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Titel der Dissertation:

The effect of allelic variation in *CRHR1* on neural correlates of cognitive-behavioral therapy in female patients with panic disorder and agoraphobia - an explorative fMRI analysis of a multicenter study on fear conditioning

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List of abbreviations

A1 / A2	Early phase of Acquisition (A1) / late phase of Acquisition (A2)
AA/AG	Haplotype of the *CRHR1* gene allele rs17689918 correlated with increased risk for Panic Disorder
AAL	Anatomical automatic labeling
ACC	Anterior cingulate cortex
ACQ	Agoraphobia Cognitions Questionnaire
ACTH	Adrenocorticotropic hormone
AD	Anxiety Disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (subgroup of glutamate receptors)
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
ASI/ASI-3	Anxiety sensitivity index / Anxiety sensitivity index -3
BA	Brodman Area
BDI-II	Becks Depression Inventory, version II
BOLD	Blood oxygenation level-dependent
BNST	Bed nucleus of the stria terminalis
CBF	Cerebral blood flow
CBT	Cognitive behavioral therapy
CGI	Clinical Global Impression
CIDI	Composite International Diagnostic Interview
$COMRO_2$	Metabolic rate of oxygen
CRH	Corticotropin releasing hormone
CRHR1	Corticotropin releasing hormone receptor 1
CRHR1	Corticotropin releasing hormone receptor gene 1
CS	Conditioned stimulus: Neutral
CS+	Conditioned stimulus: Danger cue (after pairing with the US)
$CS+_{unpaired}$	Danger cue, not simultaneously presented with the US to the subject after succesfull coupling to the US
CS-	Conditioned stimulus: Safety signal (no pairing with the US)
dAAC	Dorsal anterior cingulate cortex
dB	Decibel
dmPFC	Dorsomedial prefrontal cortex
dlPFC	Dorsolateral prefrontal cortex
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, 5^{th} Edition
E1 / E2	early phase of Extinction (E1) / late phase of Extinction (E2)
EPI	Echo planar imaging
e.g.	for example (latin: exempli gratia)
etc.	et cetera
eQTL-function	Expression quantitative trait locus function
fMRI	Functional magnetic resonance imaging
FWHM	Full Width at Half Maximum
GABA	*gamma*-Aminobutyric acid (neurotransmitter)
GG	Haplotype of the *CRHR1* gene allele rs17689918 without increased risk for Panic Disorder
GLM	General linear model

GluR1	Ionotropic glutamate receptor 1
HAM-A	Hamilton Anxiety Scale
PET	Positron emission tomography
HPA	Hypothalamic-pituitary-adrenal axis
Hz	Hertz
IFG	Inferior frontal gyrus
inf	Inferior
ISI	Insterstimulus interval
MATLAB 7.1	Mathworks Sherborn, Massacusetts, Version 7.1
MD	Major Depression
MI-7/acc/al	Mobility Inventory, 7-day version/ accompanied/ alone
mid	Middle
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
NMR	Nuclear magnetic resonance
OFC	Orbitofrontal cortex
oper	Operculum
p	probability value (p-value)
PAG	Periaqueductal gray
PANIC-NET	Multilevel and multicenter national research network funded by the Federal Ministry of Education and Research
PAS	Panic and Agoraphobia Scale
PD/A	Panic Disorder with Agoraphobia
PFC	Prefrontal cortex
PSF	Point-spread function
ROI	Region of Interest
Sig.	Significance
SIGH-A	Structured Interview Guide for the Hamilton Anxiety Scale
SNRI	Serotonine-norepinephrine reuptake inhibitors
SNFR	Signal-to-fluctuation-noise-ratio
SPM 5	Statistical Parametric Mapping, Version 5 Wellcome Trust Centre for Neu-roImaging: London, UK, 2011
SSRI	Serotonine-reuptake inhibitors
sup	Superior
Supp.	supplementary
t1 / t2	Baseline or pre-CBT (t1) / after CBT (t2)
T + / T-	Therapist guided (T +), non-therapist guided (T-)
TMT-A/B	Trail Making Test, part A and B
tri	Trigonum
US	Unconditioned stimulus, here: of aversive or fear-inducing nature (e.g. loud scream)
vlPFC	Ventrolateral prefrontal cortex

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1 INTRODUCTION

1.1 Panic Disorder and Agoraphobia

Panic Disorder (PD) represents a common Anxiety Disorder (AD) in the general population considering a life time prevalence of 2 - 4 % in adults living in the United States in America or countries in the European Union (Kessler et al., 2012; Wittchen & Jacobi, 2005). Based on the latest criteria list (A – D) by the DSM-V (American Psychiatric Association, 2013, pp. 208 - 209), distinctive diagnostic criteria of PD involve (Criterion A) recurrent and unexpected panic attacks of severe anxiety and (Criterion B) anticipatory anxiety (e.g. about future panic attacks and/or ramifications) or maladaptive modification in behavior (e.g. avoidance behavior). A panic attack is defined as a rise of acute and intense fear or discomfort emerging in a calm or anxious state in which at least four of 13 listed symptoms occur including palpitations, shaking, sweating, shortness of breath, chest pain, fear of losing control or dying, among others. Further criteria assume that (Criterion C) that the above mentioned symptoms are not caused by other medical conditions or substances and (Criterion D) are not better explained by alternative mental disorder, such as posttraumatic stress disorder, specific phobias, obsessive compulsive disorder etc. (American Psychiatric Association, 2013, pp. 208 - 209). The anticipatory anxiety (Criterion B) describes an emotional state, labeling individual preparedness and apprehension of future panic attacks and accompanying consequences while the state of panic depicts an emotional state enabling the individual to deal with an immediate traumatic event or threat (Bouton, Barlow, & Mineka, 2001).

However, anticipatory anxiety can indeed evoke or be accompanied by the individual avoiding certain situations in which the earlier panic attacks occurred or where the occurrence of panic attacks are assumed more likely to happen, thus potentiating future panic attacks (“maladaptive behavioral change”). Because of the phobic avoidance of certain places or situations that are considered to provoke panic attacks, PD is frequently accompanied by agoraphobia (PD/A). Agoraphobia is featured by anxiety or fear triggered by a variety of situations (e.g. public transportation, open or enclosed spaces) and accompanied catastrophizing thoughts that are reinforced by the apprehension or feelings of entrapment and the unavailability of help (in case of potential health-associated emergencies or the occurrence of (embarrassing) symptoms etc.). Hence, agoraphobic patients actively avoid these

feared situations (Kessler et al., 2005; American Psychiatric Association, 2013, pp. 217 - 218; Hamm, 2020). Affected patients experience substantial functional consequences in terms of impaired occupational, physical and social disability factors. Regarding comorbidity, PD/A and depressive disorders show a high comorbidity rate of 52 % (Wittchen et al., 2010). In about two third of these cases, the depression emerge simultaneously or in the course of PD/A. Other significant comorbidities include for instance a substance related disorder (e.g. alcohol use disorders), dizziness, asthma, arrhythmia or irritable bowel syndrome (American Psychiatric Association, 2013, pp. 214 – 216; Wittchen et al.,, 2010; Zimmermann et al., 2003).

In regards of the pathogenesis of PD, several theories in a behavioral, cognitive and neurobiological context have been developed. The cognitive model promote the central premise that catastrophic misinterpretation of normal internal or external cues - mainly of normal anxiety responses (e. g. palpitations, heavy breathing) – result in apprehension which in turn leads to an increase of body sensations, thus leading to a vicious circle and eventually culminating in a panic (Clark, 1986). More recent models, as the learning theory perspective of PD, extended this theory and accentuate the role of basic emotional learning by conditioning principles in the causation and preservation of PD: On the one hand, as evidential by animal models (e.g., Davis *et al.*, 2010), the learning perspective of PD suggests that acute panic and chronic anxious apprehension represent not only different emotional stages and clinical entities, but also reflect different neurobiological stages of defensive reactivity relative to the imminence or proximity of threat cues. Acute panic and escape behavior represent defensive behaviors at the time point of imminent and acute threat processing with accompanying strong autonomic arousal, whereas chronic anxious apprehension on the other hand is connected to defensive behaviors that involve the processing of proximal or subtle threat cues (e.g. body symptoms) with rather moderate autonomic reactivity. In this context, the exposure to an initial panic attack creates a basis for the development of PD by associating the initially neutral interoceptive cues (e.g. dizziness, palpitations) and exteroceptive cues (e.g. mall, escalator) that have been experienced in that panic attack, and eventually leading to an increased hypervigilance and anxious apprehension toward interoceptive cues (conditioned anxiety) respective of upcoming future panic attacks. Consequently, this conditioned anxiety potentiates future panic attacks and thus marking the starting point of the patient's spiral into PD

(Bouton et al., 2001; Hamm, 2020; Richter et al., 2012).

The emotional states of "fear" and "anxiety" differ in regard of their referral and level of autonomic arousal: "Fear" refers to an imminent threat and is often accompanied by a strong autonomic arousal (e.g., "fight or flight") while "anxiety" refers to a future threat and thus includes a relatively mild autonomic arousal (e.g., increased generalized vigilance or muscle tension) and avoidance behavior (Hamm, 2020).

Contributing factors, including temperamental (e.g., neuroticism, increased anxiety sensitivity), environmental (e.g., identifiable stressors, sexual or physical abuse in childhood) and genetic risk factors have been identified to increase the risk and vulnerability towards developing PD (Craske and Barlow, 2008, pp. 6-7; American Psychiatric Association, 2013, p. 211). Preclinical research and imaging genetics provide increasing evidence of multiple gene variants that contribute to the vulnerability of developing PD, among them are the serotonin receptor 1A (Straube et al., 2014a) monoamine oxidase A gene (Reif et al., 2014), catechol-O-methyltransferase (Kim et al., 2013), norepinephrine transporter gene (Buttenschøn et al., 2011), and the corticotropin releasing hormone receptor gene 1 (Weber et al., 2016). This study focus on the latter, the corticotropin releasing hormone receptor gene 1 (*CRHR1*), with special regard to the AA/AG-haplotype of rs17689918. In the following, the neuroanatomical model and therapy of PD will be summarized. The physiology and role of *CRHR1* will be presented in chaper 1.4.

1.1.1 Functional neuroanatomy and -physiology of Panic Disorder

Neuroanatomical models for PD, in particular a "fear network" (Gorman, Kent, Sullivan, & Coplan, 2000), have been suggested by reference to findings in animal and functional imaging studies to derive a better understanding of PD.

Based on the idea of a bi-directional and complementary model of a top-down and bottom-up processing (see fig. 1) and a hypervigilance-avoidance hypothesis, a differentiated fear network was suggested by Hofmann, Ellard and Siegle (2012) emphasizing a differential progress of information processing: An early hyper-reactivity to emotional stimuli, followed by a later regulatory activation of prefrontal including the initiation of coping strategies and finally emotion regulation. The early hyper-reactivity to emotional stimuli (within a few milliseconds after onset of stimulus)

underlies an attention bias that comprises increased attention to threat stimuli (hypervigilance), attentional avoidance and difficulty in disengagement. The reaction to emotional stimuli occurs prior to conscious awareness and is correlated with activations in brain regions dealing with emotional labeling (such as amygdala), interoception (insula) and sensory perception (visual cortex). As a result, the increased reactivity in these areas mirrors a hypervigilance and an increased attention to cues that are of internal, interoceptive and environmental nature. If an anxious individual experiences a stimulus as threatful owing to the hyperreactivity of the amygdala, other areas like the hippocampus merge this experience into an episodic memory, while the insula add interoceptive context. A further involvement of the dorsal anterior cingulate cortex (dACC) and dorsomedial prefrontal cortex (dmPFC) contributes to conscious threat appraisal and therefore cause further anxiety. The later emotion processing sets in 500ms to seconds or minutes after the onset of emotional stimuli. Proposed key brain areas are the prefrontal cortices including the orbitofrontal cortex (OFC), ventrolateral (vlPFC), dorsolateral prefrontal cortex (dlPFC) and dmPFC that play an essential role in the cognitive emotion regulation, and that in anxiety disorders more likely leads to avoidance. This bi-directional neurocircuitry involves subcortical and cortical structures in which "later stages can influence earlier stages" and "explain[s] the hypervigilance-avoidance phenomenon during the processing of certain fearful stimuli" (Hofmann et al., 2012). However, the prefrontal cortex is suggested to be "involved in both creating and mitigating negative emotion, depending on the content of the thoughts" (Hofmann et al., 2012). The vlPFC is exemplary for leading to either an increase or reduction in negative emotion depending on the neural pathway: a pathway involving the nucleus accumbens leads to less negative emotion, while the pathway involving the amygdala leads to an increase negative emotion due to reduced reappraisal success (Wager et al., 2008). In fact, more recent functional neuroimaging studies (for a review see Lai, 2019; Sobanksi & Wagner, 2017) confirm abnormal activations in an extended fear network predominantly including the brainstem, ACC, insula and the lateral/medial PFC. Activations in the amygdala, which is proposed as a central component, however is not consistently reported which is probably attributed to methodological differences or limitations and the difficulty in detecting aberrant activations due to the common thresholds in fMRI (Sobanksi & Wagner, 2017).

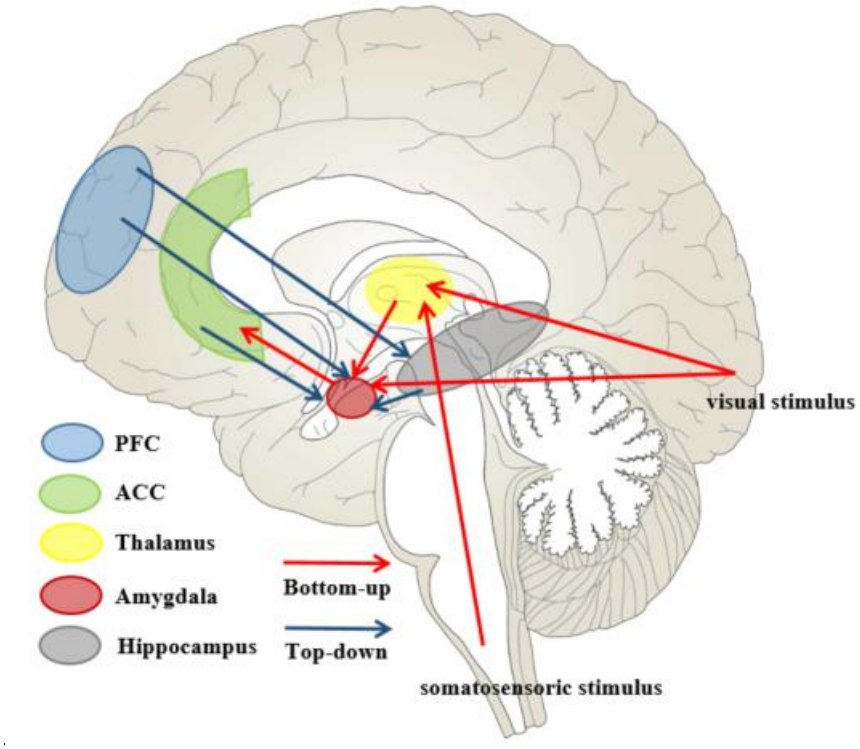


Figure 1. **Neuroanatomical model of anxiety based on Clark and Beck (2010) and Hofmann, Ellard and Siegle (2012).**

The reaction to threat stimuli comprises the activation of brain regions dealing with emotional labeling (amygdala), interoception (insula, thalamus) and sensory perceptions (such as visual stimuli) prior to conscious awareness. The increased reactivity in these areas mirrors a hypervigilance and an increased attention to cues that are of internal, interoceptive and environmental nature, while the hippocampus merges the experience of a threatful stimuli into an episodic memory (bottom-up-process). The amygdala in turn activates structures as the PFC, ACC and the hippocampus to focus on the threat stimuli and to provide cognitive ressources, thus being conscious (top-down-process). PFC: prefrontal cortex; ACC: anterior cingulate cortex. Source of the figure (modified): Yang, Kircher and Straube (2014).

Defensive behaviors are insinuated to be dynamically organized in dependence of the proximity, imminence and distance of the threat. The apprehension of possible and yet undetected or ambiguous threats elicit generalized hypervigilance, elevated autonomic arousal, anxiety, avoidance behavior and inhibition of appetitive behavior ("pre-encounter defense"), which is critically regulated by the bed nucleus of the stria terminalis (BNST) and cholinergic transmission in the thalamus and accompanied activation of the locus coeruleus. By contrast, the detection of distant threat increases the selective attention, increased level of sympathetic arousal and feeling of fear ("postencounter defense") in which rodents were shown to "freeze" and to feature a

potentiated startle reflex, mediated by activation of the central amygdala and projections to the ventral periaqueductal gray. The imminence of threat provokes active defensive behavior (fight/flight; or if not optional: tonic immobility) and the feeling of panic ("circa-strike defense"), mediated by the dorsal periaqueductal gray (in animals) and the sympathetic system (adrenaline, noradrenaline). It is therefore concluded that with increasing proximity of a threat affects a dynamic shift in the involved brain circuits: distal or ambiguous threat involves a circuit that includes predominantly the prefrontal cortex and lateral amygdala (mediating anxiety and avoidance) while the increasing proximity of threat involves predominantly mid brain circuits, a circuit encompassing the central amygdala and periaqueductal gray (mediating flight/fight or freezing) (Hamm, 2020). The physiological function of *CRHR1* and its role in Anxiety Disorder will be presented in chapter 1.4.

1.1.2 Cognitive behavioral therapy in Panic Disorder

Regarding therapy options, pharmaceutical therapy via antidepressants, in particular selective serotonin-reuptake inhibitors (SSRI), serotonin-norepinephrine reuptake inhibitors (SNRI) and cognitive behavioral therapy (CBT) represent the preferred first-line therapy in PD (Bandelow, Gruber, & Falkai, 2012, p. 91). Randomized, placebo-controlled studies support the efficacy of several SSRIs (among others fluoxetine, sertraline, citalopram) and are ascribed to have medium to large effect sizes in comparison to placebo (Bandelow et al., 2015; Bighelli et al., 2018). Psychological behavioral treatment, in particular CBT, is another frequently validated and effective treatment for PD and it has been shown that PD patients clearly benefit further by the combined treatment of SSRI/SNRI and CBT in comparison to each treatment received alone (Bandelow & Baldwin, 2020, p. 391; Bandelow et al., 2015; Carpenter et al., 2018; Van Apeldoorn et al., 2008). Early formulations by Beck & Clark in 1997 suggest a cognitive restructuring of maladaptive cognitions, including beliefs or schemas about the world, the self, the future or particular situations in order to change distress and behaviors. Upon this, various protocols have been developed to account and address different mental illnesses (e.g., Anxiety Disorders, Eating Disorders, Depression) (Beck & Clark, 1997; Hofmann et al., 2012). In the following, core principles of cognitive therapy will be summarized regarding Anxiety Disorders and

PD/A (based on Wieman et al., 2020).

Cognitive behavioral therapy combines two elements, namely cognitive therapy and behavioral therapy. Cognitive theories imply that anxiety disorders derive from distorted or biased thoughts/beliefs about psychological or physical threat. In this context, cognitive restructuring represents a common technique in which clinicians engage the patient in identifying distorted or biased beliefs/thoughts (e.g. by keeping a daily log) and learning to evaluate these and to reinstate more rational viewpoints. In addition, the patient's assumption on his ability to deal with worst-case scenarios (e.g. being critically judged by coworkers) are challenged by the clinician and brought into question. Behavioral theories highlight that fear is acquired by classical conditioning and particularly maintained by catastrophic misinterpretation and avoidance behaviors (or: operant conditioning). Therefore, behavioral therapies intent to extinct the learned fear by applying various techniques involving exposure, behavioral activation or relaxation. For anxiety disorder however, exposure techniques represent the most common approach. Exposure can be realized in different variations: by exposure to feared bodily/physiological reactions (interoceptive), exposure by imagining feared situations (imaginal), or exposure to the actual situations (in vivo). Evidence support that extinction learning represents an active learning process that is realized through repetitive exposure to feared stimuli, thus building new safety associations that eventually become stronger and compete with the initial fear association and reduce anxiety and avoidance behavior allowing the continuance of further and more frequent extinction learning (inhibitory learning). Within the exposure technique there are several strategies to optimize exposure therapy, for instance it is necessary to avoid or remove covert or overt actions that decrease anxiety on patient's behalf (so called "safety behaviors or signals", e.g. phone or medication), so that exposure success is not associated to safety behaviors/signals (Wieman et al., 2020, pp. 221–225).

In PD, the cognitive approach covers the aspect of psychoeducation and cognitive restructuring. In psychoeducation, the patient learns about the nature of PD, in particular about the misguided intense autonomic response (fight or flight) to actually harmless cues and the role of avoidance behaviors and catastrophic misinterpretations of bodily sensations on the maintenance of PD. Cognitive restructuring appears to change the patient's misinterpretations and consequences of physiological sensations, his belief on coping with panic attacks and that panic attacks

do not occur as frequently as assumed in the avoided places or situations. To account for avoidance behaviors, behavioral therapy offer exposure techniques to the feared stimuli or situation: The exposure exercises start with interoceptive exposure (exposing the patient to feared bodily sensations), then situational exposures (exposing the patient to situations or places they are avoiding) and lastly the combination of both interoceptive and situational exposure (Wieman et al., 2020, pp. 229–230).

In the following chapter, the basic principles of fMRI and Imaging genetics will be presented before ventilating the topic of the neural correlates of fear conditiong that are mostly derived by imaging data.

1.2 Principles of fMRI and imaging genetics

The advancement of technologies in the last three decades, especially in the field of magnetic resonance imaging (MRI) and computational software tools, provide the basis for noninvasive detecting of neural activity by hemodynamic responses of the brain tissue as performed in functional MRI (fMRI). In the following, the main functional principles of fMRI will be summarized below to shed light on first the physical and secondly the physiological mechanisms behind fMRI. This chapter provides background informations that are relevant for the understanding of the applied methods. This chapter is based on the informations given in Bushong & Clarke, 2013, Buxton, 2009, Faro & Mohamed, 2010 and Poldrack, Mumford, & Nichols, 2011

1.2.1 Physical principles of MRI

The physical principle of magnetic resonance imaging is grounded on the phenomenon of the field of nuclear magnetic resonance (NMR). Atomic nuclei, consisting of protons and neutrons, possess an intrinsic magnetic momentum. This magnetic momentum is featured as “angular”, thus implicating a direction of momentum or (axis of) spin, which in turn is associated with a magnetic dipole moment (see figure 4). The simultaneous work of Purcell and Bloch (Bloch, 1946; Purcell, Torrey & Pound, 1946) successfully conducted experiments showing that the spin frequency indeed rotate proportional to the exposed magnetic field and were subsequently awarded the Noble Prize in 1952. The discovery soon led to a new tool for medical imaging: the characteristic that a nucleus’ resonant frequency is directly proportional to the exposed magnetic field implicated that local variations in the magnetic field by the human body

within homogeneous magnet translate into changes of spectral lines. The application of a linear gradient field and the measuring of the distribution of frequencies allows the measure of the signal distribution in a particular sample, thus allowing to form an image (Lauterbur, 1973). The image illustrates a "map of the local transverse magnetization" of hydrogen (Buxton, 2009, p. 67). With Mansfield (Mansfield, 1977) introducing echo-planar imaging (EPI), a technique allowing prompt switching of gradients, first MRI scanners have been built in the early 1980s and representing an essential tool for diagnostic and interventions purposes. In addition to the intrinsic angular momentum of nuclei, the physical principles underlying MRI include the net

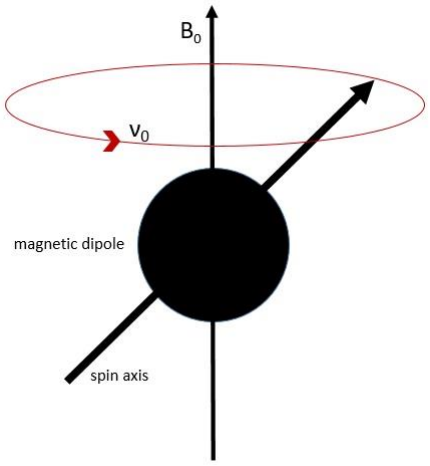


Figure 2. Precession of a magnetic dipole in a magnetic field.

The magnetic field B_0 exerts a torque on a nuclear magnetic dipole that would tend to make it align with B_0. However, because the nucleus also has angular momentum (spin), it instead precesses like a spinning top at an angle to the gravitational field. The precession frequency ν_0 is proportional to the magnetic field and is the resonant frequency of NMR. Text and adapted figure from: (Buxton, 2009b, p. 72).

magnetization, precession, free induction decay and the Fourier transformation, among others. Nuclei with even numbers of protons and neutrons consists of combined pairs that exhibit oppositely spin orientation and therefore do not feature a net spin (Buxton, 2009b, pp. 69–72). However, the single charged hydrogen nuclei consists of a single proton and is abundant in the human body making up about 80 % of all atoms (Bushong & Clarke, 2013, p. 8). In addition. The magnetic moments of the hydrogen atoms in the body are randomly oriented. However, short (milliseconds) exertion of current to the coil in a MRI scanner induces a strong magnetic field B_0 (e.g. 1,5 – 3 Tesla) which in turn causes approximately one of 1 million to align to this external magnetic field which leads to polarization, thus causing a net magnetization of

hydrogen atoms within the patient. Due to the angular momentum of protons, the alignment to the magnetic field does not occur immediately but the proton's spin axis precesses relatively to the Z-axis of the external magnetic field B_0 (Bushong & Clarke, 2013, pp. 9–10).

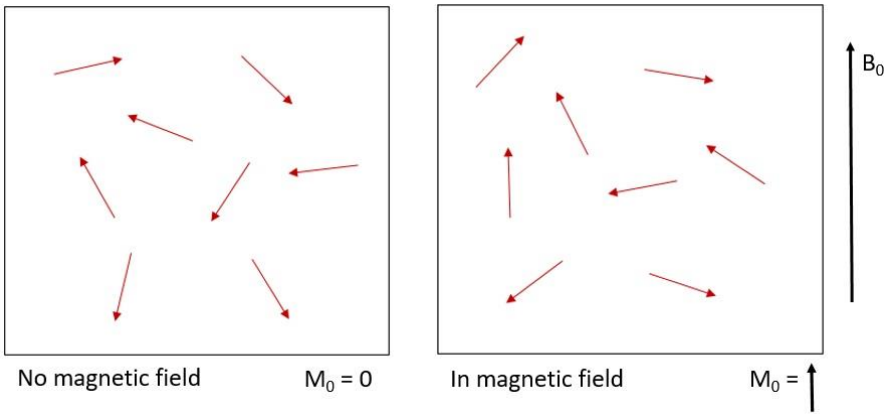


Figure 3. **Formation of an equilibrium magnetization (M0) as a result of partial alignment of nuclear magnetic dipoles.**

In the absence of a magnetic field, the spins are randomly oriented, and there is no net magnetization. When placed in a magnetic field B0, the spins partly align with the field, a relaxation process with a time constant T1 of approximately 1 s, creating a net local magnetization. Source: Text and adapted figure from: Buxton, 2009, p.74)

The frequency of precession (f_0) and the strength of the magnetic field (B_0) is proportionally related to each other. In addition to the gyromagnetic ratio (**γ**), these factors (B_0 and **γ**) constitute the Larmor equation:

$$\mathbf{f_0 = \gamma \times B_0}$$ **(Formula 1**; Bushong & Clarke, 2013, p. 11**)**

The gyromagnetic ratio (**γ**) is a constant and nucleus-specific factor, in case of hydrogen it is 42 MHz/T. Exemplary, at $B_0 = 1$ Tesla, the frequency of precession for hydrogen equals 42 MHz. In a receiving coil, the precessing net magnetization of hydrogen nuclei creates a radiofrequency signal ('free induction decay'). After shutdown of the external magnetic field, the spin of protons starts to decrease in phase coherence, which consequently leads to a decline of the radiofrequency signal ('relaxation time'). In MRI, the relaxation time can be further distinguished in two independent, but simultaneous forms: T_1 relaxation include the dissipating of magnetic energy in form of heat (transfer of energy from the spin on the surrounding atoms), thus the relative heat conductance of certain tissue crucially effect the T_1 relaxation time ('spin-lattice relaxation'). T_2 relaxation on the other hand refers to the time

constant that involves the loss of phase coherence in the spins through energetic exchange among spins ('spin-spin-relaxation' or 'transversal relaxation time'). The free induction decay represents the MR signal intensity across time. A mathematical application ('Fourier transformation') on the free induction decay leads to a NMR spectrum, which represents signal intensity across inverse time or hertz. In order to obtain an image by the NMR spectrum, the application of a further gradient magnetic field (B_x) with variation in field strength allows spatial or pixel localization by introducing a x-, y- and z-axis, thus enabling the selection of slice (z-axis), encoding of phase (y-axis) and the encoding of frequency (x-axis). The result is a three dimensional image consisting of small volume elements ('voxels') and a gray scale value representing the signal intensity. The length of time from excitation by the transmit coil and the acquisition of image is the 'echo time' ('TE'), whereas the time of two consecutive excitatory MR pulses represent the 'time of repetition' ('TR') (Bushong & Clarke, 2013, pp. 8–16; Buxton, 2009b, pp. 67–78).

1.2.2 Biological principles in fMRI

The conceptual idea that cerebral blood flow (CBF) indeed mirrors neuronal activity was first explored and presented in the late 19th century by experiments conducted by Roy and Sherrington (Roy & Sherrington, 1890) thus laying the foundations of hemodynamic-based neural imaging techniques as used in present fMRI.

The premise comprises the consideration that a local increase in CBF correlates directly with neuronal activity based on the close coupling of CBF and glucose metabolism or more specific, the cerebral metabolic rate of Glucose. Derived from this, it can therefore be assumed that changes in CBF and the metabolic rate of oxygen ($COMRO_2$) are correlated to each other. In this context, explorations via positron emission tomographic (PET) in humans revealed that activity related increases in CBF exceeded the increases in the $COMRO_2$ (activity related CBF > activity related $COMRO_2$), thus implicating that disequilibrium between changes in CBF and in the $COMRO_2$ produce an increase blood oxygenation levels in the capillaries and veins. This venous blood oxygenation level-dependent (BOLD) effect is based on fluctuations in deoxyhemoglobin concentrations, that "acts an endogenous paramagnetic contrast agent" (Faro & Mohamed, 2010, p. 3).

Derived from this BOLD-effect, alterations in local deoxyhemoglobin concentrations consequently lead to changes in the signal intensity in the MRI imaging. Therefore, apart CBF, this BOLD contrast represents a further parameter for mapping neural activity, as evidenced by several studies (Kim & Bandettini, 2010, p. 3). This hemodynamic response as shown in the BOLD-contrast underlies two basic features. First, the fast peak of neural activity (milliseconds) and the slow peak of the subsequent hemodynamic response (approx. 5 seconds) are followed by a poststimulus undershoot that remains below baseline for 15 – 20 seconds (see also fig. X). and secondly, the possibility of creating a statistical model allowing to treat the hemodynamic response as a linear time-invariant (LTI) system, which in essence describes the determination of neural activity by "adding together shifted versions of the response to a shorter train of activity" by the use of mathematical convolution (Poldrack et al., 2011, p. 2).

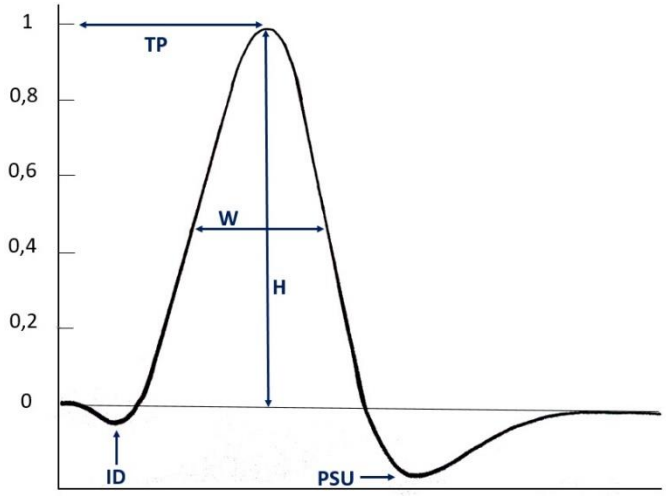


Figure 4. **Characteristics of the hemodynamic response.**
The hemodynamic response can be understood as an ideal and noiseless stimulus response due to interpersonal and intrapersonal variability. The shape of the HRF function can be described by a variety of characteristics including the time from the stimulus until peak (*TP*), height of response (*H*), the width of the HRF at half the height (*W*), poststimulus undershoot (*PSU*) and in some cases an initial dip (*ID*). The peak hight is correlated to the extend of neuronal activity, thus commonly of main interest. The initial dip is proposed to mirror early oxygen depletion prior to the actual hemodynamic. Source of text and adapted figure.: Poldrack et al., 2011, p. 72.

The property of "time invariance" signifies that a shift of a stimulus by t seconds induces a shift in the BOLD response by the same t seconds or time unit. "Linearity" on the other hand, means that a) the scale factor for the neural and BOLD response

remains the same (e.g. double magnitude in the neural response leads to a doubled BOLD response) and b) subsequent neural responses that are temporally close will result in a BOLD signal that sums the independent neural responses (implication of "additivity"). The linear and time invariant properties of BOLD signals, crucially constitute a basis for the application of statistic linear model in fMRI analysis, particularly the general lineal model (GLM). The term "general" implicates a variety of possible types of analyses e.g. correlations, F-test, one-sample and two-sample t-tests, analysis of variance or covariance (ANOVA/ANCOVA). In this, single dependent continuous variables or responses can be related to "one or more continuous or categorical independent variables, or predictors". Therefore, the GLM enables statistical parametric mapping in fMRI experiments where the X-axis comprise experimental variables and Y-axis comprise MRI data (Poldrack et al., 2011, pp. 191, 197).

When it comes to image intensity changes, several factors come into play: physiological noise (cardiac and respiratory cycles), non-physiological noise (e.g. minimal head movements or thermal noise by the instruments), and variation in the BOLD signals. To that effect, a pre-processing of the raw fMRI data ensures a decrease in various artifacts (e.g. caused by head movements), the arrangement for further statistical analyses such as group comparisons, and the statistical power. This editing of the images by SPM 5 included realignment, slice-timing, and normalization.

Slice-timing correction results from systematic differences in the acquisition time of each slice throughout the brain and therefore leading to a statistical correction. The systematic differences in the acquisition time are explained by the fact that slices are acquired one at a time (sequentially or interleaved) and thus in different points in time. Hence, a neural response appears in different brain slices resulting in different and time-delayed BOLD responses. A mismatch then occurs as a result of the statistical model assuming that all slices were acquired at the same time. A common approach to address this issue is by 'temporal interpolation': setting a reference slice (e.g. the middle slice), the rest of the slices can be time matched in relation to that reference slice. The time point of the reference slice is then used to estimate the signal amplitude for every other slice (Faro & Mohamed, 2010, p. 63; Poldrack et al., 2011, p. 41).

To address the spatial misalignment of images in the fMRI time series (e.g. due to minor head movements), a *realignment* was implemented. By setting a reference image, all the other images in the fMRI time series are realigned by rotation or

translation in three dimensions with regard to the reference image. This motion correction ensures the reduction in movements artifacts (Poldrack et al., 2011, p. 44).

For the purpose of investigating groups of individuals, anatomical variabilities in single individuals such as differences in the morphology (e.g. gyral structure) or the brain size must be taken in account. To reduce this variability in individuals a further pre-processing step ('*Normalization'*) must be applied to eventually perform analyses on a group-level. This requires a standardized, three-dimensional reference template to which the individual's EPI images are computationally aligned to. A commonly used frame is the MNI template was developed at the Montreal Neurological Institute by aligning images of healthy individuals and thus creating a mean EPI image. Hence, each EPI images of the individuals were realigned to the standardized mean EPI image of the MNI template leading to a voxel size of 2 x 2 x 2 mm^3 (Lueken et al., 2014; Poldrack et al., 2011, p. 50).

The next step in the normalized data involved the increase of the signal-to-noise ratio by reduction of small intensity artifacts or transients. This editing included *spatial smoothing* by the use of a three-dimensional Gaussian filter. In this procedure grey-scale values of each voxel were calculated from the means of the adjacent voxels, thus leading to a decrease of small intensity artifacts (e.g. single voxels) including signals that were small beforehand (e.g. in small brain regions). The images were smoothed until a 12-mm full width at half maximum (FWHM) (Poldrack et al., 2011, p. 50).

In summary, fMRI is a widespread, non-invasive and established method that contributed to the understanding of the cortical and subcortical neural networks of psychiatric disorders (e.g.anxiety disorders, schizophrenia, personality disorders), neurological disorders (e.g. movement disorders, aphasia, apraxia) and higher brain functions (e.g perception and attention) by the combined use of physical and biological principles. Standardized tasks are used to evoke and acquire local brain activation in healthy and/or affected individuals which eventually can be statistically analyzed. FMRI offer different applications as for instance diffusion tensor imaging or connectivity analysis (influence or correlation of a distinct brain region on another brain region). Not only in understanding the pathophysiology of neuropsychiatric conditions, but also the evaluation of therapeutic processes and the diagnostics and early detections of certain conditions in high-risk subjects are becoming the focus of attention. FMRI especially enabled greater insights in localising the neural correlates of fear conditioing and thus anxiety disorders in the last decades, despite the

narrowness of MRI scanners that possibly could pose a potential adversitiy for anxious individuals (Schneider & Fink, 2007, p. 2; Sehlmeyer et al., 2009).

1.2.3 Imaging Genetics

The genetic make-up of an organism and accompanying gene expression and epigenetic modulation is a crucial process to determine the structure and physiological function of proteins in the organism and thus shape a phenotype (an individual`s behavioral/clinical property). Mutations or crossing over in meiosis contribute to genetic variations (polymorphism) in individuals and thus potentially lead to altered structure and functions in protein. In addition, psychiatric disorders suggest a heritable factor to disease susceptibility (Baselmans et al, 2021; Burmeister et al, 2008), adding to the importance of further investigating the underlying mechanism that drives the susceptibility. The ability of the HPA axis to process stress (or: the processing capacity) is driven by genes that encode the related hormones and their precursors, enzymes, receptors and transporters (Shi, 2021). Modulators of the HPA axis also include the involvement of epigenetic pathways by external and internal stressors during embryo and childhood development (e.g. severe stress exposure to pregnant women or childhood trauma/abuse) that cause long-lasting or even heritable DNA methylation, which in turn can impact the processing of the stress response by the HPA axis and increase the risk for developing Anxiety Disorders in later life (Greetfeld et al., 2009; Shi, 2021). In this micro level perspective, the genetics and molecular mechanisms influence the structure and functions of hormones, transmitters and receptors in cells which have an effect and are detectable on the macro level of e.g. brain functions and behavior (Montag, 2017, p. 34). Genetic imaging is defined as a method that combines genetic data (e.g. gene variants or mutations) with imaging data (e.g. functional or structural MRI) in order to illuminate which or how brain areas are affected by a certain genetic variant (Montag, 2017, p. 43). Imaging genetics offer a technique to further investigate the neural pathways of related psychiatric disorders deriving from genetical factors, as given in gene polymorphism. The impact of genetic polymorphisms is explored by the measurement of neural activity be means of fMRI in association with an selected apropiate task for the patient or research participant as explained in chapter 2.4.

In summary, imaging genetics offer a tool to illuminate the impact of gene polymorphisms in brain activation to eventually understand their impact and neural pathways in psychiatric illness. This work aims to explore the functional role of a *CRHR1* variant rs17689918 in fear conditioning and thus the impact of the brain activation and clinical outcome of PD/A in female patients. The next chapter summarizes the acquired fMRI data on the neural correlates on fear conditioning and CBT-mediated effects on PD.

1.3 Neural correlates of fear conditioning and CBT-mediated effects

Classical fear conditioning, representing a form of associative learning, has been linked to the development and continuation of anxiety disorders (as for instance PD/AG) and is the most commonly used and powerful model in neuroimaging studies of anxiety or stress disorders (Daffre, Oliver, & Pace-Schott, 2020, p. 16; Tovote, Fadok, & Lüthi, 2015). By definition, fear conditioning "involves the pairing of a neutral stimulus with an aversive unconditioned stimulus (US)": The initially neutral stimulus "CS" becomes an aversive conditioned stimulus after repetitive pairings with the fear-inducing or aversive unconditioned stimulus (US), eventually causing anxiety with the expectation of the initial neutral "CS" that eventually turns into a danger cue "CS+", whereas another neutral stimuli remains unpaired with the unconditioned stimulus, hence representing a safety signal "CS-" (Lissek et al., 2014; Sehlmeyer et al., 2009). Consequently, this associative learning mechanism is indicative of a potential pathology and for the development of anxiety disorders considering the fact, that anxious reactions to a CS still persevere "in the absence of a CS/US contingency" (Lissek et al., 2005).

First deliberations on the role of fear conditioning in the onset and preservation of AD have been suggested for more than 90 years with the initial description of classic conditioning by Pavlov and Watson and Rayner (Pavlov, 1927; Watson & Rayner, 1920). Over the years several theoretical considerations and models have been introduced in order to elaborate on certain aspects that promote fear conditioning, for instance that a biologically substantiated preparedness and ability in creating aversive association to (external) stimuli constitutes a relevance for survival in the context of evolution, thus supporting a phylogenetic disposition to, for instance, phobic anxiety

(e.g. spiders, heights etc.) (Seligman, 1971). More specifically, other authors suggest a failing in inhibiting fear responses towards safety cues (Davis, Falls, & Gewirtz, 2000), or a higher susceptibility to increased fear learning and thus resulting in greater augmentation of the CS in absence of the US (Eysenck, 1976). In regards to the development of PD, Bouton et al proposes that the very exposure to panic attacks poses a causal factor leading to fear conditioning to intero- and exteroceptive cues: The conditioned anxiety, acting as an "anticipatory emotional state" for an upcoming panic attack however, furthermore intensifies future panic attacks. Promotive psychological or biological factors (e.g. "catastrophic misinterpretation of somatic sensations") amplify the susceptibility and vulnerability towards fear conditioning (Bouton et al., 2001). Indeed, a meta-analysis indicates that patients suffering from AD showed "greater excitatory conditioning to danger cues (CS+)" and "impaired inhibitory conditioning to safety signals" (CS-) versus healthy controls in fear conditioning (Lissek et al., 2005). Thus, experimental fear conditioning paradigms have been utilized for the investigation of AD and the exploration of underlying neural correlates via imaging studies (e.g. Yang et al., 2014 for a review). In this regard, insights on neural correlates of fear conditioning in PD/A within the context of CBT will be summarized below.

In line with the proposed neural fear circuitry (Clark & Beck, 2010; Gorman et al., 2000) animal studies highlight subcortical (amygdala, hippocampus, BNST, PAG, VTA) and medial prefrontal regions as core structures in classical fear conditioning processes (Kalisch & Gerlicher, 2014; Moustafa et al., 2013; Oliva et al., 2020; Tovote et al., 2015). In vivo human neuroimaging via positron emission tomography (PET)- and fMRI-techniques furthermore approves the previous research by identifying basic and consistent key structures such as the amygdala, dorsal and ventral ACC, insula and medial and lateral prefrontal cortices in classical fear conditioning, nonetheless of the methodological differences between imaging studies (Fullana et al., 2016; Sehlmeyer et al., 2009). With particular regards to PD, hyperactivation in fear network regions as the insula (among others, implicated in the interoception of internal homeostasis of the body), striatum (processing of salient cues), the dorsal ACC (implicated in fear acquisition and appraisal of threat), left IFG (suggested in cue detection and/or cognitive threat evaluation) and dmPFC (= "appraisal of threat") have been repeatedly shown in PD-patients. Furthermore, volumetrics deficits in the amygdala (considered

in the processing of emotionally valanced cues) and hippocampus (suggested in extinction consolidation) have been demonstrated (Pohlack et al., 2012; Kircher et al., 2013; Straube et al., 2014b; Fullana et al., 2016; Sobanksi & Wagner, 2017; Daffre et al., 2020, pp. 17–31). This conspicuous overlap of key structures in fear conditioning and the proposed fear circuitry add to the appropriateness and eligibility of classical fear conditioning paradigms for the exploration of neural substrates in anxiety disorders (Fullana et al., 2016; Shin & Liberzon, 2010).

Further fMRI studies conducted by Lueken et al (2014), approved increased bilateral activation of the IFG in patients during fear acquisition (CS+ > CS-), thus adding to the conclusion of altered top-down fear processing in PD/A patients. Beyond that, Lueken et al illustrate activation patterns in terms of bottom-up-processes involving enhanced activation in the periaqueductal gray (PAG) towards aversive stimulus (CS+) and safety signal processing (CS-) in comparison to healthy controls. The PAG is a midbrain structure is essential for the mediation of defensive reactivity when confronted with aversive stimulus or threat, hence suggesting enhanced bottom-up-processes in PD/A patients by means of increased activity in the PAG in processing threat (Lueken et al., 2014). In search for predictive neural markers for treatment response or nonresponse in CBT, Lueken et al (2013) applied a fear conditioning paradigm in PD/A patients that have been split in both groups, namely responders and nonresponders. At baseline, nonresponders show higher activations in the amygdala, hippocampus and in the right pregenual ACC when exposed to safety signals. The enhanced activations in the above identified brain areas suggest therefore dysfunctional processing of safety signals indicating lower effectiveness in comparison to responders. Even though enhanced activation in the above mentioned brain areas partially diminish after CBT, responders however, showed enhanced activation in the right hippocampus when exposed to stimulus contingencies of CS+ and CS-. In addition to this, patients with an increased inhibitory coupling of ACC and amygdala are correlated with treatment response (Lueken et al., 2013).

Kircher et al is one of the first to examine the effects of CBT on neural correlates of fear conditioning in patients suffering from PD/A. Comparing PD/AG patients and healthy controls in a fMRI fear conditioning paradigm (same paradigm as described in chapter 2.4), patients exhibit increased activity in the left IFG prior to therapy in differential conditioning (CS+ > CS-). In addition, conducted connectivity

analysis revealed an increased connectivity between the IFG and regions involved in the fear circuitry as the bilateral amygdalae, hippocampi, ACC, and the medial and lateral prefrontal cortices. After therapeutic intervention via CBT, patients show a significant activation reduction in the left IFG compared to baseline and controls. Moreover, the decrease in left IFG activation significantly correlates with the decrease in agoraphobic symptoms as measured in the MI7 (accompanied and alone). In this regard, Kircher et al proposes an "increased association of cognitive and emotional processes" in such a way that an increased attention to harm expectancy or threat (as represented in the increased activation of the left IFG) are more likely to elicit emotional responses in PD/A patients. However, the increased functional connectivity between IFG and amygdala in PD/A patients does not change by the intervention with therapy CBT in both patients and controls and therefore indicating that the modulation of this connectivity is harder to achieve or is likely detectable after a more extended time period (Kircher et al., 2013).

As mentioned earlier in chapter 1.1., gene variants indeed moderate the vulnerability of developing PD. In this regard, imaging genetics offers a method to actually detect genetically driven modulation of fear conditioning on the level of distinct neural activity in the brain. However, it remains to be shown whether and how a genetic variation of the gene *CRHR1* impacts neural activation in fear conditioning and clinical response to CBT. The following chapter therefore discusses the role of *CRHR1* in the aspects of physiology and in Anxiety Disorders.

1.4 The CRH/CRHR1-system in Anxiety Disorders

A common characteristic amongst anxiety disorders is the occurrence of fear and an inadequate stress reaction albeit no apparent stressor or actual threat present. A stress reaction is defined as a coordinated reaction to threat cues which are avoidance behavior, hypervigilance and arousal, activation of the sympathetic nervous system and the release of cortisol. Thus, the understanding of the physiology of stress reactions plays a vital role in the breakdown of Anxiety Disorders such as PD/AD (Bear, Berry, & Paradiso, 2009, p. 830; Hains & Arnsten, 2008). The hypothalamic-pituitary-adrenal axis (HPA axis or stress axis) plays a key role in the facilitation and regulation of a stress reaction next to the autonomic nervous system (sympathoneuronal and

sympatho-adrenomedullary limbs) and the release of noradrenaline and adrenaline (Deussing & Chen, 2018). In addition to this peripheral pathway with the subsequent release of cortisol in the adrenals, CRH acts as neuromodulator as well (Deussing & Chen, 2018) and mediates anxiogenic effects of stress in the brain by means of CRH receptor 1 (expressed by the gene *CRHR1*), thus reflecting a central pathway (see fig. 1.3.1).

The HPA axis, being a major and elementary neuroendocrine system, is crucial for maintaining and regulating various homeostatic systems (e.g. the cardiovascular system, metabolic system, immune system the central nervous system) and for adaption to stressful events. Stress is defined as a response (physical and behavioral) to internal or external stimuli that leads to a state of real or perceived disruption to internal homeostasis (Smith & Vale, 2006; Weber et al., 2016). The stress response includes a sequence and complex interaction of endocrine glands including the initial secretion of CRH in the hypothalamus that stimulates the release of ACTH in anterior pituitary, which in turn leads to the release of cortisol in the adrenal cortices.

The CRHR exists in two forms, CRHR1 and CRHR2, expressed by two different genes and differ in expressional pattern (Weber et al., 2016). These G protein-coupled plasma membrane receptors with an adenylyl cyclase/cAMP signaling system downstream effectors, bind CRH and urocortin with high affinity (Aguilera et al., 2004; van Pett et al., 2000). Despite CRHR1 being structurally 70 % homologous in comparison to CRHR2, the distinct N-terminus and the varying distribution of these receptors in the central and periphery nervous system indicate diverse physiological functions. CRHR1 is widely distributed in the brain, mainly in the cerebral cortex, amygdala, hippocampus, pituitary, olfactory bulb and also peripheral as for instance in adipose tissue, heart, placenta, testis, ovary and more (Hillhouse & Grammatopoulos, 2006). The CRHR1 exists in eight isoforms or variants due to alternative splicing of the *CRHR1* gene involving a deletion of one exon out from 13 existing exons (Binder & Nemeroff, 2010). These structural changes in *CRHR1* gene variants are suggested to result in “different degrees of capacity and efficiency in binding CRF and its agonists” (Hillhouse & Grammatopoulos, 2006). The primary function of CRHR1 is the binding of the corticotropin-releasing hormone (CRH) and thus the read out of the POMC gene and release of β-endorphin and adrenocorticotropic hormone (ACTH) from the pituitary gland, which leads to increased levels of cortisol (Hillhouse & Grammatopoulos, 2006).

Besides this neuroendocrine function, CRHR1 mediates also "behavioral and autonomic responses to stress" (Holsboer, 1999). Abnormalities and a stress-induced chronic hyper functioning of the CRH/CRHR1 axis have been associated with various disorders such as anxiety disorder, depression, sleep disorders, addictive behavior, chronic pain and fatigue states, allergic and autoimmune inflammatory disease and more (Hillhouse & Grammatopoulos, 2006; Refojo et al., 2011). In the following, the role of CRHR1 in Anxiety disorders -with particular regard to the *CRHR1*-gene variant rs17689918- will be summarized in the context of animal and human studies.

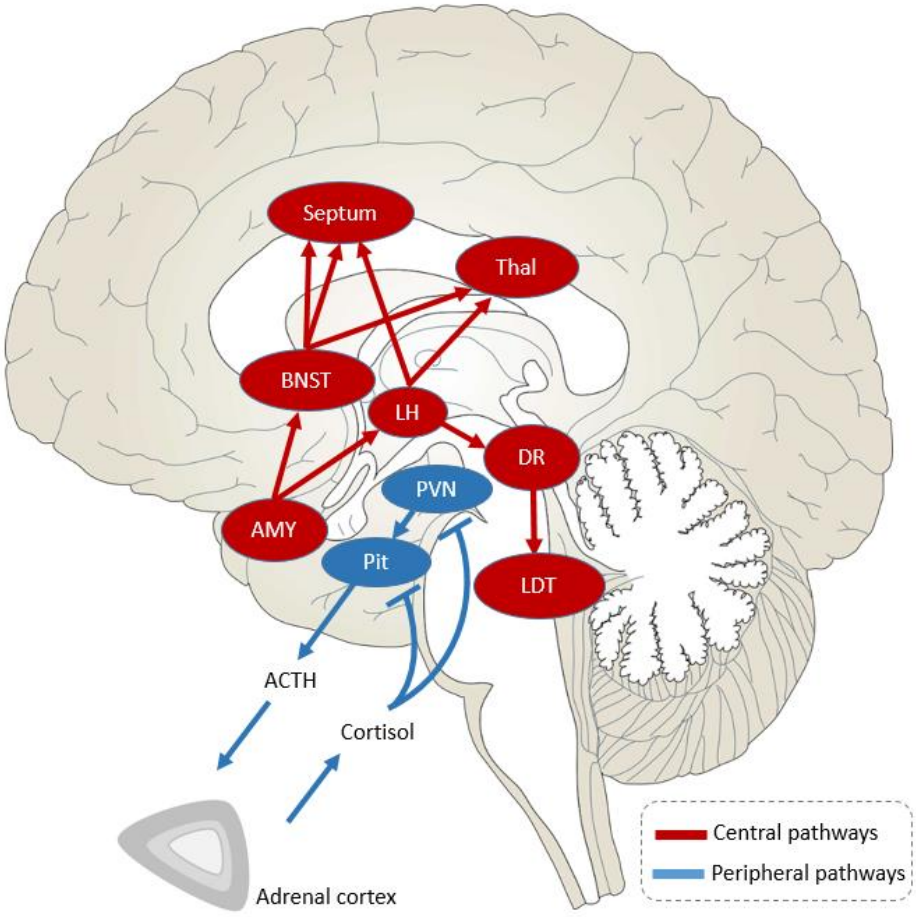


Figure 5. ***The neurotransmitter action of CRH on CRHR1.***

The corticotropin-releasing factor (CRH or CRF) is mainly produced in parvocellular neuroendocrine cells in the paraventricular nucleus (PVN) of the hypothalamus. In response to the physiological or psychological stimulation, stress or illness CRH is released into the hypophyseal portal system and reaches the anterior pituitary gland. The bonding of CRH and its receptor, CRHR1, stimulates the release of the adrenocorticotropin (ACTH) in basophilic cells in the anterior pituitary gland, which in turn stimulates the release of glucocorticoid hormones as cortisol in the adrenal cortex. Increased levels of cortisol, in turn, suppress the hypothalamic expression of CRH by binding to glucorticoid receptors in the hippocampus and hypothalamus and therefore elicits a negative feedback. In addition to this

peripheral pathway, CRH functions as a neurotransmitter and mediates anxiogenic effects of stress in the central nucleus of the amygdala and bed nucleus of the stria terminalis (BNST), which in turn generates anxiety-like behavior. The amygdala is implicated having a decisive role in the processing of "negative emotional memory" of aversive and threatful stimuli (as for instance in conditioned fear) and sends heavy projections to the BNST and lateral hypothalamus among other crucial structures as for instance the forebrain and brain stem. The BNST and lateral hypothalamus in turn send projections to areas in the brainstem (dorsal raphe, laterodorsal tegmental nucleus) and hypothalamus that elicit autonomic and behavioral responses to aversive stimuli or threat. The neurons that express CRHR1 are embedded in various neurotransmitter systems, as reported in rodent studies: Glutamatergic neurons in the hippocampus and cortex, GABAergic neurons in the reticular thalamic nucleus and globus pallidus, dopaminergic neurons in the ventral tegmental area and in the substantia nigra and lastly serotonergic neurons in the median and dorsal raphe. DR, dorsal raphe, LDT, laterodorsal tegmental nucleus, Thal, thalamus, (Berton & Nestler, 2006; Refojo et al., 2011; Sean M Smith & Vale, 2006). Source of figure: adapted from Berton & Nestler, 2006.

1.4.1 Animal Studies

Several studies have been carried out on rodents to illustrate the relevance and role of CRHR1 on stress response and anxiety. The lack of *CRHR1* in homozygous (-/-) mice mutants results in an 'increased exploratory activity' in open field tests and 'reduced anxiety-related behavior' under basal conditions. Furthermore, these CRHR1-deficient mice showed an atrophy in the medulla of the adrenal gland and a reduced release of stress-induced ACTH and corticosterone (Timpl et al., 1998). Another knockout mouse line with inactivated CRHR1 function in the anterior forebrain and limbic structures (and otherwise functional HPA-axis), exhibit reduced anxiety and a normal basal activation of their HPA system. The anxiolytic effect is therefore not dependent of the HPA activity. However, these mutants (Crhr1 loxP/loxP Camk2a-cre) show significantly elevated levels of corticotropin and corticosterone after stress exposure in comparison to *CRHR1* null mutants and therefore indicate a hypersensitivity to stress. Thus limbic CRHR1 circuitries serve as a mediator for anxiety-related behavior and is moreover essential for a feedback control of the HPA system and therefore for the adaption to stress (Müller et al., 2003). In this regard, a CRHR1 knockout in the anterior forebrain neurons and in the basolateral amygdala lead to decreased anxiety levels and furthermore emphasizes the anxiogenic role of CRHR1 in these structures (Sztainberg et al., 2010; Wang et al., 2012). In a rhesus macaque model, anxious temperament and the accompanied increased metabolic activity in predictive brain areas such as the anterior hippocampus and amygdala were shown to be affected by

CRHR1 variation prior to any adversities in childhood (Rogers et al., 2013).

In further decluttering the functional role on the CRH/CRHR1-dependent BNST activation, animal models indicate that the BNST is involved in the mediation of sustained fear rather than phasic fear (Davis et al., 2010; Schulkin et al., 2005). In laboratory models (Walker, Miles & Davis, 2009), rats who were given CRHR1-antagonist showed blocked defensive behaviors during sustained fear, while this was not the case during phasic fear. In line with this, an overexpression of the CRH gene in the BNST is shown to be correlated with heightened responses of sustained fear which presumably is "mediated by enhanced CRH receptor signaling or compensatory changes in CRH receptor density within these structures" (Sink et al., 2013).

Moreover, in a contextual conditioning paradigm it has been demonstrated that CRHR1 in the dorsal hippocampus amplify the consolidation of fear memories by means of an increased GluR1-mediated signaling in AMPA receptors, thus leading to "exaggerated fear memories" (Thoeringer et al., 2012). Therefore, the CRHR1 driven heightened responses of sustained fear via overexpression of CRH in the BNST, and the role of hippocampal CRHR1 in enhanced remote fear memory consolidation has furthermore been suggested to be crucially involved in the generalization of fear (Weber et al., 2016). Refojo et al (2011) suggests a bidirectional role of CRHR1 in terms of anxiety disorders, which on the one hand indicate that CRHR1 in glutamatergic circuits in the prefrontal cortices are linked to the neurotransmission in the amygdala and hippocampus. Therefore, mice that lack CRHR1 in glutamatergic circuits in the prefrontal cortex therefore exhibit reduced anxiety-like behavior. On the other hand, the deletion of dopaminergic neurons in the midbrain results in a reduced release of dopamine in the prefrontal cortex and thus exhibit increased anxiety-like behavior. This bidirectional model suggests "that an imbalance between CRHR1-controlled anxiogenic glutamatergic and anxiolytic dopaminergic systems" might contribute to anxiety disorders (Refojo et al., 2011).

1.4.2 Human studies

Numerous studies provide evidence for the pivotal role of *CRHR1* variants in stress-related disorders, in particular depressive and anxiety disorders in humans (Binder & Nemeroff, 2010; Florian Holsboer & Ising, 2010; Ishitobi et al., 2012; Keck et al., 2008; Smith, Goldstein, & Grant, 2016; Spijker & Van Rossum, 2012). Polymorphisms

of *CRHR1* in combination with maltreatment in childhood have been shown to diminish cortisol reactivity in adults to stress (Sumner et al., 2014). Most comprehansive evidence for the role of *CRHR1* in humans has been revealed by a recent study applying a multilevel approach evaluating allelic variations of *CRHR1* as a risk factor for panic disorder (Weber et al., 2016). This study provides the basis for the present thesis. The genotyping of 531 matched patients (suffering from PD) and healthy controls ultimately revealed nine single-nucleotide polymorphism (SNPs) of the *CRHR1* gene. Four of these nine SNPs were found to be associated with PD. Among these, the minor allele AA/AG of rs17689918 has been shown to significantly increase the risk for PD in the female subgroup. Moreover, an expression analysis of the CRHR1 has been carried out on post mortem tissue (forebrain, midbrain and amygdala) from 76 deceased individuals (female= 18; mean age 48.6 ± 12.8 years), revealing a significantly reduced expression of mRNA CRHR1 by the minor allele (AA/AG) of rs17689918 in the amygdala and forebrain. The fMRI analyses of neural activation patterns in a differential fear conditioning paradigm (as described in chapter 2.4) revealed different top-down and bottom-up processes in risk-allele carriers: On one hand, activations in bilateral (predominantly left) frontal cortices revealed reduced differential conditioning responses. On the other hand, risk allele carriers revealed increased activation in the fear processing area as the left amygdala towards safety signals. Together, these activation patterns indicate a risk-allele (AA/AG- haplotype of rs17689918) driven generalization of fear and dysfunctional safety signal processing which constitute indicative mechanisms of PD and correlation with treatment nonresponse to exposure-based therapy (see also fig.) (Weber et al., 2016).

In addition, a behavioral avoidance task, namely in a fear-provoking situation, risk-allele carriers showed a lower frequency in escape behavior during anticipation, exposure and recovery periods in comparison to non-risk genotype patients. In addition to this, risk-allele carriers exhibit lower heart rates at exposure of a fear-provoking situation, despite similar reported subjective distress in both groups (risk allele carriers and non-risk genotype patients). This dissociation of reported distress and physiological response in risk-allele carriers point towards a “phenotype characterized by fear sensitization and hence sustained fear” (Weber et al., 2016). In line with this, Mc Teague et al argues that chronic hyperarousal and dysphoria in anxiety disorders eventually attenuates the “mobilization for defensive action” due to the transitioning to a phenotype characterized by “pervasive agoraphobic apprehension and avoidance,

broad dysphoria and compromised mobilization for defensive action" (McTeague et al, 2011). In terms of psychometrics data, Weber et al demonstrated that female risk allele carrier scored higher on anxiety sensitivity (ASI, 2.42 points per A risk allele ($p = 0.040$) and both ASI3 subscales, namely cognitive (0.72 points per A risk allele; $p= 0.050$) and social concerns (1.02 points per A risk allele; $p= 0.004$) in comparison to control patients (non-risk genotype patients) (Weber et al., 2016).

In summary, it can be derived that the risk allele AA/AG of rs17689918 of the *CRHR1* gene leads to generalization of fear (as reflected in reduced differential conditioning responses in predominantly left frontal cortices) and dysfunctional safety signal processing (as reflected in increased activation in the left amygdala for safety signals) in the context of fear conditioning. However, the question that remains is whether the risk allele AA/AG of rs17689918 of the *CRHR1* gene has measureable effects on the neural correlates in fear conditioning *after* the patient's exposition to CBT.

1.5 Objectives

Based on the previous literature, Weber et al indicate that the minor A risk allele for rs17689918 significantly increases the risk for PD in females. Risk-allele carriers demonstrate reduced expression of CRHR1 in the forebrain and amygdala and congruously show impaired activation responses in the predominantly left dlPFC in differential conditioning and increased activity in the amygdala for safety signals, hence suggesting generalized fear and dysfunctional safety signal processing. This and the de-synchronicity of reported anxiety and physiological responses (rate of escape behavior and heart rate) furthermore adds to the suggestion of a phenotype that is characterized by fear sensitization and sustained fear. In addition, psychometric data suggest increased levels of anxiety sensitivity in risk-allele carriers (Weber et al, 2016). Moreover, Lueken et al demonstrated that dysfunctional processing of safety signals at baseline (as in increased activation in the amygdala for safety signals) correlates with less symptom reduction after CBT compared with responders who benefit more from CBT (Lueken et al., 2013). Successful treatment response is indicated with a normalized left IFG activation that in addition correlates with a decrease in agoraphobic symptoms (Mobility Inventory) (Kircher et al., 2013) and by increased right hippocampal activation in the context of fear conditioning (Lueken et al., 2013).

Weber et al provide baseline data (clinical and fMRI data) on risk-allele carrier (AA/AG) of rs17689918, therefore this study is exploring the following two questions: (1) Do female PD/A-patients that carry the AA/AG-haplotype of rs17689918 benefit less from CBT in comparison to female PD/A control patients with the non-risk GG-haplytpe? And (2) how do female PD/A patients, that carry the AA/AG-haplotype, respond to differential conditioning after CBT in terms of neural substrates in comparison to female PD/A control patients (carrier of the GG-haplotype)? Based on this, the following hypotheses derived are the following:

1. Hypothesis:

   A modulating effect of the risk allele on the therapeutic outcome is assumed: The female PD/A patients and risk allele carriers (= AA/AG-haplotype of the *CRHR1* gene variant rs17689918) benefit significantly less from the therapeutic intervention with CBT compared to the control group (= female PD/A patients and carrier of the non-risk GG-haplotype of the *CRHR1* gene variant rs17689918), which can be determined by means of significantly higher scores in the panic-relevant interviews/questionnaires (ASI, ACQ, PAS, BDI-II, CGI, HAMA, MI alone/accompanied) in the pre-/post comparison, thus benefiting less from CBT than the control PD/A patients (GG-haplotype). Main outcome measure was HAMA and secondary outcome measures were MI alone/accompanied, ASI, ACQ, PAS, CGI and BDI-II (Gloster et al., 2009). While we expect a consistent effect across measures, an isolated effect (or a reduction) in a particular score might convey a cue towards a specific dimensional trait of anxiety, e.g. anxiety sensitivy or agoraphobic symptoms.

2. Hypothesis:

   The presence of genetic risk (AA/AG-haplotype of the *CRHR1* gene variant rs17689918) modulates the neural processing of CS+ and CS- in the early phase of the fear conditioning experiment in the lateral prefrontal cortex. Kircher et al (2013) revealed a reduced activity in the left IFG in differential conditioning (CS+ > CS-) after therapeutic intervention with CBT, that in addition correlates with reduced clinical symptoms (MI-7). We hypothesise a significant effect of *CRHR1* gene variant rs17689918 on the CBT related

effects: risk-allele carrier show reduced CBT related changes for CS+ > CS- in the left IFG due to aberrant baseline responses in the left dlPFC compared to female PD/A-control patients (GG-haplotype) in the early fear conditioning phase. In addition, we hypothesize increased activation in the amygdalae for safety signals (CS-) compared to the control group, as demonstrated in Weber et al (2016).

2 MATERIALS AND METHODS

2.1 Participants

The patient sample was obtained from a subsample of a multicenter and randomized controlled clinical trial 'PANIC-NET' (Gloster et al., 2009) in Germany, in which eight centers participated (Aachen, Berlin-Adlershof, Berlin-Charité, Bremen, Dresden, Greifswald, Münster, Würzburg). This national research initiative was funded by the Federal Ministry of Education and Research and treated 369 unmedicated patients suffering from PD/A (100%) and comorbid Major Depression (MD, 33.2 %). PD/A and MD were diagnosed by a structural clinical interview (CIDI, Composite International Diagnostic Interview) according to DSM-IV criteria.

This fMRI-study explores participants from the same fMRI-subsample as in Weber et al (2016). However, due to loss to follow-up (drop outs) the initial sample of 19 risk-allele carriers (n= 16 females) and 29 control patients (n= 17 females) in Weber et al (2016) got reduced to a total sample of 16 risk-allele carriers (n=13 females) and 22 controls patients (n= 12 females). The fMRI sample in this study consists only of the female subsample: 13 female patients (mean age 33.7 ±8.36 years) being the risk-allele carriers (AG- and AA-haplotype) and 12 female patients (mean age 39.8 ±10.5 years) being no-risk allele carriers (GG-haplotype) and therefore representing the control sample. Initially, male patients were included in the beginning of this study. However, fMRI-analyses in the mixed female/male sample showed no significant fMRI findings. Secondly, as already reported (Weber et al., 2016) the risk-allele significantly predisposed only female patients for PD/A and not male patients. For these two reasons, male patients (n= 3 risk-allele carriers and n= 10 controls) were excluded.

Inclusion criteria were a current diagnosis of PD/A (evidenced by CIDI-Interview), a clinical-interview score > 18 on the Hamilton Anxiety Scale (HAM-A), >4 points on CGI (Clinical Global Impression), and an age of 18-65 years. All patients (18-65 years) had either normal or corrected visual acuity and were not acquainted with the experiment beforehand. Patients with other mental disorders such as schizoaffective and psychotic disorders, mental retardation, substance abuse disorders, relevant somatic comorbidities and neurological, neurodegenerative disorders were excluded. Written informed consent was obtained from each participant and the study protocol was approved by the local ethics committee according to the

declaration of Helsinki (Date of approval: 01.12.2006; Study: “Improving CBT for panic by indentifying the active ingredients and understanding the mechanism of action – a multicenter study” (ref: EK 164082006); Ethics committee: Medical faculty Carl Gustav Carus, Technische Universität Dresden; Chair: Prof. Wilhelm Kirch, applicant: Prof. Wittchen, Institut für Klinische Psychologie und Psychotherapie).

2.2 Clinical assessment

The evaluation of clinical data aims for the detection of therapy effects (pre/post comparisons), group differences (risk-allele carriers vs. controls) in respect of therapy responsiveness and to trace correlations between respective clinical data and therapy induced neuroplastic changes. Given the relatively small number of patients in this study (n= 12 risk-allele carriers; n=13 controls), the investigation has to be considered highly explorative. To increase sensitivity in this potentially underpowered sample, a variety of panic-relevant psychometric data have been used. The clinical data has been assessed by expert clinical judgement, interviews and self-reports from the patients (such as CIDI, HAM-A/SIGH-A, CGI ASI, ACQ, and BDI-II), measured by the number of panic attacks (PAS, Panic Agoraphobia Scale) and by the agoraphobic avoidance (MI, Mobility Inventory). The clinical data was assessed before and after CBT. Neuropsychological data was collected before treatment by using the Trail Making Test A and B (TMT-A/B) and digit span. These neuropsychological and clinical scores were tested for between-group differences across time (scores before CBT versus scores after CBT). For this, ANOVAs for repeated measures have been carried out considering the factors time (before/after CBT) and groups (risk-allele carrier/controls) post-hoc analysis for each risk-allele carriers and controls. The demographical data (age and educational level) and the neuropsychological data (TMT-A/B) were tested using $\chi 2$ and t-tests (two-tailed). Altogether, a threshold of $p<0.05$ indicated statistical significance. Due to the exploratory nature of these comparisons no correction had been applied. Tests were conducted by the IBM SPSS software (version 23.0, IBM Corp., Armonk, NY). In the following sections, you will find a short description of the above mentioned clinical assessments:

TMT-A/B – *Trail Making Test, part A and B.* This pencil-and-paper test is commonly used in neuropsychology to provide information on an executive function, speed of processing and scanning by sequentially connecting 25 encircled numbers (part A, e.g. 1, 2, 3 etc.) and by alternating numbers and letters by alternating (part B, e.g. 1, A, 2,

B, 3, C, etc). The score is given by the time (seconds) that was needed to complete the task (Papandonatos et al., 2015; Tombaugh, 2004).

CIDI - *Composite International Diagnostic Interview.* The computer-administered CIDI represents a standardized diagnostic interview for the assessment of mental disorders based on the criteria of ICD-10 and DSM-IV (Wittchen, 1994). The reliability and validation of this interview have been proved (Peters & Andrews, 1995; Wittchen, 1994).

HAM-A/SIGH-A - *Hamilton Anxiety Rating Scale.* The HAM-A is a clinical interview rating for the severity of anxiety symptoms (scores between 0 and 56), which are common to anxiety disorders as described in DSM IV. The SIGH-A (Structured Interview Guide for the Hamilton Anxiety Scale) represents a structured version of the HAM-A that shows a higher reliability (Shear et al., 2001)and thus has been utilized in this study.

CGI - *Clinical Global Impression.* The CGI provides a scale for an overall judgement concerning the clinical severity ranging from 1 (having no symptoms) to 7 (severe ill patients). Interviewers have been instructed to assess further symptoms (such as avoidance, anxiety, panic symptoms, anticipatory anxiety and functional level) before assessing the global rating, thus leading to a higher reliability (Kircher et al., 2013).

MI – *Mobility Inventory.* The MI measures the self-reported agoraphobic avoidance behavior by rating 27 situations from 1 (never avoid) to 5 (always avoid) both when patients are alone and accompanied by a trusted person. Furthermore, a 7-day version of the MI has been used (MI-7), which is identical to the original MI with the only difference being that patients are to report for the last 7 days (Kircher et al., 2013). The MI was found to be sensitive to change with treatment and showing high reliability (Chambless et al., 1985).

PAS - *Panic and Agoraphobia Scale.* The PAS is a questionnaire assessing the severity of the illness of patients suffering from Panic Disorder or Agoraphobia by self-report on five subscales (Panic Attacks, Agoraphobia, Anticipatory Anxiety, Disability and Worries about Health) and a scale reaching from 0 to 4 points for each of the 13 questions. The efficacy of PAS-scores concerning reliability and the sensitivity to

change have been proved (Bandelow et al., 1998).

ASI - *Anxiety Sensitivity Index.* The ASI consists of a 16-item self-reported questionnaire aiming to assess patients' beliefs on symptoms and potential social/somatic consequences related to their anxiety (Peterson & Heilbronner, 1987). The rating of each item ranges from 0 ("very little") to 4 ("very much").

ACQ – *Agoraphobic Cognitions Questionnaire.* The ACQ is a 14-item questionnaire which assesses the frequency of maladaptive cognitions related to the consequences that arises from anxiety or a panic attack. The frequency of items e.g. "I will choke to death" or "I will have a heart attack" is ranked by a scale (1 -5) ranging from "thought never occurs" (1) till "thought always occurs" (5) (Chambless et al., 1984; Khawaja, 2003).

BDI-II - *Beck Depression Inventory-II.* The BDI-II is composed of 21 questions and measures the self-reported severity of a diagnosed depressive disorder. Patients are to report for the last two weeks (Whisman, Perez, & Ramel, 2000).

2.3 Treatment

In the above mentioned 'PANIC-NET' study, PD/A patients received CBT in 12 bi-weekly sessions (each for about 100 min), which were standardized and based on a controlled treatment protocol (A. T. Gloster et al., 2009). Established manuals for patients suffering PD/A have been optimized by trained experts in order to minimize the between-therapist variability (Barlow et al., 2000).

In the first three sessions, patients received an analysis of the patient's behavior in terms of symptoms and coping, while the 4th and 5th sessions focused on establishing an exposure treatment and exercises including interoceptive exposure. The following sessions 6 to 8 conducted exposure exercises (e.g. department store, forest, and bus), which were standardized and in situ. Patients were encouraged to observe their fear and fear related symptoms, in addition to avoiding safety behaviors that relieve their anxiety, but to rather allow and endure their anxiety. The progress has been assessed in the 9th session, which was followed by two more exposure sessions (10 – 11) and this time targeting situations that are most feared by the patients. Relapse prevention,

therapeutic gains and future plans for continued exposure exercises were addressed on the last 12th session. However, the exposure sessions 6-8 and 10-11 differ by two versions of CBT, namely therapist guided (T+) and non-therapist guided (T-), among which patients got randomly assigned to. The therapist guided version and the non-therapist guided version of CBT were demonstrated to be effective. Considering the comparability of both treatments (T+ and T-) and the given fact that both groups of patients showed a significant reduction of symptoms, they have not been subdivided in this study (Gloster et al., 2009; Gloster et al., 2011; Kircher et al., 2013)

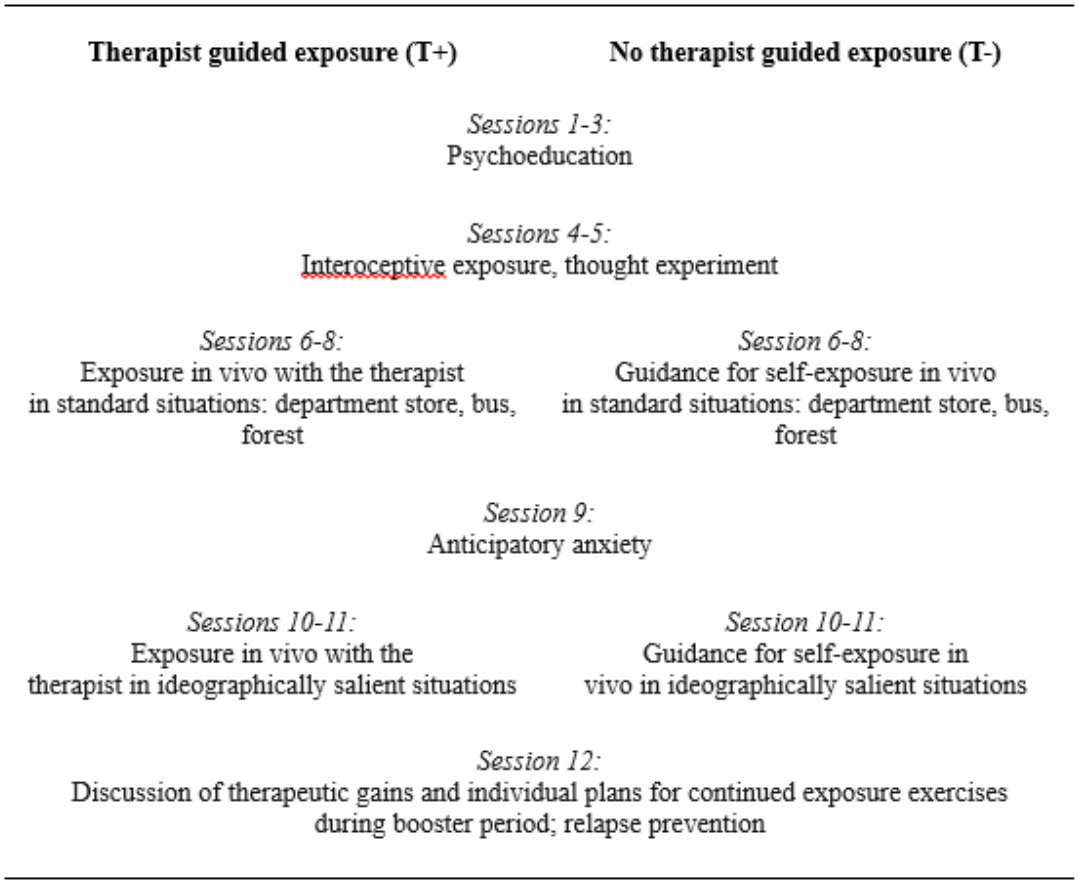


Figure 6. **Main components of the treatment procedure**

The T+ condition included a present therapist in vivo exposure exercises in the sessions 6-8 and 10-11, whereas in the T- condition, no therapist accompanied the exposure. Both, T+ and T- included the same components, the same duration and number of exposure; the frequency, patient's distress and duration have been recorded in both as the review of the self-exposures (modified figure and text from Gloster et al., 2009).

2.4 FMRI task

The fMRI task used in this study was established in an earlier study (Reinhardt et al., 2010) and represents a classical conditioning process made up of three phases: familiarization (F), acquisition (A) and extinction (E). Each of those three phases are respectively divided into an early (F1, A1, E1) and a late phase (F2, A2, E2) (Büchel et al., 1998). Squares in different colors (yellow/blue and violet/green) served as neutral stimuli, which were repeatedly used in the pre-post design and displayed for 2000 msec. The variable interstimulus interval (ISI) is 4.785 to 7.250 seconds. Another represented stimuli was an aversive white noise, serving as the unconditioned stimulus (US) and presented for 100 msec, whose volume individually adapted between 70 and 110 dB to pose a uncomfortable stimulus to the participant. The already established sound level is then also used for the second measurement after the therapy. A fixation cross is displayed in-between the stimuli.

During the five minute familiarization phase, each of the three stimuli are presented in sequence to the participant. None of the stimuli are paired with the US. In the following seven minute long acquisition phase, one square is paired pseudo-randomly with the US, resulting in a conditioned stimulus (CS+), while the other square is not and thus remaining a neutral stimulus (CS-). However, the CS+ were paired in 50 % with the US, and 50 % did not. The US was presented at 1900 msec after the onset of the CS+, as a result both stimuli ended simultaneously. The acquisition phase thus leads to fear conditioning to CS+. In the four minute lasting extinction phase, the CS+ is not coupled with the US, which also is no longer presented. Only the CS+, the fixation cross and the CS- is successively presented to the participant leading to extinction learning. Only those trials in which no US was delivered were analyzed during acquisition to avoid overlap with neuronal activation directly related to the presentation of the US.

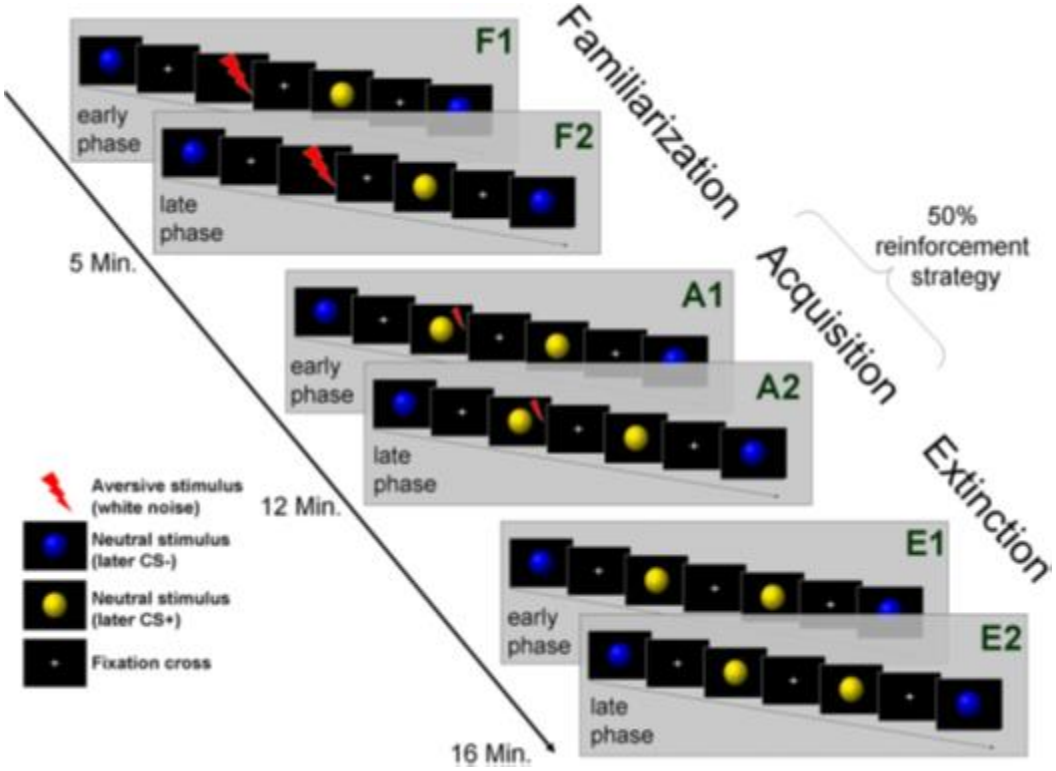


Figure 7. The conditioning paradigm

Both the familiarization phase as well as the extinction phase comprised 16 trials for the following event types: CS+, CS- and US. The acquisition phase contained 32 trials each for CS+ and CS- whereby in 16 trials (50%) the CS+ was coupled with the US. However, the remaining other 16 trials of the acquisition phase, namely the uncoupled CS+, were included in the analysis (figure slightly adapted from Kircher et al, 2013 and supplemental information).

2.5 FMRI data acquisition and analysis

2.5.1 FMRI data acquisition

In the context of the multicentered study, several scanners acquired fMRI brain images such as a 3-Tesla Siemens Trio MR scanner in Dresden (by Siemens AG, Erlangen), a 3-Tesla Philips Achieva MR scanner in Muenster and Aachen (by Philips Medical System, Best, The Netherlands) and 3-Tesla MR scanner in Berlin (by General Electric Healthcare, Milwaukee, Wisconsin). Functional images were recorded by using the following settings: EPI (echo-planar imaging), matrix size of 64 x 64 voxels, voxel size 3.6 x 3.6 x 3.8 mm, TE (echo time)= 30msec, TR (repetition time) = 2000ms, FOV (field of view) = 230mm, and 30 slices interleaved covering the whole brain and positioned parallel to the intercomissural plane (AC-PC, anterior commissure-

posterior commissure). The analysis of the magnetic resonance images have been conducted by MATLAB 7.1 (Mathworks, Sherborn, Massachusetts) using Statistical Parametric Mapping 5 (SPM 5; Wellcome Trust Center for Neuroimaging, London, UK; www.fil.ion.ucl.ac.uk). To minimize saturation effects in the acquired T1 images, the first five volumes were removed. Low-frequency fluctuations in the BOLD-signal were approached by using a 1/128 Hz high-pass filter (Kircher et al., 2013; Lueken et al., 2014)

2.5.2 Quality control and preprocessing

The necessary steps from raw data to processed data is essential for quality, validity and the subsequent, coherent statistics. Therefore, the quality control and preprocessing will be specified in the following sections.

Several editing steps had to be applied on the raw data in order to assure high quality and to minimize artifacts. The first step included the visual inspection of the unprocessed data in terms of possible extinctions or visible artifacts. In the second step, head movements above 1.5 voxel size (or: >5,7 mm in x, y or x axis) were excluded. The third and fourth step, objects to rule out deviants in the signal fluctuation. Here, the point-spread function (PSF) and the signal-to-fluctuation-noise-ratio (SNFR) come into play by applying a cut-off threshold of 2.5 standard deviations of the mean. Finally, the last step leads to the exclusion of data that showed visible artifacts in stimuli activations at $p < 0.05$ uncorrected (e. g. in the ventricles, outside or at the border of the brain) for the auditory and visual stimulus contrasted to fixation cross (baseline activation) in the familiarization phase (Kircher et al., 2013).

2.5.3 FMRI data analysis

The further statistical modeling that eventually leads to group comparisons in brain activations (here: risk-allele carriers versus controls) requires the setup of a so called first-level- and second-level analysis, which will be explained in more detail below.

The first-level analysis involved the estimation and modeling of parameters and contrasts on the level of subject-specific estimates against baseline (Poldrack et al., 2011, p. 102): Each phase (Familiarization (F), Acquisition (A) and Extinction (E)), each event type (CS-, CS+ paired, CS+ unpaired and US) and the corresponding BOLD responses were modelled by a general linear model (GLM) to eventually

analyze individual brain activations in relation to the onset of event type (e.g. CS+ or CS-). Phases were subdivided in early ('1') and late ('2') phase (F1, F2, A1, A2, E1, E2). Phase 'F2' was accounted for being used as a baseline for CS+ / CS-, since for phase 'F1' it was suggested to be biased by 'orienting reactions' (Lueken et al., 2014)

After the parameter estimates (β-values) and t-statistic images have been calculated in the first-level analysis, they serve as input for the second-level analysis (Poldrack et al., 2011, p. 102). A flexible-factorial analysis has been used in the second-level analysis to account for the between-group differences and interactions. This flexible-factorial design included 25 images of each subject, 32 conditions and the following covariates: fMRI center variables, age, education and BDI-II scores to address group differences. There were significant group differences in the clinical BDI-II scores, but fMRI analyses with or without the BDI-II scores in the covariates did not change the results of any of the patterns of brain activations.

Time	**Group**	**Conditioning**				**Extinction**			
		Early phase (A1)		Late phase (A2)		Early phase (E1)		Late phase (E2)	
Before CBT	Risk-allele carriers	CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
	Controls	CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
After CBT	Risk-allele carriers	CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
	Controls	CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-

Table 1.Schematic setup of the design matrix

The basic structure of the design matrix comprised two groups (risk-allele carrier and controls) in two different points in time, namely before and after cognitive-behavioral therapy. The phases of interest were the conditioning and extinction phase, which themselves were subdivided in an early and late phase. One full run included 8 versions of the conditions 'CS+ unpaired' and 'CS-'. This structure eventually led to 32 different versions of the conditions 'CS+ uncoupled' and 'CS-' given to two different groups at two points in time.

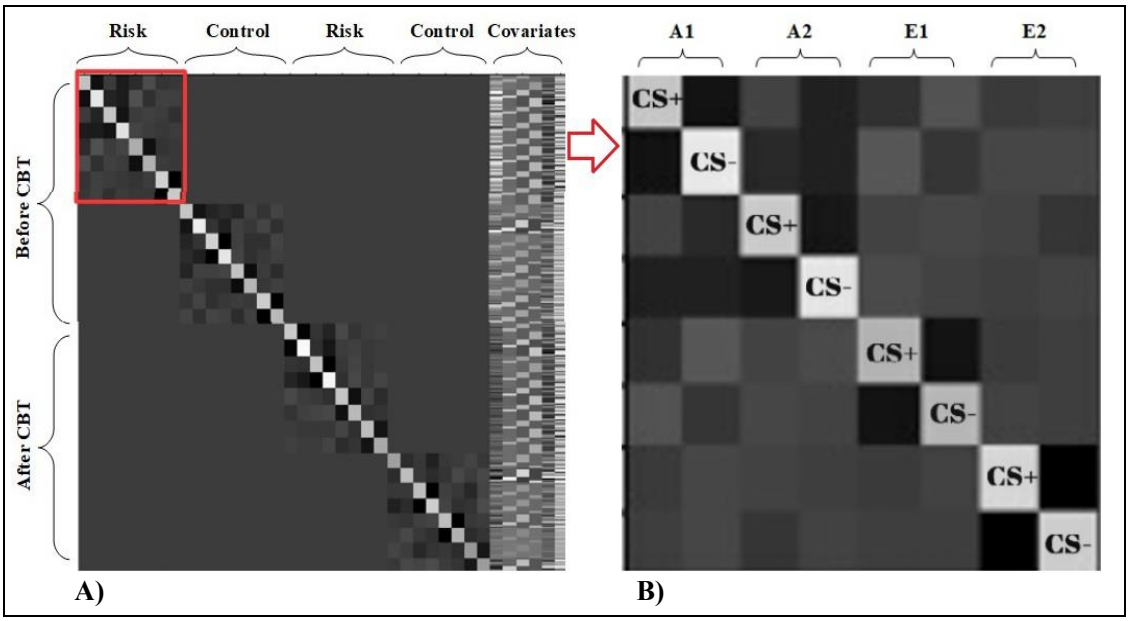


Figure 8. Second level design matrix: group analysis

The design matrix, being the basic framework of the experimental design and analysis, subdivides the participants images in 32 conditions (y-axis) and in time (x-axis).

A) The entire design matrix consists of four blocks of colums representing the groups before and after CBT: Column 1 (framed red) represents the images of the risk-allele carriers and column 2 represents the control sample, both at the time t1 (before CBT). Column 3 represents the images for the risk-allele carriers and column 4 the control sample, now both for the time t2 (after CBT). The covariates are included as regressors in the last columns, which are age, education level, study centers (Berlin, Münster, Dresden, Würzburg) and BDI scores.

B) Exemplary description for the first column block wich corresponds to the other three blocks: This block represents the images of the risk-allele carriers for each phases and conditions, which is from left to right: Acquisition phase 1 (A1) followed by Acquisition phase 2 (A2), Extinction phase 1 (E1) and finally Extinction phase 2 (E2). These 4 phases are then each subdivided into two alternating conditions 'CS+' and 'CS-', and therefore resulting in 8 conditions total per block.

2.5.3.1 Contrasts of interest

Regarding the contrasts of interest, investigations of (1) the effects of conditioning in group-by-time, group-by-CS and group-by-CS-by-time interaction and (2) the processing of safety signal (CS-) in risk-allele carriers and controls were conducted for the acquisition phase across time. Furthermore, a 'Region of Interest' (ROI) analysis was carried out in the early acquisition phase and in safety signal processing to detect smaller activations in the amygdalae that would have otherwise been undetected under the application of a cluster threshold of 142 voxels. The ROI analysis was realized by selecting the mask for amygdalae by the SPM tool “wfupickatlas”. These F contrasts

were supplemented by post hoc t-tests to account for the direction of these effects. The statistical analysis was implemented by multiple comparisons on the basis of voxel-by-voxel t-tests that eventually led to the remains of those voxels that met or exceeded a certain significance-level. In this case, multiple comparisons were conducted by a Monte Carlo simulation using $p<0.005$ (uncorrected) and a cluster extent of 142 voxels (as used in Kircher et al., 2013; Weber et al., 2016). The anatomical clusters were identified by using the attached SPM5 software tool „Anatomical automatic labeling (AAL)“. All of these above mentioned explorations were carried out on SPM 5 by the 'contrast manager' to eventually spot statistical inference in activation pattern and neural correlates in conditioning. For the purpose of visualizing the activation patterns and their directions, eigenvariate of the activation clusters were extracted and transformed into a bar chart by SPSS.

A section of contrasts have been formulated in regard to the testing of the hypotheses concerning neural activation differences in fear conditioning and in safety signal processing as formulated in chapter 1.5. These include contrasts with reference to the detection of neuroplastic changes from baseline to post-treatment assessment in the early acquisition phase (see table 2a) and safety signal processing (CS-) in early and late phases of conditioning, see table 2b. Additional explorative contrasts were conducted in further capturing potential effects in the late phases of acquisition and activation differences at baseline (t1) and at post-treatment (t2), as summarized in table 3.

Table 2. Contrasts regarding testing of the hypotheses.
a. These contrasts attempt to detect differences in brain activation the so-called fear-circuitry (involving the prefrontal-cortices, amygdala and hippocampus) in risk-allele carriers and control group in the early conditioning phase of acquisition. **b)** the contrasts concerning safety signal processing aim to detect differences in neural activation in processing CS-, that represents a safety signal. AA/AG = risk-allele carriers; GG = control group; CS+ = conditioned stimuli, uncoupled; CS- = safety signal

a) Contrasts for the detection of cerebral activation changes from baseline to post-treatment assessment in Acquisition phase 1 (early phase).
Interaction: group-by-time (AA vs GG) x (t1 vs t2)
Interaction: group x time x CS+>CS-
AA (CS+> CS-) > GG (CS+> CS-) x (t1>t2)
GG (CS+>CS-) > AA (CS+>CS-) x (t1>t2)
Post-hoc contrasts
AA: (CS+ >CS-) x (t1 > t2) *AA: (CS+<CS-) x (t1 > t2)*
GG: (CS+> CS-) x (t1 > t2) *GG: (CS+<CS-) x (t1 > t2)*
b) Contrasts for the detection of cerebral activation changes in safety signal

processing: acquisition 1 (early phase) + 2 (late phase)
Acquisition phase 1 (early phase)
Acq1: (AA: CS-) vs (GG: CS-) in t1 Acq1: (AA: CS-) vs (GG: CS-) x (t1 vs t2) Acq1: (AA: CS-) vs (GG: CS-) in t2
Acquisition phase 2 (late phase)
Acq2: (AA: CS-) vs (GG: CS-) in t1 Acq2: (AA: CS-) vs (GG: CS-) in t2 Acq2: (AA: CS-) vs (GG: CS-) x (t1 vs t2)

Table 3. Explorative contrasts.
These contrasts attempt to detect differences in neural activation in risk-allele carriers and control group in conditioning phase of acquisition at a) baseline and b) for the late acquisition phase and c) the post-treatment phase. AA/AG = risk-allele carriers; GG = control group; CS+ = conditioned stimuli, uncoupled; CS- = safety signal, t1 = at baseline, t2= at post-treatment, A1= early phase of conditioning, A2 = late phase of conditioning.

Activation differences at baseline (t1)
Acquisition phase
Main effect of group *(AA vs GG)*
Interaction effect of group-by-CS (AA vs GG) x (CS- vs CS+)
Post-hoc t contrasts for the Acquisition phases (each early/ and late)
Acq1: AA (CS+>CS-) > GG (CS+>CS-) Acq1: GG (CS+>CS-) > AA (CS+>CS-) Acq2: AA (CS+>CS-) > GG (CS+>CS-) Acq2: GG (CS+>CS-) > AA (CS+>CS-)
Neuroplastic changes from baseline to post-treatment assessment – Acquisition phase 2 (late phase)
Interaction: group-by-time
AA (CS+> CS-) > GG (CS+> CS-) x (t1>t2) GG (CS+>CS-) > AA (CS+>CS-) x (t1>t2)
AA: (CS+>CS-) x (t1 > t2) *AA: (CS+<CS-) x (t1 >t2)*
Differences at post-treatment (t2)
Acquisition phase
Main effect of group (*AA vs GG)*
Interaction effect of group-by-CS (AA vs GG) x (CS- vs CS+) **Interaction effect of group-by-CS for early phase** (AA vs GG) x (CS- vs CS+) **Interaction effect of group-by-CS for late phase** (AA vs GG) x (CS- vs CS+)
Post-hoc t contrasts for early and late phases
Acq 1: AA (CS+>CS-) > GG (CS+>CS-) Acq 1: GG (CS+>CS-) > AA (CS+>CS-) Acq 2: AA (CS+>CS-) > GG (CS+>CS-) Acq2: GG (CS+>CS-) > AA (CS+>CS-)

2.5.3.2 Correlations between cerebral activation changes and clinical outcome

To further explore a possible correlative connection between the activation changes in certain brain areas and the changes in clinical data, first the eigenvariate of a specific contrast and region were extracted to obtain the change by the means of beta values from t1 to t2. Of particular interest were contrasts and and regions that refer to the neural circuitry of fear conditioning, as the prefrontal cortices, amygdala, hippocampus, ACC, thalamus and insula. Secondly, the beta values were subtracted by calculating the beta values at 't1' minus the beta values at 't2'. The same was completed for the clinical data: the difference of clinical scores (e.g. ASI, PAS, HAM-A, etc.) before and after therapy were calculated by subtracting the scores for t1 and t2 (e.g. scores for ASI at timepoint 't1' minus scores for ASI at timepoint 't2'). In the next step, both data sets were analyzed by the software 'SPSS' for bivariate spearman's correlation using a threshold on the 0.05 level (2- tailed). Due to the exploratory nature of these comparisons no correction of multiple comparisons had been applied.

3 RESULTS

3.1 Clinical outcome

	Risk allele carriers (AA, AG-haplotype) n=13	Control patients (GG-haplotype) n=12	Statistics of between-group comparisons
Demographic characteristics			
Years of education (n (%))			
8 or less	2 (15)	0 (0)	
10	4 (31)	5 (42)	χ 2 = 2.074, p= 0.354
12 – 13	7 (54)	7 (58)	
Age*	33,7 (8.36)	39,8 (10.5)	t=-1.62, p= 0.674
Neuropsychological characteristics*			
TMT-A	26.65 (5.19)	24.08 (10.65)	t= 0.78, p= 0.445
TMT-B	14.69 (3.04)	15.42 (2.47)	t= -0.65, p= 0.521

Clinical characteristics*

	Baseline	After CBT	Baseline	After CBT	Group comparison in baseline	Treatment response	Group interaction across time
HAMA (0-56)	24.23 (5.7)	12.23 (3.11)	24.25 (5.64)	11.42 (7.6)	F(1,23)=0.013 p=0.993	**F(1,23)=77.67 p<0.001**	F(1,23)=0.087 p=0.770
CGI (1-7)	5.46 (0.66)	3.77 (0.83)	5.33 (0.65)	3.25 (1.29)	F(1,23)=0.047 p=0.630	**F(1,23)=73.9 p <0.001**	F(1,36)=0.031 p=0.86
PAS (0-57)	26.42 (6.46)	13.17 (4.48)	23.29 (11.53)	11.92 (11.52)	F(1,23)=4.96 p =0.407	**F(1,23)=57.24 p <0.001**	F(1,23)=0.403 p=0.532
MI alone (1-5)	2.43 (1.21)	1.71 (0.84)	2.32 (1.01)	1.33 (0.47)	F(1,23)=1.859 p=0.804	**F(1,23)=28.1 p <0.001**	F(1,23)=0.692 p=0.414
MI accompanied (1-5)	1.64 (0.76)	1.24 (0.52)	1.83 (1.12)	1.23 (0.43)	F(1,23)=1.006 p=0.619	**F(1,23)=10.19 p=0.004**	F(1,23)=0.437 p=0.515
ASI (0-64)	32 (8.58)	15.69 (7.64)	30.92 (10.64)	17.09 (12.02)	F(1,23)=0.047 p=0.781	**F(1,23)=59.8 p <0.001**	F(1,23)=0.36 p=0.547
ACQ	1.99 (0.38)	1.58 (0.36)	2.44 (0.58)	1.67 (0.48)	**F(1,23)=0.743 p=0.031**	**F (1,23)=46.20 p<0.001**	**F (1,23)=4.35 p=0.048**
BDI-II (0-63)	19.92 (7.71)	6.15 (3.5)	13.58 (6.88)	7.83 (6.28)	**F(1,23)=0.363 p=0.041**	**F(1,23)=65.36 p <0.001**	**F(1,23)=11.03 p=0.003**

Table 4. Demographic and clinical characteristics of the female treatment sample
Abbreviations: HAMA, Hamilton Anxiety Scale; CGI, Clinical Global Impression; PAS, Panic and Agoraphobia Scale; MI, Mobility Inventory, alone and accompanied subscale; ASI, Anxiety Sensitivity Index; ACQ, Agorpahobic Cogntions Questionnaire; BDI, Beck Depression Inventory II. A threshold of p<0.05 (uncorrected for multiple comparisons)al significance (bold).
*values are presented in: [Mean (SD)]

Both groups, the risk-allele carriers and controls did not significantly differ in demographic (as for age and level of education) or in neuropsychological characteristics (see table 3.1). for the main effects of treatment, indicate that both the risk allele-carriers and control patients exhibit significantly decreased clinical scores with respect to their clinical symptoms after cognitive-behavioral therapy in HAMA ($F(1,23)=77.67$, $p<0.001$), CGI ($F(1,23)=73.9$, $p<0.001$), PAS ($F(1,23)=57.24$, $p<0.001$), MI-7 alone ($F(1,23)=28.1$, $p<0.001$), MI-7 accompanied ($F(1,23)=10.19$, $p=0.004$), ASI ($F(1,23)=59.8$, $p<0.001$), ACQ ($F(1,23)=46.20$,$p<0.001$) and BDI-II ($F(1,23)=65.36$,$p<0.001$). This result therefore suggests that risk-allele carriers did significantly benefit from CBT despite the predisposition for fear generalization and dysfunctional safety signal processing at baseline (Weber et al, 2016). However, a group difference appeared for BDI-II with risk-allele carriers scoring significantly higher at baseline (difference in mean points: 6.34, $F(1.23) = 0.363$, $p=0.041$). However, cognitive-behavioral therapy led to a mean reduction of 13.8 points for BDI-II in risk-allele carriers and 5.8 points for controls. This difference in symptom reduction were shown to be significant for BDI-II, suggesting that CBT reduced symptoms stronger in risk-allele carriers that are concomitant with depressive disorders. In addition to that, risk-allele carriers exhibit significantly ($F(1,23) = 0.743$, $p = 0.031$) less total scores in ACQ at baseline: risk-allele carriers: 1.99 (SD 0.38) versus controls: 2.44 (SD 0.58). Moreover, control patients showed a significantly greater decrease in ACQ scores across time compared to risk carriers: mean reduction for risk-allele carriers across time: -0.41 points, for controls: -0.77 points ($F(1,23)=4.35$, $p=0.048$). The differences in the BDI-II scores have been considered in the fMRI analysis and included as a covariate factor.

3.2 Neural correlates in fear conditioning and correlations to clinical psychometric data

3.2.1 Neuroplastic changes in the early acquisition phase and in safety signal processing

In the following section the results will be presented concerning the testing of the hypotheses (second hypothesis as in chapter 1.5) on neural activation differences in fear conditioning in and on safety signal processing. These include contrasts with reference to the neuroplastic changes from baseline to post-treatment assessment in the early acquisition phase. However, chapter 3.2.2 will present results based on the explorative contrasts on the remaining phases (late acquisition phase) and points in time (each baseline and posttreatment).

3.2.1.1 Neuroplastic changes in fear conditioing from baseline to post-treatment in the early acquisition phase

The control group showed a significant higher activation in the bilateral inferior frontal gyrus (left > right), left thalamus, left ACC and left temporal gyrus in response to CS+ > CS- across time, while this was significantly reduced for the risk-allele carriers (see table 6). In addition, the normalized activation for CS+ unpaired in the right IFG, left thalamus and left ACC in the control patients was significantly correlated with the reduction of panic-related symptom as ascertained in the ASI (Anxiety Sensitivity Index). Furthermore, the normalized activation in the left ACC also correlated with the decrease in the total scores of BDI (Becks Depression Inventory) and PAS (Panic and Agoraphobia Scale). No clinical correlations were detectable for the activation changes in the left IFG and left temporal superior gyrus in control patients (see table 8).

However, a post-hoc t-contrast of the risk-allele carriers revealed a different neural response by a significant activation reduction in the left dmPFC (Brodman Area 9) and left precuneus for CS+ > CS- from baseline to post-treatment assessment (see figure 7). The activation reduction in the left dmPFC in the risk-allele carriers during differential conditioning (CS + > CS-) showed a significant correlation to the reduction of the accompanied agoraphobic avoidance (MI7T acc; see table 7 b and c). No correlation to clinical scores were detectable for the left precunes. The activation changes in the left dmPFC and precuneus in risk-allele carriers could however not been replicated in a contrast including the control group, thus indicating an unspecific effect.

A further effect when comparing both groups (AA versus GG across time, and

exclusion of CS+/CS- differenciation), the risk-allele carriers showed greater activation in a neural cluster comprising the rolandic operculum, mid cingulum and heschl's gyrus, as well as smaller clusters in the right calcarine gyrus and the left inferior frontal gyrus across time. However, these differences in activation changes in the risk-allele carriers are not reproducible in a differential conditioning T-contrast, thus representing an unweighted/unspecific increase in terms of both CS + and CS- together which does not allow a allocation towards either a neutral or aversive stimuli. Further contrasts as mentioned in table 9 and 10, partially reveal activations of these above mentioned brain areas in safety signal processing.

Table 5. Contrast results for the group differences in the brain activation (clusters) during the early phase of fear conditioning from baseline (t1) to post-treatment (t2).

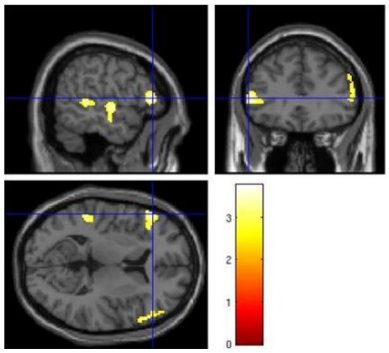
Multiple comparisons were conducted by a Monte Carlo simulation using $p < 0.005$ (uncorrected) and a cluster extent of 142 voxels. AA/AG = risk-allele carriers; GG = control group; CS+ = conditioned stimuli, uncoupled; CS- = safety signal; A1, early acquisition phase, A2 = late acquisition phase; n.s., not significant.*Local maxima is outside a labelled region. The nearest region to the local maxima is identified by the tool "AAL" in SPM 5 (Automated Anatomical Labeling).

			MNI coordinates				
Contrast and Region	**Hemis-phere**	**Voxels (Number per Cluster)**	**x**	**y**	**z**	**F or t**	**p (unc.)**
Changes in brain activation from baseline to post-treatment assessment – Acquisition phase 1 (early phase)							
Interaction: group-by-time							
(AA vs GG) x (t1 vs t2)							
Rolandic Operculum (4,47 mm from local maxima)*	left	1029	-28	-34	16	15.39	<0.001
-Cingulum mid (5,66 mm from local maxima)*			-14	-24	30	11.49	0.001
-Heschl's gyri			-34	-24	6	11.13	0.001
Calcarine gyrus (2 mm from local maxima)*	right	289	24	-54	16	13.65	<000.1
Frontal_Inf_Oper	left	191	-46	28	2	12.68	<000.1
Post-hoc t contrasts							
(AA (CS+> CS-) > GG (CS+> CS-)) x (t1>t2)	-						
(GG (CS+>CS-) > AA (CS+>CS-)) x (t1>t2)							
Inferior frontal gyrus	left	237	-54	32	8	3.78	<0.001
Superior temporal gyrus, mid temporal	left	214	-48	-34	4	3.65	<0.001
Superior temporal gyrus, sup temporal	left	274	-54	-12	-2	3.39	<0.001
Inferior frontal gyrus (BA 45)	right	282	60	26	10	3.14	0.001
AA: (CS+ >CS-) x (t1 > t2)							
Dorsomedial prefrontal cortex (BA 9)	left	219	-4	52	26	3.60	<0.001
Precuneus	left	180	0	-64	50	3.04	0.001
AA: (CS+<CS-) x (t1 >t2)	-						n.s.
GG: (CS+> CS-) x (t1 > t2)							
Superior temporal gyrus, mid temporal	left	3264	-54	-12	0	4.48	<0.001
Frontal_Inf_Tri, Inf_Oper	left	1576	-40	28	10	3.98	<0.001
Thalamus	left	167	-14	-12	8	3.53	<0.001
Rostral anterior cingulate cortex	left	151	-8	50	4	3.48	<0.001
	right	681	58	32	14	3.38	<0.001
GG: (CS+<CS-) x (t1 > t2)	-						n.s.
Region of interest (Amygdala)							
(AA (CS+> CS-) > GG (CS+> CS-)) x (t1>t2)	-						n.s.
(AA (CS+> CS-) < GG (CS+> CS-)) x (t1>t2)	-						n.s.

Table 6. Differential conditioning in the early Acquisition phase (A1) in female risk-allele carrier (AA/AG, n=13) versus female patient control group (GG, n= 12) from baseline to post-treatment.

The patient control group (GG) showed a significant reduction of activation (contrast estimates, arbitrary units) in the left IFG, right IFG and in the left superior temporal gyrus in response to CS+ > CS- from baseline to post-CBT (cluster size: 1576 voxels, p<0.001 (unc.), T = 3.98). The risk-allele carriers by contrast showed no significant change in the activation of the left IFG over time. Brain regions that are mentioned under the same contrast show the same activation pattern, only the activation cluster with the highest t-value has been pointed out in the graphics to avoid redundancy. AA/AG=risk-allele carriers; GG = control group; CS+ = conditioned stimuli, uncoupled; CS- = safety signal; IFG = inferior frontal gyrus; contrast estimates = beta values in % signal change

GG (CS+>CS-) **> AA** (CS+>CS-) x (t1>t2)



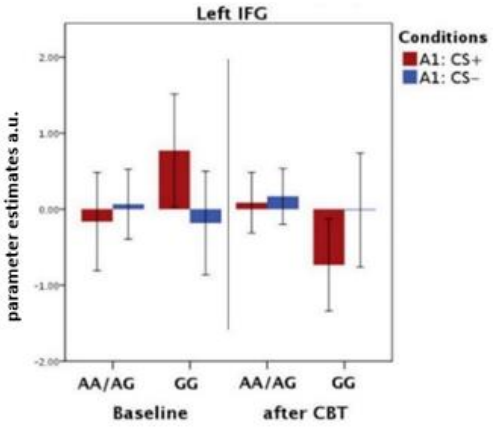
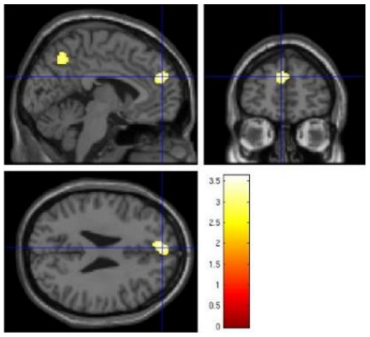
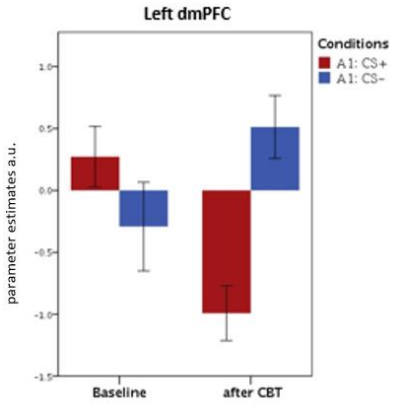


Table 7. Post-hoc analysis of differential conditioning (CS+ > CS-) in female risk-allele carriers (AA/AG, n=13) from baseline to post-treatment and clinical correlations. a. Post-hoc analysis of differential conditioning (CS+ > CS-) in female risk-allele carriers (AA/AG, n=13) from baseline to post-treatment.
Across time, risk-allele carriers differ regarding the neural response to CBT: instead of a deactivation in the left IFG as shown in the control group, they featured a different neural pathway by a significant deactivation of the left dmPFC (Brodman Area 9) and left precuneus for CS+>CS- (cluster size: 237 voxels, $p < 0.001$ (unc.), $T = 3.78$). AA/AG = risk-allele carriers; CS+ = conditioned stimuli, uncoupled in early acquisition phase (A1); CS- = safety signal in early acquisition phase (A1); dmPFC = dorsomedial prefrontal cortex; parameter estimates = extracted beta values. **b. Correlations between the therapeutic induced activation change in the left dmPFC and the reduction of panic-related symptoms in risk-allele carriers.** A bivariate (spearman's) correlation and a threshold on the 0.05 level (2- tailed) showed a significant correlation between deactivation in the left dmPFC (MNI = -4, 52, 26) in differential conditioning for CS+>CS- in early acquisition and the reduction of total MI-7 acc. in time ($p = 0.025$; 2-tailed, uncorrected). No significant correlation were detectable concerning the left precuneus. HAMA, Hamilton Anxiety Rating Scale; PAS, Panic and Agoraphobia Scale; BDI, Beck Depression Inventory-II; ASI, Anxiety Sensitivity Index; CGI, Clinical Global Impression; MI-7 al.or acc., Mobility Inventory alone or accompanied; ACQ, Agoraphobic Cognitions Questionnaire.**c. Correlation of the activation reduction (% signal change) in the left dmPFC across time and the reduction of the accompanied agoraphobic avoidance (MI7 acc. total) in risk-allele carrier across time.** The spearman correlation coefficient is $r = 0.423$. Correlation is significant at the 0.05 level (2-tailed; uncorrected*);* t1, before CBT; t2, after CBT.

a. Contrast AA/AG: CS+>CS- x t1>t2





b.

AA/AG-haplotype		HAMA	PAS	BDI	ASI	CGI	MI-7 acc.	MI-7 Alone	ACQ
Left dorsomedial prefrontal cortex	Correlation Coefficient	-.102	.300	.091	.124	-.095	.616	.115	-.084
	Significance (2-tailed)	.740	.319	.768	.687	.758	**.025**	.707	.786
Left precuneus	Correlation Coefficient	-.055	.091	.234	.228	-.202	.325	-.187	-.105
	Significance (2-tailed)	.858	.768	.442	.453	.508	.279	.541	.521
	N	13	13	13	13	13	13	13	13

c.

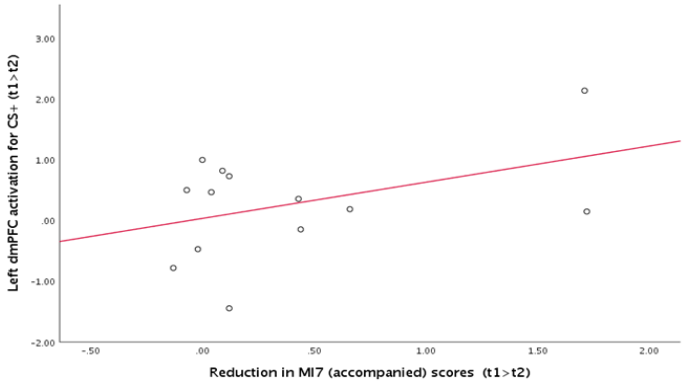
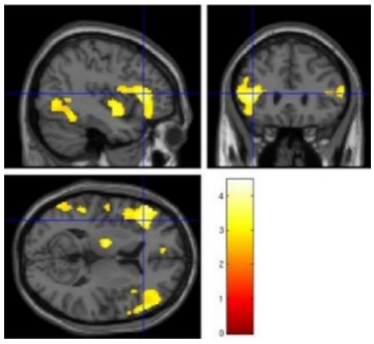
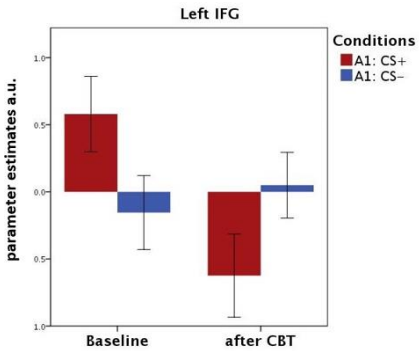


Table 8. Post-hoc analysis of differential conditioning (CS+ > CS-) in female control patients (GG, n=12) from baseline to post-treatment and clinical correlations

a.Post-hoc analysis of differential conditioning (CS+ > CS-) in female control patients (GG, n=12) from baseline to post-treatment. Across time, control patients show a normalized activation of the left IFG (largest cluster) for CS+>CS- (cluster size: 237 voxels, $p < 0.001$ (unc.), $T = 3.78$). GG = female control patients; CS+ = conditioned stimuli, uncoupled in early acquisition phase (A1); CS- = safety signal in early acquisition phase (A1); IFG = inferior frontal gyrus; contrast estimates = beta values (% signal change). Brain regions that are mentioned under the same contrast show the same activation pattern; only the activation cluster with the highest t-value has been pointed out in the graphics to avoid redundancy. **b. Correlations between the therapeutic induced change in the left IFG and the reduction of panic-related symptoms in the female control patients.** A bivariate (spearman's) correlation and a threshold on the 0.05 level (2- tailed) showed a significant correlation between deactivation in the right IFG and left thalamus in differential conditioning for CS+ (unpaired) in early acquisition with the the reduction of total ASI scores across time (p (right IFG) = 0.07 uncorrected; p (left thalamus) = 0.03 uncorrected). In addition, the normalized activation in the left ACC across time for CS+>CS- showed a significant correlation with the decrease of total scores of PAS (p=0.001), BDI (p=0.044 uncorrected) and ASI (p=0.039 uncorrected) across time. No significant correlations were shown for the deactivatoin of the left IFG or the left superior temproral gyrus with the reduction of panic-related symptoms. **c. Correlations between the therapeutic induced change in the right IFG and the reduction of panic-related symptoms in the female control patient patients.** A bivariate (spearman's) correlation and a threshold on the 0.05 level (2- tailed) showed a significant correlation between the therapeutic induced deactivation in the right IFG (MNI = 60, 26, 10) in differential conditioning for CS+(unpaired) in early acquisition and the reduction of panic-related symptoms across time. **d. Correlation of the activation reduction (% signal change) in the left thalamus across time and the reduction of the accompanied ASI total scores in the female control patients across time.** The correlation coefficient is r = 0.404. Correlation is significant at the 0.05 level (2- tailed; uncorrected*);* **e.- g. Correlation of the activation reduction (% signal change) in the left rostral ACC across time and the reduction of the accompanied ASI, BDI and PAS total scores in the female control patients across time.** The correlation coefficient is r = 0.502 for ASI, r = 0.2 for BDI and r = 0.553 for PAS. Correlation is significant at the 0.05 level (2- tailed; uncorrected).HAMA, Hamilton Anxiety Rating Scale; PAS, Panic and Agoraphobia Scale; BDI, Beck Depression Inventory-II; ASI, Anxiety Sensitivity Index; CGI, Clinical Global Impression; MI-7 al.or acc. , Mobility Inventory alone or accompanied; ACQ, Agoraphobic Cognitions Questionnaire. t1, before CBT; t2, after CBT. All results represent spearmen's correlations. Regression lines were only included for illustration purposes.

a. Contrast: GG: CS+ > CS- x t1 > t2

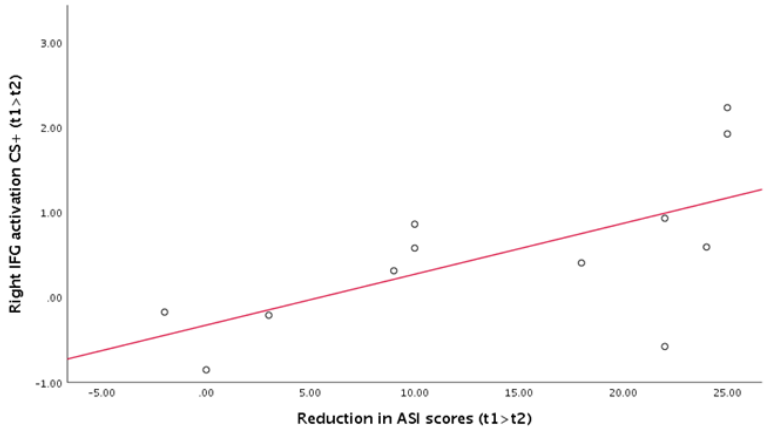




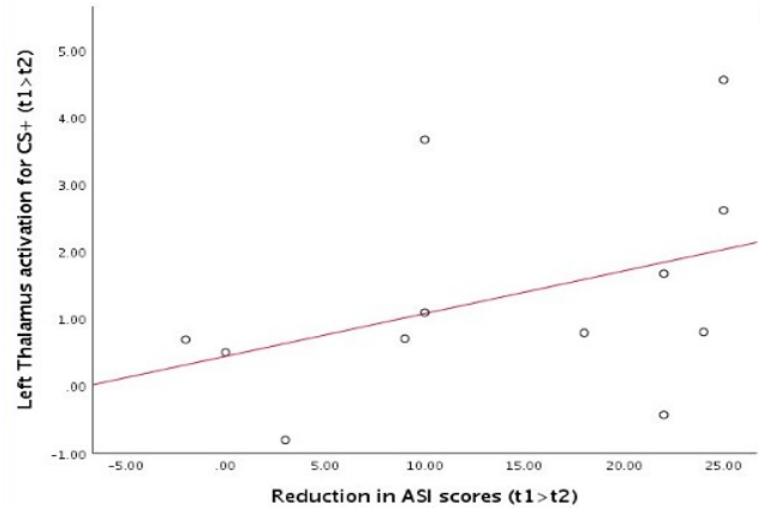
b.

GG-haplotype		HAMA	PAS	BDI	ASI	CGI	MI-7 acc.	MI-7 Alone	ACQ
Left inferior frontal gyrus	Correlation Coefficient	.175	.211	.523	.478	-.068	.372	-.014	-.021
	Significance (2-tailed)	.587	.511	.081	.116	.834	.234	.966	.948
Right inferior frontal gyrus	Correlation Coefficient	.217	.137	.168	**.735**	.146	.175	-.441	-.254
	Significance (2-tailed)	.499	.671	.601	**.007**	.650	.585	.152	.427
Left superior temporal gyrus	Correlation Coefficient	.112	.137	-.144	.569	.399	.200	-.308	.570
	Significance (2-tailed)	.729	.671	.656	.053	.198	.533	.331	.053
Left thalamus	Correlation Coefficient	.301	.288	.554	**.626**	.004	.056	-.140	-.021
	Significance (2-tailed)	.342	.364	.061	**.030**	.991	.862	.665	.948
Left rostral anterior cingulate cortex	Correlation Coefficient	.322	**.858**	**.589**	**.601**	.339	.053	.119	.556
	Significance (2-tailed)	.308	**<.001**	**.044**	**.039**	.281	.871	.713	.060
	N	12	12	12	12	12	12	12	12

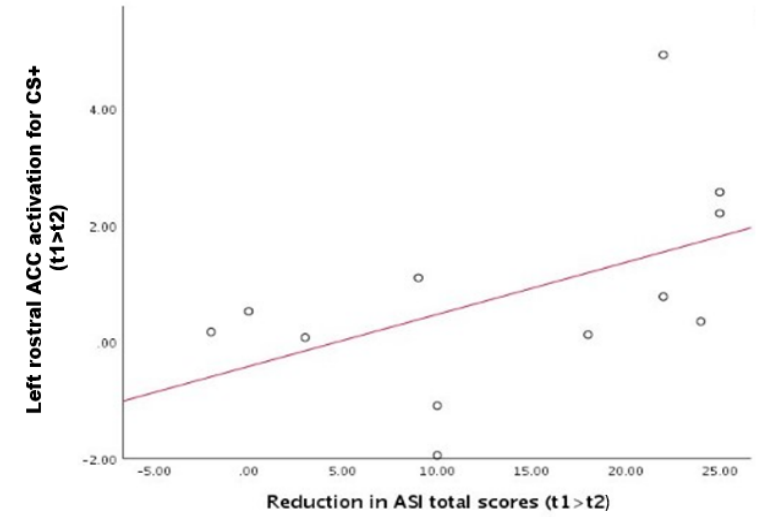
c.



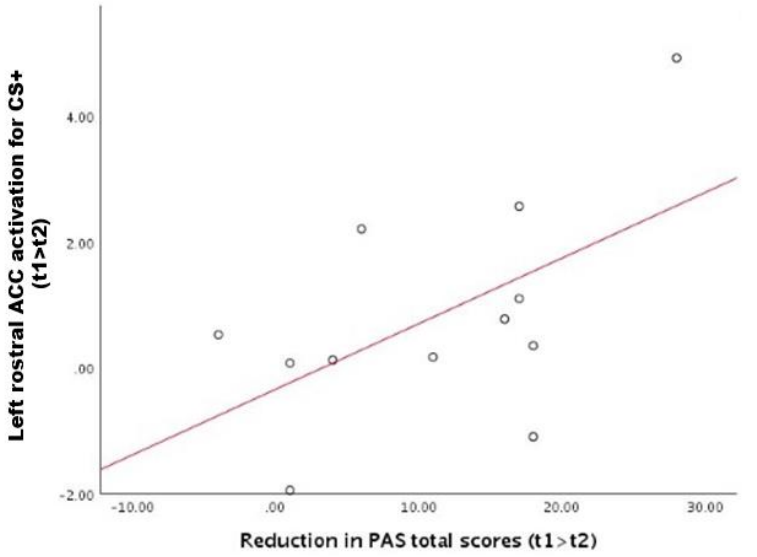
d.



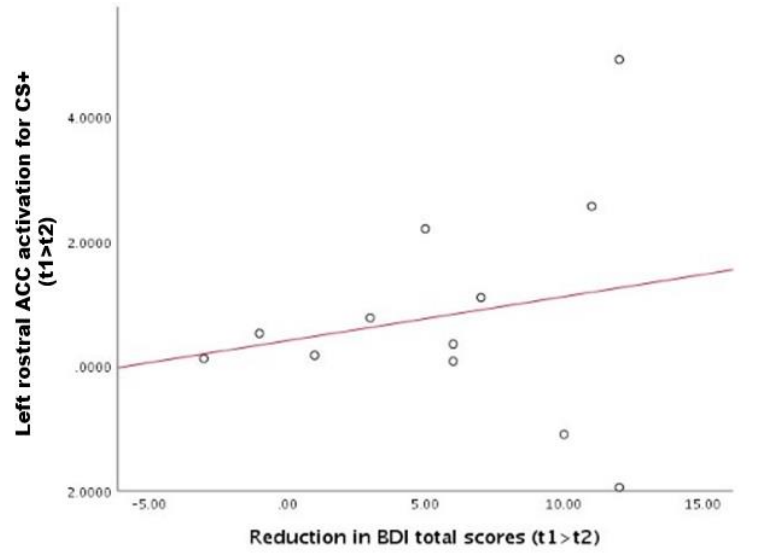
e.


Left rostral ACC activation for CS+ (t1>t2)
4.00
2.00
.00
-2.00
-5.00
.00
5.00
10.00
15.00
20.00
25.00
Reduction in ASI total scores (t1>t2)

f.


Left rostral ACC activation for CS+ (t1>t2)
4.00
2.00
.00
-2.00
-10.00
.00
10.00
20.00
30.00
Reduction in PAS total scores (t1>t2)

g.


Left rostral ACC activation for CS+ (t1>t2)
4.0000
2.0000
.0000
2.0000
-5.00
.00
5.00
10.00
15.00
Reduction in BDI total scores (t1>t2)

3.2.1.2 Safety signal processing in early and late acquisition phases

In the processing of the safety signal processing across time no activation changes in the so called "fear network" were detected. The risk-allele carriers demonstrated higher activation in the right calcarine gyrus and in a left cluster involving the rolandic operculum, precuneus and partially thalamus for CS-. Concerning the safety signal processing after therapy, the risk-allele carriers showed enhanced activation in a cluster involving the right lingual gyrus, occipital inferior gyrus, fusiform gyrus and the middle segment of the cingulum (see Table 10a). At the time of post-treatment, risk-allele carriers showed a greater activation in the right lingual gyrus and right middle cingulum for CS- after therapy compared to the control patients in the early acquisition phase (see table 10b.)

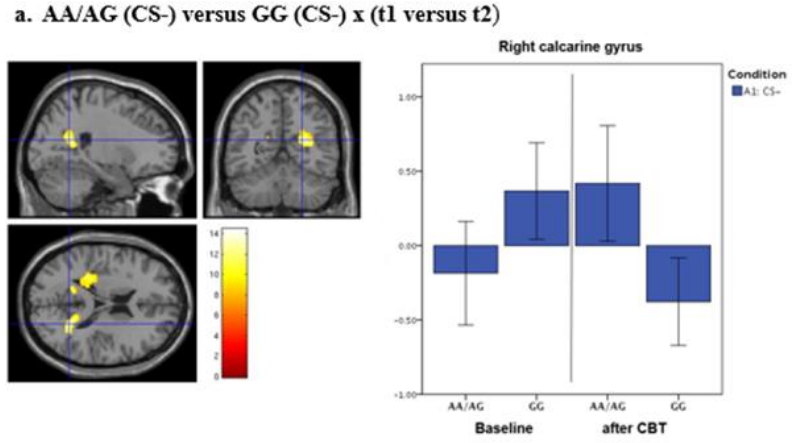
Table 9. Contrast results for group differences in the brain activation (clusters) during safety signal processing at baseline (t1) and after CBT (t2).

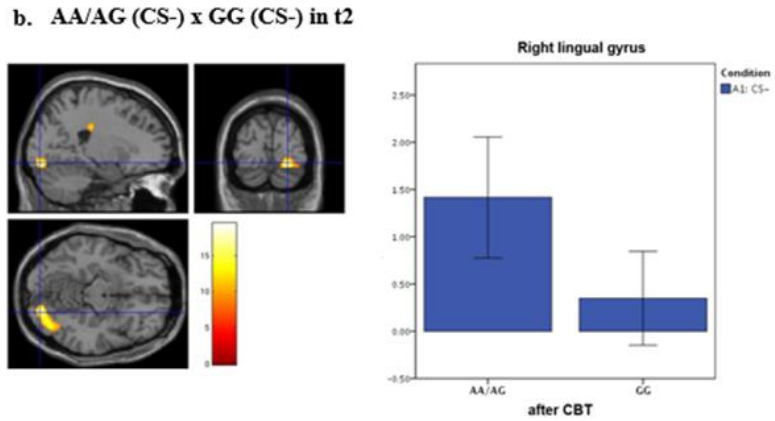
Multiple comparisons were conducted by a Monte Carlo simulation using $p < 0.005$ (uncorrected) and a cluster extent of 142 voxels. AA/AG = risk-allele carriers; GG = control group; CS- = safety signal; A1, early acquisition phase, A2 = late acquisition phase; n.s., not significant. *Local maxima is outside a labelled region. The nearest region to the local maxima is identified by the tool "AAL" in SPM 5 (Automated Anatomical Labeling).

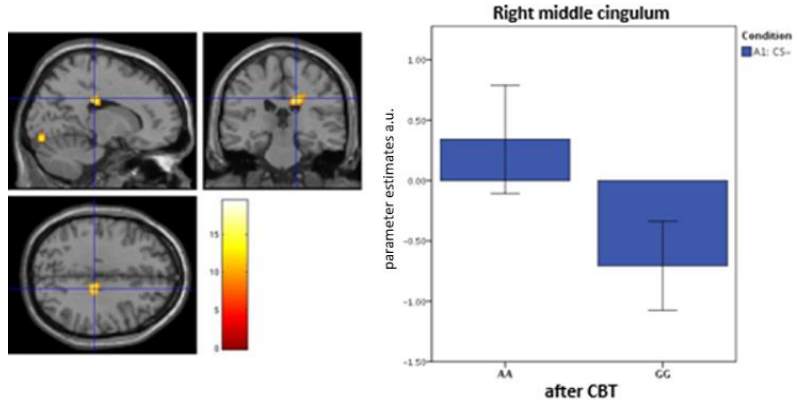
			MNI coordinates				
Contrast and Region	**Hemis-phere**	**Voxels (Number per Cluster)**	**x**	**y**	**z**	**F or t**	**p (unc.)**
Safety signal processing (Acquisition 1 (early phase) + 2 (late phase))							
Acq1: (AA: CS-) vs (GG: CS-) in t1	-						
Acq1: (AA: CS-) vs (GG: CS-) x (t1 vs t2)							
Calcarine (2mm from local maxima)	right	364	24	-56	18	14.46	<0.001
-Calcarine gyurs (4.47 mm from local maxima)*			32	-58	16	12.70	<0.001
Rolandic Operculum (4mm from local maxima)*	left	596	-28	-32	16	12.54	<0.001
-Precuneus (2mm from local maxima)*			-16	-52	16	11.64	0.001
-Thalamus (2mm from local maximum)*			-10	-30	12	9.89	0.002
Acq1: (AA: CS-) vs (GG: CS-) in t2							
Lingual gyrus	right	594	22	-88	-12	19.44	<0.001
-Occipital Inferior gyrus			38	-76	-10	16.39	<0.001
-Fusiform gyrus			42	-58	-16	8.25	0.004
Cingulum_Mid (4mm from local maxima)*	right	199	16	-26	32	16.10	<0.001
Acq2: (AA: CS-) vs (GG: CS-) in t1	-						n.s.
Acq2: (AA: CS-) vs (GG: CS-) in t2	-						n.s.
Acq2: (AA: CS-) vs (GG: CS-) x (t1 vs t2)	-						n.s.
Region of interest (Amygdala)							
Acq1: (AA: CS-) vs (GG: CS-) x (t1 vs t2)	-						n.s.
Acq2: (AA: CS-) vs (GG: CS-) x (t1 vs t2)	-						n.s.

Table 10. Safety signal processing in the early and late Acquisition phase (A2) in female risk-allele carriers (AA/AG, n=13) versus female patient control group (GG, n= 12) from baseline to post-treatment and at the time of post-treatment.

a. Safety signal processing across time in the early Acquisition phase (A1): group interaction. The risk-allele carriers showed increased activation (parameter estimates, arbitrary units) in the right calcarine gyrus for CS- from baseline to post-CBT (cluster size: 364 voxels, p<0.001 (unc.), T = 14.46). The control group exhibit a reduced activation in the right calcarine gyrus for CS- at the time of post-treatment. Brain regions that are mentioned under the same contrast show the same activation pattern, only the activation cluster with the highest t-value has been pointed out in the graphics to avoid redundancy. AA/AG = risk-allele carriers; GG = control group; CS+ = conditioned stimuli, uncoupled; CS- = safety signal; parameter estimates = beta values in % signal change. **b. Safety signal processing in the early Acquisition phase (A1) after therapy: group interaction.** The risk-allele carriers showed a higher activation (arbitrary units) in the right calcarine gyrus and right middle cingulum for CS- after therapy compared to the control patients (right calcarine gyrus: cluster size: 594 voxels, p<0.001 (unc.), T = 19.44; right middle cingulum: cluster size: 199 voxels, p<0.001 (unc.), T= 16,10; 4mm from local maxima). AA/AG = risk-allele carriers; GG = control group; CS+ = conditioned stimuli, uncoupled; CS- = safety signal; parameter estimates = beta values in % signal change.







3.2.2 Explorative contrasts at baseline, in the late acquisiton phase and at post-treatment

3.2.2.1 Differential conditioning effects at baseline

The analysis for differential conditioning responses for the risk-allele carriers (AA/AG-haplotype) and control patients (GG-haplotype) to showed no significant differences in cortical activation at baseline.

Table 11. Group differences in the brain activation (clusters) during safety signal processing at baseline (t1) and after CBT (t2).

Multiple comparisons were conducted by a Monte Carlo simulation using $p < 0.005$ (uncorrected) and a cluster extent of 142 voxels. AA/AG = risk-allele carriers; GG = control group;; CS- = safety signal; A1, early acquisition phase, A2 = late acquisition phase;, n.s., not significant

			MNI coordinates				
Contrast and Region	**Hemis-phere**	**Voxels (Number per Cluster)**	**x**	**y**	**z**	**F or t**	**p (unc.)**
Activation differences at baseline							
Acquisition phase							
Main effect of group *(AA vs GG)*							n.s.
Interaction effect of group-by-CS (AA vs GG) x (CS- vs CS+)							n.s.
Post-hoc t contrasts for the Acquisition phases (+early/late)							
Acq 1: AA (CS+>CS-) > GG (CS+>CS-) Acq1: GG (CS+>CS-) > AA (CS+>CS-) Acq2: AA (CS+>CS-) > GG (CS+>CS-) Acq2: GG (CS+>CS-) > AA (CS+>CS-)	- - - -						n.s.

3.2.2.1 Neuroplastic changes from baseline to post-treatment assessment – Acquisition phase 2

Comparing the risk-allele carriers and controls, the risk-allele carriers showed enhanced activation in the right precentral gyrus (or premotor cortex; BA 6) for CS- and a decreased activity for CS+ in differential conditioning (CS+> CS-) from baseline to post-treatment. one additional activation cluster of the left postcentral gyrus appears more active after therapy in a post-hoc t-contrast in risk-allele carriers. This activation cluster however did not appear in the interaction "group-by-time" suggesting no statistical specifity for the AA/AG group (see Table 13).

Table 12. Group differences in the brain activation (clusters) during safety signal processing at baseline (t1) and after CBT (t2).

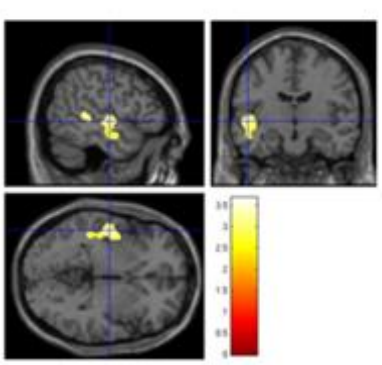
Multiple comparisons were conducted by a Monte Carlo simulation using $p < 0.005$ (uncorrected) and a cluster extent of 142 voxels. AA/AG = risk-allele carriers; GG = control group; CS- = safety signal; A1, early acquisition phase, A2 = late acquisition phase; n.s., not significant.

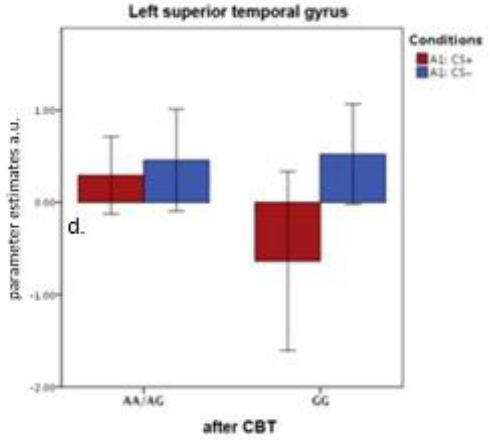
			MNI coordinates				
Contrast and Region	**Hemis-phere**	**Voxels (Number per Cluster)**	**x**	**y**	**z**	**F or t**	**p (unc.)**
Changes in brain activation from baseline to post-treatment assessment – Acquisition phase 2 (late phase)							
Interaction: group-by-time	-						
Post-hoc t contrasts							
AA (CS+> CS-) > GG (CS+> CS-) x (t1>t2) Precentral gyrus (BA 6)	 right	 208	 50	 -8	 40	 3.24	 0.001
GG (CS+>CS-) > AA (CS+>CS-) x (t1>t2)	-						
AA: (CS+>CS-) x (t1 > t2) Precentral gyrus Postcentral gyrus	 right left	 362 415	 52 -50	 -10 -12	 42 56	 3.66 3.50	 <0.001 <0.001
AA: (CS+<CS-) x (t1 >t2)	-						

Table 13. Differential conditioning in the early and late Acquisition phase (A1/A2) in female risk-allele carriers (AA/AG, n=13) versus female patient control group (GG, n= 12) at post-treatment.

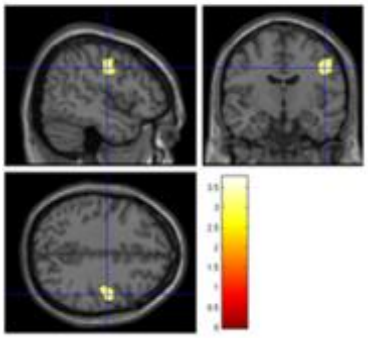
a.The control patients showed decreased activation (contrast estimates, arbitrary units) in the left superior temporal gyrus and therefore an overal differential activtiy for CS+ > CS- at post-CBT in the early Acquisition phase (cluster size: 661 voxels, p<0.001 (unc.), T = 3.68) compared to the risk-allele carrriers who did not display an overall differential activation for CS+ > CS-. **b.** The risk-allele carriers display reduced activity in a cluster involving the right post- and precentral gyrus for CS+>CS compared to the control-patients in the late acquisition phase at the time of post-treatment (cluster size: 627 voxels, p<0.001 (unc.), T = 3.77). AA/AG = risk-allele carriers; GG = control group; CS+ = onditioned stimuli, uncoupled; CS- = safety signal; parameter estimates = beta values in % signal hange.

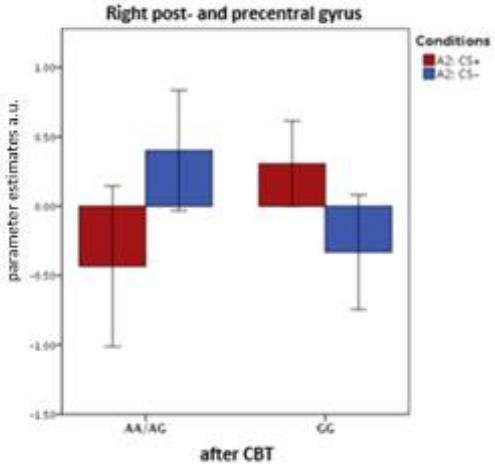
a. AA/AG (CS+>CS-) x GG (CS+>CS-) in t2





b. AA/AG (CS-) x GG (CS-) in t2





4 DISCUSSION

This study examined the effect of the minor A-risk allele of rs17689918 (AA/AG-haplotype) in female PD/A patients in comparison to female control PD/A patients (GG-haplotype) regarding the response to the therapeutic effect of CBT and accompanying changes in neuronal activation pattern in fear conditioning across time. This proposal began first with analyses of clinical scores for both groups, pre-/post-comparison for risk-allele carriers and intergroup comparisons (risk-allele carriers versus control group) by means of ANOVAs for repeated measures, secondly by a conducting a fMRI analysis of differences in neural activation clusters in a fear conditioning task (risk-allele carriers versus control group, pre-CBT versus post-CBT), and thirdly by exploration of possible correlations between CBT-induced neuronal activation changes and changes in clinical scores.

First, the female carriers of the AA/AG-haplotype exhibit significant benefit from CBT in terms of the panic-relevant psychometric scores similar to female carriers of the GG-haplotype. Minor differences regarding CBT effects on depressive (stronger effects on risk allele carrier) and maladaptive agoraphobic cognitions (stronger effect on control patients) need to be further explored in future studies. Secondly, the neural correlates of differential fear conditioning after CBT intervention differ in carriers of the AA/AG-haplotype: While control patients (GG-haplotype) showed a normalized activation in the left dlPFC, left rostral ACC and left thalamus for CS contigencies, the risk-allele carriers (AA/AG-hyplotype) strikingly did not feature this activation pattern. In a post hoc contrast from baseline to posttreatment (including only the risk-allele carriers) suggest a different response to CBT: a decrease of activation in the left dmPFC and left precuneus in differential conditioning, in which the activation reduction of the left dmPFC correlates with the decrease in the MI7 (accompanied) total scores. However, these activation changes in the left dmPFC and precuneus in risk-allele carriers could not been replicated in a contrast including the control patient group (mixed group), therefore indicating an unspecific effect.

These results however shed new light on the effect of CBT in female patients carrying the risk-allele rs17689918: On one hand, the risk-allele carriers benefit significantly from CBT in their overall panic-associated symptoms and with specific regard of their depressive symptoms (while in contrast, control patients experienced a stronger reduction of agoraphobic cognitions). On the other hand, fMRI data suggest

that CBT leads to a different, but compensatory and yet not verified neural response in risk-allele carriers when compared to non-risk patients. The details are discussed below in detail. Other than the suspected left dmPFC response in the risk-allele carrier, no other brain areas were detected in the so called “fear network”in the explorative analysis, except for the left precuneus and the co-activated sensomotoric areas (e.g. superior temporal gyrus, calcarine gyrus, lingual gyrus, premotor cortex and rolandic operculum) probably due to the accompanying cues (e.g. viusal or auditory cues) of the experimental design. No additional analysis or interpretation was conducted on areas that were not directly assigned or correlated to the “fear network” of the brain.

4.1 Clinical outcome responses of risk-allele carriers

When comparing the clinical scores of the risk-allele carriers and control patients (HAMA, CGI, PAS, MI alone/accompanied, ASI, ACQ and BDI-II), they showed significantly decreased total scores after CBT. The in-between-group interaction across time (risk-allele carrier versus control group) showed no significant difference in the reduction of total scores for HAMA, CGI, MI alone/accompanied and ASI, indicating a similar therapeutic CBT- response for these specific clinical characteristics.

However, risk-allele carriers exhibit significantly higher baseline total scores in depressive symptoms (as measured in BDI-II) on average 6.3 points more compared to the control group and a significantly higher decrease of depressive symptoms after CBT with an additional decrease of an average 8 total points in risk-allele carriers. This suggests that female risk-allele carriers experience significant more depressive symptoms than control PD/A patients at baseline, thus indicating a more aggravated clinical picture by additional depressive symptoms driven by the AA/AG-haplotype. So far no data on the specific role of the AA/AG-haplotype of *CRHR1* rs17689918 in depressive disorder is known to the author at this time. Data have evidenced the contributing role of CRHR1 in depressive disorders and the antidepressive and anxiolytic effects of CRHR1-antagonists in rodents (Binder & Nemeroff, 2010; Liu et al., 2006; Rogers et al., 2013).

In addition, PD and depression not only coincide in certain pathophysiologic pathways (for instance the HPA-axis and the serotonergic neurotransmission) and in the response to first line pharmacologic treatment via SSRI (Gorman & Coplan, 1996), but almost half of PD cases are frequently accompanied by depressive disorders

(Lueken et al., 2015). The comorbidity of depression however, does not impair the therapeutic response to CBT, but reduces anxiety and depressive symptoms effectively which is also shown in this study (Gorman and Coplan, 1996; Kessler et al, 2005; Emmrich et al., 2012; Kunas et al., 2019). Animal research also provides data that relates chronic anxiety and depression to a phenotype with generalized anxiety, and somatic hyporeactivity (e.g. Avgustinovich et al, 2005; Rygula et al., 2005; McTeague et al., 2011). Both of these traits also characterize the phenotype of female carrier of the AA/AG-haplotype that exhibit chronic anxious apprehension, fear sensitization, and reduced flight and hear rate in panic evoking situations (Weber et al., 2016), thus leading to the assumption that this phenotype can be ascribed to the risk-allele rs17689918 and accompanying increased depressive symptoms. However, in regards to the degree in which the risk-allele rs17689918 or depressive symptoms are predisposed to the above mentioned phenotype, this study points towards the risk allele rs17689918 rather than the depressive symptoms. This is because a) the change of BDI-II scores across time do not correlate with the CBT-induced activation change (left dmPFC) in risk-allele carrier and by b) the consistency of the activation change in the left dmPFC when adding the BDI-II as a covariate factor in the fMRI analysis. The fMRI-sample (males and females) in Weber et al, 2016 also differ in BDI-II scores in which risk-allele carrier scored higher than the control sample ($F= 11.30$, in supplementary table 2 in Weber et al., 2016).

Roy et al conducted analysis on male and female rs17689918 carrier (A and GG-Genotype) and concluded a correlation between depressive symptoms in male A-allele carrier and GG-allele female carrier who experienced stressful life events (Roy et al, 2018). At first sight, this does not seem consistent with the clinical data in this study, since the female A-allele carrier feature higher scores in depressive symptoms than the female control group (GG-allele carrier). The reported correlation in Roy et al was only significant at age 18 while this was not the case at age 25. The average age used in this sample is 33.7 years (SD 8.36) for the A-risk-allele carrier and 39.8 years (SD 10.5) for the GG-allele carrier (control group). Furthermore, the correlation of greater depressiveness for the GG-allele carrier was reported in direct relation to stressful life events, which are not ascertained in this study and thus making it impossible to retrace an exposition to stressful life events in the sample used (Roy et al., 2018).

In addition, risk-allele carriers exhibit significantly lower scores at baseline (on average 0.45 points less) in the clinical total scores of the Agoraphobic Cognitions Questionnaire (ACQ) compared to the control group. Both groups show significantly reduced total scores in ACQ after CBT, but risk-allele carriers exhibit a smaller decrease in total ACQ-scores after CBT when compared to the control group (difference in mean reduction across time is 0.36 points). This questionnaire is used to assess the frequency of maladaptive cognitions related to the consequences that arises from anxiety or a panic attack (Khawaja, 2003). A possible hypothesis for the less frequent maladaptive thoughts ("I am going to pass out", "I will choke to death", etc.) is the specious phenotypical experience of anxious chronic apprehension, increased depressive symptoms and generalized fear sensitization, which is distinctive for the carriers of the AA/AG-haplotype rather than acute panic with accompanied strong autonomic arousal. In addition, carriers of the AA/AG-haplotype show less of an increase in heart rate and a reduced flight escape rate in the behavioral avoidance test, which is also compatible with the reported reduced physical concerns (Weber et al., 2016). However, the statistical significance for ACQ reached borderline threshold in a relatively small sample size (n (A) = 12; n (GG) = 13) and consequently needs to be tested in a larger sample size to increase statistical validity.

Moreover, Weber et al demonstrated higher scores in the Anxiety Sensitivity Index (ASI) in female AA/AG risk-allele carriers in comparison to the non-risk genotype patients (GG) ($p = 0.040$). This could not be replicated in the sample of this study. The reason behind this is suggested in the smaller sample size in this study which is n (total) = 25 versus n (total) = 584 in Weber et al, who additionally screened 893 healthy controls (Weber et al., 2016).

In conclusion, clinical panic-relevant psychometrical data indicate that female PD/A-patients and carriers of the AA/AG-haplotype of *CRHR1* rs17689918 benefit significantly from CBT, which in addition significantly reduces the initially increased accompanied depressive symptoms in risk-allele carriers.

4.2 Neural correlates in differential fear conditioning across time

The female PD/A-control group (carriers of GG-haplotype of carriers of CRHR1 rs17689918) exhibits a normalized activation (predominantly) in the left inferior frontal gyrus (IFG) for $CS+_{unpaired}$>CS- in early differential conditioning after CBT.

This in accordance with previous studies (e.g. Kircher et al, 2011; Lueken et al, 2014; Weber et al, 2016). However, the risk-allele carriers of *CRHR1* rs17689918 (AA/AG-haplotype) did not exhibit a normalized activation in the left IFG. When analyzed in a separate (excluding the control group) post hoc analysis, the risk-allele carrier reveal a normalized activation in the left dmPFC for $CS+_{unpaired}$ in early differential conditioning after CBT. This neural response however did not reach statistical significance when analyzed in the mixed group (contrasting as group by time by CS interaction), suggesting a non-specific neural response. This diverging neural response and possible implications on will nevertheless be considered and its potential meaning for the pathophysiology and phenotype discussed below. Furthermore, the role of the AA/AG-haplotype in the pathophysiology of PD/A, gender specifity, epigenetics, and the increased depressive symptoms will be discussed below in more detail.

4.2.1 Neural correlates in fear acquisition

The CBT-related normalized activation of the (predominantly) left inferior frontal gyrus (IFG), left ACC and thalamus in the PD/A-control patients (GG-haplotype) for aversive stimuli are in line with the reported data in fear conditioning in PD/A patients (e.g. Kircher et al., 2013; Straube et al., 2014b; Sobanksi and Wagner, 2017). The left IFG is involved in cue detection and cognitive threat evaluation (e.g. risk assessment) of aversive stimuli, thus enhanced activation in the left IFG for CS+ (relatively to CS-) in fear acquisition was interpreted as an altered top-down fear processing (Lueken et al., 2014; Yang et al, 2014; Greco and Liberzon, 2016). The previous reported reduced differential conditioning responses in predominantly left and bilateral frontal cortices in risk-allele carrier before CBT (Weber et al., 2016) were however verifiable in this study with a cluster of 73 voxels in the left inferior frontal gyrus, which did not attain the significance level of 142 voxels. This may be reasoned by a smaller study sample due to dropouts: the actual female total sample size of 25 patients in this study versus the total female sample size of 33 patients as in Weber et al, 2016. Moreover, in Weber et al, 2016 this effect was reported for the combined sample that included male patients as well. In this study however, the exclusion of male patients led to a greater cluster size in the previously mentioned cortical area, which furthermore suggests to the already stated explicit effect on the female subgroup.

Risk-allele carriers in turn, exhibit decreased neural differentiation between

CS+ and CS- in differential fear conditioning in the left dmPFC at baseline, hence potentially mirroring fear generalization. After CBT, the neural differentiation between the CS-contingencies increases in the left dmPFC for the carriers of the AA/AG-haplotype which might indicates improved discrimination of neutral stimuli (CS-) and aversive stimuli (CS+). Moreover, the left dmPFC reveals a reduced activation response towards CS+ relatively to CS- after CBT. The activity in the dmPFC in humans and in a context of fear conditioning is evidenced to mirror negative "conscious appraisal of threat, with particular relevance to the subjective experience of fear and anxiety" (Fullana et al., 2016; see also Mechias et al., 2010; Kalisch and Gerlicher, 2014). The hyperactivation of the dmPFC in fear conditioning can therefore be referred to as increased or pathological appraisal of a threat, which is crucial in the development and maintenance of PD, as expressed in catastrophizing or over-interpretation/estimation of threat or bodily sensations (Beck & Clark, 1997; Kalisch & Gerlicher, 2014). This downregulation of dmPFC activity after CBT signifies a less negative reappraisal of threat situations and associated aspects (e.g. bodily symptoms during threat). One study suggests a more specific role for the dmPFC in the monitoring and judging (negative interpretation/catastrophizing) of internal changes (feelings, bodily sensations, attention) as imminent harm that arises during a threat (Maier et al., 2012). Exaggerated appraisal of a threat or bodily sensation and exaggerated worrying are suggested to be causally linked in the experience of exaggerated fear (Stapinski et al., 2010; Telch et al., 2010).

In addition, fear expression (in context of fear conditioning) by the dmPFC has been suggested by projections to the basolateral amygdala (dmPFC-amygdalar projection) in rodent studies (Corcoran and Quirk, 2007; Burgos-Robles et al., 2009). First experimental equivalence to this functional connectivity of dmPFC and amgydala has been revealed in humans too, indicating a) stronger (valence-specific) coupling of the dmPFC and amygdala during anxiety (in this regard: strongest anxiety-potentiated coupling is seen in individuals with highest levels of trait anxiety), b) that a readily or chronic coupling of dmPFC-amygdala mechanism represents the increase of vigilance towards a threat (or: attentional bias for a threat), which increases anxiety and in turn promotes the threat bias, thus constituting a top-down aversive amplification mechanism (Robinson et al., 2012). Nevertheless, the study conducted by Robinson et al did not apply a fear conditioning paradigm but a task design that required the identification of happy and fearful faces under threat or safe conditions (threat of

unpredictable shock on the foot). However, the attentional bias for threat complements the (above described) increase in exaggerated appraisal of threat and additional previous data suggests a functional equivalence of rodent's positive dmPFC-amygdala connectivity in humans in context of anxiety (Mechias et al., 2010; Etkin et al., 2011). This above mentioned dmPFC-amygdalar projection offer a possible and consistent explanation for the reduced fear expression by means of decreased activation in the left dmPFC or dmPFC-amygdala coupling for aversive contingencies ($CS+_{unpaired}$) in fear conditioning as suggested by the above mentioned previous data. However, the coupling of the left dmPFC and amygdala is not deducible by our explorative data analysis (no activation changes in the amygdala detected) and requires further testing and exploration by a functional connectivity analysis and a presumably bigger patient sample. The maladapted or pathological appraisal of threat represents a key target in CBT which offers a consistent explanation for the normalized activation in the left dmPFC (hence, normalized appraisal of threat) and accompanying positive treatment response that is reflected in the reduced clinical outcome scores: Besides exposure techniques, CBT also explicitly addresses a "cognitive restructuring" by engaging the patient first in identifying and challenging the unrealistic nature of distorted or maladaptive cognitions and misinterpretations (for instance of bodily symptoms) and second, in developing more rational and adaptive thinking and perceptions of feared situations and/or accompanying bodily symptoms (Hofmann et al., 2012; Wieman et al., 2020). This restructuring of cognitive processes is potentially expressed in reduced dmPFC activation in risk-allele carriers (Maier et al., 2012). This finding of neural response pattern (reduced activation in the dmPFC) in fear conditioning is also in line with the appraisal theory (rostral part of the dmPFC) or expression theory (posterior part of the dmPFC) concerning its function in fear conditioning as stated in earlier works (Milad et al., 2007; Etkin et al., 2011).

In addition, our data points towards a normalized activation in the left precuneus in differential conditioning in risk-allele carriers. On one hand, the precuneus is reported to be functionally involved in the default mode network and various cognitive functions including the resting-state cognition (Utevsky, Smith, & Huettel, 2014), in "self-centred mental imagery strategies" (Cavanna & Trimble, 2006), in the retrieval of episodic memory and processing of emotional inference (Henry et al., 2021; Lundstrom et al., 2003). On the other hand, the dmPFC, amygdala and the precuneus are also considered in processing uncertain future threats. It is highlighted

that the precuneus in particular is involved in the processing of self-related uncertain future threats (Geng et al., 2018). Furthermore, in the context of anxiety disorders, the precuneus correlates with state anxiety rather than trait anxiety (Saviola et al., 2020). In a study (Yuan et al., 2018) conducting a resting-state functional MRI in the context of social anxiety disorders, the abnormal functioning in the left precuneus and functional deficits in the precuneus-related network have been connected with depression and anxiety. In addition, the precuneus shows a sex-dependent connectivity, with women having greater connectivity with the medial thalamus, hippocampus, middle and anterior cingulate gyrus. Connections to the medial frontal gyrus have been documented as well (Yuan et al., 2018). However, the significance and meaning of this precuneus-medial frontal gyrus projection in the modulation of fear conditioning is unknown to the author. The normalized co-activation in the left precuneus in differential conditioning potentially mirrors reduced state anxiety and/or normalized processing of self-related uncertain future threats. The activation change from baseline to post-therapy in the left precuneus however did not correlate with any of the clinical parameters in the risk-allele patients.

The female risk-allele carriers are reported to exhibit increased activation for safety signals in the amygdala (dysfunctional safety signal processing) compared to control patients (Weber et al., 2016). This finding could not be replicated in our study, possibly due to dropouts of female patients: this study had been conducted with a total sample 25 female patients, while 33 female patients participated in Weber et al. Additionally, the measured effect in safety signal processing (t-value: 2.43, p (unc.) = 0.008, ROI analysis with a voxel threshold of p (unc.)= 0.05) is weaker compared to the differential conditioning analysis (t-value: 4.62 and p (unc.) < 0.001) in (Weber et al., 2016, see complementary methods).

In Weber et al, female PD/A - risk-allele carriers of CRHR1 rs17689918 display significantly increased total scores in the Anxiety Sensitivity Index (ASI) compared to the female control PD/A - patients. The ASI reflects the “fear of arousal-related sensations” or the “fear of fear” and is closely related to the accompanied appraisal of catastrophic misinterpretation or over-estimating and potential ramifications (Kalisch & Gerlicher, 2014; Taylor et al., 2007). In this regard, the risk-allele carriers in this study neither reveal increased dmPFC activations for CS-contingencies at baseline nor a correlation of both decreased activation reduction of the left dmPFC and ASI total scores across time. This might be attributed to the smaller sample in this study that do

not feature significantly increased ASI total scores compared to the control PD/A patients. Strikingly, the PD/A control patients show a correlation of the normalized right IFG-, left thalamus- and left ACC activation from baseline to post-CBT with their ASI total scores (the left ACC activation change from baseline to post-CBT also correlated with the reduction BDI and PAS total scores). The processing of anxiety sensitivity (as captured in the ASI) was shown to be partially correlated with ACC activations (Poletti et al., 2015). Other reports on the above mentioned specific correlations of activation change in above mentioned brain regions (ACC, left thalamus, right IFG) and psychometric data (BDI, PAS, ASI) in the context of fear conditioning is not known to the author. However, these exploratory findings need to be interpreted with caution as correlations might be driven by outliers with the highest reductions in these scores (see table 8 a.-g.). The Spearman correlation is less sensitive than the Pearson correlation to strong outliers that are in the tails of both samples. That is because Spearman's ρ limits the outlier to the value of its rank.

In regard to the phentoype of risk-allele carries, Weber et al observed a de-synchrony of reported fear and physiological responses (lower heart rate) in a behavioral avoidance test compared to PD/A-control patients (Weber et al., 2016). In further putting the role of the dmPFC in context of PD/A and the phentoype of the risk-allele carriers, further studies (Kalisch & Gerlicher, 2014; Kalisch et al., 2006; Maier et al., 2012; Raczka et al., 2010) indicate a dissociation between threat-related responses of dorsal anterior cingulate cortex dACC/dmPFC and physiological fear responses, namely heart rate and skin conductance. Yet the startle reflex has not been shown to be dissociated by neural responses of dACC/dmPFC and the tendency of the general motor response is considered rather as remote or not striking due to tenuous projections to cortical motor areas (Kalisch & Gerlicher, 2014). The proposed altered dmPFC activation in risk-allele carriers are in line with the previous reported de-synchrony of reported fear and physiological responses (lower heart rate) as in Weber et al. (2016).

In summary, risk-allele carriers show a significant response to CBT concerning overall clinical symptoms, yet the neural response differ compared to the control patient group suggesting a alternative or compensatory neural response in risk-allele carrier. A separate post-hoc analysis comprising only risk-allele carrier suggests a decreased activity in the left dmPFC (= mirroring conscious and pathological appraisal

threat) for the conditioned response $CS+_{unpaired}$>CS-, while the control patients showed a decreased activation in the left IFG (=mirroring attention/cognitions related to negative affect or threat evaluation) for the conditioned response $CS+_{unpaired}$>CS-. However, the above activation pattern in the left dmPFC in risk allele carriers failed to reach statistical significance when compared to the control patients, which eventually only allow a hypothetical assumption on how CBT affect risk-allele carrier in terms of a neural response. Other data (as shown above) indicate that threat related responses of the dACC/dmPFC are shown to be dissociated to physiological responses (e.g. heart rate, skin conductance) which is further in line with the risk-allele carrier who are characterized by a de-synchrony of reported fear and physiological response as observed in Weber et al (2016). This hypothesis remains to be proved in future studies.

4.2.2 Implications of the AA/AG-haplotype in the pathophysiology of PD/A

The expression analysis on postmortem brains of risk-allele carrier revealed significantly reduced mRNA expression for CRHR1 in the forebrain in the amygdalae (Weber et al, 2016). The direct translation of this result to females is rather restricted considering that the postmortem brains were of mixed sexes and biased towards males (58 males and 18 females). However, quantitative real time PCR and the expression quantitative trait locus function (eQTL-function) of rs17689918 (and perfect proxies) confirmed that the minor A-allele reduced the expression of CRHR1 mainly in women (Myers et al., 2007; Weber et al., 2016). In contradiction to that, Schartner et al investigated the methylation of the CRHR1 promoter by reporter gene assays in blood samples and revealed a significantly reduced methylation of CRHR1 (thus, an *increased* expression of CRHR1) in PD patients and also in healthy controls that feature high scores on anxiety symptoms (Schartner et al., 2017). The report of *decreased* CRHR1 expression in female PD/A-risk-allele carrier (Weber et al., 2016) and the *increased* expression in PD patients (Schartner et al., 2017) appear inconsistent. In further reconciling this discrepancy, Schartner et al argues that a) Weber et al stresses out the phenotype of chronic anxious apprehension while Schartner et al highlights acute panic states and accompanying intense physiological arousal in which increased transmission of CRH by increased CRHR1 expression supposedly outlines the relevant pathogenetic mechanism; b) assumptive epigenetic processes depict a compensatory mechanism in the endeavor to counteract genetic/environmental effects; c) allele-specific methylation of rs17689918 might not be applicable to the methylation

of the promoter region of *CRHR1* and therefore not dependent upon (epi-)genetic mechanisms and that lastly d) replication could possibly lead to a better understanding of seemingly contradictory findings under the considerations of various factors such as phenotype, medication, duration of illness, family anamnesis, comorbidities etc. (Schartner et al., 2017). In summary, previos and in part inconclusive work on the question of whether and how the exact expression is reduced or enhanced in female carrier of the risk-allele rs17689918 does not allow a final conclusive statement on our study samle, yet the data mentioned above suggest that rs17689918 leads to decreased expression of CRHR1 in females and might not be dependent upon (epi-)genetic mechanisms. The assumptive hypothesis of a phenotype described by a chronic anxious apprehension appear rather favorable in light of our data rather than a phenotype of acute panic states and accompanying intense physiological arousal, when considering that CBT especially reduced accompanied depressive symptoms in risk-allele carrier stronger compared to the control group: Depressive symptoms appear clinically chronic or continous rather than intermittent or in acute states with intense physiological arousal and might add to the phenotype of the risk-allele carrier. However, future studies need to further confirm the exact mechanisms underlying the AA/AG-haplotype and its effects on expression of CRHR1.

In addition, mouse models indicate a rather complex and dual role of CRHR1 glutamatergic circuits in the prefrontal cortices and dopaminergic neurons in the midbrain: A lack of CRHR1 in glutamatergic circuits in the prefrontal cortices (that are linked to neurotransmission in the amygdala and hippocampus) is correlated with reduced anxiety symptoms, while a lack of CRHR1 in midbrain dopaminergic neurons (ventral tegmental area, substantia nigra pars compacta) lead to an increase of anxiety by reduced dopaminergic neurotransmission in the projected prefrontal cortex. In this connection, the above mentioned CRH/CRHR1-driven glutamatergic and dopaminergic systems act in an antagonizing but attuned way in order to balance anxiety responses to stress or threat (Refojo et al., 2011). Lemos et al complemented this model by illuminating the crucial role of CRH/CRHR1 in the dopamine-mediated appetitive behavior and positive subjective state in the nucleus accumbens: Severe stress removes the ability for CRH to regulate dopamine effect and in turn, takes away the appetitive qualities of CRH (via reduced CRHR1 expression by a glucocorticoid signal pathway) and thus leading to a negative affective state distinguished by a negative perceptual bias or depression-like behavior (Lemos et al., 2012). Not only

severe stress, but also chronic mild stress has been shown to alter the neural encoding in dopaminergic neurons in limbic structures, which mediate depressive-like symptoms and decreased escape behavior in rodents by means of reduced recruitment in the ventral tegmental area and consequently decreased dopamine signaling in the nucleus accumbens (Tye et al., 2013). Taken together, these studies additionally point towards the involvement of neural circuits and in midbrain structures in mediating anxiety, depressive-like behavior and escape behavior: In conferring this to the phenotype of the female risk-allele carrier this data suggests the hypothesis that reduced expression of CRHR1 facilitate a shift of neurotransmission towards reduced dopaminergic neurotransmission in midbrain structures (e.g. ventral tegmental area, nucleus accumbens) and projected prefrontal cortices leading to a phenotype that is characterized by anxiety, depression-like behavior and reduced escape behavior which potentially illustrates rough approximation of the phenotype of AA/AG-rs17689918-risk allele carrier. This hypothesis of reduced dopaminergic neurotransmission in midbrain structures and projected prefrontal cortices might offer an plausible hypothesis in explaining the phenotype of the female-risk allele carriers, although activation changes in the possibly involved and above mentioned midbrain areas (e.g. ventral tegmental area, nucleus accumbens) were not detectable in our study (neither across time nor in comparison to controls). However, this hypothesis is daring due to the lack of evidence in humans and needs definitive exploration and testing in the context of humans.

Weber et al, points also to the involvement of another midbrain structure deduced from animal studies in order to account for the risk-allele carrier's phenotype that is featured by "chronic anxious apprehension" or "sustained fear" rather than phasic fear: Sustained fear has been shown to be dependent on CRH/CRHR1-related activation of the bed nucleus of the stria terminalis (BNST) (Walker et al., 2009). The specific blocking of CRHR1 inhibits defensive behaviors and sustained fear but not phasic fear, hence elucidating the involvement of the BNST in sustained fear. Congruously, the overexpression of CRH within the lateral BNST have been shown to increase sustained fear and to reduce the density of CRHR1 receptors in the BNST. Weber et al furthermore infers that overexpression of CRH to compromise anxiety reactions for threat cues and enhances remote fear memory consolidation in dorsal hippocampus (via increased GluR-1 mediated signaling), hence CRH/CRHR1-mediated activity – particularly - in the BNST have been proposed for the sustained

and generalized fear (Weber et al., 2016; see also Walker et al., 2009; Davis et al., 2010). More recent studies in rodents succeeded in linking chronic variable mild CRH-associated stress and protein kinase A-dependent CRHR1 - signaling in the (oval nucleus of the) BNST in developing chronic anxiety and maladaptive behaviors, furthermore implicating an involvement of the serotonergic neurons in the dorsal raphe nucleus (Donner et al., 2020; Hu et al., 2020). More recently, these insight have been explored and tested in humans and data indeed confirms basic perceptions in rodent studies on the role of the delayed and sustained activity of the BNST in mediating sustained fear and anticipatory anxiety (Avery, Clauss & Blackford, 2016; Buff et al., 2017; Jenks et al., 2020). Studies on humans suggest that patients with anxiety-related disorders reveal increased state functional connectivity of the BNST and the caudate in association to threat bias (while healthy controls exhibit stronger connectivity between the amygdala-thalamus/ACC) and therefore suggesting that this higher functional connectivity mirror maladaptive processes expressed by anticipatory anxiety (Jenks et al., 2020). Avery et al furthermore reported an increased functional connectivity of the BNST, insula and dmPFC during threat processing and in the expressing of "aversive emotional state during anticipation of threat" (Avery et al., 2016) and therefore providing a hypothesis for the risk-allele carriers that *CRHR1*-driven alterations of BNST activity in being a crucial underlying cause of the chronic anxious apprehension phenotype. The BNST depicts a heterogeneous structure due to its sexual dimorphism, diverse neurotransmitters (e.g GABA, serotonine), diverse receptors (e.g. adrenergic alpha-/beta receptors), sub-nuclei and various projections (in humans) to the vmPFC, dmPFC, amygdala, insula, caudate and thalamus (Lebow and Chen, 2016). However, our study could not provide evidence for the involvement of the BNST in PD/A patients since cluster activation differences in the fMRI-analysis could not be produced or captured in this study. This could be partially explained by the fairly small anatomical size of the BNST due to spatial resolution (3 Tesla), which in general has been rather challenging to explore in fMRI although technological advancement in magnetic field strength, e.g. 7 Tesla, enables a higher resolution for conducting analyses in greater detail (Avery et al., 2016; Lebow and Chen, 2016). In addition, functional connection analysis could possibly reveal further insights into the fear circuitry in risk-allele carrier.

With regards to the sex-specificity of the risk-allele AA/AG of rs17689918, association results of the AA- and AG-haplotype showed a significant association with

PD/A in the female subsample while none of these haplotypes increased the risk for PD in the male subsample (Weber et al., 2016). Weber et al proposed that males probably display a tolerance for rs17689918 driven *CRHR1*-expression and additionally hint at the extended linkage disequilibrium which is suggestive of "neighbouring risk genes of psychiatric disorder may predispose as well" (including following genes: *MAPT*, *IMP5* and *C17orf69*) and need further testing and replication (Weber et al., 2016). Moreover, the BNST being a *CRHR1*-associated brain structure, is suspected to be crucially involved in mediating sustained fear in PD/A and known for its anatomical and physiological sex-specific differences. Female humans display 76% larger structural connectivity to regions that are significantly connected to BNST. In context of hormonal influences, animal studies furthermore suggest that androgens, estrogens and progesterone interact differently concerning sustained fear and anxiety. Progesterone was shown to attenuate CRH-enhanced startle-responses and activate neurons of the BNST by means of oxytocin. Testosterone was shown to only reduce startle-responses in male rats (Lebow & Chen, 2016). In a fear conditioning paradigm estrogen was shown to interfere with the inhibition of fear in female rodents, hence indicating a greater vulnerability to adverse effects of stress compared to males (Toufexis et al., 2007). Sex hormones are suggested to critically be involved in modulating fear conditioning (e.g. Merz, Kinner and Wolf, 2018). These examples of sex-specific differences anatomy and physiology of the BNST offer a further hypothesis for future studies to explore the sex-discrepancy in the predisposing effects for PD/A of the AA/AG-Haplotype of *CRHR1* rs17689918.

Connectivity analysis revealed altered connectivity between the BNST and regions that are crucial for emotional processing and cognitive control (insula, dlPFC, dmPFC, parahippocampal and fusiform gyrus): Regarding the left dmPFC - left BNST circuit, PD-patients were shown to demonstrate attenuated causal connectivity between these regions compared to healthy controls (Pang et al., 2019). This insight enables the formulation of the hypothesis that rs17689918-risk-allele carrier display a stronger connectivity between the left dmPFC and left BNST in response to CBT compared to the PD/A control group since the BNST is considered in playing an essential role in mediating CRHR1-dependent sustained fear. However, this needs further exploration. For this purpose, a dynamic causal modelling (DCM) offer a method to allocate a causal effective connectivity between the above mentioned regions. This method require the identification of the regions of interest (here: left

dmPFC and left amygdala) in the indivdiual activation data of each subject. But since our study did not provide any (statistical) activation difference in the amygdala and in the left dmPFC in group comparisons, the applicability of this method is limited (or at least aggravated) and demands a bigger study sample in future studies.

The reduced expression of CRHR1 in the frontal cortices and amygdala act as a predisposing factor for the development of PD rather than a consequence of the latter (Weber et al., 2016). Indirect evidence supports this view: Wintermann et al demonstrated that the hypo-reactivity of the HPA-axis (reduced cortisol levels) and concomitant increased psychosocial stress increased the risk for a chronic course of PD in predominantly females. The hypothesis underlies the stress sensitization model, that start from the premise that lower cortisol levels attenuates successful adaptation and promotes therefore ineffective coping behavior which eventually magnify the potential for stressors to the susceptibility for triggering panic symptoms (Wintermann, Kirschbaum, & Petrowski, 2016). Ineffective coping to psychosocial stress (driven by low cortisol levels) is also indicated to promote depressive symptoms in females (Booij et al., 2013; Zorn et al., 2017). These findings can be hypothesized to be in line with the results of this study in terms of attenuated HPA-axis response (in this study: decreased CRHR1 expression) and PD with increased depressive symptoms in females. The applicability of the above described "stress sensitization model" however underlie following limitations: On one hand, this study did not analyze cortisol levels and on the other hand, decreased cortisol levels (as cited in previous studies) and decreased CRHR1 (in this study) display different physiological substrates in the HPA-axis and are therefore not comparable or equivalent. The applicability of the "stress sensitization model" in the context of reduced *CRHR1* expression in the frontal cortices and amygdala should be explored in future studies.

The "stress sensitization model" offers a plausible hypothesis on how reduced cerebral *CRHR1*expression in females promote the susceptibility for the development of PD. The *CRHR1*-driven alterations of BNST activity have been repeatedly involved in rodents and humans in being a crucially involved to mediate chronic anxious apprehension hence offering a promising brain structure for further exploration. Furthermore, deduced expression of *CRHR1* in rodent studies points toward a reduced dopaminergic neurotransmission in midbrain structures and projections in the prefrontal cortices being promotive for a phenotype that is featured by anxiety, depression-like behavior and reduced escape behavior, thus offering a further

hypothesis for the female risk-allele carriers that are characterized by chronic anxious apprehension, increased depressive symptoms and reduced escape rates.

4.2.3 Increased depressive symptoms in risk allele carriers

As discussed earlier (see chapter 4.1) the female risk-allele carrier exhibit increased depressive symptoms (BDI-II total scores) compared to female PD/A-control patients. This is in line with earlier findings of decreased *CRHR1*-expression in the frontal cortices of depressed patients (due to hypersecretion of CRH) (Nemeroff et al., 1988) and overall CRHR1-driven vulnerability towards depression by *CRHR1* haplotypes or gene-environmental interactions (Davis et al., 2017; Kristensen et al., 2011; Liu et al., 2006; Normann & Buttenschøn, 2020; Rogers et al., 2013). The significantly increased scores for depressive symptoms in female risk-allele carriers might give rise to the question whether the AA/AG-haplotype of *CRHR1* rs17689918 increases the risk towards depression rather than PD. On that note, Weber et al alludes to a) the linkage disequilibrium of rs17689918 in other nominally and PD-associated single nucleotide polymorphisms (SNPs; such as rs1396862 and rs1876831) and rs878886, and b) to the indirect evidence that rs17689918 also predisposes to posttraumatic stress disorder and collectively indicating no association of depressive comorbidity, thus indicative for rs17689918 to be overall PD-specific (Weber et al., 2016).

Further indirect references for the PD-specificity of the minor risk-allele of *CRHR1* rs17689918 can be derived by studies that explored the specific neural correlates mediated by comorbid major depression in PD/A-patients: PD/A-patients with comorbid depression exhibit both reduced activation in the dorsolateral prefrontal cortex (dlPFC) and insula as well as no altered safety signal processing in fear conditioning (Lueken et al., 2015). In addition, Kunas et al showed that comorbid depression did not display reduced left IFG activation but increased functional connectivity between left IFG and prefrontal and parietal cortices in fear conditioning after CBT (Kunas et al., 2019). Considering this data, on one hand risk-allele carrier indeed do not exhibit reduced activation in the left IFG or dlPFC (thus speaking for a depressive comorbid effect), but on the other hand risk-allele carrier still displays altered safety signal processing (see Weber et al., 2016), which in turn does not suit the assumption of a depressive comorbid effect in fear conditioning. In this view, risk-allele carriers might partially display altered neurofunctional substrates in fear conditioning due to comorbid depressive symptoms (particularly when expressed the

absent left IFG or dlPFC involvement in fear conditioning), but nevertheless this is inadequate to ultimately infer a straight comorbid related modulation of neural substrates in fear conditioning. For these reasons and the reasons detailed in chapter 4.1, this study points towards the PD-specificity of the AA/AG-haplotype of *CRHR1* rs17689918 of in females.

4.3 Correlation of the left dmPFC and agoraphobic avoidance (MI-7 acc.)

The female risk-allele carrier of AA/AG - rs17689918 exhibits a decreased activation in the left dmPFC for $CS+_{unpaired}$ after CBT. This change in activation pattern in the left dmPFC across time correlates significantly with the reduction of the Mobility Inventory accompanied (MI-7 acc.) scores across time. The MI-7 mirrors the self-reported agoraphobic avoidance in the last 7 days by the patients' subjective ratings in regard to what extent they would avoid depicted situations owing to their anxiety or uneasiness, either on the condition "alone" or "accompanied" by a companion. Thus, activation reduction in the left dmPFC correlates with the reduction of self-reported agoraphobic avoidance particularly in the presence of a companion ("accompanied"), but not "alone". This above described correlation in context of PD/A and the distinction between the two conditions alone versus accompanied of MI-7 will be discussed in more detail below.

Recent data in rodents provided evidence for a novel circuit encompassing projections from the dmPFC to the ventral and dorsomedial striatum (fronto-striatal circuit) being implicated in the processing of avoidance behavior (Loewke et al., 2020). In particular, the stimulation of dopaminergic type D1-type medium spiny neurons in the striatum decreased avoidance behavior whereas a stimulation of type D2-type receptors lead to an increase of avoidance behavior in rodents (LeBlanc et al., 2020; Loewke et al., 2020). Based on this above described frono-striatal circuit, the decrease of dmPFC activity for $CS+_{unpaired}$ in fear conditioning and the significant correlation to the decrease of total scores in the MI-7 acc. (mirroring self-reported agoraphobic avoidance) allows the preliminary hypothesis that the decrease of dmPFC activity for $CS+_{unpaired}$>CS- in risk-allele carriers leads to reduced stimulation of dopaminergic type D2-type medium spiny neurons in the striatum (in particular the dorsomedial striatum) and thus to a reduced avoidance behavior, and as a consequence

thereof reduced fear symptomology and avoidance behavior as measured in MI-7 acc. This hypothesis cannot be confirmed by this fMRI analysis and needs further testing by e.g. functional connectivity analysis of left dmPFC and the dorsomedial striatum. Yet, human studies provide references for the involvement of D2-type striatal neurons in avoidance in a "probabilistic selection reinforcement learning task" (Frank & Hutchison, 2010), or the involvement dorsomedial striatum in an approach-avoidance conflict paradigm (Aupperle et al, 2015). The involvement of striatum regions has been reported in predominantly delayed fear conditioning paradigms using tactile unconditioned stimuli (US) which conceptualized the role of the ventral striatum as a salience system and changes in valence (Berridge & Kringelbach, 2015; Jensen et al., 2003; Sehlmeyer et al., 2009) or for appetitive conditioning (Chase et al., 2015; Kruse et al., 2017) whereas the dorsomedial striatum is shown to be involved in the processing of goal-directed behavior (Kruse et al., 2017). So far, the translation of the above described fronto-striatal circuit in pavlovian fear conditioning in rodents into a human fear conditioning context is rather unclear and must first be explored and confirmed by future studies.

Strikingly, the dmPFC activation pattern for $CS+_{unpaired}$ >CS- seem only significantly correlated with the MI-7 *accompanied* scale, but not with MI7 *alone* scale. Key feature of agoraphobia is the anxiety that arises when being in a crowded or public space and the associated rumination that panic-like symptoms arises and potential help or an escape might be not available (Balaram & Marwaha, 2020). In this context, it seems apparent considering that the company of a trusted person in agoraphobic situations (as in scale "accompanied") indeed implicate potential help and therefore confines agoraphobic anxiety more likely rather to being alone (Hoffart et al., 2018). However, one has to consider the reservation that these two scales ("alone" and "accompanied") are highly correlated to each other (r = 0.83), hence it seems rather unlikely from a statistical perspective that only one scale is significantly correlated and the other is not (Chambless et al., 2011). In this regard, Kircher et al initially reached a similar result in which the reduction of $MI\text{-}7_{accompanied}$ total scores appeared significantly correlated to left IFG normalization across time (correl.coef: 0.444; p (unc.): 0.002), whereas this effect was relatively weak for MI-7 alone (correl.coef: 0.271; p (unc.): 0.042), thus they subsumed both scales into a total score of MI-7 in their report (Kircher *et al.*, 2013, supplementary data). We included even a smaller

subsample of the sample reported in Kircher et al, and correlations might be driven by two outliers with highest reductions in MI-7 scores. Therefore, replication with a bigger sample size for better statistical power and validity of data is necessary.

Overall, when comparing the risk-allele carriers and control patients after CBT, the activation reduction in the left dmPFC in risk-allele carriers in regard to the conditioned response ($CS+_{unpaired}$>CS-) appear to correlate with the reduction of agoraphobic symptoms or avoidance across time. Furthermore, as concluded by the suggestions of earlier studies, a fronto-striatal circuit encompassing the dmPFC and D2-type striatal neurons in the dorsomedial striatum, offer a hypothetical circuit mediating avoidance behavior which need further exploration and confirmation.

4.4 Limitations

The findings of this study include several relevant methodologic limitations and should therefore be interpreted with regard of the following restrictive factors: (1) the rather small and reduced sample size (initial fMRI sample in Weber et al, 2016: 16 risk-allele carriers and 17 controls) due to dropouts of 8 female patients (among them 4 risk-allele carriers and 5 controls) furthermore decreased the conclusiveness of the findings reported here, (2) only fear conditioning responses in the early and late acquisition phase have been explored, but not fear extinction, (3) this study is lacking functional connectivity analysis to further illuminate brain areas involved in mediating fear conditioning in the context of the *CRHR1*-risk-allele (for instance, amygdala, BNST or the striatum), (4) this study does not provide any autonomic data (e.g. heart rate or skin conductance) for further exploring the de-synchrony of reported fear and physiological responses across time (as in Weber et al, 2016), (5) the data on behavioral avoidance task was not explored for the time point after CBT to conclude whether or not the risk-allele carriers still exhibit a reduced flight escape rate and a de-synchrony of reported fear and physiological reactions (see Weber et al, 2016), (6) no (epi-)genetic exploration or expression analysis had been conducted after CBT to investigate potential molecular mechanisms that modulate the expression of CRHR1 driven by CBT, (7) the reported effects were examined only by CBT without any other control intervention, therefore an exclusive CBT-specificity cannot be concluded and similar effects by any other psychotherapeutic intervention are possible, (8) the analysis of neural activation focused on the brain areas that are frequently reported as

relevant for fear conditioning (e.g., amygdala, anterior cingulate cortex, hippocampus, insula, prefrontal cortices, bed nucleus of the stria terminalis), while other co-activated (sensomotoric) areas within the paradigm (e.g. temporal cortex, occipital cortex, motor cortex) were however not analyzed or interpreted in greater detail. (9) reported analyses were purely explorative and therefore not corrected for multiple comparisons (e.g., clinical outome measures, correlational analyses and post hoc fMRI analyses) increasing the likelihood of false-positive results.

5 CONCLUSION AND OUTLOOK

Conclusion

Female patients suffering from Panic Disorder/Agoraphobia (PD/A) and carrying the AA/AG-haplotype of the *CRHR1*-allele rs17689918 experience a significantly stronger reduction of accompanied depressive symptoms, whereas the control patients exhibited significantly stronger reductions in maladaptive agoraphobic cognitions. In addition, risk-allele carriers respond differently to CBT in regard of neural substrates and thus denoting different underlying neural response or mechanisms driven by the risk-allele. Eventually, these results lead to the final conclusion that the AA/AG-haplotype of the *CRHR1* allele rs17689918 indicate a distinct CBT response of PD/A in females on a neuro-functional level.

Outlook and clinical pertinence

The understanding of genetic moderators - as for instance gene variations of CRHR1 - is crucial for the understanding of the mechanisms that predispose the course of developing Panic Disorder and thus allowing to treat more precise and to even adapt/reinforce or to develop novel treatments in a clinical context. In the context of previous rodent and human studies on CRHR1-dependent modulation of specific neuronal circuitries, this study reinforces a distinct pathophysiological mechanisms suggesting a circuitry involving the dmPFC and amygdala, BNST and/or dorsomedial striatum in mediating the distinct phenotype of the female risk-allele carrier (chronic anxious apprehension, de-synchrony of fear and physiological responses, reduced

flight escape behavior, generalization of fear) that offer further exploration for future studies by for instance condition-dependent functional connectivity analyses and/or diffusion tension imaging to reconstruct the underlying fear circuitry. Machine learning offers a potentially beneficial tool that bridges the findings in neuroimaging studies and a translation into a useful clinical context: machine learning is based on a multivariate pattern recognition of neuroimaging data (e.g. neural characteristics in fear conditioning) on a group level that could offer a diagnostic value (e.g. classification, prognosis, identification of a comorbidity status) on a single subject level, thus enabling a novel tool for a clinical context (Hahn et al, 2015; Lueken et al, 2015; Orru et al, 2012). Future, studies should further focus on symptom-related effects of CBT by means of different psychometric scales (as for instance BDI-II or ACQ): A differenical response in these psychometric scales can –if replicated- hint to diverse mechanism of action and indicate a different underlying pathophysiology in risk-allele carriers.

6 SUMMARY

The AA/AG-haplotype of the *CRHR1* allele rs17689918 has been implicated in increasing the risk towards Panic Disorder in females. The female risk-allele carriers (AA/AG-Haplotype) that suffer from Panic Disorder/Agoraphobia (PD/A) exhibit impaired activation responses in the left dorsolateral prefrontal cortex for the conditioned response CS+>CS- and increased activation in the left amygdala for safety signals (CS-), thus mirroring a phenotype that is characterized by fear generalization and dysfunctional safety signal processing. Therefore, this study is aimed at investigating the impact of the AA/AG-haplotype of rs17689918 regarding the effect of cognitive behavioral therapy (CBT) and the underlying neural activation changes in fear conditioning across time (pre/post comparison).

For this, functional MRI (fMRI)-, clinical- and demographic data from 12 female PD/A-patients carrying the risk-allele and 13 female PD/A-controls (both medication free) were obtained from a subsample of a randomized, controlled multicenter clinical trial („PANIC-NET“). The neural correlates have been measured within a fear conditioning paradigm by fMRI and clinical data that have been ascertained respectively before and after 12 sessions of manualized CBT. Hence, clinical scores and fMRI data for both groups have been tested for between-group differences and correlations across time.

Concerning the clinical response, minor differences were observed regarding CBT effects on depressive (stronger effects on risk-allele carriers) and maladaptive agoraphobic cognitions (stronger effect on control patients). Moreover, the risk-allele carriers reveal a different neuronal response in fear conditioning from baseline to prost-treatment: the PD/A-control group exhibit a normalized activation in the left inferior frontal gyrus (IFG), left anterior cingulate cortex and thalamus for the conditioned response CS+ > CS-, which the risk-allele carriers however did not display. A post hoc analysis for risk allele carrier suggests a normalized activation in the left dorsomedial prefrontal cortex (dmPFC) and left precuneus for the conditioned response CS+ > CS-, which however did not reach a significance when compared to the control patients.

In conclusion, the AA/AG-haplotype of the *CRHR1*-risk allele rs17689918 suggests a distinct pathogenesis in PD/A and neural response to CBT in female patients suffering from PD/A, which does not interfere with the overal treatment response for

CBT compared to control patients.

7 ZUSAMMENFASSUNG

Der AA/AG-Haplotyp des *CRHR1* Allels rs17689918 erhöht signifikant das Risiko für die Entwicklung von Panikstörungen/Agoraphobie (PD/A) bei Frauen. Die Risiko-Allel-Trägerinnen des AA/AG-Haplotyps, die an PD/A leiden, zeigen hierbei beeinträchtigte Aktivierungsreaktionen im linken dorsolateralen präfrontalen Kortex für die konditionierte Reaktion CS + > CS- und eine erhöhte Aktivierung in der linken Amygdala für Sicherheitssignale (CS-), was in der Gesamtkonstellation einen Phänotyp widerspiegelt, der durch Furchtverallgemeinerung und dysfunktionale Verarbeitung von Sicherheitssignalen charakterisiert ist. Diese Studie zielt darauf ab, die Auswirkungen des AA / AG-Haplotyps von *CRHR1* rs17689918 in Bezug auf die klinische Wirksamkeit einer kognitiven Verhaltenstherapie (KVT) zu untersuchen und einhergehende Unterschiede in neuronalen Aktivitätsveränderungen (Prä/Post-Vergleich) in der Furchtkonditionierung zu detektieren.

Hierzu wurden Daten aus funktioneller MRT (fMRT) und klinischen Interviews exploriert von 12 weiblichen PD/A-Patientinnen und Trägerinnen des AA/AG-Haplotyps sowie 13 PD/A-Kontrollpatientinnen (Trägerinnen des GG-Haplotyps) aus einem subsample einer randomisierten, kontrollierten multizentrischen Studie („PANIC-NET“). Signifikante neuronale Aktivierungsveränderungen wurden mithilfe eines Furchtkonditionierungsparadigmas mittels fMRT-Bildgebung ermittelt, die gemeinsam mit den klinischen Daten jeweils vor und nach 12 Sitzungen standardisierter KVT akquiriert wurden. Die klinischen und fMRT-Daten wurden hinsichtlich Gruppenunterschiede und Korrelationen innerhalb dieser Daten zeitübergreifend (Prä-KVT versus Post-KVT) analysiert.

In Bezug auf das Ansprechen auf die KVT zeigten sich kleine Unterschiede: Trägerinnen des AA/AG-Haplotyps des *CRHR1*-Risikoallels rs17689918 profitierten stärker hinsichtlich der Reduktion ihrer depressiven Begleitsymptomatik, während die Kontrollpatienten eine stärkere Reduktion ihrer agoraphobischen Gedanken zeigten. Die KVT stellt somit eine geeignete Therapieform für Trägerinnen des AA/AG-Haplotyps dar, unabhängig seines prädisponierenden Effekts. Die Risiko-Allel-Trägerinnen zeigen nach der Therapie zudem ein abweichendes neuronales Reaktionsmuster während der Furchtkonditionierung: Während die PD/A-Kontrollgruppe eine normalisierte

Aktivierung im linken Gyrus frontalis inferior (IFG), linken anterioren cingulären Kortex und Thalamus für CS+ > CS- aufweist, zeigen die Risiko-Allel-Trägerinnen diese Aktivitätsveränderungen nicht auf. In einer separaten post-hoc Analyse zeigt sich jedoch ein Hinweis für eine normalisierte Aktivierung im linken dorsomedialen präfrontalen Kortex (dmPFC) und linken Precuneus für CS+ > CS-, welche sich aber nicht statistisch signifikant von der Veränderung in der Kontrollgruppe unterscheidet.

Zusammenfassend lässt der AA/AG-Haplotyp des *CRHR1*-Risikoallels rs17689918 auf eine distinkte Pathogenese in PD/A und Therapieansprechen auf neuronaler Ebene bei weiblichen PD/A-Patientinnen schließen, die ein Ansprechen der KVT jedoch nicht beeinträchtigt im Vergleich zu Kontrollpatientinnen.

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9 APPENDIX

9.1 List of academic teachers

Marburg

Bartsch
Bauer, Christian
Bauer, Stefan
Bauer, Uta-Maria
Baum
Becker, Annette
Becker, Katja
Becker, Stephan
Bien
Braun
Cetin
Cramer
Czubayko
Daut
Decher
Del Rey
Dettmeyer
Dodel
Donner-Banzhoff
Fröbius
Geraedts
Gress
Görg
Hegele
Hertl
Hildebrandt
Hoyer
Jansen
Kann
Kinscherf
Kircher
Knake
König
Kruse
Kühnert
Lill
Lohoff
Luster
Mahnken
Maier
Moll
Moosdorf
Müller
Neubauer
Nimsky
Oertel
Oberwinkler
Oliver
Pagenstecher
Peterlein
Plant
Reese
Renz
Richter
Rost
Rosenow
Ruchholz
Sahmland
Schäfer
Schieffer
Schu
Schulze
Schüffel
Schütz
Seifart
Sekundo
Sevinc
Sohlbach
Steiniger
Straube
Teymoortash
Thieme
Timmesfeld
Wagner
Weihe
Wilhelm
Westermann
Worzfeld
Wrocklage
Wulf
Zavorotnyy
Zemlin

Frankfurt

Weidauer
Schwarzenböck
Morlang
Schulze

Nordhorn

Henkel

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