that the impact of hydrocortisone and yohimbine should be particularly pronounced in women.

METHODS

Participants

We tested 103 healthy, young volunteers (52 women; age ($M \pm SEM$): 24.79 ± 0.36 years) who were screened for the following exclusion criteria: self-reported BMI below 19 or above 27 ($M \pm SEM$: 22.79 ± 0.19 kg/m^2), lifetime history of any neurological or mental disorders, medication intake within the 4 weeks before participation, tobacco or drug use or intake of hormonal contraceptives in women. Participants were further screened for hydrocortisone intolerance, cardiovascular disorders, including low and high blood pressure, diabetes, as well as related disorders. Female participants were not invited for participation during their menses. Participants gave written informed consent before testing and received a compensation of €35 after completing the study. This sample is part of a larger project on stress hormones and cognition, which was approved by the ethics committee of the Hamburg Medical Association.

Experimental Design and Procedure

We used a double-blind, fully crossed, placebo-controlled, between-subject design with the factors noradrenergic stimulation (placebo vs. yohimbine) and cortisol (placebo vs. hydrocortisone), resulting in four experimental groups to which participants were randomly assigned (n = 12–13 men and 12–14 women per group). To control for the diurnal rhythm of cortisol, all testing took place in the afternoon, between 12:30 and 19:00.

Physiological and Subjective Measures

After their arrival at the lab, participants completed the Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), the Trier Inventory for Chronic Stress (TICS; Schulz & Schlotz, 1999), and the State-Trait Anxiety Inventory (Spielberger, Sydeman, & Maruish, 1994) to control for depression, chronic stress, as well as state and trait anxiety. Next, measures of subjective mood (German Mood Scale [MDBF]; Steyer, Schwenkmezger, Notz, & Eid, 1994) were taken together with blood pressure measurements, using an Omron blood pressure measuring device (Omron Healthcare Europe BV, Hoofddorp, The Netherlands), as well as salivary cortisol samples. These measures were taken again 45 and 70 min after pill intake as well as after task completion (85 min after medication intake) to validate the action of the drugs. Saliva samples were collected using Salivette collection devices (Sarstedt, Germany). After collection, saliva samples were stored at −18°C. At the end of the experiment, free concentrations of cortisol were analyzed from these saliva samples using a luminescence assay (IBL, Hamburg, Germany; intra- and interassay coefficients of variance were below 10%).

Pharmacological Manipulation

Participants received orally either a placebo, 20 mg hydrocortisone, 20 mg yohimbine (an α2-adrenoceptor-antagonist leading to increased noradrenergic stimulation), or both drugs. The different pills looked identical, and

neither the participants nor the experimenter knew about the pill contents (double-blind). Both timing of administration as well as the administered dosages were chosen according to previous studies (Henckens, van Wingen, Joels, & Fernandez, 2010; Schwabe, Tegenthoff, et al., 2010; Buchanan & Lovallo, 2001).

Acquired Equivalence Paradigm

About 70 min after pill intake, participants completed a computerized version of an associative learning task with increasing difficulty, which was adapted from Myers and colleagues (2003) and served to assess memory generalization processes. In this task, participants saw differentially colored fish (consequent stimulus), each paired with a specific individual (antecedent stimulus; Figure 1), differing in age, sex, and hair color. There was a total of eight fish and eight individuals. Participants were requested to learn which fish belongs to which individual. The task consisted of an acquisition phase and a generalization phase. During the acquisition phase, participants saw one individual and two differently colored fish in each trial. They were asked to indicate by button press on a keyboard which fish belonged to the individual presented. Once participants selected a fish, a frame was placed around the fish and feedback was given about the correctness of their answer. The acquisition phase consisted of three stages: "shaping," "equivalence training," and "new consequents." During the shaping stage, participants learned four pairings between individual (antecedent stimulus) and fish (consequent stimulus). In the equivalence training stage, immediately following the shaping stage, four new individuals were presented in addition to the already familiar ones. These four new individuals were paired with the already presented fish, thus forming equivalences between two of the individuals (i.e., these individuals were associated with the same fish), which always shared two features (sex and hair color) to facilitate equivalence learning. The "new consequents" stage comprised again the four individuals shown during the shaping stage as well as the four individuals shown during the equivalence training stage; this time, however, the four individuals from the shaping stage were shown with new consequent stimuli (fish), indicating that each individual can be associated with two different fish. During each stage, a total of 24 new trials was introduced and presented together with the trials from the preceding stage. The acquisition phase was followed by the generalization phase, during which all 72 old trials from the acquisition phase were randomly intermixed with 24 new trials. In these new trials, individuals from the second stage of the acquisition phase were presented each with two of the new fish that were shown during the third acquisition stage. Thus, participants were required to use the equivalences learned in the "equivalence learning" stage to predict correctly which fish belongs to the shown individual. In the generalization phase, no feedback was given to avoid new learning effects. The generalization phase can therefore be utilized to test whether participants were able to generalize the already learned associations to new trials not shown during the acquisition phase (Myers et al., 2003; Collie et al., 2002). To further quantify participants' ability to generalize already learned associations to new stimulus pairs, a generalization score was calculated (Dandolo & Schwabe, 2016). This generalization score was calculated by subtracting the percentage correct of the 24 old trials that were presented in Stage 3 of the acquisition phase from the percentage of correct responses in the 24 new trials plus a constant of 100. The generalization score is an indispensable measure of memory generalization as it takes the interdependence of initial learning and subsequent memory generalization into account and allows us to disentangle participants' memory for the learned associations from their ability to generalize these memories to novel situations.

Statistical Analyses

Physiological and subjective parameters were analyzed using mixed-design ANOVAs with Time point of measurement as within-subject factor and Noradrenergic stimulation (placebo vs. yohimbine), Cortisol (placebo vs. hydrocortisone), and Sex (male vs. female) as between-subject factors. Performance in acquisition trials was analyzed using mixed-design ANOVAs with the Stage of the acquisition phase as within-subject factor and noradrenergic stimulation (placebo vs. yohimbine), Cortisol (placebo vs. hydrocortisone), and Sex (male vs. female) as between-subject factors. Performance in the test trials was analyzed using a univariate ANCOVA with Performance in the new trials as dependent variable and Noradrenergic stimulation (placebo vs. yohimbine), Cortisol (placebo vs. hydrocortisone), and sex (male vs. female) as independent variables. The percentage correct of the 24 old trials that were presented in Stage 3 of the acquisition phase was added as a covariate to account for initial learning performance. Generalization performance was analyzed further, using a univariate ANOVA, with the Generalization score, the critical index of memory generalization, as dependent variable and Noradrenergic stimulation (placebo vs. yohimbine), Cortisol (placebo vs. hydrocortisone), and Sex (male vs. female) as independent variables. Greenhouse–Geisser correction was applied, if required. All reported p values are two-tailed.

RESULTS

Manipulation Check

Before pill intake, systolic blood pressure and cortisol levels were comparable between groups (all $F \leq 1.921$, all $p \geq .169$; Figure 2). Diastolic blood pressure was slightly increased in the yohimbine groups at baseline ($F(1, 95) = 4.003$, $p = .048$, $\eta^2 = .040$). Subjective measures were

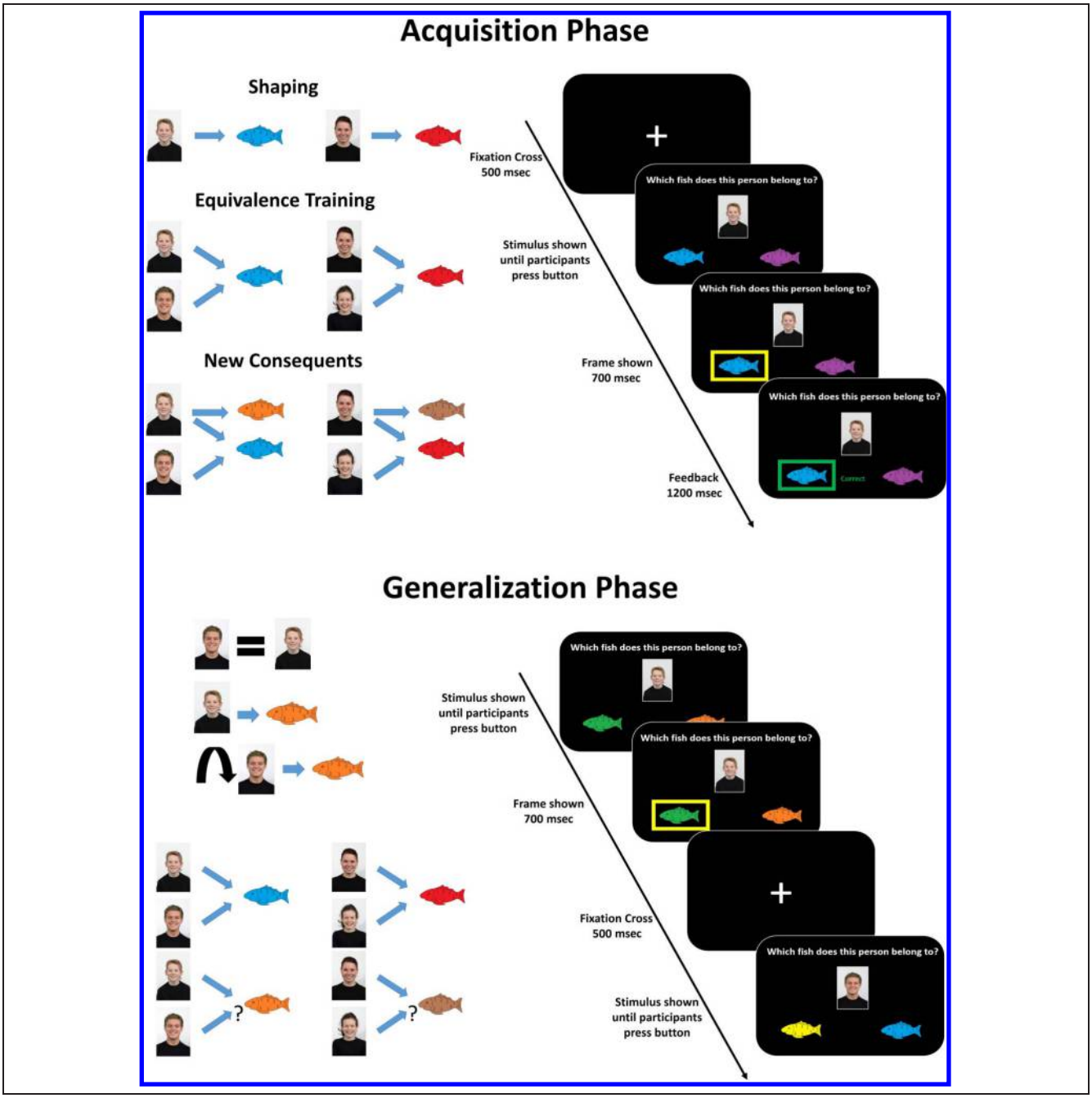

Figure 1. Associative learning task. The task has been adapted from Myers et al. (2003) and consists of two phases, the Acquisition Phase and the Generalization Phase. The Acquisition Phase comprises three stages during which participants learn the relationships between stimuli as well as equivalences. In the first phase ("shaping"), participants learn four pairings between antecedent (individual) and consequent (fish) stimuli (we show only two examples). In the second stage ("equivalence training"), four new antecedent stimuli were shown in addition to the already familiar ones. The new individuals (antecedent stimuli) were associated with the same fish (consequent stimuli) as one of the already familiar individuals, thereby creating equivalences. In the third stage ("new consequents"), the individuals from the shaping phase were associated with one additional new fish each, thus showing that one individual can be associated with two different fish. In each trial, participants were shown one antecedent stimulus (individual) and two fish and were asked to indicate, by button press, which fish is associated with the individual shown. During the acquisition phase, participants always received feedback after their answer. In the generalization phase, immediately following the third acquisition stage, participants were required to utilize their knowledge about the individual–fish associations from the previous trials and generalize across new trials. During these new trials, individuals that were introduced in the second acquisition phase were shown with two of the fish that were newly presented in the third acquisition stage. Participants were required to utilize the equivalences learned in the second acquisition stage. This time, participants also did not receive feedback after they provided an answer for each trial.

not significantly different between groups before pill intake (all $F \leq 3.324$, all $p \geq .071$; see Table 1).

Over time, there was a significant increase in systolic and diastolic blood pressure in participants who had received yohimbine (Time point of measurement × Noradrenergic stimulation: both $F \geq 7.392$, both $p \leq .001$). Forty-five minutes after pill intake as well as before the start of the associative learning task, participants who had received yohimbine showed significantly increased systolic and diastolic blood pressure, compared with those who had not received yohimbine (all $F \geq 18.166$, all $p \leq .001$). There was, however, no effect of hydrocortisone on blood pressure (Time point of measurement × Cortisol: all $F \leq 2.341$, all $p \geq .084$; see Figure 2).

Conversely, hydrocortisone intake led to a significant increase in salivary cortisol (Time point of measurement × Cortisol: $F(1.532, 145{,}539) = 47.842$, $p < .001$, $\eta^2 = .335$), whereas there was no effect of yohimbine intake (all $F \leq 2.301$, all $p \geq .117$). Forty-five minutes as well as 70 min after pill intake, participants that had received hydrocortisone showed significantly increased cortisol levels, compared with those who had not received hydrocortisone (both $F \geq 72.131$, both $p \leq .001$).

Subjective measures showed increases in negative mood as well as restlessness over time for participants who had received yohimbine (all $F \geq 2.779$, all $p \leq .050$; see Table 1), whereas there was no effect of hydrocortisone on participants' subjective assessments, neither

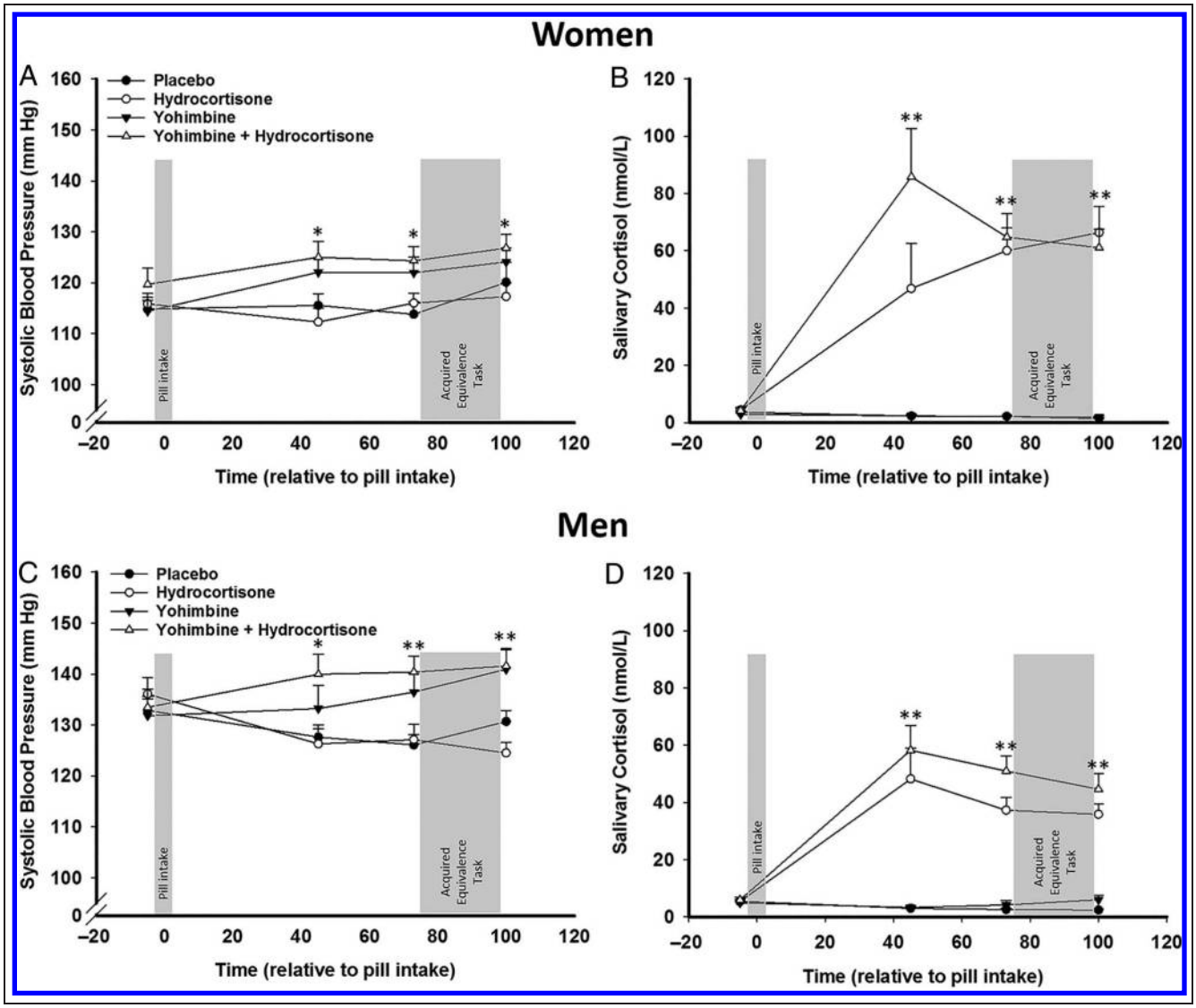

Figure 2. Effectiveness of the pharmacological manipulation in men and women. In response to administration of yohimbine alone or in combination with hydrocortisone a significant increase in (A) systolic blood pressure in women and (C) men was observed, compared with participants who received a placebo treatment or hydrocortisone alone. Hydrocortisone administration alone or in combination with yohimbine led to a significant increase in salivary cortisol levels in (B) women and (D) men, compared with participants who received either a placebo or yohimbine alone. Gray bars show time point of drug administration as well as the timing of the associative learning task. Error bars indicate *SEM*. Significances indicate the increased blood pressure in both yohimbine groups (A and C) as well as increased cortisol levels in participants that have received hydrocortisone alone or combination (B and D). *$p < .05$, **$p < .001$.

Table 1. Subjective Measures of Stress in All Groups

	Men				*Women*			
	Placebo	*Hydrocortisone*	*Yohimbine*	*Hydrocortisone + Yohimbine*	*Placebo*	*Hydrocortisone*	*Yohimbine*	*Hydrocortisone + Yohimbine*
Mood Questionnaire								
Subjective mood								
Before pill intake	34.15 (1.31)	32.83 (1.04)	35.08 (0.76)	36.15 (0.85)	34.93 (0.87)	33.62 (1.28)	34.92 (1.13)	34.00 (1.94)
45 min after pill intake	34.08 (1.21)	33.25 (1.02)	35.31 (1.02)	34.08 (1.53)	35.36 (0.67)	33.62 (1.35)	31.42 (1.91)	32.92 (1.96)
70 min after pill intake	33.54 (1.35)	32.92 (1.09)	34.92 (0.91)	33.00 (1.67)	32.93 (1.38)	33.31 (1.36)	31.50 (1.64)	32.00 (2.10)
85 min after pill intake	33.15 (1.55)	33.08 (1.24)	34.23 (0.91)	33.62 (1.30)	33.00 (1.70)	33.46 (1.10)	32.42 (1.87)	32.15 (2.41)
Wakefulness								
Before pill intake	32.15 (1.74)	30.00 (1.35)	31.92 (1.22)	32.85 (1.63)	30.07 (1.29)	28.62 (1.67)	32.50 (1.32)	31.15 (1.41)
45 min after pill intake	32.69 (1.74)	28.50 (1.66)	30.69 (1.21)	30.69 (1.32)	28.50 (0.96)	26.85 (1.81)	28.00 (1.71)	29.77 (1.82)
70 min after pill intake	32.46 (2.07)	29.75 (1.69)	29.77 (1.71)	30.92 (1.50)	25.21 (1.22)	26.08 (1.86)	27.58 (1.59)	25.92 (2.03)
85 min after pill intake	33.38 (1.69)	29.00 (1.58)	28.77 (1.70)	30.62 (1.62)	26.57 (1.11)	27.46 (1.65)	27.83 (1.47)	27.62 (2.32)
Restlessness								
Before pill intake	33.08 (0.96)	29.33 (1.34)	35.08 (0.72)	34.92 (1.03)	32.71 (1.16)	31.69 (1.04)	31.67 (1.47)	31.46 (1.90)
45 min after pill intake	34.00 (1.08)	32.75 (0.95)	34.15 (1.80)	32.08 (2.06)	32.71 (1.02)	31.69 (1.29)	28.08 (2.63)	26.85 (2.55)
70 min after pill intake	33.69 (1.18)	31.58 (1.20)	33.38 (1.21)	32.08 (1.97)	31.71 (1.72)	32.00 (1.03)	28.75 (2.29)	28.69 (2.36)
85 min after pill intake	33.08 (1.25)	30.25 (1.39)	32.85 (1.42)	33.31 (1.38)	30.93 (1.47)	31.00 (0.93)	29.75 (2.73)	29.38 (2.05)

Data represent mean scores in each category (*SE*). Higher values in subjective mood represent elevated mood, wakefulness, and reduced restlessness.

alone nor in combination with yohimbine (all $F \leq 1.537$, all $p \geq .211$).

Overall, men showed higher systolic blood pressure, wakefulness, and restlessness but lower cortisol levels compared with women (all $F \geq 5.440$, all $p \leq .022$). Importantly, however, the pattern of the physiological responses to hydrocortisone and yohimbine was very similar in men and women. Both men and women showed significant increases in systolic blood pressure and cortisol levels over time after intake of yohimbine and hydrocortisone, respectively (all $F \geq 3.554$, all $p \leq .026$; Figure 2).

Intact Acquisition Performance after Yohimbine and Hydrocortisone Intake

Participants learned the antecedent–consequent pairs very well, as reflected in an average performance of about 87% correct responses. We did not obtain a significant main effect of Acquisition stage, as participants' performance was high in all three acquisition stages ($F(1.724, 163.748) = 2.055$, $p = .138$, $\eta^2 = .021$). Acquisition performance was unaffected by drug treatment and comparable in men and women (all $F < 1.411$, all $p \geq .247$; see Table 2), which may, however, also be related to the fact that performance levels were overall relatively high.

Noradrenergic Arousal Disrupts Memory Generalization in Women

In the generalization phase, participants were required to generalize trained equivalences from the acquisition phase to new stimulus pairs. Increased noradrenergic arousal after yohimbine intake had a significant impact on memory generalization, when initial acquisition performance was

Table 2. Percentage Correct Responses in the Acquisition Stages and Generalization Phase in All Groups

	Men				*Women*			
	Placebo	*Hydrocortisone*	*Yohimbine*	*Hydrocortisone + Yohimbine*	*Placebo*	*Hydrocortisone*	*Yohimbine*	*Hydrocortisone + Yohimbine*
Acquisition Stage								
"Shaping phase"	81.73 (3.48)	88.89 (2.63)	88.14 (2.05)	86.22 (2.23)	86.61 (3.33)	82.37 (4.84)	87.50 (3.48)	88.46 (2.80)
"Equivalence training"	90.39 (1.77)	85.42 (2.10)	90.22 (2.76)	87.50 (2.34)	89.88 (1.60)	85.74 (4.74)	88.37 (1.98)	88.46 (2.28)
"New consequents"	85.79 (2.44)	86.34 (1.71)	88.03 (2.07)	87.82 (1.30)	88.49 (1.67)	83.65 (3.21)	87.15 (2.55)	87.8 (1.50)
Generalization Phase								
Old "shaping phase"	95.51 (1.45)	94.79 (2.18)	91.99 (3.82)	94.55 (1.97)	95.24 (1.68)	91.99 (3.04)	93.06 (2.20)	93.91 (1.86)
Old "equivalence training"	94.23 (2.14)	94.10 (2.26)	94.87 (1.58)	93.59 (1.30)	93.75 (2.04)	91.35 (3.73)	92.71 (1.55)	95.19 (1.69)
Old "new consequents"	88.46 (3.41)	87.85 (2.78)	86.22 (4.41)	88.78 (2.61)	83.93 (3.68)	80.13 (6.45)	88.19 (3.87)	85.90 (3.00)
New trials	70.83 (4.62)	72.92 (3.86)	77.24 (5.65)	78.53 (4.37)	83.93 (3.80)	76.92 (5.22)	77.43 (5.22)	73.08 (4.57)

Data represent mean percent correct responses (*SE*).

controlled for, and this impact differed significantly between men and women (Noradrenergic stimulation × Sex interaction: $F(1, 94)$: 7.630, $p = .007$, $\eta^2 = .075$). Yohimbine, irrespective of whether administered alone or in combination with hydrocortisone, reduced performance in the new (i.e., generalization) trials in women ($F(1, 47) = 4.551$, $p = .038$, $\eta^2 = .088$; see Table 2). In men, however, yohimbine tended even to increase generalization performance, yet this effect did not reach statistical significance ($F(1, 46) = 2.874$, $p = .097$, $\eta^2 = .059$). Cortisol on the other hand did not have an effect on generalization performance (no main effect or any relevant interactions, all $F \leq .223$, all $p \geq .638$), and neither did we obtain an interaction effect of cortisol and noradrenaline in combination with sex or without (both $F \leq .180$, $p \geq .673$).

The critical behavioral index of memory generalization was the generalization score that explicitly takes the performance for the items from the "new consequents" stage into account (see Methods). Also for this generalization score, we obtained different effects of yohimbine in men and women (Noradrenergic stimulation × Sex interaction: $F(1, 95) = 8.802$, $p = .004$, $\eta^2 = .085$). As shown in Figure 3, generalization performance was reduced in women who had received yohimbine alone or in combination with hydrocortisone ($F(1, 48) = 6.019$, $p = .018$, $\eta^2 = .111$). In men, there was again a trend for increased memory generalization after yohimbine intake ($F(1, 47) = 2.966$, $p = .092$, $\eta^2 = .059$). Cortisol did not have any significant effects on generalization performance, and we did not obtain any interactive effects between cortisol and noradrenergic stimulation, nor was there an interaction with sex (all $F \leq .345$, $p \geq .558$). Furthermore, placebo-treated women showed significantly better generalization performance than placebo-treated men ($t(25) = -3.528$, $p = .002$).

Control Variables

To control for chronic stress, depressive mood, as well as state and trait anxiety, participants completed the TICS (Schulz & Schlotz, 1999), the BDI (Beck et al., 1961), and the State/Trait Anxiety Inventory (Spielberger et al., 1994) before pill intake. We did not obtain any significant group differences in these control variables (all $F < 1.787$, $p > .185$). However, overall women had a higher BDI score ($F(1, 95) = 5.752$, $p = .018$, $\eta^2 = .057$) and tended to have a higher chronic stress level ($F(1, 95) = 3.133$, $p = .080$, $\eta^2 = .032$, see Table 3). To test whether these sex differences could explain the differential responses of men and women to noradrenergic stimulation by yohimbine, we repeated our analyses with BDI or TICS scores as covariates. Our pattern of results, however, remained unaffected by these covariates, suggesting that the obtained sex differences were not due to differences in depressive mood or chronic stress level. In particular, the interaction between sex and yohimbine intake remained significant after controlling for differences in depressive mood ($F(1, 94) = 9.014$, $p = .003$, $\eta^2 = .087$) or chronic stress ($F(1, 94) = 8.201$, $p = .005$, $\eta^2 = .080$).

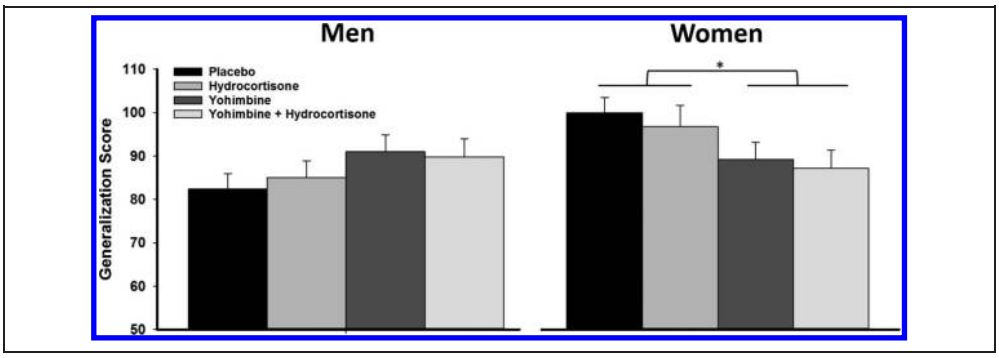

Figure 3. Impact of cortisol and noradrenaline on generalization performance. The generalization score quantifies participants' ability to generalize the acquired stimulus associations to new stimuli, with respect to their initial learning performance. A score of 100 describes an equal performance in old and new trials during the generalization phase, whereas a score below 100 illustrates a lower performance in new trials compared with old trials, pointing toward an impairment in memory generalization. Women who have received yohimbine either alone or in combination with hydrocortisone show impaired generalization performance, compared with women that have received either a placebo or hydrocortisone alone. In men, no such effect was observed. In the placebo groups, women outperformed men. Furthermore, cortisol did not affect generalization performance, neither in men nor in women. Error bars reflect *SEM*s. $*p < .05$.

Furthermore, because men tended to have higher (overall) blood pressure but lower cortisol than women, we ran our analyses again controlling for these variables. Notably, the obtained interactive influence of Noradrenergic stimulation and sex remained significant after controlling for systolic blood pressure (Noradrenergic stimulation × Sex: $F(1, 94) = 8.597$, $p = .004$, $\eta^2 = .084$), diastolic blood pressure (Noradrenergic stimulation × Sex: $F(1, 94) = 8.837$, $p = .004$, $\eta^2 = .086$), and cortisol (Noradrenergic stimulation × Sex: $F(1, 94) = 8.710$, $p = .004$, $\eta^2 = .085$). Finally, we ran our analyses again with subjective mood and restlessness as covariates. These analyses showed that these subjective changes could also not explain the differential effect of noradrenergic stimulation in men and women, that is, the Noradrenergic stimulation × Sex interaction remained (all $F \geq 7.420$, all $p \leq .008$).

DISCUSSION

Our ability to generalize across multiple discrete experiences allows us to adapt rapidly to constantly changing environments. We showed recently that this ability to generalize may be disrupted by acute stress (Dandolo & Schwabe, 2016). Here, we aimed to elucidate the role of major stress mediators (glucocorticoids and noradrenaline) in memory generalization. Our results indicate that elevated noradrenergic stimulation induced by the α2-adrenoceptor antagonist yohimbine impaired memory generalization in women, but not in men. Increased cortisol concentrations, however, did not modulate generalization performance.

Stressful events are known to be a powerful modulator of memory processes (Schwabe, Joels, Roozendaal, Wolf, & Oitzl, 2012; Sandi & Pinelo-Nava, 2007; Joels et al.,

Table 3. Control Variables in All Groups

	Men				*Women*			
	Placebo	*Hydrocortisone*	*Yohimbine*	*Hydrocortisone + Yohimbine*	*Placebo*	*Hydrocortisone*	*Yohimbine*	*Hydrocortisone + Yohimbine*
Chronic Stress								
TICS Screening Score	13.15 (2.86)	15.92 (2.22)	12.23 (2.68)	13.00 (1.66)	15.71 (2.12)	15.38 (2.10)	18.17 (2.52)	16.54 (1.99)
Depression Index								
BDI Score	5.15 (1.27)	4.42 (0.69)	4.62 (0.96)	4.08 (1.12)	6.14 (1.53)	8.08 (1.11)	5.92 (1.31)	6.00 (0.96)
State and Trait Anxiety								
State	36.08 (2.00)	36.90 (1.84)	33.08 (1.15)	32.75 (1.71)	34.62 (2.60)	33.92 (1.06)	35.55 (2.09)	35.54 (2.41)
Trait	37.23 (2.62)	38.92 (2.59)	33.62 (2.32)	32.85 (1.55)	36.64 (2.75)	39.77 (2.04)	36.64 (2.39)	33.38 (2.10)

Data represent mean scores in each category (*SE*).

2006) and noradrenaline plays a crucial role in stress-induced changes of memory. For instance, pharmacological elevations of noradrenaline levels can directly modulate memory processes (Packard & Wingard, 2004; Williams, Men, Clayton, & Gold, 1998) and a blockade of noradrenergic activation may prevent stress effects on memory (Schwabe, Hoffken, Tegenthoff, & Wolf, 2011; Schwabe, Romer, et al., 2009; Roozendaal, Okuda, Van der Zee, et al., 2006; McGaugh & Roozendaal, 2002). Our previous study showed negative correlations between autonomic nervous system activation and memory generalization performance, suggesting that (nor)adrenergic activity is also relevant for the stress-induced generalization deficit (Dandolo & Schwabe, 2016). Here, we provide direct evidence that increased noradrenergic stimulation is sufficient to disrupt memory generalization (in women). As memory generalization is thought to rely on the hippocampus (Kumaran & McClelland, 2012; Shohamy & Wagner, 2008), it might be tempting to speculate that the observed impact of yohimbine reflects a direct effect on hippocampal processing. However, we consider this interpretation relatively unlikely because there is evidence that noradrenaline rather enhances hippocampal functioning (Gray & Johnston, 1987; Stanton & Sarvey, 1985). Instead, we assume that the influence of increased noradrenergic activation was mediated by the amygdala, which then modulated hippocampal functioning (McGaugh et al., 1996). The amygdala is a primary target of noradrenergic inputs (Bouret & Sara, 2005; Jones & Moore, 1977) that is known to modulate other memory systems, including the hippocampus (Roozendaal, McEwen, & Chattarji, 2009; Roozendaal, Hui, et al., 2006; McGaugh, 2004). At the same time, recent evidence suggests that stress promotes the recruitment of a salience network, including the amygdala (Hermans, Henckens, Joels, & Fernandez, 2014), and that this recruitment of the salience network is mainly due to the action of noradrenaline (Hermans et al., 2011).

Taking the role of the amygdala into account could also provide an explanation for the observed sex differences, that is, the finding that yohimbine impaired memory generalization in women but not in men. Overall, there is substantial evidence for differences between men and women in amygdala structure and functioning (reviewed in Cahill, 2006). In particular, however, it was previously reported that the same dosage of yohimbine that we used here led to increased amygdala activation in women but to decreased amygdala activation in men (Schwabe, Hoffken, Tegenthoff, & Wolf, 2013). These differences in amygdala activation in men and women may well have resulted in a differential modulation of hippocampal functioning, translating into sex-specific effects of yohimbine on memory generalization. Such sex differences are most likely due to the action of sex hormones such as estrogen and testosterone. These hormones are known to affect limbic areas such as the hippocampus and the amygdala (Barth, Villringer, & Sacher, 2015; Matsumoto, 1991). More specifically, estrogen has been suggested to increase amygdala activity (Schiess, Joels, & Shinnick-Gallagher, 1988), and further evidence indicated that the concerted action of estrogen and noradrenaline may increase amygdala activity (McEwen & Alves, 1999; Matsumoto, 1991). Testosterone, on the other hand, has been suggested to decrease amygdala activity (Flügge, Kramer, & Fuchs, 2001). Future studies that combine pharmacological manipulations of noradrenergic and sex hormone activity are needed to test the proposed interactive influence of noradrenaline and sex hormones on memory generalization as a basis of the reported sex differences.

Whereas noradrenergic stimulation impaired memory generalization (in women), there was no effect of increased cortisol concentrations. The dose of cortisol that we administered here did also not affect amygdala processing in our previous study (Schwabe, Hoffken, et al., 2013; but see Lovallo, Robinson, Glahn, & Fox, 2010, for time-dependent effects of cortisol on amygdala resting activity), neither in men nor in women. However, glucocorticoid effects on hippocampal plasticity and activity are well documented (Henckens et al., 2010; Lovallo et al., 2010; Kim & Diamond, 2002). Moreover, we obtained previously negative correlations between stress-induced cortisol elevations and participants' capacity to generalize (Dandolo & Schwabe, 2016), although this association may have been dependent on the parallel autonomic activation as there is strong evidence that stress effects on memory processes require concurrent glucocorticoid and noradrenergic activation (Roozendaal, Okuda, de Quervain, & McGaugh, 2006; Roozendaal, Hahn, Nathan, de Quervain, & McGaugh, 2004). Yet, our present data showed also no evidence for an interactive influence of cortisol and noradrenaline. Although our results indicate that elevated glucocorticoid concentrations were not sufficient to affect memory generalization, it cannot be ruled out that they are necessary for stress-induced generalization deficits. Glucocorticoid synthesis inhibitors or pharmacological blockade of receptors for glucocorticoids could help to address this issue.

Another potential explanation for the lack of a hydrocortisone effect in the current study takes the different modes of glucocorticoid action into account. Classically, glucocorticoids were thought to exert delayed, gene-mediated effects through intracellular receptors. More recently, however, it was discovered that glucocorticoids may also act through membrane-associated receptors, inducing rapid, nongenomic effects (Joels, Sarabdjitsingh, & Karst, 2012; Karst et al., 2005). Interestingly, the rapid and delayed glucocorticoid actions have been shown to have different, perhaps even opposite, effects on hippocampal and amygdala functioning (Henckens et al., 2010, 2012; Joels et al., 2012). Although it is not fully understood yet, when exactly genomic glucocorticoid actions develop, it might well be that they are already present at about 80–100 min after hydrocortisone intake, that is, when participants performed the task. It is therefore

tempting to speculate that nongenomic and genomic glucocorticoid actions might have cancelled each other out, preventing the detection of any hydrocortisone effect.

The capacity to generalize across discrete experiences may be due to an integrative encoding process that enables later retrieval of knowledge about relations between discrete events (Shohamy & Wagner, 2008) or to an inference process during retrieval (Preston et al., 2004; Dusek & Eichenbaum, 1997). The hippocampus appears to be important for both integrative encoding and the flexible expression of memories on which inferences are based (Shohamy & Wagner, 2008; Eichenbaum, 2000). Because we administered drugs before encoding and the drugs were still active during the generalization phase, we cannot conclude whether yohimbine affected (in women) primarily encoding processes or the expression of memories (or both). The fact that we did not observe any drug effects on performance in the acquisition phase or on the retrieval of items from the acquisition phase that were tested in the generalization phase might be taken as evidence that yohimbine affected neither encoding nor simple retrieval processes but specifically the flexible expression of memories (inference) that is required for memory generalization. This conclusion, however, may be premature as there is accumulating evidence showing that, although performance may seem to be unaffected by stress or stress hormones, it is actually controlled by other, more rigid systems, the effect of which can only be seen when the flexibility of the acquired memories is probed (Schwabe & Wolf, 2009, 2012, 2013; Schwabe et al., 2007; Kim et al., 2001). Interestingly, this stress- and stress hormone-induced shift toward more rigid systems, at the expense of the hippocampus, is also mediated by the amygdala (Vogel, Fernandez, Joels, & Schwabe, 2016; Schwabe, Tegenthoff, et al., 2013; Packard & Wingard, 2004).

Stress and stress hormone effects on memory generalization processes have been reported in other studies as well. Specifically, it has been shown that stress or hydrocortisone affects the contextualization of episodic or conditioned fear memories (van Ast, Cornelisse, Meeter, Joels, & Kindt, 2013; Kaouane et al., 2012; Qin, Hermans, van Marle, & Fernandez, 2012). The conceptualization of memory generalization as reduced contextualization, however, is clearly distinct from the generalization processes examined in this study, which required participants to generalize across past experiences. Thus, although these lines of research both refer (rightfully) to memory generalization processes, the concepts differ and the findings cannot directly be related to one another. The impact of stress hormones of memory generalization or transfer processes of the individual have, to the best of our knowledge, not been tested before. The sample size of this first study examining the impact of different stress hormones on individuals' capacity to generalize across past experiences was rather moderate and future studies should test larger samples. Furthermore, future studies should include different dosages of hydrocortisone and yohimbine to test for potential dose dependencies. Including different dosages would also allow for testing whether the dosages required to modulate memory generalization capacities differ in men and women.

In summary, the present findings show that increased noradrenergic stimulation is sufficient to disrupt memory generalization in women but not in men. Memory generalization is a fundamental process that bridges discrete episodes and allows individuals to draw on past experiences to guide behavior. In addition, dysfunctional memory generalization is characteristic for several stress-related mental disorders, including schizophrenia or major depression (Gotlib & Joormann, 2010; Shohamy et al., 2010). Given that some of these disorders are much more common in women than in men, the sex differences reported here might be of particular interest. If noradrenergic activation turns out to be not only sufficient to impair memory generalization but also necessary for stress-induced generalization deficits, manipulations of noradrenergic activity, for example, by beta blockers, might be used as a tool to alleviate aberrant memory generalization in stress-related psychopathologies.

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