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Networks of fear: Functional connectivity of the amygdala, the insula and the anterior cingulate cortex in two subtypes of specific phobia

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Introduction

In the past decade, more and more findings have strengthened the hypothesis of a neural network associated with threat processing and defensive responding, the so-called fear circuit as a neural basis for fear and anxiety[1,2]. An increasing body of evidence is emerging that functional impairments of these structures feature a variety of anxiety disorders including specific phobia[3,4]. While three structures of this network – the amygdala, the anterior cingulate cortex (ACC), and the insula – are thought to play a key role in this prototypical disorder of pathological fear little is known about their functional interplay during phobogenic stimulus processing[2,4,5]. However, functional connectivity (FC) between fear-processing regions has been proposed as a meaningful diagnostic and predictive biomarker of anxiety disorders[6–10]. Moreover, dysregulated brain circuits could serve as novel targets for neuromodulation techniques[11]. Thus, exploring interactions of the ACC, the amygdala and the insula in specific phobia could provide new insights into mechanisms of pathological fear responses that may be translated into clinical utility.

Specific Phobia

Basics of Specific Phobia

Specific phobias are considered to be one of the most prevalent anxiety disorders and to be among the most common mental disorders in general[12]. Commonly mentioned 12-month prevalence rates vary between five to fifteen percent with women being more often affected than men[12–18]. According to the latest estimates for Germany, around ten percent of the adult population suffer from specific phobia – resulting in more than six million affected people[19]. Clinically, specific phobia is characterized by a prominent and persistent excessive or unreasonable fear cued by the presence or anticipation of an object or phobic situation causing almost invariably an immediate intense anxiety response and distress. This typically results in subsequent avoidance of the phobic stimulus or situation

eventually leading to significant restriction for the subject. To fulfil diagnostic criteria, the subject further has to recognize the disproportionality of the fear and to suffer at least six months from the condition[20].

Table 1. Diagnostic criteria of specific phobia*

1. Marked and persistent excessive or unreasonable fear of a specific object or situation.
2. Immediate intense anxiety response and distress produced by exposure to the phobic object or situation.
3. Subsequent avoidance of the phobic object or situation.
4. The subject recognizes the unreasonableness of the fear.
5. The condition lasts for at least six months.

*according to the DSM V (American Psychiatric Association, 2013)[20]

Five subtypes in specific phobia are distinguished: animal, natural environment, blood-injection-injury (BII), situational, and other[20]. Its etiology, however, is, as with other anxiety disorders, multifaceted – genetic vulnerability and anxiety proneness, natural preparedness, classical and operant conditioning, observational learning, verbal transmission, negative cognitive content and cognitive biases are possible contributing factors to the development of phobic fears[21,22]. Predominantly, the individual vulnerability seems to be largely innate, being based on phobia specific genetic effects as well as on additive genetic factors that provide a genetic predisposition for fearfulness[22–24]. Generally, the heritability rate of specific phobias is estimated to be as high as 30%, however varying from the individual phobia[25]. Further, unique environmental factors appear to have a triggering impact while common environmental effects are less critical[22]. Although being often regarded as benign disorders, specific phobias can cause severe and far-ranging distress leading to impairments on social functioning and everyday activities[12,16]. Additionally, subjects suffering from specific phobias exhibit a relatively high level of comorbid mood and particularly of anxiety disorders[12,26]. Unfortunately, the percentage that receives treatment is still low, although a wide array of effective therapy approaches are available[12]. Of these, in-vivo exposure based cognitive-behavioral therapy (CBT) with usually high response rates[27] is the first-line treatment according to the current German guideline for the treatment of

specific phobia[28]. Noteworthy are also promising new therapy approaches such as repetitive transcranial magnetic stimulation[11] (rTMS) or neurofeedback[29] that require a profound understanding of the underlying neural mechanisms for an appropriate therapy.

Snake Phobia

The animal phobia subtype with prevalence rates between 1% and 8% takes a leading position in the ranking of specific phobias[12,13,30]. Snake phobia (SP) – also named ophidiophobia – with a prevalence between 1.2% and 5.5% is a typical example for this type[17,30]. While self-experienced trauma is the leading cause for the development of SP with approximately 40%, more than one third of the concerned subjects cannot recall a triggering condition[23]. The relatively easy acquisition of animal phobias can be explained, among other factors, by innate preparedness[31]. This theory assumes that humans are evolutionary prepared to respond to biologically significant stimuli (e.g., snakes or saber-toothed tigers). However, there seem to exist disorder-specific genetic risk factors for the development of animal phobias, too[32]. The Snake Anxiety Questionnaire[33] (SNAQ) is a valid screening method to assess symptom severity in subjects who might suffer from SP. The diagnosis can be confirmed if relevant DSM-V criteria (e.g., by using SCID-CV) are met.

Dental Phobia

Dental phobia (DP) or odontophobia is currently assigned to the blood-injection-injury (BII) group as dental treatments are invasive medical procedures[20]. Although some authors suggest considering DP as a unique subtype[34,35], more similarities than differences are reported[36]. Having an estimated prevalence rate between 2.1% and 3.7%[17,30], many people not only suffer from DP itself but are at high risk for avoidance behavior considering dental treatment[37–39]. BII phobias show the highest heritability among all phobia subtypes and are suggested to be more than other subtypes characterized by disorder specific genetic factors[22,32]. DP is further highly associated with self-experienced trauma, while other modes of acquisition seem to play a less dominant role[23]. DP can be assessed with the Dental

Fear Survey[40] (DFS) or the Dental Anxiety Scale[41] (DAS).

Neural and Psychophysiological Basics of Specific Phobias

Neuroimaging research on specific phobia has revealed a fear processing network, encompassing the amygdala, the ACC, the insula and the thalamus[2,4,5]. However, research has predominantly focused on the animal subtype and especially on spider phobia. While it was confirmed that the neural activation pattern of spider phobia as a model disorder for animal phobias corresponds largely to SP[42], research on DP and other BII phobias is less consistent. Fear circuitry structures were reported to be active in some cases[43–45], but not in all[42,46]. So far, no study has detected amygdala hyperactivity (as widely reported for animal phobia). Instead, prefrontal and orbitofrontal regions appear to play an important role in BII phobias[42,45,47,48]. In DP, Hilbert et al. described a similar pattern to animal phobias under auditory symptom provocation pointing to stimulus dependence[44]. Further, Caseras et al. observed similar reactions in BII subjects and spider phobics during immediate stimulus processing, but not during sustained provocation[43,49].

On a psychophysiological level, SP as an animal phobia features a high activation of the sympathetic nervous system[50,51]. Contrary, the BII subtype is characterized by a biphasic vasovagal response potentially resulting in a vasovagal syncope[51,52]. In DP, the biphasic response pattern might be less pronounced and particularly fainting is less frequently observed compared to other phobias of this subgroup[35]. However, the typical diminished autonomic and hemodynamic responsiveness has been also reported in DP[42].

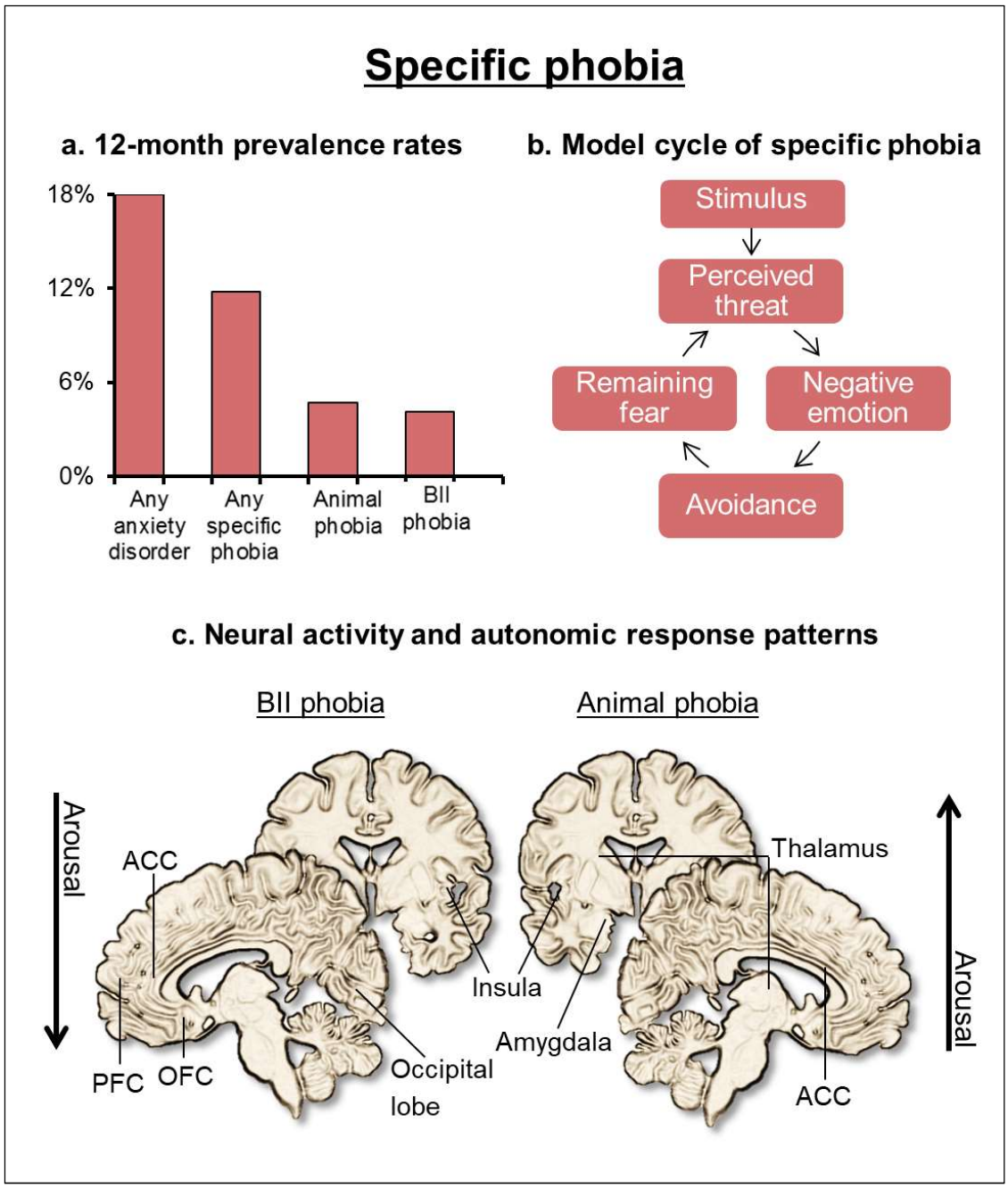


Figure 1. Specific phobia. a. 12-month prevalence of anxiety disorders in general (Kessler et al.[15]), of any specific phobia and the animal and the blood-injection-injury (BII) subtype, respectively (Oosterink, de Jongh, & Hoogstraten[17]). b. Simplified model of the preservation of phobic fears. c. Regions exhibiting altered activity[1,5] are illustrated on the left for the BII subtype, and on the right for the animal subtype. The left arrow depicts the diminished vegetative response in BII phobia, the right arrow the increased response in the animal subtype[46,51,53]. ACC: anterior cingulate cortex; OFC: orbitofrontal cortex; PFC: prefrontal cortex.

Amygdala, ACC and Insula in Specific Phobia

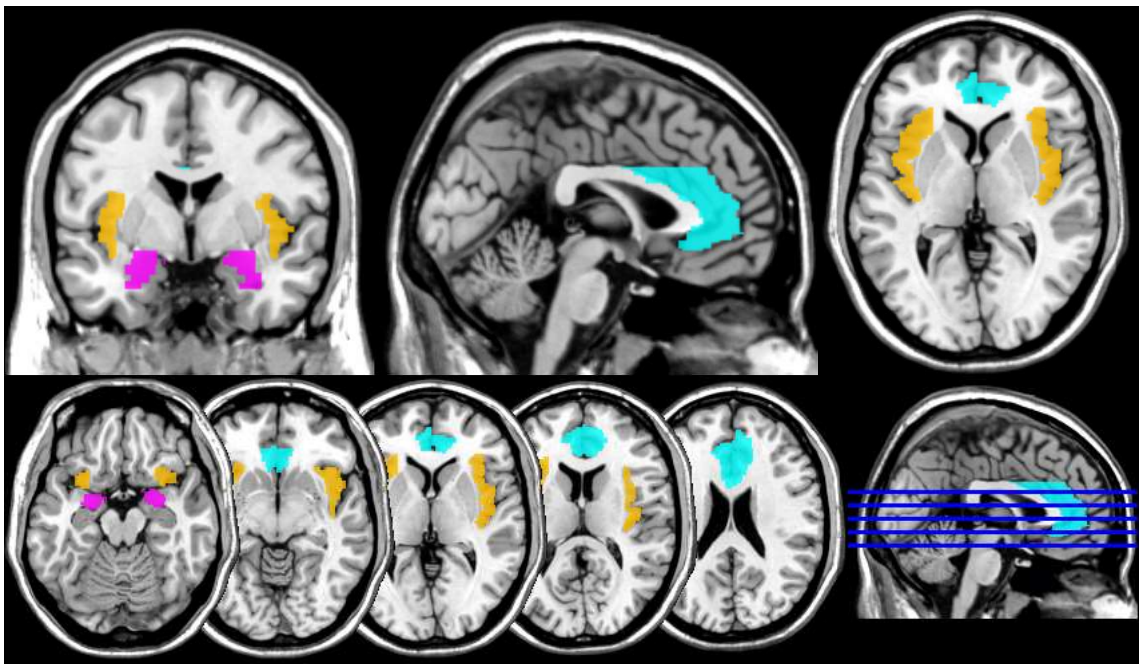


Figure 2. Amygdala, ACC and Insula. Illustration of the amygdala (violet), the anterior cingulate cortex (ACC; cyan) and the insula (orange) on an anatomic brain image in radiological convention. The regions displayed are derived from the Automated Anatomical Labeling atlas by Tzourio-Mazoyer et al.[199].

The Amygdala

The amygdala is a phylogenetically old region lying bilaterally beneath the parahippocampal gyrus in the temporal lobe[54]. It has been argued that the amygdala itself is neither a structural nor a functional entity due to its variety of distinct nuclei and subnuclei[55]. However, the amygdala is mostly subdivided into an evolutionarily primitive part containing the corticomedial group linked to the olfactory system and a newer part containing the basolateral region that is associated with the neocortex[54,55]. The amygdala as a whole has a wide array of direct and indirect connections with almost all frontal and limbic regions[56]. The lateral amygdala receives input from the thalamic sensory systems and thus is regarded as the gatekeeper of the amygdala[55]. The information gets further processed in the basomedial and the basolateral group[55]. It receives additional input from the paralimbic cortex, frontal and posterior association areas as well as from the insula[54,57]. Fibers from the basolateral and basomedial group finally reach the central group that connects the amygdala to the hypothalamus, the brainstem

and motor systems. This depicts a possible route to influence autonomic and defensive responses[54,57]. Noteworthy in this context are the intercalated cells of the amygdala exerting inhibitory control over the central and basolateral nucleus and which receive input from the infralimbic cortex [58].

The Kluver-Bucy syndrome, first documented in 1939 while examining the effects of bitemporal lesions (including the amigdaloid nuclei) in rhesus monkeys, describes a behavioral syndrome that includes hyperphagia, hypersexuality, visual agnosia and diminished fear responses[59]. This syndrome has been observed in humans, too, and many of the behavioral changes observed have been traced back to damage to the amygdala[60,61]. Since then, the amygdala has become an intensely studied brain region. To date, the amygdala's probably most prominent task is its leading role in the processing of biologically significant stimuli that could stimulate a fight-or-flight response[54,55]. The amygdala is generally involved in the recognition and immediate behavioral processing of emotional valences associated with sensory perceptions[54]. It has been proven to be necessary for Pavlovian fear conditioning[62] and to be involved in autonomic activity and frontal control and executive tasks. More precisely, it is linked to attention and explicit memory processes, reward learning, motivation and to the expression and modulation of emotional responses including fear reactions[55,63,64]. Although the amygdala is mainly associated with negative affect, it should be borne in mind that it is also active while experiencing positive emotions[65].

As in various other anxiety disorders[2,4], the amygdala has been reported to show hyperactivity in animal phobias, but not in DP[2,4,5]. In animal phobias, the amygdala has been linked to the detection of threatening phobogenic stimuli and the initiation of defensive behaviors[46]. However, a few studies failed to show any amygdala hyperactivation in this subtype, possibly due to the rapid amygdala habituation[66,67]. This is the reason why intermittent and repeated stimuli presentation are argued to be better suited for the detection of amygdala activity[5]. The increased activity has been shown to be sensitive to exposure therapy[68,69].

The Anterior Cingulate Cortex

The cingulate cortex forms a collar around the corpus callosum and is cytoarchitectonically regarded as mesocortex with a characteristically deep layer of large pyramidal neurons[54,70]. The classic model by Vogt et al. distinguishes four parts: the anterior, the mid-, the retrosplenal and the posterior cingulate cortex[70]. The ACC comprises the Brodmann areas (BA) 24, 25, 32 and 33[70] and is an important part of "Papez´s circuit"[64]. The ACC can be further subdivided in a ventral affect-related and a dorsal cognitive part[71]. The "affective" division is connected to the amygdala, periaqueductal gray, nucleus accumbens, hypothalamus, anterior insula as well as to the orbitofrontal cortex (OFC) and to further autonomic, visceromotor and endocrine systems[72,73]. Today, the affective part is often subdivided into perigenual ACC (pACC, sometime also referred to as pregenual or rostral) including BA 32 and partly inferior BA 24 and a subgenual ACC (sACC) including mainly BA 25 as well as caudal parts of BA 32 and 24 and a cingulate motor area[70]. The dorsal ACC (dACC) is assumed to represent the "cognitive" subdivision of the ACC and includes caudal BA 32 and caudal BA 24 which corresponds mainly to Vogt´s anterior midcingulate cortex[72,74]. It is connected to the lateral prefrontal cortex (BA4 6/9), the parietal cortex (BA 7), to premotor and supplementary motor areas and is included in the diffuse attentional network[64,73,75]. Naturally, there are anatomical connections between the anterior and posterior cingulate cortices as well as within the ACC, particularly BA 32 and 25 are heavily connected[64,76].

Worthy of mention is the mediating role of the ACC, which might provide an indirect route for amygdala regulation. On the basis of ample connections to the prefrontal cortices, the dACC and sACC are hypothesized to integrate and mediate input from BA 9, BA 10, BA 42 and BA 46[65]. In turn, the dACC and the sACC have the largest output to the amygdala with the sACC sending projections to every amygdala subnucleus[56,77]. Connections from the sACC are considered to be somewhat inhibitory in contrast to the projections from the pACC that seem to be excitatory and generally less dense[64,78]. Even so, the pACC has been argued to be well-situated to influence the amygdala selectively[65].

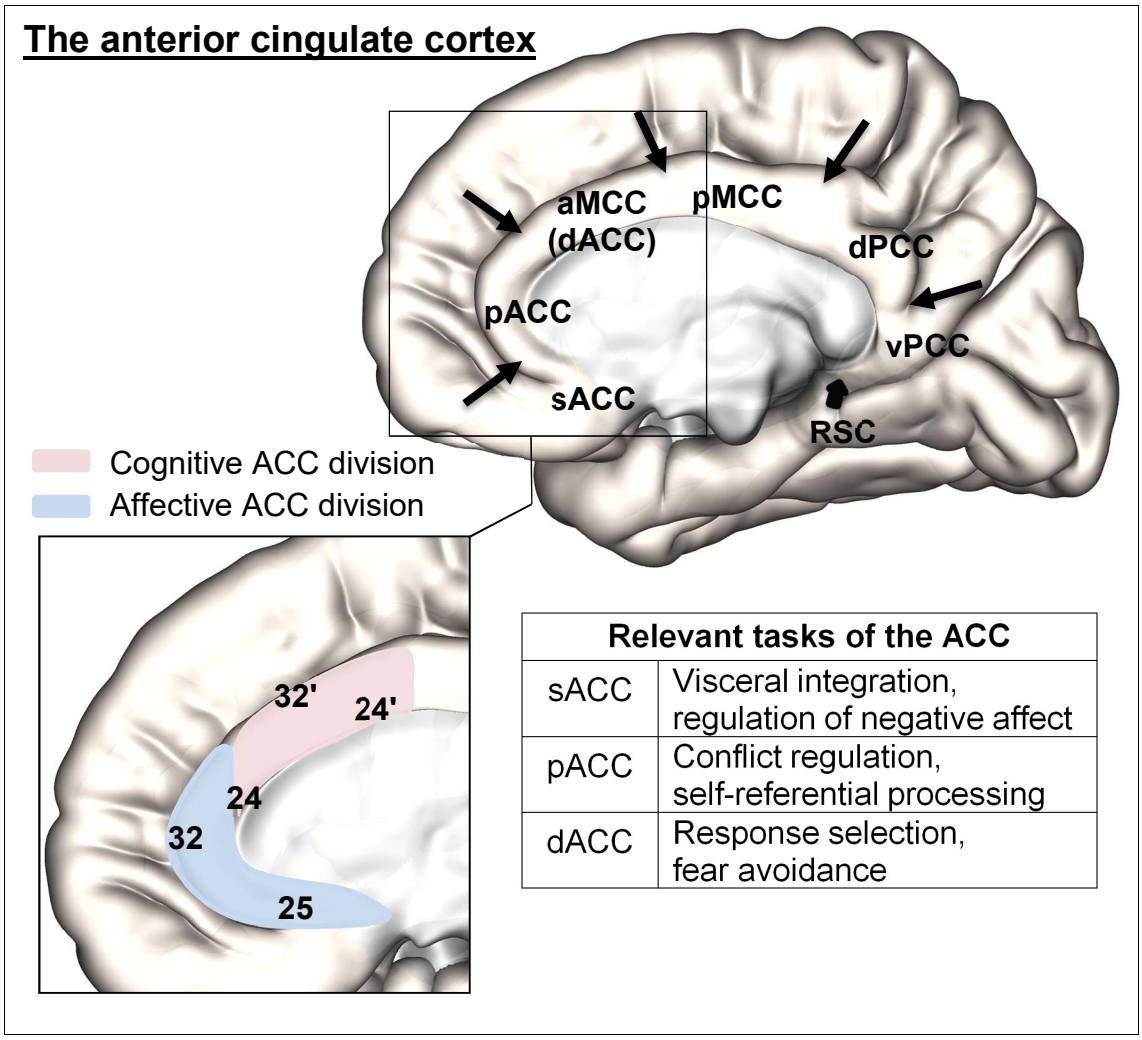


Figure 3. The anterior cingulate cortex. Top: Model of the cingulate cortex according to Vogt et al.[79]. The anterior cingulate cortex (ACC) is further divided into a pregenual ACC (pACC) and a subgenual ACC (sACC). The midcingulate cortex is divided into an anterior part (aMCC; often referred to as dorsal ACC (dACC)), and a posterior part (pMCC). Likewise, the postcingulate cortex (PCC is divided into a dorsal (dPCC) and ventral (vPCC) subdivision. Borders are marked with arrows. A model dividing the ACC in an affective and cognitive part by Bush et al.[71] is displayed on the bottom left; Brodmann areas are schematically illustrated. Relevant ACC tasks are given on the bottom on the right. Related references are given in this section. MCC: midcingulate cortex; RSC: retrosplenial cortex. The figure is based on: "Pain and emotion interactions in subregions of the cingulate gyrus." Vogt, B. A., Nat. Rev. Neurosci. 6, 533–544 (2005), with permission of Springer Nature.

Lesions in the anterior cingulate – both in animals and humans – lead to a variety of symptoms, such as apathy, motor and speech disturbances, inattention and irresponsiveness to pain. In animals, lesions are also associated with diminished avoidance learning[73,75]. Brain tumors located in the ACC have been linked to stress, adversity, and ambivalence[80]. Cingulotomy or cingulectomy, however, have been found to have a beneficial outcome on pain-related, obsessive, anxious, depressive

and aggressive aspects of behavior[73,75]. Yet, in the context of lesion studies and pathologies of the cingulate it must be considered that adjacent brain regions might be marred as well and that the symptoms might be caused by impaired fibers passing through the cingulum.

The ACC is recognized as a connector or mediator between different brain regions, thus having a central role in the coordination of affect, cognition and behavior[75,79,81]. It is generally implicated in executive functions of visceral, skeletal and endocrine systems associated with emotion control[73]. The affective part of the ACC is involved in emotional responses, such as in assessing the salience of emotion and motivational information and it has also been related to autonomic activity[72,73,79]. Specifically, the pACC is related to reflective processing and conflict regulation, to observational fear learning as well as to overcoming fear. It also seems to be pivotal for individual trait anxiety[2,81,82]. The sACC is linked to sleep, appetite, libido, autonomic and endocrine functioning and particularly to sadness and the regulation of negative effects[79,81,83]. The dACC is associated with motor functions, conflict monitoring, error detection and response selection, motivation, working memory, cognitive anticipation and pain processing[71,75]. It is also related to avoidance behavior and fear extinction[2,79,81]. Moreover, it forms a part of the attention and salience network[84,85]. Interestingly, it has been suggested that the sACC and dACC can influence antagonistically the vegetative system[86].

Hyperactivation of the ACC is regarded as a typical feature of the animal subtype, while literature on DP is unfortunately sparse[2,5,81]. The hyperactivation of the ACC in phobias is argued as corresponding to increased salience and sensitivity towards feared phobic stimuli[5]. Regarding subregions, mainly the dACC has been observed to be active[1,2,5,87]. Generally, the ACC activation seems to be responsive to treatment in specific phobia[88].

To summarize the main findings on the ACC in anxiety, it is noteworthy
(1) that it is involved in the merging of affect, cognition and behavior,
(2) that it has mainly three parts with different tasks in specific phobia and
(3) that the engagement of the ACC and its subdivisions may differ in the subtypes of specific phobia.

The Insula

The insula (also insular cortex/lobe) is a small cortical area buried deeply within the lateral sulcus lying beside the putamen[89]. The ventral anterior insula is cytoarchitectonically characterized by agranular zones. The dorsal anterior to the middle insula is dysgranular followed by a granular region from the middle to the posterior insula[90]. The insula is known to share reciprocal connections with the medial temporal, temporopolar and orbitofrontal cortices, the cingulate gyrus and the lateral prefrontal cortex[91,92]. The anterior insula shows connections to the ACC, whereas the posterior insula is connected with the middle cingulate[91]. Reciprocal connections between the insula and the different nuclei of the amygdala have also been observed[93].

The cytoarchitecture and anatomical characteristics of insula subregions are mirrored in distinct connectivity patterns, mainly pointing to an anterior emotional and a posterior sensorimotor pattern[94–96]. Particularly noteworthy is the three-system-model by Deen et al. suggesting that 1) the posterior insula (postINS) is connected to the primary and secondary somatomotor cortices, while 2) the dorsal-anterior insula (daINS) region is connected to the dACC and the regions that exert cognitive control, and that 3) the ventral-anterior insula (vaINS) is connected to the pACC (see also figure 4, p. 12).

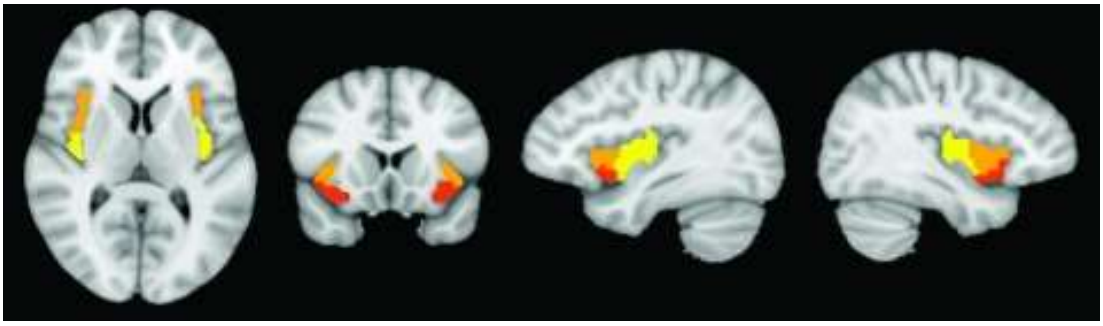
Lesions of the insula have been linked to impairments in disgust processing[97] and reduced sensitivity to the emotions of others[98], while strokes in the insula have been observed to result in somatosensory, speech and motor abnormalities[99].

The insula is a multifaceted integration region evaluating the emotional and motivational salience of varying stimuli, thus mediating between external information and internal body states[91,100–102]. It seems to function as an integrative hub in higher-order processing of sensory input and autonomic processing and mediates the integration and representation of internal bodily states of arousal[103].

The insula has been commonly observed to be hyperactive in anxiety disorders including specific phobia[4,5]. Activity may even be enhanced when subjects are instructed to focus on interceptive signals[104]. Findings for the animal subtype are more stable[2,4,5]. However, a pivotal role of the insula in DP is reasonable,

particularly due to its involvement in disgust and pain processing which are essential features of the BII subtype[105,106]. In contrast to the amygdala, the insula might be rather related to sustained than to phasic fear[67]. The hyperactivity has been shown to be responsive to treatment in specific phobia[88,107].

Insula subregions Deen et al.[96]



Insula subregions in the current analysis

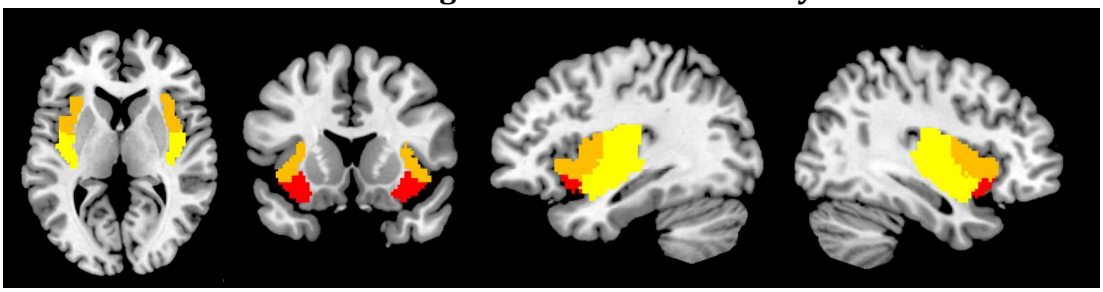


Figure 4. Insula subregions. Identified subregions by Deen et al.[97] using cluster analysis are presented in the upper row. The bottom row illustrates the insula ROIs using the Brainnetome atlas[99] in the current study. The ventral-anterior insula is shown in red, the dorsal-anterior insula in orange and the posterior insula in yellow. Images are in radiological convention. The upper image is taken from: Deen, B., Pitskel, N. B. & Pelphrey, K. A. Three systems of insular functional connectivity identified with cluster analysis. Cereb. Cortex N. Y. N 1991 21, 1498–1506 (2011), with permission of Oxford University Press.

Neural Circuits in Specific Phobia

ACC – Amygdala Connectivity

As mentioned earlier, both the ACC and the amygdala have plenty of connections with each other. Even though the connections are reciprocal, the ACC has more efferent fibers to the amygdala than vice versa. Hence, it could be suggested that the ACC is functioning as a sender that particularly regulates the amygdala[65]. Due to the variety of interconnections between the amygdala and the ACC subregions, this circuitry has been suggested as representing an important feedback-loop for

emotion regulation, e.g., overcoming fear[8,108–112], as well as for the processing of environmental stressors[113,114]. Thus, dysregulated bottom-up excitatory and top-down inhibitory control of the amygdala by the ACC/medial prefrontal cortex (mPFC) might represent a pathophysiological feature of clinically relevant anxiety[115]. This might also account for specific phobia where a large range of findings is available on frontal and amygdala activity changes during phobogenic stimuli processing[5,116]. In line with these conceptions, one study using positron emission tomography (PET) only found an inhibitory connectivity between the amygdala and the ACC in a control condition but not in the phobic anxiety condition[117]. Regarding the BII subtype, a functional magnetic resonance imaging (fMRI) study by Scharmüller et al. detected a positive coupling of the ACC and the amygdala in HC compared to DP during visual symptom provocation[118].

Amygdala – Insula Connectivity

The amygdala and the insula share widespread bidirectional anatomical connections. The anterior insula is connected to the anterior amygdala region and to the medial, cortical, accessory basal, medial basal and the lateral amygdaloid nuclei. The posterior insula shares connections with the lateral, laterobasal and central nuclei[93]. It has been suggested that the insula functions as a nexus conveying interoceptive information to the amygdala to modulate behavioral responses[100,119,120].

In resting-state examinations the amygdala was shown to exhibit a positive connectivity with the insula[121] that correlated to state anxiety[122]. Further, both regions are simultaneously active in anxiety processes[123,124]. Disturbed amygdala and posterior insula resting-state FC has been linked to behavioral and emotional dysregulation in depression[125].

Altered FC of the insula and the amygdala has been observed in several disorders (e.g., generalized anxiety disorder[126] (GAD), posttraumatic stress disorder[127] (PTSD) and major depressive disorder[128] (MDD)). Unfortunately, no study so far has examined the interplay of these two core regions in specific phobia.

ACC – Insula Connectivity

The insula has reciprocal afferent and efferent connections with the cingulum[119]. The anterior to middle insula is mainly connected to the anterior cingulate whereas the posterior insula has efferent projections to the whole cingulum with the most fibers lying in BA 24[91,92]. Given the specific functions of both regions, these connections led to the hypothesis that this circuitry is related to interoceptive markers of negative affect[129–132]. Based on resting-state examinations, a division into a ventral and a dorsal network has been made. The ventral network, consisting of both sACC and pACC and the anterior insula, is linked to emotional processes and the integration of interoceptive, emotional salient information to generate a subjective representation of the body[95,133]. On the other hand the dorsal network consisting of dorsal insula and dACC is related to cognitive control and salience[94,95,102,134,135]. Moreover, it has been suggested that the dACC and the entire insula are both involved in environmental monitoring, response selection and skeletomotory body orientation[94,96].

Regarding anxiety and anxiety-related processes, both insula and ACC have been related to attentional direction, salience processing, evaluation processes and anticipatory anxiety[101,136–139]**.** Particularly with respect to DP, it is important to consider that both regions are involved in disgust processing[140,141] as well as in pain anticipation and modulation[142], which all form critical features of the BII subtype[105,143]. Notably, structural connectivity between the pACC and the anterior insula has been found to contribute to pain vigilance and awareness[144].

ACC – insula connectivity changes have been already described in several clinical studies (e.g., MDD[145], GAD and social anxiety disorder[10] (SAD)). Though such connectivity could be expected in specific phobia, no study so far has specifically addressed this interaction.

Further Neural Circuits in Specific Phobia

Since fear generation and regulation are complicated and multifaceted processes, further neural circuits are likely to be involved in specific phobia. Various neural models of emotion regulation have been proposed, with many of them suggesting a

distinction between emotion identification, generation and simple regulation circuits on the one hand and higher order control networks on the other[146–149]. Figure 5 (p. 16) illustrates the model by Phillips et al.[148] which largely corresponds to a model of an implicit and explicit pathway of emotion regulation by Etkin et al.[149]. The model by Phillips et al. distinguishes three pathways of emotional processing: emotion identification (e.g., amygdala, thalamus, and basal ganglia), automatic emotion regulation (e.g., pACC, sACC, OFC) and voluntary emotion regulation (mainly prefrontal areas). Deficiencies in automatic regulation circuits could particularly account for SP on grounds of its strong defensive response while DP might be more characterized by impairments of voluntary emotion regulation[42,44,46]. In this respect, particularly pre- and orbitofrontal regions in DP can be expected to exhibit altered connectivity since it has been argued that these cognitive control regions are highly involved in maladaptive phobic stimuli processing in the BII subtype[150–153]. Moreover, it may particularly be assumed that regions of the so-called fear circuit (e.g., nucleus accumbens, hippocampus, the brain stem, thalamus[2]) exhibit altered FC due to frequently observed activation changes of these structures in specific phobia[2,4,5].

Two studies examining FC in DP described enhanced connectivity between the ACC and basal ganglia during phobic stimuli processing in controls only[154] as well as a generally more widespread FC in controls compared to DP[118,154]. Regarding animal phobia, one study reported enhanced functional coupling of the amygdala with the periamygdaloid area, the fusiform gyrus and the motor cortex during phobic symptom provocation, whereas the non-phobic state was characterized by negative connectivity of the amygdala with prefrontal areas[118]. Enhanced FC of the amygdala with the postcentral gyrus, middle temporal gyrus, superior occipital gyrus, middle temporal gyrus and precentral gyrus has been reported during phobic fear processing in subjects suffering from spider phobia[155]. Finally, Nakataki et al. reported increased FC between the amygdala and the fusiform gyrus which decreased under cortisol admission[156].

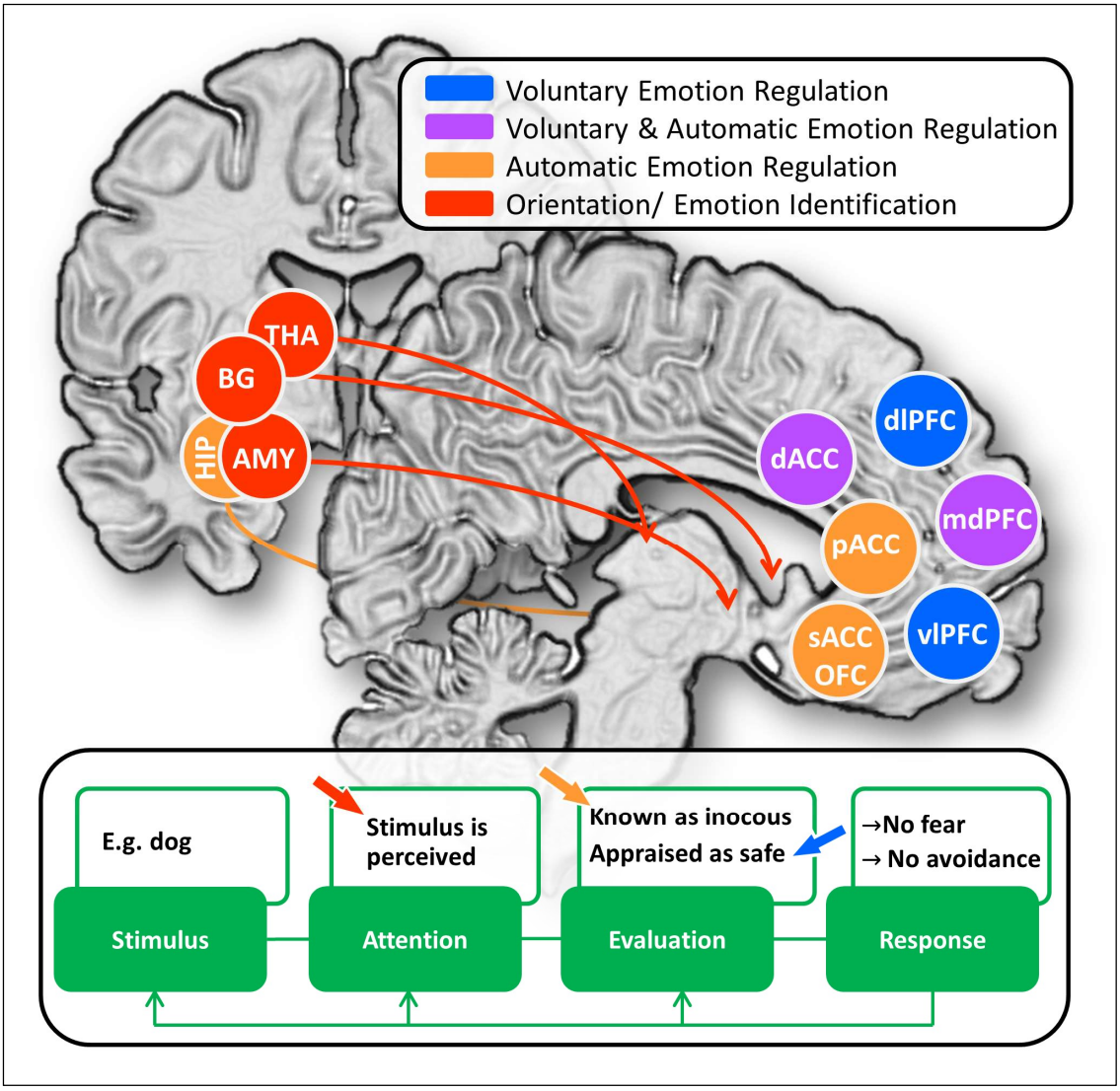


Figure 5. Neural and psychobehavioral model of emotion regulation. According to the neural model of emotion regulation by Phillips et al.[148] regions are illustrated on a coronal and axial brain slice and colored according to their primary role in emotion processing. Thin arrows depict their virtual projection on the sagittal slice. On the bottom, a psychological model of emotion regulation according to concepts of Gross[157] is exemplified for an ordinary stimulus (a dog) showing adequate responses. Thick arrows illustrate interfaces of the neural and psychological model (red: orientation/ emotion identification; orange: automatic emotion regulation; blue: voluntary emotion regulation) depicting potential points of disturbances. THA: thalamus; BG: basal ganglia; AMY: amygdala; HIP: hippocampus; sACC: subgenual anterior cingulate cortex; pACC: pregenual ACC; dACC: dorsal ACC; vlPFC: ventrolateral prefrontal cortex; mdPFC: mediodorsal PFC; dlPFC: dorsolateral PFC. The figure is based on: "A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder." Phillips, M. L., Ladouceur, C. D. & Drevets, W. C., Mol. Psychiatry 13, 833–857 (2008), with permission of Springer Nature.

Objectives and Hypotheses

Objectives

The analyses aimed at exploring FC of the amygdala, the insula and the ACC in both SP and DP as well as in a healthy control group (HC). These regions were chosen as primary research targets for the following reasons:

1) The regions are associated with emotion regulation and, in particular, with fear processing – including fear conditioning and fear extinction[2,158,159].
2) They commonly exhibit altered neural activation in specific phobia[2,4,5].
3) Further, these neural activity changes seem to be sensitive to cognitive-behavioral therapy[160].
4) Thus, exploring these functional circuits could lead to neural biomarkers of specific phobia and to the identification of new targets for neuromodulation techniques.

Due to functionally diverse subregions, the ACC was further subdivided into a subgenual, pregenual and dorsal ACC and the insula was divided into a ventral-anterior, dorsal-anterior and posterior region[95,133]. To explore the relationship between FC and clinical and behavioral outcomes, connectivity values were correlated to various questionnaire scores (e.g., SNAQ and DFS scores) and skin conductance data (SCR).

Hypotheses

I. In view of the neural activity patterns of both SP and DP as well as the previously published findings of FC in specific phobia, altered (i.e., predominantly increased positive or decreased negative) connectivity patterns in the phobic groups compared to the healthy control groups were to be expected.
   a) Provided that the ACC – amygdala circuit is primarily associated with fear inhibition appearing to be deficient in specific phobia, an increased positive or decreased negative coherence of functional connectivity was to be expected in subjects suffering from specific phobia compared to the healthy

control group.

b) Given the fact that the amygdala and the insula are both core components of specific phobia and frequently act in tandem, an increased connectivity within phobics was hypothesized.

c) Since the circuitry between the insula and the ACC has hardly been studied so far, it seems reasonable to refer to findings of exaggerated activity in specific phobia and thus to expect a heightened connectivity between these regions in phobia groups.

d) Based on the above-mentioned findings of heightened amygdala, ACC and insula activity in animal phobias and less conclusiveness in the BII subtype, particularly SP might suffer from dysregulated interplay in these regions. Hilbert et al.[44] described fewer differences between the neural activity patterns of SP and DP during auditory symptom provocation. Thus, DP was assumed to resemble more closely SP´s connectivity pattern under this kind of stimulus presentation.

II. The examination of subregions was primarily conducted to evaluate which part of the ACC is associated with amygdala inhibition during emotional processes. Here, the findings point to a pivotal role of the pACC[161] and sACC[8]. Regarding insula subregions, it was expected that the divisions of an anterior "affective" network and various posterior "cold" networks would be mirrored in the connectivity patterns in this study, too.

III. The exploratory seed-to-voxel analysis was conducted to reveal connectivity changes of the seeds with further fear-related structures in DP and SP. While SP was expected to show alterations in basal fear-processing networks, DP might be more characterized by impairments in pre- and orbitofrontal circuits.

Corresponding analytic approaches:

I. The first analysis is aimed at revealing FC within the ACC, the amygdala and the insula. Moreover, a mean FC index was calculated for each group (i.e., SP, DP and HC in D1; DP and HC in D3) and for each stimulus modality (i.e., visual and auditory stimuli). To keep the selected regions as constant as possible a region of interest (ROI) approach was employed based on regions from the Automated

Anatomical Labeling software atlas (AAL). Main findings of this analysis were recently published[162] (Please refer to: “Networks of phobic fear: Functional connectivity shifts in two subtypes of specific phobia.” Stefanescu MR*, Endres RJ*, Hilbert K, Wittchen HU, Lueken U. Neuroscience Letters, 2018 January, 1; 662:167-172. doi: 10.1016/j.neulet.2017.10.031. *shared first authorship).

II. To specify these interactions, a further ROI-to-ROI analysis was conducted using insula (vaINS, daINS, and postINS) and ACC subregions (sACC, pACC, and dACC) derived from the Brainnetome atlas.

III. Additionally, a seed-to-voxel FC analysis containing the insula, amygdala and ACC as seeds was intended to investigate further connectivity patterns of the regions concerned across the whole brain.

Material and Methods

Material

Overview

The material for the experimental procedure (subjects, raw MRI data, subjective ratings and psychophysiological data) is based on three studies that were conducted at the Institute of Clinical Psychology and Psychotherapy, Technische Universität Dresden. For this post-hoc analysis, 94 subjects were included (HC: n = 31, DP: n = 38, SP: n = 25; 9 subjects participated both in D2[46] and D3[44] as HC).

Subjects

Subjects were recruited from a student population and were preselected by an online screening on snake and dental phobia using the SNAQ and DFS. Established cut-offs of at least 20 points in the SNAQ (indicating severe SP[163]) and of 76 points in the DFS in D1 (indicating severe DP[40]), of 75 points in D2 (meaning two standard deviations above the sample mean) and of 72 in D3 (indicating moderate to severe DP[40]) were applied. Control subjects scored in the lower quartiles of both questionnaires or fewer than 33 in the DFS in D3. Subjects scoring above cut-off scores for both the DFS and SNAQ or that reported comorbidity within the last 12 months were excluded. Further exclusion criteria were either MRI-related or neurological diseases, psychotropic medication, severe mental disorders such as psychotic, bipolar, obsessive-compulsive disorder, PTSD, MDD and addiction (except to nicotine). After obtaining written informed consent, subjects were examined by using several questionnaires and clinical interviews (e.g., Composite International Diagnostic Interview[164], Anxiety Sensitivity Index[165] (ASI), Mutilation Questionnaire[166] (MQ), and Beck Depression Inventory[167] (BDI)). All studies were conducted in accordance with the ethical standards laid down in the Declaration of Helsinki and were approved by the local ethics committee of the Technische Universität Dresden. Students received either course credit or financial reward for their participation.

Experimental Procedure

The fMRI experiments were programmed on Presentation 12.0 (Neurobehavioral Systems, Albany, CA, USA) and presented by using video goggles (VisuaStim Digital, Northridge, CA, USA) and standard headphones. All subjects were instructed to focus attentively at the stimuli during their presentation.

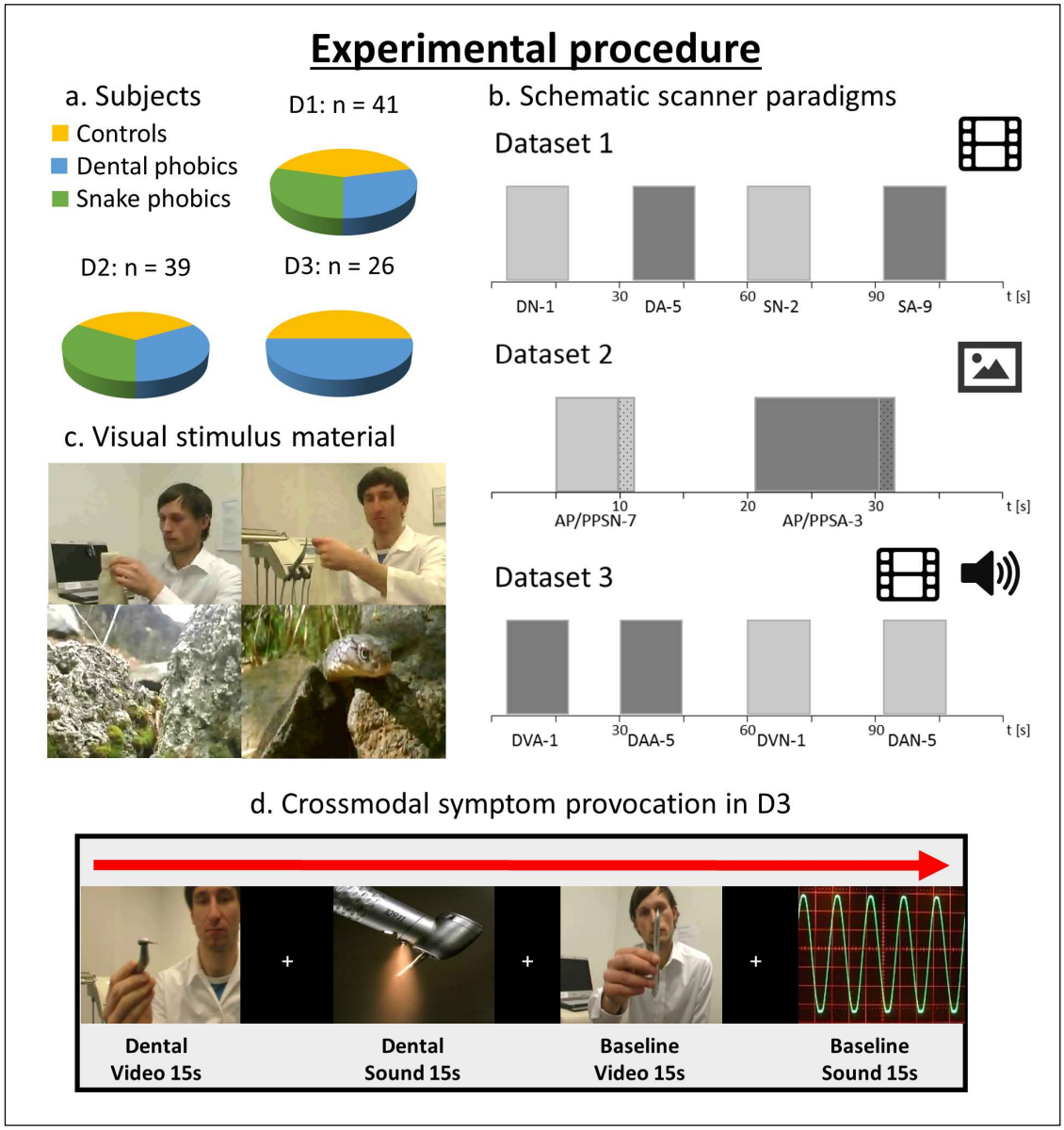


Figure 6. Overview over the experimental procedure. Number and distribution of subjects are given in circle charts in (a). (b) illustrates the scanner paradigm. (c) gives an example of the visual stimulus material (dental stimuli: upper row, snake stimuli: bottom row, anxiety conditions on the right, matched neutral conditions on the left). (d) depicts the alternating use of visual and audio stimuli in D3. DN: dental neutral; DA: dental anxiety; SN: snake neutral; SA: snake anxiety; AP/PPSN: anticipation phase/perception phase snake neutral; AP/PPSA: anticipation phase/perception phase snake anxiety; DAA: dental auditory neutral; DAN: dental auditory anxiety; DVN: dental video neutral; DVA: dental video anxiety. Graphical material of the stimuli is taken from Lueken et al.[42,168] and is used with permission of the owners.

D1: The paradigm contained 40 videos with a length of 15 seconds each that were presented in a block-design. Video sequences were based on a previously validated video set by Lueken et al.[168]. The videos depicted ecologically valid scenarios of first-person encounters with typical phobogenic stimuli such as a dental treatment (e.g., a person putting on a latex glove to prepare for treatment for the dental anxiety condition) and respective neutral conditions providing a valid baseline for non-anxiety specific processes (e.g., a person putting on a wool glove). Four stimulus conditions (snake anxiety: SA; snake neutral: SN; dental anxiety: DA; dental neutral: DN) with 10 video sequences each were used. Videos were shown in a pseudo-randomized order in which no condition was presented more than twice consecutively. A variable (11 – 19 seconds) intertrial interval separated the single videos and anxiety conditions were preceded by the respective neutral condition to avoid carry-over effects.

D2: Stimuli in D2 were presented in an event-related design to examine both anticipation and perception processes by using a cued presentation of phobic stimuli. Two pictures per condition from the above-mentioned video sequences were extracted and presented in a perception phase (PP) for 1.250 milliseconds. An anticipation phase (AP) preceded the PP and announced the following stimulus content with a letter ("Z" for dental stimuli (in German: "Zahnarzt"), "S" for snake stimuli and "N" for neutral stimuli). The AP interval varied between 5 and 10 seconds to avoid the prediction of the PP onset. An inter-stimulus interval followed with durations of 5.8, 9.3 or 11.9 seconds. Eight conditions were presented in a randomized order (AP: snake neutral (APSN), snake anxiety (APSA), dental neutral (APDN) and dental anxiety (APDA) stimuli; PP: snake neutral (PPSN), snake anxiety (PPSA), dental neutral (PPDN) and dental anxiety (PPDA)).

D3: The task was conducted in DP and HC, applying both audio and video stimuli in a block-design. Visual stimuli were based on the above-mentioned video set. Auditory stimuli were taken from a publicly available databank and included 10 dental drill sounds and 10 sinus tones as neutral conditions. The task comprised four conditions (dental audio neutral (DAN), dental audio anxiety (DAA), dental video neutral (DVN) and dental video anxiety (DVA)). The presentation order was pseudo-randomized; no condition was shown more than twice consecutively.

fMRI Data Acquisition

MRI data had been acquired using a 3-Tesla Trio-TIM MRI whole-body scanner (Siemens, Erlangen, Germany); a 12-channel head coil, goggles for visual presentation and standard headphones were used. Functional images were obtained via a T2*-weighted gradient echo-planar images sequence covering the whole brain (487 volumes in D1, 560 in D2 and D3, repetition time (TR) 2.500 milliseconds, echo time (TE) 25 milliseconds, field of view (FOV) 192 x 192 mm and matrix 64 x 64). Axial slices (D1: 43, D2 and D3: 44) were recorded with a slice thickness of 3.5 mm in D1 and 3 mm in D2 and D3 (interleaved acquisition, no gap, in-plane resolution 3 x 3 mm). Slices were recorded in a tilted angle to reduce susceptibility in inferior brain areas[169]. The first four volumes were discarded due to T1 equilibration effects. Structural images were acquired via magnetization-prepared rapid gradient-echo imaging sequence (176 sagittal slices, slice thickness 1mm, TE 2.26 ms, TR 1900 ms, flip angle 9°, FOV 256 x 256 mm and matrix 256 x 256).

Skin Conductance Data Acquisition

Available skin conductance response (SCR) data from D1 and D2 were entered in this work. SCR had been recorded applying Ag/AgCl electrodes (MES Medizintechnik, Munich, Germany) being attached to the second phalanx of the index and middle finger of the non-dominant hand. Isotonic electrode paste had been used as contact medium (Synapse, Kustomer Kinetics, Arcadia, CA, USA) and Brain Vision hard- and software for data acquisition (Brain Vision ExG Amplifier and Brain Vision Recorder, Brain Products, Munich, Germany). SCR Data had been recorded with an initial sampling rate of 1000 Hz with a low cut-off filter of 10 seconds and a high cut-off filter of 250 Hz. A Matlab-based (The MathWorks, Natick, MA, USA) application (Ledalab Version 3.3.4[170]) was employed to run a decomposition analysis. For each condition, the mean number (#NS.SCR) and sum amplitude (AMP.SCR; response criterion 0.02 µS) were calculated. SCR data were range-corrected according to Lykken[171]. Difference scores between anxiety and neutral conditions were calculated to mask fear-unrelated processes.

Methods

Analysis of Demographic and Clinical Data

Clinical and demographic sample characteristics were examined based on chi-square tests, independent t-tests and analyses of variances. The threshold of significance was set to $p < 0.05$. The demographic characteristics examined for each dataset were sex, smoking status, handedness, and age. Clinical characteristics of interest were scores in the DFS, SNAQ, and ASI. The combined sample was additionally tested on marital status, education level, scores in the BDI and MQ as well as the number of subjects with psychiatric comorbidity and general medication intake (psychotropic medication was excluded). For correlational analyses, available SCR data (#NS.SCR and AMP.NS.SCR) from D1 and D2 were included. Bivariate correlations within each group were computed between extracted β-Values as connectivity measures (i.e., "Anxiety" minus "Neutral" for dental and snake stimulus content) and markers of phobic fear (i.e., SNAQ, DFS), of autonomic arousal (i.e., SCR data), and other clinical scores (BDI, ASI, MQ). SPSS 23 was used for the computation (IBM Corp., Armonk, New York, USA, https://www.ibm.com/analytics/us/en/technology/spss). Charts were created using SigmaPlot 12 (Systat Software, Inc., San Jose, California, USA, www.systatsoftware.com) and Excel (https://products.office.com/en-us/excel). Neuroanatomical illustrations were created using MRIcron (https://www.nitrc.org/projects/mricron).

fMRI and the BOLD Contrast

fMRI is a powerful tool to examine neural activity by utilizing the interrelation between physiological function, energy metabolism and regional blood supply[172,173]. It has been widely used due to its non-invasive and safe nature, general availability and usability and its good spatial resolution[174]. It is naturally based on magnetic resonance imaging (MRI) which can generate body images by using magnetic fields, radio waves and field gradients. MRI itself is based on the principle of nuclear magnetic resonance[172,173].

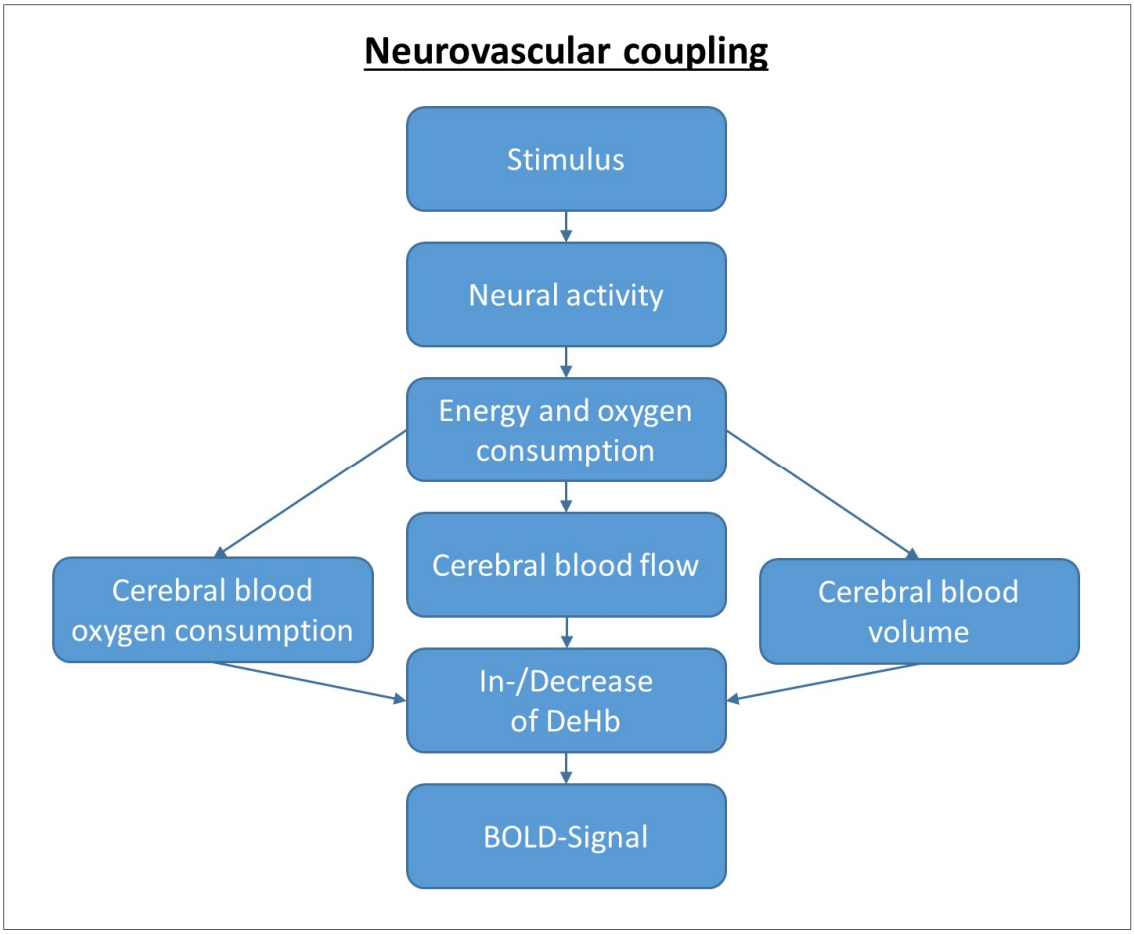


Figure 7. The BOLD response. Schematic illustration of the transformation of stimulus evoked neural activity into the BOLD signal including several physiological factors. DeHb: deoxygenated hemoglobin.

The so-called blood-oxygenation-level-dependent (BOLD) contrast reflects neuronal activity indirectly and was first demonstrated by Ogawa et al.[175,176]. To assess this measurement, the fMRI registers vascular effects in the brain that can be linked to changes in the blood oxygenation. The theory behind this is that neuronal activity causes enhanced energy and oxygen metabolism[177,178]. Despite enhanced energy consumption, that indeed is followed by an initial, short decrease of oxygenated hemoglobin, the amount of oxygenated hemoglobin increases[179]. This is caused by cerebral vessel dilation, which results in enhanced regional cerebral blood flow[179]. Thereby, the level of oxygenated hemoglobin exceeds that found in neuronal resting periods[179]. The oxygenated hemoglobin is diamagnetic and magnetically indistinguishable from brain tissue whereas deoxygenated hemoglobin exhibits four unpaired electrons and is paramagnetic[180]. Paramagnetism leads to regional gradients in the magnetic field that can be

measured. Finally, brain activity and therewith a higher ratio of oxygenated hemoglobin to deoxygenated hemoglobin leads to a stronger BOLD contrast. Technically the contrast is measured using a T2*-weighted imaging protocol due to the slower T2* relaxation time in oxygen-rich regions that leads to a better signal intensity[176].

Functional Connectivity

Principles

Connectivity analyses intend to identify and quantify the relationship between brain regions[181]. Generally, these relationships can be distinguished in 1) anatomical connectivity which measures the fiber tracks between regions, 2) functional connectivity that examines temporal dependencies of neural activation of anatomically separated regions and 3) effective connectivity which explores causal influences between brain regions[181].

The idea of functional connectivity can be traced back to the concept of functional integration that assumes the interaction of different brain regions in one task in contrast to functional segregation. It was first introduced by Friston et al. and can be defined as the statistical association among two or more anatomically distinct regions[182]. In general terms, functional connectivity refers to the organization, inter-relationship and integrated performance within neural networks and is able to depict the coupling of different brain regions[182,183]. The idea of FC is that if brain regions interact with each other, time courses of activity of these areas correlate. Stronger correlations (and thus stronger connectivity) between a seed region and a correlated brain region is thought to mirror an enhanced information exchange (e.g., resulting in mutual regulation). Its theoretical background does not enable it to depict causalities on the direction of influence (e.g., whether the interaction is driven by the seed region or by the correlated region). However, its ability to display functional interactions of distinct brain regions make it a valuable tool for hypothesis testing in examining brain networks. Further, FC has been shown to be associated with anatomical connections even though co-activity of distinct regions can be mediated by indirect structural connections[184,185]. Exploring neural networks can be done principally by hypothesis-driven methods or exploratory,

data-driven methods like independent component analysis[183,186,187]. In this work a seed-based approach has been chosen which requires a priori hypotheses for the creation of ROIs. For ROI-to-ROI connectivity, time-series of chosen regions are correlated to each other which has the benefit of limiting multiple testing[187]. Additionally in this work, a seed-to-voxel analysis has been performed. Therefore, preselected seeds are chosen and temporal correlations between the seeds´ time series are correlated separately with the time series of every other voxel across the entire brain. Both exploratory and hypothesis-driven methods can be used for resting-state examinations and task-based paradigms. In this study, task-based data were examined. This can be done by using the general linear model in the following term:

$$Y_i = X_i \cdot \beta_i + \epsilon_i$$

where, Y resembles the BOLD contrast of a voxel *i* at a given time point, X the design matrix, β the unknown activity vector and ε the error term[188,189]. So, its general assumption is a linear contribution of the experimental condition to the fMRI time courses. Therefore, a high β-Value resembles a strong reaction of the voxel to the stimulus. Finally, a measurement for FC can be obtained by creating correlation coefficients from separated voxels across the brain.

Functional Connectivity Analysis in this Work

The connectivity analyses have been carried out using the CONN toolbox (http://www.nitrc.org/projects/conn)[190]. This toolbox is a Matlab-based imaging software specialized on FC analyses and is freely available as an SPM toolbox (SPM, http://www.fil.ion.ucl.ac.uk/spm). Principally, the analysis can be divided into four steps (setup, preprocessing, first-level analysis, and second-level analysis) that are described in more detail below:

(1) The setup defines the experimental information, the file sources for structural and functional data, the ROIs and further covariates. First, basic parameters were determined, i.e., number of subjects (D1: 41, D2: 39, D3: 26), TR (D1/D2/D3: 2.5 milliseconds) and number of scanning sessions per subject (D1/D2/D3: one session per subject). Next, functional data source files and structural images were

defined. This step is followed by the definition of ROI masks. Therefore, a split approach was chosen. In the first case, ROIs were derived from the AAL atlas (http://www.gin.cnrs.fr/en/tools/aal-aal2)[191] which represents a widely used atlas in the neuroimaging community. Due to a priori hypotheses of abnormalities in the basal emotion regulation circuits in specific phobia, only three regions (i.e., amygdala, ACC and insula) were included and specifically examined.

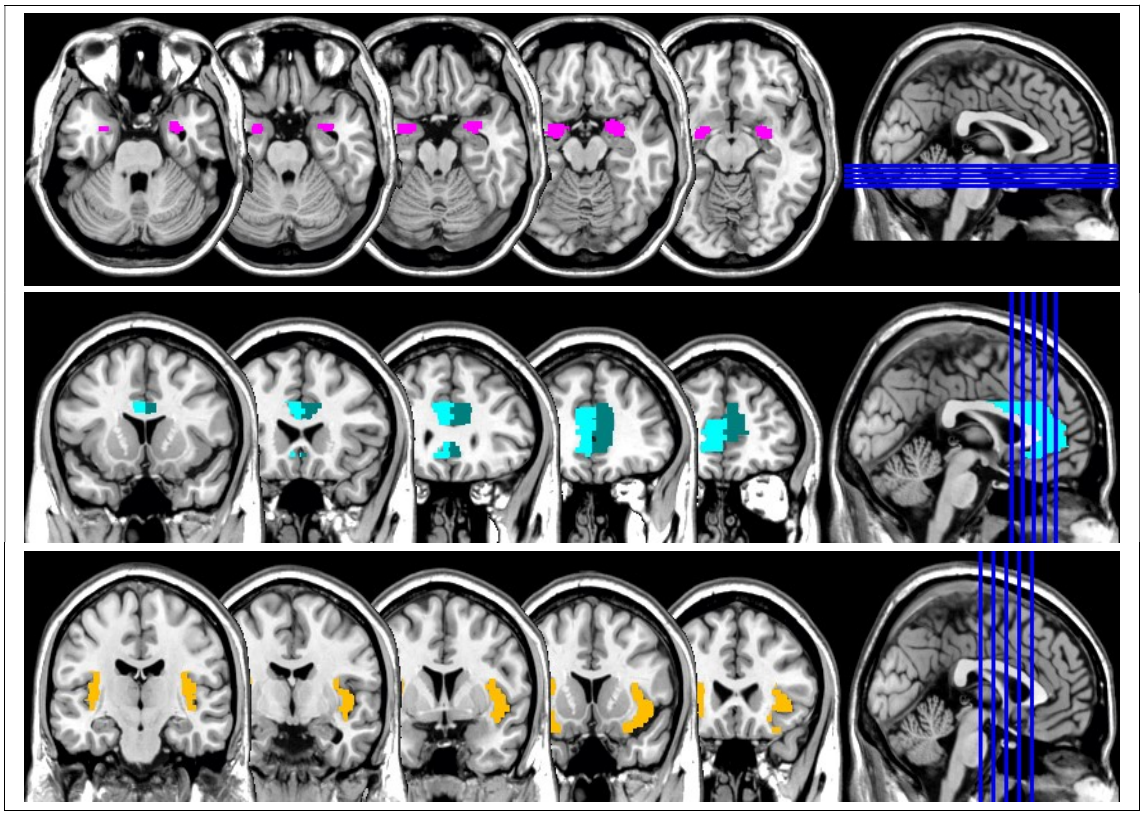


Figure 8. AAL ROIs. Regions of Interest derived from the Automated Anatomical Labeling atlas. The regions were the amygdala (upper row: violet), the anterior cingulate cortex (middle row: cyan right, green on the left) and the insula (bottom row: orange). Images are presented in radiological orientation.

However, the AAL atlas does not provide ACC and insula subregions which were further objects of interest in this project. Therefore in the second case, the analyses were conducted using the recently published Brainnetome atlas (http://atlas.brainnetome.org/bnatlas.html) that is designed specifically for connectivity analyses[192]. The ACC was subdivided into a dorsal, a perigenual and a subgenual part whereas the insula was divided into a ventral-anterior (vaINS), a dorsal-anterior (daINS) and a posterior (postINS) region.

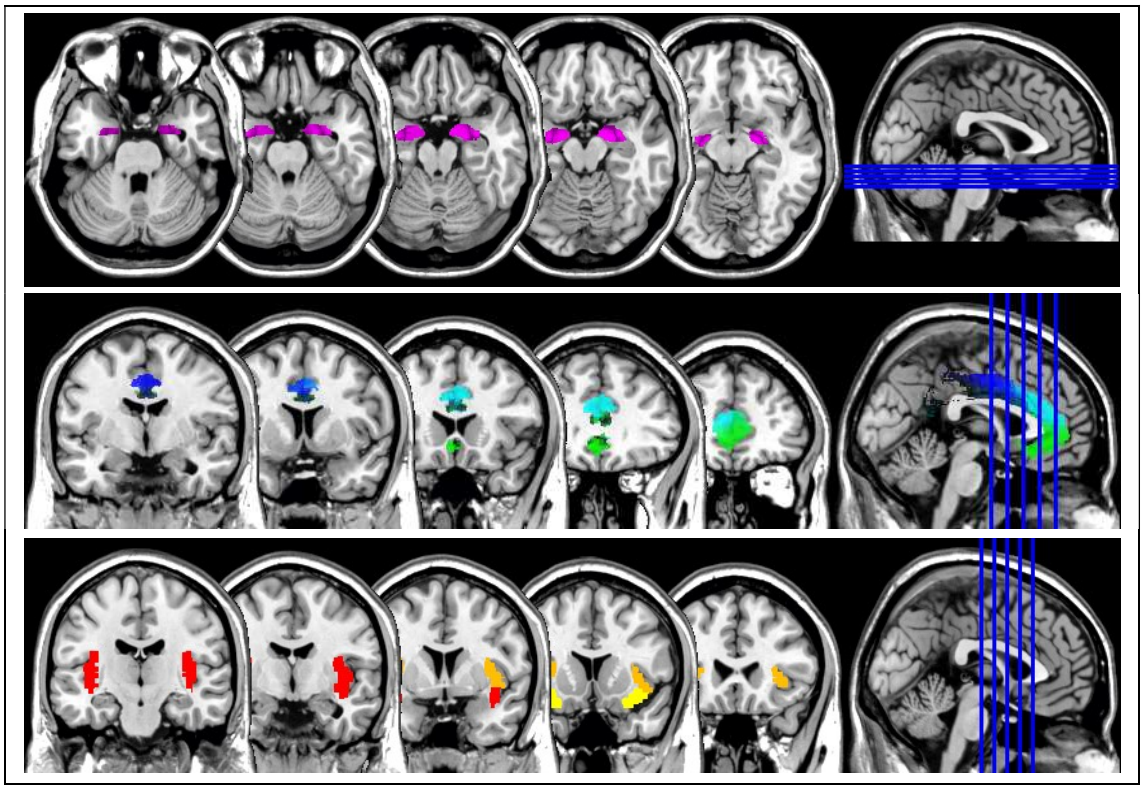


Figure 9. BNT ROIs. Regions of Interest derived from the Brainnetome atlas. Used regions were the amygdala (upper row: violet), the anterior cingulate cortex (middle row) divided into subgenual (green), rostral (cyan) and dorsal (blue) ACC and the insula (bottom row) that was divided into a ventral-anterior (yellow), dorsal-anterior (orange) and posterior (red) insula. Images are presented in radiological orientation.

Next, onset and duration of all conditions were defined. For D1 and D3 the duration of each condition was set to 15 seconds. In D2, onset and duration for each sequence were entered according to the original event-related design. Thereafter, first-level covariates (within-subject covariates) were defined, e.g., realignment parameters. Second-level covariates (between-subject covariates) were subsequently defined, e.g., groups, age, and gender.

(2) Before data can be analyzed, images must be preprocessed to increase the signal (the BOLD signal) to noise (e.g., subject movement, or scanner drift) ratio. Preprocessing encompassed the following steps:

a) Realignment to correct for movement artifacts, registering all volumes to the first volume of the time-series by applying a rigid body transformation (three translations in X, Y and Z direction and three rotations around the X, Y and Z axes).

b) Segmentation based on the estimated probability of belonging to a certain type of tissue (e.g., grey matter, white matter, cerebrospinal fluid (CSF)).
c) Coregistration that registers functional volumes of the high resolution T1 images, and normalization that warps images to a standard template brain.
d) Smoothing which approximates a Gaussian distribution. The default kernel was set out to 8 mm full-width at half maximum.

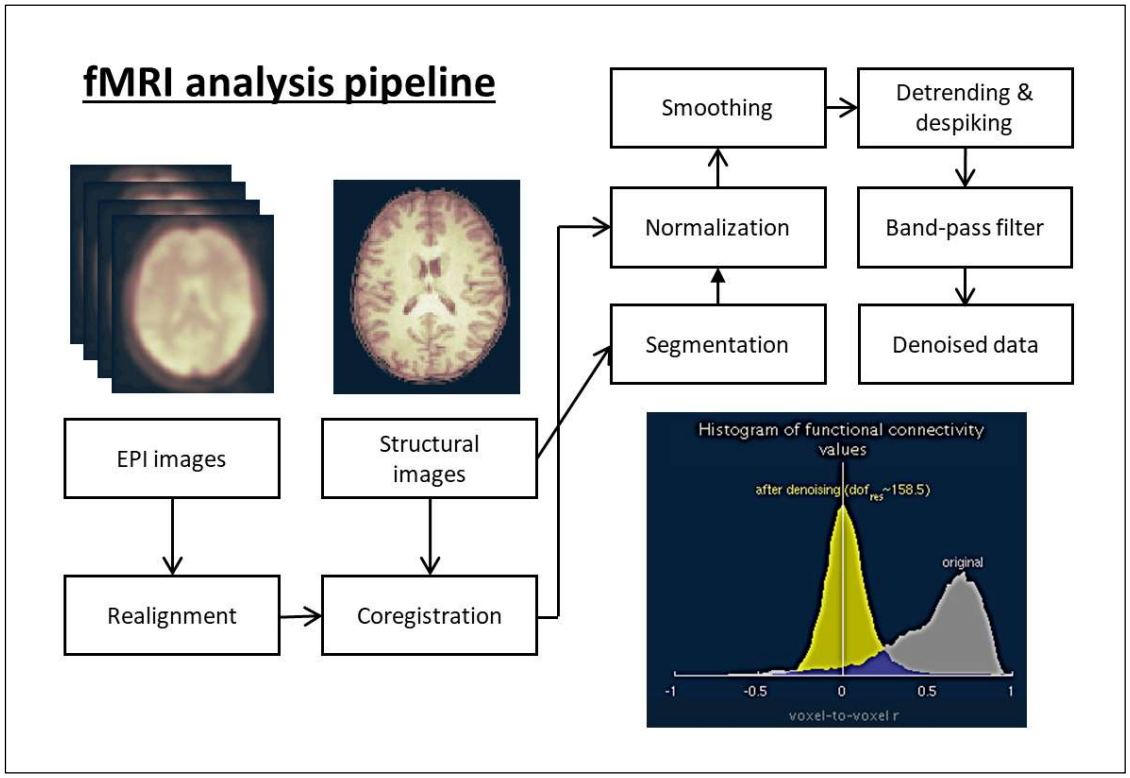


Figure 10. fMRI analysis pipeline. The figure illustrates the applied preprocessing steps. Additionally, an example time-series (top left) and a structural image (top right) are depicted. The histogram at the bottom displays the impact of denoising on the residual BOLD signal. EPI: echo-planar imaging.

Further denoising was done with the Artifact Detection Tool (ART, https://www.nitrc.org/projects/artifact_detect) that is implemented in CONN. Prominent spontaneous neural activity or inconsistent general image intensity was identified by setting a z-threshold = 3. Outliers identified were excluded. Additionally, the CONN toolbox includes the aCompCor strategy that removes distorting effects from the BOLD time-series such as white matter, CSF or main condition effects and their first temporal derivatives and other first-level covariants[193]. Finally, a band-pass filter [0.008 - 0.09 Hz] was added.

(3) Then, various sources (e.g., ROIs) for the FC analyses can be explored on a single-subject level for the first time. FC analyses can be based on regression or on correlation measurements. In this case, bivariate-regression analyses have been used. Results are given as beta-values. All scans were weighted equally, no hanning was used and the standard SPM hemodynamic response function was applied.

(4) In this step, FC can be explored by using random-effect analyses. The level of significance for ROI-to-ROI analyses was set to $P < 0.05$, corrected with the false discovery rate (FDR). The FDR has been proven to be a valid alternative to Bonferroni-type corrections in multiple testing scenarios and has been shown to be an adequate correction for fMRI, too[194,195]. Insignificant results are reported exceptionally, where close-to-significant trends (up to p (FDR) < 0.07) appeared to be noteworthy. The seed-to-voxel analysis was performed with a height threshold of $p < 0.001$ uncorrected and a cluster threshold of p (FDR) < 0.05.

Contrasts explored differed from study to study due to varying conditions. Since the main target was to obtain connectivity measures across all studies within groups, ROI-to-ROI FC within groups between all ROIs was examined with the neutral condition serving as a tailored baseline to mask anxiety unspecific effects. Additionally in D3, the impact of stimulus modality in DP was examined by comparing auditory and visual stimuli. To scan for group specific patterns, the same contrasts between phobia groups and controls were also used in the seed-to-voxel analysis. Contrasts can be generalized in the following terms:

i. Within-group contrasts:
    a. HC/DP/SP: Anxiety > Neutral
    b. DP: (Auditory: Anxiety > Neutral) > (Video: Anxiety > Video)

ii. Between-groups contrasts:
    a. Phobia groups (DP/SP) > Controls (HC): Anxiety > Neutral
    b. DP > HC: (Auditory: Anxiety > Neutral) > (Video: Anxiety > Video)

In detail, the following contrasts were examined:

i. D1: HC: (SA > SN), HC: (DA > DN), SP: (SA > SN), DP: (DA > DN), and SP > HC: (SA > SN) and DP > HC: (DA > DN).
ii. D2: PP: HC: (SA > SN), PP: HC: (DA > DN), PP: SP: (SA > SN), DP: (DA > DN), and PP: SP > HC: (SA > SN), PP: DP > HC: (DA > DN).
iii. D3: HC: (DAA > DAN), HC: (DVA > DVN), DP: (DAA > DAN), DP: (DVA > DVN), and DP: ((DAA > DAN) > (DVA > DVN)), and DP > HC: (DAA > DAN), and DP > HC: (DVA > DVN) and DP > HC: ((DAA > DAN) > (DVA > DVN)).

(5) Moreover, for the ROI-to-ROI analysis using the AAL atlas, a mean FC index was calculated on which the groups were tested by t-tests. Therefore, the overall average FC of each group (HC, DP and SP) for each condition (DA, DN, SA and SN in D1, and DAA, DAN, DVA and DVN in D3) was calculated across all the preselected bilateral ROIs (ACC, insula, amygdala) including both the unilateral and the crosslateral connectivity. Results are reported for each group individually for the contrast anxiety > neutral and between phobic groups and HC for the respective stimuli (e.g. SP > HC: (SA > SN), and DP > HC: (DA > DN)).

(6) In summary, following analyses were carried out:

1. ROI-to-ROI connectivity using the AAL atlas for D1, D2, and D3 (please see ROI-to-ROI analysis using the AAL atlas, p. 35).
2. ROI-to-ROI connectivity using the BNT atlas for D1, D2, and D3 (please see ROI-to-ROI analysis using the Brainnetome atlas, p. 44).
3. Seed-to-voxel connectivity using the AAL atlas for D1, D2, and D3 (please see Seed-to-Voxel Connectivity Analysis, p. 51).

Results

Sample Characteristics

Overview on the Combined Sample

Groups did not significantly differ regarding gender, age, and handedness, level of education or marital status. DP exhibited significant higher scores on the DFS and SP on the SNAQ. The phobic groups were also characterized by higher scores on the ASI and the BDI. HC were significantly more likely to smoke than SP (chi^2 = 6.45, p = 0.01) but not than DP (chi^2= 1.06, p = 0.30). Phobia groups did not differ in the amount of subjects with psychiatric comorbidity (DP: n = 12, SP: n = 6; chi^2 = 2.51, p = 0.64). Sample characteristics for the entire sample are given in the table below.

Table 2. Sample characteristics of the entire sample. Means (sd) except where noted.

	HC (n = 31)		**DP (n = 38)**		**SP (n = 25)**		**chi^2 (df)**	**p**
Sociodemographic characteristics								
Female [n (%)]	21	(67.7)	28	(73.6)	20	(80.0)	1.1 (2)	0.586
Smoking [n (%)]	7	(22.6)	5	(13.2)	0	(0)	6.4 (2)	0.042
Left-handed [n (%)]	0	(0)	1	(2.6)	2	(4.0)	1.1 (2)	0.565
Age (years)	23.3	(3.9)	24.4	(4.8)	23.4	(5.2)	0.6 (93)	0.525
University-entrance diploma [n (%)]	31	(100)	36*	(97.3)	24	(96.0)	4.3 (2)	0.372
Married [n (%)]	1	(3.2)	5	(13.2)	1	(4.0)	3.0 (2)	0.220
Clinical characteristics								
DFS[1]	29.0	(8.0)	79.4†	(5.5)	46.5	(13.7)	266.9 (92)	<0.01*
SNAQ[2]	4.4	(3.6)	8.8	(6.1)	23.1	(1.9)	127.5 (93)	<0.01*
ASI[3]	12.6	(6.1)	20.1	(9.9)	18.7	(8.2)	7.4 (93)	0.001
BDI[4]	2.4	(2.9)	8.7	(9.2)	5.0	(5.8)	7.4 (93)	0.001
Medication intake[5] [n (%)]	6	(19.4)	11	(29.0)	6	(24.0)	0.9 (2)	0.652

HC: healthy control group; DP: dental phobia group; SP: snake phobia group; DFS: Dental Fear Survey; SNAQ: Snake Questionnaire; ASI: Anxiety Sensitivity Index. *p > 0.0001; †n = 1 missing. [1]Dental phobics > snake phobics > controls, p < 0.001; [2]Snake phobics > dental phobics > controls, p < 0.001; [3]Phobic groups > controls, p < 0.001; [4]Phobic groups > controls, p > 0.001; [5]only non-psychotropic medication, subjects with psychotropic medication were excluded. Please note that DSF and SNAQ data relate to the date of screening used for study inclusion. ASI data relate to the date of the MRI assessment.

Sample Characteristics of D1

Groups did not differ significantly regarding gender, smoking status, handedness, age, educational status and marital status. As expected, DP scored highest on the DFS and SP on the SNAQ.

Table 3. Sample characteristics of D1. Means (sd) except where noted.

	HC (n = 17)		DP (n = 12)		SP (n = 12)		chi²/F¹ (df)	p
Female [n (%)]	12	(70.6)	9	(75.0)	9	(75.0)	0.9 (2)	0.952
Smoking [n (%)]	6	(35.3)	2	(16.7)	0	(0.0)	5.7(2)	0.059
Left-handed [n (%)]	0	(0.0)	0	(0.0)	1	(8.3)	2.5 (2)	0.29
Age (years)	23.7	(4.4)	25.5	(7.5)	25.0	(7.0)	0.4 (40)	0.702
DFS[2]	31.8	(9.5)	78.0	(7.2)	51.5	(13.5)	66.7 (39)	<0.001
SNAQ[3]	5.1	(4.0)	9.2	(6.0)	22.8	(2.4)	60.6 (40)	<0.001
ASI[4]	12.1	(6.6)	19.3	(9.1)	16.6	(5.8)	3.7 (40)	0.033

HC: healthy control group; DP: dental phobia group; SP: snake phobia group; DFS: Dental Fear Survey; SNAQ: Snake Questionnaire; ASI: Anxiety Sensitivity Index; [1]Fisher's exact test when appropriate; [2]Dental phobics > snake phobics > controls, $p < 0.001$; [3]Snake phobics > dental phobics > controls, $p < 0.001$; [4]Post-hoc tests did not reveal significant differences between groups. Please note that DSF and SNAQ data relate to the date of screening that was used for study inclusion. ASI data relate to the date of the MRI assessment.

Sample Characteristics of D2

Regarding demographic characteristics, no differences were found between groups. DP scored highest on the DFS and SP highest on the SNAQ.

Table 4. Sample characteristics of D2. Means (sd) except where noted.

	HC (n = 13)		DP (n = 13)		SP (n = 13)		chi²/F¹ (df)	p
Female [n (%)]	9	(69.3)	10	(76.9)	11	(84.6)	1.5 (2)	0.467
Smoking [n (%)]	1	(7.7)	2	(15.4)	0	(0.00)	2.2 (2)	0.329
Left-handed [n (%)]	0	(0.0)	0	(0.0)	0	(0.00)		ns
Age (years)	22.7	(3.3)	23.0	(3.4)	21.9	(1.9)	0.6 (39)	0.56
DFS[2]	25.5	(3.6)	80.3	(4.6)	41.9	(12.7)	165.7(39)	<0.001
SNAQ[3]	3.5	(2.9)	6.9	(6.3)	23.4	(1.4)	92.8 (39)	<0.001
ASI	13.3	(5.5)	18.8	(8.6)	20.6	(9.7)	3.1 (39)	0.059

HC: healthy control group; DP: dental phobia group; SP: snake phobia group; DFS: Dental Fear Survey; SNAQ: Snake Questionnaire; ASI: Anxiety Sensitivity Index. [1]Fisher's exact test when appropriate; [2]Dental phobics > snake phobics > controls, $p < 0.001$; [3]Snake phobics > dental phobics > controls, $p < 0.001$. Please note that DSF and SNAQ data relate to the date of screening that was used for study inclusion. ASI data relate to the date of the MRI assessment.

Sample Characteristics of D3

The groups did not differ regarding demographic characteristics. DP scored significantly higher on the DFS and the SNAQ. A trend for higher ASI scores remained insignificant.

Table 5. Sample characteristics of D3. Means (sd) except where noted.

	HC (n = 13)		DP (n = 13)		chi²/t (df)	p
Female [n (%)]	9	(69.2)	9	(69.2)	0.0 (1)	1
Smoking [n (%)]	1	(0.8)	1	(0.8)	0.0 (1)	1
Left-handed [n (%)]	0	(0.0)	1	(7.7)	0.0 (1)	1
Age (years)	23.2	(3.2)	24.9	(2.3)	1.6 (24)	0.308
DFS	25.8	(3.4)	79.5	(4.7)	25.8 (24)	< 0.001
SNAQ	3.8	(3.2)	10.3	(6.1)	3.8 (24)	0.066
ASI	14.5	(7.8)	22.2	(12.1)	14.5 (24)	0.054

HC: healthy controls; DP: dental phobia group; DFS: Dental Fear Survey; SNAQ: Snake Questionnaire; ASI: Anxiety Sensitivity Index. Please note that DSF and SNAQ data relate to the date of screening used for study inclusion. ASI data relate to the date of the MRI assessment.

ROI-to-ROI analysis using the AAL Atlas

Results for D1 using the AAL Atlas

Connectivity Results

Results are given in table 6 and illustrated in figure 11, 12 and 13. For the contrast (DA > DN) a negative FC between the right ACC and the left amygdala in HC (t = -3.49, p = 0.0152) was found as well as negative right amygdala – left insula FC in DP (t = -3.54, p = 0.0232). Comparisons between DP and HC did not yield any significant FC. For snake specific stimuli (SA > SN), HC exhibited a negative FC of the left amygdala with both left and right ACC (left: t = -2.75, p = 0.0358; right: t = 3.31, p = 0.0220). Conversely in SP, a positive FC between left ACC and bilateral insulae (left: t = 4.15, p = 0.0067; right: t= 3.14, t = 0.0474) and between right ACC and left insula (t = 3.85, p = 0.0067) was found. The contrast SP > HC (SA > SN) yielded a positive FC between right ACC and the left insula (t = 3.89, p = 0.0028). The left ACC was further positively connected with both insulae (left: t = 2.52, p = 0.044 and right: t = 3.89, p = 0.0028).

Table 6. ROI-to-ROI analysis of D1 using the Automated Anatomical Labeling Atlas.

Contrast	Regions	t	p (FDR[1])
Controls			
DA > DN	ACC r. – Amygdala l.	-3.49	0.0152
SA > SN	ACC r. – Amygdala l.	-3.31	0.0220
	ACC l. – Amygdala l.	-2.75	0.0358
Dental phobics			
DA > DN	Amygdala r. – Insula l.	-3.54	0.0232
Snake phobics			
SA > SN	ACC r. – Insula l.	3.85	0.0067
	ACC l. – Insula r.	3.14	0.0474
	ACC l. – Insula l.	4.15	0.0067
Dental phobics > controls			
DA > DN		n.s.	
Snake phobics > controls			
SA > SN	ACC r. – Insula l.	3.07	0.0006
	ACC l. – Insula r.	2.52	0.0444
	ACC l. – Insula l.	3.89	0.0028

l.: left; r.: right; DA: dental anxiety; DN: dental neutral; SA: snake anxiety; SN: snake neutral; ACC: anterior cingulate cortex; [1]p corrected using the false discovery rate (FDR).

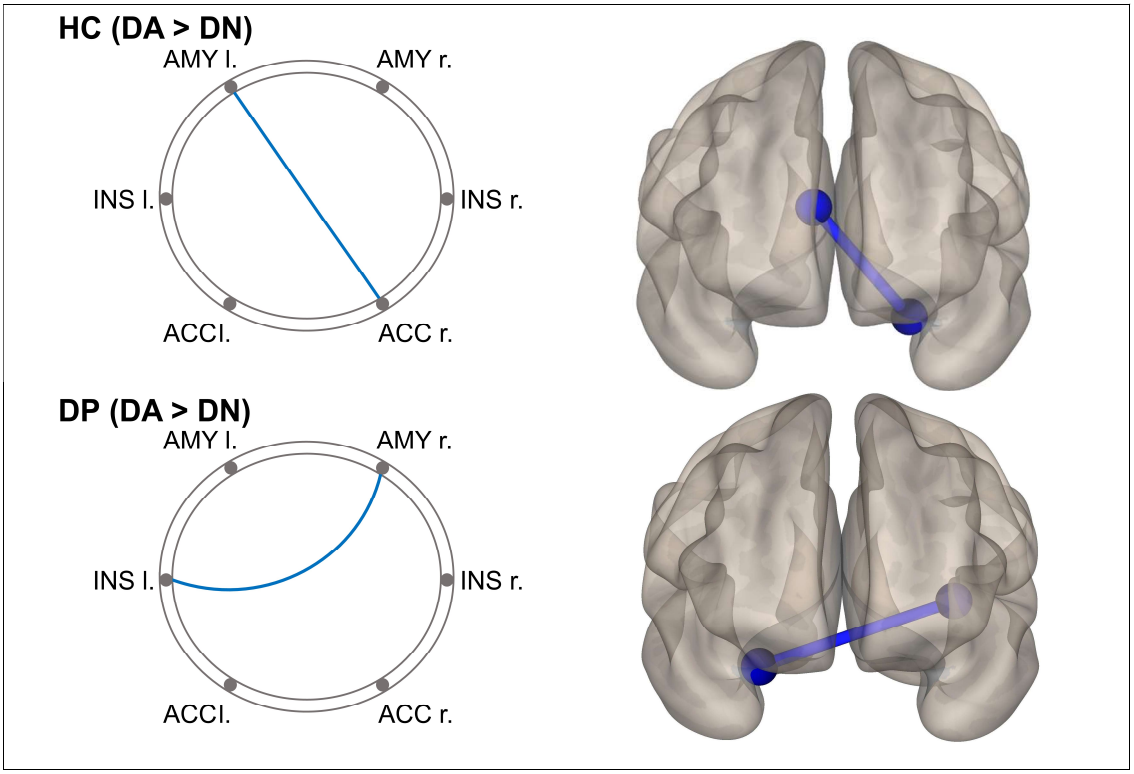


Figure 11. Functional connectivity (FC) for dental stimuli in D1. Significant FC for the contrast dental anxiety (DA) > dental neutral (DN) in controls (HC) and dental phobics (DP). Connectome rings are displayed on the left and a 3D rendered images on the right. Only negative FC (blue) was observed. AMY: amygdala; INS: insula; ACC: anterior cingulate cortex; r.: right and l.: left. Connectome rings are adapted from Stefanescu & Endres et al.[162].

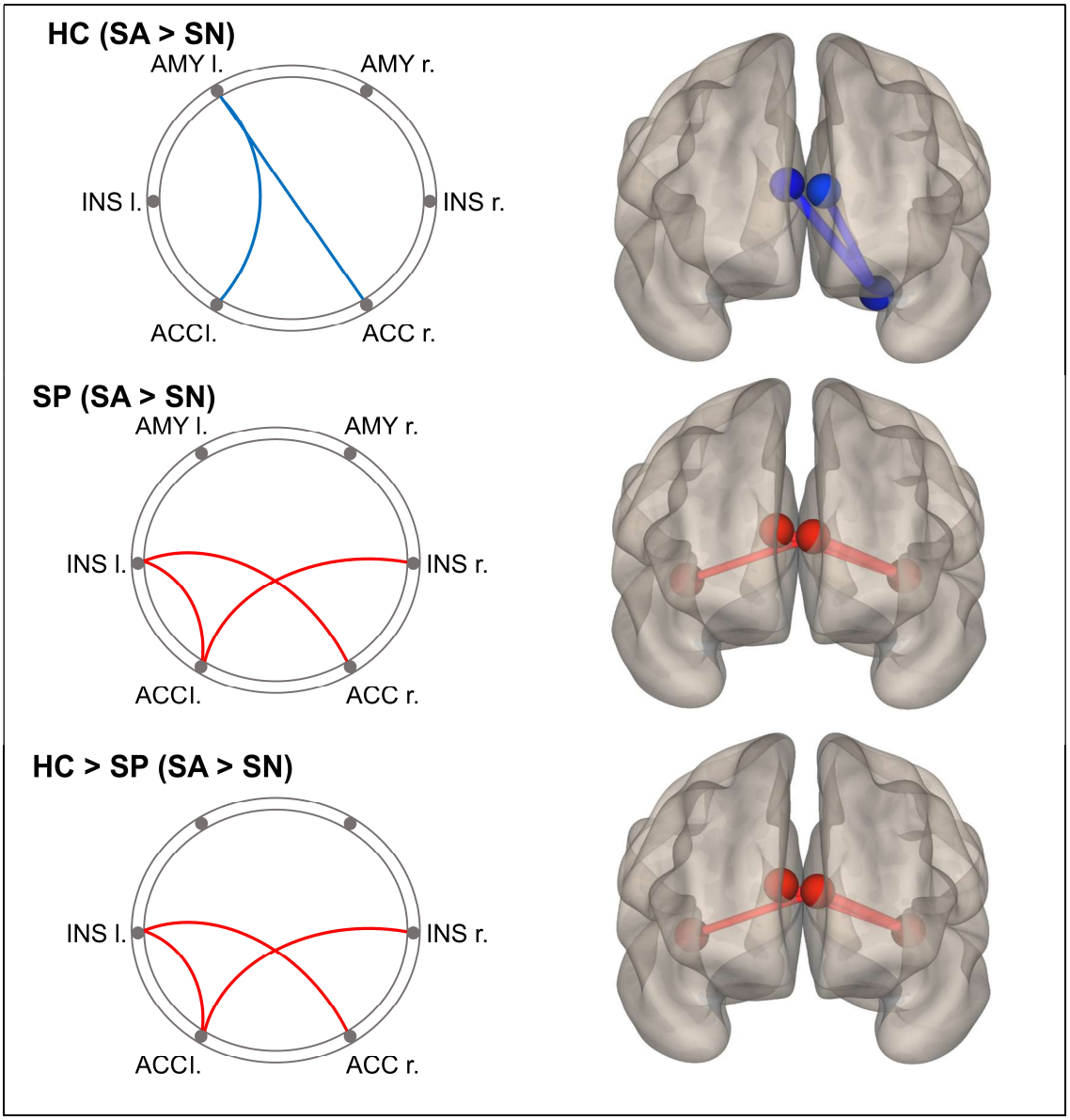


Figure 12. Functional connectivity (FC) for snake stimuli in D1. Significant FC for the contrast snake anxiety (SA) > snake neutral (SN) in controls (HC), snake phobics (SP) and between groups. Connectome rings are displayed on the left and a 3D rendered image on the right. Positive FC is shown in red and negative FC in blue. AMY: amygdala; INS: insula; ACC: anterior cingulate cortex; r.: right and l.: left. Connectome rings are adapted from Stefanescu & Endres et al.[162].

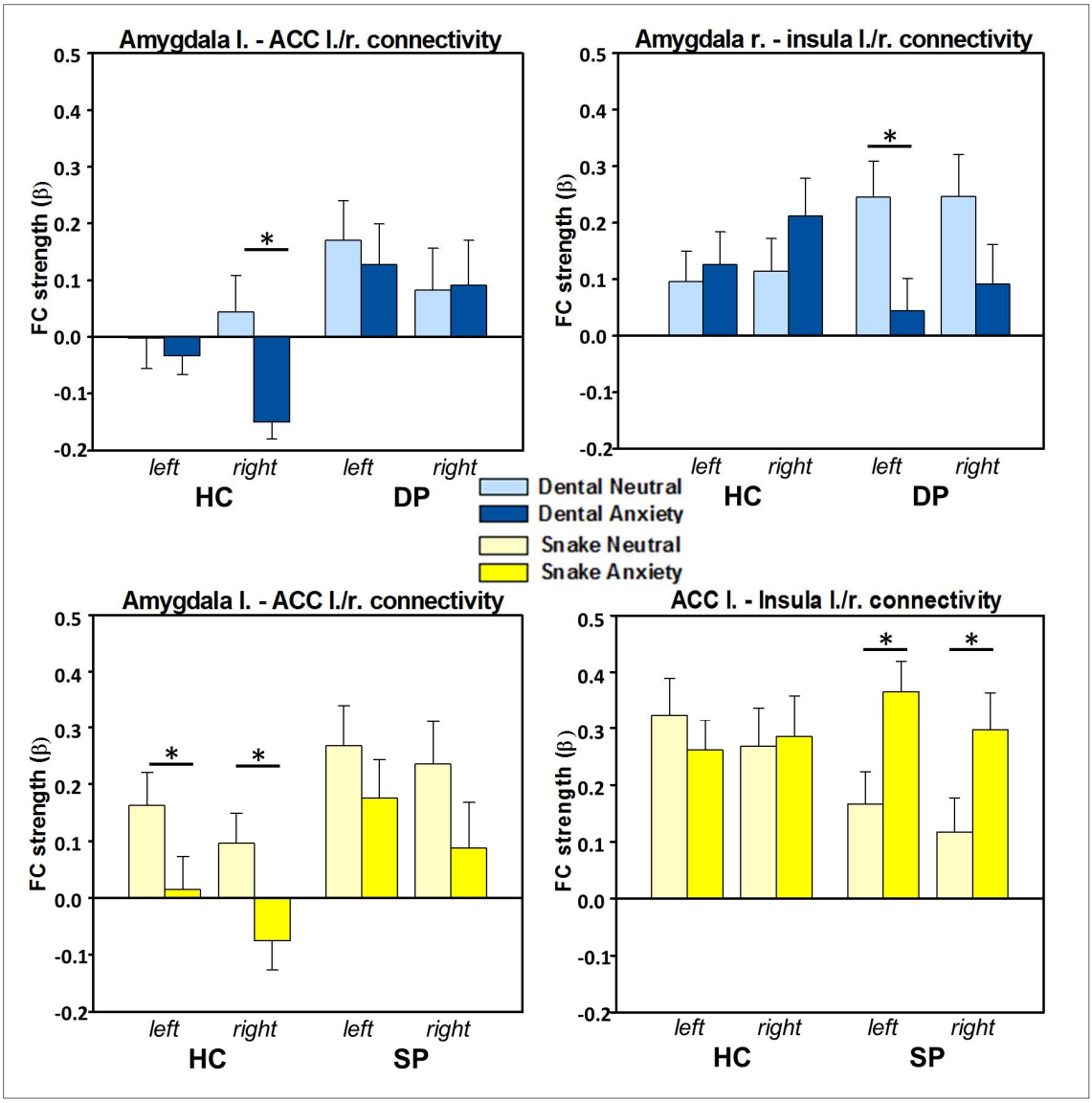


Figure 13. Connectivity strength in D1. Functional connectivity (FC, mean β-Values) for controls (HC), dental phobics (DP) and snake phobics (SP). Dental conditions are shown in blue, and snake conditions in yellow. Anxiety (dark bars) and neutral conditions (bright bars) are depicted separately. Error bars indicate the standard error of mean. *Indicates significant results at a level of p (FDR) < 0.05 for the respective anxiety > neutral contrast in groups. FC: functional connectivity; ACC: anterior cingulate cortex; l.: left; r.: right.

Mean FC Indices

Results are given in table 7 and illustrated in figure 14. The contrast SP > HC: (SA > SN) yielded a significant effect for snake specific stimuli (t = -3.69, p = 0.001). In more detail, HC were characterized by a decreased mean FC (HC: (SA > SN): t = -3.27, p = 0.003), while SP exhibited an opposing trend on a descriptive level only (t = 1.54, p = 0.138, see also figure 14). Mean FC analyses for the dental specific stimulus content did not yield any significant results.

Table 7. Mean FC indices in D1 using the Automated Anatomical Labeling Atlas.

Contrast	t	p
SA > SN		
Snake phobics > controls	**3.69**	**0.001**
Snake phobics	1.54	0.138
Controls	**-3.27**	**0.003**
DA > DN		
Dental phobics > controls	1.54	0.136
Dental phobics	-0.45	0.656
Controls	-1.44	0.164

FC: functional connectivity; SA: snake anxiety; SN: snake neutral; DA: dental anxiety; DN: dental neutral. Significant results are shown in bold.

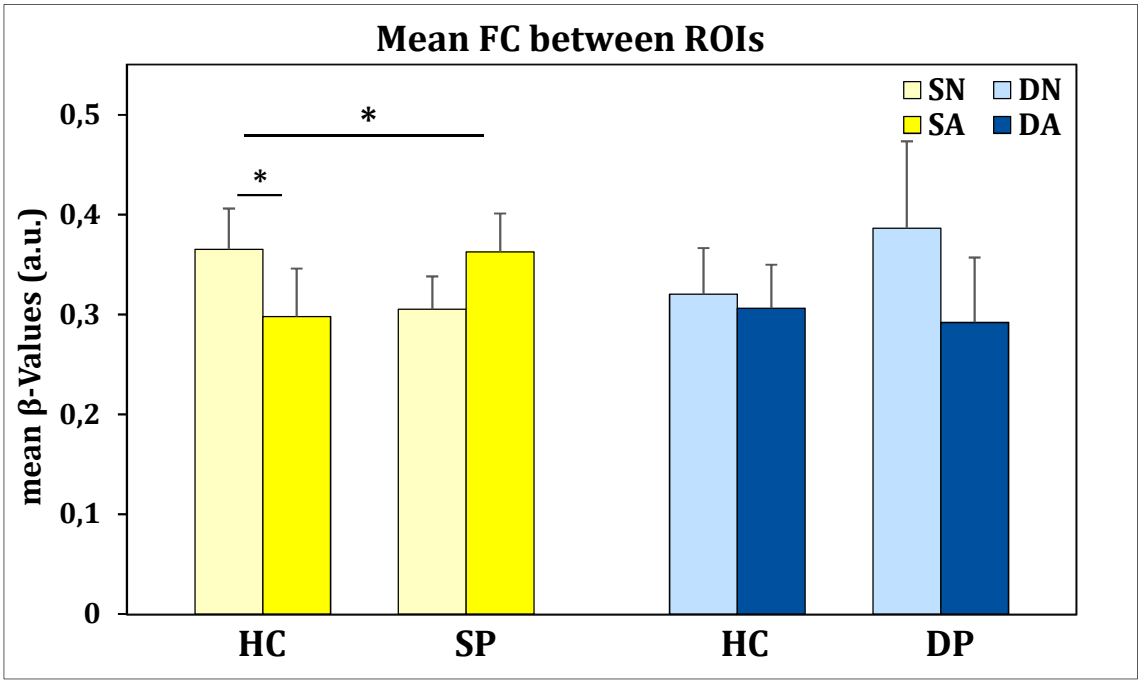


Figure 14. Mean functional connectivity (FC) in D1. Mean FC in controls (HC), snake phobics (SP) and dental phobics (DP). Yellow bars indicate snake stimuli, and blue bars dental stimuli. Anxiety conditions (dark bars) and neutral conditions (bright bars) are depicted separately. Error bars indicate the standard error of mean. *Indicates results at a level of $p < 0.05$. DP: dental phobics; SN: snake neutral, SA: snake anxiety, DN: dental neutral; DA: dental anxiety.

Relationship between Connectivity and clinical and autonomic Markers

No bivariate correlation reached significance in D1.

Results for D2 using the AAL Atlas

Connectivity Results

A positive connectivity between the left amygdala and right insula for the contrast SP > HC: PPSA > PPSN (t = -3.67, p = 0.0057) was the only significant result in this analysis.

Table 8. ROI-to-ROI analysis of D2 using the Automated Anatomical Labeling Atlas.

Contrast	Regions	t	p (FDR[1])
Controls			
PPSA > PPSN		n.s.	
PPDA > PPDN		n.s.	
Dental phobics			
PPDA > PPDN		n.s.	
Snake phobics			
PPSA > PPSN		n.s.	
Dental phobics > controls			
PPDA > PPDN		n.s.	
Snake phobics > controls			
PPSA > PPSN	Amygdala l. – Insula l.	3.67	0.0057

l.: left; r.: right; PPSA: perception phase snake anxiety; PPSN: perception phase snake neutral; PPDA: perception phase dental anxiety; PPDN: perception phase dental neutral. [1]p corrected using the false discovery rate (FDR).

Mean FC Indices

Mean FC analyses did not yield any significant results.

Relationship between Connectivity and clinical and autonomic Markers

No significant correlations were found in this analysis.

Results for D3 using the AAL Atlas

Connectivity Results

Results are given in table 9 and are illustrated in figure 15. No significant FC was found for HC. No significant FC was found for the visual contrast (DVA > DVN) in DP, either. The auditory contrast yielded a positive FC of the right insula with both right (t = 3.06, p = 0.0248) and left amygdala (t = 3.59, p = 0.0092) in DP. Positive FC between the right insula and the right ACC marginally failed to reach significance (t = 2.43, p = 0.0533). The left amygdala exhibited a positive FC to both left insula (t = 3.59, p = 0.0092) and right amygdala (t = 2.68, p 0.0333). A positive FC between the right ACC and the right insula (t = 3.21, p = 0.0373) was identified in DP for the comparison between stimulus modalities ((DAA > DAN) > (DVA > DVN)). Between-group comparisons for the auditory contrast yielded a positive FC between left ACC and right amygdala (t = 2.89, p = 0.0404).

Table 9. ROI-to-ROI analysis of D3 using the Automated Anatomical Labeling Atlas.

Contrast	Regions	t	p (FDR[1])
Controls			
DAA > DAN		n.s.	
DVA > DVN		n.s.	
Dental phobics			
DAA > DAN	Amygdala r. – Insula r.	3.06	0.0248
	Amygdala r. – Amygdala l.	2.68	0.0333
	Amygdala l. – Insula r.	3.59	0.0092
	Amygdala l. – Insula l.	3.67	0.0092
	ACC r. – Insula r.	*2.43*	*0.0533*
DVA > DVN		n.s.	
(DAA > DAN) > (DVA > DVN)	ACC r. – Insula r.	3.21	0.0373
Dental phobics > controls			
DAA > DAN	ACC l. – Amygdala r.	2.89	0.0404
DVA > DVN		n.s.	
(DAA > DAN) > (DVA > DVN)		n.s.	

DAA: dental auditory anxiety; DAN: dental auditory neutral; DVA: dental video anxiety; DVN: dental video neutral; ACC: anterior cingulate cortex. [1]p corrected using the false discovery rate (FDR). Italic: Not significant at a level of p (FDR) < 0.05.

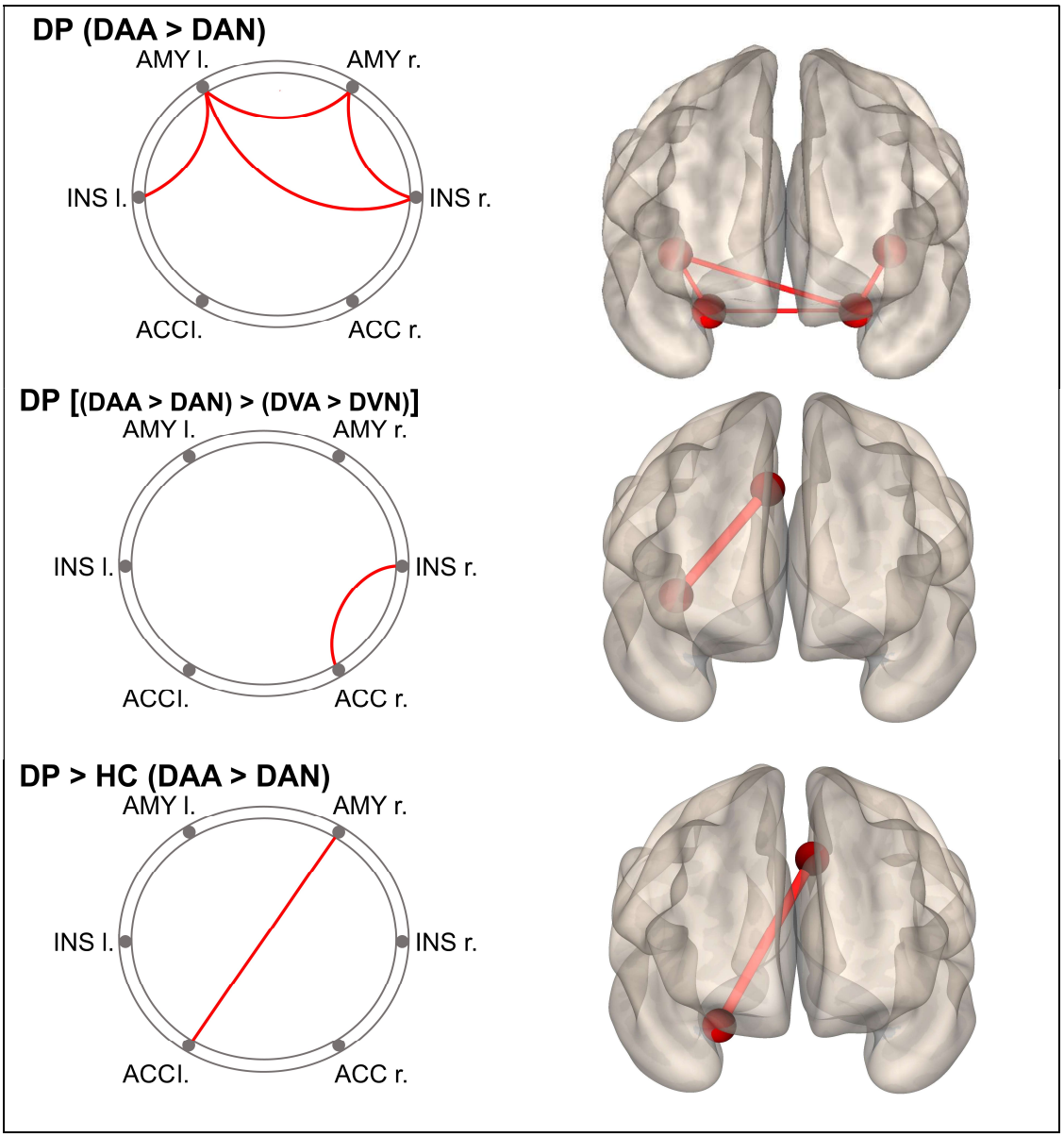


Figure 15. Functional connectivity (FC) in D3. Significant FC for dental phobics (DP) and between DP and HC (controls). Only positive FC (red) was observed. DAA: Dental Audio Anxiety; DAN: Dental Audio Neutral; DVA: Dental Video Neutral; DVN: Dental Visual Neutral; AMY: amygdala; INS: insula; ACC: anterior cingulate cortex; r.: right; l.: left. Connectome rings are adapted from Stefanescu & Endres et al.[162].

Mean FC Indices

Results are given in table 10 and are illustrated in figure 16. The contrast DP > HC: (DAA > DAN) yielded a significant effect for dental auditory stimuli ($t = 2.40$, $p = 0.022$), that was particularly driven by DP´s enhanced connectivity ($t = 2.40$, $p = 0.024$). Furthermore, contrasting stimulus modalities in DP highlighted a significant augmentation of mean FC ($t = 2.65$, $p = 0.009$). Mean FC analyses for the visual stimulus content did not yield significant results.

Table 10. Mean FC indices in D3 using the Automated Anatomical Labeling Atlas.

Contrast	t	p
DAA > DAN		
Dental phobics > controls	2.46	0.022
Dental phobics	2.40	0.024
Controls	-0.20	0.422
DVA > DVN		
Dental phobics > controls	n.s.	
Dental phobics	n.s.	
Controls	n.s.	
(DAA > DAN) > (DVA > DVN)		
Dental phobics	2.65	0.009

FC: functional connectivity; DAA: dental audio anxiety; DAN: dental audio neutral; DVA: dental visual anxiety; DVN: dental visual neutral.

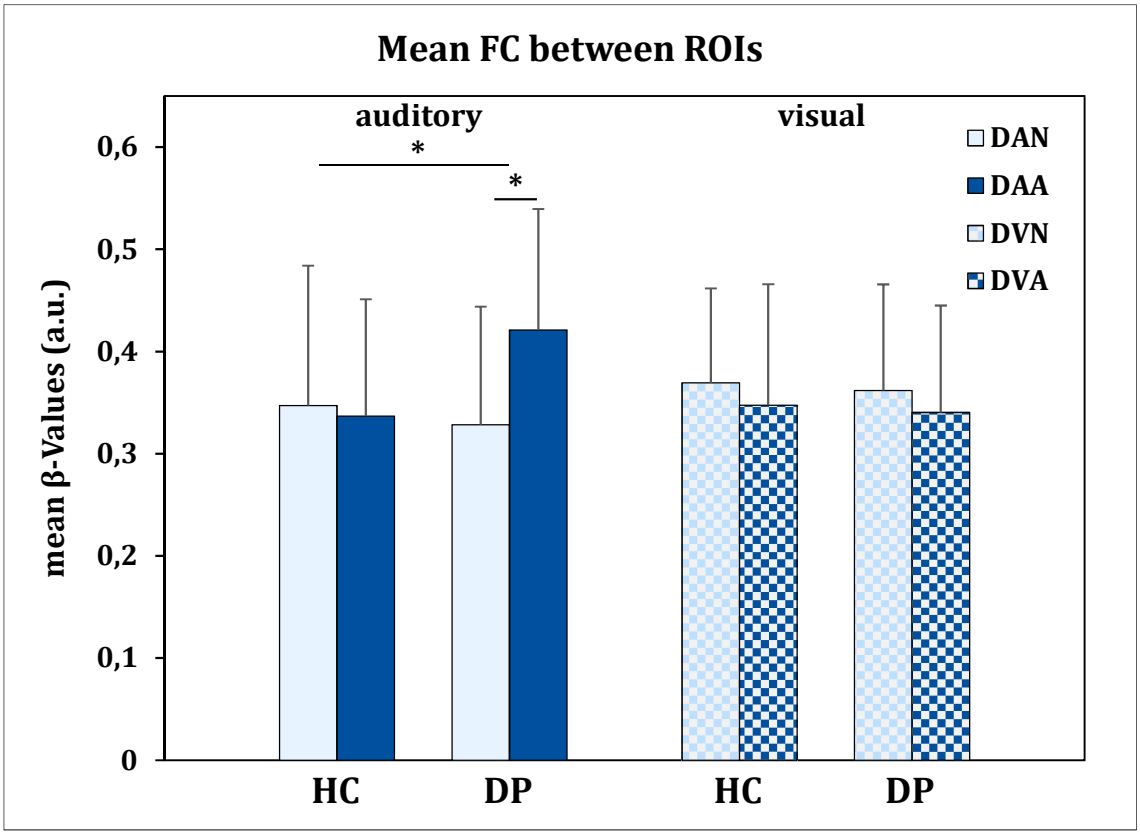


Figure 16. Mean functional connectivity (FC) in D3. Mean FC in controls (HC) and dental phobics (DP). Auditory conditions are shown on the left, and visual conditions on the right (patterned). Anxiety (dark bars) and neutral conditions (bright) are depicted separately. Error bars indicate the standard error of mean. *Indicates results at a level of $p < 0.05$. DAN: dental auditory neutral; DAA: dental auditory anxiety; DVN: dental visual neutral; DVA: dental visual anxiety.

Relationship between Connectivity and clinical Markers

No significant correlations were found for dental phobics or the control group.

ROI-to-ROI analysis using the Brainnetome Atlas

Results for D1 using the BNT Atlas

Connectivity Results

Significant results and trends up to p (FDR) < 0.7 are given in table 11 and are illustrated in figure 17, 18 and 19. The left dACC showed a negative FC to the right sACC (t = -3.58, p = 0.0324) in HC for the contrast (DA > DN) while no significant FC were found in DP. For the contrast (SA > SN) in HC, the left sACC exhibited a negative FC to both the right (t = -4.34, p = 0.0065) and to the left amygdala (t = 3.13, p = 0.0424). In SP, the left postINS was positively connected to both the right (t = 3.41, p = 0.0377), to the left sACC (t = 4.09, p = 0.0234) and to the left vaINS (t = 3.09, p = 0.0499). Further, the right sACC was negatively connected with the right amygdala (t = -3.45, p = 0.0377). The contrast for DP > HC: (DA > DN) yielded two significant patterns of positive FC of the left sACC with both the right postINS (t = 3.16, p = 0.0487) and the right vaINS (t = 3.07, p = 0.0310). For the contrast (SP > HC): (SA > SN) the right vaINS was found to be positively coupled with the left dACC (t = 2.92, p = 0.0445) and the left postINS (t = 3.55, p = 0.0179). Lastly, the left sACC showed a positive FC with the right postINS (t = 3.15, p = 0.0248).

Table 11. ROI-to-ROI analysis of D1 using the Brainnetome atlas.

Contrast	Regions	t	p (FDR[1])
Controls			
DA > DN	pACC r. – Amygdala l.	-2.95	0.0611
	dACC l. – sACC r.	**-3.58**	**0.0324**
	sACC r. – Amygdala l.	-3.04	0.0611
SA > SN	**sACC l. – Amygdala r.**	**-4.34**	**0.0065**
	sACC l. – Amygdala l.	**-3.13**	**0.0424**
Dental phobics			
DA > DN	n.s.		
Snake phobics			
SA > SN	pACC l. – postINS l.	2.74	0.0624
	sACC r. – Amygdala r.	**-3.45**	**0.0377**
	sACC r. – postINS l.	**3.41**	**0.0377**
	sACC l. – postINS l.	**4.09**	**0.0234**
	vaINS l. – postINS l.	**3.09**	**0.0449**

Table 11 (continued) ROI-to-ROI analysis of D1 using the Brainnetome atlas.

Contrast	Regions	t	p (FDR[1])
Dental phobics > controls			
	sACC l. – postINS r.	**3.16**	**0.0487**
	sACC l. – vaINS r.	**3.07**	**0.0310**
Snake phobics > controls			
SA > SN	**dACC l. – vaINS r.**	**2.92**	**0.0445**
	sACC l. – vaINS r.	2.44	0.0686
	sACC l. – postINS r.	**3.15**	**0.0248**
	vaINS r. – postINS r.	2.62	0.0602
	vaINS r. – postINS l.	**3.55**	**0.0179**

[1]p corrected using the false discovery rate (FDR). Results are given up to a level of p < 0.07. Results at a level of p < 0.05 are shown in bold. DA: dental anxiety; DN: dental neutral; SA: snake anxiety; SN: snake neutral; pACC: pregenual ACC; sACC: subgenual ACC; dACC: dorsal ACC; vaINS: ventral-anterior insula; daINS: dorsal-anterior insula; postINS: posterior insula; r.: right; l.: left.

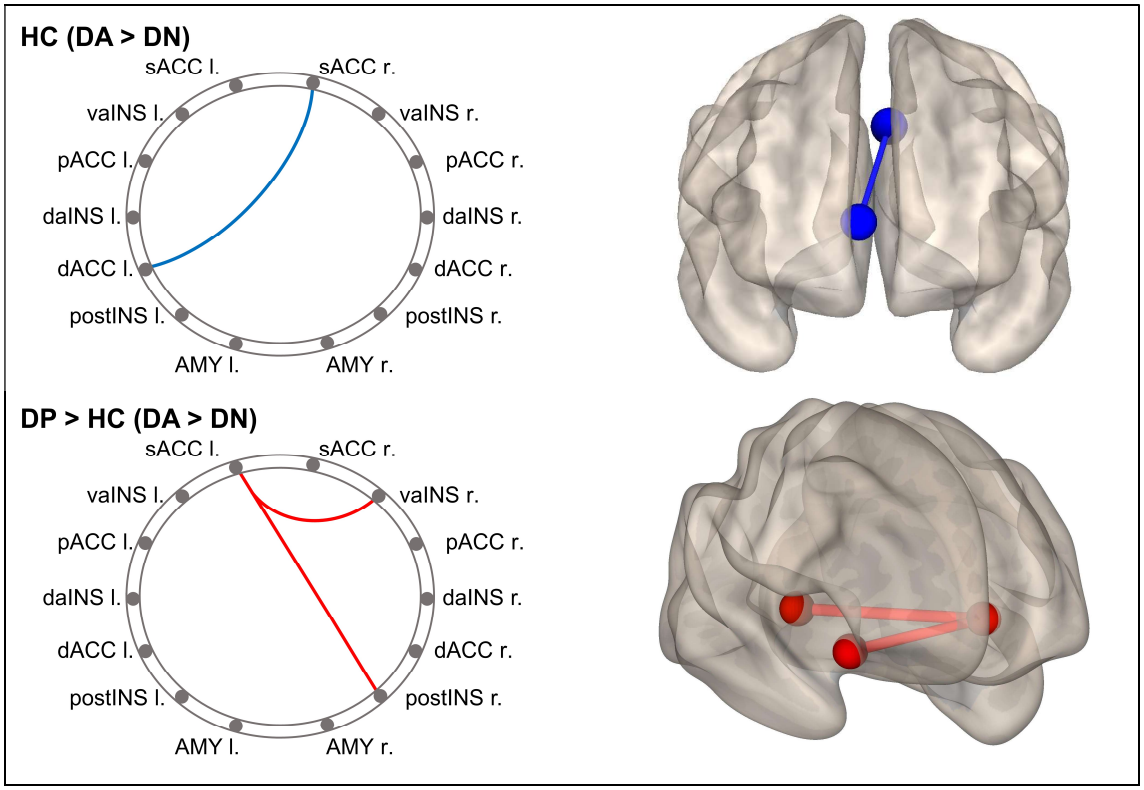


Figure 17. Function connectivity (FC) for dental stimuli in D1. Significant FC for the contrast dental anxiety (DA) > dental neutral (DN) in controls (HC) and dental phobics (DP). Connectome rings are displayed on the left and a 3D rendered image on the right. Positive FC is shown in red, and negative in blue. AMY: amygdala; vaINS: ventral-anterior insula; daINS: dorsal-anterior insula; postINS: posterior insula; sACC: subgenual anterior cingulate cortex: pACC: pregenual ACC; dACC: dorsal ACC; r.: right and l.: left.

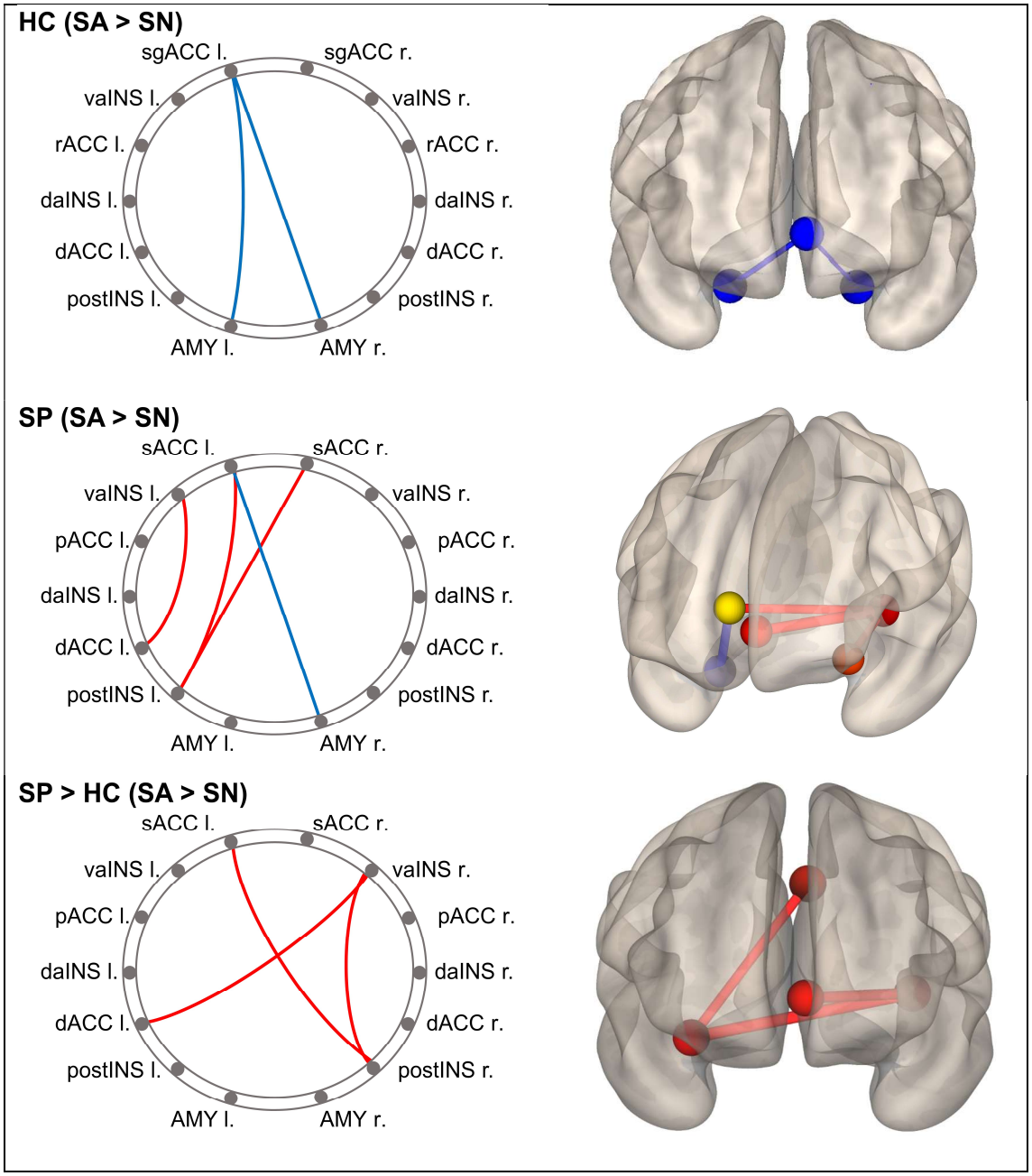


Figure 18. Functional connectivity (FC) for snake stimuli in D1. Significant FC for the contrast snake anxiety (SA) > snake neutral (SN) within controls (HC) and snake phobics (SP). Connectome rings are displayed on the left and a 3D rendered image on the right. Positive connectivity is shown in red, and negative in blue. AMY: amygdala; vaINS: ventral-anterior insula; daINS: dorsal-anterior insula; postINS: posterior insula; sACC: subgenual anterior cingulate cortex: pACC: pregenual ACC; dACC: dorsal ACC; r.: right and l.: left.

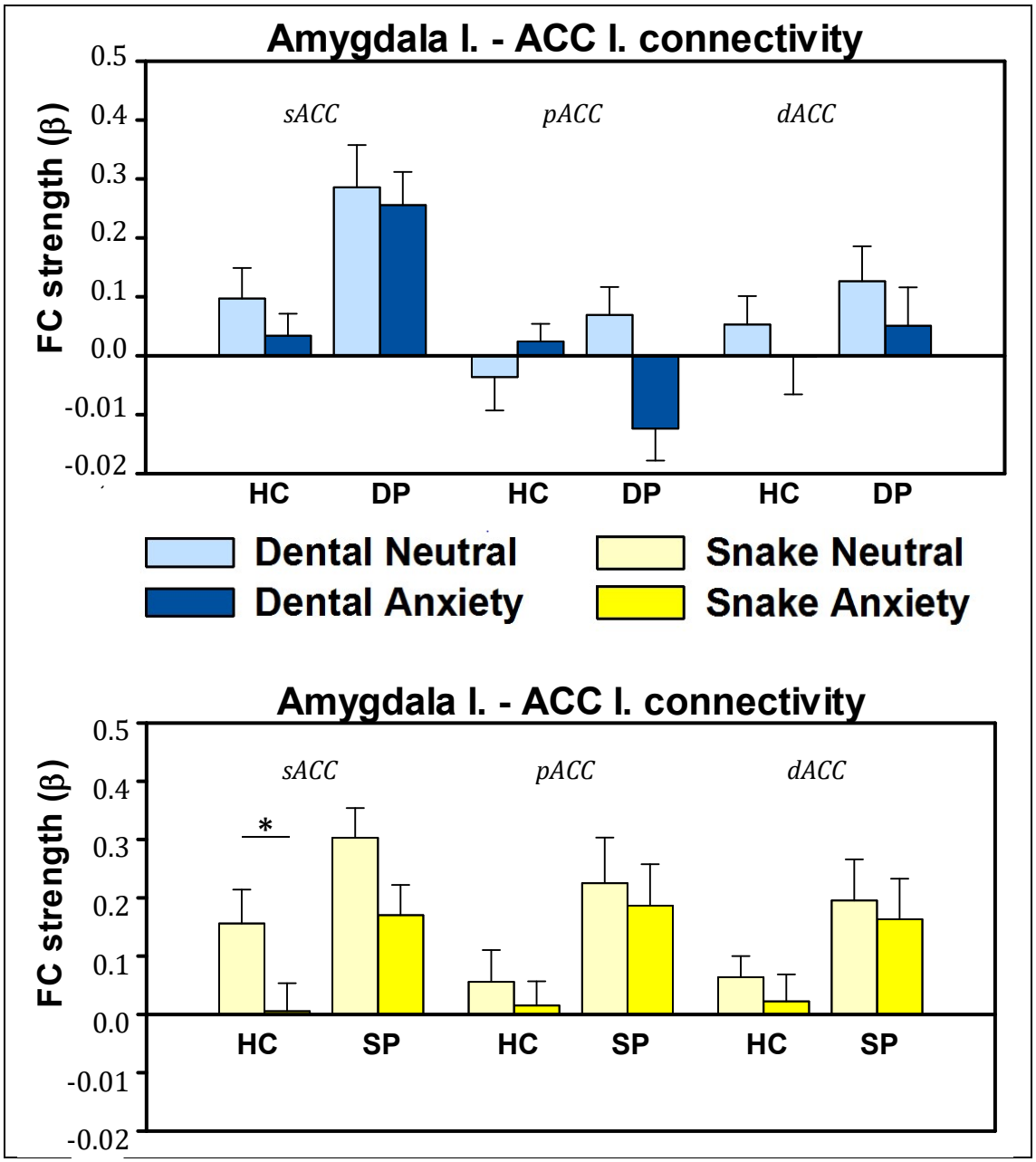


Figure 19. Connectivity strength in D1. Functional connectivity (FC, mean β-Values) for controls (HC), dental phobics (DP) and snake phobics (SP). Dental conditions are shown in blue, and snake conditions in yellow. Anxiety (dark bars) and neutral conditions (bright bars) are depicted separately. Error bars indicate the standard error of mean. *Indicates significant results at a level of p (FDR) < 0.05 for the respective anxiety > neutral contrast in groups. FC: functional connectivity; sACC: subgenual ACC, pACC: pregenual ACC; dACC: dorsal ACC; l.: left; r.: right.

Relationship between Connectivity and clinical and autonomic Markers

Exploratory correlations are given in table 12. For the contrast SP (SA > SN), left sACC – right amygdala FC correlated with AMP.NS.SCR (r = 0.630, p = 0.028) and insignificantly with #NS.SCR (r = 0.570, p = 0.053).

Table 12.
Exploratory correlations between FC and clinical and autonomic markers in D1.

Connectivity	Clinical/ autonomic marker	r	p
Controls only			
	no significant correlations		
Dental phobics only			
	no significant correlations		
Snake phobics only			
SA > SN: sACC l. – AMY r.*	#NS.SCR	0.570	0.053
SA > SN: sACC l. – AMY r.	#AMP.NS.SCR	0.630	0.028

SA: snake anxiety; SN: snake neutral; sACC: subgenual anterior cingulate cortex; AMY: amygdala; SNAQ: snake anxiety questionnaire; #NS.SCR: number of non-stimulus-specific skin conductance response; AMP.NS.SCR of non-stimulus-specific skin conductance response. *insignificant trend.

Results for D2 using the BNT Atlas

Connectivity Results

The only result reaching significance was a positive FC between left dACC and right vaINS (t = - 3.63, p = 0.0451) for the contrast SP (PPSA > PPSN).

Table 13. ROI-to-ROI analysis of D2 using the Brainnetome Atlas.

Contrast	Regions	t	p (FDR[1])
Controls			
PPSA > PPSN		n.s.	
PPDA > PPDN		n.s.	
Dental phobics			
PPDA > PPDN		n.s.	
Snake phobics			
PPSA > PPSN	dACC - vaINS	-3.63	0.0451
Dental phobics > controls			
PPDA > PPDN		n.s.	
Snake phobics > controls			
PPSA > PPSN		n.s.	

l.: left; r.: right; PPSA: perception phase snake anxiety; PPSN: perception phase snake neutral; PPDA: perception phase dental anxiety; PPDN: perception phase dental neutral. [1]p corrected using the false discovery rate (FDR). dACC: dorsal ACC; vaINS: ventral-anterior insula.

Relationship between Connectivity and clinical and autonomic Markers

No correlations were found in this analysis.

Results for D3 using the BNT Atlas

Connectivity Results

The significant results are given in table 14 and are illustrated in figure 20. No significant FC was found for controls in both the visual contrast (DVA > DVN) and the auditory contrast (DAA > DAN). DP exhibited a positive FC of the right amygdala and the right daINS (t = 3.28, p = 0.0424) and of the left amygdala and the right daINS (t = 4.05, p = 0.0208) for the auditory contrast (DAA > DAN). The visual contrast (DVA > DVN) yielded a negative connectivity of the left daINS with both the left sACC (t = -3.59, p = 0.0241) and the right sACC (t = -4.02, p = 0.0223). The left sACC was further negatively connected to the right pACC (t = -4.04, p = 0.0214). Contrasting stimulus modalities in DP yielded a positive connectivity between the right vaINS and the left postINS (t = 3.93, p = 0.0258). No significant FC was found between groups.

Table 14. ROI-to-ROI analysis of D3 using the Brainnetome atlas.

Contrast	Regions	t	p (FDR[1])
Controls			
DAA > DAN		n.s.	
DVA > DVN		n.s.	
Dental phobics			
DAA > DAN	Amygdala r. – daINS r.	3.28	0.0424
	Amygdala l. – daINS r.	4.05	0.0208
DVA > DVN	pACC r. – sACC l.	-4.04	0.0214
	sACC r. – daINS l.	-4.02	0.0223
	sACC l. – daINS l.	-3.59	0.0241
(DAA > DAN) > (DVA > DVN)	vaINS r. – postINS l.	3.93	0.0258
Dental phobics > controls			
DAA > DAN		n.s.	
DVA > DVN		n.s.	

[1]p corrected using the false discovery rate (FDR). DAA: Dental Audio Anxiety; DAN; Dental Audio Neutral; DVA: Dental Video Anxiety; DVN: Dental Video Neutral; pACC: pregenual anterior cingulate cortex; sACC: subgenual ACC; dACC: dorsal ACC; vaINS: ventral-anterior insula; daINS: dorsal-anterior insula; postINS: posterior insula.

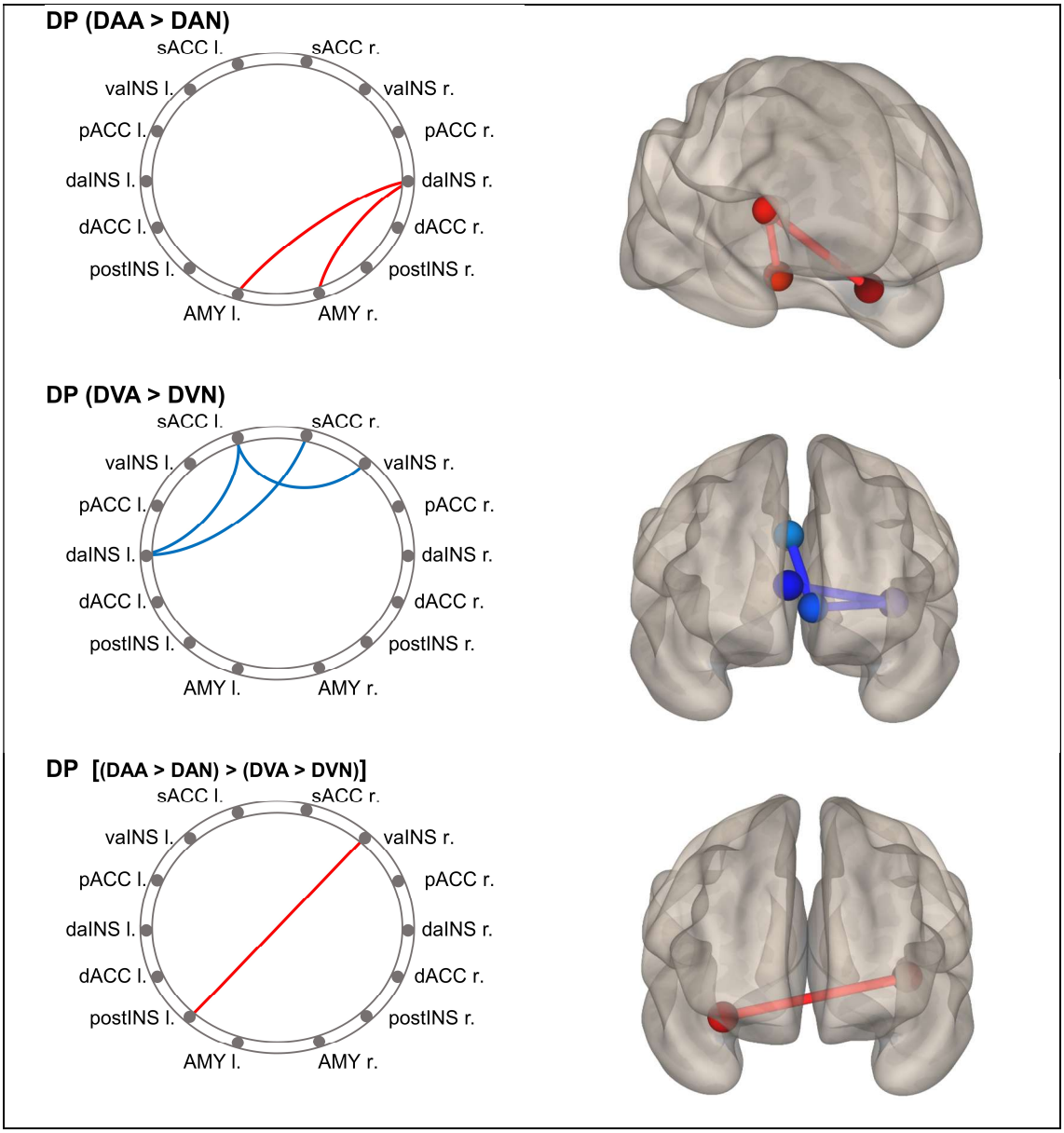


Figure 20. Functional connectivity (FC) in D3. FC for the contrasts dental audio anxiety (DAA) > dental audio neutral (DAN), dental visual anxiety (DVA) > dental visual neutral (DVN) and the crossmodal contrast. Connectome rings are displayed on the left and 3D rendered images on the right. Positive FC is shown in red, and negative in blue. AMY: amygdala; vaINS: ventral-anterior insula; daINS: dorsal-anterior insula; postINS: posterior insula; sACC: subgenual ACC: pACC: pregenual ACC; dACC: dorsal ACC; r.: right; l.: left.

Relationship between connectivity and clinical Markers

Exploratory analyses for the visual contrast yielded a correlation of right pACC – left sACC FC with DFS scores ($r = -0.606$, $p = 0.028$).

Table 15. Exploratory correlations between FC and clinical markers in D3.

Connectivity	Clinical marker	r	p
Controls only			
	no significant correlations		
Dental phobics only			
DVA > DVN: pACC right – sACC left	DFS scores	-0.606	0.028

DVA: dental visual anxiety; DVN: dental visual neutral; pACC: perigenual anterior cingulate cortex; sACC: subgenual anterior cingulate cortex; DFS: Dental Fear Survey. FC, functional connectivity.

Seed-to-Voxel Connectivity Analysis

Seed-to-Voxel Analysis Results for D1

Clusters are given in table 16 and are displayed in figure 21. No connectivity was found for the dental contrast. Using the left ACC as seed, the snake contrast (SP > HC): (SA > SN) yielded one positively connected cluster in the left hemisphere which contained 167 voxels in the insula, 66 voxels in the temporal pole (TP) and 58 in the planum polare. Positively connected voxels to the left insula were found in the left and right paracingulate gyrus (108 respectively 10), the ACC (85) and the subcallosal cortex (13). The negatively connected cluster covered the left fusiform gyrus (26), the cerebellum (24 voxels) and the left inferior temporal gyrus (21). No significant clusters were found for the amygdala seed.

Table 16. Seed-to-voxel functional connectivity in D1.

Seed	Side	+/–	X	Y	Z	Regions	Voxels	p (FDR)
Dental phobics > controls (DA > DN)								
						n.s.		
Snake phobics > controls (SA > SN)								
ACC	r.					n.s.		
ACC	l.	+	-40	-2	-8	Insula/TP/planum temporale	372	0.0006
AMY	r.					n.s.		
AMY	l.					n.s.		
Insula	r.					n.s.		
Insula	l.	+	-6	38	28	Paracingulate gyrus	145	0.0191
		+	-6	34	0	ACC	116	0.0237
		-	-42	-56	-22	Fusiform gyrus/ cerebellum/ ITG	91	0.0374

DA: Dental Anxiety; DN: Dental Neutral; SA: Snake Anxiety; SN: Snake Neutral; ACC: anterior cingulate cortex; AMY: amygdala; ITG: inferior temporal gyrus; TP: temporal pole. Regions with at least 20 voxels in a cluster are reported.

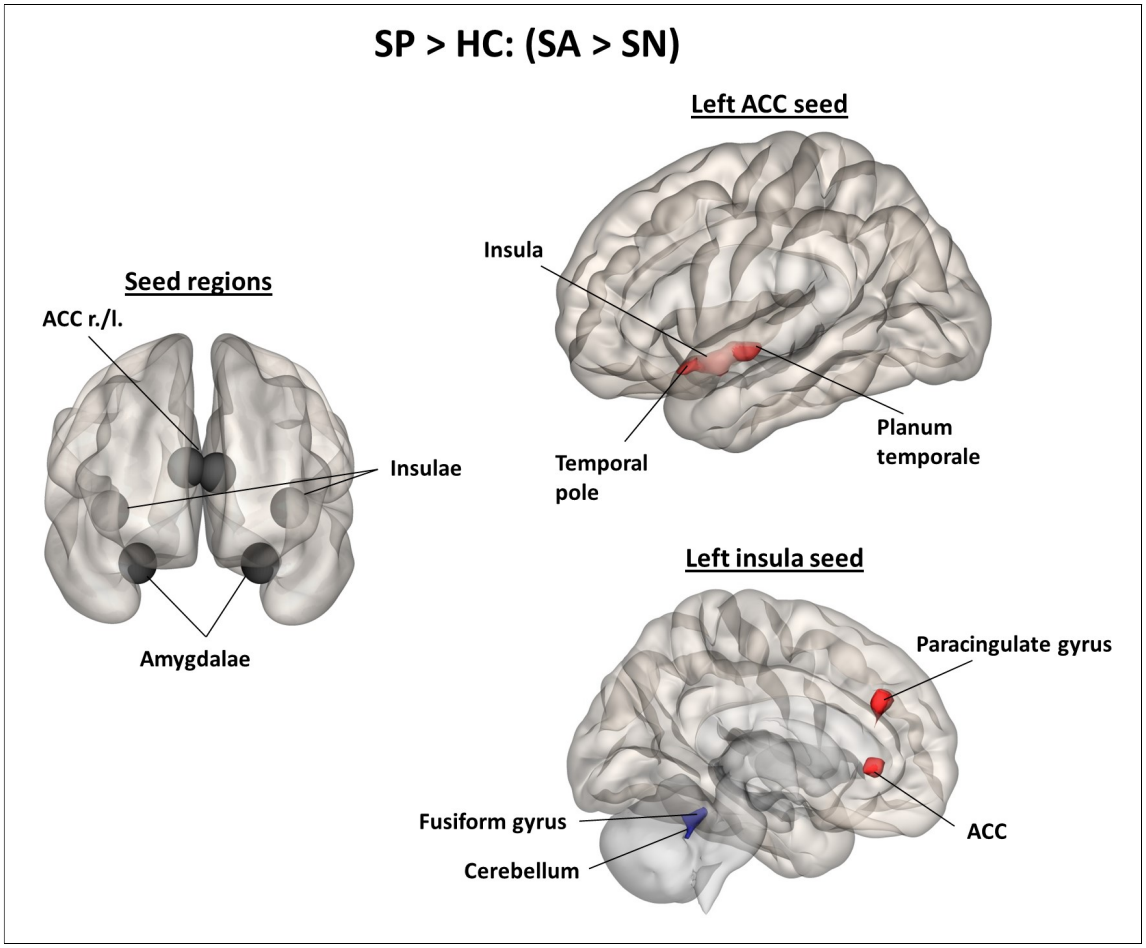


Figure 21. Cluster illustration for D1. Significantly connected clusters for the contrast (SP > HC): (SA > SN) for the left ACC (top) and for the left insula seed (bottom). Positively connected clusters are shown in red, and negatively connected clusters in blue. Regions with at least 20 voxels in significantly connected clusters are labeled. Seed regions are displayed on the left.

Seed-to-Voxel Analysis Results for D2

No significant clusters emerged in this analysis for the contrasts (SP > HC): (PPSA > PPSN) and (DP > HC): (PPSA > PPSN).

Seed-to-Voxel Analysis Results for D3

Clusters are given in table 17 and illustrated in figure 22. No clusters were found for the auditory contrast. The contrast (DP > HC): (DVA > DVN) yielded a positive FC of the left ACC seed and the right supramarginal gyrus (SMG, 110 voxels), right parietal operculum (PO, 49) and the left supplementary motor area (SMA, 44) whereas it was negatively connected with the right frontal pole (FP, 105), right OFC (81), bilateral TP (80 and 64) and the right paracingulate gyrus (49). The right insula was connected positively with the right superior temporal gyrus (33) and the right TP (17) while negatively correlated voxels were found in the right FP (142). The left insula was positively connected to the left SMG (142). Comparing stimulus modality between groups highlighted a positive FC of the right amygdala with the right paracingulate cortex (66) and the right superior frontal gyrus (18).

Table 17. Seed-to-voxel connectivity in D3 between dental phobics and controls.

Seed	Side	+/–	X	Y	Z	Regions	Voxels	p (FDR)
Dental phobics > controls (DAA > DAN)								
						n.s.		
Dental phobics > controls (DVA > DVN)								
ACC	r.					n.s.		
ACC	l.	+	44	-38	24	SMG/ parietal operculum	319	0.0006
		-	6	52	22	Frontal pole/OFC	182	0.0076
		-	44	20	-22	Temporal pole	147	0.0135
		+	-8	-2	42	SMA	122	0.0378
		-	-42	20	-28	Temporal pole	99	0.0218
AMY	r.					n.s.		
AMY	l.					n.s.		
Insula	r.	-	14	62	28	Frontal pole	160	0.0111
		+	64	8	0	Superior temporal gyrus	33	0.0214
Insula	l.	+	-56	-36	44	Supramarginal gyrus	142	0.0248
Dental phobics > controls ((DAA > DAN) > (DVA > DVN))								
ACC	r.					n.s.		
ACC	l.					n.s.		
AMY	r.	+	4	24	48	Paracingulate gyrus	66	0.0129
AMY	l.					n.s.		
Insula	r.					n.s.		
Insula	l.					n.s.		

DAA: Dental Audio Anxiety; DAN: Dental Audio Neutral; DVA: Dental Video Anxiety; DVN: Dental Video Neutral; AMY: amygdala; OFC: orbitofrontal cortex; SMA: supplementary motor area; SMG: supramarginal gyrus. Regions with at least 20 voxels in a cluster are reported.

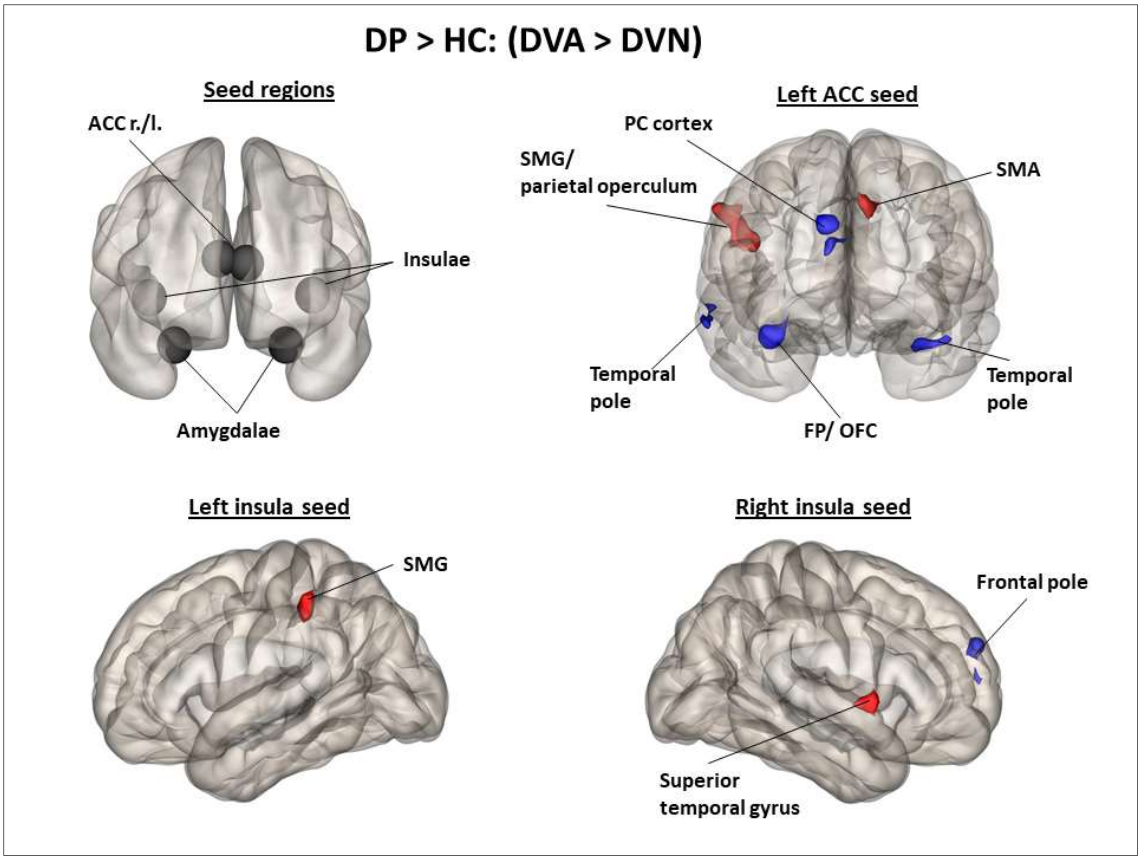


Figure 22. Cluster illustration for D3. Significant clusters for the contrast dental phobics (DP) > controls (HC): dental visual anxiety (DVA) > dental visual neutral (DVN) are displayed for the left ACC and the left and right insula seeds. Further, all seed regions are depicted. Positively connected clusters are shown in red, and negatively connected clusters in blue. FP: frontal pole; PC cortex: paracingulate cortex; SMG: supramarginal gyrus; SMA: supplementary motor area. Regions with at least 40 voxels in significantly connected clusters are labeled.

Discussion

The current work had the aim of exploring connectivity alterations during fear processing in specific phobia. The main findings are:
1) controls showed an inhibitory functional coupling between the ACC and the amygdala during phobogenic stimulus processing that was particularly driven by the sACC subregion,
2) phobia groups were predominantly characterized by increased connectivity between fear-processing structures,
3) in DP, this effect was specific for auditory, but not visual stimulation, possibly reflecting their prime phobogenic stimulus modality, and lastly
4) in DP further superordinate cognitive regions (e.g., pre- and orbitofrontal cortices) exhibited altered FC patterns during phobogenic stimulus presentation.

In the following section, each analysis (ROI-to-ROI analyses using the AAL atlas, ROI-to-ROI analyses using the BNT atlas, and the Seed-to-Voxel Connectivity Analyses) will be briefly discussed. Finally, the findings will be put into context of current literature on emotion regulation and possible implications for clinical practice will be discussed.

ROI-to-ROI analyses using the AAL atlas

This investigation had the objective of exploring and describing connectivity changes potentially contributing to impaired fear regulation within well-known emotion-related brain structures in two subtypes of specific phobia during phobic stimulus processing, including both visual and auditory stimuli. The major findings were:
1) controls exhibited a negative coupling particularly between the ACC and the amygdala during phobic symptom provocation,
2) phobic groups were mainly characterized by an excitatory connectivity between the regions examined, and

3) in DP, this phenomenon was specific for auditory, but not for visual, stimulation, potentially mirroring DP´s primary phobogenic stimulus modality.

In D1, a negative coupling during phobogenic stimulus processing (i.e., (SA > SN), and (DA > DN)) between the ACC and the amygdala was found in the control group only. This connectivity pattern might be assumed to reflect inhibitory top-down control over the amygdala by the ACC during stimulus processing, providing a basis for appropriate emotional responses[196–199]. Contrary, SP exhibited an increased positive FC of the ACC and the insula and significant higher mean FC than controls. In DP, however, a negative FC between the amygdala and insula was observed while no differences on mean FC could be found. Even though the assumption of an excitatory coupling of amygdala and ACC in phobia groups – particularly in SP due to the frequently reported prominent amygdala activity – was not positively confirmed, the blunted connectivity in SP might reflect a functional decoupling during phobic stimulus processing in agreement with the findings of Åhs and colleagues[117]. The increased mean FC is further in line with the hypothesis that excitatory connectivity loops in fear-regulative regions might represent a pathophysiological feature of the animal subtype during phobogenic stimulus processing.

In D2, a relatively sparse pattern of differential connectivity was observed. Owing to the post-hoc nature of this work, particularly D2 seemed to be underpowered for the connectivity analyses. Notably, D2 featured an event-related design that is generally less powerful in detecting neural activity compared to block-designs[200]. Eventually, the contrast (SP > HC): (PPSA > PPSN) revealed a negative connectivity between the amygdala and insula in the PP. Due to the relatively short stimulus presentation time of 1.25 seconds in the PP this might rather depict early co-activity in stimulus perception than mutual information exchange (i.e., emotion regulation processes such as reappraisal require longer time periods[188]). Thus, this result must be viewed critically due to the different methodological approach.

D3 included DP and HC only, but applied both visual and auditory stimuli. During auditory stimulus processing, between-group comparisons (DP > HC): (DAA > DAN) yielded a positive connectivity between the amygdala and insula. Worthy of note, this connectivity was negative in D1 during visual stimulus presentation.

Furthermore, a trend emerged (p (FDR) = 0.0533) for a positive connectivity between the insula and the ACC, thus reflecting results from D1 for SP. Directly contrasting stimulus modalities (i.e., auditory > visual stimuli) augmented this connectivity. Consistent with that, DP was characterized by a connectivity shift towards increased mean FC during auditory stimulus processing pointing to stimulus dependence in this phobia. Although phobic symptom provocation was successful with both stimulus modalities in D3 (measured by subjective ratings), the two differed from the point of view of their pain-inducing effects and overall valence, with the auditory modality being rated more painful and aversive in general[44]. In line with this, the auditory stimuli have been assumed to be the major fear sensation quality in this particular phobia[105,202]. Hence, the use of auditory stimulus material may contribute to a stronger FC possibly reflecting enhanced, inappropriate emotion regulation during more intense states of fear.

In sum, the findings point to a critical role of the ACC – amygdala circuit for sufficient fear regulation during phobogenic stimulus presentation. Moreover, SP was generally marked by an excitatory coupling of fear-processing regions. Regarding DP, a FC shift was observed during auditory stimulus presentation which could mirror primary phobogenic stimulus quality. Lastly, the current results are consistent with the original study by Hilbert et al. that suggested more commonalities between the examined phobias during auditory symptom provocation than during visual symptom provocation[44].

ROI-to-ROI analyses using the BNT atlas

The objective of the ROI-to-ROI analysis was to specify the connectivity patterns of the ACC and the insula subregions, given their functionally diverse engagement. The main findings were that:

1) the inhibitory connectivity between the ACC and amygdala in controls was predominantly driven by the sACC,

2) in phobics, particularly in SP, enhanced connectivity was found outside the proposed ventral “affective” and dorsal “cold” ACC – insula networks (e.g., between sACC and postINS, and between dACC and vaINS),

3) the altered FC in DP of the insula was driven by the daINS both for the auditory and the visual stimulus modality.

Controls in D1 exhibited negative connectivity patterns for both snake and dental specific contrasts predominantly between the sACC and the amygdala. This points to a crucial impact of the sACC in the inhibitory route between the ventromedial PFC and the amygdala[204]. Further, in controls for the contrast (DA > DN) the dACC was found to correlate negatively with the sACC while the pACC exhibited a negative connectivity with the amygdala. This corresponds well to the assumption that the ACC subregions and the amygdala form an important feedback-loop for emotional conflict regulation on the basis of a ventral/dorsal distinction of the ACC subregions[8,133,161]. Due to the strong connections with the DLPC, the ACC could function as an indirect route for higher-order cognitive control over the amygdala[65,108]. FC disruptions in this circuit as observed for DP for the contrast (DVA > DVN) between the pACC and the sACC could therefore account for the irrationality of phobic fear responses. Moreover, this analysis could specify that the insula FC in DP emerged mainly from the anterior insula. This is well in line with the role of this region in pain processing and in cognitive control which both form critical components of BII phobias[105,106,134,205].

SP, however, during phobogenic stimulus processing was characterized by enhanced FC between the sACC and the postINS and between the postINS and the vaINS. The enhanced postINS engagement might reflect augmented perceptions of vegetative arousal in the animal subtype. Further, between-group comparisons (SP > HC) showed a positive coupling of the vaINS with the dACC and between insula subregions (postINS – vaINS). These findings seem to conflict with the assumption of an "affective" ventral and a "cold" dorsal ACC – insula network[94,95]. Therefore, abnormal high joint engagement of these regions outside of established functional circuits might be argued to represent the merging of affective, limbic processes with sensorimotor integration and executive processes. In this concept, affective and bodily information streams could affect and potentiate each other inadequately resulting in biased emotional responses and abnormally high psychophysiological outflow.

In D2, the only significantly differentiating FC pattern emerged for the contrast SP: (PPSA > PPSN) and showed a negative coupling of dACC and vaINS which contrasts with the mainly positive patterns in D1. However, the same limitations must be taken into consideration when drawing conclusions here.
Taken together, this analysis highlighted the importance of the ventral "affective" ACC division for amygdala regulation. Connectivity changes regarding the insula were predominantly traced back to its anterior part. Moreover, connectivity alterations were not only observed in commonly established networks but also outside these circuits which could represent the inappropriate merging of different information streams.

Seed-to-Voxel Connectivity Analyses

The seed-to-voxel analyses using the ACC, amygdala and insula as seeds were applied to explore further FC patterns across the whole brain. The following main results were observed:
1) In SP, clusters associated with the ACC seed were found in the insula and in temporal regions,
2) DP was characterized by negatively connected clusters in pre- and orbitofrontal regions,
3) the result under 2) above applied only for the visual stimulus modality while no clusters were found for the auditory conditions.

In D1, the ACC was positively connected with voxels in the insula, planum temporale and TP in SP during snake specific stimulus processing. These findings largely mirror results from the a priori defined ROI-to-ROI analyses on a whole-brain level. Further comments will concentrate on the temporal regions since the insula has been already discussed (please see p. 55 and p. 57). Due to its adjacent location to the amygdala and the OFC and widespread connections to limbic and paralimbic regions, the TP has been argued as belonging to an extended limbic system[206]. It has been linked to face recognition and theory of mind and was further argued as forming preprocessed perceptual information into visceral emotional responses[207,208]. Moreover, emotional states like anxiety, fear and

disgust have been associated with TP activity[208]. These characteristics make an involvement in SP possible although its precise role remains speculative. The other connected area – the planum temporale – is a region traditionally related to language processing and is clinically associated with schizophrenia and dyslexia[209]. Anatomically, it is situated on the superior temporal gyrus which has been reported before to show joint activity with the mPFC[210] and was related to emotion regulation in general[147]. Thus, an explanation for this connectivity could be an increased alertness resulting in upregulated environmental monitoring, e.g., a more attentive hearing, during states of fear. This seems reasonable especially for animal phobias that are marked by a high vegetative arousal and anticipatory anxiety. The insula showed (besides to the ACC; please see p. 55 and p. 57) a positive connectivity to the left paracingulate area – an area being only present in 30% to 60% of all humans[211,212]. Lying adjacent to the cingulate sulcus it has been argued as expanding the ACC, namely BA 24 and 32[74,111,211]. Furthermore, the cluster detected in this analysis was located close to the sACC ROI of the subregion analysis. Worthy of note, the sACC showed also a positive FC with the insula. Thus, it is not surprising that this adjacent region shares a similar connectivity pattern. The insula was additionally negatively connected with voxels in the fusiform cortex, the inferior temporal gyrus and the cerebellum. The cerebellum participates emotional processes such as anxiety, although findings are inconsistent on whether it shows hyper- or hypoactivity[213–215]. Since little is known about these regions in fear processing so far, their contribution to specific phobia remains rather speculative.

In D3, the contrast (DP > HC) surprisingly did not yield any significant clusters for the auditory stimulus modality, even though this contrast showed strong connectivity patterns in the ROI-to-ROI analyses. In the visual contrast, the insula showed a positive FC with two small clusters in the superior temporal gyrus and the TP and a bigger in the SMG. Whereas the TP is supposedly involved in the integration of perceptual inputs into visceral emotional responses, the insula integrates interoception with emotional salience to form a subjective representation of the body[101] which makes joint work plausible. The positively connected SMG is considered as a part of the default mode network (DMN)[216] and

the mirror neuron system[217]. As association cortex, it helps integrating several information streams and is involved in higher-level executive functions[218]. Therefore, the insula and the SMG are both engaged in the processing of tactile and pain stimuli and are well-located to integrate different information streams in phobogenic stimulus processing. The FP is corresponding to BA 10[219] and was the only region showing a negative FC with the insula. It has been reported to be crucial for initial assessment of coexisting alternatives and subsequent decision-making[220–222]. The disrupted connectivity of the FP and insula might represent malfunctioning interactions between superordinate frontal regions over top-down controlled regions.

The ACC seed was positively connected to the SMG. The ACC and the SMG are engaged in a range of integration and higher-order tasks which probably explains its enhanced connectivity during fear processing. Likewise, the ACC showed a positive FC to the SMA, which has been related to emotion regulation[147]. Additionally, the ACC is implicated in movement control and is involved in generating the readiness potential[223] and both regions work together during movement preparation[224]. Thus, it is possible that these regions work together to prepare flight attempts requiring movement preparing areas like the SMA – particularly in the initial phase of the diphasic psychophysiological response of BII phobias[225]. Lastly, the parietal operculum exhibited a positive correlation with the ACC. This region is closely associated with the insula and is involved in pain processing[226–228], somatosensory and visual integration[228], and – at least in rodents – implicated in associative Pavlovian conditions and the generation and maintenance of associative emotional memory[229,230]. Moreover, the engagement of the parietal operculum in pain processing is considered be closely related to the cognitive-evaluative stimulus appraisal[231]. In DP, FC of these regions could be particularly associated with the integration of pain aspects. Negatively correlated regions with the ACC encompassed the FP, the OFC, the TP and the paracingulate gyrus. Unlike for snake stimuli, the latter two exhibited a negative FC in this analysis. The FP lacks dense connections to the amygdala which is why the cingulate has been suggested as an indirect access to the amygdala for the it[56]. Thus, disrupted connectivity between the ACC and the FP might be followed by

biased stimulus evaluation and solution strategies eventually leading to enhanced amygdala activity. The OFC is involved in a wide range of tasks including sensory integration, decision-making, and especially the representation of emotional value of reinforcers and the evaluation of expected outcomes (e.g., punishments) as well as the subsequent stimulus-reinforcement association learning and extinction learning[232,233,234]. The engagement of the OFC is considered to display an example for the evaluation-based fear response in DP indicating particularly the relevance of cognitive control and appraisal in the BII subtype[42,44,151,152]. Therefore, disturbance of OFC – ACC connectivity might be followed by biases such as failed extinction learning of innocuous situations (e.g., dental treatments) and unreasonably high anticipatory anxiety.

To further explore stimulus modality influence on a whole-brain level, auditory and visual stimuli were compared directly between groups. Hereby, a positive FC between the amygdala seed and the paracingulate cortex as well as the superior frontal gyrus could be observed. No enhanced connectivity was measured for auditory information processing regions. Instead, the superior frontal gyrus (SFG) is a prefrontal region that is associated mainly with working memory[235]. The FC with the paracingulate gyrus as a paralimbic region extends the pattern of strongly connected limbic regions in the auditory stimulus condition.

In summary, the seed-to-voxel analysis largely confirms the findings of the previous analyses on a whole-brain level by revealing altered FC particularly within the a priori defined ROIs and anatomically adjacent regions (e.g., the parietal operculum, the paracingulate gyrus, temporal pole). Regarding DP, it further strengthens assumptions that phobic fear during visual symptom provocation emerges predominantly from impairments on higher-level cognitive processing resulting in dysregulated top-down controlled regions.

Combined Discussion

Brain Network Dysfunctions in Specific Phobia

The conducted analyses focused on brain regions that are closely related to both phobic fear processing and emotion regulation in general and was aimed at revealing fundamental networks contributing to phobic fear. Both ROI-to-ROI analyses provide preliminary evidence for altered, predominantly upregulated coupling between the examined ROIs in SP and a connectivity shift in DP during auditory stimulus presentation. These findings were amended by the seed-to-voxel analyses that pointed to the involvement of frontal and temporal regions, particularly in DP.

Together, current results can be embedded in a theoretical framework of emotion processing that links disturbances in functional brain circuits to inappropriate fear responses. Stein et al. proposed an extended prefrontal network consisting of the ACC, OFC, Insula and dlPFC that is associated with amygdala regulation as a crucial basis for emotional regulation[236]. Except the dlPFC, all regions were found to exhibit FC dysregulations in this work. A further important framework is provided by Philipps et al. dividing emotional processes in emotion identification and automatic and voluntary emotion regulation[148] (see also figure 23, p. 64) . In this context, the observed patterns of augmented FC of SP and for DP under auditory stimulus presentation can be viewed as impairments in the automatic, bottom-up processes of emotion regulation. This is a well-suited concept particularly for SP that is characterized by evolutionary conserved limbic fear responses[46].

The FC patterns of DP during visual stimulus processing prompt the suggestion that imbalanced top-down control by pre- and orbitofrontal regions might account for the deficient stimulus control. Thus, DP might rather suffer from impaired voluntary emotion regulation processes in contrast to SP. This suits its complex structure (e.g., including multifaceted social aspects[237]) and highlights the importance of cognitive reappraisal strategies and their conscious application as coping skills in this subtype[238].

However, it should be considered that other networks might be engaged in further aspects of fear processing (e.g., visual stimulus detection). Interestingly, the ACC

and the insula have also been considered to represent central hubs in such relevant large-scale networks (e.g., the salience network[102,239] or the default mode network[240]).

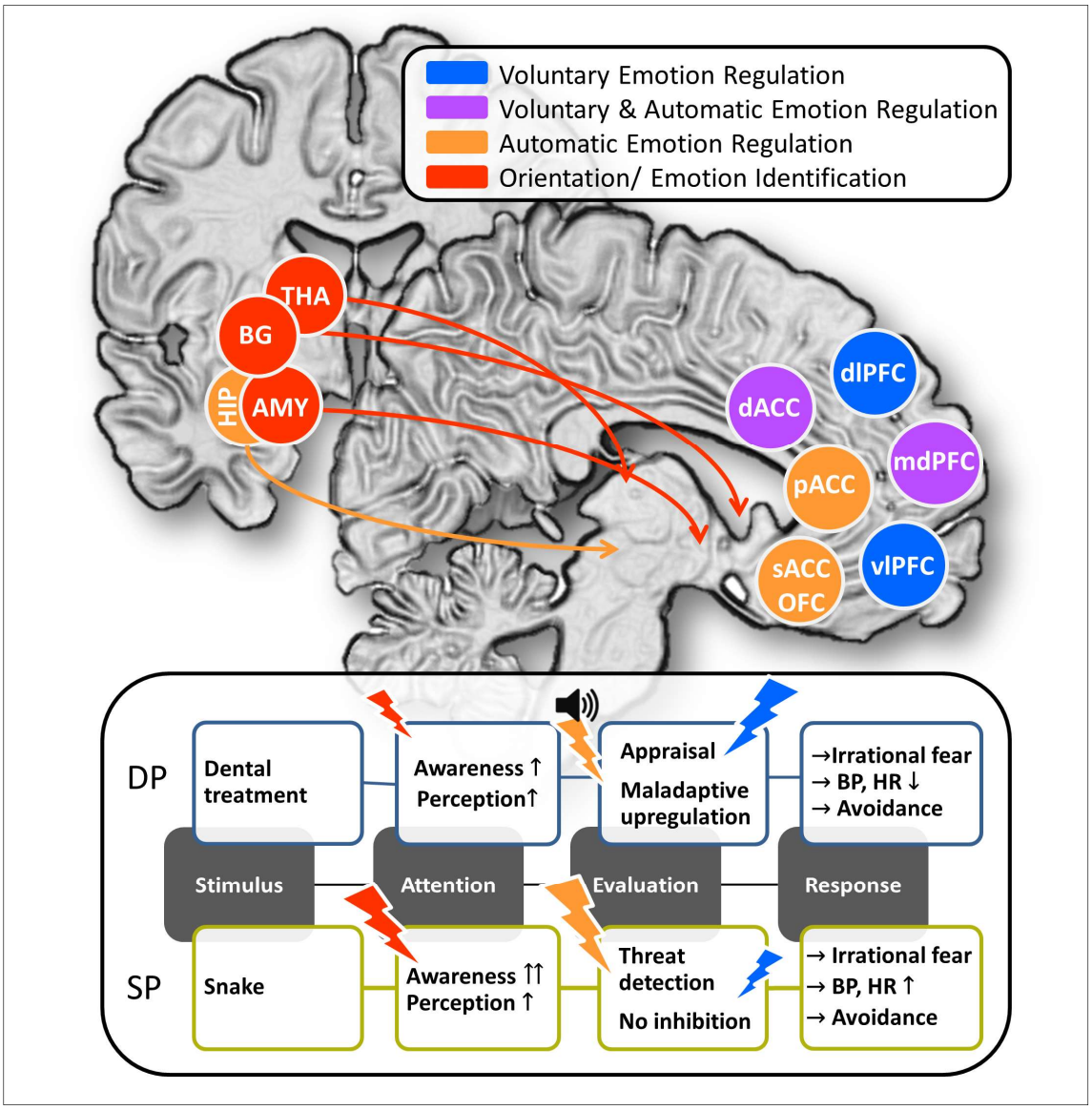


Figure 23. Emotion regulation in specific phobia. Following the neural model of emotion regulation of Phillips et al.[148], emotion-related regions are illustrated on a coronal and sagittal brain slice and colored corresponding to their primary role in emotion processing. Arrows depict their virtual projection on a sagittal slice. A psychological model of emotion regulation according to Gross[157] is adjusted for phobogenic stimuli processing in dental phobia (DP, upper row) and snake phobia (SP, bottom row). Flashes depict interferences of neural processes on the psychological model (red: orientation/emotion identification; orange: automatic emotion regulation; blue: voluntary emotion regulation). THA: thalamus; BG: basal ganglia; AMY: amygdala; HIP: hippocampus; sACC: subgenual anterior cingulate cortex; pACC: pregenual ACC; dACC: dorsal ACC; vlPFC: ventrolateral prefrontal cortex; mdPFC: mediodorsal PFC; dlPFC: dorsolateral PFC. The figure is based on: "A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder." Phillips, M. L., Ladouceur, C. D. & Drevets, W. C., Mol. Psychiatry 13, 833–857 (2008), with permission of Springer Nature.

Translating current Research Findings into clinical Utility

The circuits examined have been previously observed to exhibit functional dysregulations in further typical anxiety disorders such as social anxiety disorder[241–243] or panic disorder[9]. This, however, might not be surprising since comorbidity among mental disorders is common[12,26,167] and might be explained by shared pathophysiological factors[244,245]. Among others, this has been a reason for the initiation of the Research Domain Criteria Project[246] (RDoC) by the US National Institute of Mental Health (https://www.nimh.nih.gov/research-priorities/rdoc/index.shtml). This project provides a new dimensional and unbiased framework for psychological research and aims at exploring basic mechanisms of mental functioning from normal to abnormal. Within the RDoC framework the current work examined the construct of "Acute Threat (Fear)" by focusing on relevant neural circuits in specific phobia. In this context, FC alterations found in the previous analyses and particularly ACC – amygdala circuitry dysfunctions might depict an intermediate phenotype of phobic fear responses. Since disorder-specific genetic effects have already been proposed for BII and animal phobias[22,32], the question arises if specific gene variations might be associated with FC changes in DP and SP as already reported for example in PD[247]. However, future studies – ideally family and high-risk studies – are needed to specify if connectivity disturbances depict characteristic risk factors for the development of specific phobias or if they represent unspecific concomitant features of several anxiety disorders.

Better knowledge of the neural pathophysiology of specific phobia could bear the potential to improve clinical treatment by two means. First, FC-based biomarkers could be used to predict therapy response and to identify subjects that are at high risk to obtain no benefit from standard treatment. This approach has been successfully applied to exposure-based CBT in PD with agoraphobia where non-responders where characterized by relatively higher excitatory ACC – amygdala connectivity[9]. Due to commonalities of therapeutic approaches and neural patterns this may account for specific phobia, too. In specific phobia, the current first-line treatment is also an exposure-based cognitive-behavioral therapy (CBT) with

relatively high response rates[27,28]. The extinction achieved hereby could be partially conveyed through changes in ACC – amygdala FC. Theoretically this is based on top-down control of the amygdala in terms of inhibitory ACC – amygdala FC[108,109] which is considered as a key mechanism of effective CBT[248]. As exposure-based therapy still is associated with drop-out rates up to 10-20% and a relatively high treatment refusal due to its confronting nature[27], directly targeting this circuit offers a second starting point to improve treatment for specific phobias. Relevant brain-based treatments in this context are neurofeedback and rTMS. Although the ACC – amygdala circuit has not been targeted specifically in specific phobia, it was shown that FC of these regions can principally be modulated by neurofeedback training[249]. Up to now, neurofeedback has been recently applied successfully for the first time in spider phobia by using the dlPFC and the insula to provide feedback[29]. Considering rTMS, two studies have applied a single-session intermittent theta burst stimulation over the left dlPFC in specific phobia[250,251]. While these attempts failed to observe beneficial effects, targeting the ACC – amygdala circuit might result in a more beneficial outcome. For instance, treatment response to exposure therapy in acrophobia could be improved by using the ACC as stimulation site[252], encouraging its application in specific phobia, too.

Limitations

Several limitations need to be considered when evaluating these findings. Although three studies were analyzed to provide replicated evidence, the single samples were relatively small. This could have limited the ability to detect small scale effects. Particularly, the studies seemed to be underpowered to relate findings of altered FC to demographic and clinical scores using Pearson correlations. Further, the sample included SP and DP with comorbid disorders. Even though the comorbidity load was comparable across phobic groups, a contribution of comorbidity cannot be excluded. The task design possibly influenced FC findings due to differences regarding immediate and prolonged fear processing. Whereas event-related designs and shorter stimulus presentation periods appear to be more suitable to detect activity and connectivity within regions with short-time activity during immediate stimulus processing like the amygdala[46,67,253], block-

designs and longer stimulus presentation periods seem to be more appropriate with respect to regions that exhibit longer activity such as the insula[42,44,67]. Moreover, neutral conditions were closely matched to anxiety conditions; thus, carry-over effects cannot be excluded. Further, anxiety conditions might provoke subliminal fear responses in controls due to a relatively high prevalence of aversiveness in the general population to dental and snake stimuli[17] which could have limited the ability to find significant results in inter-group comparisons. Further, the individual pathophysiology in specific phobia might vary due to different genetic risk factors, acquisition modes and coping strategies. Exploring these interdependencies principally require large sample sizes with subgroups that may be followed-up longitudinally.

Lastly, only two types of specific phobia were investigated, namely the animal and BII type. The present body of evidence is largely biased by investigating the animal subtype while virtually no studies are available on the situational or environmental subtype. Based on the initial findings depicting differences between the animal and BII subtype, significant differences in the neural substrates and networks within the other subtypes could be anticipated. A proof of evidence, however, is still lacking and should be targeted by future studies.

Conclusion

The present results highlight the relevance of brain circuit dysfunctions in the pathophysiology of specific phobia. Particularly the inhibitory functional coupling between the ventral ACC and the amygdala seems to be crucial for appropriate fear responses. In contrast, SP was characterized by an excitatory coupling of fear-processing regions which suits the concept of evolutionary conserved bottom-up fear responses in the animal subtype. In DP, enhanced FC was only observed during auditory stimulus presentation indicating stimulus dependency. During visual stimulation however, FC changes of pre- and orbitofrontal regions were found. This strengthens suggestions that DP strongly depends on evaluation-based fear responses. Findings might be of clinical use given the extending evidence highlighting a pivotal role of the ACC in amygdala inhibition, thus providing a

reasonable target for experimental treatment techniques such as neurostimulation or neurofeedback. In sum, the findings enlarge our knowledge of connectivity shifts in specific phobia that may confer risk for exaggerated fear reactivity as a probable basis of pathological forms of fear and anxiety.

Summary

Abstract

Neuroimaging research has highlighted the relevance of well-balanced functional brain interactions as an essential basis for efficient emotion regulation. In contrast, abnormal coupling of fear-processing regions such as the amygdala, the anterior cingulate cortex (ACC) and the insula could be an important feature of anxiety disorders. Although activity alterations of these regions have been frequently reported in specific phobia, little is known about their functional interactions during phobogenic stimulus processing.

To explore these interrelationships in two subtypes of specific phobia – i.e., the blood-injection-injury subtype and the animal subtype – functional connectivity (FC) was analyzed in three fMRI studies. Two studies examined fear processing in a dental phobia group (DP), a snake phobia group (SP) and a healthy control group (HC) during visual phobogenic stimuli presentation while a third study investigated differences between auditory and visual stimuli presentation in DP and HC.

Due to a priori hypotheses of impaired interactions between the amygdala, the ACC and the insula, a first analysis was conducted to explore the FC within these three regions of interest. Based on emerging evidence of functionally diverse subregions, the ACC was further divided into a subgenual, pregenual and dorsal ACC and the insula was divided into a ventral-anterior, dorsal-anterior and posterior region. Additionally, an exploratory seed-to-voxel analysis using the amygdala, ACC and insula as seeds was conducted to scan for connectivity patterns across the whole brain.

The analyses revealed a negative connectivity of the ACC and the amygdala during phobogenic stimulus processing in controls. This connectivity was predominantly driven by the affective ACC subdivision. By contrast, SP was characterized by an increased mean FC between the examined regions. Interestingly, this phenomenon was specific for auditory, but not visual symptom provocation in DP. During visual

stimulus presentation, however, DP exhibited further FC alterations of the ACC and the insula with pre- and orbitofrontal regions.

These findings mark the importance of balanced interactions between fear-processing regions in specific phobia, particularly of the inhibitory connectivity between the ACC and the amygdala. Theoretically, this is assumed to reflect top-down inhibition by the ACC during emotion regulation. The findings support the suggestion that SP particularly is characterized by excitatory, or missing inhibitory, (para-) limbic connectivity, reflecting an overshooting fear response based on evolutionary conserved autonomic bottom-up pathways. Some of these characteristics applied to DP as well but only under the auditory stimulation, pointing to stimulus dependency. DP was further marked by altered pre- and orbitofrontal coupling with the ACC and the insula which might represent disturbances of superordinate cognitive control on basal emotion processes. These observations strengthen the assumption that DP is predominantly based on evaluation-based fear responses.

In conclusion, the connectivity patterns found may depict an intermediate phenotype that possibly confers risks for inappropriate phobic fear responses. The findings presented could also be of clinical interest. Particularly the ACC – amygdala circuit may be used as a predictive biomarker for treatment response or as a promising target for neuroscience-focused augmentation strategies as neurofeedback or repetitive transcranial magnetic stimulation.

Zusammenfassung

Neurowissenschaftliche Erkenntnisse der letzten Jahre verdeutlichten die Relevanz intakter neuronaler Netzwerke als Grundlage adäquater Emotionsregulationsmechanismen. Funktionelle Dysregulationen zwischen angstverarbeitenden Regionen wie der Amygdala, der Insula oder dem anterioren cingulären Cortex (ACC) könnten hingegen einen wichtigen pathophysiologischen Mechanismus von Angststörungen darstellen. Obwohl Aktivitätsunterschiede dieser Regionen wiederholt für spezifische Phobien beschrieben wurden, sind deren funktionelle Interaktionen während phobischer Stimulusverarbeitung kaum erforscht.

Zur Untersuchung dieser Interaktionen in zwei Subtypen der spezifischen Phobie – dem Blut-Spritzen-Verletzungs-Typus und dem Tier-Typus – wurden im Rahmen dieser Arbeit funktionelle Konnektivitäts-Analysen (FK) anhand dreier fMRT-(funktionelle Magnetresonanztomographie) Studien durchgeführt. Zwei Studien untersuchten die neurale Verarbeitung visueller phobischer Stimuli in einer dentalphobischen Gruppe (DP), einer schlangenphobischen Gruppe (SP) sowie einer Kontrollgruppe (KG). Ergänzend verglich eine dritte Studie den Einfluss visueller und akustischer Stimuli für die DP und eine KG.

Basierend auf der a priori-Hypothese einer veränderten FK zwischen der Amygdala, der Insula und dem ACC wurden deren spezifische Konnektivitätsmuster untersucht. Aufgrund funktionell unterschiedlicher Subregionen erfolgte eine Untergliederung des ACC in eine subgenuale, perigenuale und dorsalen Region. Analog dazu wurde die Insula in eine ventral-anteriore, dorsal-anteriore und posteriore Region unterteilt. Um darüberhinausgehender Konnektivitätsmuster über das gesamte Gehirn zu ermitteln, wurde eine abschließende Seed-to-Voxel-Analyse mit den Seeds Amygdala, Insula und ACC durchgeführt.

In der Auswertung zeigte sich eine negative FK der Amygdala und des ACC während phobischer Stimulusverarbeitung in der KG, die insbesondere auf die ventrale Division des ACC zurückzuführen war. Die phobischen Gruppen hingegen waren im Vergleich zu der Kontrollgruppe durch eine erhöhte Konnektivität der

untersuchten Regionen gekennzeichnet. Dieser Effekt war bei der DP spezifisch für die akustische Stimulusmodalität. Bei visueller Stimuluspräsentation zeigten sich hingegen veränderte Konnektivitätsmuster des ACC und der Insula mit prä- und orbitofrontalen Regionen.

Insbesondere die negative FK der Amygdala und des ACC, die theoretisch auf einer top-down-Inhibition des ACC über die Amygdala basiert, erscheint einen wichtigen Bestandteil einer effektiven emotionalen Kontrolle darzustellen. In beiden phobischen Gruppen fehlte diese Inhibition. Die erhöhte FK (para-)limbischer Konnektivität der SP könnte hingegen die verstärkte Rekrutierung autonomischer bottom-up-Prozesse als zugrundeliegendem Mechanismus der überschießenden und irrationalen Angstreaktion repräsentieren. Diese Charakteristika konnten in der DP nur für die akustische Stimulusmodalität beobachtet werden. Während der visuellen Stimuluspräsentation war die DP durch Dysregulationen prä- und orbitofrontaler Regionen gekennzeichnet, welche eine beeinträchtigte kognitive Kontrolle über grundlegende Emotionsprozesse widerspiegeln könnte. Dies entspricht der Annahme, dass die DP vor allem durch evaluationsbasierte Furchtreaktionen gekennzeichnet ist, während in der SP als Vertreter des Tier-Typus evolutionär konservierte, limbische Prozesse dominieren.

Zusammenfassend bestätigen die Ergebnisse die Bedeutung funktioneller Netzwerke in der spezifischen Phobie, wobei die gefundenen Konnektivitätsmuster einen intermediären Phänotyp darstellen könnten, der möglicherweise das Risiko für das Auftreten dysfunktionaler phobischer Angstreaktionen vermittelt. Von klinischem Interesse ist vor allem die Amygdala – ACC-Vernetzung, die als prädiktiver Biomarker für das Therapieansprechen genutzt oder im Rahmen neuromodulatorischer Therapieansätze wie dem Neurofeedback oder der repetitiven transkraniellen Magnetstimulation gezielt angesteuert werden könnte.

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Abbreviations

#NS.SCR	Mean Number of Non-specific Skin Conductance Response Fluctuations
ACC	Anterior Cingulate Cortex
aMCC	Anterior Midcingulate Cortex
AMP.SCR	Sum Amplitude of Non-Specific Skin Response Fluctuations
AMY	Amygdala
ANOVA	Analysis of Variance
ART	Artifact Detection Tool
ASI	Anxiety Sensitivity Index
BA	Brodmann Area
BDI	Beck Depression Inventory
BG	Basal Ganglia
BII	Blood-Injection-Injury Subtype of Specific Phobia
BNT	Brainnetome Atlas
CBT	Cognitive Behavioral Therapy
CSF	Cerebrospinal Fluid
DA	Dental Anxiety
DAA	Dental Auditory Anxiety
dACC	Dorsal Anterior Cingulate Cortex
daINS	Dorsal-Anterior Insula
DAN	Dental Auditory Neutral
df	Degrees of Freedom
DFS	Dental Fear Survey
dlPFC	Dorsolateral Prefrontal Cortex
DN	Dental Neutral
DP	Dental Phobia
dPCC	Dorsal Posterior Cingulate Cortex
DSM	Diagnostic and Statistical Manual of Mental Disorders
DVA	Dental Visual Anxiety
DVN	Dental Visual Neutral
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FP	Frontal Pole
GAD	General Anxiety Disorder
HC	Healthy Controls
INS	Insula
l.	Left
MCC	Midcingulate Cortex
MDD	Major Depressive Disorder
mdPFC	Mediodorsal Prefrontal Cortex
mPFC	Medial Prefrontal Cortex
MQ	Mutilation Questionnaire
MRI	Magnetic Resonance Imaging
OFC	Orbitofrontal Cortex
pACC	Perigenual Anterior Cingulate Cortex
PCC	Posterior Cingulate Cortex
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
pMCC	Posterior Midcingulate Cortex
postINS	Posterior Insula
PTSD	Posttraumatic Stress Disorder
r.	Right
RDoC	Research Domain Criteria
ROI	Region-of-Interest
RSC	Retrosplenial Cortex
rTMS	Repetitive Transcranial Magnetic Stimulation

SA	Snake Anxiety
sACC	Subgenual Anterior Cingulate Cortex
SAD	Social Anxiety Disorder
SCID-CV	Structured Clinical Interview for DSM Disorders (Clinical Version)
SCR	Skin Conductance Response
SMA	Supplementary Motor Area
SMG	Supramarginal Gyrus
SN	Snake Neutral
SNAQ	Snake Anxiety Questionnaire
SP	Snake Phobia
TE	Echo Time
THA	Thalamus
TMS	Transcranial Magnetic Stimulation
TP	Temporal Pole
TR	Repetition Time
vaINS	Ventral-Anterior Insula
vPCC	Ventral Posterior Cingulate Cortex

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