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Der Einfluss des Serotonintransporter-Promotor-Allels auf magnetoenzephalografische Korrelate der Angstkonditionierung

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The Influence of the Serotonin-Transporter-Promotor-Allele on Magnetoencephalographic Correlates of Fear-Conditioning

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Content

1. Introduction 1

1.1 Conditioning and Fear Condition 1

1.2 Serotonin, the Serotonin Transporter Polymorphism and Anxiety Disorders 5

1.3 Magnetoencephalography 9

1.4 ERF Components 10

1.5 Working Hypothesis and Leading Question 12

2. Materials and Methods 14

2.1 Participants 14

2.2 Stimuli and Experimental Design 14

2.3 Data Acquisition 17

2.4 Data Analysis 18

2.4.1 Rating Data 18
2.4.2 Event-Related Fields 18

3. Results 19

3.1 Rating Data 19

3.1.1 Valence 19
3.1.2 Expectancy 20

3.2 Event Related Fields 22

3.2.1 Main Effect of Conditioning on All Subjects 22
3.2.2 Genotype Group x Condition Interaction 22
3.2.3 Within Genotype Group Analysis 23
3.2.4 Within Condition Analysis 26

4. Discussion 28

4.1 Rating 28

4.2 Main Conditioning Effect 28

4.3 Conditioning x Genotype Group Interaction 29

4.3.1 Conditioning Effects within Genotype Groups 29

4.3.2 Genotype Group Effects within Conditions 32

4.4 Limitations 34

5. Conclusion 36

6. Summary 37

7. Zusammenfassung 38

8. Abbreviations 39

9. References 40

10. Appendix 49

10.1 MEG Sensor Layout 49

10.2 Significant Channels of the Genotype Group x Condition Interaction Analysis 50

10.3 Significant Channels of the Conditioning Effects Within Genotype Groups 51

10.4 Significant Channels of the Genotype Group Effects Within Conditions 52

10.5 Cluster Meanvalues and Standard Errors of the Genotype Group x Condition Interaction 53

10.6 Cluster Barplots of the Meanvalues and Standard Errors of the Genotype Group x Condition Interaction 53

10.7 Facial Stimuli 54

10.8 Previous Publication 55

11. Danksagung 56

12. Lebenslauf 57

13. Eidesstattliche Versicherung 58

1. Introduction

Learning is an essential process in human development as it not only facilitates survival and preservation of the species but also enhances the efficiency of actions and enables the creation of civilisation. Nevertheless, sometimes learning provides the reasons for the development of anxiety-related psychological disorders (Lissek et al. 2004). There is evidence that serotonin moderates psychological condition (Berger et al. 2009), and research found polymorphisms in serotonin-related alleles (Lesch et al. 1996).
Because the present MEG study discusses the influence of genetic variants of the serotonin-transporter-promoter-allele on fear conditioning, the description of recent knowledge about conditioning and its possible association with anxiety disorders is likely.

1.1 Conditioning and Fear Condition

Conditioning is one type of learning and at the beginning of the 19th century Ivan Pavlov accidently discovered conditioned reflexes on dogs. *Pavlovian* or *classical conditioning* results in the association that a neutral stimulus (NS) predicts the appearance of another stimulus (unconditioned stimulus = UCS) and thus elicits a conditioned response (CR). When the initial NS is able to evoke a CR without being paired with the UCS, it becomes the conditioned stimulus (CS) (Pavlov 1927, Maren 2001). In *fear conditioning* or *aversive conditioning* an unpleasant stimulus is used as the UCS (e.g. electric shocks or loud noise) (see figure 1).

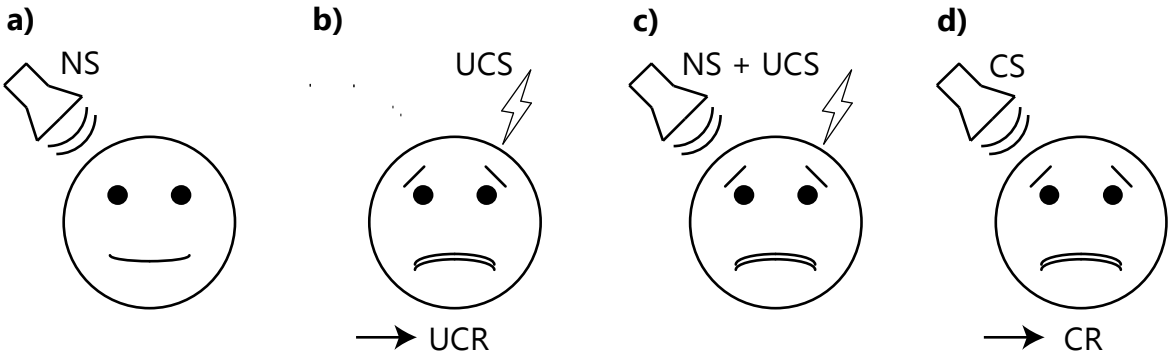

Figure 1. Fear conditioning. a) Before conditioning the neutral stimulus (NS, e.g. a tone) does not elicit a specific reaction. **b)** An aversive stimulus (unconditioned stimulus = UCS, e.g. a shock) elicits an unconditioned response (UCR, e.g. fear). **c)** During condition the NS is paired with the UCS. **d)** After conditioning, the NS becomes the conditioned stimulus (CS) and elicits a conditioned response (CR) even in the absence of the UCS.

1. Introduction

During the past 20 years, a lot of research using the techniques of electroencephalography (EEG), magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI) was done to investigate the neuronal processes underlying conditioning. Early works describe a visually elicited neuromagnetic CR in the primary somatosensory cortex, which precedes the UCS (Wik et al. 1997). Rats express conditioned fear before and during the expected UCS (Burman and Gewirtz 2004). Neuronal correlates of the CR are weaker than those of unconditioned responses (UCR, response following the UCS) (Skrandies and Jedynak 2000). Stimulus reinforcement can influence duration and amplitude of conditioned electrophysiological responses. Animal studies reveal its appearances already after two to six CS-UCS-pairings (Galambos and Sheatz 1962). Performing aversive conditioning with facial stimuli, Dolan and colleagues (2006) measured event related fields (ERFs) peaking at 150 ms preceding the typical face response at 170 ms. In line with other authors he assumes an accelerated processing of stimuli with acquired emotional value (Sams et al. 1997, Deffke et al. 2007). Source modeling suggests ventral occipital generator regions (Dolan et al. 2006), while Moratti and Keil (2009) describe conditioned differential activity in right occipital areas and bilateral supplementary motor areas. FMRI research reveals differential activation of anterior cingulate cortex (ACC) and anterior insula (Büchel et al. 1998). Auditory sensory, frontal and parietal cortical regions are involved in conditioned and amplified processing of affective tones (Bröckelmann et al. 2011). The broad range of experimental paradigms differing in stimulus type, timing procedures and measurements might cause those differences in research results.

The influence of contingency (quota of CS-UCS pairings) and the awareness of it on fear conditioned brain activity are still controversially discussed (Sehlmeyer et al. 2009). Bröckelmann and colleagues (2011) published a novel acoustic multiCS-paradigm and describe that conditioned subjects are unaware of contingencies after only six different CS-UCS combinations were used. Associative experience, rather than expectancy of an UCS, increases steady state visual evoked fields (ssVEFs, “reflect widely distributed functional networks oscillating coherently at the driving stimulus frequency and are sensitive to attentional and complex cognitive processes” (Moratti and Keil 2005)) in occipital and supplementary motor areas. Therefore, the authors underline an eased motor preparation and sensory processing even in unaware subjects (Moratti and Keil 2009). In contrast, Marschner and colleagues (2008) associate higher shock expectancy with enhanced ACC

and anterior insula activity. As anticipatory anxiety involves both areas, the linkage of CS-UCS awareness to physiological fear responses as human startle and skin conductance response is coherent (Hamm and Weike 2005). Both responses are dependent on amygdala activity but the startle response rather indexes fear learning even in unaware subjects, while changes in skin conductance only appear in aware subjects (Hamm and Weike 2005). Other authors suggest more activity in right amygdala in unaware subjects and postulate no necessity of contingency awareness for successful conditioning (Büchel et al. 1998, Büchel and Dolan 2000).

In a meta-analysis Mechias and colleagues (2010) underline a "core fear network" consisting of more occipital parts of the dorsal ACC (dACC) and dorsomedial prefrontal Cortex (dmPFC). Independent of a paradigm working with *instructed* (participants are told the contingencies of stimuli) or *classical* fear conditioning, both brain areas were activated. There is evidence for enhanced brain activity in more rostral parts of dmPFC and dACC in aware subjects compared to more posterior activation in unaware subjects. Because the rostral activity habituates, it is evaluated as "gate to consciousness" (Mechias et al. 2010). As acceleration of heart rate pattern and rather than awareness of stimulus contingency is paired with enhanced ssVEFs in visual and parietal cortex, Moratti and Keil (2005) suggest an activation of the fear system during aversive conditioning (Moratti and Keil 2005, Moratti et al. 2006).

Beyond the subject's intrinsic properties (as contingency awareness and heart rate pattern), the experimental paradigm can also influence the CR. Firstly, the time interval between the onsets of CS and UCS influences the CR (Burman and Gewirtz 2004). Whereas *delay conditioning* (UCS overlaps the CS or follows it immediately) leads to a more rapid learning, associations established by *trace conditioning* (with a gap up to many seconds between the two stimuli) are slower to be extinguished and characterized by stronger participation of working-memory mechanisms and hippocampal activity (Sehlmeyer et al. 2009). Secondly, physical stimulus features affect conditioned neuronal responses and the timing of neuromagnetic responses. Differential processing of acoustic signals with an acquired emotional value begins as early as 20-50 ms after stimulus onset and thus earlier than visual CS combined with olfactory UCS resulting in differential processing at 50-80 ms (Dolan et al. 2006, Bröckelmann et al. 2011, Miskovic and Keil 2012, Steinberg et al. 2012). Thirdly, the CS can be either a *discrete* cue (e.g. a face picture) or a *context* cue

(e.g. a test chamber). These use different pathways to reach the amygdala. Whereas context conditioning involves multisensory areas and the hippocampus in signal transduction, cue conditioning makes use of direct primary sensory areas from cortex and thalamus (Maren 2001, Alvarez et al. 2008). Seemingly, the study design regarding stimulus type of CS and UCS does not influence the activation of insula, ACC and amygdala (Sehlmeyer et al. 2009).

The amygdala, located in the anterior medial temporal lobe, has a key role in mediating discrete fear conditioning (Büchel et al. 1998, Hamm and Weike 2005, Alvarez et al. 2008, Marschner et al. 2008). It is organized in functional subsystems. The basolateral complex receives afferent cords from subcortical and cortical brain regions and channels processed information to multiple areas. The central complex interacts with the autonomic and somatic fear response systems (Büchel and Dolan 2000, Davis and Shi 2000, Maren 2001, Hamm and Weike 2005). Many authors describe a habituation of amygdala activation over time and stress its role at the beginning of the emotional association learning process (Büchel et al. 1998, Büchel and Dolan 2000, Marschner et al. 2008, Petrovic et al. 2008, Mechias et al. 2010). The modulation of amygdaloid activity is associated with plastic modifications in early conditioning stages and a role in discriminating incoming stimuli (Büchel et al. 1998, Sehlmeyer et al. 2009). When it processes information on a sub-cortical level, the amygdala is faster in encoding emotional qualities than the complex cortex network (Büchel and Dolan 2000). There is a correlation between the amygdala activity and the skin conductance response as both adapt during the conditioning process (Büchel et al. 1998, Petrovic et al. 2008). However, when the CS is paired with an aversive UCS ($CS+_{paired}$), there is no habituation in amygdala activity (Büchel and Dolan 2000). Taken together, the reported findings emphasise the involvement of the amygdala in evaluating the emotional value of the CS and its fundamental role in appropriate fear acquisition and expression.

Learning and fear conditioning serve protective purposes but too much anxiety can lead to psychiatric diseases. Disorders of anxiety and fear are linked to interferences in conditioning (Maren 2001, Gross and Hen 2004, Mineka and Zinbarg 2006). Lissek and colleagues (2004) describe the relation of fear learning to pathological anxiety. They support their arguments with the impact of exposure therapy in therapeutic treatment and the increased incidents of anxiety disorders among trauma and combat survivors. Findings

indicate elevated negative arousal to CS+ and safety cues (CS-, stimulus never paired with UCS) in anxiety patients. A higher overall fear level in patients compared to controls is concluded. Research assumes higher excitatory conditioning to threat cues and an affected inhibitory control to CS- (Lissek et al. 2004, Lau et al. 2008). The over-responding to a CS and the generalization of fear to stimuli that resemble the CS or barely relate at all (overgeneralization) might contribute to the development of pathological anxiety. Patients with panic disorders and patients with generalized anxiety disorders exhibit an overgeneralization of CS related stimuli (Lissek et al. 2010, Lissek et al. 2014).

1.2 Serotonin, the Serotonin Transporter Polymorphism and Anxiety Disorders

The neurotransmitter serotonin (5-HT) contributes to the regulation of several vegetative body functions (e.g. sleep pattern, body temperature, appetite, algesia, bowel movements(Berger et al. 2009)) and it is linked to diverse psychiatric disorders such as depression and anxiety disorders (Berger et al. 2009, Nordquist and Oreland 2010). 5-HT homeostasis is essential for the development of normal anxiety modulating circuits (Gross and Hen 2004). As one of the first neurotransmitters during embryonic development, it also acts as a neurotrophic factor for crest, heart and CNS cells. The ability of certain neurons to re-uptake serotonin from the synaptic cleft affects cortex patterning and the regulation of neuron development in rodents (Nordquist and Oreland 2010). In the central nervous system the hormone is mainly released in the raphe nucleus, while its various excitatory and inhibitory receptors can be found in many cerebral areas (e.g. amygdala, prefrontal cortex, white matter, cerebellum) (Frazer and Hensler 1999). Next to the variable 5-HT receptor activity, serotonergic effects are influenced by the serotonin transporter (5-HTT) which removes serotonin from the synaptic cleft back into the cell (Nordquist and Oreland 2010).

Heils and colleagues (1996) described a polymorphism in the promoter region of the serotonin transporter allele (5-HTTLPR). This polymorphism was found in a Guanine-Cytosine-rich area of chromosome 17q11.2 and features a long (L) and a short (S) variant. In a Caucasian population about 19% are homozygous for the S/S and 32% for the L/L variant while there are about 49% S/L carrier (Lesch et al. 1996). The deletion of 44 base

pairs leads in vitro to less transcriptional activity, less basal activity of the 5-HTT and thus to less 5-HT re-uptake (Heils et al. 1996). Post-mortem studies show an association between genotype, serotonin transporter mRNA levels and 5-HTT binding (Little et al. 1998). In vivo positron emission tomography studies do not reveal gene dependent differing 5-HTT binding in the adult midbrain (e.g. Shioe et al. 2003). However, in 2014 Little and colleagues published a longitudinal prospective study showing reduced left hippocampal volumes in adolescent S-allele carrier, which were associated with increased risks of experiencing a first onset of a major depressive disorder. They suggest that those structural differences might partly cause associations between the 5-HTTLPR and depressive disorders.

Studying a population sample, Lesch and colleagues (1996) were the first to discover a link between the polymorphism and anxiety-related neuroticism scores. The influence of genetic variations on anxiety scores and 5-HTT levels in the adult brain is still a matter of controversial discussion (Nordquist and Oreland 2010). In a sib-pair analysis, individuals with S-alleles scored higher in harm avoidance, which is an anxiety-related sub-dimension in a tri-dimensional personality questionnaire (Mazzanti et al. 1998). Other authors link S-allele carriers to higher neuroticism scores and not to higher harm avoidance scores (Greenberg et al. 2000), or link the S-allele to both qualities (Lesch et al. 1996, Hariri and Holmes 2006, Canli and Lesch 2007). Terracciano and colleagues (2009) used the same assessments as Lesch and colleagues in 1996 (NEO-PI-R = Revised NEO Personality Inventory (Mccrae and Costa 2004)) and tested about 4000 male and female homogenous participants. They reject a main effect of the polymorphism on neuroticism scores.

Nevertheless, several factors moderate this effect. Interestingly, the gender seems to influence scoring behaviour in different psychological assessment tasks. As tested with various questionnaires, male S/S carriers scored higher compared to male S/L carriers, while this pattern was reversed in female samples (Du et al. 2000, Mizuno et al. 2006). Greenberg and colleagues (2000) did not find differences between the S/L and S/S- allele carrier but supported higher neuroticism scores in S-carrier compared to homozygous L-carrier on a female sample. Next to gender differences, the missing linear association between the genetic variation and extreme scorers (Sirota et al. 1999) may partly cause the controversial influence of the polymorphism on neuroticism scores. Using a sample of 900 subjects, Sirota and colleagues (1999) compared the actual and the theoretical power to

detect associations between the 5-HTTLPR and scoring, but found a non-linear pattern in their data.

The 5-HTTLPR is not only associated with scoring behaviour but also seems to be involved in the development of anxiety disorders by affecting mood regulating brain circles. Animal experiments, which illuminate the impact of the less active transporter in S-carriers with 5-HTT knockout mice, indicate a down-regulation of inhibitory 5HT1A receptors and an up-regulation of the excitatory 5HT2C receptor. This leads to increased depression-like behaviour, hyper-excitability, reduced 5-HT homeostasis, elevated impulsiveness and reduced aggressiveness in the knockout mice (Hariri and Holmes 2006, Nordquist and Oreland 2010).

Macaques with the less active S-allele exhibit an even more elevated emotionality when they suffered a stressful life event (SLE) like growing up motherless (Gross and Hen 2004, Nordquist and Oreland 2010). Those animals have similar modifications in brain architecture to humans (Jedema et al. 2010, Nordquist and Oreland 2010).Caspi and colleagues (2003) underline the connection between SLEs and the occurrence of a depression in human S-allele-carrier. SLEs might influence human amygdala activity and cause different effects on the polymorphism groups. Compared to non-carriers, S-allele-carriers with more SLEs exhibit stronger amygdaloidal activity for emotion-related tasks like watching negative stimuli (Heinz et al. 2000) and less activity of left amygdala during the extinction phase in an conditioning experiment (Hermann et al. 2012). Children with S-S-alleles, who suffered SLEs show impaired coping strategies (Cline et al. 2015). Duncan and Keller (2011) critically review the gene-by-environment interaction in psychiatry. The authors estimate the quote of positive replication attempts to be less than 27%. They caution against a strong publication bias and underpowered studies.

However, a review by Domschke and Dannlowski (2010) did not find a major role of the 5-HTTLPR in panic disorders, but they conclude an impact of the 5-HTT polymorphism on anxiety-related traits. The authors outline an uncoupled feedback mechanism between amygdala and ACC in S-allele-carrier. Patients with social anxiety disorders and at least one S-allele show elevated depression scores as well as increased state and trait anxiety scores, which are accompanied by increased blood flow in right amygdala. The S-allele might lead to dysfunctional emotional processing. Less grey matter connecting amygdala and sub-genual ACC (a circuit known for emotional regulation) might explain the

amygdaloidal dysregulation in S-carrier (Canli and Lesch 2007). Klucken and colleagues (2015) also describe exaggerated amygdala activity in S-carrier and conclude that the less functioning allele is associated with an elevated acquisition of fear learning. A decreased activity of amygdala and orbitofrontal cortex (OFC), and aggrieved fear learning (reduced SCR and expectancy ratings) were found in a functional magnetic resonance imaging (fMRI) conditioning experiment for subjects with reduced tryptophan levels. Because serotonin is built out of the essential amino acid tryptophan, rather the long more active 5-HTTLPR is mimicked by dietary tryptophan depletion (Attar et al. 2012).

Using fMRI to elucidate amygdala responsiveness to subliminally presented pictures of happy and sad faces, Dannlowski and colleagues (2010) describe a gene by valence interaction in S-carriers for negative stimuli only; while Belsky and colleagues (2009) show evidence that there might be "increased sensitivity" for positive stimuli as well. They mention a "for-better-and-for-worse pattern" in the S-group. It would not only lead to worse aspects of psychological disorders when the S-carrier underwent negative experiences, but also had protective effects on the S/S-allele-carriers in the absence of negative stimuli. Additionally, a recent study of Haase and colleagues (2015) associates the S-allele with stronger positive emotional expressions. In EEG studies differences in the P300 (associated with cognitive information processing (Van Dinteren et al. 2014)) and mismatch negativity (MMN: a signal, inattentively evoked by change in frequency, intensity or duration (Naatanen et al. 1993)) were found. Homozygous L-carriers exhibit a decreased P300 and increased MMN compared to S-subjects. Consequently, the high aggression index in L/L-subjects is supposed to be evoked by their elevated sensitivity and lower control (Sysoeva et al. 2009).

The combination of 5-HTTLPR with other polymorphisms that influence fear conditioning (e.g. COMTval58met, a gene coding for a dopamine-degrading enzyme) could increase the risk for the development of anxiety disorders (Lonsdorf et al. 2009, Wendt et al. 2015). However, the increased startle response and emotionality in S-allele-carrier may also lead to improved cognition. Higher activation in the ACC accompanies the "loss of control over emotion" and could facilitate better integration of feedback information leading to improved probabilistic and temporal processing (Homberg and Lesch 2011).

As with the gene-environment-interaction, the influence of the 5-HTTLPR on psychiatric disorders is doubted. A European multicenter case-control study including almost 2000

participants failed to identify a significant association between affective disorders (unipolar and bipolar) and the 5-HTTLPR (Mendlewicz et al. 2004). On the contrary, Schinka and colleagues (2004) stress the dependency of the "small but real effect" of the S-allele on anxiety and neuroticism scores on the specific psychological measurements. In addition, the majority of the mentioned inconsistencies in different studies may be due to differences in paradigm and subject criteria (Hariri and Holmes 2006, Canli and Lesch 2007).

Even though the topic is still controversial (Mendlewicz et al. 2004, Terracciano et al. 2009, Duncan and Keller 2011) the polymorphism in the serotonin transporter promoter region led to several neurophysiologic findings in the last 20 years. Because elevated neuroticism scores are linked to anxiety disorders (e.g. Bienvenu et al. 2007) and the S-allele is linked with higher ratings on those, it might be a risk factor for the development of anxiety-related diseases.

1.3 Magnetoencephalography

Learning and fear are cognitive processes and emotional sensations, which induce electrophysiological potentials and magnetic fields in the brain. An excitatory postsynaptic potential causes certain transmitters to reach the synaptic cleft and to depolarise the postsynaptic membranes of the activated neurons. Those neurons change their surface potentials by opening and closing various ion channels, which allow certain cations to enter the soma. These processes create negativity outside the apical dendrites and result in an intra- and extracellular current that is surrounded by a magnetic field (Klinke 2005). Postsynaptic signals with voltage between 100 µv and 10 mV and duration of 5 up to 100 ms can sum up spatially and in time. Cortical pyramidal cells arrange regularly with the same spatial orientation and lie perpendicular to the cortical surface. The postsynaptic potentials of many of those neurons fulfil the requirements to accumulate to a stronger signal. Still in the range of femtotesla ($fT = 10^{-15}$), and thus 100 million times smaller than the earth's magnetic field, a magnetoencephalograph is able to measure those neuronal magnetic fields outside the skull.

The device contains Superconducting Quantum Interference Devices called "SQUIDs", which are located in a helmet and cooled down to -270°C to establish the super-conduction. Magnetic fields initiate a voltage drop or even a pole reversal in the SQUIDs

(Pollok and Schnitzler 2010). The SQUIDs are either magnetometer or gradiometer. The former is made up of only one coil, which measures the absolute magnetic field. The latter suppresses electromagnetic perturbations by detecting the magnetic field gradient between two coils. In axial gradiometers, the coils are of opposing windings and aligned along the centre line, while the coils of planar gradiometer are eight-shaped (Papanicolaou 1995, Pollok and Schnitzler 2010).

Compared to neurovascular measurements like fMRI, the advantage of the neurophysiologic method is a high temporal resolution. While hemodynamic measurements are able to display the sources of activity, neurophysiological methods only allow source estimation. Because current always flows through the path of least resistance, it causes a distortion in EEG signals. Magnetic fields are independent of the tissue density and consequently have a much higher spatial resolution than EEG measurements (Hillyard and Kutas 2002). When choosing the MEG, one must consider the increased technical expense. A magnetic shielded measure chamber is necessary and there are expenses for the liquid helium to cool down the SQUIDs. Because magnetic fields run around the dipole, they can best be measured when the dipole is parallel to the scalp. Consequently, MEG is only able to detect activity in the brain sulci where the pyramidal cells lie parallel to the skull.

1.4 ERF Components

When neuronal communication is time-locked to motor, sensory or cognitive events, it leads to event-related potentials (ERPs) and the correlated event-related fields (ERFs) (Hillyard and Kutas 2002). Event-related fields represent the processing of distinct stimuli and the preparation of the subjects' response to it (Pollok and Schnitzler 2010). The averaging of many trials improves the signal-to-noise ratio and extracts the ERFs. Different components compose the ERFs and ERPs and lead to peaks and troughs in the averaged MEG and EEG signal. Commonly the components are named by their signal polarity (N or P), their spatial appearance (e.g. posterior or anterior), and time of appearance. Since the early MEG publications of Cohen in 1968, research learned a lot about the distinct brain processes, which compose the components.

When a subject receives a stimulus, it activates attentional networks. Depending on the

stimulus modality (visual, auditory or olfactory), attention enhances ERFs over frontal, parietal and sensory cortex areas (Bröckelmann et al. 2011). Auditory conditioning studies found emotional modulation of signals at early time intervals of 50-80 ms and 130-180 ms. Those appear even in the absence of contingency awareness (Steinberg et al. 2012). Visual-spatial attention involves occipital cortex areas, the inferior temporal and posterior parietal lobe. Attended stimuli enlarge the N1 (150-190 ms) component (Hillyard and Kutas 2002, Thom et al. 2014). For auditory stimuli its magnetic counterpart the N1m (100-130 ms) is located in the auditory cortex and appears earlier than for visual stimuli (Bröckelmann et al. 2011).

The early posterior negativity (EPN) appears between 120 and 350 ms over occipito-temporal sensors and is an electro-encephalographic, bilateral component, which reflects the encoding of emotional stimuli (Schupp et al. 2003, Herold 2008, Thom et al. 2014). Its magnetic counterpart (EPN-M) provides a biphasic pattern with polarity reversal for both hemispheres. It separates in an early (120-170 ms) and late (220-310 ms) time window and characterizes the visual processing stream of emotional relevant pictures. While the first interval rather covers occipito-parieto-temporal sensors, the later activation is more anterior over temporal regions. Emotional association leads to a prioritized processing of pictures (Peyk et al. 2008).

The M170 (150-200 ms) is a face specific, rather posterior response, reflecting the recognition of global face configuration and the individual face identification. It is located in bilateral areas of the temporal cortex and fusiform gyrus (Lu et al. 1991, Liu et al. 2002, Deffke et al. 2007).

EEG research supposes an involvement of the P200m in working memory processes (Wolach and Pratt 2001, Lefebvre et al. 2005, Freunberger et al. 2007). It usually appears within 180-270 ms in centro-frontal as well as in parieto-occipital areas. In visual language experiments, there is evidence that the P200 depicts item encoding and feature detection (Luck and Hillyard 1994, Shaul 2007). Compared to the auditory EEG component, sparse research has been done on the visual P200 and its magnetic counterpart. Results from auditory research on the P200m component underline its ability to adapt to contingency reversal (Kluge et al. 2011). Correct stimulus integration might be dependent on the P200.

The positive EEG component P300 with latency at about 300 ms displays higher cognitive information processing (Van Dinteren et al. 2014). As described by Johnsen (1986) in a

triarchic model, three dimensions influence the P300: (1) the subjective probability (larger amplitudes when lower target or event expectation), (2) the stimulus meaning (P300 amplitude is sensitive to task complexity, stimulus complexity and stimulus value) and (3) the information transmission (the amount of transmitted information and necessary attention). Despite a lot of research on the P300 has been done and the P300 might picture a specific aspect of cognitive processing, Luck (2005) stresses the absence of a clear consensus about the exact process that underlies the P300.

The further processing of emotional relevant and affective stimuli elicits the late positive potential (LPP) over centro-parietal sensors. Despite the positive shift can already start at 200 ms (Cuthbert et al. 2000) many authors examine the LPP between 400 and 800 ms (Schupp et al. 2003, Choi et al. 2014, Thom et al. 2014, Alomari et al. 2015, Rostami et al. 2016).

Because the present MEG study focuses on early effects of conditioning, I mentioned only components, which happen within 500 ms and might be affected by the experimental paradigm.

1.5 Working Hypothesis and Leading Question

In the previous paragraphs, I illustrated fear conditioning and its involvement in the development of anxiety disorders. With the persistence of anxious responses to a CS even when there is no CS-UCS contingency, conditioning might be associated with the development of anxiety-related disorders (Maren 2001, Lissek et al. 2004). There are questionnaires to measure the anxiety-related personality traits like neuroticism (e.g. the NEO-PI-R (Mccrae and Costa 2004). The serotonin transporter might influence those scores. Carriers of the short variant of the serotonin transporter promotor allele tend to reveal elevated scores (Hariri and Holmes 2006, Canli and Lesch 2007).

The current experiment links the known association of conditioning and anxiety disorders with the polymorphism in the serotonin transporter. With the use of MEG and a delay conditioning paradigm, the experiment aims to investigate the influence of 5-HTTLPR on the process of fear conditioning (see figure 2).

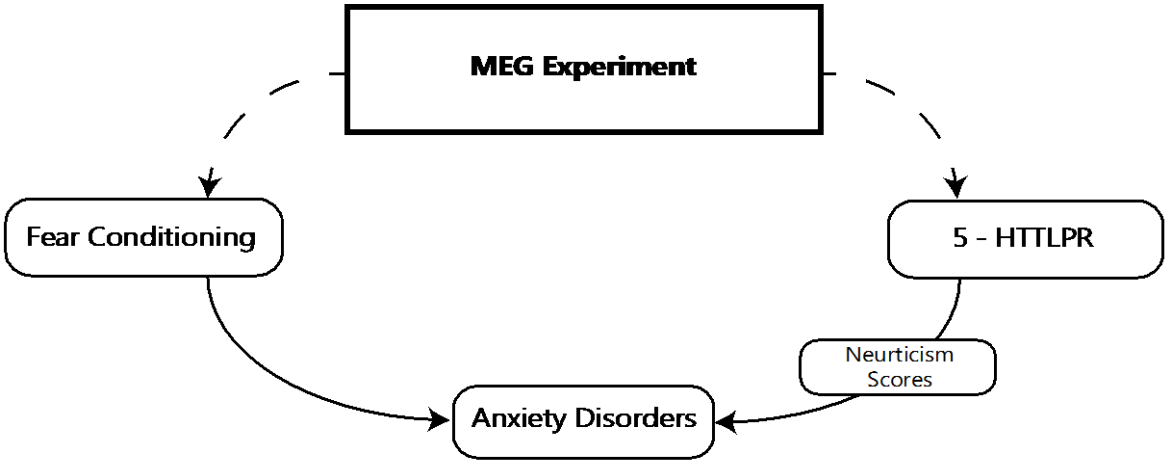

Figure 2. Placement of the present MEG study in the context to anxiety disorders (AD). Fear conditioning is involved in the development of AD. The 5-HTTLPR is supposed to influence neuroticism scores. Elevated neuroticism scores are a risk factor for the development of AD.

MEG recordings were obtained while male subjects differing in their 5-HTT gene were fear conditioned to human faces by applying electric shocks on their fingertips.

I examined the influence of conditioning and genetic variation on behavioural data.

Conditioning should lead to a more negative stimulus association to CS+ compared to CS-. In addition, valence and expectancy ratings for the stimuli might differ between the S- and L-group.

Because hemodynamic dependant research describes an enlargement of amygdala activity for affective stimuli in S-carrier (Canli and Lesch 2007, Dannlowski et al. 2010, Homberg and Lesch 2011), one could hypothesise stronger ERFs to CS+ in S-carrier compared to the L-group.

Conditioning embodies elements of memory and emotional processing (Büchel et al. 1998). Consequently, I expect differences between the genotype groups in MEG components that are involved in stimulus processing. Attention associated N1m component, components referred to emotional processing like EPN-M or LPP, or the working memory associated P200 component could differ between the groups during and after the conditioning processes.

I characterize the influence of the 5-HTTLPR on the spatial and temporal neurophysiologic correlates of fear conditioning. To my knowledge no research that used the temporal and

spatial advantages of the MEG technique was performed to enlighten the influence of 5-HTTLPR on fear conditioning. I try to extend the knowledge about the neuronal basis that underlies the vulnerability for the development of anxiety related disorders. An extension of the current understanding about the neurophysiological basis of anxiety disorders leads a step further toward its more effective prevention and treatment

2. Materials and Methods

2.1 Participants

Forty-nine male volunteers between the ages of 18 and 48 years (mean age = 28.3, SD = 5.6) participated in this study. To exclude possible gender effects on conditioning (Cahill 2006, Milad et al. 2006), only male volunteers were included. The volunteers underwent genotyping for a polymorphism in the promotor region of the serotonin transporter and were separated into S-group (SS- and SL-carrier) and L-group (homozygous LL-carrier). All of them had normal or corrected to normal visual acuity, reported no history of psychiatric disorders, their mother tongue had to be German and they had to be right-handed. Subjects were recruited from the database of the Department of Systems Neuroscience in the University Medical Centre Hamburg-Eppendorf and were paid €13 per hour for participation. All participants provided written informed consent approved by the ethics committee prior to MEG-recording.

Due to technical problems with the electrical stimulus generator, five subjects were excluded from the analysis. One further subject was excluded due to extensive artefacts caused by a metal implant in the right forefinger. Data from two more participants were discarded due to high alpha band activity caused by tiredness. Finally, I analyzed the data from 41 subjects.

2.2 Stimuli and Experimental Design

For visual stimulation six male faces with neutral facial expression from the Karolinska Directed Emotional Faces database (Lundqvist et al. 1998) were selected and presented in

pseudo-randomized order. Consequently, each face was shown 16 times per block. Using a projector (Sanyo PLC-XP51, resolution: 1024x768, refresh rate: 60 Hz) located outside the measuring chamber and a mirror system, the visual stimuli were presented on a screen, which was mounted 54 cm from the subject's face. The interstimulus interval jittered between 1100-1650 ms. A small white fixation cross, which indicated the centre point between the eyes of the presented faces, remained in the middle of the screen to avoid eye movement.

In order to find the appropriate number of stimuli for framing the process of conditioning with my experimental design, four pilot experiments with different numbers of individual faces (4, 6, 12, 20) were performed (number of participants ranged between 3 and 5). As revealed by behavioural data, the usage of six different faces induced the desired learning curve, which reflects the conditioning effect. Three distinct faces served as CS+ and CS- each and the assignment was randomized over subjects. Half of the CS+ were paired with an electric pain stimulus, which served as the UCS. The painful stimulus appeared 450 ms after visual stimulus onset and lasted 50 ms (see figure 3). For the experiment a 50% partial reinforcement strategy was applied. Consequently the conditioned stimuli were combined with the pain stimulus in 50% of their presentations ($CS+_{paired}$), whereas the other half of the CS+ trials was not combined with a pain stimulus ($CS+_{unpaired}$). The remaining three faces were never presented with a pain stimulus (CS-).

In 10% of all trials, two consecutive questions followed the face presentation to measure the expectancy of an UCS and the valence of the face. The subjects rated their sympathy ("Wie angenehm war Ihnen das letzte Gesicht?" = How pleasant did you perceive the last presented face?) and the expectation of a painful stimulus ("Wie sehr erwarteten Sie einen Schmerzreiz ?" = How much pain (if any) did you expect?) on a visual analogue scale. Using a MEG-compatible response box, the subjects could move a cross on a line towards their desired answer ("unangenehm/ angenehm" = unpleasant/ pleasant; "gar nicht/ auf jeden Fall" = not at all/ definitely). The questions never followed a $CS+_{paired}$ trial and the answers were encoded into numbers between 0 and 100.

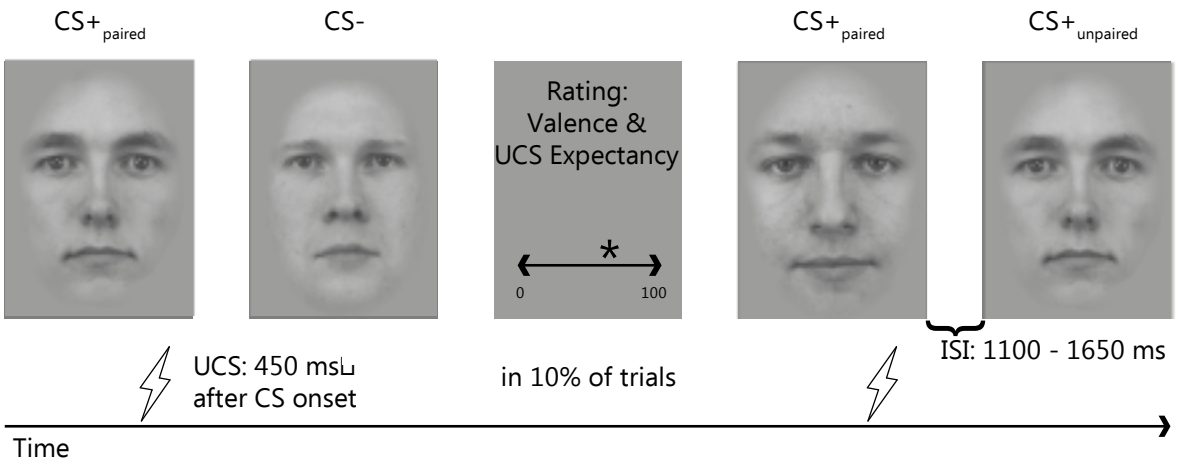

Figure 3. Schematic representation of the experimental paradigm. The facial stimuli were presented for 500 ms. 450 ms after CS onset the UCS was presented for 50 ms in the $CS+_{paired}$ trials. In 10% of the trials, the CS was followed by a rating trial. The ISI jittered between 1100 ms and 1650 ms. Abbrevations: CS, conditioned stimulus; UCS, unconditioned stimulus; ISI, interstimulus interval.

After the elucidation, the subject's individual pain threshold was determined by applying electric intracutaneous stimuli with ascending intensity of 0.01 mA steps. Participants were instructed to rate these stimuli on a scale between zero ("no sensation") and 100 ("worst imaginable pain"). On this scale, a value of forty labels the lower pain threshold. This intensity is comparable to the sensation evoked by pulling a skin hair. In order to measure the individual pain threshold, I applied a staircase procedure: the amplitude of the electrical stimulus was increased in steps of 0.02 mA until the threshold was reached for at least three consecutive stimuli; then the intensity was lowered again. The stimulus intensity was restricted to a maximum of 0.6 mA. The mean electric current reaching the pain threshold was multiplied by 1.5 to reach above-threshold stimulation and account for the disturbing effect of pain habituation. The stimulus generator (RSG0405, Ibrro) sent four pulses of 2.5 ms duration per pulse and 5 ms inter-pulse interval. After removing the upper layer of epidermis by the use of a round bur, two strips of plaster tape fixed the intracutaneous electrode on the distal phalanx of the left middle finger. The counter ring electrode was attached with electrode cream (Hellige®) to the most proximal part of the same finger.

There was an individually terminated practice run without any measurement and painful stimulation. The subjects could train the usage of the MEG- compatible response box, not to blink during face presentation and to move as little as possible.

The Experiment consisted of eight blocks, each separated by a pause screen that participants could terminate themselves. Overall, 768 visual stimuli were presented to each subject. Thus, 384 CS-, 192 $CS+_{paired}$ and 192 $CS+_{unpaired}$ stimuli were shown in random order. To ensure habituation to the experimental environment and the visual face stimuli, the first block was presented without painful stimulation. Due to the often-reported tiredness, I divided the recording session into two consecutive sessions including a 5 min break in-between from the twentieth participant onwards. Depending on the length of the breaks between the blocks, the experiment lasted about 55 minutes.

2.3 Data Acquisition

The MEG data were acquired using a 275-channel whole head magnetometer (Omega 2000, CTF Systems Inc., Port Coquitlam, Canada) with axial gradiometers. It was installed at the Institute of Neurophysiology at the University Medical Centre Hamburg-Eppendorf. An online 300 Hz low-pass filter was applied while continuously recording the data at a sampling rate of 1200 Hz. Due to technical problems with the squids, four sensors (LF21, RO11, LT52, and LT31) were excluded from the recording. Within the magnetically shielded and sound attenuated testing cabin the light and the air conditioning were switched on. The experiment was controlled using the software Presentation® (Neurobehavioral Systems, Albany, CA, USA) for visual and pain stimulation, acquisition of button presses, and timing. For offline artefact detection an electrooculogram was recorded. Therefore, two electrodes were placed above and below the eye on the musculus orbicularis occuli. Three further electrodes recorded the eye movements. One was attached between the eyebrows, two others on the left and right cheekbone. In order to detect spike potentials caused by microsaccades an occipital electrode was applied. The electrocardiogram with one electrode below the midpoint of the right collarbone and another in the midclavicular line below the left costal arch completed artefact detection. Three further coils (left and right ear, and nasion) helped to localize the head position relative to the MEG Squids. The Treatment of the skin with alcohol and abrasive gel (ABRALYT 2000, EasyCAP) before placing the electrodes minimised the noise within the signals. They were fixed with an electrode paste (EC2 electrode cream, Grass) and additional plaster strips (Durapore™, 3M Health Care).

2.4 Data Analysis

2.4.1 Rating Data

Using Matlab7.10.0 (R2010a, MathWorks, Natick, MA) the encoded answers were sorted for the two conditions and for both addressed topics (valence/ expectancy). Firstly, I averaged all values of each subject in every category and calculated the mean for each condition. In another calculation, I split the whole data sets into eight blocks (five rating trials per condition per block) and identified the mean rating of every subject for each block. Then those block values were averaged over all subjects to analyze the overall progress of the ratings (see figure 4). T-tests and a 2x2x8 ANOVA [condition (CS-/ CS+) x genotype group (short/ long) x block (1-8)] determined significant differences between conditions at 0.05 significance.

2.4.2 Event-Related Fields

Matlab7.10.0 (R2010a, MathWorks, Natick, MA, USA) and the open source toolboxes FieldTrip (Oostenveld et al. 2011, http://www.ru.nl/fcdonders/fieldtrip) were used for the analysis of the event-related fields (ERFs). Trials containing $CS+_{paired}$ stimuli were excluded from the analysis, because I was interested in studying the conditioned response and the electric stimulus caused massive artefacts.

The first step of the analysis extracted data epochs (-500 to 500 ms, around visual stimulus onset), which were baseline corrected (-100 to 0 ms) for each participant. To illustrate the influence of conditioning on the ERFs I did not include the first block (with no presentation of a painful stimulus) in the MEG analysis and finally analysed 336 CS-, 168 $CS+_{paired}$, 168 $CS+_{unpaired}$ trials.

The trials that contained artefacts due to muscle activity and eye blinks were rejected semi-automatically. Trials containing signal jumps, which exceeded the z-transformed value of 50, and those, which contained artefacts caused by passing cars were also excluded from further analysis. Artefact rejection removed on average 16.7% (range: 2.6% to 59.1%) of the trials in each subject. An extended infomax independent component analysis (weight change $<10^{-7}$ as stop criterion) reduced further artefacts such as horizontal eye movements

or electrocardiographic activity (see Schneider et al. 2008).

In a subsequent step the data were bandpass filtered between 0.5 and 170 Hz (Butterworth filter, low-pass filter order 4, high-pass filter order 3) and the noise caused by the power supply was notch filtered (50, 100, 150 Hz, Butterworth filter, filter order 4). Afterwards the sampling rate was reduced to 400 Hz.

I applied a two-step analysis to identify the regions and time of interest (Schupp et al. 2003, Bröckelmann et al. 2011) for the main conditioning effect and the genotype group x condition interaction ([(CS+ short) - (CS- short)] vs. [(CS+ long) - (CS- long)]). In a first step, I averaged event-related magnetic fields for each condition, sensor and individual, and calculated the differences ([CS+] – [CS-]). Then I performed a statistical method introduced by Guthrie and Buchwald (1991) in which point-wise running t-tests between the conditions were calculated at each sensor for the entire timeframe. To reduce type I errors, intervals of at least ten consecutive sampling points (equal to a period of 25 ms) and clusters with at least 8 neighbouring channels that met an alpha criterion of 0.05 were considered significant (Bröckelmann et al. 2011). This analysis did not find a main effect but identified two time windows of interest for the comparison of the differences (150–250 ms, 400-500 ms). In the second step, I collapsed the significant clusters according to spatial and temporal characteristics. A post hoc repeated measures 2x2 analysis of variance (ANOVA) (condition [CS-/ CS+] x genotype group [short/ long]) was calculated to characterize the effect size of the genotype group x condition interactions.

Finally, I computed grand mean topographic maps and ERFs for all conditions and groups and marked the found significant cluster (see figures 5-7).

3. Results

3.1 Rating Data

3.1.1 Valence

Grand mean valence ratings tested for a main effect of conditioning revealed a less pleasant evaluation for the CS+ (M ± SEM = 40.8 ±1.6) compared to the CS- faces (M ± SEM = 47.3 ± 1.8, $t(40) = 2.43$, $p < 0.05$ (0.02), SD = 14.76, paired sample t-test) on the

visual analogue scale (with values of 1-100). Also, the 2x2x8 ANOVA [condition (CS-/ CS+) x genotype group (short/ long) x block (1-8)] identified a main effect of conditioning on the valence rating ($F(1,40) = 6.86$, $p = 0.013$) and a condition x block interaction ($F(7,273) = 3.38$, $p = 0.002$), while genetic variation did not affect the valence ratings ($F(1,40) = 0.21$, $p = 0.65$). As shown in figure 4a, both genetic groups rated all faces nearly equal on the valence scale during the first block (CS-: short: M ± SEM = 44.8 ± 3.1, long: M ± SEM = 43.6 ± 2.0; CS+: short: M ± SEM = 42.9 ± 2.7, long: M ± SEM = 43.6 ± 2.4). The valence for the presented faces started to differ significantly in block four ($t(40) = 2.39$, $p = 0.02$) with a block mean value of 45.4 (SEM = ± 2.2) for CS- and 38.8 (SEM = 2.2) for CS+ faces. Despite the values of 47.6 (SD = ± 2.6) for CS- and 41 (SD= ±1.8) for CS+ faces the difference only tends towards significance in block seven ($t(40) = 1.91$, $p = 0.064$, SD = 22.17). After the last block the difference between the conditions is highly significant ($t(40) = 3.23$, $p = 0.0013$, SD = 18.75; CS-: M ± SEM = 51 ± 2.4; CS+: M ± SEM = 41 ± 1.9) with a valence increase for CS- and a decrease for CS+ faces.
While the genotype groups did not affect the valence ratings, analysis of the whole subject sample discovered less pleasant CS+ evaluation.

3.1.2 Expectancy

There was a higher grand mean expectancy for a painful stimulus after CS+ (M ± SEM = 47.4 ± 2.7) compared to the CS- faces (M ± SEM = 29.4 ± 3.3, $t(40) = -4.98$, $p < 0.001$ ($1.354e^{-005}$), SD = 21.74, paired sample t-test) on the visual analogue scale (values of 1-100). Again, genetic variation did not affect the rating for expectancy ($F(1,40) = 0.124$, $p = 0.727$, 2x2x8 ANOVA [condition (CS-/ CS+) x genotype group (short/ long) x block (1-8)]). Figure 4b illustrates the UCS expectancy ratings for both conditions and genotype groups, which is increasing over blocks. Whereas the expectancy of a painful stimulus is low for both conditions during the first block (CS+: M ± SEM = 11.29 ± 2.51; CS-: M ± SEM = 12.92 ± 2.80), there is an increased expectation for both conditions during the second block. This is already significantly higher for the CS+ faces ($t(40) = -4.23$, $p > 0.001$, paired t-test). The further gain of expectancy after the following third block is higher for the CS+ (M ± SEM = 50.4 ± 3.2) than for the CS- faces (M ± SEM = 33.8 ± 4.0). Within the blocks 4-8, expectancy ratings are approaching stable values for the CS-

and CS+ condition. After the eighth block, the expectation for a painful stimulus after a CS- and a CS+ differs about 20 points on the scale from 0 to 100.

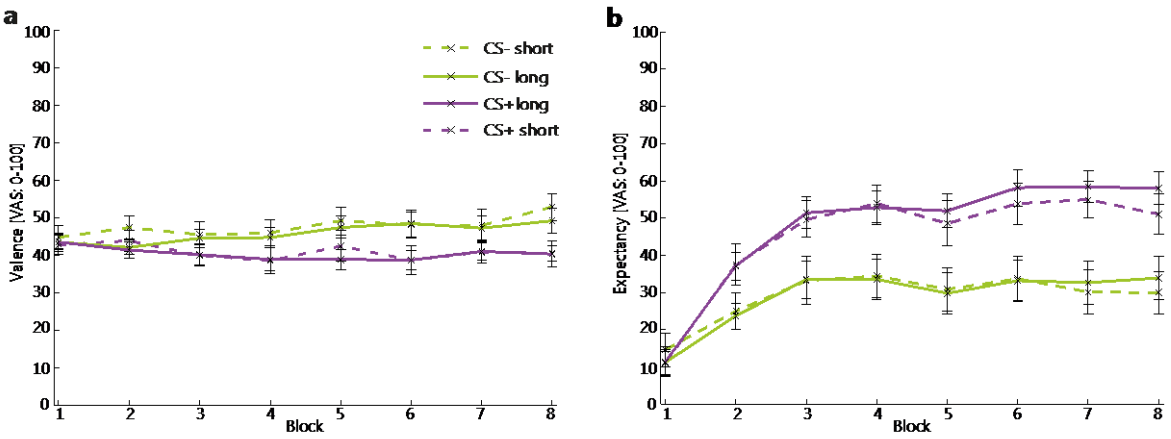

Figure 4. Rating of the valence for differently conditioned faces and the expectancy of a painful stiumulus.
a) Averaged valence ratings of genetically typed subjects (short 5-HTTLPR: n = 20; long 5-HTTLPR: n = 21) for each block. Due to conditioning CS+ were rated less pleasant over blocks. **b)** Averaged UCS expectancy ratings of all subjects. Due to conditioning the expectancy for a CS+/UCS pairing increased over time. Ratings ranged from 0 (unpleasant/ expected not at all) to 100 (pleasant/ expected in any case). Errorbars reflect the SEM.

The 2x2x8 ANOVA [condition (CS-/ CS+) x genotype group (short/ long) x block (1-8)] supported the strong main effect of conditioning on the expectancy of a painful stimulus ($F(1,39) = 25.895$, $p < 0.001$). The factor block influenced the expectancy ratings significantly ($F(7,273) = 50.36$, $p < 0.001$) and a condition x block interaction ($F(7,273) = 13.39$, $p < 0.001$) was identified.

3.2 Event Related Fields

3.2.1 Main Effect of Conditioning on All Subjects

The sensor level analysis including all subjects and not considering genetic variations did not indicate statistically significant differences in the ERFs between the CS+ and CS- conditions.

3.2.2 Genotype Group x Condition Interaction

3.2.2.1 Early Time-Window

Between 150 and 250 ms the 2 x 2 ANOVA (genotype groups x conditions) between subjects revealed significant conditioning by genotype group interactions within a left temporo-occipital sensor region ($F(1,40) = 24.98$, $p < 0.001$, $eta2 = 0.39$) and a right centro-frontal sensor cluster ($F(1,40) = 14.03$, $p = 0.001$, $eta2 = 0.264$) (see figure 5). For the left cluster the ERF waveforms showed two peaks for the L-group and a negative curve for the S-group, while polarities were reversed on the right cluster.
Consequently, for the left cluster the mean difference ([CS+] – [CS-]) of the homozygous long allele carrier was positive ($M \pm SEM = 6.44 \pm 1.99$), whereas it was negative for S-group ($M \pm SEM = -7.67 \pm 2.07$). Again, the pattern was reversed for the right cluster (short: $M \pm SEM = 6.61 \pm 2.07$; long: $M \pm SEM = -5.92 \pm 2.0$).

3.2.2.2 Late Time-Window

The later time window elicited a significant left parietal ($F(1,40) = 7.91$, $p = 0.008$, $eta2 = 0.169$) and a right front-centro-temporal sensor region ($F(1,40) = 13.20$, $p = 0.001$, $eta2 = 0.253$) (see figure 5). The ERF waveforms had the same polarity as in the early time window, and the mean of the L-group ($M \pm SEM = 4.0 \pm 2.67$) was more positive than for the S-group ($M \pm SEM = -6.4 \pm 2.55$) on the left hemisphere. On the right hemisphere the mean of the L-group was more negative ($M \pm SEM = -3.49 \pm 1.7$) than for the S-group ($M \pm SEM = 8.22 \pm 2.77$).
Additionally the 2 x 2 ANOVA identified a significant effect of the factor genetic variation on the difference amplitudes (left: $F (1,40) = 9.07$, $p = 0.005$; right: $F (1,40) = 6.16$, $p =$

0.017).

Within both time windows the differences ([CS+] – [CS-]) on the left clusters were positive for L-group and negative for the S-group, whereas it was vice versa for the right sensor cluster

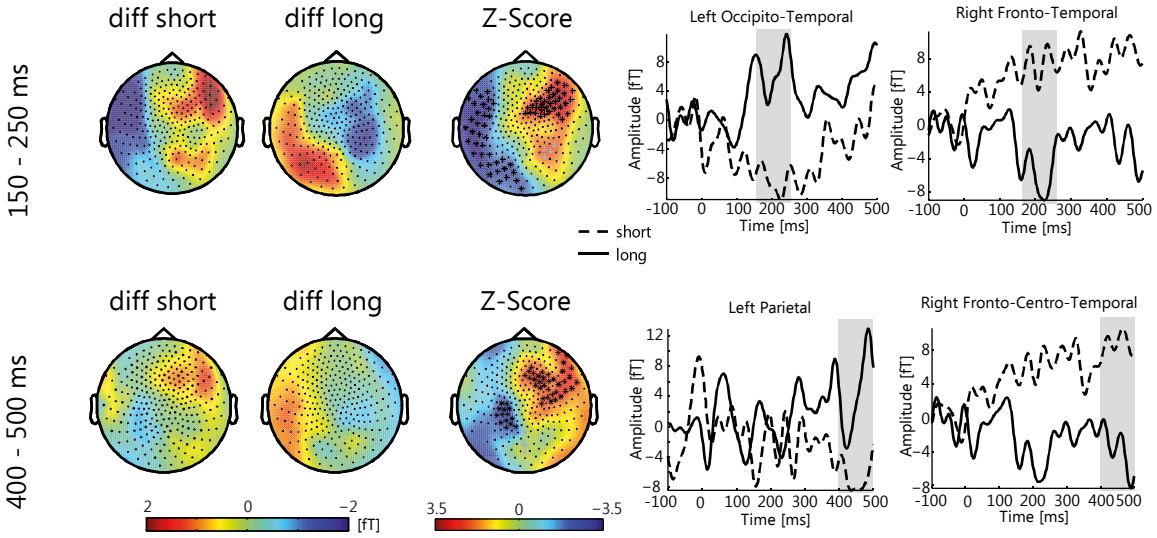

Figure 5. Topografic maps of the differences ([CS+] - [CS-]) within the different genotype groups (short/ long 5-HTTLPR), Z-Score topographies ([short] - [long 5-HTTLPR allele]) and grand mean ERFs averaged across the significant channels (marked with *) for the early and the late time window. Grey * mark channels, which were excluded from further ERF analysis.

3.2.3 Within Genotype Group Analysis

3.2.3.1 Early Time Window

Within the first time-window (150-250 ms) statistics for the CS- versus CS+ contrast (CS- short vs. CS+ short/ CS- long vs. CS+ long) revealed left temporal and right fronto-temporal effects within the S-group and effects over left temporo-occipital and right centro-fronto-parietal sensor regions for L-group. The traces of the ERFs of both groups picture two peaks within the early time window (see figure 6).

The S-group's waveforms of the left and right cluster are quite similar but contrary in

polarity. The mean over the left channels appeared to be significantly higher for the CS- condition (M ± SEM = 9.44 ± 3.69) than for the CS+ condition (M ± SEM = -1.84 ± 5.65). The right sensor region showed a stronger negativity for the CS- condition (M ± SEM = -14.67 ± 7.75) compared to the CS+ condition (M ± SEM = -4.88 ± 7.9) within the S-group. In contrast to the S-group, the mean over left sensors within the L-group was higher for the CS+ (M ± SEM = 7.96 ± 8.38) compared to the CS- condition (M ± SEM = 0.67 ± 8.57). The mean over right sensor clusters was increased for the CS- (M ± SEM = 4.6 ± 6.87) compared to the CS+ (M ± SEM = -2.71 ± 6.91).
Between 150 ms and 250 ms the topographies of both groups suggest a bilateral distribution of ERFs with opposing magnitudes for the comparisons between conditions, groups, and hemispheres.

3.2.3.2 Late Time-Window

Figure 6 depicts the conditioning effect in the late time window (400-500 ms) located left central and right fronto-temporal within the S-group and only one left temporal within the L-group. The ERF waveforms depict no clear peaks but differ clearly between the two conditions.
Within the S-group the mean for CS- condition is positive for the left (CS-: M ± SEM = 3.64 ± 3.99; CS+: M ± SEM = -2.77 ± 3.67) and negative for the right (CS-: M ± SEM = -8.9 ± 2.32; CS+: M ± SEM = -0.89 ± 3.29) cluster. The L-group displayed larger amplitude for the CS+ (M ± SEM = 5.77 ± 5.31) than for the CS- (M ± SEM = 15.15 ± 6.33) trials on the left temporal cluster.

Both genetic groups showed effects of conditioning on the ERFs, however, differences between conditions were of opposite direction (the absolute mean value was higher for CS- than for CS+ in the S-group, while it was vice versa in the L-group).

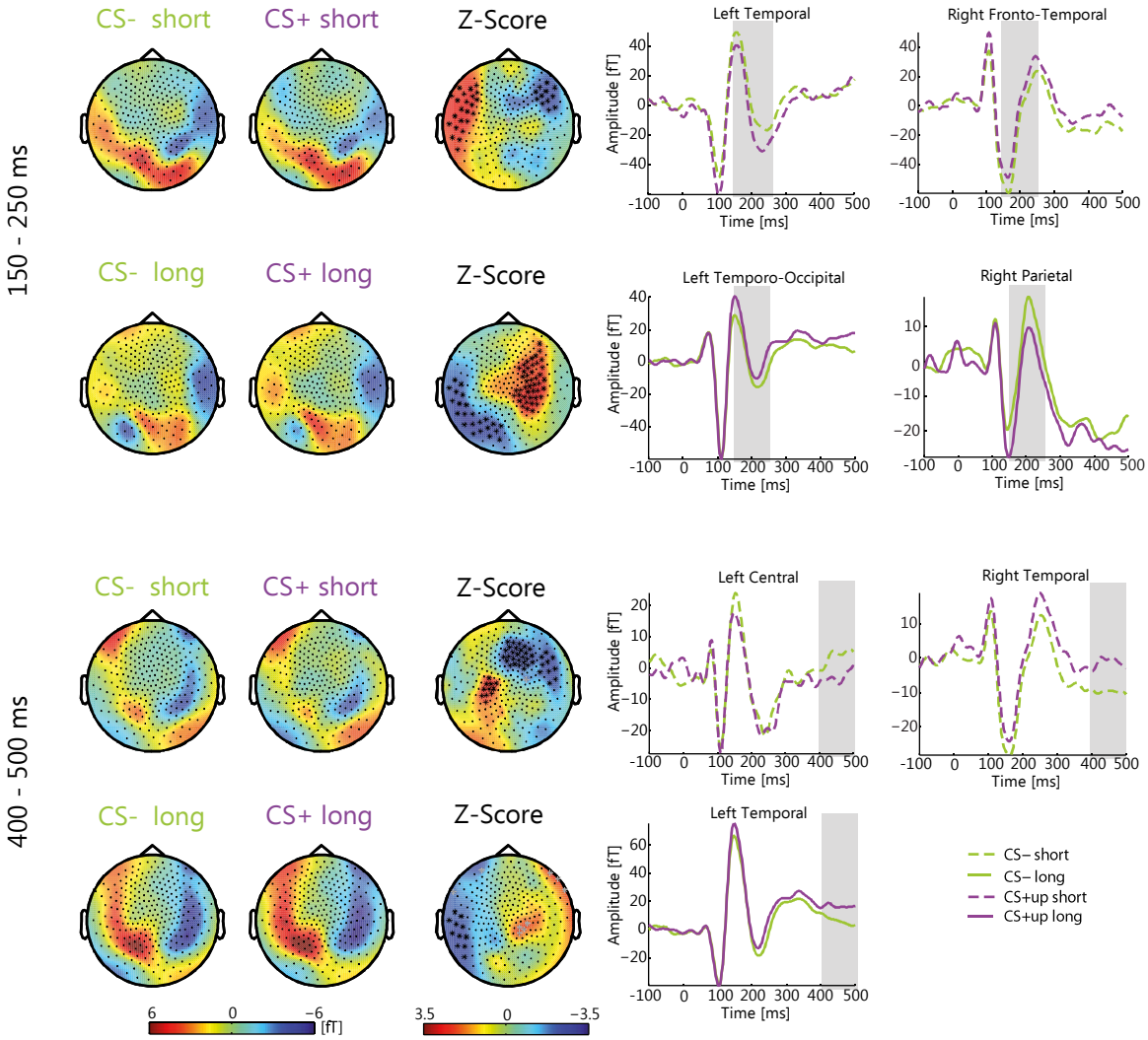

Figure 6. Topografic maps of the conditioning effect (CS- vs. CS+) within the different genotype groups (short/ long 5-HTTLPR allele), Z-Score topographies ([CS+] - [CS-]) and grand mean ERFs averaged across the significant channels (marked with *) for the early and the late time window. Grey * mark channels, which were excluded from further ERF analysis.

3.2.4 Within Condition Analysis

3.2.4.1 Early time window

Figure 7 depicts a significant frontal sensor cluster for the CS- condition (CS- short vs. CS- long) and a significant left frontal sensor region for the CS+ condition (CS+ short vs. CS+ long) both depicting a peak. The ERF waveforms had similar features for both groups but were more shifted towards positivity in the L-group. The mean value of the L-group for CS- (M ± SEM = 8.94 ± 3.66) and for CS+ stimuli (M ± SEM = 10.23 ± 4.3) is significantly higher than for the S-group (CS-: M ± SEM = -4.0 ± 2.4; CS+: M ± SEM = -10.82 ± 4.80).
The ERF waveforms demonstrate a more positive signal for the L-group compared to a more negative graph for the S-group.

3.2.4.2 Late Time-Window

For the late time window a left parietal sensor cluster was found within the CS- condition once more showing a more positive ERF for the L-group (M ± SEM = 24.79 ± 4.68) compared to the S-group (M ± SEM = 6.09 ± 3.98). Left parietal and right fronto-parieto-temporal significant sensor clusters were observed in the CS+ condition. Within the left cluster the L-group showed a stronger positive (M ± SEM = 29.5 ± 5.97) and within the right a stronger negative ERF waveform (M ± SEM = -20.21 ± 4.39) compared to the S-group (left: M ± SEM = 3.11 ± 3.77; right: M ± SEM = -1.17 ± 3.56).
Evaluation of the late time window implies larger amplitudes of the ERFs of the L-group.

Within condition analysis of the ERF waveforms as well as their mean over significant channels and time windows indicated that signals of the L-group were larger than those of the short allele carriers.

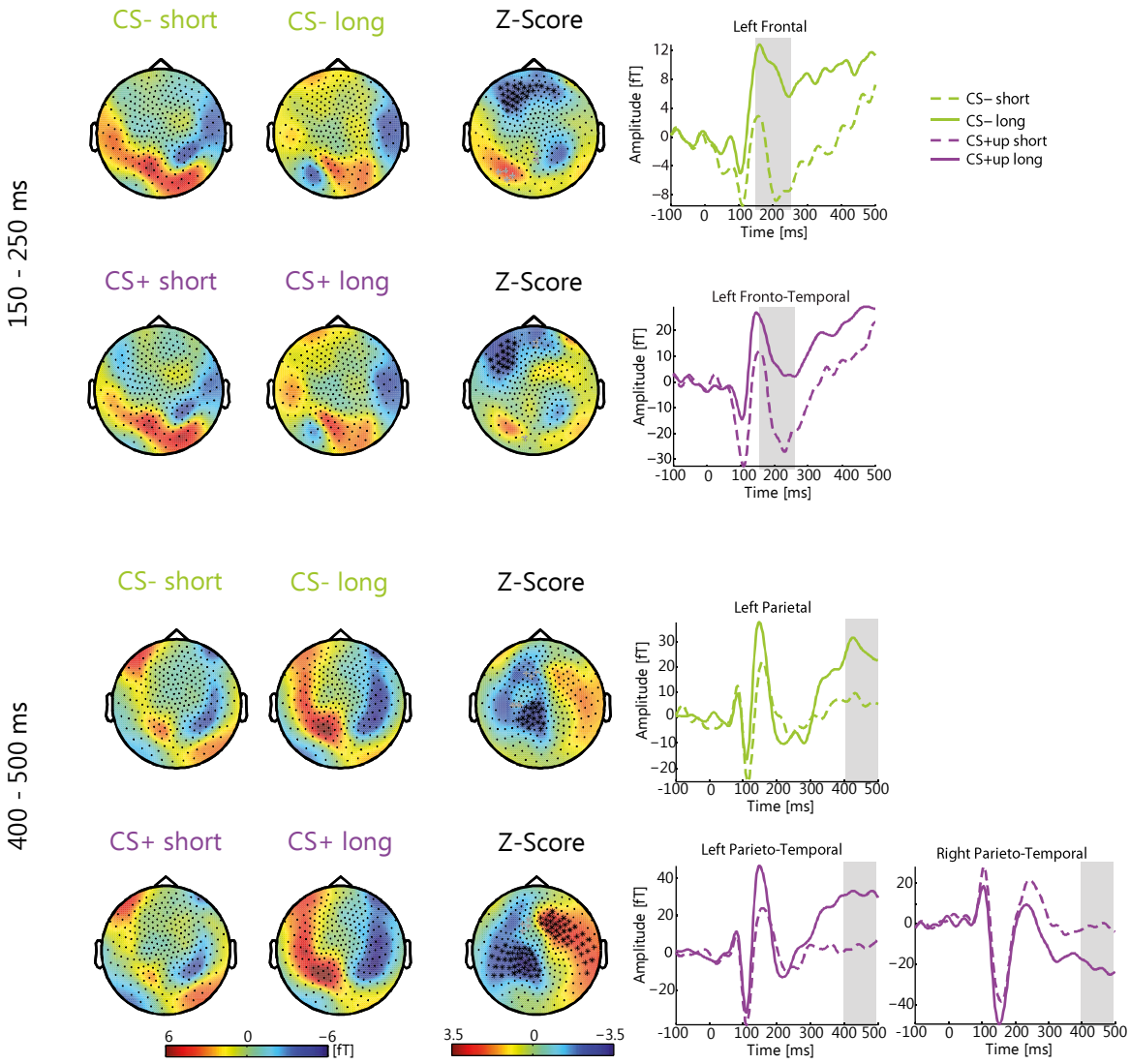

Figure 7. Topografic maps of the genotype group effect (short vs. long 5-HTTLPR) within the different conditions (CS-/ CS+), Z- Score topographies ([short] - [long 5-HTTLPR allele]) and grand mean ERFs averaged across the significant channels (marked with *) for the early and the late time window. Grey * mark channels, which were excluded from further ERF analysis.

4. Discussion

The short variant of the 5-HTTLPR might be associated with an enlarged vulnerability for anxiety disorders (Hariri and Holmes 2006, Lissek and Grillon 2010). I investigated the influence of the 5-HTTLPR on neuromagnetic correlates of fear conditioning and found different ERFs in the comparisons between and within the 5-HTTLPR genotype groups. Contrary to my expectation and earlier fMRI publications (see Canli and Lesch 2007, Dannlowski et al. 2010, Homberg and Lesch 2011), the S-allele-carriers had reduced ERF amplitudes for the CS+ compared to the CS-. In the comparison between the genotype groups the low-expressing 5-HTTLPR group was associated with weaker ERFs compared to the L-group. The ERFs differ starting at about 150 ms after stimulus presentation and shortly before the UCS has been expected. The following section discusses those findings in more detail.

4.1 Rating

Behavioural data revealed that the experimental paradigm successfully induced conditioning effects. Conditioning led to an increasing expectancy of a painful stimulus and a less pleasant evaluation for the CS+ stimuli. In line with the results of Klucken and colleagues (2015), the genotype groups did not differ on the behavioural level. This result supports the notion that the influence of the 5-HTTLPR might be too modest to affect a rather complex rating phenotype (Canli and Lesch 2007). Next to this quite broad phenotype, the influence of other unconsidered trait related polymorphisms and developmental conditions might obscure 5-HTTLPR related effects (Gross and Hen 2004, Domschke and Dannlowski 2010).

4.2 Main Conditioning Effect

Contrary to my expectation, the sensor level analysis including all subjects did not reveal a main conditioning effect. Interestingly, the findings of the present interaction analysis resemble the sensors and time windows revealed by previous MEG conditioning research

that did not focus on the 5-HTTLPR polymorphism (Dolan et al. 2006, Kluge et al. 2011). Furthermore, in the within group comparison the mean ERFs are maximal for the CS- in the S-group, while it is vice versa for the L-group with maximal CS+ mean values. In addition, the groups have inverted polarities in the within condition analysis. Those reversing averages might extinguish the main conditioning effect when all subjects are included. Moreover, the 50-50 ratio between the S- and L–group, with an elevated rate of homozygous L-carrier, which does not display a normal distributed population sample (Lesch et al. 1996), might cause the absent main conditioning effect.

4.3 Conditioning x Genotype Group Interaction

As described earlier, the statistical analysis revealed a conditioning by genotype group interaction, which allowed post hoc testing for a closer look on the influences. In the following section, I present a more detailed discussion about those interactions.

4.3.1 Conditioning Effects within Genotype Groups

Analysis of the ERFs within the genetically different groups detected a bilateral ERF distribution for both groups in the early time window (150-250 ms). Contrary to my expectations, the S-group has higher mean values for the CS- compared to the CS+. Previous neuroimaging publications demonstrated increased activity of amygdala and ACC in response to aversive stimuli in the S-group (Canli and Lesch 2007, Dannlowski et al. 2010, Homberg and Lesch 2011). These different results might be caused by the earlier mentioned different capabilities of MEG and fMRI to measure distinct physiological processes of brain activity. However, one MEG conditioning study is in line with the just mentioned results. An auditory conditioning paradigm, which did not distinguish between S- and L-group, caused enhanced CS- compared to CS+ ERFs. Because in conditioning experiments auditory cues are processed more rapidly than visual ones (Miskovic and Keil 2012), the differences appear earlier (85-115 ms) (Kluge et al. 2011) than those of the present study.

In contrast to the S-group, the L-group shows enlarged ERF mean values for CS+ compared to the CS-. The early and the later time window depict these opposing results (S:

higher CS- mean values; L: higher CS+ mean values). These findings substantiate that the genotype groups process aversive and safety stimuli differently. One would expect an enlarged sensitivity for aversive stimuli in the S-group (Canli and Lesch 2007, Dannlowski et al. 2010, Homberg and Lesch 2011) but sensor level analysis found that activity for CS+ peaked in the L-group. This might provide the basis for the accurate processing of aversive stimuli in the L-group. Preferred CS+ processing might lead to the L-group's milder manifestation of anxiety-related psychiatric disorders compared to S-carrier (Hariri and Holmes 2006, Lissek et al. 2010).

In accordance with the findings in the L-group, previous conditioning research also saw enhanced occipito-parieto-temporal activity between 130 and 190 ms for CS+ compared to CS-. The authors used facial and odorous stimuli. They discussed a parietal and frontal axis that is supposed to reflect the involvement of attentional networks (Steinberg et al. 2012). Bröckelmann and colleges (2011) also observed the involvement of posterior sensors in auditory conditioning but again, these auditory effects appear earlier (100-130 ms) than the my visual effects. Their dipole reconstruction suggests the sources in auditory cortex. Because the effects happen to appear in the N1m time window, the authors hypothesize the activity to reflect prioritized processing of relevant stimuli. Both publications associate their posterior results with attentional networks mediating conditioning effects. Supported by this notion, effects of emotional learning on attention might also affect the early bilateral ERFs differing between the conditions within the genotype groups in the current study. The effects of the attentional networks might contribute to elevated fear expression in S-carrier. As the L-group's ERFs demonstrate differences at more posterior sensors, they might involve more attention-associated networks than the S-group. This additional attention might be necessary for correct stimulus classification and gives advantage to the L-group.

Another temporo-occipital component that overlaps with my time window of significant differences within the groups is the early posterior negativity (EPN). Indeed, the component is associated with processing of emotional stimuli (Schupp et al. 2003, Herold 2008, Thom et al. 2014). The biphasic pattern of its magnetic counterpart (EPN-M) is separated in an early more occipito-parieto-temporal (120-170 ms) and a late more anterior over temporal regions (220-310 ms) time window (Peyk et al. 2008). In the S-group, the CS- and CS+ ERFs differ on rather frontal sensors, compared to the L-group. The more

frontal effects might suggest enlarged differences in the later part of EPN-M in S-carrier. Hence, they could demonstrate delayed differences in visual processing stream compared to the L-group. The selective attention, which is guided by emotional features (Peyk et al. 2008) in S-carriers, may be disabled. This might lead to a misclassification of stimuli and an enlarged vulnerability to anxiety-related disease in subjects that have the low functioning allele.

A more detailed look on the data reveals two peaks reversed in polarity within the early significant time window. The first peak appears before and the other after 200 ms. The former covers a time window known for face responsive activity. Mimicking the M170 the differences in those peaks around 150 ms might suggest conditioning induced effects on face processing. Previous work demonstrated auditory conditioned face responses over right occipito-central sensors. Those were earlier and decreased for CS+ compared to CS- (Dolan et al. 2006). While the S-group exhibits decreased CS+ amplitudes compared to CS-, it was vice versa in the L-group. Again, those differences in physiological effects that reflect stages in face perception like face recognition and identification (Liu et al. 2002) might contribute to the S-group's reduced ability to process aversive stimuli. The enlarged CS- associated ERFs in the S-carriers thus could even represent the overgeneralization of CS in anxious subjects (Lissek et al. 2010).

The first significant time window not only covers the M170 but also includes P200m (180-270 ms). Conveyed to the presented data significant differences of the working memory associated P200 could affect stimulus classification, which might be necessary for adequate responses to emotional stimuli. Patients with anxiety disorders are impaired in the proper reaction to a certain stimulus (e.g. Maren 2001). Differences in working memory processes that lead to impaired stimulus classification might affect S-carriers and lead to their enlarged vulnerability for anxiety disorders.

The rather frontal differences in the S-groups ERFs shortly before UCS omission are in line with the results of Skrandies and Jedynak (2000). In their EEG conditioning experiment subjects were visually conditioned with median nerve stimulation. Visually evoked potentials appeared at rather anterior sensors after conditioning. The LPP (416-456 ms) over centro-parietal sensors appears within the late time window found in the within group analysis. This component relates to the further processing of emotional relevant stimuli (Schupp et al. 2003, Thom et al. 2014). Therefore, the S-group's enlarged centro-

frontal ERF for CS- instead of CS+ is surprising. The missing signal increase for aversive stimuli might explain their restricted ability to process emotional stimuli successfully.
In the analysis within the genotype groups, it becomes evident that conditioning affects components like the M170 and the EPN-M, which deal with face processing, attentional networks and the processing of emotional stimuli. The maximal ERF mean values, which are opposing between the conditions, might reflect divergent prioritised processing of CS+ and CS- within the 5-HTTLPR groups or indicate impaired inhibitory processes to safety signals in participants.

4.3.2 Genotype Group Effects within Conditions

The waveform analysis identified different peaks between the groups 150 ms after stimulus onset. Those signals resemble the waveforms that were found for conditioned facial stimuli (Dolan et al. 2006). However, despite the early genotype group differences (150-250 ms) cover time intervals of face perception and processing, they are unlikely to reflect those processes. Because the differences appear in left and frontal ERFs, they are not in line with the literature. Authors rather describe the right hemisphere (Watanabe et al. 1999, Alvarez et al. 2008) and occipito-temporal cortex to be relevant in face processing (Sams et al. 1997, Sato et al. 1999, Deffke et al. 2007).
Contrary to the L-group with similar mean values for both conditions, the S-group's CS- mean value is closer to baseline than the CS+ mean value. This is likely to reflect weaker frontal CS- ERFs in the S-group than in the L-group. Prefrontal cortex areas are associated with the emotional categorization of experienced stimuli (Steinberg et al. 2013). The S-group's decreased frontal CS- ERFs might depict a process of reduced and consequently misled emotional classification of safety signals. Deficient stimulus integration might lead to elevated anxiety-related scores. As patients with anxiety disorders exhibit impaired inhibitory conditioning to safety cues (Lissek et al. 2004), reduced inhibitory processes might also cause anxiety-raising effects in the S-group. The finding of elevated fear levels for both stimulus types, CS+ and CS-, in adolescents with anxiety disorders (Lau et al. 2008) supports this assumption.
Next to reduced CS- ERFs in the S-group, one could discuss elevated ERF mean for the CS- in the L-group. As mentioned earlier, the prefrontal categorisation of stimuli and thus

the correct classification of CS- as a safety signal might benefit the better coping with stressful events in the L-group. Furthermore, the reported group effects might also reflect the rather frontal, later part of the EPNm (Peyk et al. 2008). As this component is involved in emotional differentiation of stimuli, it might also contribute to a more appropriate stimulus classification and processing in the L-group.

While the S-group demonstrates a reduced mean value for the CS- condition in the early time window compared to the L-group, carriers of the low-expressing 5-HTT variant exhibit no deflection shortly before the UCS is expected. The L-group shows parieto-temporal ERFs differing significantly from the S-group between 400 and 500 ms for both conditions, which already rise at 300 ms. Rats express fear before and at the same time the UCS appears (Burman and Gewirtz 2004), hence this time period might be associated with crucial fear processing. Again, the late group effect might also reflect differences in the LPP (associated with the processing of emotionally relevant stimuli) (Schupp et al. 2003). In addition, the significant differences between the S- and L-groups coincide with the learning associated global field power increasing over rather frontal sensors at 360 ms in visual EEG experiments (Skrandies and Jedynak 2000). Taken together, the late L-group's ERFs represent differences in the processing of emotional stimuli. Compared to the L-group, the S-group shows no increase in peak amplitude. This supports the notion that the S-carrier might be impaired in emotional stimulus processing. Consequently, they exhibit elevated fear related scores and behaviour. Lissek and colleagues (2004) propose a combination of weaker inhibitory and stronger excitability associations among patients. As the signals are rather weaker in the S-group compared to the L-group, they might reflect reduced inhibitory skills resulting in enhanced excitability.

The present findings partly contradict previous studies reporting decreased potentials in homozygous L-carriers compared to subjects with the S/L and S/S variant. In an EEG experiment, the authors describe an increased P300 (280-320 ms) in the S-group. They correlate their finding with enlarged cognitive resources contributing to information processing in S-carrier (Sysoeva et al. 2009). However, the fact that the present study did not detect those group differences might be explained by the P300 not being a conditioning associated component. Consequently, the experimental paradigm did not elicit differences in the P300 or its magnetic counterpart.

Also inconsistent with my findings, Hindi Attar and colleagues reported a signal reduction

in participants that indirectly mimic rather the high-functioning long variant of the 5-HTTLPR with reduced 5-HT levels. Dietary 5-HT depleted participants demonstrated reduced amygdala and OFC activity in a conditioning fMRI experiment. Also detecting reduced skin conductance and lower expectancy rating, the authors reported impaired fear learning in subjects with reduced 5HT-levels (Attar et al. 2012). Contrary to the present study one could deduce a signal enlargement in S-carrier from the reported fMRI study. Many researchers, using Neuroimaging studies, report an elevated amygdala activity in S-carrier (Canli and Lesch 2007, Dannlowski et al. 2010, Hermann et al. 2012). Nevertheless, the amygdala activity is rapidly adapting during conditioning processes (Büchel and Dolan 2000, Marschner et al. 2008) and the present experimental paradigm is not able to detect those amygdala differences between groups.

The elevated parieto-temporal late amplitudes (400-500 ms) of the L-group for both stimuli compared to the S-group might be the key finding in the group comparison. They appear in the time range of the LPP and might depict the L-group's high level cognitive processing of the stimuli in the working memory (Thom et al. 2014).

Taken together with the other findings, ERFs of the genotype group comparison within the conditions substantiate advantages in emotional processing in L-carrier. Reduced capabilities in fear processing might lead to reduced fear coping of S-carrier and cause their elevated vulnerability for anxiety disorders.

4.4 Limitations

Lissek and colleges (2004) stress that differences between anxiety patients and controls are primarily apparent when looking at a simple, single cue paradigm and not at discrimination studies differing between CS+ and CS-. Because my analysis did not compare against baseline level, the type of paradigm might have masked conditioning effects.

Because gender and sex hormones differ with the menstruation circle and influence learning processes (Cahill 2006, Dalla and Shors 2009), I decided to use male participants exclusively. Despite the limited generalisability for the described results, the usage of only one gender is common in conditioning research (Sato et al. 1999, Petrovic et al. 2008, Sysoeva et al. 2009, Attar et al. 2012). Furthermore, there is evidence for a sex-dependent influence of 5-HTTLPR on psychological ratings (Du et al. 2000, Mizuno et al. 2006,

Adrian et al. 2015). Because no MEG results of 5-HTTLPR dependant conditioning effects were published, I needed to restrict the sample to better focus and clarify results.

Furthermore, the present study neither considered possible gene-environment interactions (Canli and Lesch 2007, Nordquist and Oreland 2010), nor paid attention to other genetic variants of the 5-HTTLPR. There is a single nucleotide polymorphism in the L-allele that leads to the separation of L_A und L_G. The L_G variant is supposed to function as the S-allele (Nordquist and Oreland 2010), and those subjects could have been considered as S-group participants. Further conditioning experiments could also attend possible gene x gene interaction. There are more polymorphisms in genes concerning the 5-HT balance. Indeed polymorphism in the monoamine oxidase A (Gross and Hen 2004) and the tryptophan hydroxylase-2 (Hermann et al. 2012) might interact with the 5-HTTLPR and could cause different results in fear conditioning.

Because there is evidence that 5-HTTLPR influences appear rather during development (Nordquist and Oreland 2010), future research could examine how age influences the anxiety- polymorphism association.

Working with healthy participants is a common problem in respect of clinical significance. As to my knowledge this is the first MEG study, that examines the influence of 5-HTTLPR on ERFs, this limitation needed to be taken. Further research could deal with the influence of the 5-HTTLPR on fear conditioning in patients with anxiety disorders and compare the results to those of the present study with healthy participants.

5. Conclusion

In conclusion, the present study provides evidence that there are neuromagnetic differences in aversive conditioning within and between the genotype groups. The sensor level analysis of the physiological data provides evidence that mechanisms of emotional stimulus and face processing might contribute to those effects. Impairment in attention, face recognition, and working memory related components might lead to an increased vulnerability for anxiety disorders in S-carrier. A better understanding of the underlying processes of anxiety disorders may promote their treatment. The experimental paradigm may not picture the expected enhanced excitatory conditioning to threat cues, but suggests incorrect stimulus classification caused by affected emotion processing and impaired inhibitory control to CS- in S-carrier (Lissek et al. 2004, Lau et al. 2008). If disabled inhibitory processes might be part of the pathogenesis of anxiety related disorders, the treatment might approach this point. Future work could address the female gender and the influences of traumatic life events on the ERFs.

6. Summary

Conditioning associates a certain event with a distinct stimulus. This conditioned stimulus (CS) predicts the event and its appearance elicits a conditioned response (Pavlov 1927, Maren 2001). This kind of learning might influence the pathogenesis of anxiety disorders (Maren 2001, Lissek et al. 2004). Psychological tests of personality traits may determine a person's vulnerability for those diseases. Previous work associates anxiety related traits with a polymorphism in the serotonin transporter promoter allele (5-HTTLPR), which has a short (S) and a long (L) genotype (Lesch et al. 1996). Consequently, one might examine the relation between the 5-HTTLPR and the development of anxiety disorders.

This work uses magnetoencephalography to examine fear conditioned, event-related, magnetic fields (ERFs) of 5-HTTLPR genotyped participants (S-allele-carrier, homozygote L-allele-carrier). During the conditioning experiment, three of the six facial stimuli occasionally were paired with a painful stimulus (CS+ condition) while the other faces never appeared with an additional stimulus (CS- condition). The sensor-level analysis revealed early (150-250 ms) and late (400-500 ms) genotype group x condition interactions. Those differences appeared in components that are associated with face (N170) and emotion processing (EPNm, LPP), attention (N1m), and working memory (P200). The analysis within the S-group revealed rather weaker ERFs for the CS+ compared to CS-. The comparison between the S-group and the homozygous L-group demonstrated also lower ERFs for the S-carrier. Those signals might represent inhibitory processes and their reduction in the low functioning S-group might trigger their increased vulnerability for anxiety disorders. Future therapy strategies could enforce such inhibitory processes or train the patient's attention to classify safety cues (CS-) correctly.

7. Zusammenfassung

Konditionierung verbindet das Auftreten eines bestimmten Ereignisses mit einem gewissen Stimulus. Dieser konditionierte Stimulus (CS) sagt dann das Ereignis voraus und dessen Erscheinen führt zu einer erlernten, konditionierten Reaktion (Pavlov 1927, Maren 2001). Diese Form des Lernens könnte an der Pathogenese einiger Angststörungen beteiligt sein (Maren 2001, Lissek et al. 2004). Die Vulnerabilität für diese Erkrankungen kann mittels psychologischer Tests bestimmt werden. Untersuchungen fanden Assoziationen zwischen diesen Befragungen und einem Polymorphismus im Serotonin-Transporter, welcher eine lange (L) und eine kurze (S) Variante des Promotor-Allels zeigt (Lesch et al. 1996). Infolgedessen stellt sich die Frage nach dem Einfluss des Polymorphismus im Promotor Allel des Serotonin Transporters (5-HTTLPR) auf die Entwicklung einer Angststörung.

Die vorliegende Arbeit nutzt Magnetoenzephalografie, um die ereigniskorrelierten, magnetischen Felder (ERFs) von 5-HTTLPR genotypisierten Probanden (S-Allel-Träger, homozygote L-Allel-Träger) zu untersuchen. Im Konditionierungsexperiment wurden drei von sechs Gesichtsstimuli in 50% der Präsentationen mit einem intrakutan applizierten, elektrischen Reiz gepaart (CS+ Bedingung), während den anderen Gesichtern nie ein Schmerzreiz folgte (CS- Bedingung). Auf Sensoren-Ebene sind frühe (150-250 ms) und späte (400-500 ms) Interaktionen zwischen den genetischen Gruppen und den Konditionierungsbedingungen gefunden worden. Die Unterschiede betreffen MEG-Komponenten, welche sowohl mit der Gesichts- (N170) und der Emotionsverarbeitung (EPNm, LPP), mit Aufmerksamkeit (N1m) als auch mit dem Arbeitsgedächtnis (P200) assoziiert werden. Der Vergleich innerhalb der genetisch verschiedenen Gruppen hat in der S-Gruppe eher schwächere Felder für die CS+ im Gegensatz zu den CS- gezeigt. Der Vergleich zwischen der S- und der homozygoten L-Gruppe zeigt ebenfalls schwächere ERFs für S-Allel-Träger. Diese Signale könnten inhibitorische (hemmende) Prozesse darstellen. Die Abmilderung der Hemmung in S-Allel-Trägern könnte eine gesteigerte Anfälligkeit für Angsterkrankungen bewirken. Genau dort, läge der Ansatzpunkt für künftige Therapiestrategien. Diese könnten sich mit der Stärkung inhibitorischer Prozesse beschäftigen oder auch die Aufmerksamkeit der Patienten auf die korrekte Einordnung der CS- (Sicherheitsstimuli) erhöhen.

8. Abbreviations

5-HT	-	serotonin
5-HTT	-	serotonin transporter
5-HTTLPR	-	serotonin transporter promotor linked polymorphism
ACC	-	anterior cingulate cortex
ANOVA	-	analysis of variance
CS	-	conditioned stimulus
CS+	-	conditioned stimulus occasionally paired with UCS
CS-	-	conditioned stimulus never paired with UCS
$CS+_{paired}$	-	CS+ always paired with UCS
$CS+_{unpaired}$	-	CS+ not paired with UCS
dACC	-	dorsal, anterior cingulate cortex
dmPFC	-	dorso-medial prefrontal cortex
EEG	-	electroencephalogrphy
EPN	-	early posterior negativity
EPN-M	-	magnetic counterpart of the EPN
ERF	-	event related fields
ERP	-	event related potentials
F	-	F-score, Fischer score
fMRI	-	functional magnetic resonance imaging
L	-	long allele
LPP	-	late positive potential
M	-	arithmetic medium
MEG	-	magnetoencephalography
NS	-	neutral stimulus
NEO-PI-R	-	Revised NEO Personality Inventory
S	-	short allele
SD	-	standard deviation
ssVEF	-	steady state visual evoked fields
UCS	-	unconditioned stimulus
UCR	-	unconditioned response

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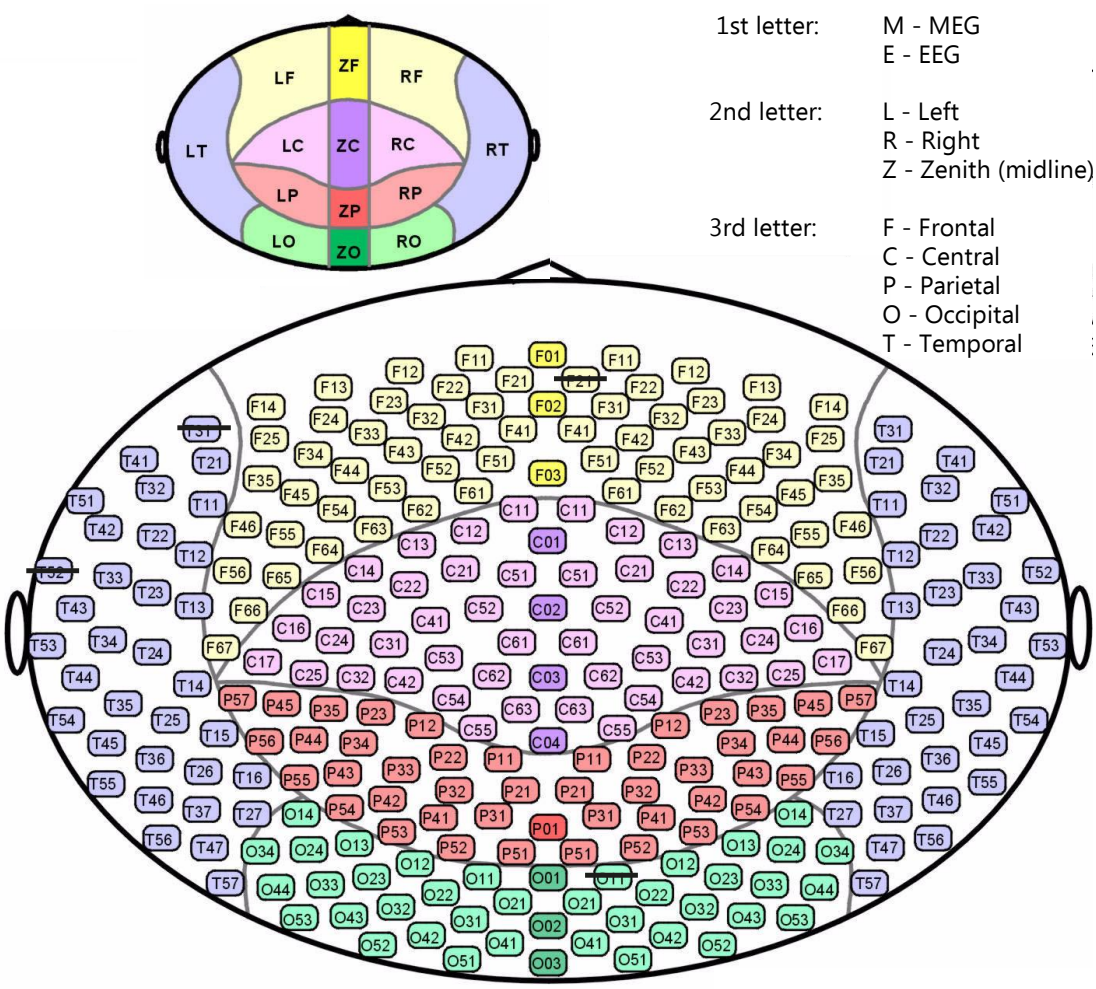
10. Appendix

10.1 MEG Sensor Layout



Note: 1st and 2nd letters ('ML','MR','MZ') omitted for clarity.

Channel count:

	Frontal	Central	Parietal	Occpital	Temporal	Totals
Z (Midline)	3	4	1	3	D	11
L (Left)	31	24	22	19	32	129
R (Right)	33	24	22	18	34	131
Totals	59	52	46	40	66	271

10.2 Significant Channels of the Genotype Group x Condition Interaction Analysis

	Cluster	*Channel*								
150-250 ms	left	LF46	LO23	LO24	LO32	LO33	LO34	LO42	LO43	LO44
		LO51	LO53	LT11	LT12	LT13	LT21	LT22	LT23	LT24
		LT25	LT32	LT33	LT34	LT35	LT36	LT37	LT41	LT42
		LT43	LT44	LT45	LT46	LT47	LT51	LT53	LT54	LT55
		LT56	LT57							
	right	RC13	RC14	RC15	RC16	RC21	RC22	RC23	RC24	RC31
		RC41	RC51	RC52	RF35	RF45	RF46	RF55	RF56	RF63
		RF64	RF65	RF66	RF67	RT11	RT12	RT13	RT14	RT21
		RT22	RT23	RT24	RT32	RT33	RT42	ZC02		
400-500 ms	left	LC25	LC32	LP23	LP33	LP34	LP35	LP41	LP42	LP43
		LP44	LP45	LP54	LP55	LP56				
	right	RC11	RC12	RC13	RC14	RC15	RC16	RC17	RC22	RC23
		RC25	RC32	RF52	RF54	RF55	RF56	RF61	RF62	RF63
		RF64	RF65	RF67	RP23	RP45	RP57	RT13	RT14	RT22
		RT23	RT24	RT31	RT32	RT33	RT34	RT41	RT42	RT43

Abbreviations: L=Left; R= Right, F=Frontal, P=Parietal, C=Central, T=Temporal, O=Occipital

10.3 Significant Channels of the Conditioning Effects Within Genotype Groups

	Genotype Group	*Cluster*	*Channel*							
150-250 ms	short	left	LF25	LF35	LF46	LT11	LT12	LT13	LT21	LT22
			LT23	LT24	LT32	LT33	LT34	LT35	LT41	LT42
			LT43	LT44	LT45	LT51	LT53	LT54	LT55	
		right	RF46	RF56	RT11	RT12	RT13	RT22	RT23	RT24
			RT32	RT33	RT34	RT42				
	long	left	LO22	LO23	LO24	LO32	LO33	LO34	LO42	LO43
			LO44	LT34	LT35	LT36	LT37	LT43	LT44	LT45
			LT46	LT47	LT53	LT54	LT55	LT56	LT57	
		right	RC14	RC15	RC16	RC17	RC22	RC23	RC24	RC25
			RC31	RC32	RC55	RF46	RF55	RF56	RF64	RF65
			RF67	RO13	RO14	RP11	RP12	RP22	RP23	RP32
			RP33	RP34	RP35	RP41	RP42	RP43	RP44	RP45
			RP53	RP54	RP55	RP56	RP57	RT11	RT12	RT13
			RT14	RT15	RT16	RT21				
400-500 ms	short	left	LC24	LC25	LC32	LC42	LP23	LP34	LP35	LP44
			LP45	LP55	LP56					
		right	LC11	RC11	RC12	RC13	RC14	RC15	RC21	RC22
			RC23	RC32	RC51	RF41	RF42	RF43	RF44	RF45
			RF51	RF52	RF53	RF54	RF55	RF61	RF62	RF63
			RF64	RF65	RF67	RT12	RT13	RT22	RT23	RT24
			RT25	RT33	RT34	RT35	ZC01	ZF03		
	long	left	LT34	LT35	LT36	LT43	LT44	LT45	LT46	LT51
			LT53	LT54	LT55	LT56				

Abbreviations: L=Left; R= Right, F=Frontal, P=Parietal, C=Central, T=Temporal, O=Occipital

10.4 Significant Channels of the Genotype Group Effects Within Conditions

	Condition	*Cluster*	*Channel*							
150-250 ms	CS-	frontal	LF13	LF14	LF22	LF23	LF24	LF25	LF31	LF32
			LF33	LF34	LF35	LF41	LF42	LF43	LF44	LF45
			LF51	LF52	LF53	LF54	LF55	LF63	RF31	RF32
			RF33	RF34	RF41	ZF02				
	CS+	frontal	LF13	LF14	LF23	LF24	LF25	LF33	LF34	LF35
			LF44	LF45	LF46	LF54	LF55	LT11	LT12	LT21
			LT22	LT32						
400-500 ms	CS-	parietal	LC54	LC55	LC63	LP11	LP12	LP21	LP22	LP23
			LP31	LP32	LP33	LP34	LP41	LP42	LP43	LP51
			LP53	LP54	LP55	ZC04	ZP01			
	CS+	left	LC25	LC32	LC42	LC54	LC55	LF67	LO14	LP11
			LP12	LP21	LP22	LP23	LP31	LP32	LP33	LP34
			LP35	LP41	LP42	LP43	LP44	LP45	LP51	LP52
			LP53	LP54	LP55	LP56	LP57	LT14	LT15	LT16
			LT24	LT25	LT26	LT35	LT36	LT37	LT46	
		right	RC12	RC13	RC14	RC15	RC16	RC17	RC22	RC23
			RC24	RF44	RF46	RF53	RF54	RF55	RF56	RF61
			RF62	RF63	RF64	RF65	RF66	RF67	RP57	RT12
			RT13	RT14	RT23	RT24	RT25	RT34	RT35	RT36
			RT44	RT45	RT46	RT54	RT55	RT56	ZP01	

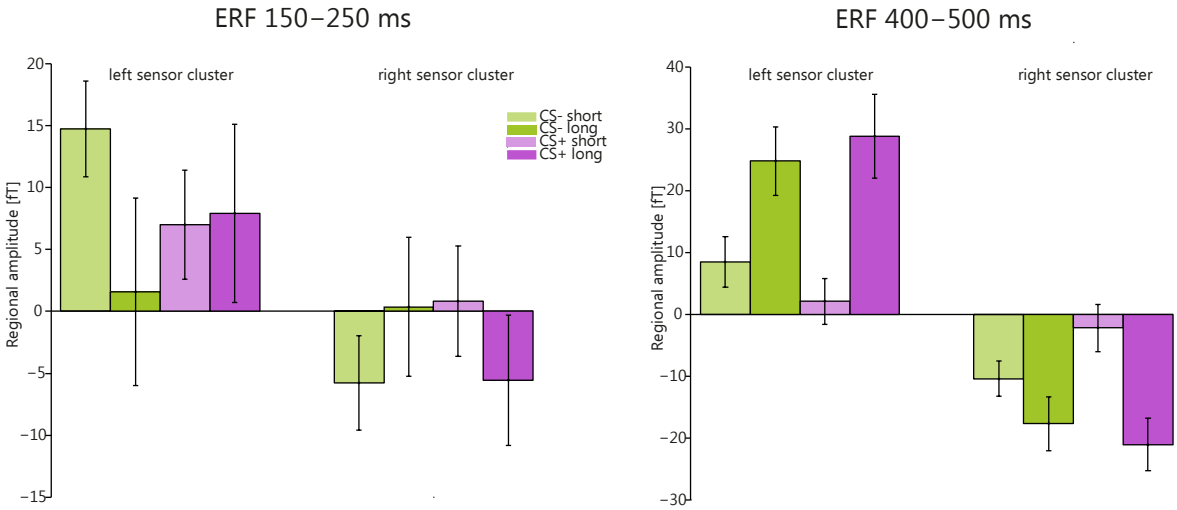
Abbreviations: L=Left; R= Right, F=Frontal, P=Parietal, C=Central, T=Temporal, O=Occipital

10.5 Cluster Meanvalues and Standard Errors of the Genotype Group x Condition Interaction

		short				***long***			
	Cluster	CS-		CS+		CS-		CS+	
		M	SEM	M	SEM	M	SEM	M	SEM
150-250 ms	left	14.35	± 3.92	6.68	± 4.42	1.01	± 7.75	7.46	± 7.37
	right	-5.77	± 3.82	0.84	± 4.44	0.38	± 5.63	-5.55	± 5.27
400-500 ms	left	8.49	± 4.11	2.09	± 3.67	24.78	± 5.52	28.78	± 6.73
	right	-10.59	± 2.96	-2.37	± 3.96	-18.12	± 4.49	-21.66	± 4.46

Note: M = Meanvalue, SEM = Standart error of mean

10.6 Cluster Barplots of the Meanvalues and Standard Errors of the Genotype Group x Condition Interaction



Barplots with errorbars for the early (150-250 ms) and late (400-500 ms) time window with mean ERF data of different conditions at left and right significant sensor clusters found in the analysis of differences ([(CS+ short) - (CS- short)] vs. [(CS+ long) - (CS- long)]). Errorbars reflect SEM. Values are taken from the channels of the sensor clusters shown in Appendix 10.2.

10.7 Facial Stimuli

10.8 Previous Publication

Some parts of this study have previously been published in abstract form:

Schneider TR, Tramm J, Engel AK (2012) Changes of neuronal synchronization in the high gamma-band predicting aversively conditioned stimuli. Society for Neuroscience Abstract Viewer and Itinerary Planner 42.

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12. Lebenslauf

(entfällt aus datenschutzrechtlichen Gründen)

13. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.
Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.