Temporal predictability of threat: Evaluation of differential involvement of amygdala and BNST, and relevance for therapy response prediction in spider phobia

Zeitliche Vorhersagbarkeit von Bedrohung: Evaluation der unterschiedlichen Aktivierung von Amygdala und BNST sowie die Relevanz für die Vorhersagbarkeit von Therapieerfolg bei der Spinnenphobie

Doctoral thesis for a doctoral degree

at the Graduate School of Life Sciences,

Julius-Maximilians-Universität Würzburg,

Section Neuroscience

Niklas Siminski

from

Hannover, Germany

Würzburg**,** 2021

Submitted on: 15.04.2021

Office stamp

Members of the Thesis Committee

Chairperson: Prof. Dr. Carmen Villmann

Primary Supervisor: Prof. Dr Martin Herrmann

Supervisor (Second): Prof. Dr Grit Hein

Supervisor (Third): Prof. Dr Matthias Gamer

Date of Public Defence: ……

Date of Receipt of Certificates: ……

Acknowledgement

Zunächst würde ich mich gerne bei Prof. Martin Herrmann für die gute fachliche und persönliche Zusammenarbeit bedanken, dass ich immer im Büro vorbeikommen konnte und mir immer geholfen wurde, wenn ich mal nicht weiterwusste. Dies hat mir die Tätigkeit als wissenschaftlicher Mitarbeiter von Anfang an sehr erleichtert. In diesem Zuge bedanke ich mich auch bei Prof. Grit Hein und Prof. Matthias Gamer für die konstruktive Zusammenarbeit im Komitee. Des Weiteren möchte ich mich beim gesamten Würzburger Spider-VR Team bedanken, mit denen ich gemeinsam mit sehr viel Spaß und Freude das Projekt gestalten durfte. Aber auch bei den anderen vielen Kollegen bedanke ich mich für die lustigen Gespräche in der Mittagspause und das Gefühl mit Freunden zusammen zu arbeiten.

Ein letzter Dank gebührt meiner Familie und meinen Freunden, die mir immer zur Seite stehen.

List of contents

List of figures

List of tables in the main text

List of tables in appendices

Abbreviations

ACC	Anterior cingulate cortex
ADS-K	The short version of the German depression scale
ANOVA	Analysis of variance
BAT	Behavior avoidance test
BNST	Bed nucleus of stria terminalis
BOLD	Blood oxygen-level-dependent
CM	Centromedial amygdala
dACC	Dorsal anterior cingulate cortex
fMRI	Functional magnetic resonance imaging
fMRT	Funktionelle Magnetresonanztomographie
FOV	Field of view
FU	Follow up
GAD	Generalized anxiety disorder
GLM	General linear model
HRF	Hemodynamic response function
ITI	Inter trial interval
NPS	Neuropeptide S
NPSR1	Gencode for Neuropeptide S receptor
OCD	Obsessive-compulsive disorder
PSWQ	Penn State Worry Questionnaire
PTSD	Post-traumatic stress disorder
ROI	Region of interest
SPQ	Spider phobia Questionnaire
STAI-S	State-Trait-Anxiety Inventory State-Anxiety Scale
STAI-T	State-Trait-Anxiety Inventory Trait-Anxiety Scale
TE	Echo time
TR	Repetition time
VRET	Virtual reality exposure treatment

Abstract

Predictability of threat is one of the key modulators of neural activity in fear and anxiety-related threat processes and there is a considerable number of studies focusing on the exact contribution of centromedial amygdala and Bed nucleus of stria terminalis (BNST) in animals as well as in humans. In this research field, some studies already investigated the differential involvement of both areas during temporally predictable and unpredictable threat processes in humans. However, these studies showed several limitations e.g. small sample size, no predictable threat conditions, no separation of anticipation and confrontation processes, which should be addressed in future studies. Furthermore, evidence for group-based inter-individual differences of amygdala and BNST activity during predictable and unpredictable threat processes have not been studied extensively.

Several studies suggest a relevant role of the amygdala and BNST activity in phobic processes in patients with specific phobia, but no study so far has investigated the exact contribution of centromedial amygdala (CM) and BNST during temporally predictable and unpredictable threat processes in specific phobia.

This thesis consisted of three studies and aimed to evaluate the exact contribution of CM and BNST during temporally predictable and unpredictable threat anticipation and confrontation with the use of an optimized functional magnetic resonance imaging (fMRI) paradigm, which aimed to solve methodological limitations of recent studies. Study 1 used a large sample of healthy participants who were grouped based on *NPSR1* genotype, and study 2 and study 3 used a sample of patients with spider phobia. In sum, the results of all three studies indicated, that BNST is more relevant for anticipation processes as compared to the CM. Contrary, during the confrontation phase the CM displays a greater relevance for threat confrontation processes.

In recent years, various studies have investigated the extent to which treatment success can be predicted in patients with anxiety disorders based on pre-treatment fMRI activity. Therefore, this was investigated for the first time in study 3 in patients with spider phobia during temporally predictable and unpredictable threat processes. Results indicated that independent of temporal predictability lower anterior cingulate cortex (ACC) activity during threat anticipation and engaged BNST during threat confrontation might be benefitting factors for successful therapy response in spider phobia.

Zusammenfassung

Die Vorhersagbarkeit der Bedrohung ist einer der wichtigsten Modulatoren der neuronalen Aktivität bei angst- und furchtbezogenen Bedrohungsprozessen, und es gibt eine beträchtliche Anzahl von Studien, die sich mit der unterschiedlichen Beteiligung der zentromedialen Amygdala (CM) und des Bed nucleus of stria terminalis (BNST) während dieser Prozesse sowohl bei Tieren als auch beim Menschen beschäftigen. In diesem Forschungsfeld untersuchten bereits einige Studien die exakte Beteiligung beider Areale während zeitlich vorhersehbarer und unvorhersehbarer Bedrohungsprozesse beim Menschen. Diese Studien wiesen jedoch einige Limitationen auf (z.B. geringe Stichprobengröße, keine vorhersagbaren Bedrohungsbedingungen, gemeinsame Analyse der Aktivierung über Antizipations- und Konfrontationsphasen hinweg), die in zukünftigen Studien verbessert werden sollten.

Zudem gibt es bisher wenige Studien, welche die unterschiedliche Beteiligung der Amygdala und des BNST während Bedrohungsprozessen auf Grundlage gruppenbasierter inter-individueller Unterschiede untersucht haben. Mehrere Studien deuten auf eine erhöhte Aktivierung sowohl der Amygdala als auch des BNST während phobischer Angstprozesse bei Patient*Innen mit einer spezifischen Phobie hin. Allerdings hat bisher jedoch noch keine Studie die Aktivierung der CM und des BNST während zeitlich vorhersagbarer und unvorhersagbarer phobischer Bedrohungsprozesse bei Patient*Innen direkt verglichen.

Unter Verwendung eines optimierten funktionelle Magnetresonanztomographie (fMRT)-Paradigmas, das darauf abzielte, methodische Probleme der bisherigen Studien zu lösen, wurde in dieser Arbeit die unterschiedliche Aktivierung von CM und BNST während zeitlich vorhersehbarer und unvorhersehbarer Bedrohungsantizipation und -konfrontation in einer großen Stichprobe gesunder Teilnehmer, die anhand des *NPSR1*-Genotyps gruppiert wurden (Studie 1), und Patient*Innen mit einer Spinnenphobie untersucht (Studie 2 und Studie 3). In Summe zeigten die Ergebnisse aller drei Studien, dass im Vergleich zur CM der BNST für Antizipationsprozesse relevanter ist. Hingegen zeigte die CM in der Konfrontationsphase mit einer Bedrohung eine größere Aktivierung als der BNST.

In den letzten Jahren haben verschiedene Studien untersucht, inwieweit der Behandlungserfolg bei Patient*Innen mit einer Angststörung anhand der fMRT-Aktivität vor der Behandlung vorhergesagt werden kann. Daher wurde in Studie 3 dies erstmals bei Patient*Innen mit einer Spinnenphobie während zeitlich vorhersehbarer und unvorhersehbarer Bedrohungsprozesse untersucht. Die Ergebnisse deuteten darauf hin, dass eine geringere Aktivität im anterioren cingulären Cortex (ACC) während phobischen Antizipationsprozessen

und eine höhere Aktivierung im BNST während der Konfrontation mit phobischen Stimuli günstige Faktoren für den Therapieerfolg bei Spinnenphobie sein könnten.

1 Introduction

The author H.P. Lovecraft wrote in his "Supernatural Horror in Literature" as early as 1927, republished in a new version of Lovecraft (2013) on page 1: "*The oldest and strongest emotion of mankind is fear, and the oldest and strongest kind of fear is fear of the unknown*". It demonstrates the long history of the interest in fear and anxiety processes and their manifestations in humans. These processes are not only present in literature, but also in a scientific environment. Fear and anxiety processes have become a major research field combining several different disciplines such as psychology, medicine, or biology. Over the years, research suggested two different defense systems, depending on the predictability of threat: fear and anxiety (LeDoux and Pine, 2016). The two terms are often used interchangeable (Fox and Shackman, 2019), but are considered to be conceptionally distinguishable (Davis et al., 2010; LeDoux and Pine, 2016). Fear, also named "phasic fear", is a short-lasting response to an imminent threat and elicits a fight-or-flight reaction. Contrary, anxiety, also named "sustained fear", is future-orientated, long-lasting, and the response to a less specific, less predictable, or more distal threat (Davis et al., 2010).

Among these several different disciplines, a considerable amount of studies investigated neuronal differences and similarities of human fear and anxiety-related threat processes (Chavanne and Robinson, 2020; LeDoux and Pine, 2016). The so-called extended amygdala including the central and medial nuclei of the amygdala as well the bed nucleus of stria terminalis (BNST) are considered as key regions for these processes (Alheid, 2003; Avery et al., 2016; Clauss, 2019; Davis et al., 2010; Fox and Shackman, 2019; Knight and Depue, 2019; Lebow and Chen, 2016; Shackman and Fox, 2016). However, the exact contribution of the amygdala and BNST in these processes is widely discussed and somewhat unclear yet (Fox and Shackman, 2019). According to the often-cited model of Davis et al. (2010), phasic threat processes are mediated by projections of the medial part of the central nucleus of the amygdala, whereas sustained threat processes result from projections of the lateral central amygdala to the lateral part of BNST. The BNST inhibits the signaling of the medial central nucleus of the amygdala and therefore allows a transition from phasic to sustained responses. Recent evidence in rodents supports the model of Davis et al. (2010), as it highlights the important role of BNST in temporally unpredictable threat processes (Goode and Maren, 2017; Goode et al., 2019). Another recent review emphasizes a functional separation of the amygdala and BNST (Knight and Depue, 2019). Accordingly, BNST is found to be relevant e.g. in the detection of a potential threat and maintaining hypervigilance processes, whereas the amygdala reacts preferentially to acute danger.

However, several pieces of evidence show controversial results to the statement, that BNST is limited to sustained processes. It has been shown on a neurochemical basis, that BNST is also involved in discrete threat processing (Gungor and Pare, 2016). In line with that, there is evidence for BNST and amygdala activity during the confrontation with a threat also in human fMRI (Brinkmann et al., 2018; Lebow and Chen, 2016; Mobbs et al., 2010). In a very recent systematic review of Chavanne and Robinson (2020) authors found a similar activity based on correlational analyses during predictable (in sense of fear conditioning) and unpredictable threat anticipation processes in several regions including the BNST area. Although this new review evaluated the fMRI activity in thousands of humans, the authors mentioned that activity in the very small amygdala and BNST regions is often not addressed adequately by whole-brain analyses. Furthermore, the authors of the review noted, that included studies used different fMRI paradigms. This raises the need for more amygdala and BNST specified research to clarify in which modalities of threat amygdala and BNST are activated.

Specific phobia is the most prevalent anxiety disorder (eg. Jacobi et al., 2014), and several fMRI studies indicated engaged amygdala and BNST activity during phobic anticipation and confrontation processes (Chavanne and Robinson, 2020; Del Casale et al., 2012; Etkin and Wager, 2007; Hoppe et al., 2021; Ipser et al., 2013; Linares et al., 2012; Munsterkotter et al., 2015; Peñate et al., 2017; Straube et al., 2007). However, no study so far directly compared the exact contribution of BNST and amygdala in temporally predictable and unpredictable threat processes in a sample with specific phobia.

Although exposure therapy is effective in specific phobia (Bandelow et al., 2015), only 50 percent respond positively to the treatment (Loerinc et al., 2015), raising the question of interindividual differences in the ability for getting successful treatment. In the last decades, a considerable number of studies investigated the potential of fMRI activity for the prediction of therapy response before the treatment (eg. Lueken et al., 2016; Santos et al., 2019). However, no study investigated the prediction of therapy outcome of exposure therapy in specific phobia based on fMRI activity temporally predictable and unpredictable threat processes.

This dissertation contains three empirical studies leading to the aim to contribute to the understanding of modalities of differential involvement of amygdala and BNST during temporally predictable and unpredictable threat processes in a healthy sample (study 1) as well as in specific phobia (study 2 and study 3). Furthermore, in study 3, pretreatment fMRI activity during temporally predictable and unpredictable threat processes will be evaluated concerning the prediction of therapy response to exposure therapy in spider phobia. For these purposes, in

chapter 2.1 an overview of amygdala and BNST activity of recent studies in healthy samples as well patients with anxiety disorders will be provided. Afterward, in chapter 2..2 clinical aspects of specific phobia and the exposure therapy in specific phobia will be clarified. After chapter 2, implications for the three studies will be offered in chapter 3 and then the empirical studies follow in chapter 4, which are generally discussed in chapter 5. The thesis ends with a short summarizing conclusion in chapter 6.

2 Theoretical background

2.1 Differential involvement of amygdala and BNST in human predictable and/or unpredictable threat processes

According to Davis et al. (2010), the fear response arises on cues, which are connected to predictable events or to the threat itself. Contrary, the anxiety response might be elicited with cues or contexts, which are connected to unpredictable, diffuse, or distal events. This chapter will give an overview of recent evidence of the activity of the amygdala and BNST during predictable and unpredictable threat processes in healthy samples (2.1.1) as well as in clinical samples (2.2.2).

2.1.1 Involvement of amygdala and BNST activity during predictable and/or unpredictable threat processes in healthy samples

A considerable number of studies evaluated amygdala and/or BNST activity in healthy samples during predictable and/or unpredictable threat processes. However, these studies differed in kind of paradigm or methodology. An overview of some of the methods is provided in Siminski et al. (2021). The main difference between the analyses is whether the activity in BNST and amygdala are directly compared or not. The first allows assuming differential involvement (Shackman and Fox, 2016). Therefore, in the following the results of studies that did not directly compare the activity of amygdala and BNST will be presented (2.1.1.1) and afterward, the evidence of studies with direct comparisons of activity in the amygdala and BNST will be presented (2.1.1.2).

2.1.1.1 Evidence from studies that did not compare the activity of the amygdala and BNST directly in healthy samples

Several studies investigated amygdala and BNST activity in human fMRI with predictable or unpredictable threat processes but used different paradigms. Three of these studies used a contextual paradigm in virtual reality (Alvarez et al., 2011; Alvarez et al., 2015; Andreatta et al., 2015). During an unpredictable context, where threatening stimuli might occur, there is evidence for higher activity in the amygdala as well in BNST (Alvarez et al., 2011; Alvarez et al., 2015; Andreatta et al., 2015). However, only Alvarez et al. (2011) used also a predictable context, where an aversive event followed after a three seconds tone and found elevated activity of the amygdala during the tone in the predictable condition. The three studies indicate that

contextual paradigms can initiate different activities in the amygdala and BNST regarding predictability. However, the studies do not evaluate longer temporally predictable anticipation periods (Alvarez et al., 2011) or do not consider a predictable threat condition at all (Alvarez et al., 2015; Andreatta et al., 2015). Furthermore, analyses of contexts include anticipation and confrontation processes, which limits conclusion for each phase alone. Therefore, a considerable number of studies used event-related paradigms with cued anticipation periods to explicitly analyze amygdala and BNST activation patterns in anticipation and/or confrontation. One study in healthy subjects (n = 38) investigated phasic and sustained activation patterns of the amygdala and BNST during the anticipation period of temporally unpredictable threats (Herrmann et al., 2016). The phasic activity was defined as the activation during the onset (first second) of the anticipation period, whereas sustained activity displayed the activity over the whole anticipation period. The study found phasic amygdala and sustained BNST activity during anticipation of aversive sounds relative to the anticipation of neutral sounds (Herrmann et al., 2016). However, it did not evaluate activity in the confrontation phase. This was conducted by two other studies, which used confrontation phases with temporally unpredictable occurrences (Brinkmann et al., 2018; Sarinopoulos et al., 2010). In a study by Sarinopoulos et al. (2010) 40 healthy participants had to anticipate six to ten seconds either a predictable aversive confrontation, a predictable neutral confrontation, or an unpredictable confrontation. The kind of condition was cued at the beginning of each trial. Results showed higher amygdala activity to the aversive confrontation after the unpredictable cue in comparison to the predictable threat cue. However, the study did not evaluate BNST activity. This was conducted by Brinkmann et al. (2018) in which 93 healthy subjects were confronted with short-lasting aversive or neutral pictures (800ms), which were presented with a temporally unpredictable onset (duration of anticipation ranged from 1280 ms to 18960 ms). The valence of confrontation was not cued, hence evaluation of activity during anticipation was not possible. Participants showed higher activity of amygdala and BNST during the aversive confrontation relative to the neutral confrontation. This shows that there is evidence for BNST activity also during confrontation phases.

As these studies did not use temporally predictable conditions, the question stays unanswered whether BNST activity is only engaged during temporally unpredictable threat processes or is also engaged during temporally predictable threat processes. Therefore, some studies analyze the activity of the amygdala and/or BNST during temporally predictable and unpredictable threat processes. During an exploratory whole-brain analysis Shankman et al. (2014) found higher amygdala activity during a temporally predictable threat block relative to

the temporally unpredictable threat block but did not conduct explicit region of interest (ROI) analyses of amygdala and BNST. The effect was not confirmed in the study of Somerville et al. (2013), which found engaged amygdala activity on the onset of aversive confrontation independent of predictability. Additionally, BNST showed engaged activity during aversive relative to neutral blocks as well during unpredictable relative to predictable blocks.

In sum, the results of this section indicate that BNST and amygdala are both activated in a state of unpredictability, but the amygdala also showed engaged activity during predictable anticipation processes. Therefore the results in this subchapter are in line with the model provided by Davis et al. (2010). However, following the recommendations of Shackman and Fox (2016) it is necessary to compare the activity of the amygdala and BNST directly to prove differential activity during predictable and unpredictable threat processes. Therefore, in the next section results of studies will be presented which include a factor region in the analysis.

2.1.1.2 Evidence from studies that compared activity amygdala and BNST directly in healthy samples

There is a subset of studies that compared the activity of the amygdala and BNST directly in different threat processes. The studies use mixed methods, which are summarized in table 1 and an overview has been published already in the work of Siminski et al. (2021). During anticipation of threat, two studies highlighted the relevance of BNST in comparison to the amygdala during temporally unpredictable threat anticipation (Klumpers et al., 2017; Naaz et al., 2019). Another study showed that higher engagement of BNST during threat anticipation does not depend on threat predictability (Pedersen et al., 2019). However, there is also evidence for similar activity of amygdala and BNST during threat anticipation processes (Clauss et al., 2019; Hur et al., 2020). During the confrontation phases, the results of studies are also not concurring. There is evidence of higher amygdala activity relative to the BNST during temporally unpredictable threat confrontations (Klumpers et al., 2017). However, another study in healthy participants found higher involvement of BNST during an ambiguous threat confrontation relative to an explicit threat confrontation, whereas the amygdala did not show differential activity during ambiguous threat confrontation and explicit threat confrontation (Naaz et al., 2019). One study identified inter-individual differences regarding social anxiety in a mixed sample of healthy participants and patients with diverse anxiety disorders (Clauss et al., 2019). In individuals with high social anxiety, BNST was more involved compared to the amygdala during an ambiguous confrontation with fear faces relative to the ambiguous

confrontation with neutral faces. In individuals with low social anxiety, the amygdala was more involved compared to the BNST during the ambiguous confrontation with fear faces relative to the ambiguous confrontation with neutral faces. Therefore, depending on the degree of anxiety-related symptoms, BNST and amygdala might show different involvement. This raises the need for more research of interindividual differences in differential involvement of BNST and amygdala during temporally predictable and unpredictable threat anticipation and confrontation.

To summarize, studies that directly compare activity showed mixed results in anticipation phases as well as during confrontation phases. However, the studies did show several limitations which should be addressed by future studies (see table 1), including small sample sizes, the non-evaluation of confrontation phases, or limitations that ground on nature of the used fMRI-paradigms in the studies, e.g. no temporally predictable conditions, different durations of anticipation periods during the predictable and unpredictable anticipation, or no use of intertrial stimulus interval.

Table 1. Overview of studies that analyzed amygdala and BNST activity with direct activity comparisons

sample size	a summary of fMRI paradigm	limitations
Klumpers et al. (2017), Study 1		
108	- cued anticipation of shocks: two cues (4 sec): threat (shocks in one-third of trials), safe (no shock) - inter-trial interval = 11-13 sec - each cue was presented 18 times	no temporally predictable anticipation of threat
Klumpers et al. (2017), Study 2		
70	- cued anticipation of shocks: two cues (6-12 sec): threatening cue (temporally unpredictable administration of shocks) and safe cue (no shock) - inter-trial interval = 8-12 sec - each cue was presented 42 times	no temporally predictable anticipation of threat
Clauss et al. (2019)		
44*	- three cues: one cue indicated a temporally unpredictable aversive event onset (10 trials), another cue a temporally unpredictable neutral event onset (10 trials), and the third a temporally unpredictable unknown event onset (20 trials; 10 aversive and 10 neutral trials)	no temporally predictable anticipation of threat

Table 1. (continued)

Naaz et al. (2019)		
20	- explicit condition (4 sec, 24 trials): the explicit cue (500 msec) was directly followed by the predictable confrontation	short anticipation periods
	- ambiguous condition (8 sec, 48 trials): the ambiguous cue (500 msec) was followed by a black screen (jittering from 500 msec to 5000 msec) and the unpredictable confrontation (2000 msec)	different durations of anticipation in the explicit and ambiguous condition
		small sample size
Pedersen et al. (2019)		
28	- hybrid block design with four blocks (temporally predictable threat, temporally predictable safety, temporally unpredictable threat, temporally unpredictable safety)	no inter-stimulus interval between prior confrontation and next anticipation period
	- eight blocks (two repetitions of each type):	
	- total: 26 images per condition	
Hur et al., 2020)		
99	- event-related fMRI paradigm with a temporally predictable confrontation (anticipation period always lasting 18.75 seconds) and temporally unpredictable confrontation (anticipation period ranging from 8.75 seconds to 30 seconds; mean = 18.75 seconds)	No evaluation of confrontation phases
		No evaluation of main effect predictability

Note. * this sample consists of 35 healthy participants and 9 participants diagnosed with an anxiety disorder; sec = seconds; msec = milliseconds. This table is a modified version of table A 1 published by Siminski et al. (2021)[1].

[1] As an author of this article, I can reuse the article in full or in parts in a thesis, providing it is not used for commercial purpose. This right includes the right to reuse tables. According to the permission guidelines of Elsevier, no written permission is necessary (https://www.elsevier.com/about/policies/copyright/permissions). The guidelines requests to provide the DOI-link leading to the original article. Therefore, the original work can be found here: https://doi.org/10.1016/j.bbr.2020.112883.

2.1.2 Involvement of amygdala and BNST activity during predictable and/or unpredictable threat processes in clinical samples

A considerable amount of studies is investigating activity patterns of the amygdala and BNST during temporally unpredictable anticipation processes in samples with an anxiety disorder relative to a healthy control group. In patients with a generalized anxiety disorder (Buff et al., 2017), or panic disorder (Brinkmann et al., 2017a), the amygdala was more activated in the clinical samples relative to the control groups during the onset of the anticipation period (phasic activation). On the other side, the BNST was more activated during the whole period of anticipation (sustained activation) in patients relative to the healthy control group. These two studies used a threat paradigm with aversive and neutral sounds and therefore no disorder-specific confrontations. Other studies investigated amygdala and BNST activity also in temporally unpredictable anticipation processes of disorder-specific confrontation and found mixed results. There is evidence for higher phasic amygdala activity on anticipation onset but not higher sustained BNST activity over the whole duration of anticipation of phobic confrontation in a sample with blood-injection-injury phobia relative to a control group (Brinkmann et al., 2017c), or higher phasic BNST and amygdala activity during anticipation onset in patients with a social anxiety disorder during anticipation of a social threat task (Figel et al., 2019). In another study that only analyzed activity during the whole duration of the anticipation period, BNST activity was increased during spider picture anticipation in patients with spider phobia relative to the control group, but this study did not find elevated amygdala activity (Straube et al., 2007). All these studies did not use a predictable threat condition. This was considered in a study by Munsterkotter et al. (2015), which analyzed amygdala and BNST activity during predictable and unpredictable threat processes in a sample with spider phobia patients and a healthy control group. The study used a symptom provocation paradigm with an unpredictable condition, where spider pictures might occur, a predictable condition, where spiders will certainly occur, and a no-fear control condition with no spiders being presented. Results indicated higher amygdala activity during the predictable condition in patients with spider phobia relative to a control group and higher BNST activity during the unpredictable condition in patients relative to the control group. However, given the nature of the paradigm, Munsterkotter et al. (2015) did not evaluate activity during anticipation periods and confrontation periods separately. Furthermore, the study did not directly compare the activity of the amygdala and BNST. Therefore, no study yet directly compared the activity of the amygdala and BNST during temporally predictable and unpredictable threat anticipation and confrontation processes in a sample with an anxiety disorder relative to a control group.

2.2 Specific phobia

In Germany, anxiety disorders have a 12-month prevalence of 15.4 percent (Jacobi et al., 2014). Among the anxiety disorders, specific phobia is the most prevalent anxiety disorder with a 12-month prevalence of 10.3 percent (Jacobi et al., 2014). There are five categories of specific phobic objects and situations: animals, natural environment, blood-injection-injury, situational, and others (American Psychiatric Association, 2013), with the subtype "animals" as the most prevalent specific phobia (Becker et al., 2007; Wardenaar et al., 2017). According to the diagnostics of specific phobia, patients must express marked and persistent excessive or unreasonable fear of specific objects or situations at least for the last six months. Exposure to this phobic object or situation leads to an immediate intense anxiety response and distress. Therefore, patients avoid these phobic objects or situations, even knowing, that this fear is unreasonable (American Psychiatric Association, 2013).

In a study by Trumpf et al. (2010), women with specific phobia showed a higher risk to develop other mental illnesses including other anxiety disorders, major depression, or any somatoform disorder. This is supported by a recent report of Wardenaar et al. (2017), which indicates, that more than 60 percent of the specific phobia cases reported at least one other lifetime disorder, i.e. mood disorder (34.3%), anxiety disorder (41.2%), substance use disorder (15.9%), and impulse control disorder (17.4%). Specific phobia preceded the other disorders in most of the cases (71.6% - 92.2%). Furthermore, in an older Spanish population, having a specific phobia was associated with less quality of life (Ausín et al., 2020). These several pieces of evidence emphasize the clinical impact of the specific phobia.

2.2.1 Exposure therapy and the prediction of therapy success in specific phobia

Exposure to the phobic material has been shown as the comparably best and effective option for the therapy in specific phobias (Bandelow et al., 2015; Bandelow et al., 2014). The general procedure during exposure therapy is the confrontation in sensu or in vivo with the phobic stimuli until the distress decreased (Wechsler et al., 2019). In specific phobia, an intensive one-time session is already effective (Davis et al., 2012). Exposure might be also conducted in virtual reality with comparable efficiency (Botella et al., 2017; Suso-Ribera et al., 2019; Wechsler et al., 2019). Furthermore, virtual reality exposure was described as more tolerable than in vivo exposure (Thng et al., 2020).

However, only around 50 percent of patients with specific phobia respond positively to cognitive behavior therapy (Loerinc et al., 2015). Therefore, in a recent systematic review by Böhnlein et al. (2020) factors were investigated which predicted exposure success in specific phobias. Before therapy, high motivation, high self-efficacy, and low trait anxiety were related to better therapy success. Other positive factors during exposure therapy on a physiological level were high cortisol levels and heart rate variation, and sleep. On the methodological level the evoking disgust additionally to anxiety, avoiding relaxation, focusing on cognitive changes, context variation, and the use of memory-enhancing drugs were positively associated with therapy success. Böhnlein et al. (2020) suggested that these factors are in line with the fear inhibition model described in the work of Craske et al. (2014). According to these authors, exposure therapy should violate expectations, lead to recognition of non-occurrence of the feared event which enables new learning processes. Furthermore, to enhance the fear inhibition, the number of safety behaviors and signals should be decreased, and variable stimuli and contexts should be used.

A few systematic reviews investigated already the potential of pre-treatment fMRI activity for the prediction of therapy response in anxiety disorders (Chakrabarty et al., 2016; Lueken et al., 2016; Santos et al., 2019). However, conclusive statements for specific phobias based on these reviews are limited, as not so many studies investigating the prediction of therapy response in specific phobia were included in the systematic reviews. Recently, two studies investigated the prediction of therapy response or therapy success-related behavior concerning fMRI activity in specific phobia. In one study, decreased amygdala activity to repeating spiders predicted avoidance behavior in sense of larger approach behavior during an incentive conflict task (Björkstrand et al., 2020). Furthermore, the activity of the ventromedial prefrontal cortex during early extinction processes predicted a better outcome of a group-based single exposure session in specific phobia during the post-measurement phase (Lange et al., 2020). These two studies indicate the relevance of pretreatment fMRI activity for the prediction of therapy success in specific phobia. No study so far evaluated the predictive potential of pre-treatment fMRI activity during predictable and unpredictable threat processes in specific phobia.

3 Summary of theoretical background and implications for this dissertation

There is a considerable amount of studies focusing on the exact contribution of amygdala and BNST during threat processes in animals as well as in humans. In this research field, some studies already investigated the involvement of both areas during temporally predictable and unpredictable threat processes in humans. However, these studies showed several limitations, which will be addressed in this dissertation. Evidence for group-based inter-individual differences of amygdala and BNST activity during predictable and unpredictable threat processes has not been studied in detail. Therefore, in study 1 an optimized fMRI paradigm was used to evaluate the differential involvement of CM and BNST in a large healthy sample (n = 109). Furthermore, study 1 aimed to investigate individual differences based on the *NPSR1* genotype, as no study investigated the modulative role of genetics in the differential involvement of CM and BNST.

Specific phobia is one of the most prevalent anxiety disorders (Jacobi et al., 2014). No study so far investigated the differential involvement of CM and BNST in specific phobia relative to a control group in temporally predictable and unpredictable threat processes. Therefore, in study 2 the fMRI paradigm was adapted from study 1 to spider phobia relevant material and evaluated the differential involvement of CM and BNST during temporally predictable and unpredictable threat processes in a sample of patients with spider phobia (n = 21) compared to an age – and gender-matched healthy control group (n = 21).

No study so far investigated pre-treatment activity during temporally predictable and unpredictable threat processes for therapy response prediction in specific phobia. Therefore, the aim of study 3 was to investigate the predictive potential for exposure therapy response based on pre-treatment fMRI activity during temporally predictable and unpredictable threat processes in 67 patients. Furthermore, study 3 aimed to validate the analysis approach of study 1 and study 2 and evaluated the differential involvement of CM and BNST in a larger sample of patients with spider phobia.

4 Empirical studies

4.1 Study 1: BNST and amygdala activation to threat: effects of temporal predictability and threat mode and *NPSR1*

This chapter bases on a modified version of the publication of Siminski et al. (2021)[2]. My contributions were also published in the publication of Siminski et al. (2021): Conceptualization, Formal analysis, Writing – original draft, Visualization, Validation.

4.1.1 Introduction in study 1

The differential involvement of amygdala and BNST during threat processes are not completely understood and more research is necessary to investigate under which circumstances amygdala and/ or BNST are activated during threat processes. Results of a recently published study in rodents suggested that BNST is activated when an anticipated shock is temporally distant (Goode et al., 2019). This is in line with other studies in humans, where temporal uncertainty was associated with long-lasting startle responses (Bennett et al., 2018; Hefner et al., 2013). In chapter 2.1 of this dissertation, a summary was given of all studies investigating amygdala and BNST activity during temporally predictable and unpredictable threat processes. However, these studies showed several limitations. Furthermore, no study so far investigated the differential involvement of the amygdala and BNST in an event-related anticipation paradigm with longer anticipation periods and temporally predictable and unpredictable confrontation.

Genetic differences account for a substantial part of the inter-individual variation of fear and anxiety, and the gene encoding of the neuropeptide S receptor (*NPSR1*) in this context is a relevant candidate (Pape et al., 2010; Schiele et al., 2020). In pharmacological rodent studies administration of neuropeptide S (NPS) is associated with anxiolytic effects in behavior tests as well with higher arousal (Ionescu et al., 2012; Leonard et al., 2008; Lukas and Neumann,

[2] Parts of introduction, methods, results, and discussion in this chapter are concurring in a modified version to the cited article. As an author of this article, I can reuse the article in full or in parts in a thesis, providing it is not used for commercial purpose. This right includes the right to reuse all figures and tables. According to the permission guidelines of Elsevier, no written permission is necessary (https://www.elsevier.com/about/policies/copyright/permissions). The guidelines requests to provide the DOI-link leading to the original article. Therefore, the original work can be found here: https://doi.org/10.1016/j.bbr.2020.112883

2012; Rizzi et al., 2008; Tillmann et al., 2019; Vitale et al., 2008; Xu et al., 2004; Zoicas et al., 2016). NPS consists of 20 amino acids and shows sequence homology in humans, mice, and rats (Pape et al., 2010). NPS Precursor mRNA is mainly expressed in the brain stem (Xu et al., 2007). NPS binds to an excitatory G_q and G_s-protein-coupled receptor (NPSR1) (Pape et al., 2010). *NPSR1* mRNA is widely expressed in the central nervous system with the highest expressions in the cortex, thalamus, hypothalamus, and amygdala as well as with moderate expressions in the BNST (Leonard and Ring, 2011; Reinscheid et al., 2005; Xu et al., 2007). Furthermore, the distribution of *NPSR1* mRNA is homologous across mice and rats (Clark et al, 2011). The human gene coding for the neuropeptide S receptor is located on chromosome 7p14. It contains an A/T non-synonymous single nucleotide polymorphism (rs324981) with the T-allele (107Ile) increasing *NPSR1* expression and NPS efficacy at the receptor about 10-fold (Anedda et al., 2011; Reinscheid et al., 2005). The frequency in Europe for the T-allele is 46% and for the A-allele 54% (Auton et al., 2015). This T-allele has been associated with heightened heart rate and higher symptom intensity during a behavioral avoidance test in patients with panic disorder (Domschke et al., 2011). Furthermore, the T-allele has been linked to increased fear ratings in pavlovian conditioning (Raczka et al., 2010) and increased cortisol responses to acute psychosocial stress as well as higher subjective stress levels during stress anticipation (Kumsta et al., 2013). Imaging genetic studies showed reduced prefrontal activity (Guhn et al., 2015; Tupak et al., 2012), as well higher amygdala activity in T-allele carriers during threat confrontation (Dannlowski et al., 2011; Gechter et al., 2019; Streit et al., 2014). Only one study investigated the potential modulation effects of *NPSR1* rs324981 on amygdala activity during threat anticipation (Gechter et al., 2019). They used an fMRI paradigm with a short-lasting anticipation cue of 250 ms and could not reveal differences in amygdala activity between T-allele carriers and homozygote AA-carriers during threat anticipation (but during threat confrontation). As abnormal neural correlates of threat anticipation have been recently linked with several anxiety disorders (Brinkmann et al., 2017a; Brinkmann et al., 2017b; Buff et al., 2017), more research should be conducted to analyze the neural basis of threat anticipation and *NPSR1* genotype.

The present study aims to clarify the differential involvement of the CM and BNST during anticipation of temporally predictable and unpredictable threats and threat confrontation. We used the CM as ROI for the amygdala because this ROI includes the central nucleus and medial nucleus of the amygdala, which are both parts of the extended amygdala (Alheid, 2003). Furthermore, Pedersen et al. (2019) also used the CM for their analyses. Therefore, this study is more comparable to the analyses of Pedersen et al. (2019). However, it must be noted, that

the central nucleus and medial nucleus of the amygdala are also involved in distinct functional processes (Swanson and Petrovich, 1998). Animal data suggest an involvement of CM in predictable and unpredictable processes and BNST involvement only in unpredictable processes (Davis et al., 2010). However, based upon recent fMRI data in humans, this study expects during a cue, indicating the valence of the upcoming confrontation, higher activity in CM and BNST during aversive cue relative to the neutral cue (Herrmann et al., 2016; Naaz et al., 2019; Pedersen et al., 2019). Based on the results of Klumpers et al. (2017), this study expects higher BNST activity during unpredictable threat anticipation relative to the CM. Further, this study expects CM activity is higher activated in predictable as compared to unpredictable threat anticipation (Naaz et al., 2019; Shankman et al., 2014), but BNST shows higher activity during unpredictable threat anticipation relative to predictable threat anticipation (Naaz et al., 2019). During threat confrontation, both CM and BNST are expected to show enhanced activity to the unpredictable compared to the predictable threat confrontation (Naaz et al., 2019; Sarinopoulos et al., 2010), but CM is expected to show higher activity during unpredictable confrontation relative to the BNST (Klumpers et al., 2017). No study so far has investigated the modulatory potential of *NPSR1* rs324981 genotype on the neural correlates of predictable vs. unpredictable threat anticipation and confrontation. However, based on reported associations between amygdala activity and *NPSR1* variation (Dannlowski et al., 2011; Gechter et al., 2019; Streit et al., 2014), the present study additionally evaluates the effects of *NPSR1* rs324981 genotype on CM activity during these predictable and unpredictable threat processes. Furthermore, no study has so far evaluated the impact of *NPSR1* gene variation on BNST activity in humans. As it has been shown that *NPSR1* is expressed in rodent BNST (Leonard and Ring, 2011; Xu et al., 2007) and given the relevance of *NPSR1* rs324981 and BNST in the etiology of anxiety (Goode and Maren, 2017; Pape et al., 2010), possible modulation effects will also be investigated. Building upon recent results of Gechter et al. (2019), no modulation is expected in the CM for the cue phase and threat anticipation. Based on the association between the T-allele and panic disorder and higher sustained BNST activity during unpredictable threat anticipation, this study expects to observe higher BNST activity in *NPSR1* rs324981 T-allele carriers relative to AA-carriers during unpredictable threat anticipation as well as higher subjective ratings of unpredictable threat anticipation cues (Brinkmann et al., 2017a; Domschke et al., 2011). During the confrontation phase, *NPSR1* rs324981 T-allele carriers might show elevated CM and BNST activity during unpredictable threat confrontation (Brinkmann et al., 2018; Dannlowski et al., 2011; Gechter et al., 2019; Naaz et al., 2019; Sarinopoulos et al., 2010; Streit et al., 2014).

4.1.2 Methods of study 1

4.1.2.1 Subjects

This study comprised one hundred and twenty-three healthy individuals of Caucasian descent. Fourteen participants had to be excluded from analyses because of head movements (moved > 3 mm/°) or technical problems during fMRI acquisition. Accordingly, the final sample consisted of 109 healthy individuals (38 males, 71 females, mean age = 27.19 ± 6.28 years). A sensitivity analysis conducted with G*Power by Faul et al. (2007) showed, that this sample size can detect an effect size of $f = .27$ with a power of 80 percent and an alpha level of 0.05. All participants were right-handed, had no history of mental disorders, were medication-free, and had normal or corrected-to-normal vision and hearing. Participants were genotyped for *NPSR1* rs324981 (see 4.1.2.2). Genotype groups did not differ in sex ratio ($\chi^2(1) = 0.727$, $p = .394$), age, and self-reported anxiety and depression (Table 2), assessed with the State-Trait Anxiety Inventory (Spielberger, 2010), the short version of the German depression scale (Hautzinger et al., 2012) and the Penn State Worry Questionnaire (Meyer et al., 1990). Participants were recruited from a pool of individuals who showed a general interest in participating in scientific studies or who participated already in previous non-BNST-fMRI focussing studies of the university hospital of Würzburg. Before including in the study, participants received a detailed description of the study aims and procedures and gave written informed consent in line with the Declaration of Helsinki (World Medical Association, 2013). The ethics committee of the medical faculty of the University of Würzburg approved the study (ethics vote number: 82/12).

Table 2: Sample characteristics of study 1

	NPSR1 rs324981					
	AT,TT (*n* =64)		AA (*n* =45)			
variable	***M***	***SD***	***M***	***SD***	***t***	***p***
age	27.77	7.37	26.33	4.18	-1.18	.242
STAI -S	35.25	5.77	33.64	6.41	-1.37	.175
STAI- T	34.92	8.06	34.43	7.09	-0.33	.745
ADS-K	7.11[†]	4.78	6.60	4.87	-0.54	.589
PSWQ	40.70	9.15	40.73	10.45	0.02	.987

Note: AT,TT , T-allele carriers of the *NPSR1* rs324981 polymorphism; AA, AA genotype carriers of the *NPSR1* rs324981 polymorphism; STAI-S and STAI-T, State-Trait Anxiety Inventory (Spielberger, 2010); ADS-K, the short version of the German depression scale (Hautzinger et al., 2012); PSWQ, Penn State Worry Questionnaire" (Meyer et al., 1990). [†]because of missing data *n* = 63

4.1.2.2 Genotyping

The sample was genotyped for the *NPSR1* rs324981 polymorphism according to previously published protocols (Guhn et al., 2015; Schiele et al., 2020; Tupak et al., 2012). In sum, there were 45 AA genotype carriers, 46 AT genotype carriers, and 18 TT genotype carriers. Genotypes were grouped as follows according to previous studies in fear- and threat-related phenotypes (Beste et al., 2013; Dannlowski et al., 2011; Domschke et al., 2011; Guhn et al., 2015; Ruland et al., 2015; Tupak et al., 2012): AA (*n* = 45 [41.30%]), AT,TT (*n*=64 [58.70%]).

4.1.2.3 Paradigm

All participants went through an instructed threat-anticipation paradigm during fMRI (Fig 1). Pictures from the International Affective Picture System (Lang and Bradley, 1999), twenty aversive and neutral each, coupled with matched sounds from the International Affective Digitized Sounds by Bradley and Lang (2007) and from freesound by Font et al. (2013) were presented as threatening confrontation and neutral confrontation. First, the valence of the stimuli presented after an anticipation phase was cued by the letters "A" and "B" (two seconds in duration). The letters were counterbalanced across participants. After that, a second cue was

presented which displayed an orange or blue schematic watch. Colors symbolized the predictability of stimuli' occurrence (confrontation) and were counterbalanced across participants, too. In the predictable condition, the onset of the pictures and sounds were at twelve o´clock position, in the unpredictable condition, the stimuli confrontation could appear at any moment. Anticipation intervals differed in duration: three seconds (one trial), ten seconds (one trial), 16 seconds (seven trials), and 20 seconds (one trial), resulting in a mean anticipation period of 14.5 seconds. Then, the pictures and sounds were presented simultaneously for four seconds. Between trials, a white fixation cross was displayed on grey background for 15 seconds (ITI). This long ITI mitigates a potential carry-over from the prior confrontation phase. In total, participants underwent 40 trials and so the experiment lasted approximately 23 minutes. After fMRI measurements, participants were asked to rate the anticipation phase on a nine-point Likert scale (Self-Assessment Manikin; Bradley and Lang (1994)) assessing the dimension's unpleasantness (1 = very pleasant to 9 = very unpleasant), anxiety (1 = not anxiety-inducing to 9 = highly anxiety-inducing), and arousal (1 = not arousing to 9 = highly arousing). Three-way-factor analyses of variance (ANOVA) with mixed measures for group (AT,TT / AA), valence (aversive / neutral), and predictability (unpredictable / predictable) were calculated. Analyses were conducted using SPSS software (Version 25.0. Armonk, NY: IBM Corp.) for each rating. An alpha level of $p < .05$ was considered statistically significant.

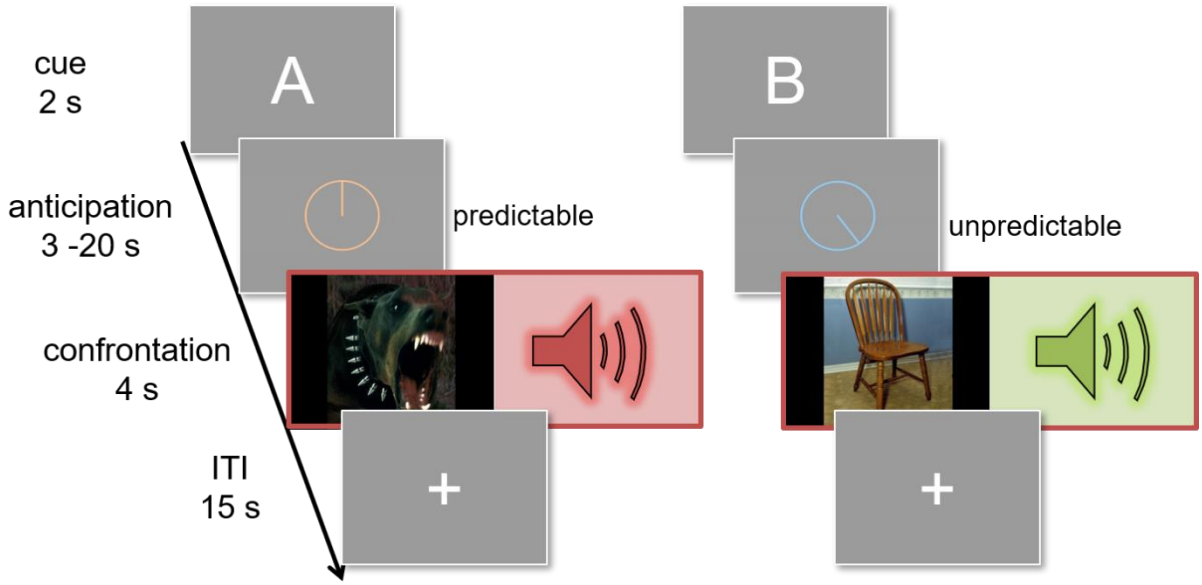

Fig 1. Experimental design of study 1.

The letter cued the valence (threat vs. neutral) of the confrontation stimuli. The color of the watch either signaled the exact moment of the impending confrontation stimulus (predictable) or did not (unpredictable).

4.1.2.4 MRI data

Structural and functional MRI data were acquired with a 3 Tesla magnetic resonance scanner ("Magnetom Skyra", Siemens, Medical Solutions, Erlangen, Germany). Structural brain images were acquired with a high-resolution T1-weighted scan with 176 slices (thickness= 1mm, echo time (TE) = 2.26 ms, repetition time (TR) = 1900 ms). Functional data was collected with a T2*-weighted echo-planar sequence (TE = 30 ms, flip angle = 90°, matrix = 64 x 64, field of view (FOV) = 230 mm², TR = 2480 ms) consisting of 580 volumes. Each volume consisted of 37 axial slices (thickness = 3.5 mm, gap = 0 mm, in-plane resolution = 3.6 x 3.6 mm, slice order = ascending).

Data were processed using Brain Voyager QX (BVQX) software (Version 3.6; Brain Innovation, Maastricht, The Netherlands). Analogous to the previous work of Herrmann et al. (2016), the first four volumes were discarded from each run to ensure that steady-state tissue magnetization was sufficient. Next, data were controlled for excessive head movement > 3 mm/° in any direction. Data preprocessing comprised correction for slice time errors and movement artifacts, co-registration from functional data to anatomical data, normalization to Talairach space (Talairach, 1988), sinc interpolation and spatial smoothing (5mm full-width at

half-maximum Gaussian kernel) as well as temporal filtering (high pass filter: 5 cycles per run; low pass filter: 2.8 s; linear trend removal).

The preprocessed data were analyzed using multiple linear regression of the signal time course at each voxel. The expected blood oxygen-level-dependent (BOLD) signal change for each condition was modeled with a canonical double-gamma hemodynamic response function (HRF). A general linear model (GLM) was calculated. The GLM consists of the cue phase, the anticipation phase, and the confrontation phase. Predictors of interest were valence (aversive vs. neutral) for all phases and predictability (predictable vs. unpredictable) for the anticipation and confrontation phase. A full-duration was used for each regressor (cue phase = 2s; anticipation period = 3-20s; confrontation phase = 4s). Additionally, the six movement parameters were modeled as predictors of no interest. Percent-standardized predictor estimates based on voxel-wise statistical maps for each participant were calculated. In the first-level analysis, a random-effects analysis with adjustment for autocorrelation following a global AR (2) model across the individual predictor estimates for planned contrasts was performed. Analyses were restricted to a-priori defined regions of interest (ROIs). The bilateral centromedial amygdala ROI (CM) based on maximum probability maps of Amunts et al. (2005) included in the anatomy toolbox (Eickhoff et al., 2005). The bilateral BNST ROI was based on probabilistic maps provided by Torrisi et al. (2015). ROIs were converted in Talairach coordinates via ICBM2tal (Lancaster et al., 2007). Figure 2 displays the ROIs on the averaged T1-scan of participants.

For statistical analyses, for each participant averaged beta values for each regressor within the ROIs were extracted. For the cue, a three-way-factor analysis of variance (ANOVA) with mixed measures for *NPSR1* genotype (AT,TT / AA), region (CM, BNST), and valence (aversive/neutral) as factors and averaged beta values as the dependent variable were calculated. For the anticipation phase and the confrontation four-way-factor analyses of variance (ANOVA) with mixed measures for genotype (AT,TT / AA), region (CM, BNST), valence (aversive/neutral), and predictability (unpredictable/ predictable) as factors and averaged beta values as the dependent variable were calculated. Calculations were conducted using SPSS software (Version 25.0. Armonk, NY: IBM Corp). An alpha level of $p < .05$ was considered statistically significant. For exploratory whole-brain analysis, this study performed a non-parametric cluster-based permutation based on an in-house MATLAB script (Release 2015b; The MathWorks, Inc., Natick, Massachusetts) to control over the familywise alpha level (Eklund et al., 2016). Similar to other recent published studies this study set up the voxel-level threshold for $p < .005$ and conducted 1000 permutations (Brinkmann et al., 2018; Buff et al.,

2017; Figel et al., 2019). This study performed this analysis for following contrasts: cue aversive > cue neutral (and vice versa); predictable anticipation > unpredictable anticipation (and vice versa), aversive anticipation > neutral anticipation (and vice versa); predictable > unpredictable confrontation (and vice versa); aversive > neutral confrontation (and vice versa). To evaluate the interaction effect of predictability and valence, this study also compared the valence differences of the predictable condition > unpredictable condition (and vice versa), each in the anticipation phase and the confrontation phase.

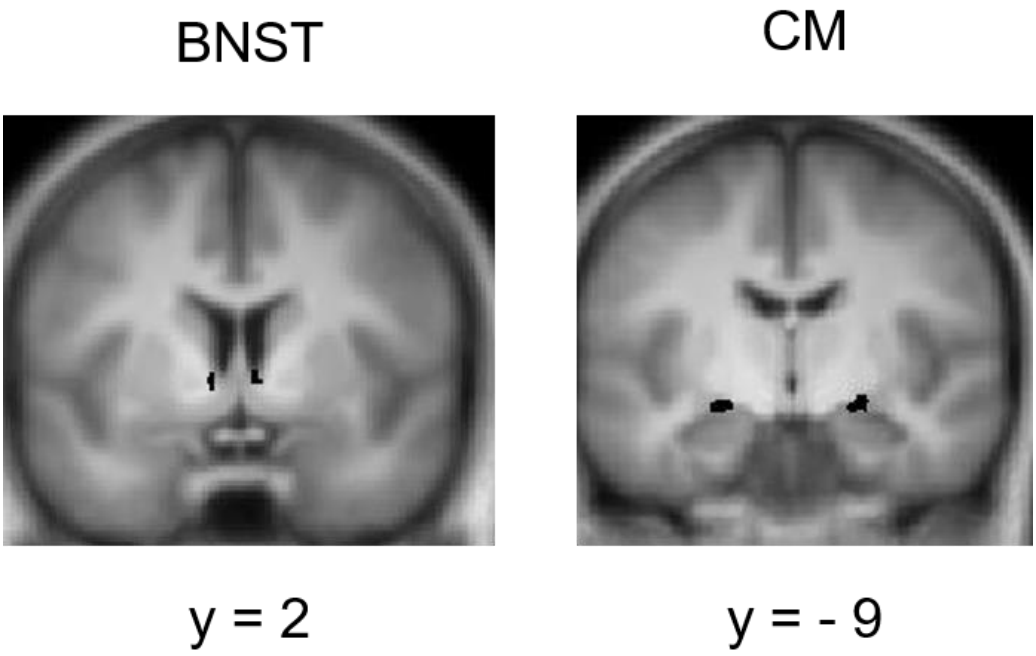

Fig 2. BNST and CM ROIs of study 1.

left: BNST ROI; right: CM ROI. ROIs are overlaid on the averaged T1 scan.

4.1.3 Study 1: results

4.1.3.1 Ratings of anticipation cues

Three-way-factor analyses of variance (ANOVA) with mixed measures for *NPSR1 genotype* (AT,TT / AA), valence (aversive/neutral anticipation) and predictability (unpredictable/predictable anticipation) were calculated for self-reported ratings of unpleasantness, arousal, and anxiety. For the ratings of unpleasantness and arousal there were significant main effects of valence (unpleasantness: $F(1,107) = 138.02$, $p < .001$, $\eta_p^2 = 0.563$;

arousal: $F(1,107) = 169.20$, $p < .001$, $\eta_p^2 = 0.613$) and predictability (unpleasantness: $F(1,107) = 33.35$, $p < .001$, $\eta_p^2 = 0.238$; arousal: $F(1,107) = 37.67$, $p < .001$, $\eta_p^2 = 0.260$). Participants rated unpredictable and aversive cues as more unpleasant and more arousing. Analyses for anxiety ratings indicate also significant main effects of predictability ($F(1,107) = 19.51$, $p < .001$, $\eta_p^2 = 0.155$) and valence ($F(1,107) = 111.90$, $p < .001$, $\eta_p^2 = 0.514$) as well as a significant interaction of valence and predictability ($F(1,107) = 13.88$, $p < .001$, $\eta_p^2 = 0.116$). Aversive cues were rated as more anxiety-inducing compared to neutral cues when they were unpredictable relative to when they were predictable ($t(107) = 3.80$, $p < .001$, $d = 0.734$). Regarding the modulation of *NPSR1*, analyses of unpleasantness ratings revealed a significant interaction of predictability and *NPSR1* genotype ($F(1,107) = 4.34$, $p = .040$, $\eta_p^2 = 0.039$). T-carriers rated unpredictable cues as more unpleasant relative to predictable cues compared to AA-carriers (T-carriers: $M = 0.83$, $SD = 1.17$; AA-carriers: $M = 0.39$, $SD = 0.95$; $t(107) = -2.084$, $p = .040$, $d = 0.413$). All other effects were not significant (Table A 1, appendices of study 1). Descriptive statistics of ratings are displayed in Figure 3.

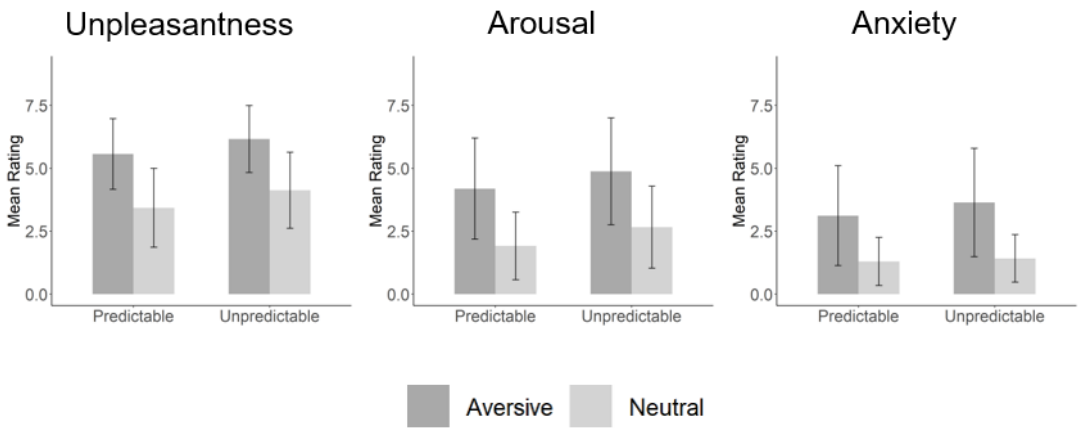

Fig 3. Means and standard deviations of self-reported ratings of anticipation cues of study 1.

4.1.3.2 ROI Analyses

4.1.3.2.1 Cue presentation

Three-way-factor analyses of variance (ANOVA) with mixed measures for *NPSR1* genotype (AT,TT / AA), region (CM / BNST) and valence (aversive/neutral cue) as factors and averaged

beta values of the ROI as dependent variable were calculated. Analyses revealed significant main effects of region ($F(1, 107) = 41.76$, $p < .001$, $\eta_p^2 = 0.281$) and valence ($F(1, 107) = 40.00$, $p < .001$, $\eta_p^2 = 0.273$). These effects were qualified by significant region*valence interaction ($F(1, 107) = 4.57$, $p = .035$, $\eta_p^2 = 0.041$). Posthoc analysis revealed higher BNST activity compared to CM during aversive cues relative to neutral cues ($t(108) = 2.10$, $p = 0.038$, $d_z = 0.20$; Fig4). All other comparisons were not significant (Table A 2, appendices of study 1).

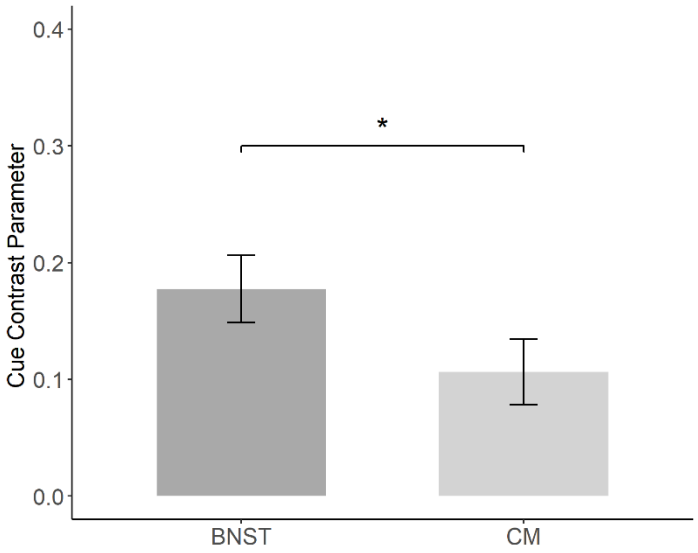

Fig 4. Significant region*valence interaction during cue presentation in study 1.

Bar graphs display the estimated contrast parameter of aversive compared to neutral cue stratified for *region* (BNST vs CM) (mean ± SE; * $p < .05$). BNST showed higher activity during aversive as compared to neutral cue ($t(108) = 2.10$, $p = 0.038$, $d_z = 0.20$).

4.1.3.2.2 Anticipation period

Four-way-factor analyses of variance (ANOVA) with mixed measures for *NPSR1* genotype (AT,TT / AA), region (CM / BNST), valence (aversive/neutral anticipation) and predictability (unpredictable/ predictable anticipation) as factors and averaged beta values of the ROI as the dependent variable were calculated. Analyses revealed a significant main effect of region ($F(1, 107) = 97.90$, $p < .001$, $\eta_p^2 = 0.478$). BNST showed higher activity during the anticipation phase relative to the CM. This is qualified by a significant region*predictability**NPSR1* genotype interaction during the anticipation phase ($F(1,107) = 5.03$, $p = .027$, $\eta_p^2 = 0.045$). BNST was more activated relative to CM in *NPSR1* T-allele carriers compared to AA-carriers during

unpredictable anticipation as compared to predictable anticipation ($t(107) = 2.24$, $p = .027$, $d = 0.45$; Fig5). All other comparisons were not significant (Table A 3, appendices of study 1).

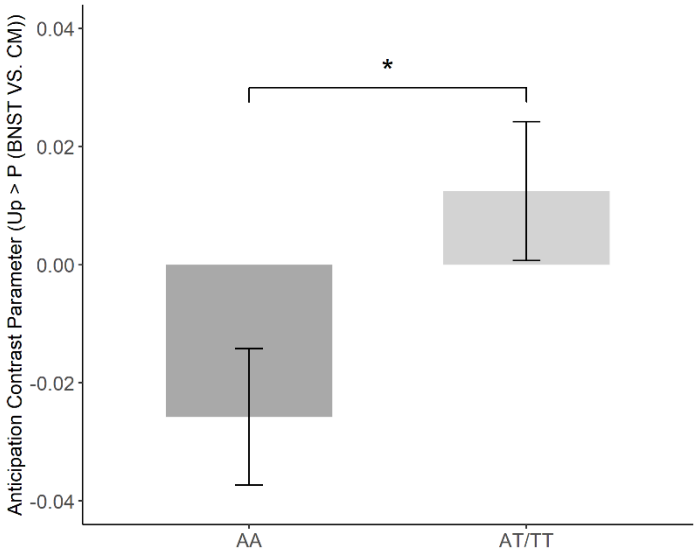

Fig 5. Significant interaction of region*predictability*NPSR1 genotype during anticipation period in study 1.

Up = unpredictable; P = predictable. Bar graphs display the estimated contrast parameter of BNST activity compared to CM activity during unpredictable anticipation relative to predictable anticipation stratified for *NPSR1* genotype (AA vs AT,TT) (mean ± SE; * $p < .05$). T-allele carriers showed higher BNST activity compared to CM activity during unpredictable anticipation relative to predictable anticipation ($t(107) = 2.24$, $p = .027$, $d = 0.45$).

4.1.3.2.3 Confrontation period

Four-way-factor analyses of variance (ANOVA) with mixed measures for *NPSR1* genotype (AT,TT / AA), region (CM / BNST), valence (aversive/neutral confrontation) and predictability (unpredictable/predictable confrontation) as factors and averaged beta values of the ROI as dependent variable were calculated. Analyses revealed a main effect of region ($F(1,107) = 40.22$, $p < .001$, $\eta_p^2 = 0.273$). During confrontation CM showed higher activity relative to the BNST (Fig. 6). Further, analyses revealed a significant main effect of predictability $F(1,107) = 16.67$, $p < .001$, $\eta_p^2 = 0.135$). BNST and CM activity was higher during unpredictable confrontation relative to the predictable confrontation (Fig. 6). Furthermore, analyses showed a main effect of valence ($F(1,107) = 36.01$, $p < .001$, $\eta_p^2 = 0.252$). Aversive confrontation led to

higher activity relative to neutral confrontation (Fig. 6). This effect was qualified by a significant interaction of region and valence ($F(1,107) = 29.81$, $p < .001$, $\eta_p^2 = 0.212$). Subsequent post-hoc analysis revealed that CM is more activated in comparison to the BNST during aversive confrontation relative to neutral confrontation ($t(108) = 5.41$, $p < .001$, $d_z = 0.52$; Fig7). Furthermore, during confrontation analyses showed a significant region*_NPSR1_ genotype interaction ($F(1,107) = 4.06$ $p = .047$, $\eta_p^2 = 0.037$). Post hoc tests revealed no difference in either BNST activity ($p > .389$) or CM activity ($p > .215$), but _NPSR1_ AA-allele carriers exhibit higher CM activity relative to the BNST during confrontation in comparison to the T-carriers ($t(107) = 2.01$, $p = .047$, $d = 0.392$; Fig. 8). All other comparisons were not significant (Table A 4, appendices of study 1).

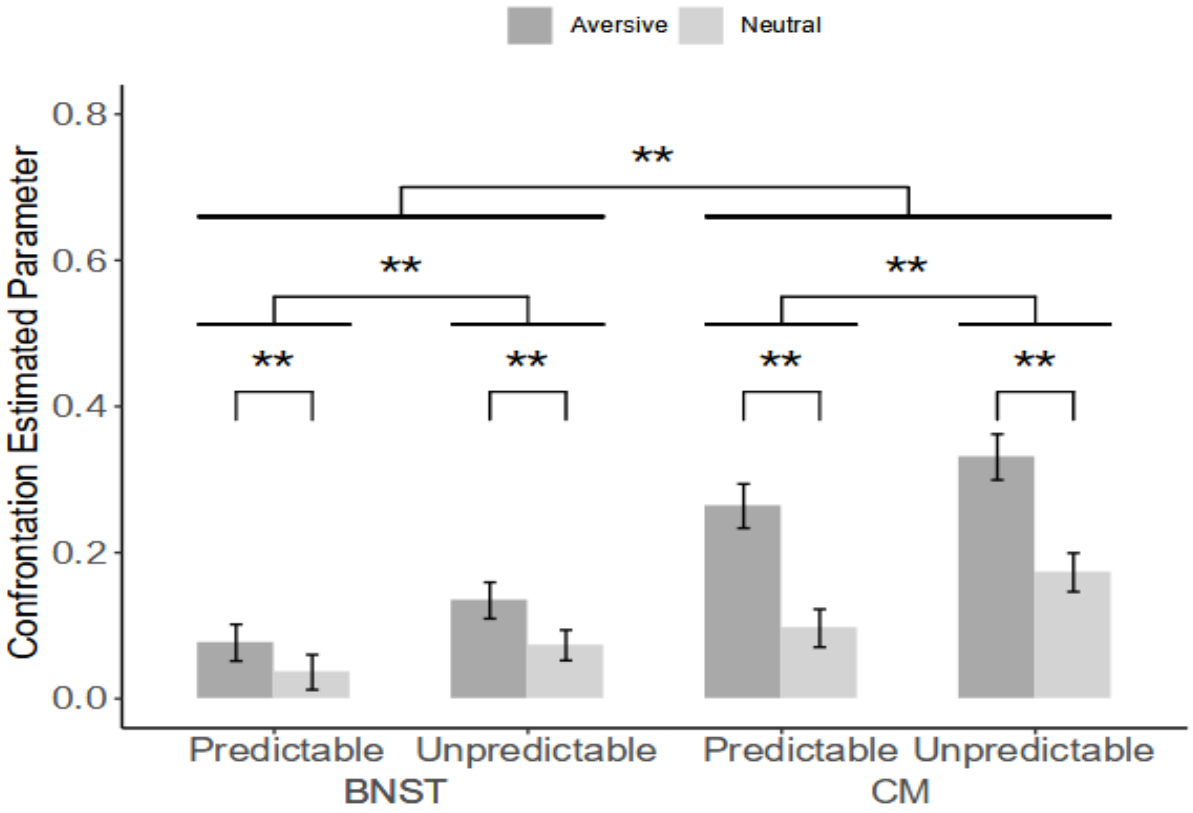

Fig 6. Main effects of region, predictability, and valence during the confrontation period in study 1.

Bar graphs display estimated parameter for aversive and neutral/predictable and unpredictable stimulus confrontation condition stratified for region (BNST vs CM) (mean ± SE; * $p < .05$, ** $p \leq .001$). All ROIs showed higher activity during the unpredictable confrontation and aversive confrontation. Overall confrontation conditions, CM showed higher activity relative to the BNST.

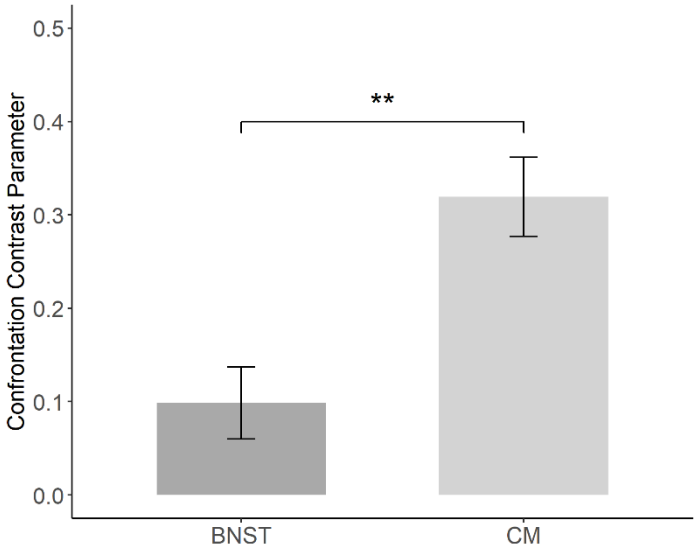

Fig 7. Significant region*valence interaction during threat confrontation period in study 1.

Bar graphs display the estimated contrast parameter of aversive compared to neutral stimulus confrontation stratified for region (BNST vs CM) (mean ± SE; ** p< .001). *CM* showed higher activity during aversive as compared to neutral confrontation (t(108) = 5.41, p < .001, d_z = 0.52).

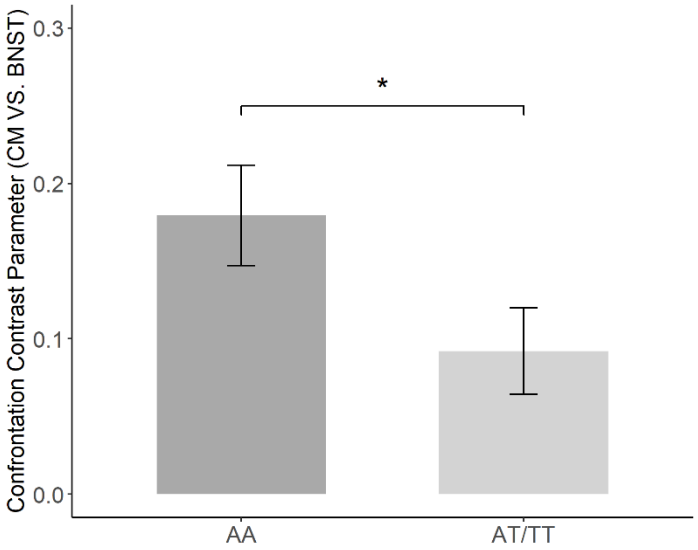

Fig 8. Significant interaction of region*NPSR1 genotype during confrontation period in study 1.

Bar graphs display the estimated contrast parameter of CM activity relative to BNST activity during confrontation stratified for *NPSR1* genotype (AA vs AT,TT) (mean ± SE; * $p < .05$). AA-allele carriers showed higher CM activity relative to BNST activity during confrontation in comparison to the T-allele carriers ($t(107) = 2.01$, $p = .047$, $d = 0.392$).

4.1.3.2.4 Exploratory whole-brain analyses

For exploratory whole-brain analysis, several contrasts were calculated during each phase. Significant clusters were found in the contrasts: cue aversive > cue neutral, predictable anticipation (aversive vs. neutral) > unpredictable anticipation (aversive vs. neutral), unpredictable confrontation > predictable confrontation, aversive confrontation > neutral confrontation, neutral confrontation > aversive confrontation, as well for the predictable confrontation (aversive vs. neutral) > unpredictable confrontation (aversive vs. neutral). The Results of the exploratory whole-brain analysis are presented in Table A 5 (appendices of study 1).

4.1.4 Discussion study 1

4.1.4.1 Differential involvement of CM and BNST during threat anticipation and confrontation

The present study aimed to investigate the differential involvement of BNST and the CM during predictable and unpredictable threat anticipation and confrontation. Both BNST and the CM showed activation in response to the cues (that cued the valence of the upcoming stimulus confrontation). In both ROIs, activity was elevated in response to the aversive as compared to the neutral cue. However, BNST shows stronger activity during the aversive cue relative to the neutral cue compared to the CM. This adds to the evidence that BNST is not only limited to sustained anticipation responses but is also involved in phasic threat responses (Figel et al., 2019; Fox and Shackman, 2019). Results are in line with a study by Klumpers et al. (2017), which indicated a stronger activity of BNST relative to the central amygdala in threat anticipation. These suggestions are supported by the results during the anticipation phase, as BNST was more activated relative to the CM independent of the condition of anticipation.

However, analyses did not reveal any effect of valence during the anticipation period. Other studies indicated sustained BNST activity during aversive threat anticipation (Herrmann et al., 2016; Pedersen et al., 2019). Therefore, the results of the present study are surprising. An explanation could be given by the nature of the study design. Studies that found sustained activity did use one cue, shorter anticipation periods, or analyzed activity during whole blocks (see Table 1). The design of the present study comprised two cues, one indicating the valence of upcoming confrontation and one for the information about temporal characteristics of the confrontation presentation. As this study observed a valence effect during the cue indicating the valence, but not during the cue indicating the moment of presentation, this points out that in the paradigm of this present study processing of threat is more related to phasic threat processes than to sustained threat processes. Furthermore, there was no effect of predictability or interaction of predictability and valence on BNST and CM activity during the anticipation period. This is surprising, as a recent study indicated higher BNST activity during unpredictable threat anticipation and higher amygdala activity during predictable threat anticipation (Naaz et al., 2019). However, in that study, predictable and unpredictable threat anticipation differed in length of the anticipation period and the anticipation period was very short. The present study parallelized the durations of the anticipation intervals in predictable and unpredictable conditions with longer anticipation periods. Another study by Pedersen et al. (2019) did also

not find an effect of predictability during anticipation. This study and the present study used a sample of healthy subjects. Other studies found higher BNST activity during unpredictable threat anticipation in clinical samples relative to a healthy control group (Brinkmann et al., 2017a; Brinkmann et al., 2017b; Buff et al., 2017). Therefore, future studies should investigate the differential involvement of CM and BNST during temporally predictable and unpredictable threat anticipation in a sample with an anxiety disorder.

Analyses during threat confrontation revealed the main effects of predictability and valence. BNST and CM showed enhanced activity during the confrontation with temporally unpredictable conditions. BNST results are consistent with recent animal and human studies (Goode et al., 2019; Naaz et al., 2019). BNST has been connected with the processing of salient information (Avery et al., 2016). Higher BNST activity during unpredictable confrontation results from this attribute. CM results are in line with Sarinopoulos et al. (2010), who also found amygdala activity on unpredictable aversive pictures. Imaging studies that used paradigms based only on temporally unpredictable confrontation without comparison to predictable confrontation also discerned higher amygdala and BNST activity (Brinkmann et al., 2018; Klumpers et al., 2017). Taken together, these studies and the results of the present study indicate that during the confrontation with aversive or neutral stimuli, temporal unpredictability modulates the amygdala and BNST activity. The present study observed CM and BNST activation during the confrontation with aversive vs. neutral stimuli, which is in line with previously published studies indicating the amygdala and BNST activity in brief threat processing (Brinkmann et al., 2018; Gungor and Pare, 2016; Shackman and Fox, 2016). This effect was stronger in CM than in BNST, which is in line with Klumpers et al. (2017), who showed also higher CM activity in threat confrontation. During threat confrontation, this study could also not discover an interaction of predictability and valence. Other studies who found differences in either CM or BNST used a paradigm, wherein the unpredictable condition the valence of the event was also unpredictable, not only the temporal aspects (Naaz et al., 2019; Sarinopoulos et al., 2010). In the present study, the valence of confrontation was always surely predictable, whereas the moment of presentation differed in terms of predictable or unpredictable conditions. This could indicate that there are different activity patterns concerning predictability mode.

4.1.4.2 Involvement of NPSR1 rs324981 during temporally predictable and unpredictable threat processes

This is the first study evaluating *NPSR1* rs324981 genotype modulation on the CM and BNST activity in predictable and unpredictable threat anticipation and confrontation. This study discovered differential involvement of BNST relative to the CM during unpredictable anticipation relative to the predictable anticipation in T-carriers compared to the homozygote AA-carriers. In line with this result, T-carriers rated unpredictable anticipation cues as more unpleasant relative to the predictable cues compared to the AA-carriers. During anticipation higher BNST activity has been associated with anxiety disorders (Brinkmann et al., 2017a; Brinkmann et al., 2017b; Buff et al., 2017). Furthermore, unpredictability is a core modulator of neural anxiety-related processes (Grupe and Nitschke, 2013). Therefore a stronger involvement of BNST during unpredictable anticipation relative to predictable anticipation in comparison to the CM could be an important factor for the development of anxiety disorders, as the T-allele has been linked to panic disorder (Beste et al., 2013; Domschke et al., 2012; Domschke et al., 2011; Gottschalk and Domschke, 2016; Okamura and Reinscheid, 2007). During threat confrontation, this study was not able to replicate recent published higher amygdala activity in T-carriers (Dannlowski et al., 2011; Gechter et al., 2019; Streit et al., 2014), but found a region*_NPSR1_* interaction. The activity difference between CM and BNST was smaller in T-carriers compared to AA-carriers. This could indicate a general higher engagement of fear network during the processing of confrontation in T-carriers. Taken together, results indicate an involvement of the NPS system in fear and anxiety-related threat processes, as the more active T-allele seems to increase sensitivity to unpredictability during anticipation and to result in a generally higher engagement of the fear network during confrontation conditions. However, given the polygenic nature of imaging genetic phenotypes, the individual effect sizes of single genetic variants in modulating neural phenotypes may be more modest than originally reported (cf. Bogdan et al., 2017). Posthoc power analyses conducted with G*Power (Faul et al., 2007) indicated an achieved power of 0.53 - 0.62 for the presently described analyses including the *NPSR1* genotype. Therefore, the present results regarding genetic modulation effects are to be considered preliminary and warrant replication in larger, sufficiently powered samples applying the same study design and analysis methodology. Additionally, while the chromosomal region 7p14-15 encompassing the *NPSR1* locus has been previously found to be linked to panic disorder in genome-wide surveys (Crowe et al., 2001; Knowles et al., 1998; Logue et al., 2003), genome-wide association studies (GWAS) did not associate *NPSR1* rs324981 with fear and anxiety-related phenotypes so far.

Hence, conclusive statements about the relevance of *NPSR1* rs324981 for fear and anxiety-related processes cannot be derived from the present state of the literature including the present study.

4.1.5 Conclusion Study 1

In summary, the present study demonstrated, that BNST and CM are both involved in fear-related processes, but show threat mode-specific differential involvement, as BNST is more activated when focusing on phasic threat anticipation processes and CM is stronger activated when focusing on threat confrontation. Further, this study showed that temporal unpredictability modulates CM and BNST activity during the confrontation.

4.2 Study 2: Centromedial amygdala shows greater activation during spider confrontation relative to the BNST in patients with spider phobia

Data of study 2 were collected at the Institute of Medical Psychology and Systems Neuroscience of the University of Münster and belongs to the project I am participating (SFB TRR 58, C07). My contribution to this study contains the development of the study goal, full data analysis, data interpretation and validation, visualization of study results, and writing of the original draft of the manuscript.

4.2.1 Introduction of study 2

Chapter 2.1 of this dissertation presented methods and results of recent studies that found amygdala and/or BNST activity during temporally predictable and unpredictable threat processes. However, these studies showed some limitations which were addressed in study 1 of this dissertation. Results of study 1/Siminski et al. (2021) indicated a higher involvement of BNST relative to the CM during a short-lasting anticipation cue and a higher involvement of CM during threat confrontation relative to the BNST. No study so far investigated the activation interaction of CM and BNST in a sample with an anxiety disorder relative to a control group.

Spider phobia belongs to the specific phobia disorder, which was introduced in chapter 2.2 of this dissertation. Both amygdala and BNST showed elevated activity in spider phobics relative to a healthy control group concerning predictability (Munsterkotter et al., 2015; Straube et al., 2007). Amygdala was more active during predictable spider condition (Munsterkotter et al., 2015), whereas BNST was more activated during unpredictable phobic conditions (Munsterkotter et al., 2015; Straube et al., 2007). However, during a four seconds-lasting video showing an approaching spider, the activity of the dorsal amygdala and BNST was also elevated in healthy subjects (Mobbs et al., 2010). This indicates an involvement of both areas in anticipation and confrontation with spiders in humans and raises the question under which threat modes both areas are differentially activated in healthy subjects and patients with spider phobia.

Building upon recent studies which evaluated differential involvement of CM and BNST in predictable and unpredictable threat anticipation and confrontation processes (Hur et al., 2020; Naaz et al., 2019; Pedersen et al., 2019; Siminski et al., 2021), this study aims to directly compare CM and BNST activity for the first time in patients with an anxiety disorder

(spider phobia) in comparison to an age- and gender-matched control group. During a short-lasting cue, indicating if the participant has to anticipate either a spider or a bird, this study expected higher involvement of the BNST in comparison to the CM during the spider cue relative to the bird cue in patients compared to healthy controls (Siminski et al., 2021). During the anticipation phase, this study expected patients to show higher BNST activity during the unpredictable threat anticipation relative to the unpredictable bird anticipation in comparison to the predictable threat anticipation relative to the predictable bird anticipation (Munsterkotter et al., 2015; Straube et al., 2007). During the confrontation period, this study expected that unpredictability leads to higher activity in both regions (Siminski et al., 2021). Furthermore, this study expected that in patients the CM is more involved during the confrontation with spiders relative to the confrontation with birds than the BNST as compared to healthy controls (Siminski et al., 2021).

4.2.2 Methods of study 2

4.2.2.1 Participants

This sample consisted of 37 patients with specific phobia (animal subtype: spiders) and 34 healthy participants. Patients were recruited from a sample of a larger study, see Schwarzmeier et al. (2020) for more detailed information (this is a bicentric study including the center Münster and Würzburg in Germany; patients in the present study were taken from the center in Münster as data of the healthy participants were also collected in Münster). In that study, patients diagnosed with a specific phobia of the animal subtype (spider phobia) assessed with the structured clinical interview for DSM-IV (SCID-IV) (Wittchen et al., 1997) were included. They had to reach the critical score of a minimum of 20 points according to Öst (1996) in the spider phobia questionnaire (SPQ; Hamm, 2006; Klorman et al., 1974). Furthermore, they had to be right-handed, had a normal or corrected-to-normal vision, were aged between 18 and 65 years, had Caucasian descent, were fluent in the German language, and were motivated to participate in a highly controlled behavioral exposure delivered via virtual reality (VR). Exclusion criteria were a lifetime diagnosis of other comorbid anxiety disorders (panic disorder, agoraphobia, social phobia, and generalized anxiety disorder), obsessive-compulsive disorder (OCD), posttraumatic stress disorder (PTSD), severe major depression, borderline personality disorder, bipolar I disorder, psychotic disorders, substance dependence (except nicotine), or acute suicidality. Patients with a comorbid mild to moderate depression (unless currently treated) and other specific phobias of the animal subtype were able to participate in that study

if spider phobia was the primary diagnosis. Furthermore, patients with current (psycho)pharmacological treatment, current or past psychotherapy, neurological diseases, pregnant women, and if they fulfilled MRI-related exclusion criteria were excluded. Healthy participants were also right-handed, aged between 18 to 50 years, not pregnant, free of neurological disorders, free of psychiatric disorders in their lifetime, free of psychopharmacological treatment, free of current or past psychotherapy, had a normal or corrected-to-normal vision, and were screened for substance dependence. Further, healthy participants denied fear of spiders or in case they confirmed the fear, they underwent also the SPQ and were included if they did not reach the critical score of 20 points.

In the first step, three patients had to be excluded because of movement over three mm/°. Furthermore, four patients and three healthy controls were excluded, because they did not press the button at least once during the attention task. Also, one healthy control was excluded because of enlarged ventricles relative to the other participants. After these steps, the sample consisted of 30 patients and 30 healthy controls. However, according to Stuart et al. (2013) these resulting groups were unbalanced in the distribution of age (patients: M = 29.27, SD = 10.18; healthy controls: M = 23.4, SD = 3.58) and sex (patients: 24 women, 6 men; healthy controls: 17 women, 13 men), as the standardized differences across the groups were larger than the maximum recommended critical standardized difference of 0.25 (age: standardized difference = 0.72; sex: standardized difference = 0.52; calculation of standardized differences were based on previous studies of Flury and Riedwyl (1986) as well of Austin (2009)). Therefore, to have comparable, equally powered groups, this study used the R matching toolbox matchIT to match the groups for age and sex distribution (Ho et al., 2018). For that purpose, this study conducted a matching based on the nearest neighbour method (relevant arguments of the matchit function: discard = “both”, model = “logit”, method = “nearest”, m.order = “random”, reestimate = “false”, caliper = 0.25, ratio = 1). The final sample consisted of 21 healthy participants and 21 patients. Groups did not differ in sex distribution and age, but in self-reported fear assessed with the State-Anxiety-Inventory (STAI-S), indicating higher subjective fear in patients before the fMRI assessment (Table 3). The study complies with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Study goals were explained to the participants and written informed consent was given. The ethics committee of the medical faculty of the University of Muenster approved this study.

Table 3. Sample characteristics of study 2

variable	healthy controls (n = 21)		patients (n = 21)		statistics	
					χ^2 *(1)*	*p*
sex	6 m, 15 w		5 m, 16 w		0.12	.726
	M	*SD*	*M*	*SD*	*t(40)*	*p*
age	23.81	3.63	24.14	5.04	-0.25	.807
STAI - S	32.24	6.66	37.86	10.66	-2.05	.047
SPQ	-	-	21.76	1.89	-	-

Note: STAI-S, State-Trait-Anxiety Inventory State-Anxiety Scale (Laux et al., 1981); SPQ, the German translation of the Spider phobia Questionnaire (Hamm, 2006; Klorman et al., 1974).

4.2.2.2 Paradigm

The paradigm was adapted to spider phobic relevant material from a recently published study by Siminski et al. (2021). All participants went through an instructed threat-anticipation paradigm during fMRI (Fig 9). The stimuli set was used from previous studies and consisted of 20 pictures of spiders and 20 pictures of birds (Lipka et al., 2014; Lipka et al., 2011). In the beginning, the valence of the stimuli (spider or bird) presented after an anticipation phase was cued by the letters “A” and “B” (cue, Fig. 9). Following that, a second cue was presented which displayed an orange or blue schematic watch (anticipation, Fig. 9). Colors indicated the predictability of the occurrence of stimuli. The letters and colors of the watches were counterbalanced across participants. In the predictable condition, the onset of the stimuli confrontation was, when the pointer reached the twelve o´clock position, in the unpredictable condition, the stimuli confrontation could appear at any moment. Different anticipation periods were used for both the predictable and unpredictable conditions: 3 seconds (one trial), 10 seconds (one trial), 16 seconds (seven trials), and 20 seconds (one trial), resulting in a mean anticipation period of 14.5 seconds. Then, the spiders or birds were presented for four seconds each (confrontation, Fig 9). Spider and bird pictures were also counterbalanced across the conditions. To control for visual attention, participants had to press a button during the confrontation phase when the picture changed (part of the spider/bird picture got cut out; four times in total, once in every condition). Between trials, a white fixation cross was displayed on a grey background, jittering from 10 to 20 seconds. In total, participants underwent 40 trials and the experiment lasted approximately 23 minutes.

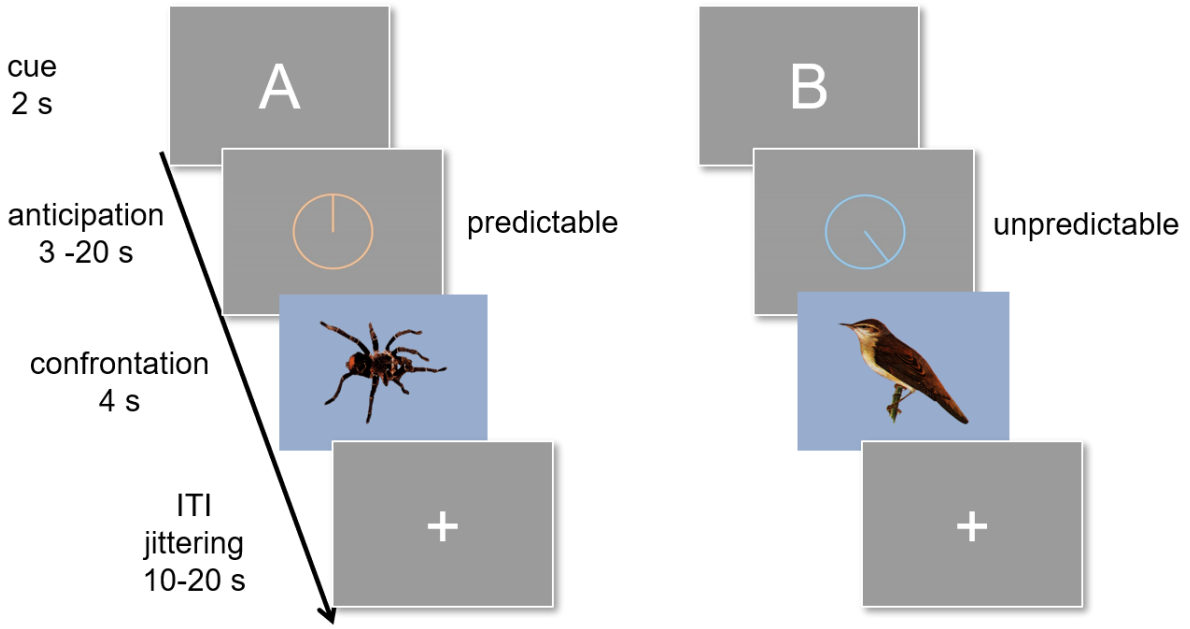

Fig 9. Experimental paradigm of study 2.

ITI = Inter trial interval. The letter cued the valence (spider vs. bird) of the pictures. The color of the watch either signaled the exact moment of the upcoming pictures (predictable) or did not (unpredictable).

4.2.2.3 Subjective ratings of anticipation cues and pictures

After fMRI measurements, participants were asked to rate the anticipation cues and the pictures on a nine-point Likert scale (Self-Assessment Manikin; Bradley and Lang (1994)) assessing the dimension's pleasantness (1 = very unpleasant to 9 = very pleasant) and emotional arousal (1 = not arousing to 9 = highly arousing) as well as their subjective anxiety (1 = not anxiety-inducing to 9 = highly anxiety-inducing). Three-way-factor ANOVAs with the between factor group (patients / hc) and the within factors valence (spider/neutral anticipation) and predictability (unpredictable/predictable anticipation) were calculated for anticipation cues. For picture ratings, two-way-factor ANOVAs with the between factor group (patients / hc) and the within factor valence (spider/neutral confrontation) were calculated. Analyses were conducted using SPSS software (Version 26.0. Armonk, NY: IBM Corp.) for each rating. An alpha level of $p < .05$ was considered statistically significant.

4.2.2.4 Acquisition of fMRI data

Structural and functional MRI data were collected with a 3 Tesla magnetic resonance scanner ("Magnetom Skyra", Siemens, Medical Solutions, Erlangen, Germany). Structural brain images were collected with a high-resolution T1-weighted scan with 192 sagittal slices (thickness= 1mm, matrix = 256 x 256, TE = 2.28 ms, TR = 2130 ms). Functional data were acquired with a T2*-weighted echo-planar sequence consisting of 610 volumes (42 slices, thickness = 3mm, field of view = 216 mm, matrix = 72 x 72, flip angle = 90°, TE = 30 ms, TR = 2300 ms).

4.2.2.5 Pre-processing of fMRI data

Data were pre-processed using SPM 12 (https://www.fil.ion.ucl.ac.uk/spm). Analogous to the previous work of Herrmann et al. (2016), the first five volumes were discarded from each run to ensure that steady-state tissue magnetization was sufficient. Next, data were corrected for movement artifacts (registered to the first image), slice time corrected (middle slice as reference slice), co-registered from anatomical to functional data, segmented, and normalized with a voxel size of 3 mm to the standard MNI space. In the last step of pre-processing data were spatially smoothed (5mm full width at half-maximum Gaussian kernel).

4.2.2.6 Statistical analysis of fMRI data

During the first-level analysis, for every person, a GLM was calculated to model a canonical HRF. The GLM included all phases: the cue phase (spider cue vs neutral cue; full duration of 2 s), the anticipation phase (predictable/unpredictable spider/neutral anticipation; full duration jittering from 3 to 20 s), and the confrontation phase (predictable/unpredictable spider/neutral confrontation; full duration of 4 s). Six movement parameters were modeled as predictors of no interest. Furthermore, a mask threshold of 0.8, a high pass filter cut-off of 128 seconds, and a first-order regression order were used as standard. Analyses were restricted to a-priori defined regions of interest (ROIs). Similar to Siminski et al. (2021), this study extracted the bilateral centromedial amygdala ROI (CM) based on maximum probability maps of Amunts et al. (2005) included in the anatomy toolbox (Eickhoff et al., 2005) and the bilateral BNST ROI based on probabilistic maps provided by Torrisi et al. (2015). ROI masks were reoriented to the individual's space using the coregister (Reslice) option. Figure 10 displays the reoriented ROIs on the averaged T2-scan of participants.

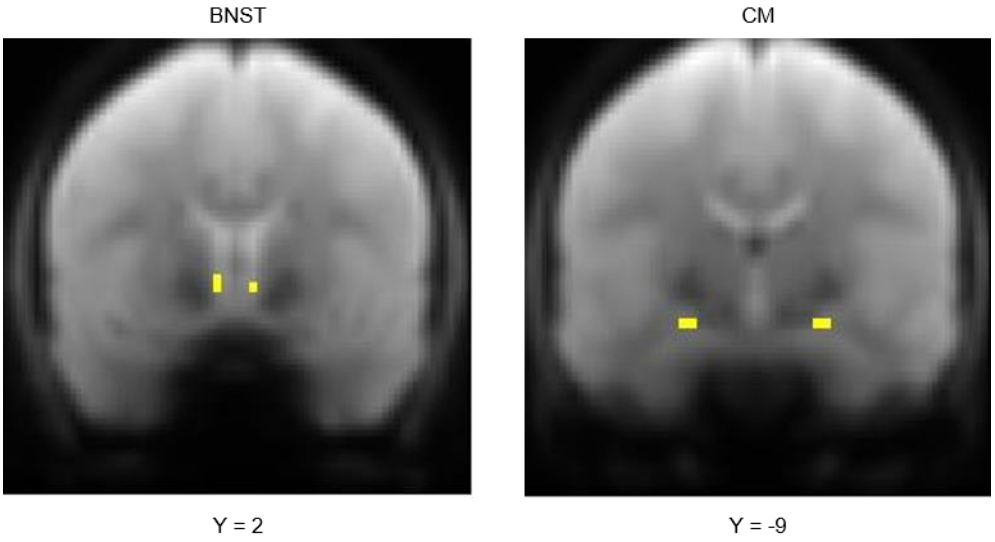

Fig 10. Anatomical ROIs overlaid on the average functional scan of study 2.

left: BNST ROI; right: CM ROI; BNST = bed nucleus of the stria terminalis; CM = centromedial amygdala; ROI = Region of interest

Similar to Klumpers et al. (2017), this study used MarsBar (http://marsbar.sourceforge.net/) to extract average beta values of all predictors of interest for both ROIs. For the cue, a three-way-factor ANOVA with the between factor group (patients / hc) and the within factors region (CM, BNST), and valence (spider/neutral) as factors and averaged beta values as the dependent variable were calculated. For the anticipation phase and the confrontation phase four-way-factor ANOVA with the between factor group (patients / hc) and the within factors region (CM, BNST), valence (spider/neutral), and predictability (unpredictable/ predictable) as factors and averaged beta values as the dependent variable were calculated. Calculations were conducted using SPSS software (Version 26.0. Armonk, NY: IBM Corp). An alpha level of $p < .05$ was considered statistically significant.

Furthermore, whole-brain analyses were conducted for exploratory analyses. To identify group differences in the cue phase, a two-sample t-contrast was conducted in the contrast cue spider vs. cue bird. For the anticipation phase and the confrontation phase, full factorial models were calculated. This study focused on the interaction effects with the factor group. Therefore, analyses were restricted to the interactions of group*predictability, group*valence, and group*predictability*valence. To correct for multiple corrections, a threshold-free Cluster enhancement method (TFCE) with FWE correction ($p_{FWE} < 0.05$) and 5000 permutations was

used, as this is a more sensitive and less arbitrary procedure (Smith and Nichols, 2009). TFCE analyses were conducted with the TFCE-toolbox (http://dbm.neuro.uni-jena.de/tfce).

4.2.3 Results of study 2

4.2.3.1 Subjective ratings

4.2.3.1.1 Subjective ratings of anticipation cues

Three-way-factor ANOVAs with the between factor group (patients / hc) and the within factors valence (spider/neutral anticipation) and predictability (unpredictable/predictable anticipation) were calculated for self-reported ratings of pleasantness, arousal, and anxiety. For the ratings of pleasantness and arousal there were significant main effects of predictability (pleasantness: $F(1,40) = 6.64$, $p = .014$, $\eta_p^2 = .142$; arousal: $F(1,40) = 5.34$, $p = .026$, $\eta_p^2 = .118$). Unpredictable cues were rated as more unpleasant and more arousing then predictable cues. Analyses also revealed main effects of valence in each self-reported rating (pleasantness: $F(1,40) = 30.69$, $p < .001$, $\eta_p^2 = .434$; arousal: $F(1,40) = 39.88$, $p < .001$, $\eta_p^2 = .499$; anxiety: $F(1,40) = 36.931$, $p < .001$, $\eta_p^2 = .480$). These were qualified by interactions of valence*group (pleasantness: $F(1,40) = 30.69$, $p < .001$, $\eta_p^2 = .434$; arousal: $F(1,40) = 39.88$, $p < .001$, $\eta_p^2 = .499$; anxiety: $F(1,40) = 36.93$, $p < .001$, $\eta_p^2 = .480$). Analyses of interactions revealed, that patients rated anticipation cues of spiders as more unpleasant ($t(40) = 4.56$, $p < .001$, $d = -1.409$), as more arousing ($t(40) = -5.42$, $p < .001$, $d = 1.672$), and more anxiety inducing ($t(40) = -5.16$, $p < .001$, $d = 1.591$) relative to the neutral anticipation cues in comparison to the healthy controls (see Figure 11). All other effects were not significant (Table A 6, appendices of study 2).

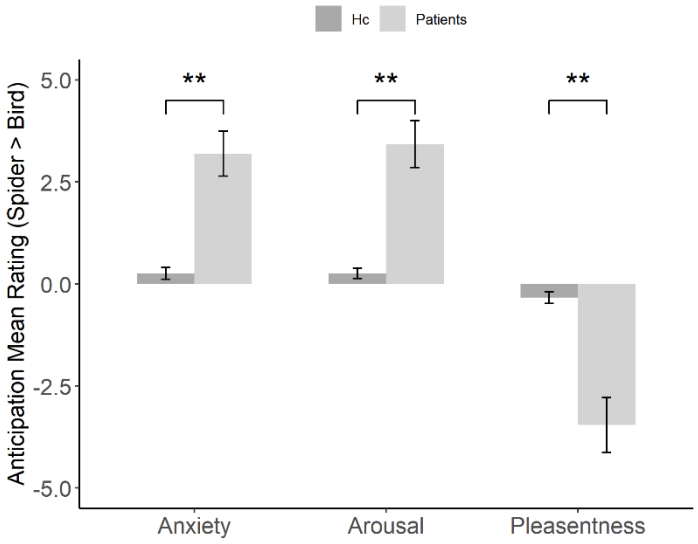

Fig 11. Valence*group interactions of the anticipation cues in study 2.

Hc = healthy controls. Bar graphs display the estimated contrast of spider anticipation ratings > bird anticipation ratings (mean ± SE; ** p< .001). Patients rated anticipation cues of spiders of as more anxiety inducing (t(40) = -5.16, p < .001, d = 1.591) , as more arousing (t(40) = -5.42, p < .001, d = 1.672) and less pleasant (t(40) = 4.56, p < .001, d = -1.409) relative to the anticipation cues of birds as compared to the healthy controls.

4.2.3.1.2 Subjective ratings of pictures

Two-way-factor ANOVAs with the between factor group (patients / hc) and the within factor valence (spider/neutral confrontation) were calculated for self-reported ratings of pleasantness, arousal, and anxiety. Analyses revealed main effects of valence in each self-reported rating (pleasantness: F(1,40) = 118.49, p < .001, η_p^2= .748; arousal: F(1,40) = 193.63, p < .001, η_p^2= .829; anxiety: F(1,40) = 167.41, p < .001, η_p^2= .807). These were qualified by interactions of valence*group (pleasantness: F(1,40) = 27.61, p < .001, η_p^2= .408; arousal: F(1,40) = 117.55, p < .001, η_p^2= .746; anxiety: F(1,40) = 103.77, p < .001, η_p^2= .722). Analyses of interactions revealed, that patients rated pictures of spiders as more unpleasant (t(40) = 5.26, p < .001, d = -1.409), as more arousing (t(40) = -10.84, p < .001, d = 1.672), and more anxiety inducing (t(40) = -10.19, p < .001, d = 1.591) relative to the bird pictures in comparison to the healthy controls (see Figure 12).

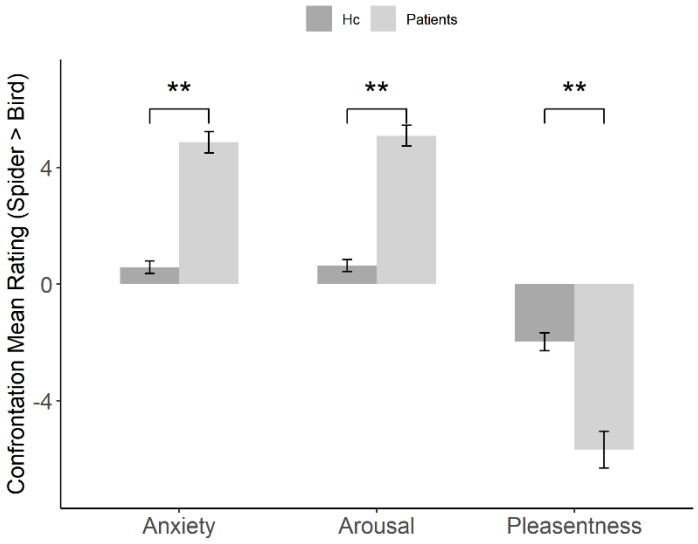

Fig 12. Valence*group interactions of the ratings of pictures in study 2.

Hc = healthy controls. Bar graphs display the estimated contrast of spider confrontation > bird confrontation (mean ± SE; ** p< .001). Patients rated pictures of spiders as more unpleasant (t(40) = 5.26, p < .001, d = -1.409), more arousing (t(40) = -10.84, p < .001, d = 1.672) and more anxiety inducing (t(40) = -10.19, p < .001, d = 1.591) relative to the pictures of birds as compared to the healthy controls.

4.2.3.2 fMRI data

4.2.3.2.1 Cue presentation

A three-way-factor ANOVA with the between factor group (patients /hc) and the within factors region (CM / BNST) and valence (spider cue/bird cue) as factors and averaged regressor beta values of the region of interest (ROI) as the dependent variable were calculated. The ANOVA revealed a significant main effect of region (F(1, 40) = 12.85, p < .001, η_p^2= 0.243). BNST was more active over both cues relative to the CM. Furthermore, the ANOVA revealed a main effect of valence (F(1, 40) = 14.16, p < .001, η_p^2= 0.262). Participants showed higher CM and BNST activity during the spider cue relative to the bird cue. All other comparisons were not significant (Table A 7, appendices of study 2).

4.2.3.2.2 Anticipation period

A four-way-factor ANOVA with the between factor group (patients /hc) and the within factors region (CM / BNST), valence (spider/bird anticipation), and predictability (unpredictable/ predictable anticipation) as factors and averaged regressor beta values of the ROI as the dependent variable were calculated. The ANOVA revealed a significant main effect of region ($F(1, 40) = 30.11$, $p < .001$, $\eta_p^2 = 0.429$). BNST showed stronger activation across all anticipation conditions relative to the CM. Further, the ANOVA revealed a significant main effect of valence ($F(1, 40)$ 5.21, $p = .047$, $\eta_p^2 = 0.095$). Both regions showed stronger activity during spider anticipation relative to neutral anticipation. Furthermore, the ANOVA revealed a group*predictability interaction ($F(1, 40) = 6.18$, $p = .017$, $\eta_p^2 = 0.134$). Healthy controls showed higher CM and BNST activity during predictable anticipation relative to unpredictable anticipation in comparison to the patients ($t(40) = 2.49$, $p = .017$, d = 0.77 , Fig.13). All other comparisons were not significant (Table A 8, Appendices of study 2).

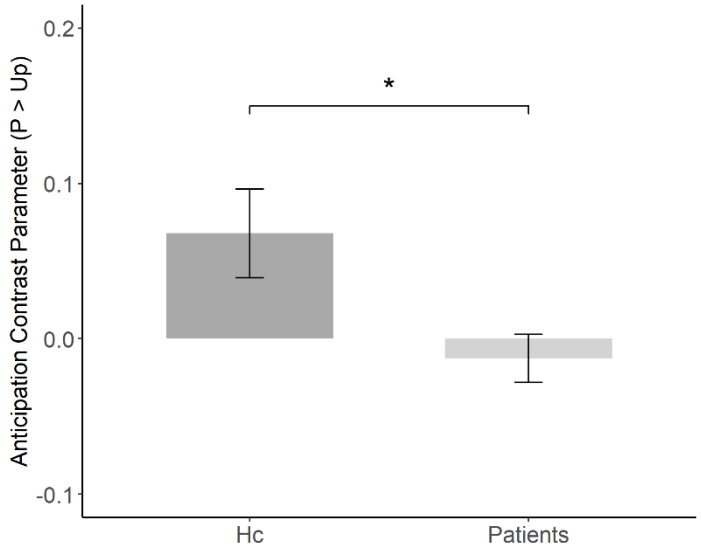

Fig 13. Significant group*predictability interaction during anticipation period in study 2.

p = predictable anticipation; up = unpredictable anticipation; Bar graphs display the estimated contrast parameter of predictable anticipation compared to the unpredictable anticipation stratified for group (hc vs. patients) (mean ± SE; * $p < .05$). Healthy controls showed higher CM and BNST activity during predictable anticipation relative to unpredictable anticipation in comparison to the patients ($t(40) = 2.49$, $p = .017$, d = 0.77).

4.2.3.2.3 Confrontation period

A four-way-factor ANOVA with the between factor group (patients /hc) and the within factors region (CM / BNST), valence (spider/bird confrontation), and predictability (unpredictable/ predictable confrontation) as factors and averaged regressor beta values of the ROI as the dependent variable were calculated. The ANOVA revealed a main effect of valence ($F(1,40) = 8.91$, $p = .005$, $\eta_p^2 = 0.182$), and an interaction of valence*region ($F(1,40) = 12.59$, $p = .001$, $\eta_p^2 = 0.239$, which were qualified by an interaction of region*predictability*valence*group ($F(1,40) = 5.72$, $p = .022$, $\eta_p^2 = 0.125$). A separate explorative follow-up ANOVA for patients revealed a main effect valence ($F(1,20) = 7.50$, $p = .013$, $\eta_p^2 = 0.273$), which was qualified by an interaction of valence and region ($F(1,20) = 13.07$, $p = .002$, $\eta_p^2 = 0.395$). Analysis of valence*region interaction showed, that in patients CM is more involved relative to the BNST during spider confrontation compared to the bird confrontation ($t(20) = 3.62$, $p = .002$, $d_z = 0.79$; Fig.14). Analyses in patients did not reveal a significant interaction of region*predictability*valence($F(1,20) = 0.80$, $p = .381$, $\eta_p^2 = 0.039$). However, the explorative follow-up ANOVA for the control group revealed a significant region*predictability*valence interaction ($F(1,20) = 5.41$, $p = .031$, $\eta_p^2 = 0.213$). Explorative post-hoc t-tests showed higher engagement of CM compared to the BNST in the predictable spider confrontation relative to the predictable bird confrontation ($t(20) = 3.24$, $p = .004$, $d_z = 0.71$; Fig 15), whereas no differences revealed in the unpredictable confrontation ($t(20) = 0.19$, $p = .851$, $d_z = .04$; Fig 15). All other comparisons of the four-way-factor ANOVA were not significant (Table A 9, appendices of study 2).

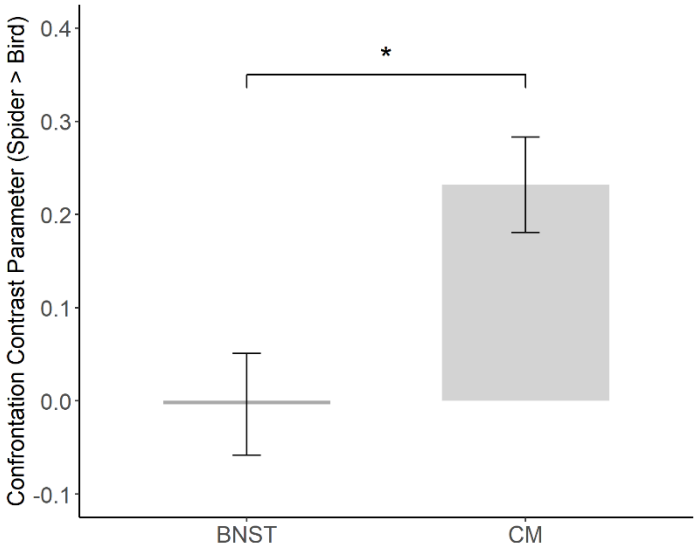

Fig 14. Significant region*valence interaction during threat confrontation in patients in study 2.

Bar graphs display the estimated contrast parameter of spiders compared to bird confrontation stratified for region (BNST vs CM) (mean ± SE; * $p < .05$). CM showed higher activity during spiders as compared to bird confrontation ($t(20) = 3.62$, $p = .002$, $d_z = 0.79$).

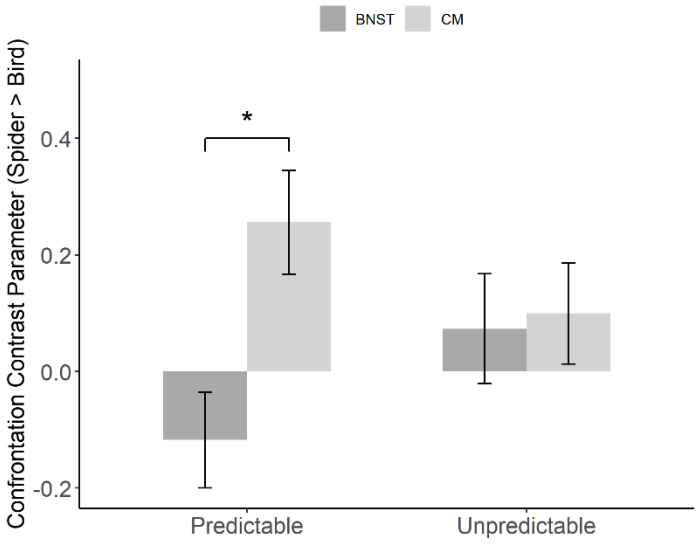

Fig 15. Significant region*predictability*valence interaction during threat confrontation in healthy controls in study 2.

Bar graphs display the estimated contrast parameter of spiders compared to bird confrontation stratified for region (BNST vs CM) and predictability (predictable vs. unpredictable) (mean ± SE; * $p < .05$). Healthy controls showed higher CM activity relative to the BNST during the predictable spider confrontation as compared to predictable bird confrontation ($t(20) = 3.24$, $p = .004$, $d_z = 0.71$), but showed no differential involvement of CM and BNST during the unpredictable spider confrontation relative to the unpredictable bird confrontation ($t(20) = 0.19$, $p = .851$, $d_z = .04$).

4.2.3.2.4 Exploratory whole-brain group comparisons

For the contrast cue spider vs. cue bird, and the group interactions (group*predictability, group*valence, group*predictability*valence) in the full factorial model of the anticipation phase as well in the full factorial model of confrontation phase, the exploratory whole-brain analyses revealed no significant clusters.

4.2.4 Discussion of study 2

The present study aimed to evaluate the differential involvement of CM and BNST during temporally predictable and unpredictable anticipation and confrontation of disorder-specific threat stimuli in patients with spider phobia relative to age and gender-matched control group. On a behavioral level, unpredictable cues were rated as more unpleasant and more arousing

than predictable cues. Furthermore, patients as compared to the healthy controls rated phobic anticipation cues and phobic confrontation as more unpleasant, more arousing, and more anxiety-inducing relative to the neutral anticipation/confrontation. On a neural basis, healthy controls showed higher CM and BNST activity during predictable anticipation relative to the unpredictable anticipation in comparison to the patients. Furthermore, in patients, CM shows stronger involvement compared to the BNST during phobic confrontation relative to the neutral confrontation. In healthy controls, this effect has been shown only in the predictable confrontation condition whereas in the unpredictable condition no differentiation between CM and BNST appeared. Results indicate different neural activity during phobic threat processing in patients with spider phobia and a healthy control group.

4.2.4.1 Involvement of centromedial amygdala and BNST during anticipation processes

During the cue presentation and the anticipation phase, BNST was more active over all conditions relative to the CM in each phase. This is in line with other studies highlighting the relevance of BNST during anticipation processes relative to the amygdala (Klumpers et al., 2017; Pedersen et al., 2019; Siminski et al., 2021). During the cue presentation, CM and BNST showed higher activity during the phobic cue relative to the neutral cue. Likewise, during the anticipation phase CM and BNST were also more activated during phobic anticipation relative to the neutral anticipation. This shows that CM is also involved in long-lasting phobic anticipation processes. However, patients and the control group did not differ regarding valence processing. Higher CM and BNST activity during spider anticipation in both groups indicate, that spiders might be an aversive stimulus in general and could also elicit defense reactions in non-phobics. However, other studies found higher BNST activity in patients with spider phobia relative to a control group in different states of threat unpredictability (Munsterkotter et al., 2015; Straube et al., 2007). Straube et al. (2007) found higher BNST activity during blocks of temporally unpredictable anticipation periods and Munsterkotter et al. (2015) found higher BNST during a condition, where pictures were presented and a few of them could be pictures of spiders. In the present study, an event-related fMRI paradigm with temporally predictable and temporally unpredictable threat anticipation periods was. Possibly, different forms of threat anticipation/confrontation paradigms recruit also different BNST functions and therefore modulate BNST activity. Future studies should compare different conditions of predictable and unpredictable threat anticipation in spider phobia to evaluate the impact of these methodological differences. Furthermore, future studies should investigate differential CM and BNST

involvement in temporally unpredictable but also predictable threat anticipation in patients with other anxiety disorders, as other studies showed engaged activity of the amygdala and BNST during states of temporally unpredictable anticipation (Brinkmann et al., 2017a; Brinkmann et al., 2017c; Buff et al., 2017; Figel et al., 2019).

Healthy controls showed higher activity in both regions during predictable anticipation relative to the unpredictable anticipation in comparison to the patients. One hypothesis was recently provided by Hur et al. (2020). These authors found a relatively larger activity during temporally certain threat anticipation relative to the uncertain threat anticipation in the BNST and the dorsal amygdala in healthy participants. These authors suggested, that temporally predictable threat anticipation could encourage a "reactive, stimulus-bound cognitive mode", as these conditions deliver information of the exact onset of upcoming events. Analyses of the present study did not reveal a predictability*valence interaction during anticipation periods, but this stimulus-bound cognitive mode could be also present independent of the valence of the anticipation in the control group. A review highlights threat unpredictability as a key modulator of neural activity in anxiety disorders (Grupe and Nitschke, 2013), and other studies found higher amygdala and BNST activations in anxiety disorder patients in different states of temporally unpredictable threat anticipation (Brinkmann et al., 2017a; Brinkmann et al., 2017c; Buff et al., 2017; Figel et al., 2019). This larger reactivity in anxiety disorders to threat unpredictability could also lead to a reduced differentiation between the predictable and unpredictable anticipation processes in patients in the present study. However, as the present study did not expect a greater differentiation of predictable conditions in contrast to unpredictable anticipation conditions in healthy controls relative to the patients, future studies should explicitly test this hypothesis.

4.2.4.2 CM and BNST involvement during confrontation phase

This study is amongst the first studies reporting differential involvement of the CM and BNST during the confrontation with threatening stimuli in an anxiety disorder. Patients showed higher CM involvement compared to the BNST during phobic confrontation relative to the neutral confrontation independent of the predictability, whereas in healthy controls this greater involvement was only present in the predictable condition. Higher activity to spider pictures relative to the bird pictures during the predictable condition in healthy controls is in line with a study of Mobbs et al. (2010), which found higher dorsal amygdala and BNST activity during a four seconds video of a tarantula getting closer to participants foot. However, these researchers

did not directly compare the activity of the amygdala and BNST. Higher activity of CM during a phobic confrontation is also in line with several reviews highlighting hyperactive amygdala as a common observation in phobic neural activity (Chavanne and Robinson, 2020; Del Casale et al., 2012; Etkin and Wager, 2007; Ipser et al., 2013; Linares et al., 2012; Peñate et al., 2017).

Greater involvement of CM relative to the BNST during threat confrontation is in line with recent studies, indicating stronger involvement during threat confrontation phases of the amygdala relative to the BNST in healthy subjects (Klumpers et al., 2017; Siminski et al., 2021). Another study that found greater BNST activity during the threat confrontation relative to the neutral condition in healthy samples did not directly compare the amygdala and BNST activity (Brinkmann et al., 2018). With a medium effect, the present study shows, that the CM is more relevant for the processing of phobic confrontation relative to the BNST, as the activity of CM and BNST during threat confrontation (relative to a neutral confrontation) in specific phobia patients were directly compared.

However, one study did conduct also a region*mode analysis in the confrontation phase in a healthy sample and found elevated BNST activity during an unpredictable confrontation relative to a predictable threat confrontation (Naaz et al., 2019). These researchers used a threat confrontation condition, where an aversive stimulus might occur. In the present study, a threat confrontation condition was used, where threat always occurred, but only the moment of appearance differed depending on condition. In future studies, it is necessary to investigate, if BNST and amygdala show differential involvement under different phobic confrontation modes.

4.2.4.3 Limitations

Although results offer a solid basis for further clinical research, there are some limitations to consider, which could be addressed by future studies. Our results are limited to spider phobia. Hence, future studies should replicate our study in samples with other anxiety disorders. Another limitation is, that there is no SPQ-value for every healthy participant. However, in the present study patients and healthy participants did differ strongly in the ratings of phobic and neutral stimuli indicating a successful recruitment procedure. Because of the matching procedure, the resulting sample is quite small, which leads to a loss in the power of the sample. Future studies should use larger samples to validate our results.

4.2.5 Conclusion of study 2

Keeping the limitations in mind, these results contribute to a better understanding of the separate roles of the centromedial amygdala and BNST during predictable and unpredictable threat anticipation and confrontation processes. The BNST seems to be more relevant for anticipation processes (independent of predictability or valence), whereas the CM seems to be more relevant for threat confrontation in patients with specific phobia (independent of predictability).

4.3 Study 3: Lower ACC activity during spider anticipation and higher BNST activity during spider confrontation predicts therapy response in spider phobia

Data of study 3 were collected at the University Hospital of Würzburg and belongs to the project I am participating (SFB TRR 58, C07). My contribution to this study contains the data collecting, development of the study goal, full data analysis, data interpretation and validation, visualization of study results, and writing of the original draft of the manuscript.

4.3.1 Introduction in study 3

Anxiety disorders are the most prevalent psychiatric disorder (Wittchen et al., 2011). Exposure therapy is the chosen therapy for the treatment of anxiety disorders (Bandelow et al., 2014). However, only 50 percent of the patients respond successfully (Loerinc et al., 2015). Understanding underlying neural individual differences between responder and non-responder of an exposure therapy could reveal potential biomarkers and therefore optimize individual therapy success. A few systematic reviews already investigate pre-treatment biomarkers based on fMRI activity. In a systematic review of Chakrabarty et al. (2016), the anterior cingulate cortex (ACC), anterior insula (AI), and the amygdala revealed to be a potential prognostic biological marker for predicting therapy response in major depression disorder as well as some anxiety disorders (including OCD and PTSD). These authors summarized, that higher AI and higher dorsal ACC (dACC) pre-treatment activity as well as reduced activity in the amygdala, and pregenual/subgenual ACC is associated positively with therapy response in anxiety disorders. This is in line with another cross diagnostical systematic review in anxiety disorders that highlight the activation of ACC, amygdala, insula, and the hippocampus as predictors of therapy success in anxiety disorders (Santos et al., 2019). However, in another review of Lueken et al. (2016), only ACC shows consistent results across different studies analyzing pre-treatment activity in anxiety disorder, as this was the only region with more positive results relative to null results.

No study investigated brain activity during temporally predictable and unpredictable threat anticipation and confrontation concerning therapy response prediction. Furthermore, only a few studies to date have examined the prediction of treatment outcome in specific phobia using pre-treatment fMRI data (Lange et al., 2020). Therefore, this study aims to identify pre-treatment fMRI activity differences in temporally predictable/unpredictable threat processes between responder of exposure therapy in spider phobics relative to the non-responder of this therapy in ACC, Amygdala, AI, and hippocampus. Given the relevance of the centromedial

amygdala in predictable and unpredictable threat processes, this study focused on the centromedial part of the amygdala (Davis et al., 2010; Pedersen et al., 2019; Siminski et al., 2021). Furthermore, recent studies highlight the relevance during unpredictable threat processes of the BNST in spider phobia (Munsterkotter et al., 2015; Straube et al., 2007). A recent study found higher pre-treatment activity in BNST to threatening pictures is associated with a greater reduction of clinical hypervigilance after an attention bias modification in a sample with several transdiagnostic disorders (anxiety disorders, PTSD, OCD) (Price et al., 2018). As the BNST is a key region for anxiety disorders (e.g. Knight and Depue, 2019; Lebow and Chen, 2016; Shackman and Fox, 2016), and no study so far evaluated the relevance of BNST for exposure therapy response prediction, this study will also evaluate these effects.

Basing on the mentioned systematic reviews and recent studies in the field of predictable and unpredictable threat processes in spider phobia, this study expected activation in ACC, AI, CM, BNST, and Hippocampus can differentiate between therapy responder and non-responder (Chakrabarty et al., 2016; Lueken et al., 2016; Munsterkotter et al., 2015; Santos et al., 2019; Siminski et al., 2021; Straube et al., 2007). Furthermore, this study used the same fMRI paradigm of study 2. Therefore, for further evaluation and validation of the results of study 1 and study 2, this study aimed to analyze differential involvement of CM and BNST in anticipation and confrontation processes in a larger sample of patients with spider phobia. It was assumed, that BNST and CM show no difference in activity during the cue phase and anticipation phase, but the CM showed stronger involvement during spider confrontation relative to the neutral confrontation (study 2).

4.3.2 Methods of study 3

4.3.2.1 Study design of study 3

In that prospective longitudinal study patients with specific phobia underwent in total five to six meetings (see Fig. 16 for a schematic overview of study design). Before inviting for the first meeting, interested participants were screened for exclusion and inclusion criteria during a telephone call. The first meeting was a baseline assessment including a clinical interview, the conduction of the behavior avoidance test (BAT) with a real bird spider (see also in chapter 4.3.2.3 for more information), and the collection of psychometric data (including the SPQ) and blood samples. Afterward, patients had to undergo two different MRI assessments at two

meetings. Then patients underwent a massed one-session exposure therapy in virtual reality (VRET). After the VRET, patients underwent a post-assessment and a 6-month follow-up (FU) assessment after the post measurement. During the post-assessment and the FU assessments, patients also had to undergo the BAT, and psychometric (including the SPQ) and genetic data were collected. A few participants also underwent an optional MRI visit after the post-assessment. For a broader overview of which psychometric data were collected during each assessment, see also in the study protocol of Schwarzmeier et al. (2020).

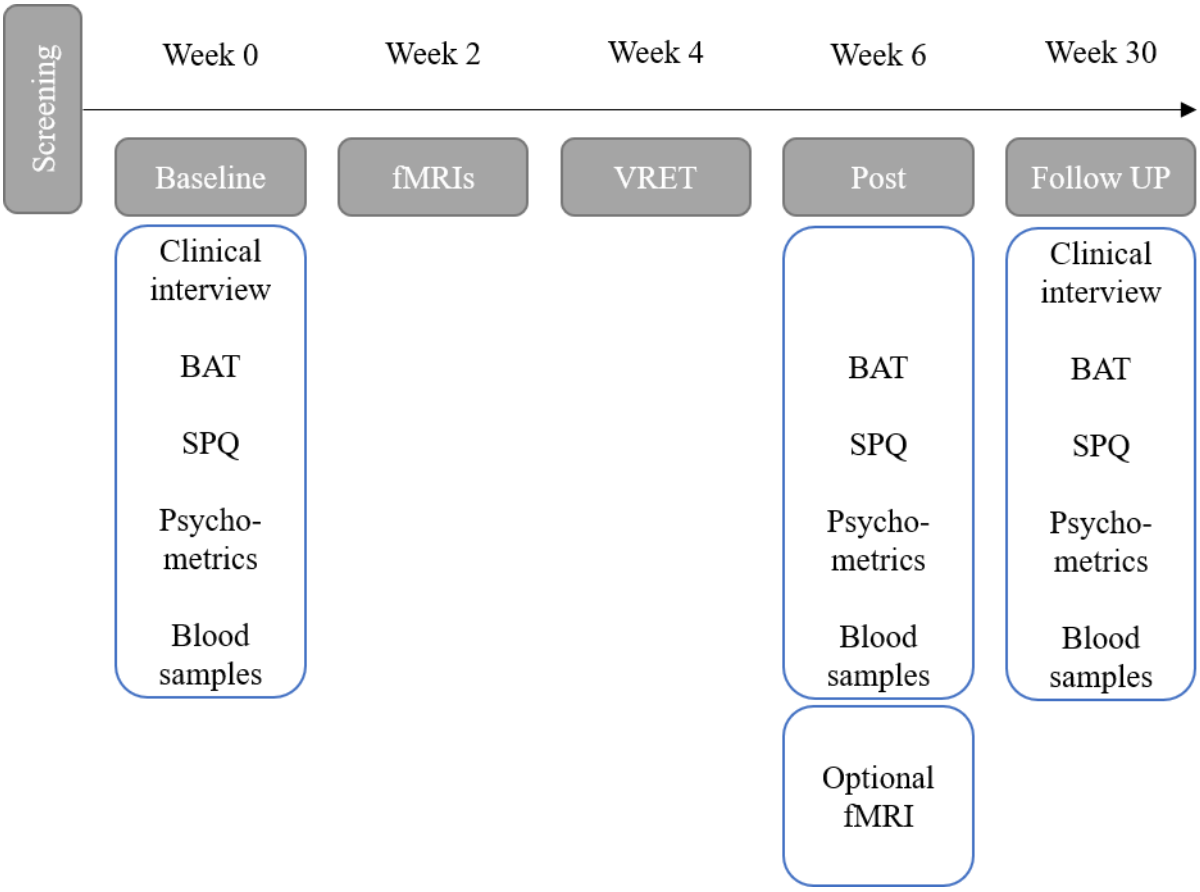

Fig 16. Schematic overview of study design of study 3.

BAT = Behavior Avoidance test; SPQ = Spider phobia questionnaire; VRET = Virtual reality exposure treatment. Participants had to visit five to six meetings, whereas three meetings (baseline assessment: symptom severity, fMRI) were conducted before the therapy session in VRET and two to three meetings were conducted after the VRET to evaluate short-term therapy effects (Post) as well long term effects (Follow up).

4.3.2.2 Treatment of study 3

At the end of the last fMRI assessment, patients were handed a psychoeducational manual adapted from Herrmann et al. (2017) which the patients had to read before the treatment. In this manual information was provided about the function of anxiety, etiology of anxiety, and factors that lead to a maintaining of anxiety. Furthermore, patients were given information about the therapy rationale of the exposure therapy. Directly before the massed one-time exposure in VR the therapist and the patients briefly summarized the psychoeducational manual and patients had to write down their expectations of what would happen during the exposure. The VRET (VT+ research systems, VTplus GmbH, Würzburg) session consisted of five standard scenarios, which were ideally completed by every patient (Fig. 17). Experimental control was established using the VR-software CyberSession (CS-Expo 5.6, VTplus GmbH, Würzburg, Germany; see www.cybersession.info for detailed information). The virtual environment was generated by a Source SDK (Source SDK 2013 Multiplayer, Valve Corporation, Bellevue, Washington, USA) based modification (VrSessionMod 0.6, VTplus GmbH, Würzburg, Germany) and displayed via a Z800 3D head-mounted display (HMD; eMagin, NY, USA). Patients were able to move around the VR environment using a gaming controller.

In the beginning, patients had to get used to the VR environment. Patients were placed in a classroom (see Fig. 17a). Afterward, the first scenario was explained. Before each scenario, the scenario was explained what should be done and the subjective fear on a scale from "0 = no fear at all" to "100 = extremely strong fear." was asked. Then patients had to do the task and were repeatedly asked how the subjective level of anxiety is. If they reach a subjective anxiety score of 20 or less or gave three times in a row the same fear value, patients were placed to the start position of the first scenario, and then the next scenario was explained. In the first scenario, there was a small black spider in a box at the end of the room and the patients had to go as close as possible to the box and band over it to watch the spider carefully (see Fig. 17b). During the second scenario, an oversized spider hanged under a door. Patients had to go through the door and then turn around and go as close as possible back to the spider and look up to the spider (see Fig. 17c). The task of the third scenario was to approach a crawling big spider on the floor and stand directly before it. Afterward, patients had to bend down to the spider (see Fig. 17d). During the fourth scenario, two spiders were placed in the room (one crawling on the floor, the other one was behind a table at the wall). Patients had to approach firstly to the spider at the wall as close as possible, without controlling for the other spider. Afterward, the patients had to focus only on the spider on the floor (see Fig. 17e). In the fifth scenario, four big spiders crawled on the floor and patients had to approach two spiders at the end of the room and focus

only on them without looking behind them to the other spiders in the room (see Fig. 17f). After the VRET, patients were asked to evaluate whether their pre-therapy expectations had been met. Afterward, patients received the Igroup Presence Questionnaire (IPQ; (Schubert, 2003)) for measuring the sense of presence experienced in a virtual environment. The maximum time of exposure duration was 2.5 hours.

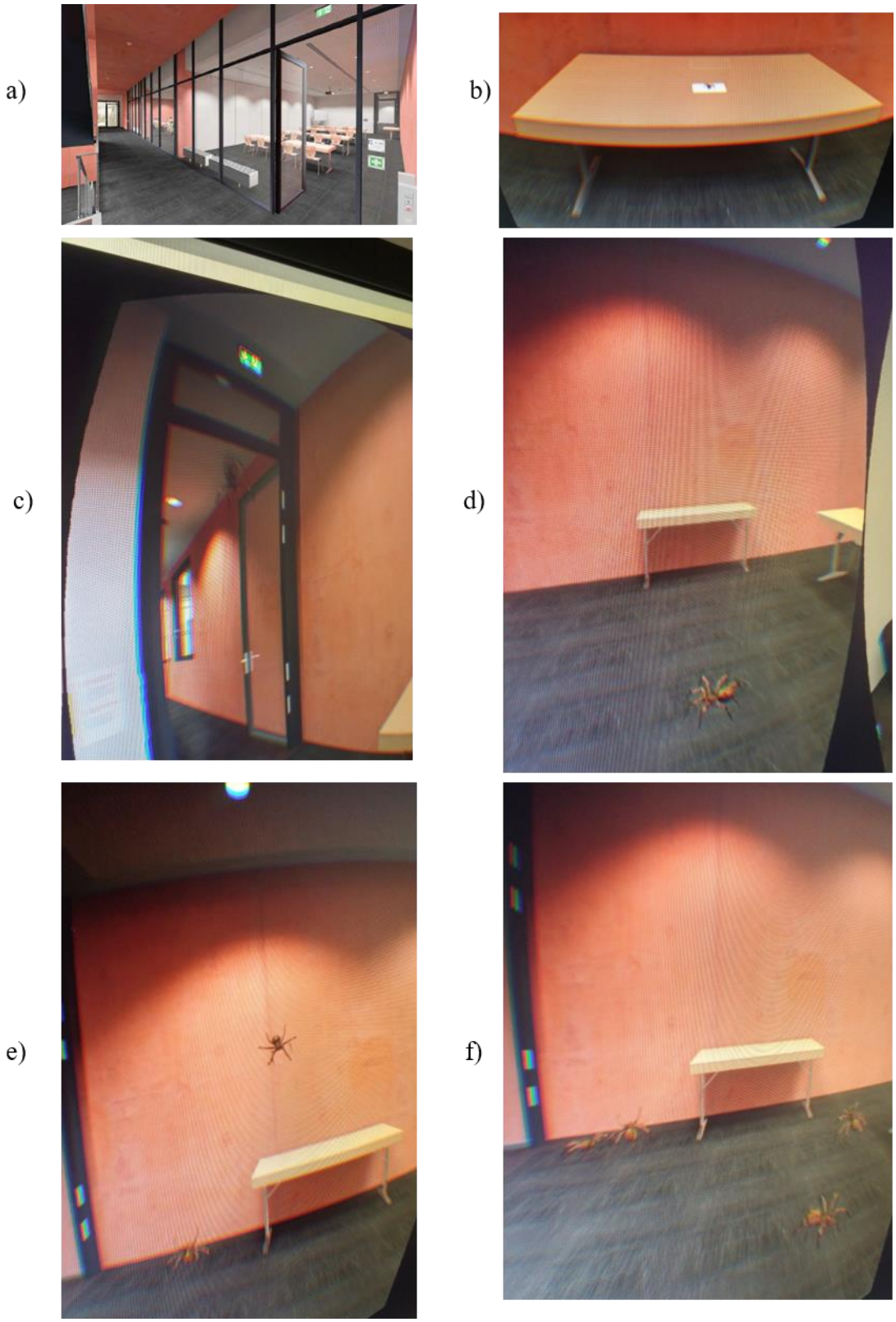

Fig 17. Examples of VRET used by this study (VT+Expo2 Spider, © VTplus GmbH).

a) the classroom where exposure to spiders took place. b) first scenario c) second scenario; d) third scenario; e) fourth scenario; f) fifth scenario.

4.3.2.3 Clinical assessments

To define the clinical severity of spider phobia, two measurements were chosen based on the work of Schwarzmeier et al. (2020). One measurement was the German translation of the Spider Phobia Questionnaire SPQ (Hamm, 2006; Klorman et al., 1974) to evaluate spider phobia on a dimensional level of psychopathology. The questionnaire consists of 31 items (the maximum score per item is 1). The English version shows a high internal consistency of 0.91 and a test-retest correlation of 0.94 (Muris and Merckelbach, 1996) (Muris & Merckelbach, 1996). The sum score was used to define the dimensional outcome. Another measurement was on a behavioral level using a Behavior avoidance test (BAT, Fig. 18.). In the BAT, the spider was placed in a box at the end of a three-meter-long table. The patient sat at the other end of the table and had to pull the box toward him/her with his/her right hand using a crank (see Fig 18). The final distance in cm between the spider and the patient was used to define the behavioral outcome.

Fig 18. Example of Behaviour avoidance test (BAT) used in study 3.

Picture was provided by Schwarzmeier et al. (2020) [3]. The spider was placed in a box on the left side of the table. The participants sit on the other side of the table and had to pull the box toward him/her with his/her right hand using a crank.

4.3.2.4 Definition of therapy response

Based on the work of Schwarzmeier et al. (2020) classification as a responder depended on a significant reduction in two criteria outcomes. Firstly, the patient has to show a reduction of the subjective symptom severity of 30 percent from the baseline meeting to the FU meeting using

[3] I am a co-author of this article. As it is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, it is possible to reuse material in any form, unless it is not used for commercial purposes and the original work is cited.

the German version of the SPQ (Hamm, 2006; Klorman et al., 1974). An in vivo behavior avoidance test (BAT) was used to determine the second outcome criterion. A reduction of 50 percent from baseline meeting to FU meeting of the final distance between the spider and the patient was considered sufficient reduction to be classified as a responder. In this analysis presented here, patients were classified as a responder if they fulfill both criteria (30 percent reduction from baseline to FU in the SPQ and 50 percent of the final distance in BAT from baseline to FU).

To evaluate group-based differences in the success of therapy, two-way factor ANOVAs with the between factor group (non-responder / responder) and the within factor time point (Baseline / Follow up), and the criterium outcome SPQ sum score/ BAT final distance as the dependent variable were calculated. Calculations were conducted using SPSS software (Version 26.0. Armonk, NY: IBM Corp). An alpha level of $p < .05$ was considered statistically significant. To correct for multiple tests, post-hoc analyses of significant group*time points interactions were Bonferroni corrected.

4.3.2.5 Sample characteristics of study 3

The patients from study 3 were taken from a larger project described elsewhere (Schwarzmeier et al., 2020). Diverse opportunities were used to recruit patients including flyers, posters, social media, local advertisements, university recruitment systems, specialized outpatient centers, and medical practices. Inclusion and exclusion criteria were already described in study 2, but see also Schwarzmeier et al. (2020) for more detailed information. In the analysis of study 3, only patients participating in the University hospital of Würzburg were included. In total 82 patients underwent all assessments (see Fig. 19 for detailed information of exclusion of patients during the study). During data analysis, 15 participants were excluded because of incomplete fMRI data, excessive head motion over 3 mm/°, and/or if they did not press the button during the attention task at least one time (like study 2). Therefore, the final sample consist of 28 non-responder and 39 responder. Groups did not differ in age and sex distribution, SPQ Baseline sum score, BAT Baseline final distance, and the scales of the IPQ indicating a similar sense of experience in the virtual environment (table 4). The study is in line with the declaration of Helsinki and the study protocol has been approved by the Ethics Committees of the Medical Faculties at Würzburg University (proposal number 330/15). The participants revealed an explanation of total study protocol and gave afterwards the written informed consent. The study has been registered at ClinicalTrials.gov (ID: NCT03208400).

Table 4. Sample characteristics of study 3

variable	non-responder (n = 28)		responder (n = 39)		statistics	
					χ^2 (1)	*p*
sex	(3 m, 25 w)		(4 m, 35 w)		0.004	.952
	M	*SD*	*M*	*SD*	*t(65)*	*p*
age	29.29	10.17	30.23	10.58	0.37	.715
SPQ_baseline	23.04	2.24	22.69	1.89	0.68	.500
BAT_baseline	176.70	54.20	160.54	63.56	1.09	.280
SPQ_FU	15.46	2.89	11.82	2.28	5.77	.001**
BAT_FU	110.95	49.89	35.21	31.68	5.80	.001**
SPQ_delta	0.33	0.13	0.48	0.10	5.30	.001**
BAT_delta	0.35	0.22	0.80	0.16	9.45	.001**
IPQ_PRES	4.69	1.22	4.74	1.31	-0.21	.837
IPQ_SP°°	22.56	3.94	22.11	4.42	0.42	.673
IPQ_INV°°	13.29	6.08	14.90	6.20	-1.03	.306
IPQ_REAL°	13.29	4.24	12.97	4.46	0.38	.702

Note: SPQ, the German translation of the Spider phobia Questionnaire (Hamm, 2006; Klorman et al., 1974); BAT, Behaviour avoidance test; SPQ_baseline, SPQ sum score at baseline assessment; BAT_baseline, the final distance between spider and patient in BAT during baseline assessment; SPQ_FU, SPQ sum score at follow up assessment; BAT_FU, the final distance between spider and patient in BAT during follow up assessment; SPQ_delta, percentual SPQ reduction from baseline SPQ sum score to follow up SPQ sum score; BAT_delta, the percentual reduction from the final distance between spider and patient in BAT from baseline to follow up; IPQ, Igroup Presence Questionnaire (Schubert, 2003); IPQ_PRES, general presence item of IPQ; IPQ_SP, spatial presence scale of IPQ; IPQ_INV, Involvement scale of IPQ; IPQ_REAL, Experienced realism scale of IPQ. °, df = 64, because of one missing item; °°, df = 63, because of two missing items; **, $p < .001$.

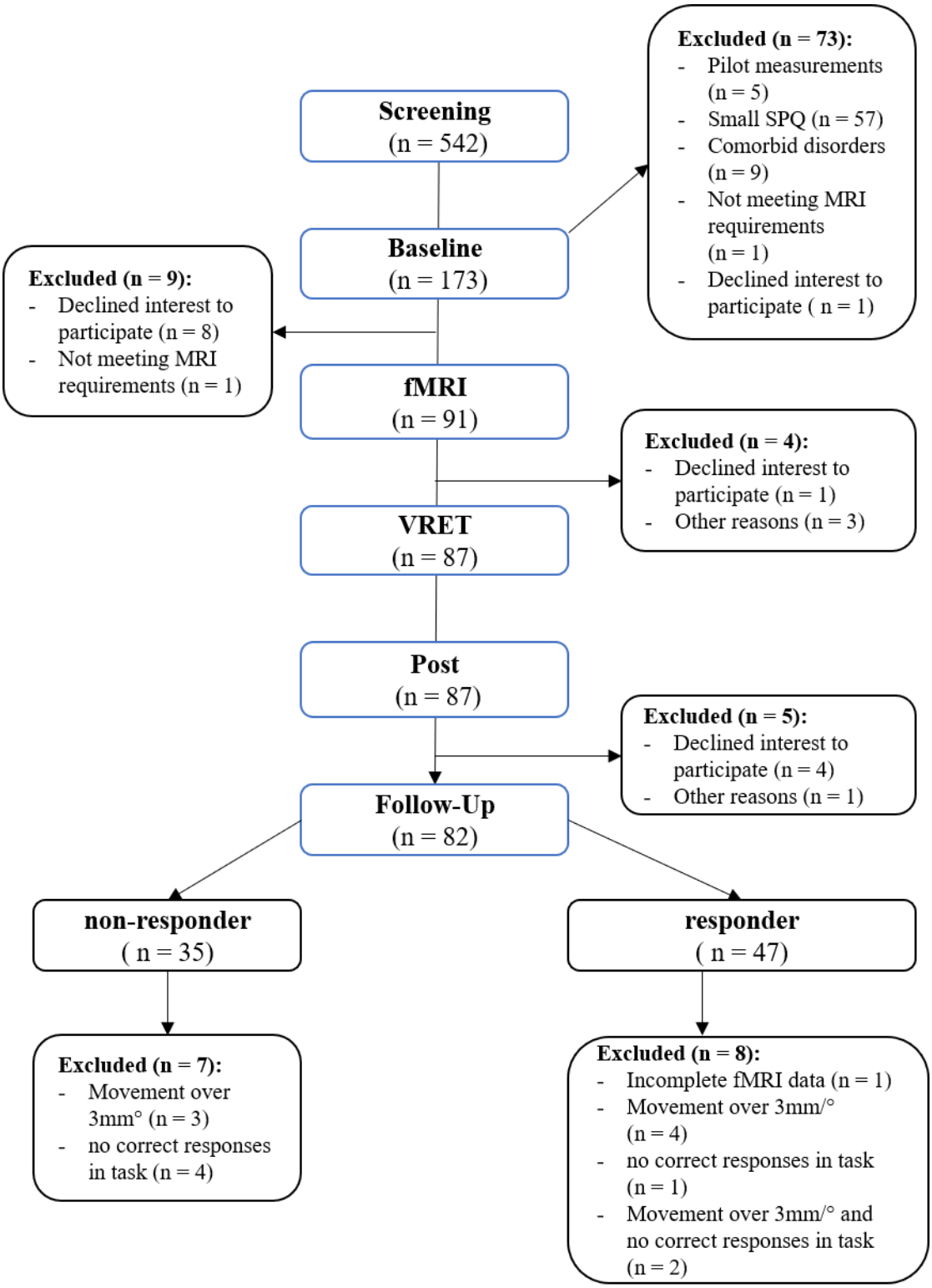

Fig 19. CONSORT diagram of data analysis in study 3.

4.3.2.6 fMRI data collection and analysis

In study 3 the same fMRI paradigm, subjective ratings of the paradigm, fMRI acquisition, and fMRI pre-processing were used as described in study 2.

Analyses were restricted based on specific regions, which has been shown relevant for therapy response prediction (Chakrabarty et al., 2016; Lueken et al., 2016; Santos et al., 2019), or (temporally) predictable/unpredictable threat processes (Munsterkotter et al., 2015; Siminski et al., 2021; Straube et al., 2007). The bilateral ACC and the bilateral Hippocampus masks were created based on the AAL atlas included in the WFU toolbox (Maldjian et al., 2003; Tzourio-Mazoyer et al., 2002). The AI mask was similar to a recently published study and kindly shared by the authors (Strosche et al., 2021). The centromedial amygdala mask was based on maximum probability maps (Amunts et al., 2005) and is included in the SPM Anatomy toolbox (Eickhoff et al., 2005). The BNST mask was provided by Torrisi et al. (2015). Furthermore, whole-brain analyses were conducted for exploratory analyses. To identify group differences in the cue phase, for each ROI analysis and the whole brain analysis a two-sample t-contrast was conducted in the contrast cue spider vs. cue bird. For the anticipation phase and the confrontation phase, for each ROI analysis and the whole brain analysis full factorial models were calculated. This study focused on the interaction effects with the factor group. Therefore, analyses were restricted to the interactions group*predictability, group*valence, and group*predictability*valence. To correct for multiple corrections, we used a free threshold Cluster enhance method (TFCE) with FWE correction ($p_{FWE} < 0.05$) and 5000 permutations. TFCE analyses were conducted with the TFCE-toolbox (http://dbm.neuro.uni-jena.de/tfce). For exploratory post-hoc analyses of interaction effects, the estimated parameters were extracted from the significant clusters of the interaction effects and then further analyzed in SPSS software (Version 26.0. Armonk, NY: IBM Corp). An alpha level of $p < .05$ was considered statistically significant.

To evaluate the differential involvement of CM and BNST during the three phases of interest, estimated betas for each ROI for each parameter were extracted using MarsBar (http://marsbar.sourceforge.net/). For the cue phase an ANOVA with the within factors region (CM / BNST), valence (spider/bird), and the estimated parameter for each ROI as a dependent variable was calculated. For the anticipation phase and the confrontation phase an ANOVA with the within factors region (CM / BNST), predictability (predictable/unpredictable), valence (spider/bird), and the estimated parameter for each ROI as a dependent variable were calculated. Calculations were conducted using SPSS software (Version 26.0. Armonk, NY: IBM Corp). An alpha level of $p < .05$ was considered statistically significant.

4.3.3 Results of study 3

4.3.3.1 Group-based evaluation of therapy success

To evaluate group-based differences of therapy success of the VRET two-way factor ANOVAs with the between factor group (non-responder / responder) and the within factor time point (baseline / follow up) and the outcome criterium (SPQ sum score/ BAT final distance in cm) as dependent variables were calculated. For both outcome criteria there were main effects of time point and a significant group*time point interaction as well. Post-Hoc analyses with Bonferroni-correction indicated, that both groups showed significant reductions in SPQ sum score (non-responder: $t(27) = 12.25$, $p < .001$, $d_z = 2.315$; responder: $t(38) = 24.73$, $p < .001$, $d_z = 3.960$; Fig. 20), and the final distance in BAT (non-responder: $t(27) = 6.53$, $p < .001$, $d_z = 1.234$; responder: $t(38) = 14.06$, $p < .001$, $d_z = 2.251$; Fig. 20). However, responder showed a lower SPQ sum score and a lower final distance in BAT as compared to the non-responder during the follow up (SPQ: $t(65) = 5.77$, $p < .001$, $d = 1.431$; BAT: $t(65) = 5.80$, $p < .001$, $d = 1.439$; Fig. 20). Furthermore, responder showed a greater reduction in SPQ sum score, as well as in the final distance in BAT from the baseline to the follow up relative to the non-responder (SPQ: $t(65) = 4.48$, $p < .001$, $d = 1.111$; BAT: $t(65) = 3.70$, $p < .001$, $d = 0.918$).

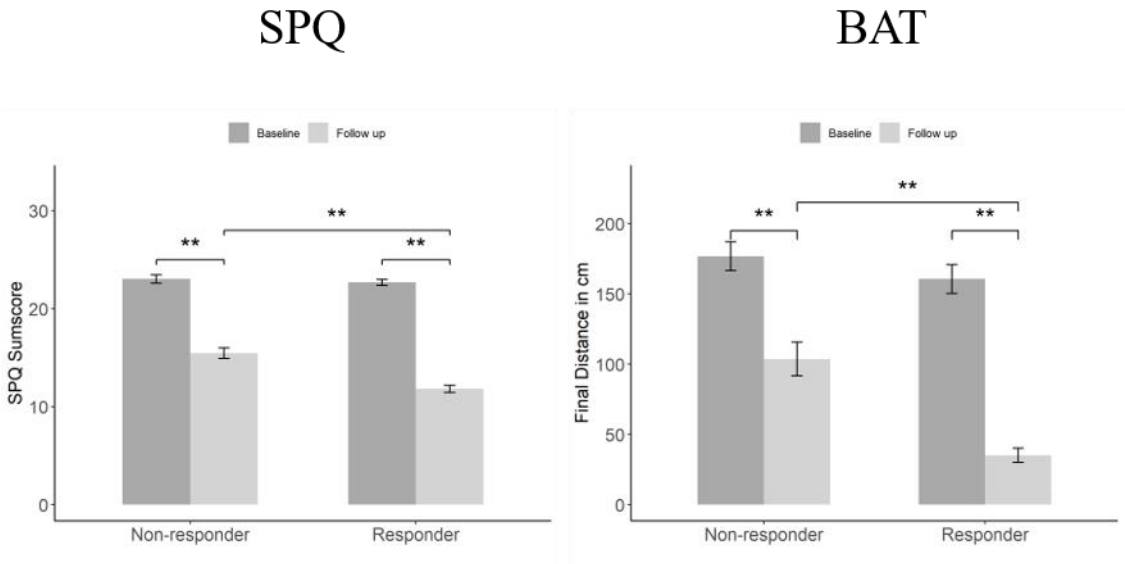

Fig 20. SPQ sum scores and final distances of BAT during baseline and follow up in study 3.

Bar graphs for SPQ (left) indicated the sum score of SPQ. Bar graphs for BAT (right) indicated the final distance in cm between the spider and the participant stratified for groups and time point (mean ± SE; * $p < .001$).

4.3.3.2 Subjective ratings

4.3.3.2.1 Subjective ratings of anticipation cues

Three-way-factor ANOVAs with the between factor group (non-responder / responder) and the within factors valence (spider/neutral anticipation) and predictability (unpredictable/predictable anticipation) were conducted for self-reported ratings of pleasantness, arousal, and anxiety. For the ratings of pleasantness and arousal there were significant main effects of predictability (pleasantness: $F(1,65) = 8.92$, $p = .004$, $\eta_p^2 = .121$; arousal: $F(1,65) = 13.91$, $p < .001$ $\eta_p^2 = .176$). Unpredictable cues were rated as more unpleasant and more arousing then predictable cues. Analyses also revealed main effects of valence in each self-reported rating (pleasantness: $F(1,65) = 111.95$, $p < .001$, $\eta_p^2 = 0.633$; arousal: $F(1,65) = 102.57$, $p < .001$, $\eta_p^2 = 0.612$; anxiety: $F(1,65) = 108.19$, $p < .001$, $\eta_p^2 = 0.625$). Spider cues were rated as more unpleasant, more arousing and more anxiety-inducing as bird cues (Fig. 21). All other effects were not significant (Table A 10, appendices of study 3).

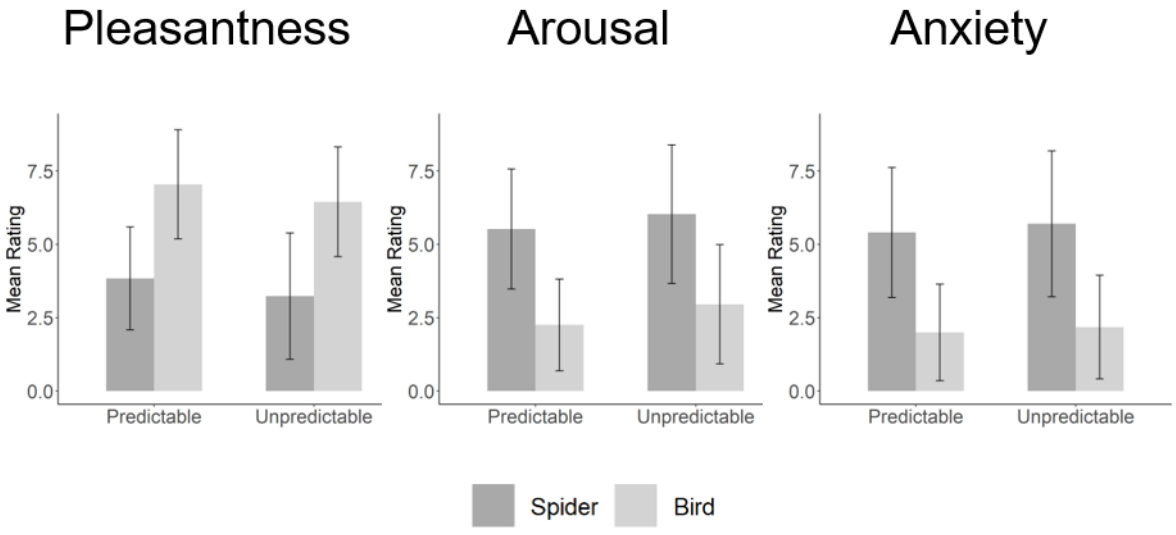

Fig 21. Means and standard deviations of self-reported ratings of anticipation cues of study 3.

4.3.3.2.2 Subjective ratings of pictures

Two-way-factor ANOVAs with the between factor group (non-responder / responder) and the within factor valence (spider/neutral confrontation) were calculated for self-reported ratings of pleasantness, arousal, and anxiety. Analyses revealed main effects of valence in each self-reported rating (pleasantness: $F(1,65) = 418.54$, $p < .001$, $\eta_p^2 = 0.881$; arousal: $F(1,65) = 380.57$, $p < .001$, $\eta_p^2 = 0.854$; anxiety: $F(1,65) = 353.44$, $p < .001$, $\eta_p^2 = 0.8475$). Spider pictures were rated as more unpleasant, more arousing and more anxiety-inducing as bird pictures (Fig. 22). All other effects were not significant (Table A 11, appendices of study 3).

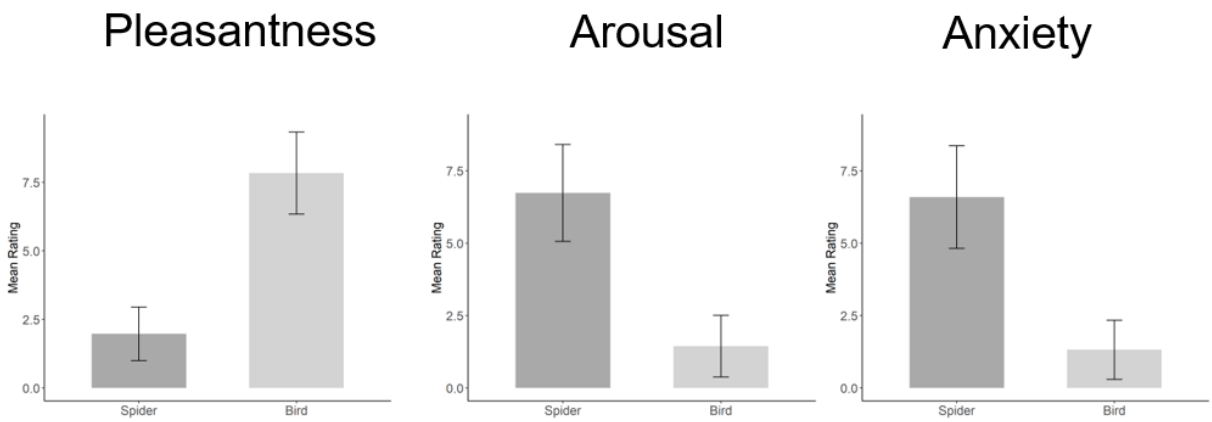

Fig 22. Means and standard deviations of self-reported ratings of confrontation pictures of study 3.

4.3.3.3 fMRI data: ROI analyses for prediction of therapy response during cue phase

ROI Analyses revealed significant group differences in the ACC in the contrast cue spider vs cue bird (Fig. 23; Cluster 1: k = 2, $p_{FWE} = .042$, TFCE = 74.97, peak coordinates = -12 35 20; Cluster 2: k = 2, $p_{FWE} = .045$, TFCE = 72.87; peak coordinates = 12 35 5; Cluster 3: k = 3, $p_{FWE} = .045$, TFCE = 72.17; peak coordinates = -3 32 17). In all three clusters, non-responder showed higher ACC peak voxel activity during spider cues relative to the bird cues (Fig 23). No further effects were revealed in the ROI analyses or the exploratory whole-brain analyses during the cue phase.

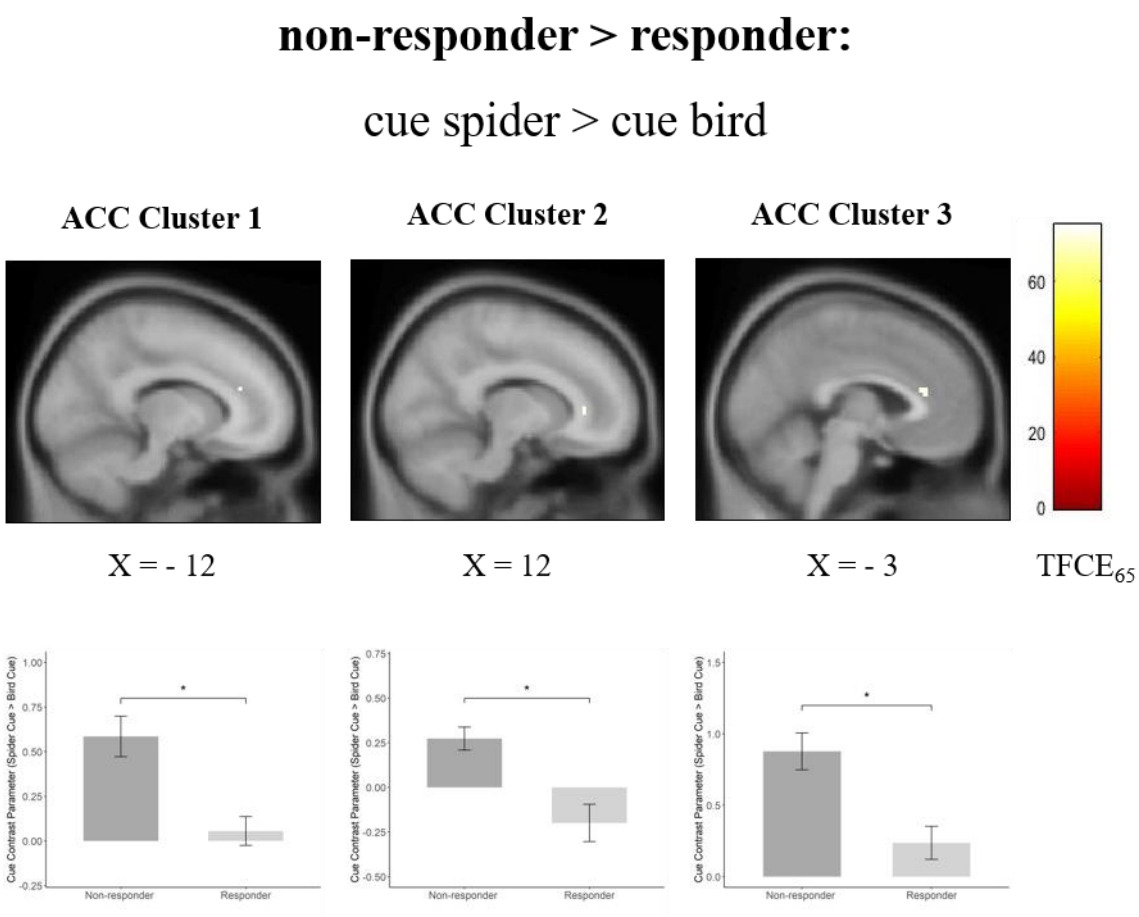

Fig 23. Significant clusters in the ROI analyses during cue phase in study 3.

Clusters are overlaid on MNI template 152 T1. Bar graphs display the peak voxel estimated contrast parameter of spider cue compared to bird cue stratified for group (non-responder vs responder) (mean ± SE; * p_{FWE} < .05). Non-responder showed higher ACC activity during spider cue relative to the bird cue in all clusters (Cluster 1: k = 2, p_{FWE} = .042, TFCE = 74.97, peak coordinates = -12 35 20; Cluster 2: k = 2, p_{FWE} = .045, TFCE = 72.87; peak coordinates = 12 35 5; Cluster 3: k = 3, p_{FWE} = .045, TFCE = 72.17; peak coordinates = -3 32 17).

4.3.3.4 fMRI data: ROI analyses for prediction of therapy response during the anticipation phase

ROI Analyses revealed a significant cluster in ACC for the group*valence interaction (Fig. 24; Cluster: k = 2, p_{FWE} = .029, TFCE = 4550.93, peak coordinates = -12 41 -1). Explorative post hoc analysis showed that non-responder showed higher ACC activity during spider anticipation relative to the neutral anticipation as compared to the responder (t(65) = 4.06, p < .001, d =

1.007). No further effects revealed in the ROI analyses or the exploratory whole-brain analyses during the anticipation phase.

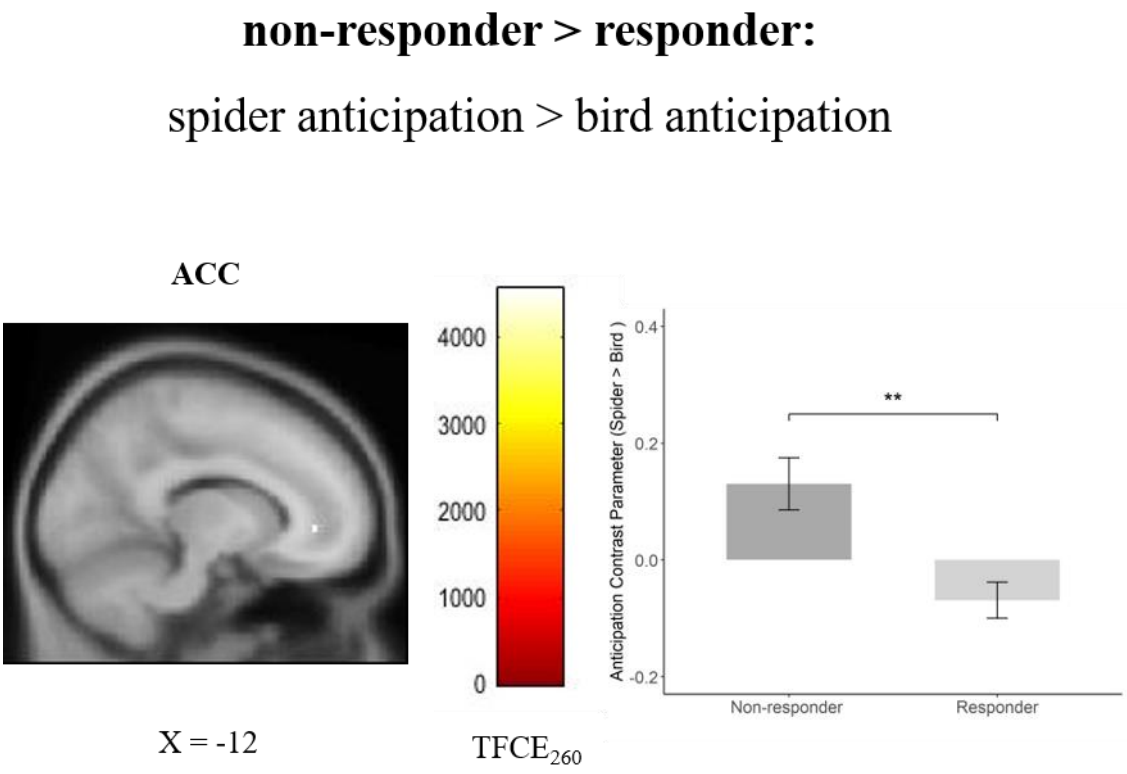

Fig 24. Significant cluster in ACC during anticipation phase in study 3.

Cluster is overlaid on MNI template 152 T1. Bar graphs display the cluster estimated contrast parameter of spider anticipation compared to bird anticipation stratified for group (non-responder vs responder) (mean ± SE; * $p < .001$). Analyses revealed a significant cluster for the interaction of group*valence (Cluster: k = 2, $p_{FWE} = .029$, TFCE = 4550.93, peak coordinates = -12 41 -1). Explorative post hoc analysis indicated that non-responder showed higher ACC activity during spider anticipation relative to the bird anticipation as compared to the responder ($t(65) = 4.06$, $p < .001$, d = 1.007).

4.3.3.5 fMRI data: ROI analyses for prediction of therapy response during confrontation phase

ROI Analyses revealed a significant group*valence interaction in BNST (Fig. 25; Cluster: k = 1, $p_{FWE} = 0.046$, TFCE = 14.44, peak coordinates = 6 2 -1). Explorative post hoc analyses revealed, that responder and non-responder did not differ in BNST activity during spider confrontation ($t(65) = -1.47$, $p = .148$, d = -0.364), bird confrontation ($t(65) = 0.80$, $p = .425$, d

= 0.198), as well in the difference of spider confrontation relative to the bird confrontation (t(65) = -1.52, *p* = .132, d = -0.377). However, BNST showed higher activity in responder during spider pictures relative to bird pictures (t(38) = 2.61, *p* = .013, d = 0.418; Fig.25), but in non-responder BNST did not show a different activity during spider confrontation and bird confrontation (t(27) = 0.26, *p* =.800, d = 0.049; Fig.25).

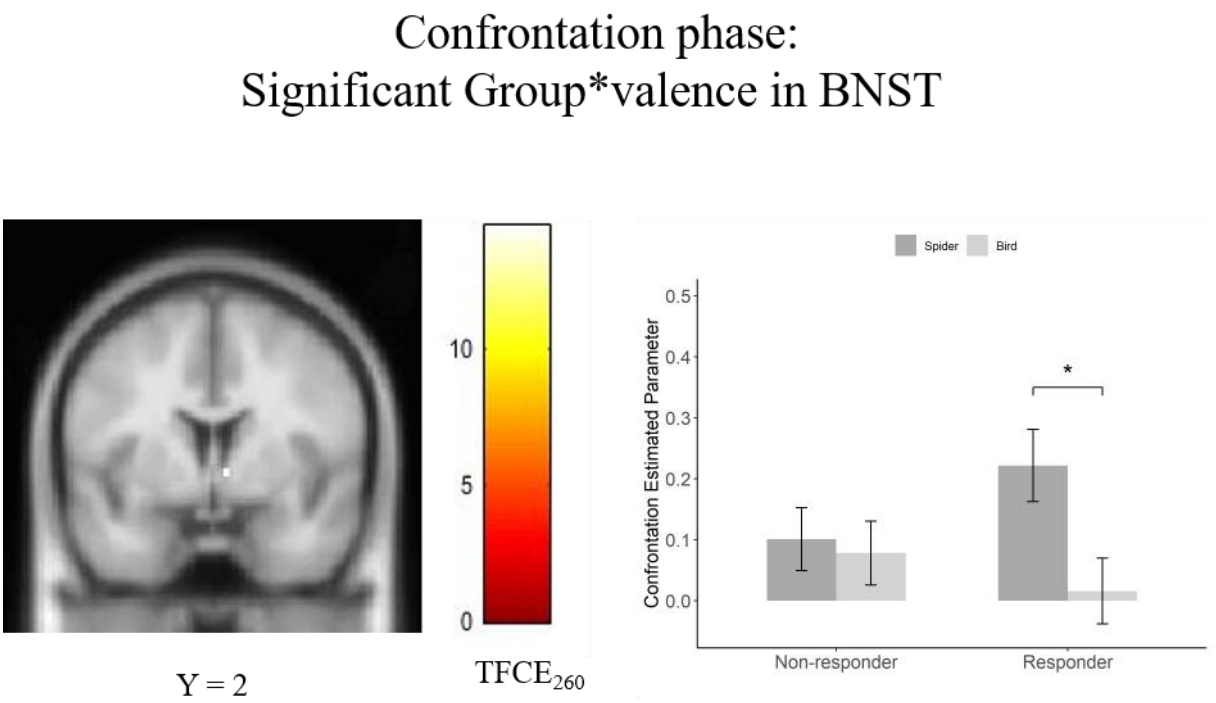

Fig 25. Significant cluster in BNST during confrontation phase in study 3.

Cluster is overlaid on MNI template 152 T1. Bar graphs display the cluster estimated parameter of spider and bird confrontation stratified for group (non-responder vs responder) (mean ± SE; * $p < .05$). Analyses revealed a significant cluster for the interaction of group*valence (Cluster: k = 1, p_{FWE} = 0.046, TFCE = 14.44, peak coordinates = 6 2 -1). Explorative post hoc analyses revealed, that BNST showed higher activity in responder during spider pictures relative to bird pictures (t(38) = 2.61, *p* = .013, d_z = 0.418), but in non-responder BNST did not show a different activity during spider confrontation and bird confrontation (t(27) = 0.26, *p* =.800, d_z = 0.049).

4.3.3.6 fMRI data: Involvement of CM and BNST in the cue phase, anticipation phase, and confrontation phase

Group differences in each phase in CM and BNST activity are already assessed in the prior analysis. In this chapter, differential involvement of CM and BNST during each phase is in the focus of analysis and no further group evaluation was conducted as no direct hypotheses are provided for the factor group concerning differential involvement of CM and BNST.

4.3.3.6.1 Cue phase:

A two-way-factor ANOVA with the within factors region (CM / BNST) and valence (spider cue/bird cue) and the estimated betas of each ROI as the dependent variable was calculated. Analyses revealed a significant main effect of region ($F(1, 66) = 13.72$, $p < .001$, $\eta_p^2 = 0.172$). BNST was more active over both cues relative to the CM. Furthermore, analyses revealed a main effect for valence ($F(1, 66) = 34.54$, $p < .001$, $\eta_p^2 = 0.344$). Patients showed higher CM and BNST activity during the spider cue relative to the bird cue. The region*valence interaction was not significant ($F(1, 66) = 3.48$, $p = .066$, $\eta_p^2 = 0.050$).

4.3.3.6.2 Anticipation phase:

A three-way-factor ANOVA with the within factors region (CM / BNST), predictability (predictable anticipation / unpredictable anticipation), and valence (spider anticipation/bird anticipation) and the estimated betas of each ROI as the dependent variable was calculated. Analyses revealed a significant main effect of region ($F(1, 66) = 17.70$, $p < .001$, $\eta_p^2 = 0.211$). BNST was more active over all conditions relative to the CM. Furthermore, analyses revealed a main effect for valence ($F(1, 66) = 24.59$, $p < .001$, $\eta_p^2 = 0.271$). Patients showed higher CM and BNST activity during the spider anticipation relative to the bird anticipation. No further effect was significant (Table A 12, appendices of study 3).

4.3.3.6.3 Confrontation phase

A three-way-factor ANOVA with the within factors region (CM / BNST), predictability (predictable confrontation / unpredictable confrontation), and valence (spider pictures / bird pictures) and the estimated betas of each ROI as the depended variable was calculated. Analyses revealed a main effect for valence ($F(1, 66) = 33.49$, $p < .001$, $\eta_p^2 = 0.337$), which is qualified

by a significant interaction of region and valence F(1, 66) = 40.21, $p < .001$, $\eta_p^2 = 0.379$). CM showed higher activity compared to the BNST during spider confrontation relative to the bird confrontation (t(66) = 6.34, $p < .001$, $d_z = 0.76$; Fig. 26). No further effect was significant (Table A 13, appendices of study 3).

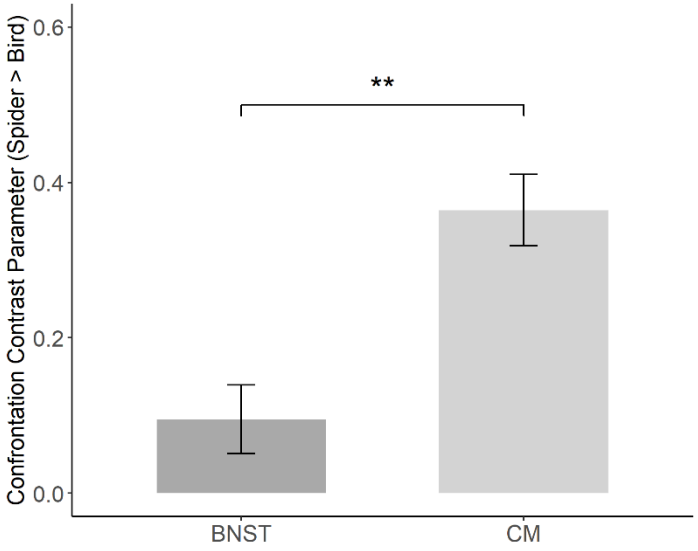

Fig 26. Significant region*valence interaction during threat confrontation in study 3.

Bar graphs display the estimated contrast parameter of spiders compared to bird confrontation stratified for region (BNST vs CM) (mean ± SE; ** $p < .001$). CM showed higher activity during spiders as compared to bird confrontation (t(66) = 6.34, $p < .001$, $d_z = 0.76$).

4.3.4 Discussion of study 3

This is the first study, which evaluates the therapy response prediction based on pre-treatment fMRI activity during temporally predictable and unpredictable threat processes in spider phobia. Analyses revealed that non-responder showed higher ACC activity during spider cues relative to the bird cue as well during spider anticipation relative to the bird anticipation compared to the responder. However, the responder showed higher BNST activity during spider confrontation relative to the bird confrontation, but the non-responder did not show differences in BNST activity regarding threat processing.

4.3.4.1 Pre-treatment activation and prediction for therapy response in spider phobia

Results during the cue phase and anticipation phase are in line with recent systematic reviews indicating the potential of ACC activity towards therapy response prediction (Chakrabarty et al., 2016; Lueken et al., 2016; Santos et al., 2019). Higher ACC activity in non-responder during the phobic cue phase and phobic anticipation phase is in line with another study investigating the long-term prediction potential of pre-treatment fMRI activity in patients with social anxiety (Månsson et al., 2015). In that study, non-responder showed higher dACC activity to self-referential criticism. Furthermore, in that study dACC activity showed the highest accuracy for therapy response prediction. Another study analyzing pre-treatment fMRI activity in patients with panic disorder found also higher activity in the right pregenual ACC in non-responder to a safety signal (Lueken et al., 2013). In a recent systematic review analyzing neural activity in anxiety disorders relative to control groups and fear-induced paradigms in healthy samples, higher ACC activity has been found as a core region for anxiety disorders including specific phobia, as well for healthy samples (Chavanne and Robinson, 2020). In line with this review, elevated ACC activity was shown in spider phobics relative to a control group during temporally unpredictable spider anticipation (Straube et al., 2007), as well in a study with healthy subjects, which found higher ACC activity during different states of temporally unpredictable anticipation periods (Herrmann et al., 2016). Therefore, higher ACC activity during spider anticipation phases relative to the bird anticipation phases might indicate an inappropriate neural apprehension in non-responder. However, there is also evidence for higher ACC activity in responder in patients with social anxiety (Frick et al., 2020; Klumpp et al., 2017), or in earlier studies with GAD (Nitschke et al., 2009; Whalen et al., 2008). One reason for the mixed results might be followed by the type of fMRI task which the participants had to underwent. In the present study, patients were confronted with disorder-specific stimuli, whereas the other studies did not use disorder-specific stimuli. However, these mixed results indicate a need for replication in other studies with spider phobia to confirm higher ACC activity in non-responder during phobic anticipation processes.

This is the first study that found BNST activity concerning the prediction of exposure therapy in specific phobia. Responder showed higher BNST activity during spider confrontation relative to the bird confrontation. Results are in line with a recent study that found higher pretreatment BNST activity during initial responses to threatening pictures were related to a greater reduction of clinical hypervigilance after an attention bias modification in patients with transdiagnostic disorders (anxiety disorders, PTSD, OCD) (Price et al., 2018). Higher BNST activity during the phobic confrontation in responder supports the emotional processing

theory of Foa and Kozak (1986), which suggests, that the fear network needs to be sufficiently activated during threat confrontation to allow modifications of behavior through the exposure therapy. However, there is also evidence that did not confirm emotional processing theory as the underlying mechanism for the success of exposure therapy (e.g Rupp et al., 2017). Therefore, also because post hoc analysis for the group*valence interaction in BNST was on an exploratory level, further research in spider phobia is necessary to validate higher pretreatment BNST activity during the phobic confrontation as a benefitting factor for a better outcome of exposure therapy.

Besides these effects, no further effect was found in other regions. One reason might be that both groups showed a reduction of symptoms and on average non-responder fulfill also the criteria of sufficient therapy response in the SPQ criterium outcome according to Schwarzmeier et al. (2020). Therefore, this might lead to fewer different brain activity patterns between both groups. Furthermore, predictability does not play a relevant role in the prediction of therapy response in spider phobia. Other studies found higher amygdala and BNST activity in patients with panic disorder or GAD during temporally unpredictable threat anticipation (Brinkmann et al., 2017a; Buff et al., 2017). Therefore, future studies should evaluate therapy response prediction of the activity of the amygdala and BNST concerning threat predictability in other anxiety disorders.

4.3.4.2 Differential involvement of CM and BNST during threat processes in spider phobia

A further aim of this study was to replicate the analysis method of study 1 and study 2 in a larger sample with patients with specific phobia. Results are in line with study 2 indicating higher involvement of BNST during anticipation processes relative to the CM independent of valence and predictability mode. Likewise, in study 1 and study 2, during confrontation CM was more active compared to the BNST during spider confrontation relative to the bird confrontation. Therefore this study confirms suggested theories of higher relevance of BNST in anticipation processes and higher relevance of CM in confrontation processes (Klumpers et al., 2017; Siminski et al., 2021).

4.3.4.3 Limitations of study 3

Results of the present study are only limited to spider phobia, which results in a need for replication in other anxiety disorders. Furthermore, analyses were restricted to fMRI-related

pre-treatment prediction and therefore did not consider other relevant factors contributing to the therapy response (Lueken et al., 2016). In this study, the choice of treatment was an exposure therapy conducted in one session with virtual reality. Therefore, predictions are only limited to this kind of treatment.

4.3.5 Conclusion of study 3

Study 3 confirmed the results of study 2 regarding the differential involvement of BNST and CM in threat processes in patients with spider phobia. Regarding the prediction of therapy response, lower pretreatment ACC activity during threat anticipation phases and engaged BNST activity during threat confrontation might be a beneficial biomarker for therapy success in spider phobia. However, considering the explorative post hoc analysis of interaction effects, these results need to be replicated in future studies.

5 General discussion

Fear is supposed to be the response to predictable threats, whereas anxiety displays the response to unpredictability (Davis et al., 2010). This thesis aims to contribute to the understanding of the dissociative interactive roles of CM and BNST during fear and anxiety-related processes. Several studies investigated the involvement of the amygdala and BNST during temporally predictable and unpredictable threat processes but showed several limitations (Clauss et al., 2019; Hur et al., 2020; Klumpers et al., 2017; Naaz et al., 2019; Pedersen et al., 2019). Therefore, a new fMRI paradigm was developed which aimed to address these limitations, which was used in the three studies of the dissertation. In study 1 this thesis evaluated this differential involvement in a large healthy sample (n = 109). Furthermore, study 1 aimed to investigate individual differences based on *NPSR1* genotype, as no study investigated the modulative role of genetics in differential involvement of CM and BNST. Results indicated higher BNST activity compared to the CM during the threat cue relative to the neutral cue. Contrary, during confrontation CM showed higher involvement compared to the BNST during threat confrontation relative to the neutral confrontation. Modulation analyses by *NPSR1* rs324981 genotype indicated higher BNST activity relative to the CM in unpredictable anticipation relative to predictable anticipation in T-carriers compared to AA-carriers. Additionally, AA-carriers showed higher differential involvement of CM and BNST during all confrontation conditions compared to the T-carrier.

No study so far evaluated direct activity comparisons of CM and BNST in a sample with an anxiety disorder relative to healthy controls during temporally predictable and unpredictable threat anticipation paradigm. Therefore, in study 2 the paradigm of study 1 was adapted to spider phobia relevant stimuli and patients with spider phobia were compared to age and gender-matched healthy controls. Healthy controls showed higher CM and BNST activity during the predictable anticipation relative to the unpredictable anticipation compared to the patients. Furthermore, the BNST was more activated relative to the CM during each anticipation phase. However, patients showed higher CM activity compared to the BNST during spider pictures relative to the bird pictures, whereas healthy controls did show this higher engagement only during the predictable condition.

Recent studies highlight the potential of fMRI activity for the prediction of therapy response in anxiety disorders (Chakrabarty et al., 2016; Lueken et al., 2016; Santos et al., 2019). No study so far investigated pre-treatment activity during temporally predictable and unpredictable threat processes for therapy response prediction. Therefore, the aim of study 3 was to investigate the

predictive potential for exposure therapy response not only of CM and BNST activity, but also other relevant areas in the brain, which are relevant for therapy response prediction (Chakrabarty et al., 2016; Lueken et al., 2016; Santos et al., 2019). Results indicate, that during the cue presentation phase and anticipation phase ACC activity predicts therapy response. Non-responder showed higher activity during the spider cue/anticipation relative to the bird cue/anticipation in comparison to the responder. However, the responder showed elevated BNST activity during spider confrontation, but the non-responder did not show this differential BNST activity. Furthermore, study 3 aimed to validate the analysis approach of study 1 and study 2 and investigated the differential involvement of CM and BNST also in a larger sample with spider phobia patients and replicated results of study 2.

In the following, the results of the three studies in the thesis for each of the three relevant phases will be discussed separately. Afterward, limitations of this thesis and a general outlook will be provided.

5.1 CM and BNST involvement during the cue presentation in healthy subjects and patients with spider phobia

This phase displays a short-lasting anticipation period (2 seconds), where an individuum must anticipate either an aversive event or a neutral event. This cue was always coupled with the appearance of the expecting event. This makes it comparable to the full reinforced fear acquisition phases in fear conditioning studies or some phases of studies in the field of the amygdala and BNST activation during predictable and unpredictable threat processes: the phasic condition in the study of Alvarez et al. (2011), the explicit condition in (Naaz et al., 2019), or the phasic analyses of several studies investigating the onset of a temporally unpredictable threat anticipation period (Brinkmann et al., 2017a; Brinkmann et al., 2017c; Buff et al., 2017; Figel et al., 2019; Herrmann et al., 2016).

Both regions showed engaged activity during threat processing in all three studies. In rodents, BNST was supposed to be more activated in a state of ambiguity (Goode et al., 2019), also recently supported again in mice suggesting higher activity of anterior ventral BNST during partially reinforced cues relative to the fully reinforced cues (Glover et al., 2020). However, in a recent study, it has been shown, that BNST is also involved in strong initial conditioning processes in rodents (Williams and Lattal, 2020). In line with that, Figel et al. (2019) observed engaged phasic activation during temporally unpredictable threat anticipation

in BNST in patients with social anxiety relative to a control group. Therefore, the results of the dissertation add new insights into the functional role of BNST in human threat processing, as during this phase, the event was always coupled with an aversive or neutral event and therefore leads to a state of predictable threat, equivalent to a fully reinforced fear acquisition. The central nucleus of the amygdala is supposed to play a key role in forward fear conditioning (Ressler et al., 2020). In line with that, studies in healthy humans and patients with anxiety disorders indicate a strong involvement of amygdala during onset of anticipation period (Brinkmann et al., 2017c; Buff et al., 2016; Herrmann et al., 2016). Therefore, higher engagement of centromedial amygdala in the three studies of this dissertation is in line with recent evidence of humans and animals and highlights the relevance of centromedial amygdala in cue related threat processes.

In all three studies, BNST showed a general higher involvement during this phase compared to the CM independent of the valence of the cue. Therefore, this thesis offers new insights into the interplay of CM and BNST during anticipation processes. BNST is more involved in general during short-lasting anticipation cues. In study 1 analysis revealed a region*valence interaction, indicating stronger threat differentiation in BNST relative to the CM. This interaction could not be replicated in study 2 and study 3. This could indicate that the stronger involvement of BNST relative to the CM in threat differentiation depends on the kind of confrontation which is anticipated. Furthermore, no differential involvement of CM and BNST was found based on group-based inter-individual differences, whether on the genetic kind (study 1) nor the clinical kind (study 2). Future research might consider investigating the activity of the amygdala and BNST during short anticipation cues in other anxiety disorders or other group-based comparisons in healthy samples.

5.2 CM and BNST involvement during anticipation phase in healthy subjects and patients with spider phobia

This phase followed the cue phase and lasted three seconds to 20 seconds. Cues presented the type of temporal predictability. During the temporally predictable cue, participants knew exactly the moment of threat presentation. Contrary, during the temporally unpredictable cue, participants did not know when the threat presentation will be, but they knew, that the threat presentation will happen for sure. Analyses of the anticipation phase are comparable to the analysis of the anticipation period of Pedersen et al. (2019), the analysis of Hur et al. (2020), or when only focusing on the unpredictable conditions to the analyses investigating activation of

amygdala and BNST during whole periods of temporally unpredictable threat anticipation (e.g Brinkmann et al., 2017a; Buff et al., 2017; Figel et al., 2019; Herrmann et al., 2016; Straube et al., 2007), as all these studies used anticipation cues which were always followed by the coupled event.

The analyses of the three studies only revealed the main effects of valence in study 2 and study 3. This could indicate that prolonged activity of CM and BNST towards valence depends also on the confrontation as well on the sample characteristics. In study 1 a sample of 109 healthy participants were anticipating an aversive picture and sound combination, whereas in the second study 21 patients with spider phobia and 21 healthy controls, and in the third study 69 patients with spider phobia anticipated spider pictures or bird pictures. Using the same paradigm in other anxiety samples or healthy samples (e.g. shocks) might clarify the modulation of the prolonged activity of BNST and CM towards valence.

In none of the three empirical studies in this dissertation significant main effects predictability or a significant predictability*valence interactions were observed in the analyses of anticipation phases. This is similar to other studies (Hur et al., 2020; Pedersen et al., 2019). Hur et al. (2020) did not evaluate a main effect of predictability, but also found no difference in the dorsal amygdala and BNST activity during predictable threat anticipation and unpredictable anticipation. In a study by Pedersen et al. (2019) also no main effect of predictability revealed during the anticipation period. Like the three studies in this dissertation, both studies also used a paradigm with temporally unpredictable/predictable threats, but the valence of threat was always sure. Contrary to that, Naaz et al. (2019) showed that BNST was more active during an anticipation period of an ambiguous confrontation in comparison to the explicit anticipation period, whereas the basolateral amygdala was more engaged during the explicit anticipation in comparison to the ambiguous confrontation. These described findings result from analyses of the anticipation periods. When focusing on whole blocks of predictable and unpredictable threats there is more evidence for the modulation of activity through the factor predictability. Somerville et al. (2013) found a significant main effect of predictability in BNST in sense of higher activity during blocks within temporally unpredictable events relative to blocks with temporally predictable events, although another study with a similar fMRI paradigm by Pedersen et al. (2019) could not replicate this main effect during whole blocks. Other studies that found different activation patterns of the amygdala and BNST used different forms of predictable/unpredictable threat. In two studies BNST was more active during an unpredictable threat context (Alvarez et al., 2011; Alvarez et al., 2015). In another study, there

was a clear dissociation of the amygdala, which was more engaged in patients with spider phobia during the predictable symptom provocation task, whereas the BNST was engaged during the unpredictable symptom provocation task (Munsterkotter et al., 2015). These studies suggest the higher engagement of BNST during unpredictable prolonged threat responses, as in these studies analyses of whole blocks were conducted including anticipation processes and confrontation processes. All in all, these evidences and the results of this thesis lead to the hypothesis that it does not matter for the threat processing if the threat is temporally predictable or unpredictable when focusing only on the anticipation period of temporally predictable and unpredictable threat, where the valence was always surely predictable. This hypothesis is only valid for the anticipation period, whereas for whole blocks or in ambiguous valence conditions the factor predictability seems more relevant. Future studies might consider evaluating the differential involvement during anticipation of different threat forms in relation to temporal predictability and threat mode (surely predictable in its valence vs. ambiguous in its valence). However, there is already one study that compared amygdala and BNST activity in a paradigm with either temporally unpredictable full reinforced anticipation period as well during a temporally unpredictable anticipation period of an ambiguous event, but this study did not evaluate temporally predictable conditions (Clauss et al., 2019). Results of this study suggest higher BNST activity relative to the amygdala only during the ambiguous anticipation period but not during the full reinforced anticipation period.

Results of study 1 and study 2 showed interindividual differences concerning the factor predictability. Study 1 discovered differential involvement of BNST relative to the CM during unpredictable anticipation relative to the predictable anticipation in T-carriers compared to the homozygote AA-carriers. Therefore, higher engagement of BNST relative to the CM during unpredictable anticipation processes can be modulated through inter-individual differences. Also, in study 2 a group-based difference was observed concerning predictability. The healthy control group showed higher activity during predictable anticipation processes relative to the unpredictable processes. The two group interactions support theories highlighting the relevance of unpredictability for anxiety disorders (Grupe and Nitschke, 2013), but results reveal novelty as no study so far investigated genetic modulations of differential involvement of CM and BNST during threat processes nor investigated different activity pattern between patients with spider phobia and a healthy control group concerning predictability. Therefore, the group-predictability interactions during the anticipation phase of this thesis offer a basis for further investigation in other genotype studies or other samples with different anxiety disorders to validate these interactions.

Like the cue phase, in all three studies, BNST showed higher engagement over all anticipation conditions relative to the CM. Therefore, BNST seems to be more relevant in general anticipation processes without a threat focus relative to the CM independent of the duration of the anticipation period.

5.3 CM and BNST involvement during confrontation phase in healthy subjects and patients with spider phobia

This phase displays a confrontation of four seconds, which is followed directly after the anticipation period. Participants did know the valence of confrontation for sure, whereas the exact moment of confrontation presentation differed depending on the condition. Analysis of this phase is comparable to studies investigating the activity of amygdala and BNST during confrontation periods following a temporally predictable and/or unpredictable anticipation period (Brinkmann et al., 2018; Klumpers et al., 2017; Naaz et al., 2019; Pedersen et al., 2019; Sarinopoulos et al., 2010; Somerville et al., 2013).

In all three studies of this dissertation CM was more active relative to the BNST during threat confrontation relative to the neutral confrontation. This is in line with Klumpers et al. (2017), that also showed similar effects during an unpredictable confrontation, where the threat might occur relative to the neutral confrontation. In the three studies used in this dissertation, the valence of confrontation was always sure. Therefore, it is possible to assume, that CM is more relevant for threat differentiation processes, at least for the confrontation mode used in this dissertation, as three comparable studies showed engaged activity of CM relative to BNST.

Only study 1 showed a main effect of predictability, and none of the studies showed a predictability*valence interaction except the explorative post-hoc analysis of the control group in the second study, that found a region*predictability*valence interaction. Other studies investigating the main effects of predictability in confrontation phases did also not reveal significant main effects of predictability (Pedersen et al., 2019; Somerville et al., 2013). However, another study found higher BNST activity during the ambiguous threat relative to the explicit threat (Naaz et al., 2019). Likewise, to the anticipation period, different involvement of BNST and CM during the confrontation period might depend on the threat mode, as during ambiguous threat BNST might be involved, but during threat, which is always predictable in its valence, BNST is not more involved depending on the predictability. This hypothesis should explicitly be tested in future studies. Furthermore, patients in study 2 and study 3 did not show

differential activity towards predictability during the phobic confrontation, this leads to the assumption that predictability does not play a relevant role in phobic processing in patients with spider phobia. To strengthen this assumption, future studies should directly compare reactivity in aversive confrontation (e.g. shocks or aversive pictures and sounds), and phobic confrontation concerning temporal predictability in patients with spider phobia.

Furthermore, inter-individual differences of CM and/or BNST activity were found during the confrontation phase in all three studies. In study 1, AA-carriers showed higher differentiation between CM and BNST over all conditions. This demonstrates, that more research should evaluate interindividual differences in CM/BNST activity interplay, as only one other study also investigated group-based differences in the differential involvement of CM and BNST in a sample with mostly healthy participants (Clauss et al., 2019). In study 2, healthy controls showed higher engagement of CM relative to the BNST only in predictable threat differentiation processes, but not during the unpredictable threat differentiation, whereas in patients CM was more active relative to the BNST during spider pictures as compared to neutral pictures independent of predictability. This raises the assumption that predictability is not relevant for spider phobia during the confrontation but results of study 2 need to be replicated in future studies as post-hoc-analyses of study 2 were on an explorative basis. In study 3 no inter-individual differences regarding the differential involvement of CM and BNST were evaluated but the factor group was analyzed in each ROI separately. Responder showed engaged activity during spider pictures relative to neutral pictures in BNST, but non-responder did not show this differential activity in BNST. CM activity was not predictive at all. This was the first study that evaluated BNST activity in the prediction of exposure therapy response in patients with an anxiety disorder. Therefore, these explorative results offer a basis for further investigation in other anxiety disorders and indicate to add BNST investigation in the ROI analyses of future studies.

5.4 Limitations and general outlook

Besides the limitations already mentioned in the studies, there are also some general limitations of the dissertation. The BNST ROI is very small and therefore a 3-Tesla scan might not be the best procedure to measure BNST activity. However, there is evidence for BNST activity in comparable fMRI paradigms in other studies using a 3-Tesla scan for data acquisition (Brinkmann et al., 2018; Brinkmann et al., 2017a; Buff et al., 2017; Figel et al., 2019), but future studies should consider using a data acquisition with a higher resolution. The mask of

Torrisi et al. (2015), which was used in this dissertation is appropriate for investigations in the 7-Tesla magnet field (Torrisi et al., 2015). Therefore, future studies could directly build upon studies conducted in this thesis and enable comparable analyses to this dissertation.

Results of the three studies are limited to the specific group characteristics of samples. Study 1 investigated inter-individual differences based on the *NPSR1* genotype. This genotype has not been associated with anxiety disorders in GWAS studies. Therefore, future studies could investigate inter-individual differences based on genotypes, which has been found relevant also in GWAS studies (e.g Levey et al., 2020). Study 2 and study 3 analyzed activity in patients with spider phobia. Therefore, assumptions are only valid for spider phobia and need more replication in other anxiety disorders or other specific phobias.

Furthermore, the fMRI paradigm used in this dissertation is limited to assumptions in threat processes, where participants know the valence of confrontation. Additionally, following the recommendation of Shackman and Fox (2016) it would not be appropriate to compare anticipation processes and confrontation processes in this thesis, as confrontation and anticipation periods differed in duration. Therefore, a definitive statement about differential involvement of BNST and CM during the anticipation phase and the confrontation phase would not be appropriate. A development of a new fMRI paradigm (see Fig. 27 for an example), which solves these limitations, could provide new insights into differential involvement of CM and BNST in threat processes.

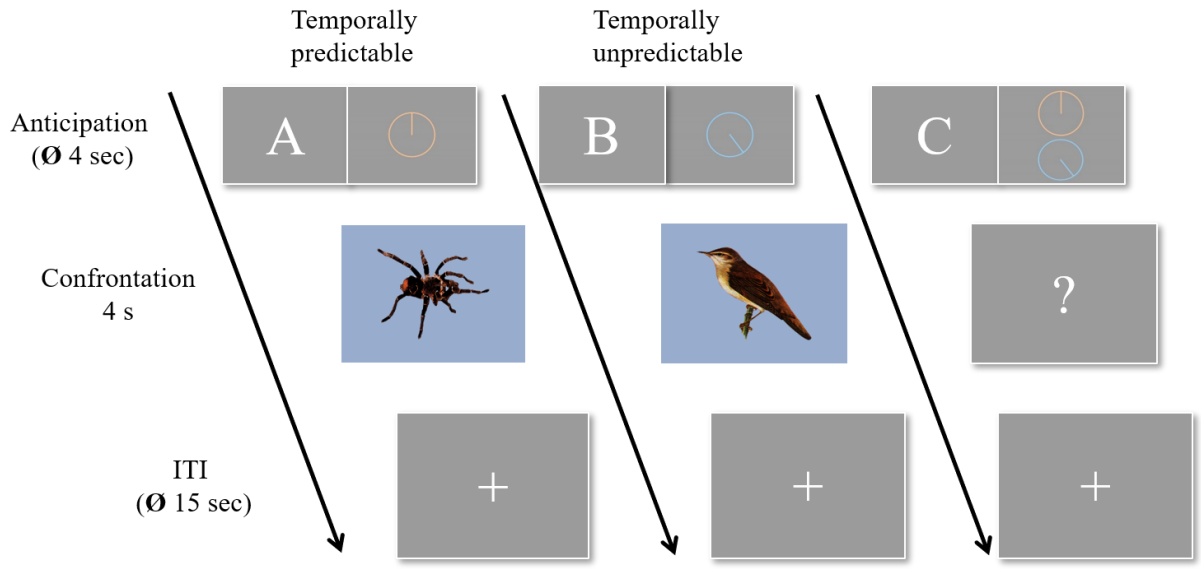

Fig 27. An optimized fMRI threat anticipation paradigm for future studies based on the fMRI paradigm used in study 2 and study 3.

sec = seconds. During the first phase (average 4 seconds), participants see a combination of a letter, that offers information about the valence, and a watch, that offers information about the moment of appearance of the confrontation. During the confrontation phase, the confrontation can be either predictable aversive, predictable neutral, or unpredictable in its valence in line with other studies (eg. Clauss et al., 2019; Sarinopoulos et al., 2010). As anticipation and confrontation phases are equal in the mean duration, activity of CM and BNST could be directly compared during confrontation and anticipation depending on valence (aversive, neutral, unpredictable) and temporal predictability (temporally predictable vs. temporally unpredictable).

6 Conclusion of this dissertation

In sum, this thesis offers new insights into the different contributions of CM and BNST during threat processes. The results demonstrate that BNST is more relevant for anticipation processes independent of the duration of the anticipation period, valence, and predictability of confrontation as compared to the CM. Contrary, during the confrontation phase the CM displays a greater relevance for threat confrontation. This assumption of functional difference needs further proof in other anxiety disorders or other confrontation modes (e.g. fully vs. partly reinforced). Future studies should also evaluate direct activity comparisons of anticipation and confrontation periods with similar mean durations to do conclusive assumptions of different contributions in anticipation and confrontation processes. Furthermore, BNST did show predictive potential for therapy outcome in spider phobia, resulting in an opportunity for an investigation into other anxiety disorders.

7 Appendices of study 1

Table A 1. Subjective ratings of anticipation cues three-way mixed measures ANOVA of study 1

Ratings		*F*-Value	*p*-Value	η_p^2
Unpleasantness	Predictability	33.35	**.001****	**0.238**
	Predictability**NPSR1*	4.34	**.040***	**0.039**
	Valence	138.02	**.001****	**0.563**
	Valence**NPSR1*	0.35	.557	0.003
	Predictability*Valence	0..43	.515	0.004
	Predictability*Valence**NPSR1*	0.057	.781	0.001
Arousal	Predictability	37.68	**.001****	**0.260**
	Predictability**NPSR1*	0.20	.658	0.002
	Valence	169.20	**.001****	**0.613**
	Valence**NPSR1*	1.99	.161	0.018
	Predictability*Valence	0.094	.760	0.001
	Predictability*Valence**NPSR1*	0.029	.865	0.000
Anxiety	Predictability	19.51	**.001****	**0.155**
	Predictability**NPSR1*	1.39	.241	0.013
	Valence	111.90	**.001****	**0.514**
	Valence**NPSR1*	0.34	.564	0.003
	Predictability*Valence	13.88	**.001****	**0.116**
	Predictability*Valence**NPSR1*	0.00	.988	0.000

Note: Factor predictability includes two levels (predictable and unpredictable); Factor valence includes two levels (aversive and neutral); Factor *NPSR1* includes two levels (*NPSR1* rs324981 AA vs. AT,TT genotypes); df for all effects = 1, 107.* $p < .05$, ** $p \leq .001$.

Table A 2. Cue response three-way mixed measures ROI ANOVA of study 1

Effect	*F*-Value	*p*-Value	η_p^2
Region	41.76	**.001****	**0.281**
Region**NPSR1*	0.76	.784	0.001
Valence	40.00	**.001****	**0.272**
Valence**NPSR1*	1.60	.209	0.015
Region*Valence	4.57	**.035***	**0.041**
Region*Valence**NPSR1*	0.20	657	0.002

Note. Factor region includes two levels (CM and BNST); Factor valence includes two levels (aversive and neutral cue); Factor *NPSR1* includes two levels (*NPSR1* rs324981 AA vs. AT,TT genotypes); df values for all effects = 1, 107. * $p < .05$, ** $p \leq .001$.

Table A 3. Anticipation response four-way mixed measures ROI ANOVA of study 1

Effect	*F*-Value	*p*-Value	η_p^2
Region	97.90	**.001****	**0.478**
Region**NPSR1*	1.91	.170	0.018
Predictability	0.06	.804	0.001
Predictability**NPSR1*	0.07	.793	0.001
Valence	3.55	.062	0.032
Valence**NPSR1*	1.68	.198	0.015
Region*Predictability	0.61	.437	0.006
Region*Predictability**NPSR1*	5.03	**.027***	**0.045**
Region*Valence	1.77	.186	0.016
Region*Valence**NPSR1*	0.45	.502	0.004
Predictability*Valence	0.45	.502	0.004
Predictability*Valence**NPSR1*	0.19	.668	0.002
Region*Predictability*Valence	0.15	.700	0.001
Region*Predictability*Valence**NPSR1*	0.08	.773	0.001

Note. Factor region includes two levels (CM and BNST); Factor predictability includes two levels (predictable and unpredictable anticipation); Factor valence includes two levels (aversive and neutral anticipation); Factor *NPSR1* includes two levels (*NPSR1* rs324981 AA vs. AT,TT genotypes); df values for all effects = 1, 107. * $p < .05$, ** $p \leq .001$.

Table A 4. Confrontation response four-way mixed measures ROI ANOVA of study 1

Effect	*F*-Value	*p*-Value	η_p^2
Region	40.22	**.001****	**0.273**
Region**NPSR1*	4.16	**.044***	**0.037**
Predictability	16.67	**.001****	**0.135**
Predictability**NPSR1*	0.40	.530	0.004
Valence	36.01	**.001****	**0.252**
Valence**NPSR1*	0.50	.483	0.005
Region*Predictability	1.42	.237	0.013
Region*Predictability**NPSR1*	1.33	.252	0.012
Region*Valence	28.81	**.001****	**0.212**
Region*Valence**NPSR1*	0.12	.735	0.001
Predictability*Valence	0.03	.856	0.000
Predictability*Valence**NPSR1*	0.14	.708	0.001
Region*Predictability*Valence	0.54	.466	0.005
Region*Predictability*Valence**NPSR1*	1.27	.263	0.012

Note. Factor region includes two levels (CM and BNST); Factor predictability includes two levels (predictable and unpredictable confrontation); Factor valence includes two levels (aversive and neutral confrontation); Factor *NPSR1* includes two levels (*NPSR1* rs324981 AA vs. AT,TT genotypes); df values for all effects = 1, 107. * $p < .05$, ** $p \leq .001$.

Table A 5. Significant results of exploratory whole-brain analyses for cue presentation, anticipation phase, and confrontation phase of study 1

	x	y	z	t(max)	k
Cue presentation					
Aversive > Neutral					
Right Insula	36	19	6	4.38	250
Left Cingulate Gyrus	-4	26	27	5.83	552
Right Caudate	19	-6	19	4.08	408
Left Pyramis	-18	-64	-29	4.58	70
Anticipation phase					
P (aversive – neutral) > UP (aversive – neutral)					
Left Middle Occipital Gyrus	-34	-86	9	3.89	144

Table A 5. (continued)

Confrontation phase					
UP > P					
Left Anterior Cingulate	-6	29	25	3.80	386
Aversive > Neutral					
Right Middle Occipital Gyrus	42	-70	9	7.74	499
Right Thalamus	3	-28	3	8.99	806
Left Medial Frontal Gyrus	-8	46	31	5.90	261
Left Insula	-32	12	-2	5.62	79
Left Middle temporal Gyrus	-45	-67	7	8.07	288
Neutral > aversive					
Right Postcentral Gyrus	62	-17	24	-4.54	145
Left Inferior Parietal Lobule	-55	-22	30	-5.30	2267
Right Middle Frontal Gyrus	42	41	17	-4.02	263
Left Middle Frontal Gyrus	-24	4	40	-4.28	93
Left Precentral Gyrus	-46	22	36	-4.71	297
UP (aversive – neutral) > P (aversive – neutral)					
Right Superior temporal Gyrus	45	-18	8	4.27	98
Right Middle Frontal Gyrus	29	62	22	3.83	74
Left Thalamus	-9	-23	10	5.40	12
Right Culmen	3	-48	-21	4.48	76
Left Superior temporal Gyrus	-40	-33	17	4.48	120
Left Culmen	3	-48	-21	4.48	76
P (aversive – neutral) > UP (aversive – neutral)					
Right Parahippocampal Gyrus	24	-43	-3	-5.94	101

Note. P, predictable; UP, unpredictable; (x,y,z), Talairach coordinates of maximally activated voxel; t(max), t value for maximally activated voxel; k, number of voxels in the activated cluster

8 Appendices of study 2

Table A 6. Subjective ratings of anticipation cues ANOVA of study 2

Ratings		*F*-Value	*p*-Value	η_p^2
Unpleasantness	Predictability	6.644	**.014***	**0.142**
	Predictability*Group	.291	.593	0.007
	Valence	30.692	**.001****	**0.434**
	Valence*Group	20.834	**.001****	**0.342**
	Predictability*Valence	.367	.548	0.009
	Predictability*Valence*Group	1.309	.259	0.009
Arousal	Predictability	5.376	**.026***	**0.118**
	Predictability*Group	.449	.507	0.011
	Valence	39.876	**.001****	**0.499**
	Valence*Group	29.359	**.000****	**0.423**
	Predictability*Valence	4.526	**.040***	**0.102**
	Predictability*Valence*Group	3.831	.057	0.087
Anxiety	Predictability	1.61	.212	0.039
	Predictability*Group	.273	.604	0.007
	Valence	36.931	**.001****	**0.480**
	Valence*Group	26.575	**.001****	**0.399**
	Predictability*Valence	1.274	.266	0.031
	Predictability*Valence*Group	2.72	.107	0.064

Note: Factor predictability includes two levels (predictable and unpredictable); Factor valence includes two levels (aversive and neutral); Factor *group* includes two levels (patients and hc); df values for all effects = 1, 40.* $p < .05$, ** $p \leq .001$.

Table A 7. Cue response ROI ANOVA of study 2

Effect	F-Value	p-Value	η_p^2
Region	12.86	**.001****	**0.243**
Region*Group	0.32	.575	0.008
Valence	14.16	**.001****	**0.262**
Valence*Group	1.990	.166	0.047
Region*Valence	2.21	.145	0.052
Region*Valence* Group	1.48	.231	0.036

Note. Factor region includes two levels (CM and BNST); Factor valence includes two levels (aversive and neutral cue); Factor group includes two levels (patients and hc); df values for all effects = 1, 40. ** $p \leq .001$.

Table A 8. Anticipation response ROI ANOVA of study 2

Effect	F-Value	p-Value	η_p^2
Region	30.11	**.001****	**0.429**
Region* Group	0.56	.460	0.014
Predictability	2.91	.096	0.068
Predictability* Group	6.18	**.017***	**0.134**
Valence	4.21	**.047***	**0.095**
Valence* Group	1.31	.260	0.032
Region*Predictability	0.13	.725	0.003
Region*Predictability* Group	0.02	.902	0.000
Region*Valence	3.88	.056	0.088
Region*Valence* Group	3.68	.062	0.084
Predictability*Valence	1.61	.212	0.039
Predictability*Valence* Group	2.40	.130	0.057
Region*Predictability*Valence	0.23	.634	0.006
Region*Predictability*Valence* Group	0.30	.589	0.007

Note. Factor region includes two levels (CM and BNST); Factor predictability includes two levels (predictable and unpredictable anticipation); Factor valence includes two levels (spider and neutral anticipation); Factor group includes two levels (patients and hc); df values for all effects = 1, 40. * $p < .05$, ** $p \leq .001$.

Table A 9. Confrontation response ROI ANOVA of study 2

Effect	F-Value	p-Value	η_p^2
Region	3.44	.071	0.079
Region* Group	1.18	.284	0.029
Predictability	0.05	.829	0.001
Predictability* Group	0.00	.990	0.000
Valence	8.91	**.005***	**0.182**
Valence* Group	0.32	.576	0.008
Region*Predictability	0.97	.332	0.024
Region*Predictability* Group	0.11	.748	0.003
Region*Valence	12.59	**.001****	**0.239**
Region*Valence* Group	0.08	.774	0.002
Predictability*Valence	0.48	.490	0.012
Predictability*Valence* Group	0.16	.696	0.004
Region*Predictability*Valence	1.69	.201	0.041
Region*Predictability*Valence* Group	5.72	**.022***	**0.125**

Note. Factor region includes two levels (CM and BNST); Factor predictability includes two levels (predictable and unpredictable confrontation); Factor valence includes two levels (spider and neutral confrontation); Factor group includes two levels (patients and hc); df values for all effects = 1, 40. * $p < .05$, ** $p \leq .001$.

9 Appendices of study 3

Table A 10. Subjective ratings of anticipation cues ANOVA of study 3

Ratings		*F*-Value	*p*-Value	η_p^2
Pleasantness	Predictability	8.92	**.004***	**0.121**
	Predictability*Group	1.26	.267	0.019
	Valence	111.95	**.001****	**0.633**
	Valence*Group	2..10	.152	0.031
	Predictability*Valence	0.04	.839	0.001
	Predictability*Valence*Group	1.54	.219	0.023
Arousal	Predictability	13.91	**.001***	**0.176**
	Predictability*Group	1.02	.317	0.015
	Valence	102.57	**.001****	**0.612**
	Valence*Group	0.88	.353	0.013
	Predictability*Valence	0.41	.522	0.006
	Predictability*Valence*Group	0.07	.792	0.001
Anxiety	Predictability	2.62	.110	0.039
	Predictability*Group	0.21	.648	0.003
	Valence	108.19	**.001****	**0.625**
	Valence*Group	0.31	.583	0.005
	Predictability*Valence	0.06	.814	0.001
	Predictability*Valence*Group	0.88	.352	0.013

Note: Factor predictability includes two levels (predictable and unpredictable anticipation); Factor valence includes two levels (spider and bird anticipation); Factor *group* includes two levels (non-responder and responder); df values for all effects = 1, 65.* $p < .05$, ** $p \leq .001$.

Table A 11. Subjective ratings of confrontation pictures ANOVA of study 3

Ratings		F-Value	p-Value	η_p^2
Pleasantness	Valence	481.54	**.001****	**0.881**
	Valence*Group	0.70	.407	0.011
Arousal	Valence	380.57	**.001****	**0.854**
	Valence*Group	0.82	.370	0.012
Anxiety	Valence	353.44	**.001****	**0.845**
	Valence*Group	1.16	.285	0.018

Note: Factor valence includes two levels (spider and bird confrontation); Factor *group* includes two levels (non-responder and responder); df values for all effects = 1, 65. ** $p \leq .001$.

Table A 12. Anticipation response four-way mixed measures ROI ANOVA of study 2

Effect	*F*-Value	*p*-Value	η_p^2
Region	17.70	**.001****	**0.211**
Predictability	3.37	.071	0.049
Valence	24.59	**.001****	**0.271**
Region*Predictability	0.04	.848	0.001
Region*Valence	2.49	.119	0.036
Predictability*Valence	2.70	.105	0.039
Region*Predictability*Valence	0.04	.853	0.001

Note. Factor region includes two levels (CM and BNST); Factor predictability includes two levels (predictable and unpredictable anticipation); Factor valence includes two levels (spider and bird anticipation); df values for all effects = 1, 66. ** $p \leq .001$.

Table A 13. Confrontation response four-way mixed measures ROI ANOVA of study 3

Effect	*F*-Value	*p*-Value	η_p^2
Region	2.75	.102	0.040
Predictability	0.07	.792	0.001
Valence	33.49	**.001****	**0.337**
Region*Predictability	0.16	.900	0.000
Region*Valence	40.21	**.001****	**0.379**
Predictability*Valence	0.02	.897	0.000
Region*Predictability*Valence	0.70	.407	0.010

Note. Factor region includes two levels (CM and BNST); Factor predictability includes two levels (predictable and unpredictable confrontation); Factor valence includes two levels (spider and bird pictures); df values for all effects = 1, 66. ** $p \leq .001$.

References

Alheid, G.F., 2003. Extended amygdala and basal forebrain. Ann N Y Acad Sci 985, 185-205.
Alvarez, R.P., Chen, G., Bodurka, J., Kaplan, R., Grillon, C., 2011. Phasic and sustained fear in humans elicits distinct patterns of brain activity. NeuroImage 55, 389-400.
Alvarez, R.P., Kirlic, N., Misaki, M., Bodurka, J., Rhudy, J.L., Paulus, M.P., Drevets, W.C., 2015. Increased anterior insula activity in anxious individuals is linked to diminished perceived control. Transl Psychiatry 5, e591-e591.
American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (5th), Arlington.
Amunts, K., Kedo, O., Kindler, M., Pieperhoff, P., Mohlberg, H., Shah, N.J., Habel, U., Schneider, F., Zilles, K., 2005. Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. Anatomy and Embryology 210, 343-352.
Andreatta, M., Glotzbach-Schoon, E., Mühlberger, A., Schulz, S.M., Wiemer, J., Pauli, P., 2015. Initial and sustained brain responses to contextual conditioned anxiety in humans. Cortex 63, 352-363.
Anedda, F., Zucchelli, M., Schepis, D., Hellquist, A., Corrado, L., D'Alfonso, S., Achour, A., McInerney, G., Bertorello, A., Lördal, M., Befrits, R., Björk, J., Bresso, F., Törkvist, L., Halfvarson, J., Kere, J., D'Amato, M., 2011. Multiple Polymorphisms Affect Expression and Function of the Neuropeptide S Receptor (NPSR1). PLOS ONE 6, e29523.
Ausín, B., Muñoz, M., Castellanos, M.Á., García, S., 2020. Prevalence and Characterization of Specific Phobia Disorder in People over 65 Years Old in a Madrid Community Sample (Spain) and its Relationship to Quality of Life. 17, 1915.
Austin, P.C., 2009. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Statistics in medicine 28, 3083-3107.
Auton, A., Abecasis, G.R., Altshuler, D.M., Durbin, R.M., Abecasis, G.R., Bentley, D.R., Chakravarti, A., Clark, A.G., Donnelly, P., Eichler, E.E., Flicek, P., Gabriel, S.B., Gibbs, R.A., Green, E.D., Hurles, M.E., Knoppers, B.M., Korbel, J.O., Lander, E.S., Lee, C., Lehrach, H., Mardis, E.R., Marth, G.T., McVean, G.A., Nickerson, D.A., Schmidt, J.P., Sherry, S.T., Wang, J., Wilson, R.K., Gibbs, R.A., Boerwinkle, E., Doddapaneni, H., Han, Y., Korchina, V., Kovar, C., Lee, S., Muzny, D., Reid, J.G., Zhu, Y., Wang, J., Chang, Y., Feng, Q., Fang, X., Guo, X., Jian, M., Jiang, H., Jin, X., Lan, T., Li, G., Li, J., Li, Y., Liu, S., Liu, X., Lu, Y., Ma, X., Tang, M., Wang, B., Wang, G., Wu, H., Wu, R., Xu, X., Yin, Y., Zhang, D., Zhang, W., Zhao, J., Zhao, M., Zheng, X., Lander, E.S., Altshuler, D.M., Gabriel, S.B., Gupta, N., Gharani, N., Toji, L.H., Gerry, N.P., Resch, A.M., Flicek, P., Barker, J., Clarke, L., Gil, L., Hunt, S.E., Kelman, G., Kulesha, E., Leinonen, R., McLaren, W.M., Radhakrishnan, R., Roa, A., Smirnov, D., Smith, R.E., Streeter, I., Thormann, A., Toneva, I., Vaughan, B., Zheng-Bradley, X., Bentley, D.R., Grocock, R., Humphray, S., James, T., Kingsbury, Z., Lehrach, H., Sudbrak, R., Albrecht, M.W., Amstislavskiy, V.S., Borodina, T.A., Lienhard, M., Mertes, F., Sultan, M., Timmermann, B., Yaspo, M.-L., Mardis, E.R., Wilson, R.K., Fulton, L., Fulton, R., Sherry, S.T., Ananiev, V., Belaia, Z., Beloslyudtsev, D., Bouk, N., Chen, C., Church, D., Cohen, R., Cook, C., Garner, J., Hefferon, T., Kimelman, M., Liu, C., Lopez, J., Meric, P., O'Sullivan, C., Ostapchuk, Y., Phan, L., Ponomarov, S., Schneider, V., Shekhtman, E., Sirotkin, K., Slotta, D., Zhang, H., McVean, G.A., Durbin, R.M., Balasubramaniam, S., Burton, J., Danecek, P., Keane, T.M., Kolb-Kokocinski, A., McCarthy, S., Stalker, J., Quail, M., Schmidt, J.P., Davies, C.J., Gollub, J., Webster, T., Wong, B., Zhan, Y., Auton, A., Campbell, C.L., Kong, Y., Marcketta, A., Gibbs, R.A., Yu, F., Antunes, L., Bainbridge, M., Muzny, D., Sabo, A., Huang, Z., Wang, J., Coin, L.J.M., Fang, L., Guo, X., Jin, X., Li, G., Li, Q., Li, Y., Li, Z., Lin, H., Liu, B., Luo, R., Shao, H., Xie, Y., Ye, C., Yu, C., Zhang, F., Zheng, H., Zhu, H., Alkan, C., Dal, E., Kahveci, F., Marth, G.T., Garrison, E.P., Kural, D., Lee, W.-P., Fung Leong, W., Stromberg, M., Ward,

A.N., Wu, J., Zhang, M., Daly, M.J., DePristo, M.A., Handsaker, R.E., Altshuler, D.M., Banks, E., Bhatia, G., del Angel, G., Gabriel, S.B., Genovese, G., Gupta, N., Li, H., Kashin, S., Lander, E.S., McCarroll, S.A., Nemesh, J.C., Poplin, R.E., Yoon, S.C., Lihm, J., Makarov, V., Clark, A.G., Gottipati, S., Keinan, A., Rodriguez-Flores, J.L., Korbel, J.O., Rausch, T., Fritz, M.H., Stütz, A.M., Flicek, P., Beal, K., Clarke, L., Datta, A., Herrero, J., McLaren, W.M., Ritchie, G.R.S., Smith, R.E., Zerbino, D., Zheng-Bradley, X., Sabeti, P.C., Shlyakhter, I., Schaffner, S.F., Vitti, J., Cooper, D.N., Ball, E.V., Stenson, P.D., Bentley, D.R., Barnes, B., Bauer, M., Keira Cheetham, R., Cox, A., Eberle, M., Humphray, S., Kahn, S., Murray, L., Peden, J., Shaw, R., Kenny, E.E., Batzer, M.A., Konkel, M.K., Walker, J.A., MacArthur, D.G., Lek, M., Sudbrak, R., Amstislavskiy, V.S., Herwig, R., Mardis, E.R., Ding, L., Koboldt, D.C., Larson, D., Ye, K., Gravel, S., The Genomes Project, C., Corresponding, a., Steering, c., Production, g., Baylor College of, M., Shenzhen, B.G.I., Broad Institute of, M.I.T., Harvard, Coriell Institute for Medical, R., European Molecular Biology Laboratory, E.B.I., Illumina, Max Planck Institute for Molecular, G., McDonnell Genome Institute at Washington, U., Health, U.S.N.I.o., University of, O., Wellcome Trust Sanger, I., Analysis, g., Affymetrix, Albert Einstein College of, M., Bilkent, U., Boston, C., Cold Spring Harbor, L., Cornell, U., European Molecular Biology, L., Harvard, U., Human Gene Mutation, D., Icahn School of Medicine at Mount, S., Louisiana State, U., Massachusetts General, H., McGill, U., National Eye Institute, N.I.H., 2015. A global reference for human genetic variation. Nature 526, 68-74.

Avery, S., Clauss, J., Blackford, J., 2016. The human BNST: functional role in anxiety and addiction. Neuropsychopharmacology 41, 126.

Bandelow, B., Reitt, M., Röver, C., Michaelis, S., Görlich, Y., Wedekind, D., 2015. Efficacy of treatments for anxiety disorders: a meta-analysis. Int Clin Psychopharmacol 30, 183-192.

Bandelow, B., Wiltink, J., Alpers, G.W., Benecke, C., Deckert, J., Eckhardt-Henn, A., Ehrig, C., Engel, K., Falkai, P., Geiser, F., 2014. Deutsche S3-Leitlinie Behandlung von Angststörungen.

Becker, E.S., Rinck, M., Türke, V., Kause, P., Goodwin, R., Neumer, S., Margraf, J., 2007. Epidemiology of specific phobia subtypes: findings from the Dresden Mental Health Study. European Psychiatry 22, 69-74.

Bennett, K.P., Dickmann, J.S., Larson, C.L., 2018. If or when? Uncertainty's role in anxious anticipation. Psychophysiology 55, e13066.

Beste, C., Konrad, C., Uhlmann, C., Arolt, V., Zwanzger, P., Domschke, K., 2013. Neuropeptide S receptor (NPSR1) gene variation modulates response inhibition and error monitoring. NeuroImage 71, 1-9.

Björkstrand, J., Agren, T., Frick, A., Hjorth, O., Furmark, T., Fredrikson, M., Åhs, F., 2020. Decrease in amygdala activity during repeated exposure to spider images predicts avoidance behavior in spider fearful individuals. Transl Psychiatry 10, 292.

Bogdan, R., Salmeron, B.J., Carey, C.E., Agrawal, A., Calhoun, V.D., Garavan, H., Hariri, A.R., Heinz, A., Hill, M.N., Holmes, A., Kalin, N.H., Goldman, D., 2017. Imaging Genetics and Genomics in Psychiatry: A Critical Review of Progress and Potential. Biological psychiatry 82, 165-175.

Böhnlein, J., Altegoer, L., Muck, N.K., Roesmann, K., Redlich, R., Dannlowski, U., Leehr, E.J., 2020. Factors influencing the success of exposure therapy for specific phobia: A systematic review. Neuroscience & Biobehavioral Reviews 108, 796-820.

Botella, C., Fernández-Álvarez, J., Guillén, V., García-Palacios, A., Baños, R., 2017. Recent Progress in Virtual Reality Exposure Therapy for Phobias: A Systematic Review. Current Psychiatry Reports 19, 42.

Bradley, M.M., Lang, P.J., 1994. Measuring emotion: The self-assessment manikin and the semantic differential. J Behav Ther Exp Psychiatry 25, 49-59.

Bradley, M.M., Lang, P.J., 2007. The International Affective Digitized Sounds (2nd Edition; IADS-2):Affective ratings of sounds and instruction manual, Gainesville:University of Florida.

Brinkmann, L., Buff, C., Feldker, K., Neumeister, P., Heitmann, C.Y., Hofmann, D., Bruchmann, M., Herrmann, M.J., Straube, T., 2018. Inter-individual differences in trait anxiety shape the functional connectivity between the bed nucleus of the stria terminalis and the amygdala during brief threat processing. NeuroImage 166, 110-116.
Brinkmann, L., Buff, C., Feldker, K., Tupak, S.V., Becker, M.P.I., Herrmann, M.J., Straube, T., 2017a. Distinct phasic and sustained brain responses and connectivity of amygdala and bed nucleus of the stria terminalis during threat anticipation in panic disorder. Psychol Med 47, 2675-2688.
Brinkmann, L., Buff, C., Neumeister, P., Tupak, S.V., Becker, M.P., Herrmann, M.J., Straube, T., 2017b. Dissociation between amygdala and bed nucleus of the stria terminalis during threat anticipation in female post-traumatic stress disorder patients. Human Brain Mapping 38, 2190-2205.
Brinkmann, L., Poller, H., Herrmann, M.J., Miltner, W., Straube, T., 2017c. Initial and sustained brain responses to threat anticipation in blood-injection-injury phobia. Neuroimage: Clinical 13, 320-329.
Buff, C., Brinkmann, L., Bruchmann, M., Becker, M.P.I., Tupak, S., Herrmann, M.J., Straube, T., 2017. Activity alterations in the bed nucleus of the stria terminalis and amygdala during threat anticipation in generalized anxiety disorder. Social cognitive and affective neuroscience 12, 1766-1774.
Buff, C., Brinkmann, L., Neumeister, P., Feldker, K., Heitmann, C., Gathmann, B., Andor, T., Straube, T., 2016. Specifically altered brain responses to threat in generalized anxiety disorder relative to social anxiety disorder and panic disorder. Neuroimage: Clinical 12, 698-706.
Chakrabarty, T., Ogrodniczuk, J., Hadjipavlou, G., 2016. Predictive Neuroimaging Markers of Psychotherapy Response: A Systematic Review. Harv Rev Psychiatry 24, 396-405.
Chavanne, A.V., Robinson, O.J., 2020. The Overlapping Neurobiology of Induced and Pathological Anxiety: A Meta-Analysis of Functional Neural Activation. Am J Psychiatry, appiajp202019111153.
Clauss, J., 2019. Extending the neurocircuitry of behavioural inhibition: a role for the bed nucleus of the stria terminalis in risk for anxiety disorders. Gen Psychiatr 32, e100137.
Clauss, J.A., Avery, S.N., Benningfield, M.M., Blackford, J.U., 2019. Social anxiety is associated with BNST response to unpredictability. Depression and Anxiety 0.
Craske, M.G., Treanor, M., Conway, C.C., Zbozinek, T., Vervliet, B.J.B.r., therapy, 2014. Maximizing exposure therapy: An inhibitory learning approach. 58, 10-23.
Crowe, R.R., Goedken, R., Samuelson, S., Wilson, R., Nelson, J., Noyes Jr, R., 2001. Genomewide survey of panic disorder. American Journal of Medical Genetics 105, 105-109.
Dannlowski, U., Kugel, H., Franke, F., Stuhrmann, A., Hohoff, C., Zwanzger, P., Lenzen, T., Grotegerd, D., Suslow, T., Arolt, V., Heindel, W., Domschke, K., 2011. Neuropeptide-S (NPS) receptor genotype modulates basolateral amygdala responsiveness to aversive stimuli. Neuropsychopharmacology 36, 1879-1885.
Davis, M., Walker, D.L., Miles, L., Grillon, C., 2010. Phasic vs Sustained Fear in Rats and Humans: Role of the Extended Amygdala in Fear vs Anxiety. Neuropsychopharmacology 35, 105.
Davis, T.E., Ollendick, T.H., Öst, L.-G., 2012. Intensive one-session treatment of specific phobias. Springer.
Del Casale, A., Ferracuti, S., Rapinesi, C., Serata, D., Piccirilli, M., Savoja, V., Kotzalidis, G.D., Manfredi, G., Angeletti, G., Tatarelli, R., Girardi, P., 2012. Functional neuroimaging in specific phobia. Psychiatry Research: Neuroimaging 202, 181-197.
Domschke, K., Klauke, B., Winter, B., Gajewska, A., Herrmann, M.J., Warrings, B., Mühlberger, A., Wosnitza, K., Dlugos, A., Naunin, S., Nienhaus, K., Fobker, M., Jacob, C., Arolt, V., Pauli, P., Reif, A., Zwanzger, P., Deckert, J., 2012. Modification of caffeine effects

on the affect-modulated startle by neuropeptide S receptor gene variation. Psychopharmacology 222, 533-541.
Domschke, K., Reif, A., Weber, H., Richter, J., Hohoff, C., Ohrmann, P., Pedersen, A., Bauer, J., Suslow, T., Kugel, H., Heindel, W., Baumann, C., Klauke, B., Jacob, C., Maier, W., Fritze, J., Bandelow, B., Krakowitzky, P., Rothermundt, M., Erhardt, A., Binder, E.B., Holsboer, F., Gerlach, A.L., Kircher, T., Lang, T., Alpers, G.W., Ströhle, A., Fehm, L., Gloster, A.T., Wittchen, H.U., Arolt, V., Pauli, P., Hamm, A., Deckert, J., 2011. Neuropeptide S receptor gene — converging evidence for a role in panic disorder. Mol Psychiatry 16, 938–948.
Eickhoff, S.B., Stephan, K.E., Mohlberg, H., Grefkes, C., Fink, G.R., Amunts, K., Zilles, K., 2005. A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. NeuroImage 25, 1325-1335.
Eklund, A., Nichols, T.E., Knutsson, H., 2016. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. Proceedings of the National Academy of Sciences 113, 7900.
Etkin, A., Wager, T.D., 2007. Functional Neuroimaging of Anxiety: A Meta-Analysis of Emotional Processing in PTSD, Social Anxiety Disorder, and Specific Phobia. American Journal of Psychiatry 164, 1476-1488.
Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A., 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods 39, 175-191.
Figel, B., Brinkmann, L., Buff, C., Heitmann, C.Y., Hofmann, D., Bruchmann, M., Becker, M.P.I., Herrmann, M.J., Straube, T., 2019. Phasic amygdala and BNST activation during the anticipation of temporally unpredictable social observation in social anxiety disorder patients. Neuroimage: Clinical 22, 101735.
Flury, B.K., Riedwyl, H., 1986. Standard Distance in Univariate and Multivariate Analysis. The American Statistician 40, 249-251.
Foa, E.B., Kozak, M.J., 1986. Emotional processing of fear: Exposure to corrective information. Psychological Bulletin 99, 20-35.
Font, F., Roma, G., Serra, X., 2013. Freesound technical demo. Proceedings of the 21st ACM international conference on Multimedia, pp. 411-412.
Fox, A.S., Shackman, A.J., 2019. The central extended amygdala in fear and anxiety: Closing the gap between mechanistic and neuroimaging research. Neuroscience Letters 693, 58-67.
Frick, A., Engman, J., Alaie, I., Björkstrand, J., Gingnell, M., Larsson, E.-M., Eriksson, E., Wahlstedt, K., Fredrikson, M., Furmark, T., 2020. Neuroimaging, genetic, clinical, and demographic predictors of treatment response in patients with social anxiety disorder. Journal of Affective Disorders 261, 230-237.
Gechter, J., Liebscher, C., Geiger, M.J., Wittmann, A., Schlagenhauf, F., Lueken, U., Wittchen, H.-U., Pfleiderer, B., Arolt, V., Kircher, T., Straube, B., Deckert, J., Weber, H., Herrmann, M.J., Reif, A., Domschke, K., Ströhle, A., 2019. Association of NPSR1 gene variation and neural activity in patients with panic disorder and agoraphobia and healthy controls. Neuroimage: Clinical, 102029.
Glover, L.R., McFadden, K.M., Bjorni, M., Smith, S.R., Rovero, N.G., Oreizi-Esfahani, S., Yoshida, T., Postle, A.F., Nonaka, M., Halladay, L.R., Holmes, A., 2020. A prefrontal-bed nucleus of the stria terminalis circuit limits fear to uncertain threat. eLife 9.
Goode, T.D., Maren, S., 2017. Role of the bed nucleus of the stria terminalis in aversive learning and memory. Learning & Memory 24, 480 -491.
Goode, T.D., Ressler, R.L., Acca, G.M., Miles, O.W., Maren, S., 2019. Bed nucleus of the stria terminalis regulates fear to unpredictable threat signals. eLife 8, e46525.
Gottschalk, M.G., Domschke, K., 2016. Novel developments in genetic and epigenetic mechanisms of anxiety. 29, 32-38.

Grupe, D.W., Nitschke, J.B., 2013. Uncertainty and anticipation in anxiety: an integrated neurobiological and psychological perspective. Nat Rev Neurosci 14, 488-501.
Guhn, A., Domschke, K., Müller, L., Dresler, T., Eff, F., Kopf, J., Deckert, J., Reif, A., Herrmann, M., 2015. Neuropeptide S receptor gene variation and neural correlates of cognitive emotion regulation. Social cognitive and affective neuroscience 10, 1730 - 1737.
Gungor, N.Z., Pare, D., 2016. Functional Heterogeneity in the Bed Nucleus of the Stria Terminalis. Journal of neuroscience 36, 8038-8049.
Hamm, A., 2006. Spezifische Phobien. Hogrefe Göttingen.
Hautzinger, M., Bailer, M., Hofmeister, D., Keller, F., 2012. ADS (2012)–Allgemeine Depressionsskala (2012). Tests Info. Hogrefe, Göttingen.
Hefner, K.R., Moberg, C.A., Hachiya, L.Y., Curtin, J.J., 2013. Alcohol stress response dampening during imminent versus distal, uncertain threat. Journal of abnormal psychology 122, 756-769.
Herrmann, M.J., Boehme, S., Becker, M.P., Tupak, S.V., Guhn, A., Schmidt, B., Brinkmann, L., Straube, T., 2016. Phasic and sustained brain responses in the amygdala and the bed nucleus of the stria terminalis during threat anticipation. Hum Brain Mapping 37, 1091-1102.
Herrmann, M.J., Katzorke, A., Busch, Y., Gromer, D., Polak, T., Pauli, P., Deckert, J., 2017. Medial prefrontal cortex stimulation accelerates therapy response of exposure therapy in acrophobia. Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation 10, 291-297.
Ho, D., Imai, K., King, G., Stuart, E., Whitworth, A., 2018. Package 'MatchIt'. Version.
Hoppe, J.M., Holmes, E.A., Agren, T., 2021. Exploring the neural basis of fear produced by mental imagery: imaginal exposure in individuals fearful of spiders. Philos Trans R Soc Lond B Biol Sci 376, 20190690.
Hur, J., Smith, J.F., DeYoung, K.A., Anderson, A.S., Kuang, J., Kim, H.C., Tillman, R.M., Kuhn, M., Fox, A.S., Shackman, A.J., 2020. Anxiety and the neurobiology of temporally uncertain threat anticipation. The Journal of Neuroscience, JN-RM-0704-0720.
Ionescu, I.A., Dine, J., Yen, Y.C., Buell, D.R., Herrmann, L., Holsboer, F., Eder, M., Landgraf, R., Schmidt, U., 2012. Intranasally administered neuropeptide S (NPS) exerts anxiolytic effects following internalization into NPS receptor-expressing neurons. Neuropsychopharmacology 37, 1323-1337.
Ipser, J.C., Singh, L., Stein, D.J., 2013. Meta-analysis of functional brain imaging in specific phobia. Psychiatry Clin Neurosci 67, 311-322.
Jacobi, F., Höfler, M., Strehle, J., Mack, S., Gerschler, A., Scholl, L., Busch, M.A., Maske, U., Hapke, U., Gaebel, W., Maier, W., Wagner, M., Zielasek, J., Wittchen, H.U., 2014. Psychische Störungen in der Allgemeinbevölkerung. Der Nervenarzt 85, 77-87.
Klorman, R., Weerts, T.C., Hastings, J.E., Melamed, B.G., Lang, P.J., 1974. Psychometric description of some specific-fear questionnaires. Behavior Therapy 5, 401-409.
Klumpers, F., Kroes, M.C.W., Baas, J.M.P., Fernandez, G., 2017. How Human Amygdala and Bed Nucleus of the Stria Terminalis May Drive Distinct Defensive Responses. Journal of neuroscience 37, 9645-9656.
Klumpp, H., Fitzgerald, J.M., Kinney, K.L., Kennedy, A.E., Shankman, S.A., Langenecker, S.A., Phan, K.L., 2017. Predicting cognitive behavioral therapy response in social anxiety disorder with anterior cingulate cortex and amygdala during emotion regulation. Neuroimage: Clinical 15, 25-34.
Knight, L.K., Depue, B.E., 2019. New Frontiers in Anxiety Research: The Translational Potential of the Bed Nucleus of the Stria Terminalis. 10.
Knowles, J.A., Fyer, A.J., Vieland, V.J., Weissman, M.M., Hodge, S.E., Heiman, G.A., Haghighi, F., de Jesus, G.M., Rassnick, H., Preud'homme-Rivelli, X., Austin, T., Cunjak, J., Mick, S., Fine, L.D., Woodley, K.A., Das, K., Maier, W., Adams, P.B., Freimer, N.B., Klein,

D.F., Gilliam, T.C., 1998. Results of a genome-wide genetic screen for panic disorder. American Journal of Medical Genetics 81, 139-147.
Kumsta, R., Chen, F.S., Pape, H.-C., Heinrichs, M., 2013. Neuropeptide S receptor gene is associated with cortisol responses to social stress in humans. Biological Psychology 93, 304-307.
Lancaster, J.L., Tordesillas-Gutiérrez, D., Martinez, M., Salinas, F., Evans, A., Zilles, K., Mazziotta, J.C., Fox, P.T., 2007. Bias between MNI and Talairach coordinates analyzed using the ICBM-152 brain template. 28, 1194-1205.
Lang, P.J., Bradley, M.M., 1999. International affective picture system (IAPS): Instruction manual and affective ratings. The center for research in psychophysiology, University of Florida.
Lange, I., Goossens, L., Michielse, S., Bakker, J., Vervliet, B., Marcelis, M., Wichers, M., van Os, J., van Amelsvoort, T., Schruers, K., 2020. Neural responses during extinction learning predict exposure therapy outcome in phobia: results from a randomized-controlled trial. Neuropsychopharmacology 45, 534-541.
Laux, L., Glanzmann, P., Schaffner, P., Spielberger, C.D., 1981. Das State-Trait-Angstinventar. Hogrefe, Göttingen
Lebow, M.A., Chen, A., 2016. Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders. Mol Psychiatry 21, 450-463.
LeDoux, J.E., Pine, D.S., 2016. Using Neuroscience to Help Understand Fear and Anxiety: A Two-System Framework. American Journal of Psychiatry 173, 1083-1093.
Leonard, S.K., Dwyer, J.M., Sukoff Rizzo, S.J., Platt, B., Logue, S.F., Neal, S.J., Malberg, J.E., Beyer, C.E., Schechter, L.E., Rosenzweig-Lipson, S., Ring, R.H., 2008. Pharmacology of neuropeptide S in mice: therapeutic relevance to anxiety disorders. Psychopharmacology 197, 601-611.
Leonard, S.K., Ring, R.H., 2011. Immunohistochemical localization of the neuropeptide S receptor in the rat central nervous system. Neuroscience 172, 153-163.
Levey, D.F., Gelernter, J., Polimanti, R., Zhou, H., Cheng, Z., Aslan, M., Quaden, R., Concato, J., Radhakrishnan, K., Bryois, J., Sullivan, P.F., Stein, M.B., 2020. Reproducible Genetic Risk Loci for Anxiety: Results From ~200,000 Participants in the Million Veteran Program. American Journal of Psychiatry 177, 223-232.
Linares, I.M., Trzesniak, C., Chagas, M.H., Hallak, J.E., Nardi, A.E., Crippa, J.A., 2012. Neuroimaging in specific phobia disorder: a systematic review of the literature. Braz J Psychiatry 34, 101-111.
Lipka, J., Hoffmann, M., Miltner, W.H.R., Straube, T., 2014. Effects of Cognitive-Behavioral Therapy on Brain Responses to Subliminal and Supraliminal Threat and Their Functional Significance in Specific Phobia. Biological psychiatry 76, 869-877.
Lipka, J., Miltner, W.H.R., Straube, T., 2011. Vigilance for Threat Interacts with Amygdala Responses to Subliminal Threat Cues in Specific Phobia. Biological psychiatry 70, 472-478.
Loerinc, A.G., Meuret, A.E., Twohig, M.P., Rosenfield, D., Bluett, E.J., Craske, M.G., 2015. Response rates for CBT for anxiety disorders: Need for standardized criteria. Clin Psychol Rev 42, 72-82.
Logue, M.W., Vieland, V.J., Goedken, R.J., Crowe, R.R., 2003. Bayesian analysis of a previously published genome screen for panic disorder reveals new and compelling evidence for linkage to chromosome 7. Am J Med Genet B Neuropsychiatr Genet 121b, 95-99.
Lovecraft, H.P., 2013. Supernatural horror in literature. The Palingenesis Project (Wermod and Wermod Publishing Group).
Lueken, U., Straube, B., Konrad, C., Wittchen, H.U., Ströhle, A., Wittmann, A., Pfleiderer, B., Uhlmann, C., Arolt, V., Jansen, A., Kircher, T., 2013. Neural substrates of treatment response to cognitive-behavioral therapy in panic disorder with agoraphobia. Am J Psychiatry 170, 1345-1355.

Lueken, U., Zierhut, K.C., Hahn, T., Straube, B., Kircher, T., Reif, A., Richter, J., Hamm, A., Wittchen, H.-U., Domschke, K., 2016. Neurobiological markers predicting treatment response in anxiety disorders: A systematic review and implications for clinical application. Neuroscience & Biobehavioral Reviews 66, 143-162.
Lukas, M., Neumann, I.D., 2012. Nasal application of neuropeptide S reduces anxiety and prolongs memory in rats: social versus non-social effects. Neuropharmacology 62, 398-405.
Maldjian, J.A., Laurienti, P.J., Kraft, R.A., Burdette, J.H., 2003. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. NeuroImage 19, 1233-1239.
Månsson, K.N.T., Frick, A., Boraxbekk, C.J., Marquand, A.F., Williams, S.C.R., Carlbring, P., Andersson, G., Furmark, T., 2015. Predicting long-term outcome of Internet-delivered cognitive behavior therapy for social anxiety disorder using fMRI and support vector machine learning. Transl Psychiatry 5, e530-e530.
Meyer, T.J., Miller, M.L., Metzger, R.L., Borkovec, T.D., 1990. Development and validation of the penn state worry questionnaire. Behaviour Research and Therapy 28, 487-495.
Mobbs, D., Yu, R., Rowe, J.B., Eich, H., FeldmanHall, O., Dalgleish, T., 2010. Neural activity associated with monitoring the oscillating threat value of a tarantula. Proceedings of the National Academy of Sciences 107, 20582-20586.
Munsterkotter, A.L., Notzon, S., Redlich, R., Grotegerd, D., Dohm, K., Arolt, V., Kugel, H., Zwanzger, P., Dannlowski, U., 2015. Spider or No Spider? Neural Correlates of Sustained and Phasic Fear in Spider Phobia. Depress Anxiety 32, 656-663.
Muris, P., Merckelbach, H., 1996. A comparison of two spider fear questionnaires. J Behav Ther Exp Psychiatry 27, 241-244.
Naaz, F., Knight, L.K., Depue, B.E., 2019. Explicit and Ambiguous Threat Processing: Functionally Dissociable Roles of the Amygdala and Bed Nucleus of the Stria Terminalis. Journal of cognitive neuroscience 31, 543-559.
Nitschke, J.B., Sarinopoulos, I., Oathes, D.J., Johnstone, T., Whalen, P.J., Davidson, R.J., Kalin, N.H., 2009. Anticipatory Activation in the Amygdala and Anterior Cingulate in Generalized Anxiety Disorder and Prediction of Treatment Response. American Journal of Psychiatry 166, 302-310.
Okamura, N., Reinscheid, R.K., 2007. Neuropeptide S: A novel modulator of stress and arousal. Stress 10, 221-226.
Öst, L.-G., 1996. One-session group treatment of spider phobia. Behaviour Research and Therapy 34, 707-715.
Pape, H.-C., Jüngling, K., Seidenbecher, T., Lesting, J., Reinscheid, R.K., 2010. Neuropeptide S: a transmitter system in the brain regulating fear and anxiety. Neuropharmacology 58, 29-34.
Pedersen, W.S., Muftuler, L.T., Larson, C.L., 2019. A high-resolution fMRI investigation of BNST and centromedial amygdala activity as a function of affective stimulus predictability, anticipation, and duration. Social cognitive and affective neuroscience 14(11), 1167-1177.
Peñate, W., Fumero, A., Viña, C., Herrero, M., Marrero, R.J., Rivero, F., 2017. A meta-analytic review of neuroimaging studies of specific phobia to small animals. The European Journal of Psychiatry 31, 23-36.
Price, R.B., Cummings, L., Gilchrist, D., Graur, S., Banihashemi, L., Kuo, S.S., Siegle, G.J.J.J.o.c., psychology, c., 2018. Towards personalized, brain-based behavioral intervention for transdiagnostic anxiety: Transient neural responses to negative images predict outcomes following a targeted computer-based intervention. 86, 1031.
Raczka, K.A., Gartmann, N., Mechias, M.L., Reif, A., Büchel, C., Deckert, J., Kalisch, R., 2010. A neuropeptide S receptor variant associated with overinterpretation of fear reactions: a potential neurogenetic basis for catastrophizing. Mol Psychiatry 15, 1067.

Reinscheid, R.K., Xu, Y.-L., Okamura, N., Zeng, J., Chung, S., Pai, R., Wang, Z., Civelli, O., 2005. Pharmacological Characterization of Human and Murine Neuropeptide S Receptor Variants. Journal of Pharmacology and Experimental Therapeutics 315, 1338 -1345.
Ressler, R.L., Goode, T.D., Evemy, C., Maren, S., 2020. NMDA receptors in the CeA and BNST differentially regulate fear conditioning to predictable and unpredictable threats. Neurobiol Learn Mem 174, 107281.
Rizzi, A., Vergura, R., Marzola, G., Ruzza, C., Guerrini, R., Salvadori, S., Regoli, D., Calo, G., 2008. Neuropeptide S is a stimulatory anxiolytic agent: a behavioural study in mice. British Journal of Pharmacology 154, 471-479.
Ruland, T., Domschke, K., Schütte, V., Zavorotnyy, M., Kugel, H., Notzon, S., Vennewald, N., Ohrmann, P., Arolt, V., Pfleiderer, B., Zwanzger, P., 2015. Neuropeptide S receptor gene variation modulates anterior cingulate cortex Glx levels during CCK-4 induced panic. European Neuropsychopharmacology 25, 1677-1682.
Rupp, C., Doebler, P., Ehring, T., Vossbeck-Elsebusch, A.N., 2017. Emotional Processing Theory Put to Test: A Meta-Analysis on the Association Between Process and Outcome Measures in Exposure Therapy. 24, 697-711.
Santos, V.A., Carvalho, D.D., Van Ameringen, M., Nardi, A.E., Freire, R.C., 2019. Neuroimaging findings as predictors of treatment outcome of psychotherapy in anxiety disorders. Prog Neuropsychopharmacol Biol Psychiatry 91, 60-71.
Sarinopoulos, I., Grupe, D.W., Mackiewicz, K.L., Herrington, J.D., Lor, M., Steege, E.E., Nitschke, J.B., 2010. Uncertainty during Anticipation Modulates Neural Responses to Aversion in Human Insula and Amygdala. Cerebral Cortex 20, 929-940.
Schiele, M.A., Herzog, K., Kollert, L., Schartner, C., Leehr, E.J., Böhnlein, J., Repple, J., Rosenkranz, K., Lonsdorf, T.B., Dannlowski, U., Zwanzger, P., Reif, A., Pauli, P., Deckert, J., Domschke, K., 2020. Extending the vulnerability-stress model of mental disorders: three-dimensional NPSR1 × environment × coping interaction study in anxiety. Br J Psychiatry, 1-6.
Schubert, T., 2003. The sense of presence in virtual environments: A three-component scale measuring spatial presence, involvement, and realness. Zeitschrift für Medienpsychologie 15, 69-71.
Schwarzmeier, H., Leehr, E.J., Böhnlein, J., Seeger, F.R., Roesmann, K., Gathmann, B., Herrmann, M.J., Siminski, N., Junghöfer, M., Straube, T., Grotegerd, D., Dannlowski, U., 2020. Theranostic markers for personalized therapy of spider phobia: Methods of a bicentric external cross-validation machine learning approach. International Journal of Methods in Psychiatric Research 29, e1812.
Shackman, A.J., Fox, A.S., 2016. Contributions of the Central Extended Amygdala to Fear and Anxiety. Journal of neuroscience 36, 8050-8063.
Shankman, S.A., Gorka, S.M., Nelson, B.D., Fitzgerald, D.A., Phan, K.L., O'Daly, O., 2014. Anterior insula responds to temporally unpredictable aversiveness: an fMRI study. Neuroreport 25, 596-600.
Siminski, N., Böhme, S., Zeller, J.B.M., Becker, M.P.I., Bruchmann, M., Hofmann, D., Breuer, F., Mühlberger, A., Schiele, M.A., Weber, H., Schartner, C., Deckert, J., Pauli, P., Reif, A., Domschke, K., Straube, T., Herrmann, M.J., 2021. BNST and amygdala activation to threat: Effects of temporal predictability and threat mode. Behavioural Brain Research 396, 112883.
Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: Addressing problems of smoothing, threshold dependence and localisation in cluster inference. NeuroImage 44, 83-98.
Somerville, L.H., Wagner, D.D., Wig, G.S., Moran, J.M., Whalen, P.J., Kelley, W.M., 2013. Interactions Between Transient and Sustained Neural Signals Support the Generation and Regulation of Anxious Emotion. Cerebral Cortex 23, 49-60.
Spielberger, C.D., 2010. State-Trait Anxiety Inventory. The Corsini Encyclopedia of Psychology, pp. 1-1.

Straube, T., Mentzel, H.-J., Miltner, W.H.R., 2007. Waiting for spiders: Brain activation during anticipatory anxiety in spider phobics. NeuroImage 37, 1427-1436.
Streit, F., Haddad, L., Paul, T., Frank, J., Schäfer, A., Nikitopoulos, J., Akdeniz, C., Lederbogen, F., Treutlein, J., Witt, S., Meyer-Lindenberg, A., Rietschel, M., Kirsch, P., Wüst, S., 2014. A functional variant in the neuropeptide S receptor 1 gene moderates the influence of urban upbringing on stress processing in the amygdala. Stress 17, 352-361.
Strosche, A., Zhang, X., Kirsch, M., Hermann, D., Ende, G., Kiefer, F., Vollstädt-Klein, S., 2021. Investigation of brain functional connectivity to assess cognitive control over cue-processing in Alcohol Use Disorder. Addiction Biology 26, e12863.
Stuart, E.A., Lee, B.K., Leacy, F.P., 2013. Prognostic score-based balance measures can be a useful diagnostic for propensity score methods in comparative effectiveness research. Journal of clinical epidemiology 66, S84-S90.e81.
Suso-Ribera, C., Fernández-Álvarez, J., García-Palacios, A., Hoffman, H.G., Bretón-López, J., Baños, R.M., Quero, S., Botella, C., 2019. Virtual Reality, Augmented Reality, and In Vivo Exposure Therapy: A Preliminary Comparison of Treatment Efficacy in Small Animal Phobia. Cyberpsychology, Behavior, and Social Networking 22, 31-38.
Swanson, L.W., Petrovich, G.D., 1998. What is the amygdala? Trends in Neurosciences 21, 323-331.
Talairach, J., 1988. 3-dimensional proportional system; an approach to cerebral imaging. co-planar stereotaxic atlas of the human brain. Thieme, 1-122.
Thng, C.E.W., Lim-Ashworth, N.S.J., Poh, B.Z.Q., Lim, C.G., 2020. Recent developments in the intervention of specific phobia among adults: a rapid review. F1000Res 9.
Tillmann, S., Skibdal, H.E., Christiansen, S.H., Gøtzsche, C.R., Hassan, M., Mathé, A.A., Wegener, G., Woldbye, D.P.D., 2019. Sustained overexpression of neuropeptide S in the amygdala reduces anxiety-like behavior in rats. Behavioural Brain Research 367, 28-34.
Torrisi, S., O'Connell, K., Davis, A., Reynolds, R., Balderston, N., Fudge, J.L., Grillon, C., Ernst, M., 2015. Resting state connectivity of the bed nucleus of the stria terminalis at ultra-high field. Human Brain Mapping 36, 4076-4088.
Trumpf, J., Margraf, J., Vriends, N., Meyer, A.H., Becker, E.S., 2010. Specific phobia predicts psychopathology in young women. Social Psychiatry and Psychiatric Epidemiology 45, 1161-1166.
Tupak, S., Reif, A., Pauli, P., Dresler, T., Herrmann, M., Domschke, K., Jochum, C., Haas, E., Baumann, C., Weber, H., Fallgatter, A., Deckert, J., Ehlis, A.-C., 2012. Neuropeptide S receptor gene: Fear-specific modulations of prefrontal activation. NeuroImage 66, 353-360.
Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. NeuroImage 15, 273-289.
Vitale, G., Filaferro, M., Ruggieri, V., Pennella, S., Frigeri, C., Rizzi, A., Guerrini, R., Calò, G., 2008. Anxiolytic-like effect of neuropeptide S in the rat defensive burying. Peptides 29, 2286-2291.
Wardenaar, K.J., Lim, C.C.W., Al-Hamzawi, A.O., Alonso, J., Andrade, L.H., Benjet, C., Bunting, B., de Girolamo, G., Demyttenaere, K., Florescu, S.E., Gureje, O., Hisateru, T., Hu, C., Huang, Y., Karam, E., Kiejna, A., Lepine, J.P., Navarro-Mateu, F., Oakley Browne, M., Piazza, M., Posada-Villa, J., ten Have, M.L., Torres, Y., Xavier, M., Zarkov, Z., Kessler, R.C., Scott, K.M., de Jonge, P., 2017. The cross-national epidemiology of specific phobia in the World Mental Health Surveys. Psychol Med 47, 1744-1760.
Wechsler, T.F., Kümpers, F., Mühlberger, A., 2019. Inferiority or Even Superiority of Virtual Reality Exposure Therapy in Phobias?—A Systematic Review and Quantitative Meta-Analysis on Randomized Controlled Trials Specifically Comparing the Efficacy of Virtual Reality

Exposure to Gold Standard in vivo Exposure in Agoraphobia, Specific Phobia, and Social Phobia. 10.
Whalen, P.J., Johnstone, T., Somerville, L.H., Nitschke, J.B., Polis, S., Alexander, A.L., Davidson, R.J., Kalin, N.H., 2008. A Functional Magnetic Resonance Imaging Predictor of Treatment Response to Venlafaxine in Generalized Anxiety Disorder. Biological psychiatry 63, 858-863.
Williams, A.R., Lattal, K.M., 2020. Involvement of the bed nucleus of the stria terminalis in initial conditioning and rapid reconditioning following extinction of contextual fear. Behav Neurosci 134, 177-186.
Wittchen, H.-U., Wunderlich, U., Gruschwitz, S., Zaudig, M., 1997. SKID I. Strukturiertes Klinisches Interview für DSM-IV. Achse I: Psychische Störungen. Interviewheft und Beurteilungsheft. Eine deutschsprachige, erweiterte Bearb. d. amerikanischen Originalversion des SKID I.
Wittchen, H.U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., Olesen, J., Allgulander, C., Alonso, J., Faravelli, C., Fratiglioni, L., Jennum, P., Lieb, R., Maercker, A., van Os, J., Preisig, M., Salvador-Carulla, L., Simon, R., Steinhausen, H.C., 2011. The size and burden of mental disorders and other disorders of the brain in Europe 2010. European Neuropsychopharmacology 21, 655-679.
Xu, Y.-L., Gall, C.M., Jackson, V.R., Civelli, O., Reinscheid, R.K., 2007. Distribution of neuropeptide S receptor mRNA and neurochemical characteristics of neuropeptide S-expressing neurons in the rat brain. Journal of Comparative Neurology 500, 84-102.
Xu, Y.-L., Reinscheid, R.K., Huitron-Resendiz, S., Clark, S.D., Wang, Z., Lin, S.H., Brucher, F.A., Zeng, J., Ly, N.K., Henriksen, S.J., de Lecea, L., Civelli, O., 2004. Neuropeptide S: A Neuropeptide Promoting Arousal and Anxiolytic-like Effects. Neuron 43, 487-497.
Zoicas, I., Menon, R., Neumann, I.D., 2016. Neuropeptide S reduces fear and avoidance of con-specifics induced by social fear conditioning and social defeat, respectively. Neuropharmacology 108, 284-291.

Appendices of dissertation

Publication list:

1. Schwarzmeier, H., Leehr, E.J., Böhnlein, J., Seeger, F.R., Roesmann, K., Gathmann, B., Herrmann, M.J., **Siminski, N.,** Junghöfer, M., Straube, T., Grotegerd, D., Dannlowski, U., 2020. Theranostic markers for personalized therapy of spider phobia: Methods of a bicentric external cross-validation machine learning approach. International Journal of Methods in Psychiatric Research 29, e1812. https://doi.org/10.1002/mpr.1812
2. **Siminski, N.,** Böhme, S., Zeller, J.B.M., Becker, M.P.I., Bruchmann, M., Hofmann, D., Breuer, F., Mühlberger, A., Schiele, M.A., Weber, H., Schartner, C., Deckert, J., Pauli, P., Reif, A., Domschke, K., Straube, T., Herrmann, M.J., 2021. BNST and amygdala activation to threat: Effects of temporal predictability and threat mode. Behavioural Brain Research 396, 112883. https://doi.org/10.1016/j.bbr.2020.112883

Curriculum vitae

Affidavit

I hereby confirm that my thesis entitled *Temporal predictability of threat: Evaluation of differential involvement of amygdala and BNST, and relevance for therapy response prediction in spider phobia* is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Place, Date Signature

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation *Zeitliche Vorhersagbarkeit von Bedrohung: Evaluation der unterschiedlichen Aktivierung von Amygdala und BNST sowie die Relevanz für die Vorhersagbarkeit von Therapieerfolg bei der Spinnenphobie* eigenständig, d.h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Ort, Datum Unterschrift