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An fMRI study in outpatients

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Stellingen

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On the neural basis of emotion processing in depression and anxiety. An fMRI study in outpatients

van

Liliana Ramona Demenescu

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1. Perception of facial expressions of emotion elicit to a large extent the same neural mechanism in outpatients with depression and anxiety disorders as in healthy volunteers.

Chapter 3 of this thesis

2. The use of medication alone may not explain the normalization of neural response to emotional stimuli, but there may be an interaction of illness severity and antidepressant.

Chapter 3 and 4 of this thesis

3. Anxiety, but also depression may be characterized by aberrant neural response during emotional attribution to words.

Chapter 4 of this thesis

4. Aberrant connectivity of brain areas may be a trait marker for affective disorders. Neuroticism, which may be considered as starting point for the development of depression or anxiety, seems to modulate functional brain areas connectivity during emotional processing.

Chapter 5 of this thesis

5. Anxiety disorders, in remission or with mild–to–moderated illness severity, may be associated with distinct brain areas connectivity even if there are no gross abnormalities in the neural response during emotional processing.

Chapter 6 of this thesis

6. It is too often forgotten that our results always speak about a model, that our data is rather statistical and approximate than an absolute, objective truth. Our experimental setup and our methods constitute our model, and the slightest change in either of them can have a major influence on our understanding.

7. Comorbidity of depression and anxiety may be considered a distinct diagnosis.

Contents

Chapter 1

Anteloquy

The subject of this thesis lies in the field of neuropsychology and is concerned with the study of neural basis of emotions in relation to anxiety and depression. This study makes use of functional neuroimaging [1], which allows for non-invasive identification of patterns of human brain activity associated with perceptual, cognitive, emotional and behavioral processes. This introduction will briefly describe the background and rationale for the study. First, I will introduce the subject of emotion processing and brain function. I will then describe major depressive disorder and the anxiety disorders that will be included in our investigations. Finally, I will provide an overview of the different chapters.

In neuropsychology, functional neuroimaging techniques are used to gain a better understanding of brain-behavior relationships. The aim is to link specific psychological processes to anatomical areas and physiological processes in the brain [1].
In recent years, functional magnetic resonance imaging (fMRI) has been a very successful method of studying different cognitive and emotional functions in normal and abnormal behavior [2]. Together with imaging methods like positron emission tomography (PET) and electroencephalography (EEG), fMRI has contributed to elucidating the role of different neural networks in the brain and in relation to different emotions and cognitive functions and also in exploring the functional abnormalities related to different diagnostic entities. In fMRI, one measures the hemodynamic response, *i.e.*, changes in blood flow, related to neural activity in, for example, the brain or the spinal cord of biological organisms [1]. The main advantages of this technique are its low invasiveness, the possibility to record activity in all regions of the brain and the high spatial resolution.

The wide implications of emotions in the social and private life, and the far-reaching consequences of dysfunctions in emotional processing, have made emotion one of the most widely studied psychological processes [3, 4]. The study of emotion is important not only for understanding its nature, but also for identifying the neural mechanisms involved in the perception of the different types of emotion [5]. In other words, not only the bodily mechanisms of emotional experience are important, but also the neural mechanisms responsible for the perception of emotions in others. The latter play a key role in social interactions. Moreover, the identification of neural mechanisms underlying emotional processing may help to understand psychiatric disorders resulting from dysfunctions in emotional processing.
It is well known by now, that different emotions may generate activity in multiple areas across the brain [6, 7]. Furthermore, research has emphasized the interaction between emotion and cognition and suggested that emotions influence memory, learning, attention and perception processes [8]. For a clear picture of the neural networks involved in emotional processes, and for understanding their complex interactions with other psychological processes, high resolution images are required of the neural networks involved. Using fMRI, neural activity in all anatomical locations of the brain can be recorded at good spatial and temporal resolution (images

[1] A *psychological process* is defined herein as a change which takes place inside the nervous system of an organism, *i.e.*, a *biological process*, and has implications in the behavior of the organism.

are usually acquired every 1-4 seconds and the *voxels* in the resulting image represent cubes of tissue with an edge length of 2-4 millimeters [1]), a quality that has made fMRI, the method of choice for the study of human emotions and their neural networks.
Studies in humans and animals have been trying to depict the *emotional brain* [2], to understand how emotions are generated by the brain. Classical studies considered that the *limbic system* [3], which involves the following structures: hippocampus, mammillary bodies, thalamus, cingulate and parahippocampus brain areas, represents the core-system of emotions [10]. However, more modern research has revealed that different aspects of emotional processing also involve areas of the brain [11, 9] that mediate other functions beyond emotional processing [12]. For example, the dorsal medial prefrontal cortex and the ventrolateral prefrontal cortex are part of the neural circuitry involved in experience of emotion, but are also related to cognitive processes [7]. Barrett and colleagues [7] suggested that emotion experience and cognition represent a "gradient", rather than two independent systems which can interact with each other. Further, it has been suggested that emotional experience plays an important role in the judgement of perceived emotional stimuli ([13], p. 596-618). Recent functional neuroimaging studies have provided additional support for this neural model of emotional processing [14, 15]. Thus, in addition to amygdala and hippocampus, which were already shown as being involved in emotional processing, the prefrontal cortex (PFC) and the anterior cingulate cortex (ACC) have also been shown to be activated during emotional processing ([13], p. 8-24). Additional studies [6, 5, 15] have shown that different types of emotion are associated with activity in specific brain areas. Fear-related emotions were found to specifically activate the amygdala, sadness was associated with increased activation in the subcallosal cingulate cortex, disgust was related to insula activation, positive emotions – and again, disgust (see the above discussion) – were linked to basal ganglia activation, whereas the medial PFC was suggested to be a shared region activated across different emotions.
The amygdala [4] is considered the key component of the neural mechanism of emotion. Although early studies considered that the amygdala is the core neural structure of fearful emotion, latter studies reported amygdala responsiveness also to positive and neutral facial expressions, suggesting a more general role of this brain area in processing salient visual stimuli rather than in emotion processing *per se* [16]. Furthermore, it has been shown that amygdala response to facial expressions is modulated by attention: *e.g.*, implicit or "automatic" processing of facial expressions elicited stronger amygdala response, relative to explicit or "conscious" processing of emotional facial expressions [17]. In addition, researchers have emphasized the role of the amygdala in different forms of psychopathology [18, 19, 20]. In order to identify the abnormalities in the neural response associated with psychopathological disorders, we used an implicit emotional perception task, but also an explicit emotional processing paradigm.
Brain regions work like a network of interconnected areas, rather than like isolated areas reserved solely for the processing of specific inputs. It is therefore important to understand how these regions are connected. As the amygdala is considered to play an important role in emotional processing, neuroimaging studies have recently examined the anatomical and functional connectivity of this region. Until recently, evidence regarding anatomical connectivity of the amygdala with other brain areas was based only on animal studies. These studies have reported that the amygdala has anatomical connections with several forebrain areas including ventromedial prefrontal cortex, insula, temporal cortex, thalamus and basal ganglia, and this system was associated with emotional behavior and mood [21]. Functional neuroimaging studies in humans have recently shown that the amygdala in the human brain is part of a complex network of brain regions, including prefrontal regions, anterior cingulate cortex, insula and thalamus [22]. The study of this neural network may help us to better understand the neural mechanism of emotion and cognitive processes in the healthy brain and in psychopathological disorders.

Given the complexity of neural mechanism related to emotional processing, it is not surprising that emotional dysfunctions characterize a variety of psychopathological syndromes. An emotional dysfunction may be caused by a disturbance in one or more components of the emotional processing system, such as differences in cognitive style (negative cognition may influence the evaluation process such that ambiguous emotional stimuli are interpreted as being more negative), aberrant emotional experience or differences in physiological responses to

[2]The term "emotional brain" was used for the first time by LeDoux, in 1996, to define the neural circuitry of emotion [9].
[3]The limbic system is a set of brain structures that lies on both sides (left and right hemisphere) under the cerebrum forming the inner border of the cortex. The term limbic comes for Latin *limbus* meaning "border" or "belt".
[4]The amygdala is a small structure of the brain, has an almond-shape and its located deep in the medial temporal lobe of the brain.

emotional stimuli [23].

In the present thesis, we will focus on two psychological disorders characterized by emotional disturbances: 1) major depressive disorder and 2) anxiety disorders. Anxiety and depression have a high prevalence in the general population and are related to a decline in the quality of life. Yearly, aproximatively 7% of the adult population suffers from a depressive disorder and 18% suffers of anxiety disorders [24]. According to the World Health Organization, mood disorders rank among the top ten of diseases causing worldwide burden and it is speculated that in 2020 depression will be on the second place (http://www.who.int/en/).
Identifying abnormalities in brain mechanisms involved in emotional processing in patients with depression and anxiety disorders may be of a paramount importance in the effort to improve the treatment of these disorders. Among these patients suffering from depression or anxiety, those coming from primary mental health care are the most numerous and hence, identifying their emotional processing abnormalities could lead to an improvement of their treatment and to a faster reintegration.
The main goal of the present work is to delineate the neural mechanism of emotional processing in community-based outpatients with depression and anxiety disorders. The major strength of this study is the large number of participants and the fact that our findings, presented in this thesis, may be representative for the clinical outpatients diagnosed with major depression and anxiety in the general population.
Major depressive disorder (MDD) is characterized by persistent negative mood accompanied by diminished interest or pleasure, motor and mental speed, appetite, libido, disturbance in attention, anhedonia, feelings of guilt and suicidal ideation [25]. These symptoms lead to poor social skills and major difficulties in community integration. For example self-focused attention and negative perceptual bias influence cognitive and perceptual components of social behavior and are related to poor social skills [26]. Furthermore, patients with MDD were found to have an impairment in discriminating facial expressions of emotions in others, an impairment which is likely to interfere with their ability to accurately respond to socio-emotional signals [23]. These findings show that a better understanding of specific emotional dysfunction and the etiology of MDD is crucial in developing treatments and therapies.
Given the broad spectrum of emotional, cognitive, but also behavioral dysfunctions it is not surprising that MDD is associated with abnormalities in many brain areas [27]. Mayberg [28] hypothesized that depression is associated with hypoactivity of dorsal limbic (anterior and posterior cingulate) and neocortical areas (PFC, premotor and parietal cortex), and hyperactivity of ventral paralimbic regions (subgenual cingulate, anterior insula, hypothalamus and caudate). Abnormalities in the neural response of the dorsal regions were associated with impaired cognitive processes, poor task performances involving selective or directed attention, whereas the ventral system was hypothesized to mediate vegetative and somatic symptoms of depression [28]. Additionally, depression was associated with prolonged amygdala responses during emotional processing, which may be related to negative affect [29]. Amygdala hyperactivation to emotional stimuli has been reported by a number of previous studies, suggesting its important role in major depressive disorder [30, 31, 32]. Nevertheless, other studies failed to report amygdala hyperactivation to emotional stimuli in depressed patients [33, 34, 35]. These inconsistencies in findings may be caused by differences in experimental design, such as task demands and stimulus type. Our aim is to identify the neural pattern of activation to facial expressions of emotions and verbal emotional stimuli in community-based MDD outpatients.
Anxiety disorders are characterized by exaggerated fear response relative to innocuous stimuli, *e.g.*, phobia, or spontaneous fear response in the absence of a true threat [25]. Neuroimaging studies suggested that anxiety is associated with amygdala hyperactivation and PFC hypoactivation [36]. Rauch suggested that anxiety in general may be associated with dysfunctions in the anterior paralimbic cortex, sensory cortex and deep brain structures, *e.g.*, amygdala, hippocampus, striatum and brainstem nuclei ([13], p. 963-975). Just as depression, anxiety has also been associated with aberrant amygdala activation during threat-related stimuli [19]. However, the neural mechanism of emotional processing specific to anxiety disorders is poorly understood and the delineation of this mechanism in a large sample of outpatients is an aim of this work.
As mentioned above, affective disorders may be characterized by amygdala hyperactivation to negative emotional stimuli. Robust evidence showed that *neuroticism* is a key predictor of psychological disorders, *e.g.*, phobia, panic disorders and other anxiety disorders, and correlates also with a high risk for depression [37, 38, 39]. Neuroticism is considered a personality trait characterized by tendencies to worry and to be anxious [40, 41]. Eysenck suggested that neuroticism is associated with "high levels of *visceral brain* (amygdala, hippocampus, cingulate, septum and hypothalamus) activity" ([13], p. 933). Recently, fMRI studies have indicated that

neuroticism (and related personality traits) modulates the neural response of the amygdala and frontal brain regions during emotional processing [42, 43]. As mentioned previously, the amygdala has functional connections with frontal regions and this coupling is important for the integration of emotion and cognition [12, 44]. However, little is known about the functional connectivity between the limbic system and the frontal areas modulated by neuroticism. Thus, elucidating the neural basis of individual differences in emotional processing related to neuroticism is the third and last aim of this thesis. The findings may offer an insight into the neural mechanism associated with high risk for affective disorders.

The research presented in this thesis is part of the longitudinal study *Netherlands Study of Anxiety and Depression* (NESDA) for investigating the course of depression and anxiety disorders. The main aim of NESDA is to determine the factors that influence the development and long-term prognosis of depression and anxiety [45]. Among the anxiety disorders, social phobia was reported to be poor and with a low recovery rates, and panic disorders has a low to no improvement rates [45].
The prevalence of depression and anxiety disorders is increased in the community – primary care, specialized health care institutions –, whereas the most severe and longstanding disorders are more likely to be hospitalized in mental health care. Given the high prevalence of depression and anxiety comorbidity and the severity of the symptoms within these patients, the NESDA study focused not only on depression and anxiety alone, but also on their comorbidity, which may gives a better insight of this "state". Thus, the aim of the NESDA study is to provide information about on the course of psychiatric disorders in the community and primary care settings [45].
Almost three thousand participants, with and without psychopathological symptoms, were selected to participate in the NESDA study from primary mental health care and specialized mental health institutions. From this pool of subjects, almost three hundred subjects were included in the NESDA-fMRI study. Three main centers were involved in this project: University Medical Center Groningen, Amsterdam Medical Center and Leiden University Medical Center.

The structure and the composition of the present thesis can be summarized as follows: In order to determine if depressed or anxious patients have an impairment in the discrimination of emotional facial expressions, we review the studies on the recognition of facial expressions of emotion in patients with depression and anxiety disorders (Chapter 2). In Chapter 3, we investigate the neural correlates of implicit perception[5] of facial expression in patients with depression and anxiety disorders relative to healthy participants. Chapter 4 examines the neural mechanism of explicit emotional processing[6] during attribution of valence to words in depressed and anxious patients. The task used in Chapter 4 may be well defined as an *emotional cognitive* task [7].
In the second part of this thesis, we study the functional connectivity during perception of facial expressions and the effect of neuroticism on this functional connectivity in healthy participants (Chapter 5). We further examine, in Chapter 6, the brain mechanism of emotion perception involved in social phobia and panic disorder, not only looking at regional brain activity but also at the functional connectivity between areas involved in emotion processing. This approach may give a clearer picture of the neural mechanisms involved in anxiety disorders, as not only specific brain regions related to aberrant emotional processing are investigated but, additionally, the functional connectivity of the brain areas that are subserving the processing of emotional stimuli.
Finally, the last part of the present thesis provided a conclusion of the empirical findings presented in this thesis. Additionally a critical observation on the methods and suggestions for future research are proposed.

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[5] *Implicit emotional processing* is considered to be unconscious or without conscious awareness in processing of emotional stimuli and may be associated with increased activation in subcortical regions.

[6] *Explicit emotional processing* involves cognitive processes, such as attention, language processing – reading a word –, emotional evaluation, which may involved memory process.

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Chapter 2

Impaired attribution of emotion to facial expressions in anxiety and depression

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Abstract

Background: Recognition of others' emotions is an important aspect of interpersonal communication. In depression, a significant emotion recognition impairment has been reported. It remains unclear whether the ability to recognize emotion from facial expressions is also impaired in anxiety disorders. There is a need to review and integrate the published literature on emotional expression recognition in anxiety disorders and depression.
Method: A detailed literature search was used to identify studies on explicit emotion recognition in patients with anxiety disorders and depression compared to healthy participants. Eighteen studies provided sufficient information to be included. The differences on emotion recognition impairment between patients and controls (Cohen's d) with corresponding confidence intervals were computed for each study.
Results: Over all studies, adults with anxiety disorders had a significant impairment in emotion recognition (d = -0.35). In children with anxiety disorders no significant impairment of emotion recognition was found (d = -0.03). Depression was associated with an even larger impairment in recognition of facial expressions of emotion (d = -0.58).
Conclusion: Results from the current analysis support the hypothesis that adults with anxiety disorders or depression both have a deficit in recognizing facial expressions of emotion, and that this deficit is more pronounced in depression than in anxiety.

2.1 Introduction

The ability to identify and interpret facial expressions of emotion is essential in human communication and social interaction. Ekman and Friesen [1] concluded that six facial expressions are universal across cultures: happy, angry, sad, anxious, disgusted and surprised, and each of them is characterized by a particular facial muscular pattern.

Discrimination of emotion from facial expressions has been the focus of a number of psychological studies over the past decades, and was later complemented by neurobiological findings [2, 3]. The specific way in which an individual processes and interprets emotional information can be a causal factor in the development or maintenance of emotional disturbances. Studies in subjects with emotional disorders, such as depression and anxiety, aim to understand the relation between emotional processing and psychopathology.

Emotional dysfunctions (*e.g.*, difficulty in understanding emotions, difficulty in changing how one feels) are related to poor social functioning and can be considered as important features of psychopathology [4, 5]. Poor social and interpersonal relations could arise from a deficit in the ability to read signals of interpersonal threat or safety. Psychopathological variables may explain the variance in the accuracy of recognition of facial expressions of emotion.

Over the last two decades, research regarding emotional facial expressions in anxiety and depression has focused on two areas: attentional bias and the ability to recognize emotions, with much more consideration devoted to the former. Several types of cognitive bias have been described in social phobia, generalized anxiety disorder and panic disorder. Biases involve attention, judgment, interpretation, imagery and memory (for a review, see: Hirsch and Clark [6], Lang and Sarmiento [7], Clark and McManus [8]). Anxiety disorders have been associated with a selective attentional bias toward threatening stimuli, whereas in depression a selective attentional bias has been observed for negative emotional stimuli such as those related to sadness, loss and failure [9, 10].

The aim of the present review was to determine, by conducting a comprehensive meta-analytical synthesis of previous studies, the magnitude of the impairment in facial emotion recognition associated with anxiety disorders and depression. Anxious patients are thought to direct their attention toward threat-related stimuli and to avoid extended attention toward these stimuli [11]. Depression is characterized by negative affects and cognitive impairments [12], which may lead to difficulties in recognition of emotion from facial expressions. In light of this, we hypothesized that there would be a small difference for anxiety patients when compared to healthy controls in emotion recognition as they do not have substantial cognitive deficits. In contrast, we expected to find a larger impairment in emotion recognition in depressed patients.

2.2 Methods

2.2.1 Screening procedures and inclusion criteria

The Web of Science (ISI) and PubMed databases were searched for the period 1980 – 2009. The search was performed using "anxiety" and "depression" combined with "emotion", "facial expressions", "recognition", "discrimination", "labeling" as search terms. Additionally, more specific terms were used such as "social phobia" (SP), "generalized anxiety disorder" (GAD), "panic disorder" (PD), "posttraumatic stress disorder" (PTSD) and "major depressive disorder" (MDD).

173 studies on anxiety disorders and 208 studies on depression were identified. Subsequently, title and abstract of the articles were screened for possible inclusion in the analysis. The identified studies were included if they met the following criteria: the diagnosis of major depressive disorder or anxiety disorders was made according to the DSM-III or DSM-IV criteria. Secondly, each study had to deal with a group of adults or children experiencing depression or anxiety disorders and a control group. Third, behavioral measures of emotional facial expressions discrimination had to be reported with sufficient statistical information for the computation of the effect size (d-value). This implies that means and standard deviations, t-values or F-values and the relevant means, and exact p-values had to be reported. Lastly, only studies published in English were included.

2.2.2 Data analysis

For each study the effect size (Cohen's d) was calculated for the difference in emotion recognition performance between the patient group and the control group. The d was calculated as the difference between the two group means, patient group minus control group and divided by the pooled standard deviation [13]. When means and standard deviation were not given, d-values were computed from F-values or t-values. The effect size was computed using the program developed by D. Wilson (http://mason.gmu.edu/ dwilsonb/ma.html). The direction of the effect size was negative if the performance of the patient group in discrimination of facial expression was worse than the control group.

Table 2.1: Meta-analytic results for facial expressions of emotion discrimination in children with anxiety disorders, adults with anxiety disorders and adults with depression.

	k	*d*	95% CI	Z	p*	I^2	Q	p**
Anxiety - Children	5	-0.03 (0.08)	-0.30, 0.24	-0.21	0.831	0.00	2.99	0.56
Anxiety - Adults	5	-0.35 (-0.51)	-0.61, -0.10	-2.69	0.007	22.30	5.15	0.27
Depression - Adults	8	-0.58 (-0.42)	-0.79, -0.36	-5.17	0.000	33.75	10.57	0.16

k - number of studies included in the meta-analysis; *d* - effect size (estimated effect size after correction of publication bias in parentheses); CI - confidence intervals; p* - indicates the statistical significance of association Z; p** - indicates the significance of Q-statistic; I^2 - indicator of heterogeneity.

After computing the effect size for each study, a meta-analytic method was used (Comprehensive Meta-analysis program, www.meta-analysis.com). The combined effect size was calculated with the corresponding confidence intervals (95%) indicating the magnitude of the effect across all studies. The Z-values and p-values provide an indication of the statistical significance of the association. In addition, the *Q*-statistic was calculated [14] as an indicator of homogeneity. A significant *Q*-statistic points to heterogeneity of the effects across studies. As the *Q* test is reported to be susceptible to the number of studies included in the meta-analysis, the *I*-squared [15] was also calculated. *I*-squared is an index of heterogeneity describing the percentage of non-chance inconsistency. *I*-squared of 25% indicates low, 50% moderate and 75% high heterogeneity [15].
Publication bias was tested using the Duval and Tweedie's trim and fill, and by inspecting the funnel plot. The Duval and Tweedie's trim and fill is a nonparametric method which concerns a simple funnel plot-based method of testing and adjusting for publication bias in meta-analysis, by using the ranks of the absolute values of the observed effect sizes and the signs of those effect sizes around the global effect size [16].

2.3 Results

2.3.1 Search results

Out of 381 identified studies, twenty-eight studies were potentially eligible for inclusion based on screening of the title and abstract, and the full text version of each of these manuscripts was further evaluated. Six studies were excluded because of insufficient data needed to calculated the effect size [17, 18, 19, 20, 21, 22]. Two studies [23, 24] were excluded as they examined facial emotion recognition in non-clinical participants (high and low anxiety). One study [25] was excluded because it did not include a patient group. One study was excluded because of significant differences in age between the patient group and the control group [26]. Two studies were excluded because of methodological differences [27, 28]. Ten studies targeting facial expressions of emotion discrimination in anxiety disorders and eight studies in depression met our inclusion criteria. Characteristics of the included studies and data are provided in Table 2.2 for the studies on anxiety disorders and Table 2.3 for the studies on depression.

2.3.2 Meta-analysis results

The results of meta-analysis of emotion recognition in anxiety disorders and depression are displayed in Table 2.1. Results from the meta-analysis suggest that children with anxiety disorders do not have an impairment (p = 0.831, d = -0.03) of recognition of facial expressions of emotion (Table 2.1). The effect size after adjustment for possible publication bias using Duval and Tweedie's trim and fill method remained insignificant (d = 0.08, 95% confidence interval: -0.16 to 0.32). In adults with anxiety disorders the meta-analysis showed a significant impairment (p = 0.007) with a medium magnitude (d = -0.35, 95% confidence interval: -0.61 to -0.10) of facial emotion recognition. There was a low variability among the effect sizes (I^2 = 22.30). Correcting this for publication bias still resulted in a robust estimated effect size of d = -0.51 (95% confidence interval: -0.73 to -0.29). The overall effect size was larger than if we ignored a possible publication bias. More specifically, Duval and Tweedie's trim and fill suggested that there may be two missing studies with a negative effect (impaired emotion recognition in anxiety disorders compared to controls).
The meta-analysis of studies on depression showed significant impairment of emotion recognition with a medium overall effect size of d = -0.58 (p < 0.001). There was a moderate variability among the effect sizes (I^2 = 33.75, Q = 10.57, p = 0.16). Once corrected for publication bias the relationship between the impairment of emotion recognition and depression remained robust, although this overall effect size was reduced (d

= -0.42, 95% confidence interval: -0.62 to -0.23) relative to that estimated from the original data.

2.4 Discussion

The purpose of this study was to investigate whether and to what extent anxiety disorders and depression are associated with impaired recognition of emotion in others. The results from the current analysis support the hypothesis that adults with depression or anxiety disorders have an impaired recognition of facial expression of emotion, as substantiated by the medium effect size. This effect was not observed in children with anxiety disorders.

The present meta-analysis shows that children with anxiety disorders do not have an overall emotion recognition deficit, but the possibility of a mild emotion specific deficit cannot be discarded. This could not be investigated in the present meta-analysis because of a lack of data. Easter et al. [29] found that children with anxiety compared to controls had more difficulties interpreting the emotional expressions of adults than of children. They suggested that this deficit reflects a disorder-specific dysfunction.

Additionally, behavioral studies in children with anxiety suggested an emotion specific deficit. For example, Simonian et al. [30] using pictures of facial affect of adults portraying happiness, sadness, anger, fear, surprise and disgust reported that children with social phobia had difficulties relative to controls in identifying sadness, happiness and disgust. Ellis et al. [17] reported that children with anxiety disorders have difficulties in recognizing anger and disgust, and that on 11% of the occasions they misidentified anger as disgust. However, other studies did not find an emotion recognition deficit in children with anxiety disorders compared to healthy children [31, 18, 32, 19]. The inconsistencies between studies may be explained by sample characteristics, such as a variety of anxiety disorder diagnoses (*e.g.*, social or specific phobia, generalized anxiety disorder, separation anxiety or posttraumatic stress disorder), whereas others included children with only one diagnosis, such as social phobia [33, 30]. Another explanation for these discrepancies may be differences in the instruction to subjects, such as labeling the emotion depicted in a photograph versus recognition of the emotion named in the story read by the examiner, from a set of photographs [17], but also stimulus material used.

Furthermore, the present meta-analysis revealed a moderate impairment of facial emotion recognition in adults with anxiety disorders. The underlying mechanism for this impairment is unknown, but attentional biases might be involved. Indeed, an emotion specific impairment has been suggested in association with anxiety disorders. For example, Kessler et al. [34] and Mohlman et al. [35] found that socially anxious patients, as compared to healthy participants, had a tendency to misclassify neutral expressions as angry. On the other hand, a high sensitivity has been found in recognizing negative facial expression [36]. Surcinelli et al. [23] reported that non-clinical participants with a high trait anxiety have a better recognition of fearful faces. A possible explanation might be that anxious subjects have a negative bias, such that they misinterpret neutral expressions as displaying a negative emotion [37, 38, 39]. This impairment may also be triggered by the presence of emotional dysregulation in anxiety disorders [40]. As most studies in the present meta-analysis did not report emotion specific recognition scores, we were not able to systematically investigate possible biases.

Regarding depression, we also found a moderate overall emotion recognition impairment. Thus, patients with depression may be compromised in recognizing emotions of other people from facial expressions. This may contribute to social dysfunction, as it has been well established that emotion recognition contributes to proficient social functioning [41]. One explanation for this perceptual impairment might be the presence of cognitive deficits associated with depression, as suggested by Persad and Polivy [42]. Leppanen et al. [43] reported that depressed patients may have an impairment in recognizing neutral faces and a tendency to interpret happy faces as neutral, and interpreting this as a negative shift in emotion recognition [44, 45, 46]. Thus, deficits in recognition of facial expressions of emotion in depression may be determined by patients' negative emotional experience as well as by the assessment of their internal mood state. Depression is characterized by negative cognitions (worthlessness, self-criticism, hopelessness) and consequently their evaluation of external stimuli, including facial expressions, might be more negative than in healthy subjects [47, 4].

Emotion recognition has been shown to be related to social functioning [48]. Indeed, neuroimaging studies have reported that to a large extent regions involved in emotional processing are also part of the neural network responsible for social cognitive processes [49]. Keightley et al. [50] reported that prefrontal cortical structures, amygdala and inferior temporal cortex (fusiform gyrus) have a critical role in emotion recognition processes. At the neural level, anxiety disorders are associated with amygdala and insula hyperactivation during perception of threat-related emotions [51]. In depression abnormal cerebral blood

flow (CBF) has been shown in amygdala, anterior cingulate cortex (ACC), ventral striatum, anterior insula and prefrontal cortex [52, 53]. Thus, neuroimaging studies can offer an identification of pathological mechanisms associated with affective disorders.

Limitations Factors which might influence our results are the small sample size, differences in stimulus material, and duration of illness which may have an impact on the outcome of emotion recognition tasks and cognitive tests. A second limitation concerns the fact that there were differences in the degree of severity of the anxiety disorders, not all of them being clinical patients. Another limitation is that we could not distinguish between individual emotions, because most studies did not report adequately detailed data to permit such comparisons. It would be of interest to investigate whether depressed patients are selectively more impaired for certain expressions (*e.g.*, happy) than others (*e.g.*, sad), as was suggested by Surguladze et al. [22]. Medication use might have been a confounding variable because manipulation of the serotonin system, which is a common antidepressant treatment, produces specific alterations in the ability to recognize fear [54].

In summary, we reviewed behavioral studies indicating the relevance of facial expressions of emotion to anxiety disorders and depression. The present findings suggest a global deficit in recognition of different types of emotions, which was more pronounced in depression than in anxiety disorders. These emotion recognition deficits may contribute to compromised social functioning in these disorders.

Table 2.2: The characteristics of included studies in anxiety disorders.

Reference	Subjects	Mean age (years)	Psychopathological measures	Anxiety rating score	Characteristics of the task	Effect size: Cohen's *d* (95% CI)
Melfsen and Florin [33]	17 social anxiety; 15 controls	10.24; 10.07; Range: 8-12	SPAI-C	M (s.d.) = 25.72 (8.21); M (s.d.) = 5.23 (2.09)	72 pictures with neutral, positive (joyful) or negative (angry, disgusted, sad) facial expressions, black and white, half of them showing adults and half children (Matsumoto and Ekman, 1988).	0.06 (-0.43 to 0.55)
Simonian et al. [30]	15 social phobia; 14 controls	12.2; 11.0; Range: 9-15	SPAI-C	Score 18 (social phobia) Score 15 (control group)	36 slices from pictures of facial affect, black and white, consisting of adult faces, displaying six emotions (happiness, anger, sadness, fear, surprise and disgust).	-0.24 (-0.97 to 0.49)
Easter et al. [29]	15 anxiety disorder (11 met criteria for GAD, 8 with SP, 3 with SAD, 4 had comorbid major depression); 11 controls.	13.1; 12.5	Kiddie-Schedule for Affective Disorders and Schizophrenia for School Age Children (K-SADS-PL). Pediatric Anxiety Rating Scale.		Child facial expression and adult facial expressions subtests of the DANVA, consisting of 24 photographs of either children's or adults faces displaying happy, sad, angry and fearful.	-0.48 (-1.28 to 0.31)
Manassis and Young [32]	14 children with anxiety disorders; 10 healthy control children.	10.5; 10.4; Range: 8-12	Anxiety Disorders Interview Schedule for DSM-IV: Child and Parent versions (ADIS-C/P)		DANVA2: 24 item series of adult facial expressions depicting happiness, sadness, anger and fear.	-0.11 (-0.68 to 0.47)
Allen et al. [31]	20 children with anxiety disorders (GAD, separation anxiety disorder, specific phobia, PTSD); 19 control children.	9; 8.9; Range: 7-15	Revised Child Manifest Anxiety Scale (RCMAS); ADIS-C/P		Photographic images of facial emotion expressions depicting: surprise, anger, happiness, fear, disgust, sadness.	0.29 (-0.28 to 0.88)
Winton et al. [38]	13 anxious[1]; 11 controls[2]	20.6; 22.7	Fear of Negative Evaluation Scale (FNE), Social Avoidance and Distress scale (SADS), Beck Depression Inventory (BDI)	M(s.d.) = 22.5(3.6) on FNE, M(s.d.) = 8.5(8.2) on SADS, M(s.d.) = 8.0(5.2) on BDI; M(s.d.) = 5.3(2.5) on FNE, M(s.d.) = 2.3(2.7) on SADS, M(s.d.) = 3.2(4.1) on BDI.	40 slides of negative facial emotional expressions (anger, sadness, disgust, contempt, and fear) and 40 slides displaying neutral expressions (Matsumoto and Ekman, 1988).	-0.39 (-1.20 to 0.42)
Mohlman et al. [35]	26 GSAD (4 dysthymic disorder, 2 GAD, 2 panic disorder, 1 MDD, 6 specific phobias); 26 controls.	21.46; 21.08	FNE, Social Phobia Scale (SPS, Mattick and Clarke, 1998), State Trait Anxiety Inventory (STAI), BDI	GSAD criteria score above 20 on FNE, Controls scored below 9 on FNE.	Facial expressions depicted: neutral, happy, sad and angry at different affective intensities (25%, 50% and 100%)	-0.22 (-0.76 to 0.32)
Kessler et al. [34]	37 PD outpatients; 43 controls.	37.8; 36.4	State Trait Anxiety Inventory (STAI), BDI.		FEEL test, portrait pictures, conditions: anger, sadness, disgust, happiness, fear, surprise (Kessler et al., 2002).	-0.74 (-1.20 to -0.30)
Corcoran et al. [55]	36 PD; 36 controls. The patients were recruited from anxiety disorder specialty clinics.	34	Anxiety Disorders Interview Schedule for DSM-IV; Structured Clinical Interview for DSM-IV; Yale-Brown Obsessive-Compulsive Scale; BDI.		Black and white photographs depicting anger, disgust, fear and sadness (Ekman and Friesen, 1979).	0.04 (-0.54 to 0.62)

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Table 2.2: The characteristics of included studies in anxiety disorders.

Reference	Subjects	Mean age (years)	Psychopathological measures	Anxiety rating score	Characteristics of the task	Effect size: Cohen's *d* (95% CI)
Campbell et al. [56]	N = 12 generalized social phobia (GSP); N = 28 healthy controls (HC)	31.9; 30.4	Liebowitz Social Anxiety Scale (LSAS); State-Trait Anxiety Inventory (STAI); BDI	GSP: M(s.d.) = 90.6 (26.4) on LSAS, M(s.d.) = 45.8 (11.4) on STAI and M(s.d.) = 15.8 (10.6) on BDI; HC: M(s.d.) = 11.5 (10.8) on LSAS, M(s.d.) = 22.6 (4.3) on STAI and M(s.d.) = 1.8 (2.5) on BDI	24 emotional faces selected from Matsumoto and Ekman (1988) set depicting: happiness, disgust and anger.	-0.19 (-0.87 to 0.49)

[1] high score on the Fear of Negative Evaluation Scale (FNE); [2] low score on the FNE; SPAI-C – Social Phobia and Anxiety Inventory for Children, EPQ-J – Extraversion Scale of the Junior Eysenck Personality Questionnaire; DANVA – Diagnostic Analysis of Nonverbal Accuracy; GAD –Generalized Anxiety Disorder; GSAD – Generalized Social Anxiety Disorder; PD – panic disorder; FEEL test – Facially Expressed Emotion Labeling;

Table 2.3: The characteristics of included studies on major depressive disorder.

Reference	Subjects	Mean age (years)	Psychopathological measures	Depression ratings score	Characteristics of the task	Effect size: Cohen's *d* (95% CI)
Rubinow and Post [57]	17 inpatients (7 bipolar I, 5 bipolar II, 5 unipolar), 31 controls.	39; 31	Bunney-Hamburg Depression Scale (B-HDS), Research Diagnostic Criteria (Spitzer et al, 1978)	7 on B-HDS in inpatients	48 photographs of faces: sad, fearful, happy, angry, disgusted, surprised and interested (Ekman et al., 1973).	-1.01 (-1.64 to -0.38)
Leppanen et al. [43]	18 depressed patients, 18 controls	45.1; 44.7; Range: 23-59	BDI; Positive and Negative Affect Scale.	M(s.d.) = 36.8 (9.6) on BDI in depressed subjects, M(s.d.) = 11.1. (8.4) on BDI in controls; M(s.d.) = 25.2 (7.0) on positive affect and 19.7 (9.2) on negative affect in depression, M(s.d.) = 31.7 (5.0) on positive and 11.8 (2.6) on negative affect in controls.	96 trials. Male and female models with happy, sad and neutral expressions selected from Ekman and Friesen (1976).	-1.00 (-1.70 to -0.31)
Zuroff and Colussy [46]	15 depressed inpatients (7 dystihymic disorder, 5 MDD, 3 adjustment disorder with depressed mood), 15 controls	37	BDI, D-30 scale from the MMPI	M = 14 on BDI and M = 79.5 on D-30 in depression; M = 1.8 on BDI and M = 44.8 on D-30 in controls.	32 black and white prints of adult male and female faces (Izard, 1971). Eight emotions: happiness, anger, surprise, disgust, shame, fear, sadness and interest.	-0.70 (-1.44 to 0.04)
Persad and Polivy [42]	16 DCS[1] , 16 NDCS[2], 16 DPP[3].	26.50; Range: 18-53.	BDI	M = 16.19 on BDI in DCS, M = 3.75 on BDI in NDCS. -diagnosis of MDD on Axis I, according to DSM-III, a cutoff score of 22 or higher on BDI in DPP.	Facial affective booklet, consisting of a set of 14 photographed facial expressions developed by Ekman (1976); expressed emotions: fear, anger, surprise, contempt, happiness, sadness and indifference.	-0.62 (-1.36 to 0.13)
Hale [47]	48 depressed subjects (28 outpatients received antidepressant medication, 15 received benzodiazepine medication); 48 controls.	38; 41; Range: 20-69.	BDI	M(s.d.) = 27.8 (7.1) on BDI in depression., M(s.d.) = 2.7 (2.6) on BDI in control group.	12 schematic facial expressions.	-0.52 (-0.93 to -0.11)
Archer et al. [58]	12 depressed inpatients; 12 controls	56.19; 48.04	Hospital diagnosis criteria based on the DSM-III Manual.		Facial expressions of emotion (Ekman and Friesen, 1976) depicted: happy, sad, frightens, angry, surprised, and disgust. Emotions were presented in pairs, subject had to indicate which of the two faces was expressing the target word presented on the screen.	-0.52 (-1.10 to 0.06)
Surguladze et al. [22]	27 depressed inpatients and outpatients; 29 controls.	46.9; 43	BDI ; Hamilton Depression Rating Scale (HAMD).	M(s.d.) = 33 (9.9) depressed patients and M(s.d.) = 3.1 (3.5) controls on BDI; M(s.d.) = 16.9 (5.5) depressed patients on HAMD.	10 facial expressions from standardized series Young et al., (2002), displaying happy, sad and neutral expressions.	-1.21 (-2.47 to 0.05)

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Table 2.3: The characteristics of included studies on major depressive disorder.

Reference	Subjects	Mean age (years)	Psychopathological measures	Depression ratings score	Characteristics of the task	Effect size: Cohen's *d* (95% CI)
Kan et al. [59]	16 depressed inpatients; 20 controls	50.9; 59.0	Hamilton Depression Rating Scale (HDRS); Zung self rating depression scale (SDS)	M(s.d.) = 18.3 (8.64) on HDRS and M(s.d.) = 60.0 (15.3) on SDS in depressed patients.	Videotaped facial expression from neutral to emotion to neutral. Six basic emotions: happy, sad, angry, fearful, surprise and disgust were presented.	0.15 (-0.43 to 0.73)

[1]DCS – depressed college students, [2]NDCS – nondepressed college students, [3]DPP – depressed psychiatric patients.

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Chapter 3

Neural correlates of perception of emotional facial expressions in outpatients with mild-to-moderate depression and anxiety

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Abstract

Background: Depression has been associated with limbic hyperactivation and frontal hypoactivation in response to negative facial stimuli. Anxiety disorders have also been associated with increased activation of emotional structures such as amygdala and insula. This study examined to which extent activation of brain regions involved in perception of emotional faces is specific to depression and anxiety disorders in a large community-based sample of outpatients.
Methods: An event-related functional magnetic resonance imaging paradigm was employed including angry, fearful, sad, happy and neutral facial expressions. One hundred eighty-two outpatients (59 depressed, 57 anxiety and 66 comorbid depression-anxiety) and 56 healthy controls selected from the Netherlands Study of Depression and Anxiety were included in the present study. Whole-brain analyses were conducted. In addition, the temporal profile of the amygdala activation was investigated.
Results: Facial expressions relative to scrambled faces activated the amygdala and fusiform gyrus in depressed patients with or without anxiety and healthy controls, but this was less evident in patients with anxiety disorders. Response shape of the amygdala did not differ between groups. Depressed patients showed dorsolateral prefrontal cortex hyperactivation in response to happy faces, compared to healthy controls.
Conclusion: We suggest that this may reflect increased attention to mood-incongruent stimuli. The lack of strong differences in neural activation to negative emotional faces, relative to healthy controls, may be characteristic of the mild-to-moderate severity of illness in this sample and may be indicative of a certain cognitive-emotional processing reserve.

3.1 Introduction

Facial expressions are essential for social communication for they provide information concerning emotional states and intentions of others. Depression has been considered a disorder of emotion and its regulation [1]. A high prevalence of depressive and anxiety disorders has been observed in primary care settings [2]. Moreover, 63 % of patients with panic disorder and 35 % of patients with social phobia were reported to have at least one episode of major depression [3].
Both depression and anxiety disorders have been associated with neurophysiological abnormalities regarding emotion perception [4, 5]. In depressed patients, stronger amygdala activation has been reported in response to negative emotional stimuli [6, 7, 8, 9]. In addition, it has been suggested that depressed patients have a sustained amygdala response during processing of negative emotional stimuli, compared to healthy volunteers [10]. In response to positive stimuli ventral striatum hypoactivation was reported in depressed patients compared to controls [11]. The authors suggested that the lack of ventral striatum activation may reflect anhedonia, *i.e.*, the reduced capacity to experience pleasure [11].
With regard to anxiety disorders, amygdala hyper-responsiveness was reported to negative facial expressions [12, 13, 14], whereas other studies reported amygdala hyperactivation to neutral [15, 16] or positive [17] facial expressions. Therefore, it is not clear if amygdala hyper-responsiveness in anxiety disorders is specific to threat-related stimuli or also to positive and ambiguous facial expressions.
In addition to these abnormalities observed during emotion processing, Mayberg [18] has hypothesized that hypoactivation in dorsal neocortical areas (anterior and posterior cingulate, prefrontal, premotor and parietal cortex) and hyperactivation in ventral paralimbic areas (subgenual cingulate, anterior insula, hypothalamus and caudate) may characterise depression. Phillips et al. [19] proposed a model that is consistent with Mayberg [18], but which is more comprehensive, involving deficient cortico-limbic interactions in depression. Specifically, depression is assumed to be associated with hyperactivation of limbic regions responsible for emotion identication and generation of emotional behavior, including subgenual cingulate gyrus, ventrolateral prefrontal cortex (PFC), amygdala, anterior insula, ventral striatum and thalamus, and hypoactivation of dorsal regions, important for emotion regulation, including dorsomedial and dorsolateral prefrontal cortices [19]. In anxiety disorders, Etkin and Wager [14] concluded that hyperactivation of the amygdala and insula may constitute a common pathway in social anxiety disorders and specic phobia. Emotion dependent abnormal amygdala activation has thus been reported in both depression and anxiety disorders, although mainly in reaction to syndrome-specific emotional stimuli. Taken together, it appears that there are both distinct and common neural substrates underlying processing of various emotional information in depression and anxiety disorders. We would therefore expect that the presence of depression and anxiety diagnoses have a differential impact on the neural response to emotional stimuli. However, no study to date has focused on emotion processing in depression and anxiety, while explicitly controlling for their comorbidity.
The present study is part of the Netherlands Study of Depression and Anxiety (NESDA), a multisite cohort study aimed to provide an insight into the long-term course of depression and anxiety disorders in patients selected from the general practices and the mental health organisations [20]. Hence, the aim of the present study was to identify the areas involved in perception of facial expressions of emotion in large community-based samples of outpatients with depression, anxiety and depression-anxiety comorbidity relative to healthy participants. We further tested for differences in the temporal amygdala response to facial expressions between groups. Outpatients with anxiety-depression comorbidity were included in order to investigate the possible implications of comorbidity on the neural mechanisms involved in emotion perception.
Based on the literature, we hypothesised amygdala and ventral anterior cingulate cortex (ACC) hyperactivation in response to negative emotional expressions in depressed outpatients compared with healthy controls. In response to happy faces we expected ventral striatum hypoactivation in depressed outpatients compared to healthy controls. In anxiety disorders, amygdala hyperactivation was expected in response to angry, fearful and neutral faces.

3.2 Methods

3.2.1 Participants

The present work is a multicentre study, which involved University Medical Center Groningen (UMCG), Amsterdam Medical Center (AMC) and Leiden University Medical Center (LUMC). This study was approved by the Ethical Review Boards of each participating centre. Participants were selected from the Netherlands Study of Depression and Anxiety (NESDA, Penninx et al. [20]). After receiving written information, each participant gave written informed consent. Participants did

not receive any compensation for their participation in this study.

Exclusion criteria – a diagnosis of other Diagnostic and Statistical Manual of Mental Disorders-IV [21] axis I disorders than major depression, social phobia and panic disorder and generalised anxiety disorder such as psychotic disorder or dementia, current alcohol or substance abuse, presence or history of major internal and neurological disorder with potential central nervous system sequelae; current use of beta-blockers; hypertension >180/130 mm Hg; age over 57 years; and MRI incompatible implants or tattoos.

We included 68 outpatients with major depressive disorder (MDD), 61 outpatients with anxiety disorders (Anx) – with panic disorder with/without agoraphobia, generalised anxiety disorder and/or social phobia –, 78 outpatients with depression-anxiety comorbidity (DAC) and 60 healthy controls (HC). All diagnoses were made prior to the scanning session by trained clinical staff on the basis of Composite International Diagnosis Interview - lifetime version 2.1 - [22], in accordance with DSM-IV criteria. The HC had never met the criteria for any DSM-IV disorder. Functional MRI data from 4 Anx patients, 9 MDD patients, 12 DAC patients and 4 HC participants were discarded because of technical problems during scanning, *e.g.*, head movement artifacts (> 3 mm on any axis) or incomplete coverage of the temporal lobe. Table 1 represents the demographic characteristics of the samples. The groups were matched on age ($F[3,234]=1.71$, $p=0.15$), gender ($\chi^2[3]=2.94$, $p=0.40$) and handedness ($\chi^2[3]=0.08$, $p=0.99$), but not on years of education ($F[3,234)=13$, $p<0.05$). Post-hoc tests using Bonferroni correction ($\alpha_{crit}=0.0167$) indicated that HC had signicantly longer education than MDD ($t[113]=4.76$, $p<0.001$), Anx ($t[111]=3.45$, $p=0.001$) and DAC ($t[120]=6.322$, $p<0.001$) patients.

Fifty-four patients were using selective serotonin reuptake inhibitors: citalopram 20-60 mg (16 patients), paroxetine 20 mg (30 patients), sertraline 50 mg (two patients), fluoxetine 20 mg (three patients) and fluvoxamine 50-100 mg (three patients). Ten patients used the serotonin norepinephrine reuptake inhibitor venlafaxine 75-225 mg. Three patients used benzodiazepines infrequently (3 times 2 tablets weekly, or within 48 hours prior to the scanning): oxazepam 40 mg (two patients) and diazepam 20 mg (one patient).

At the day of scanning, before the scanning session, all participants were evaluated by means of a battery of standardised questionnaires and structured interviews: Montgomery-Åsberg Depression Rating Scale (MADRS; [23]), Beck Anxiety Inventory (BAI; [24]) and Fear Questionnaire (FQ; [25]).

3.2.2 Faces paradigm

The paradigm employed in the present study was based on the event-related emotional paradigm used by Wolfensberger et al. [26]. Color photographs of angry, fearful, sad, happy, and neutral facial expressions, as well as a control condition consisting of scrambled faces were presented to all participants. The photographs were selected from the Karolinska Directed Emotional Faces System [27], representing standardised facial expressions of emotions expressed by amateur actors. Twenty-four stimuli were selected for each of five facial expressions, comprising twelve female and twelve male faces. Each particular face was not presented more than four times. The control condition (scrambled faces) was presented eighty times. The experimental paradigm was presented using E-prime software (Psychological Software Tools, Pittsburgh, PA, USA). In order to reduce anticipatory effects, an event-related design was employed. This entailed a pseudo-random presentation of a total of 200 stimuli against a black background. Each photograph was shown on the screen for 2.5 s, with an interstimulus (black screen) interval varying between 0.5 and 1.5 s. The images were projected onto a translucent screen at the end of the scanner bed, visible via a mirror above the participants head. All participants were instructed to indicate each face's gender by pressing one of two buttons with the index finger of the left or right hand on two magnet-compatible button boxes. During the presentation of scrambled faces, participants had to press left or right buttons in conformity with the instruction presented on the screen, *i.e.*, an arrow pointing to the left or to the right. The reaction time was recorded. The Faces paradigm was administered as part of a functional scanning session, involving a planning task, a memory task and a resting state scan, the results of which will be reported elsewhere.

3.2.3 MRI data acquisition

Images were acquired on a Philips 3T MR-scanner. SENSE-8 (UMCG and LUMC) and SENSE-6 (AMC) channel head coils were used for radio frequency transmission and reception. For each participant a series of 310 echo planar imaging (EPI) volumes sensitive to blood oxygenation level dependent effect were obtained, entailing a T2*-weighted gradient echo sequence (repetition time [TR]=2300 ms, echo time [TE]=28.0 ms at UMCG and TE=30.0 ms at AMC and LUMC, flip angle 90) using axial whole-brain acquisition, with an interleaved slice acquisition order. The EPI volumes were acquired at 39 slices at UMCG and 35 slices at AMC and LUMC (0 mm gap, 3

mm thickness). The matrix sizes were: 64x64 voxels at UMCG and 96x96 voxels at AMC and LUMC. The in-plane resolution was 3x3 mm at UMCG and 2.29x2.29 mm at AMC and LUMC. The images were acquired parallel to the anterior-posterior commissure plane. A T1-weighted anatomical MRI was also acquired for each subject (TR=9 ms, TE=3.5 ms, matrix size 256x256, voxel size 1x1x1 mm).

3.2.4 Data analysis

The behavioral data were analysed in SPSSv.16.0 (SPSS Inc., Chicago, Il) to test for an effect of group on reaction time employing an analysis of variance (ANOVA). In case a signicant effect was identied, post-hoc tests were conducted using Bonferroni correction.
Functional imaging data were pre-processed and analysed using Statistical Parametric Mapping software (SPM5) implemented in Matlab v.7.1.0 (The MathWorks Inc., MA, USA). Before pre-processing, manual origin setting was performed to the anterior commissure on the EPI volumes. Temporal and spatial correction of the data included slice timing correction, spatial realignment to the first image, co-registration between the anatomical and mean EPI images, spatial normalisation to the standard Montreal Neurological Institute (MNI), resampling into a 3x3x3 mm grid, and spatial smoothing using a Gaussian kernel (8 mm full-width at half-maximum).
In order to remove low-frequency temporal noise, a high-pass filter was applied, with a cut-off of 128 s, to the fMRI time-series. A canonical haemodynamic response function (HRF), with the temporal derivative (TD) and the dispersion derivative (DD) [28] was used in a general linear model and parameter estimates were generated for each voxel, for each condition. For each subject, weighted contrasts were computed (angry>scrambled, fearful>scrambled, sad>scrambled, happy>scrambled and neutral>scrambled).
A five (conditions) by four (groups) repeated measures analysis of covariance (ANCOVA) was conducted on weighted contrasts generated at single-subject level, with centers, age and education (years) added as nuisance factors. The main effect of condition is reported at a threshold of $p<0.05$ corrected for Family Wise Error (FWE), and groups differences were inspected at $p<0.001$ (uncorrected) and the cluster surviving $p<0.05$ corrected are reported.
In order to test for effects of medication on the neural response to facial expressions, further analysis was performed excluding medicated patients. A total of 176 participants were included in this analysis (56 HC, 45 MDD, 39 Anx and 36 DAC) employing a five by four repeated measure ANCOVA as described earlier. An additional ANCOVA was performed to test for differences between 62 medicated (14 MDD, 18 Anx and 30 DAC) and 120 unmedicated patients.
To identify brain regions associated with illness severity, regression analyses were performed within each group of patients using MADRS, BAI and FQ scores as regressors.
The temporal prole of amygdala activation was investigated employing a region of interest (ROI) approach, using an amygdala anatomical mask [29]. Beta values (HRF, TD and DD), for each subject, within each region, were extracted using MarsBar [30]. The mean and standard deviation of the haemodynamic response shape within each group and for each condition was reconstructed and plotted for visual inspection. Further, for each subject and for each response curve the maximum amplitude and the corresponding time point of the peak amygdala response were calculated in the haemodynamic response function and imported into SPSSv16.0. Group effects on these parameters were investigated with non-parametric tests (Kruskal-Wallis [H] and Mann-Whitney [U] as post-hoc test).

3.3 Results

3.3.1 Characteristics of the groups

Results of demographic and psychometric assessments of the participants are shown in Table 5.1. A signicant group effect was present for MADRS ($F[3,231]=58.02$, $p<0.001$), BAI ($F[3,209]=52.01$, $p<0.001$) and FQ ($F[3,223]=37.01$, $p<0.001$) scores. Post-hoc tests using Bonferroni correction ($\alpha_{crit}=0.0167$) indicated that DAC patients scored signicantly higher on MADRS compared to MDD ($t[122]=5.47$, $p<0.001$) and Anx ($t[119]=5.54$, $p<0.001$). Depressed patients had mild-to-moderate depressive symptoms (MADRS score between 9 and 34; [31]). Anx patients showed greater anxiety severity compared with MDD (BAI: $t[112]=3.93$, $p<0.001$, FQ: $t[107]=4.64$, $p<0.001$). Patients with DAC scored signicantly higher on BAI and FQ compared with Anx (BAI: $t[119]=2.54$, $p=0.012$) and MDD (BAI: $t[121]=7.58$, $p<0.001$, FQ: $t[118]=5.40$, $p<0.001$).
No group effect was found on reaction time during presentation of angry ($F[3,5492]=0.411$, $p=0.745$), fearful ($F[3,5481]=0.656$, $p=0.579$), happy ($F[3,5461]=1.206$, $p=0.306$), neutral ($F[3,5458]=1.802$, $p=0.144$) and sad facial expressions ($F[3,5491]=0.773$, $p=0.509$). The mean reaction times for each condition within each group are presented in Table 3.2.

Table 3.1: Demographic and clinical characteristics of the groups (n represents the number of participants). HC – healthy controls, MDD – major depression, Anx – anxiety disorder, DAC – depression-anxiety comorbidity. Mean (and standard deviation) are presented.

Groups	Age (SD)	Education years (SD)	Right-Handed (%)	Female (%)	SSRIs users (%)	BAI (SD)	FQ (SD)	MADRS (SD)	AgeOnset MD (SD)	AgeOnset ANX (SD)
HC (n=56)	39.75 (9.67)	14.68 (2.65)	91.1	60.7	0	2.32 (2.73)	9.65 (7.61)	1.36 (2.43)	-	-
MDD (n=59)	36.24 (10.79)	12.29 (2.72)	89.8	66.1	23.7	7.81 (6.15)	20.26 (13.01)	11.16 (8.66)	24.93 (10.47)	-
Anx (n=57)	35.74 (9.44)	12.74 (3.28)	91.2	75.4	31.6	13.86 (9.89)	35.21 (20.13)	10.89 (8.74)	-	18.16 (10.67)
DAC (n=66)	36.42 (11.25)	11.47 (2.91)	90.9	65.2	45.5	18.14 (8.85)	36.79 (19.49)	19.55 (8.38)	23.88 (11.8)	18.11 (10.61)

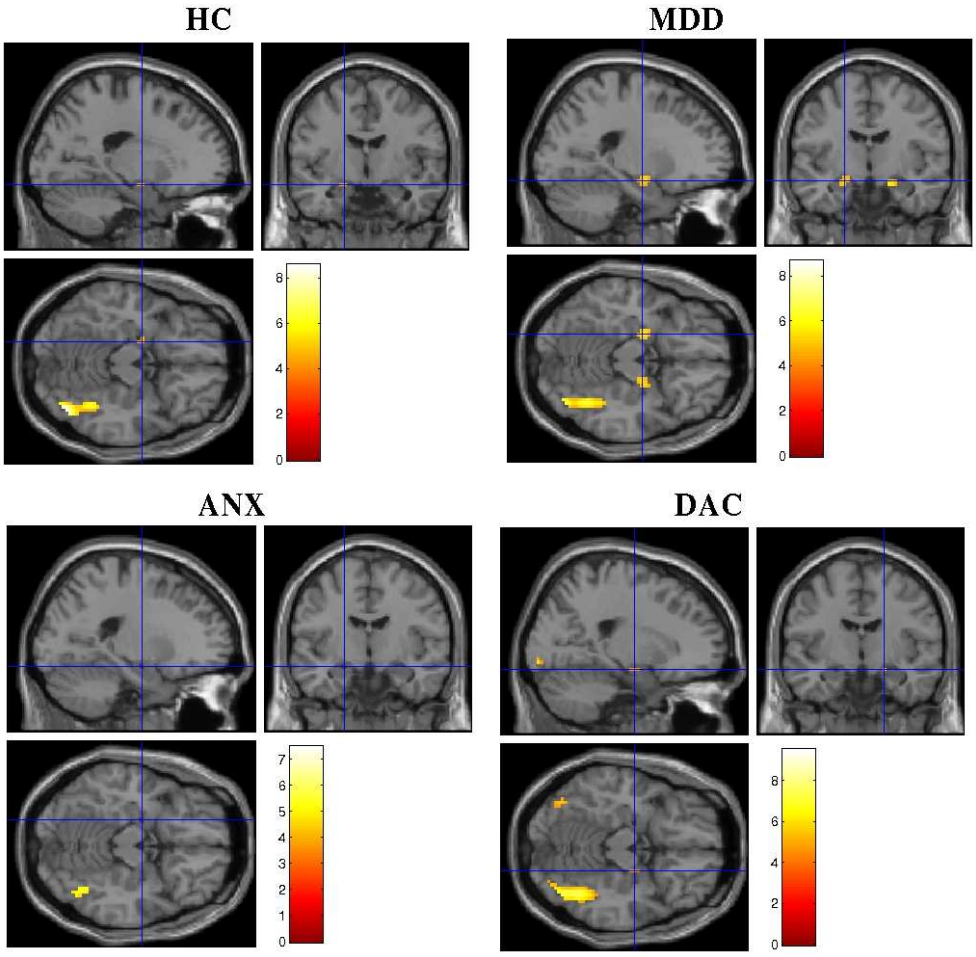

Figure 3.1: Main effect of viewing photographs of faces (>scrambled) within each group ($p<0.05$ FWE). Main activations were in the fusiform gyrus and amygdala. Color bar indicates t-value. HC – healthy, MDD – major depression, Anx – anxiety disorder, DAC – depression-anxiety comorbidity. See also table Table A.1.

Table 3.3: Anatomical regions showing significant between-group differences in activation in response to facial expressions. MNI coordinates, p<0.001 uncorrected. R – right hemisphere, L – left hemisphere, k – cluster size in voxels.

Group	Condition	Region	Side	Coordinate			Z-value	k	p corrected
				x	y	z			cluster–level
MDD>HC	Happy > scrambled	Superior Frontal gyrus (BA10)	R	21	51	3	4.70	60	0.033
		Middle Frontal gyrus (BA 9)	R	27	36	30	5.90	264	<0.005
	Neutral > scrambled	Posterior Cingulate	L	-12	-60	9	4.51	66	0.024
HC>Anx	Happy > scrambled	Middle Temporal gyrus	R	48	-66	6	4.13	90	0.007
		Globus Pallidus	R	12	0	0	4.44	57	0.040
		Putamen	R	21	3	3	4.39		

Note: : BA - Brodmann area; ACC - Anterior Cingulate Cortex; Side: L - left, R - right; HC healthy, MDD - major depression, Anx - anxiety disorder, DAC – depression-anxiety comorbidity.

Table 3.2: Behavioral data: mean reaction time (and standard deviation) in msec.

Groups	Reaction time				
	angry	fearful	happy	neutral	sad
HC	731.79	768.30	758.07	787.31	761.61
(n=56)	(283.19)	(325.03)	(305.57)	(335.40)	(310.34)
MDD	732.96	774.93	763.70	770.81	768.63
(n=59)	(286.78)	(317.36)	(318.24)	(321.48)	(305.14)
Anx	741.91	761.86	758.84	759.66	750.76
(n=57)	(296.16)	(324.64)	(307.57)	(312.42)	(306.95)
DAC	740.09	759.23	777.64	765.98	761.08
(n=66)	(295.37)	(319.59)	(319.80)	(319.10)	(300.52)

Note: HC – healthy, MDD – major depression, Anx – anxiety disorder, DAC – depression-anxiety comorbidity.

3.3.2 Imaging data

Main effect of task within group – Viewing facial expressions (>scrambled faces) elicited fusiform gyrus activation within each group of participants (p<0.05, FWE corrected). Amygdala activation to facial expressions (>scrambled) was found in MDD, DAC patients and HC (p<0.05, FWE corrected, Figure 3.1). In anxiety patients, amygdala activation to facial expressions (>scrambled) was not found at p<0.05 FWE corrected, but was present at uncorrected p<0.005 (right Z=2.59, left Z=2.64).

Full details (coordinates and Z-values) of group activation maps to facial expressions (>scrambled) are presented in the Appendix (Table A.1).

Condition by group interaction – A significant group by condition interaction effect was found in the right dorsal PFC extending to anterior cingulate cortex (x=27, y=39, z=30, Z=5.54) and left Rolandic operculum (x=-39, y=-6, z=15, Z=3.66).

Between-group comparisons – No significant differences in the neural response to negative (angry/fearful or sad) faces versus scrambled were found between MDD and HC. Patients with MDD showed right superior frontal gyrus extending into middle frontal gyrus hyperactivation in response to happy (>scrambled) faces, compared with HC (Figure 3.2, Table 3.3). In response to neutral (>scrambled) faces, greater left posterior cingulate cortex (PCC) activation was found in MDD compared to HC (Table 3.3).

Anx patients showed right lentiform nucleus hypoactivation to happy>scrambled faces, compared to HC (Figure 3.3, Table 3.3), whereas no significant differences in activation to angry, fearful, happy or neutral (>scrambled) faces were observed in Anx compared to HC.

Like Anx, DAC patients did not show significant differences in the neural response to any facial expressions (>scrambled) compared to HC.

Medication effects

After excluding medicated patients, the analysis of demographic and clinical characteristics showed a significant group effect on age (F[3,172]=2.85, p<0.05), years of education (F[3,172]= 8.97, p<0.05), MADRS (F[3,170]=37.55, p<0.005), BAI (F[3,172]=19.56, p<0.005) and FQ (F[3,172]=16.97, p<0.005). No significant group effect was found on gender (χ^2[3]=3.13, p=0.37) or handedness (χ^2[3]=0.87, p=0.83). In the Appendix (Table A.3) psychometric measures of medicated and unmedicated patient groups are presented.

Unmedicated MDD patients showed right middle frontal gyrus extending into cingulate cortex (BA32) hyperactivation to happy>scrambled faces relative to HC (Table 3.4). In response to neutral>scrambled faces greater right medial frontal gyrus activation was found in unmedicated MDD patients relative to HC. No significant differences in activation to negative (>scrambled) faces were found between MDD and HC.

No significant differences in the neural response to any facial expression (>scrambled) were found between unmedicated Anx and HC.

Unmedicated DAC patients showed right ACC hyper-

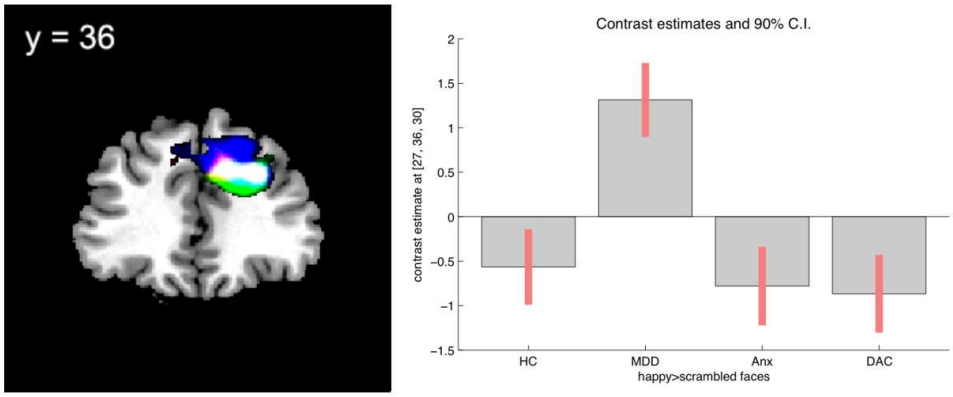

Figure 3.2: Right frontal cortex activation to happy facial expressions (>scrambled) in MDD compared to HC (red)/Anx (blue)/DAC (green). White represents the overlapping of the clusters. HC - healthy, MDD - major depression, Anx - anxiety disorder, DAC - depression-anxiety comorbidity.

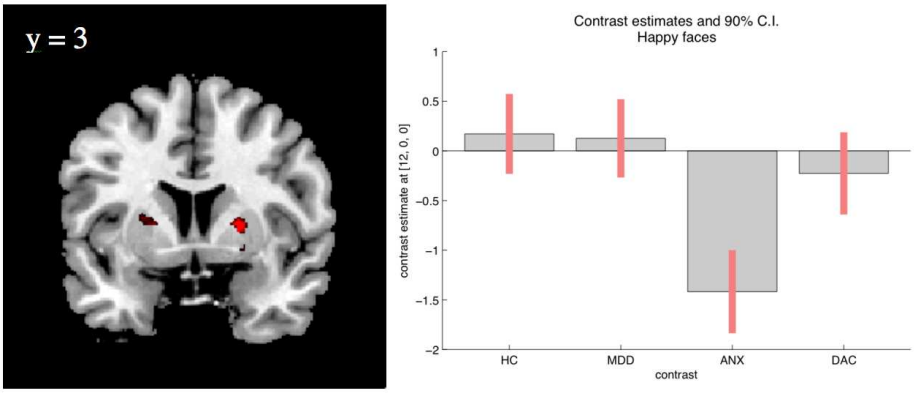

Figure 3.3: Increased right putamen activation to happy facial expressions (>scrambled) in HC compared to Anx outpatients ($p<0.001$ uncorrected with an extended threshold of 50 continuous voxels). HC - healthy, MDD - major depression, Anx - anxiety disorder, DAC - depression-anxiety comorbidity.

Table 3.4: Anatomical regions showing significant difference in activation, in response to facial expressions between unmedicated and medicated patients, and HC. MNI coordinates, $p < 0.001$ uncorrected. R – right hemisphere, L – left hemisphere, k – cluster size in voxels.

Group	Condition	Region	Side	Coordinate			Z-value	k	p corrected
				x	y	z			cluster–level
unmed MDD > HC	Happy > scrambled	Middle Frontal gyrus (BA 9)	R	27	36	30	5.56	217	<0.005
		Cingulate cortex	R	12	33	30	4.75		
		Inferior Parietal lobule (BA 40)	L	-42	-51	39	3.83	71	0.019
		Insula (BA 13)	R	36	3	15	3.78	71	0.019
	Neutral > scrambled	Medial Frontal gyrus (BA 9)	R	12	39	33	3.62	64	0.028
unmed DAC > HC	Happy > scrambled	ACC	R	9	33	9	5.24	61	0.033
unmed > med MDD	Happy > scrambled	Precentral gyrus (BA 6)	L	-42	-9	33	4.61	171	<0.005
med > unmed Anx	Happy > scrambled	Postcentral gyrus (BA 43)	L	-51	-15	15	4.06	59	0.044
med > unmed DAC	Happy > scrambled	Medial Frontal gyrus (BA 8)	L	-12	24	45	4.04	65	0.032

Note: : BA – Brodmann area; ACC – Anterior Cingulate Cortex; Side: L – left, R – right; HC – healthy, MDD – major depression, Anx – anxiety disorder, DAC – depression-anxiety comorbidity.

activation in response to happy faces, compared to HC (Table 3.4).

Unmedicated versus medicated outpatients – Medicated patients did not differ on depression and anxiety severity from unmedicated patients ($p<0.05$, Table A.3). Differences in the neural response to facial expressions between medicated and unmedicated patients are presented in Table 3.4.

Correlations of activation with illness severity

No significant correlation was found between amygdala activation and illness severity in patients groups. However, we did observe a significant correlation between left fusiform gyrus activation to angry and fearful (>scrambled) faces and depression severity in MDD patients. In the Appendix (Table A.2) are listed the regions showing significant correlation with illness severity.

Amygdala response shape

Figure 3.4 displays the response shape of the amygdala (mean and standard deviation) during viewing facial expressions for each group. There was no group effect on left or right amygdala amplitude and on the time of the maximum peak (all p values >0.05).

3.4 Discussion

In the present study, we examined neural responses during implicit emotion processing in a large number of outpatients diagnosed with MDD, Anx and DAC disorders. Fusiform gyrus activation to facial expressions (>scrambled) was found within each group. Amygdala activation was found to all facial expressions (>scrambled) in MDD, DAC and HC. Thus, a common neural network was implied in the perception of facial expressions across all groups. All these regions have been previously reported to be involved in processing facial expressions of emotions [32, 33, 34].

In contrast to our expectations and to some of the previous studies, which reported amygdala hyperactivation to negative emotional stimuli in patients with major depression [7, 6, 8] and anxiety [4, 35], in our study we failed to observe significant differences in amygdala response to facial expressions in outpatients with depression and/or anxiety relative to HC. This was the case for both medicated and unmedicated outpatients. Thus, although antidepressant medication has been shown to dampen the putatively excessive activation of the amygdala in depression [36, 8, 37] and anxiety disorders [38, 39], medication status could not explain the lack of group differences in amygdala activation in the present study.

However, our results are in agreement with previous findings in depression which suggest that there is no amygdala difference in depressed patients compared to HC: Gotlib et al. [40], Lawrence et al. [41], Almeida et al. [42], Lee et al. [43], Norbury et al. [44]. A

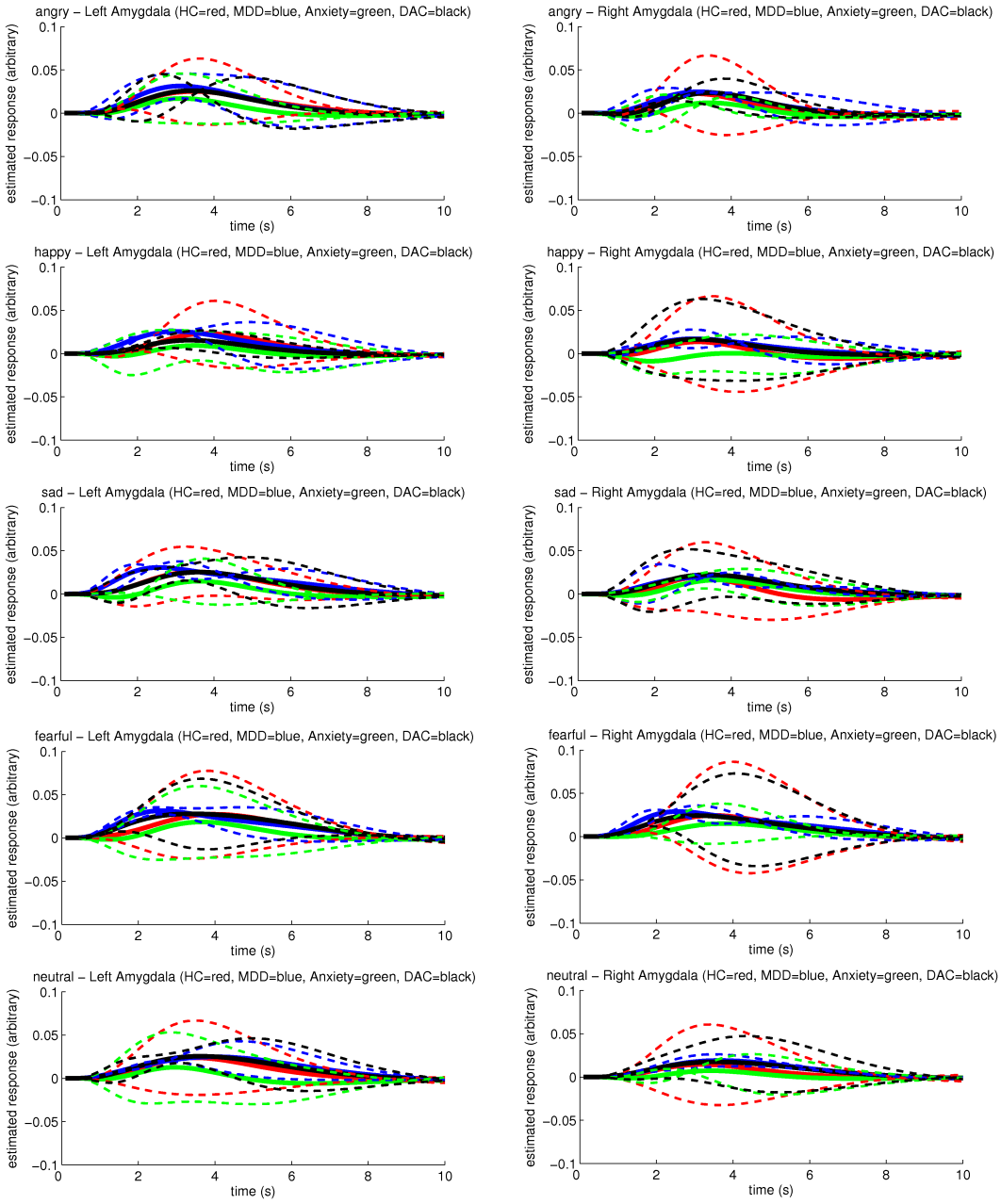

Figure 3.4: Amygdala response shape within each group and for each facial expression. Solid lines indicate group average for one ROI for one group, dashed lines are confidence intervals (SD). There were no significant group differences in peak height of activation nor in peak time of activation. HC – healthy, MDD – major depression, Anx – anxiety disorder, DAC – depression-anxiety comorbidity

meta-analysis of functional brain activation to negative stimuli in depressed patients also failed to find amygdala hyperactivation [45]. In the studies that did not observe differences in amygdala activation between depressed patients and controls, the severity of the depression was diverse (recovered: Norbury et al. [44], moderate: Almeida et al. [42], Gotlib et al. [40], severe: Lawrence et al. [41], Lee et al. [43]), as was medication use (all unmedicated: Norbury et al. [44], some medicated: Almeida et al. [42], Gotlib et al. [40], Lee et al. [43], all medicated: Lawrence et al. [41]). Nonetheless, the findings presented herein fit well and add to the aforementioned studies.

For severe depression, amygdala response showed abnormalities in previous studies [8, 6, 36] and medication may down-regulate the amygdala response [8, 36, 41]. In our case, for mild-moderate depression, with and without medication, no aberrant amygdala activation was observed, whereas for a group of patients with severe or moderate depression and medication use, no aberrant amygdala response was found by Lawrence et al. [41], Lee et al. [43], Almeida et al. [42], Gotlib et al. [40]. Taken together, these studies suggest that amygdala response is influenced both by illness severity and medication and that there is an interaction effect between illness severity and medication. Antidepressant may down-regulate amygdala response in severe depressed patients, but not in patients with mild-moderate illness severity. This conclusion is supported by recent study [46] reporting that antidepressants are associated with reduced illness severity only in severe depression.

The fact that some studies do find amygdala hyperactivation and some do not may be due to methodological factors such as task design (subliminal presentation of emotional faces [8] or task demands, *e.g.*, unattended versus attend faces [7], passive viewing [6] and implicit processing by asking for gender discrimination [40, 41]. Straube et al. [35] reported that implicit (photography versus schematic faces) processing of angry facial expression elicited significantly larger amygdala activation in social phobics relative to control participants, whereas explicit processing of angry faces elicited larger amygdala activation in both groups. Because we used an implicit or incidental facial affect-processing task where participants were not explicitly instructed to pay attention to facial expressions, we cannot exclude the possibility that participants may have consciously processed facial expressions. Hence, in the present study, the lack of significant group differences in amygdala activation to facial expressions may partially be an effect of the employed task, *i.e.*, facial expressions elicited amygdala hyperactivation in all groups.

Analysis of the amygdala response shape to facial expressions corroborated our findings that amygdala activation was not abnormal in patient groups. Although a comparatively early response of the left and the right amygdala activation to fearful faces was apparent in MDD patients (Figure 3.4), this difference was not significant compared with HC or other patient groups. Two other studies did find a difference in amygdala response shape: sustained amygdala activation in response to negative emotions in seven depressed patients [10] and a delayed amygdala response to angry, fearful and happy faces in fourteen patients with generalised social phobia [13]. There are several factors which may contribute to this discrepancy, for example sample heterogeneity in terms of disease severity, the fact that patients in those two studies were more severely affected, but also differences in the experimental design (words versus pictures).

In the present study, there was no significant correlation between illness severity and the magnitude of the amygdala response in outpatients. A positive correlation between fusiform gyrus activation to fearful and angry faces and depression severity was observed in MDD outpatients. We suggest that this pattern of activation to angry and fearful faces is symptom-related in depression. In a previous study [47] larger fusiform gyrus activation to sad faces was reported in patients with severe depression compared to healthy volunteers suggesting an attentional bias toward sad emotion in major depression.

Contrary to our hypothesis, ventral striatum hypoactivation to happy faces was not observed in MDD outpatients compared to HC. However, whereas Surguladze et al. [47] and Lawrence et al. [41] reported putamen hypoactivation in response to happy faces in nineteen MDD patients relative to controls, other authors [48, 49, 10] reported no significant differences in ventral striatum activation to positive stimuli in depression, consistent with the present findings. A possible explanation for these inconsistencies may be the sample characteristics (severe versus mild depression, and sample size). Nevertheless, right putamen hypoactivation in response to happy faces was observed in Anx outpatients compared with HC. Activation of the basal ganglia, including the ventral striatum and putamen, in response to positive emotional stimuli has been reported previously [32] suggesting its role in reward-related processes [50]. Previously it has been reported that decreased gray matter volume in the right putamen is associated with anxiety severity in panic disorder [51]. Abnormal putamen function may be characteristic for anxiety disorders.

We did, however, observe right superior and middle

frontal gyrus hyperactivation in response to happy (>scrambled) faces in MDD outpatients compared to HC. Larger middle frontal gyrus, cingulate cortex (BA32) activation to happy faces was also found in unmedicated MDD outpatients relative to HC. We suggest that this stronger activation for happy faces may reflect increased attention to mood-incongruent stimuli. Alternatively, it could be argued that MDD patients suppress positive emotions, and suppression of emotion has been shown to involve the right lateral frontal cortex [52]. Consistent with this interpretation, a recent meta-analysis of emotional reactivity in MDD showed that reactivity to positive emotional stimuli was even stronger reduced than reactivity to negative stimuli [53]. Unmedicated MDD also showed medial frontal gyrus hyperactivation in response to neutral faces. Similar to the present study, Frodl et al. [54] reported increased DLPFC during implicit emotional processing and failure of deactivation of this region during explicit emotional processing in patients with depression. They suggested that depressed patients activated this region more in order to meet task demands.

Similar to MDD patients, unmedicated DAC patients compared to HC showed dorsal ACC hyperactivation to happy faces. The dorsal ACC activation has been suggested to mediate conflicts between emotion and cognition [55] and is associated with attentional processing of emotional information [56]. Taken together, we may conclude that dorsal PFC hyperactivation in depressed outpatients with or without anxiety suggests increased processing demands for mood-incongruent stimuli.

Limitations and strengths: As was previously mentioned, the MDD patients included in the present study had mild-to-moderate depression severity (34 depressed outpatients were in remission [MADRS score<12 [57]], Appendix), which may have limited the sensitivity of our design. Although we analysed the potential contribution of medication status, this might still be a confounder in the present study, as we did not study type or medication dosage. A major strength of the present study concerns the large sample size, relative to previous studies, which lends confidence to the nontrivial suggestion that altered DLPFC function is a key feature of the neurobiology of depression. In conjunction with this, the sample analysed in this study is likely to represent a community outpatient population, the most prevalent community in mental health care practice.

Conclusions: The present study demonstrates that perception of facial expressions elicits a common neural response in community outpatients with mild-to-moderate depression and/or anxiety, and healthy controls. The lack of group differences in amygdala activation to emotional facial expressions may be characteristic of a community-based sample of outpatients with depression and/or anxiety. However, we did observe diagnosis specific activations for MDD, including dorsal PFC hyperactivation in response to happy facial expressions, which may reflect increased processing demands for mood-incongruent stimuli. Antidepressant and illness severity may influence amygdala response to emotional stimuli in major depression. Moreover, medication use seems to influence the neural response associated with cognitive control of emotion in MDD and DAC outpatients.

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A Facial expressions of emotion

In addition to the main analysis presented in the main text, we tested for group differences during emotional facial expressions (angry/fearful/happy/sad) relative to neutral faces. A four (condition: angry>neutral, fearful>neutral, happy>neutral and sad>neutral) by four (groups: MDD, ANX, DAC and HC) repeated measures ANCOVA was conducted with center (Amsterdam and Leiden as dummy variables), age and years of education defined as nuisance factors.
We observed increased right middle temporal gyrus activation in response to happy>neutral in HC compared to ANX and DAC patients. No significant difference was found between MDD and HC participants.

Amygdala temporal profile – data analysis
The temporal prole of amygdala activation was investigated employing a region of interest (ROI) approach, using an amygdala anatomical mask [29]. Beta values (HRF, TD and DD), for each subject, within each region, were extracted using MarsBar [30]. The mean and standard deviation of the haemodynamic response shape within each group and for each condition was reconstructed and plotted for visual inspection. Further, for each subject and for each response curve the maximum amplitude and the corresponding time point of the peak amygdala response were calculated in the haemodynamic response function and imported into SPSSv16.0. Group effects on these parameters were investigated with non-parametric tests (Kruskal-Wallis [H] and Mann-Whitney [U] as post-hoc test).

Illness severity
Depression severity – Out of 59 outpatient diagnosed with major depression severity 39 were in remission (Mean = 4.88 ± 3.93, MADRS score < 12 [57]). From DAC outpatients group 12 had a MADRS score < 12 (Mean = 8.67 ± 2.27).
Anxiety severity – Out of 57 outpatients diagnosed with anxiety disorders (PD and/or SP, and GAD), 45 had a BAI score < 21 (Mean = 9.91 ± 5.49), 7 had moderate trait anxiety (22 < BAI score < 35: Mean = 25.29 ± 2.75) and four had BAI score > 36. 44 outpatients diagnosed with depression and anxiety comorbidity (DAC) had low trait anxiety (BAI score < 21, Mean = 13.61 ± 5.38), 19 had moderate trait anxiety (22 < BAI score < 35, Mean = 26.21 ± 3.73) and 2 had high trait anxiety (BAI score > 36).

Table A.1: Significant brain areas activation in each group in response to viewing facial expressions (compared with scrambled faces). MNI coordinates, p<0.05 FWE. R – right hemisphere, L – left hemisphere, k – cluster size in voxels.

Group	Facial expression	Region	Side	Coordinate			Z-value
				x	y	z	
HC	all faces>scrambled	fusiform gyrus	R	39	-48	-24	>8
		amygdala	L	-18	-6	-15	4.78
	angry>scrambled	fusiform gyrus	R	39	-48	-21	6.60
	fearful>scrambled	fusiform gyrus	R	39	-48	-24	7.80
		amygdala	L	-18	-9	-15	5.32
	happy>scrambled	fusiform gyrus	R	39	-51	-21	>8
		fusiform gyrus	L	-39	-60	-18	5.15
	neutral>scrambled	fusiform gyrus	R	39	-48	-24	7.06
	sad>scrambled	fusiform gyrus	R	39	-48	-24	7.58
	emotional faces>neutral	middle temporal gyrus	L	-57	-60	3	4.92
		precentral gyrus	R	42	-18	54	4.87
MDD	all faces>scrambled	fusiform gyrus	R	39	-48	-21	>8
			L	-39	-51	-21	5.50
		amygdala	L	-21	-9	-15	5.55
			R	18	-9	-18	5.49
	angry>scrambled	fusiform gyrus	R	39	-48	-21	7.23
		amygdala	L	-21	-6	-15	4.93
	fearful>scrambled	fusiform gyrus	R	39	-48	-21	7.63
			L	-42	-54	-21	4.93
		amygdala	L	-21	-9	-15	6.46
			R	21	-9	-15	5.67
	happy>scrambled	fusiform gyrus	R	39	-45	-21	>8
			L	-42	-54	-21	6.12
		medial frontal gyrus	R	21	33	30	5.72
		postcentral gyrus	R	45	-21	54	5.06
	neutral>scrambled	fusiform gyrus	R	39	-48	-21	7.74
		amygdala	R	21	-9	-18	5.22
			L	-21	-9	-15	4.49
	sad>scrambled	fusiform gyrus	R	39	-54	-21	7.25
			L	-39	-51	-21	5.07
		amygdala	R	18	-9	-15	5.31
	emotional faces>neutral	postcentral gyrus	R	45	-24	54	5.33
Anx	all faces>scrambled	fusiform gyrus	R	39	-54	-21	7.39
	angry>scrambled	fusiform gyrus	R	39	-51	-21	6.01
	fearful>scrambled	fusiform gyrus	R	39	-54	-21	6.04
	happy>scrambled	fusiform gyrus	R	39	-54	-21	>8
	neutral>scrambled	fusiform gyrus	R	39	-48	-21	6.28
	sad>scrambled	fusiform gyrus	R	39	-54	-21	6.41
	emotional faces>neutral	–	–	–		–	–
DAC	all faces>scrambled	fusiform gyrus	R	39	-51	-21	>8
			L	-42	-54	-21	5.74
		amygdala	R	21	-9	-15	4.87
	angry>scrambled	fusiform gyrus	R	39	-51	-21	>8
			L	-39	-51	-21	5.07
	fearful>scrambled	fusiform gyrus	R	39	-51	-21	>8
			L	-39	-51	-21	5.76
		amygdala	R	21	-6	-15	5.68
	happy>scrambled	fusiform gyrus	R	39	-51	-21	>8
			L	-39	-78	-12	5.71
	neutral>scrambled	fusiform gyrus	R	39	-51	-21	>8
		amygdala	R	21	-9	-15	4.62
	sad>scrambled	fusiform gyrus	R	39	-51	-21	>8
			L	-42	-54	-21	5.51
		inferior frontal gyrus	R	48	24	21	5.26
	emotional faces>neutral	–	–	–		–	–

Note: HC – healthy participants, MDD – patients with major depression disorder, Anx – patients with anxiety disorders, DAC – patients with depression-anxiety comorbidity.

Table A.2: Anatomical regions showing an effect of illness severity. MNI coordinates, p<0.001 uncorrected. R – right hemisphere, L – left hemisphere, k – cluster size in voxels.

Group / Condition	Measure	Region	Side	Coordinate x	y	z	Z-value	k	p corrected cluster–level
MDD									
angry > scrambled	MADRS	Lingual gyrus	L	-9	-72	-9	4.17	318	<0.005
	MADRS	Fusiform gyrus	L	-18	-45	-12	4.03	59	0.025
fearful > scrambled	MADRS	Fusiform gyrus	L	-24	-75	-6	3.97	53	0.031
Anx									
happy > scrambled	BAI	Insula	L	-36	-12	15	4.58	70	0.011
	BAI	Angular gyrus	R	42	-60	36	3.61	80	0.006
	MADRS	Superior Temporal gyrus	R	51	-12	-9	3.96	61	0.020

Note: : MDD – major depression, Anx – anxiety disorder, MADRS – Montgomery-Åsberg Depression Rating Scale; BAI – Beck Anxiety Inventory.

Table A.3: Psychometric measures of medicated and unmedicated patients. Mean (standard deviation).

Group	MADRS	BAI	FQ
Anx			
med. (n=18)	11.28 (9.16)	11.44 (8.07)	35.56 (22.60)
unmed (n=39)	9.63 (10.80)	13.00 (16.26)	29.18 (24.35)
DAC			
med. (n=30)	19.43 (7.09)	17.60 (8.56)	38.80 (21.06)
unmed. (n=36)	19.64 (9.42)	18.06 (9.20)	31.89 (20.16)
MDD			
med. (n=14)	13.71 (7.22)	10.57 (7.55)	23.29 (16.39)
unmed. (n=45)	10.34 (8.99)	5.38 (11.73)	17.38 (15.26)

Note: : MADRS – Montgomery-Åsberg Depression Rating Scale; BAI – Beck Anxiety Inventory, FQ – Fear Questionnaire, MDD – major depression, Anx – anxiety disorder, DAC – depression-anxiety comorbidity, med – medication users, unmed – medication free.

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Chapter 4

Amygdala activation during emotional evaluation of words in anxiety disorders and depression

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Abstract

Background: Anxiety disorders and depression are characterized by increased emotional reactivity that has been associated with amygdala hyperactivation. However, few studies have examined the neural response during processing of emotional words in patients with anxiety disorders and depression, and findings have been inconsistent. We hypothesized increased amygdala activation to words evaluated as negative by anxious and depressed patients.
Method: Outpatients and healthy controls were recruited from the Netherlands Study of Depression and Anxiety. Sixty outpatients with anxiety disorders, 65 outpatients with depression, 77 outpatients with comorbid depression and anxiety and 57 healthy controls rated the emotional valence of words in an event-related functional magnetic resonance imaging (fMRI) task.
Results: Anxiety patients showed elevated right amygdala activation in response to negatively evaluated (>neutral) words. Depressed patients with or without anxiety comorbidity showed no significant differences in response to emotional words relative to healthy controls. A positive association between amygdala activation to negative (>neutral) words and MADRS score was found in depressed patients.
Conclusions: The present findings indicate that comorbid depression and anxiety cannot to be regarded as a simple summation of depression and anxiety. Our findings also support the hypothesis of aberrant amygdala activation during the processing of emotional words in anxiety disorders. In depression aberrant amygdala activation is associated with illness severity.

4.1 Introduction

Affective disorders such as anxiety disorders and depression have a high incidence in the general population and are both characterized by dysregulation of emotional processing [1]. In addition, depression and anxiety disorders are frequently comorbid in primary care [2].

Neuropsychological studies have reported anxiety disorders to be associated with an information-processing bias [3], and depression to be associated with a negative attentional bias to mood-congruent stimuli (sad faces) [4, 5]. Hence, anxiety is associated with a fast attentional bias, whereas depression is characterized by a later, strategic bias [4]. These biases may result from abnormal perceptual processing, such as enhanced attention toward emotionally relevant stimuli [6]. At the neural level, this bias may be associated with hyperactivation of the amygdala [6], a key structure in perceptual value judgments and generating emotional responses.

In healthy participants, functional neuroimaging studies have highlighted the role of the amygdala during explicit emotional evaluation Lee and Siegle [7]. While most studies have employed graphical stimuli, *e.g.*, emotional facial expressions and pictures from the International Affective Picture System, amygdala activation has also been observed in response to semantic stimuli (see for a review Sergerie et al. [8]). For example, Maddock et al. [9] and Tabert et al. [10] reported amygdala hyperactivation in response to negative words, but Hamann and Mao [11] observed amygdala hyperactivation to negative as well as positive words. A potential advantage of semantic stimuli is that the set from which they can be taken is very large, thereby circumventing the problem of rapid habituation of the amygdala due to stimulus repetition, which is difficult to avoid when using standardized picture sets. In addition, emotional signals, transmitted either visually or verbally, play an important role in human experience and behavior.

Abnormalities in detecting, interpreting and reacting to emotional stimuli characterize many forms of psychopathology. In patients with anxiety disorders and depression, limbic structures – including the amygdala – have been reported to show aberrant activation during emotion processing [12, 13]. Previous studies in patients with anxiety disorders have reported increased amygdala activation in response to emotional pictures and in anticipation of aversive stimuli, compared to healthy participants [14, 15, 16]. A recent meta-analysis of functional neuroimaging studies [17] confirmed the involvement of amygdala in anxiety disorders. It should be noted, however, that several studies failed to find abnormalities in amygdala activation in anxiety disorders [18, 19] and depression [20, 21].

In depression, an increased amygdala activation, as compared to the pattern in healthy controls, has been reported in response to personally relevant words, suggesting increased emotional reactivity [22, 23]. However, other studies using emotional words did not find significantly increased amygdala activation to negative words in depressed patients, relative to healthy participants [20, 21].

To a large extent, these inconsistencies in the findings in these affective disorders may be explained by task differences, *e.g.*, conscious or masked forms of emotional processing. In a recent meta-analysis [7], it has been suggested that "the amygdala is [only] involved in the initial emotional processing of explicit evaluation" which may also be relevant for explaining incongruent findings. Furthermore, differences in medication status may have confounded previous results, since it has been reported that antidepressants may normalize amygdala response to emotional stimuli in patients with depression [12].

In our text dominated society, words are a common vehicle for emotional communication, of similar importance as facial expressions [24]. Remarkably, so far only a few studies have examined the neural response during emotional evaluation of words in patients with depression and/or anxiety disorders. In the present study, we aimed to investigate to what extent the amygdala is involved in processing word valence in patients diagnosed with anxiety disorders (Anx) and/or major depressive disorder (MDD) compared to healthy controls (HC), using functional magnetic resonance imaging (fMRI). Patients with depression–anxiety comorbidity (DAC) were included in order to determine whether the comorbid state can be conceptualized as a summation of depression and anxiety. We hypothesized that MDD and/or Anx patients would both exhibit increased amygdala activation to negatively evaluated words. In response to positive words we assumed that depressed and anxious patients would have less amygdala activation. At the behavioral level, we expected that MDD, Anx and DAC patients would categorize more words as negative. Finally, we predicted that the use of medication in depressed and/or anxiety patients would attenuate differences in amygdala activation.

4.2 Methods and Materials

4.2.1 Participants

Participants included in the present study were selected from the Netherlands Study on Depression and Anxiety (NESDA, [25]), a multicenter, longitudinal cohort study. Three centers were involved in this project: University Medical Center Groningen (UMCG), Amsterdam Medical Center (AMC) and Leiden University Medical Center (LUMC). The study was approved by the Ethical Review Boards of each participating center. Prior to the scanning procedure, written informed consent was obtained from every participant. All participants were native Dutch speakers.

Inclusion criteria – Patients: (1) a Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV) diagnosis of major depressive disorder (MDD) and/or anxiety (social anxiety disorder [SAD] and/or panic disorder with or without Agoraphobia [PD] and general anxiety disorder [GAD] which was not an exclusion criterion) within the last six months prior to the interview, (2) a confirmation of the diagnosis with the Composite International Diagnosis Interview - lifetime version 2.1 [26] conducted by trained clinical staff. Controls: (1) did not meet the criteria for a DSM-IV diagnosis, no current and lifetime DSM-IV diagnosis, (2) confirmed absence of diagnosis as above. In all cases, diagnostic assessment took place approximately two months prior to the scanning. Participants were recruited from different health care settings: community, primary care and specialised mental health care.

Exclusion criteria – (1) a diagnosis other than MDD, SAD, PD, or GAD on axis I disorders or a history of bipolar disorders, (2) current alcohol or substance abuse, (3) a history of major neurological disorder, (4) current use of psychotropic medication other than stable use of SSRIs or infrequent benzodiazepines, i.e., equivalent to 2 x 10 mg oxazepam three times a week or use within 48 hours prior to scanning, (5) older than 57 years and (6) MRI incompatible implants or tattoos.

Of the subjects participating in this MRI study, overall, 34 subjects were excluded from the analysis because of technical problems during scanning, poor image quality, or excessive head movement (more than 3 mm on any axis). Our final sample consisted of 60 outpatients with anxiety disorders (Anx), 65 outpatients with major depression disorder (MDD), 77 outpatients with depression and anxiety comorbidity (DAC) and 57 healthy controls (HC). Regarding the Anx group, 73.3% had a diagnosis of SAD, 28.3% had PD without Agoraphobia, 31.7% had PD with Agoraphobia and 23.3% had additionally GAD. In the comorbid depression and anxiety group, besides MDD diagnosis present in all these patients, co-occurrence of anxiety diagnosis is as follows: 53.2% had a diagnosis of SAD, 16.9% had PD without Agoraphobia, 33.8% had PD with Agoraphobia and 61% had additionally GAD. Fourteen MDD, 16 Anx and 23 DAC patients were using medication at the time of the study.

Symptom severity was assessed on the scanning day using the Montgomery-Åsberg Depression Rating Scale (MADRS, [27]), the self-rated Beck Anxiety Inventory (BAI, [5]) and the Fear Questionnaire (FQ, [28]).

4.2.2 Stimuli and paradigm

We employed an event-related fMRI design with verbal stimuli displayed using E-prime software (Psychological Software Tools, Pittsburgh, PA, USA) during the scanning session. The stimuli were projected on a transparent screen at the end of the scanner bed, visible through a mirror above the participant's head. Instructions were presented on the screen at the beginning of the task. A response button device was positioned near the right hand of the participant.

The stimuli consisted of 120 study words [29] and 40 control words. Stimuli were presented pseudo-randomly in 20 blocks of eight words, each block consisting of two negative words, two positive words, two neutral words and two control words. The task was paced by the participants, but words were never displayed for longer than 5 s. During each stimulus, response options were indicated at the bottom of the screen. For the study words participants were requested to evaluate the emotional valence of the word. The study words were divided in three categories: 40 positive, 40 neutral and 40 negative, and matched for length (ranging from three to twelve letters) and frequency of occurrence in the Dutch language. Participants pressed the left button with the index finger if they evaluated the word as being positive, the middle button with the middle finger if the word was judged as neutral or the right button with the ring finger if the word was assessed as negative.

The control condition was chosen based on earlier research by Stark and Squire [30] indicating that an unconstrained rest condition may not be an optimal baseline condition because of reflective mental processes. The 40 control words were "left", "middle" and "right" (in Dutch) and participants were instructed to press the corresponding button.

4.2.3 fMRI data acquisition

Image acquisition was performed in all three centers (UMCG, LUMC and AMC) on a 3 Tesla Philips MR– scanner. Volumes of 39 slices at UMCG and 35 slices at LUMC and AMC, acquired parallel to the anterior commissure – posterior commissure plane (3 mm thickness, no gap, in plane resolution 3x3 mm at UMCG and 2.29x2.29 mm at AMC and LUMC, matrix size: 64x64 at UMCG and 92x92 at AMC and LUMC), were obtained using a T2*-weighted gradient echo sequence (TR=2300 ms, TE=28 ms at UMCG and TE=30 ms at LUMC and AMC) sensitive to blood oxygenation level dependent (BOLD) effect. A high-resolution anatomical scan was obtained with a sagittal 3D gradient-echo T1-weighted sequence (TR=9 ms, TE=3.5 ms, matrix 256x256, voxel size 1x1x1 mm, 170 slices).

4.2.4 Preprocessing

Imaging data analysis was conducted using SPM5 (Statistical Parametric Mapping; Welcome Department of Cognitive Neurology, London, UK) software implemented in Matlab v.7.1 (The MathWorks Inc., MA, USA). The time series were corrected for differences in slice acquisition time and spatially realigned. The structural T1 image was co-registered to the mean EPI obtained after realignment. Subsequently, T1 and EPIs were spatially normalized into standard Montreal Neurological Institute (MNI) stereotactic space and resampled to a 3x3x3 mm voxel size. Finally, the EPI volumes were smoothed using an 8 mm full-width at half maximum Gaussian kernel.

4.2.5 Statistical analysis

Voxel-wise fixed-effects contrast images were calculated at the single subject level. Event-related responses were convolved with a canonical hemodynamic response function (modulated by reaction times) and its time and dispersion derivatives [31]. Positive, negative and neutral words were classified according to each subject's evaluation. Control words were implicitly modeled in the design. For each subject, weighted contrasts were computed for "positive>neutral words" and "negative>neutral words". The resulting contrast images were entered into a second level analysis employing repeated measures 4 (groups) by 2 (contrast: positive>neutral and negative>neutral words) analysis of covariance (ANCOVA), with centers (dummy variables), age and years of education included as nuisance factors. The main effects are reported at $p<0.05$ with family–wise error (FWE) correction for multiple comparisons, whereas group differences were inspected at $p<0.001$ uncorrected and the reported clusters are those surviving a corrected cluster–level. Because the amygdala was of special interest in the present study, a small volume correction using a bilateral anatomical amygdala mask defined with WFU-pickatlas [32] was applied (SVC, $p<0.05$ FWE).
In addition, we repeated the random effect analysis testing for group differences, in order to determine possible medication effects. In this later analysis we included 126 medication free patients (44 MDD, 40 Anx and 42 DAC) and 56 HC. We further examined, differences between unmedicated and medicated patients within each patient group.
Lastly, voxel-wise regression analyses between the symptoms severity and the neural response during emotional evaluation of words were conducted using multiple regression analysis with a threshold of $p<0.001$ uncorrected and the clusters surviving the threshold $p<0.05$ corrected at cluster-level are reported. This analysis was performed for the contrasts "positive>neutral" and "negative>neutral" words within each patient groups (MDD, Anx and DAC) with center, age and years of education included as nuisance factors. Due to missing data of MADRS, BAI or FQ scores, some patients (four MDD, eleven Anx and fourteen DAC) were excluded from this analysis.
Behavioral data analyses (emotional categorization of words and reaction time) were conducted using repeated measures ANOVA (SPSS v.16.0) with group as between-subject factor and valence (positive, negative and neutral words categorization) or reaction time (RT) as within-subjects factors.

4.3 Results

4.3.1 Characteristics of the samples and behavioral data

Demographic and clinical data are listed in Table 4.1. Groups did not differ on gender ($\chi^2(3,259)=2.618$, $p=0.454$) and handedness ($\chi^2(3,259)=3.750$, $p=0.710$). Groups differed with regard to age ($F[3,255]=2.720$, $p<0.05$) and years of education ($F[3,255]=7.231$, $p<0.001$). Post-hoc Bonferroni tests showed that HC were significantly older than Anx patients ($p<0.05$). Also, HC had significantly more years of education than MDD and DAC patients ($p<0.05$). Significant differences were found on illness severity (MADRS, BAI and FQ scores) between HC and patient groups (all $p<0.05$). Regarding differences on illness severity between patient groups, the DAC patients scored

Table 4.1: Demographic and clinical characteristics of the samples (n represents the number of participants). Mean and standard deviation (S.D.) are given. MDD – major depressive disorder, Anx – anxiety disorders, DAC – depression and anxiety comorbidity, HC – healthy controls.

	MDD (n=65)	Anx (n=60)	DAC (n=77)	HC (n=57)
Mean age (S.D.)	36.29 (10.26)	35.35 (9.49)	36.87 (10.81)	40.40 (10.07)
Mean years of education (S.D.)	12.51 (2.84)	12.88 (3.30)	11.86 (3.29)	14.32 (2.76)
Female (%)	64.6	74.6	68.8	61.4
Right handed (%)	90.8	91.7	92.2	91.2
SSRIs users (%)	32.30	33.33	44.15	0.00
Mean MADRS (S.D.)	13.14 (9.03)**	10.17 (8.38)**	19.69 (8.76)**	1.35 (2.38)
Mean BAI (S.D.)	8.67 (8.29)**	13.48 (9.14)*	17.80 (8.84)*	2.46 (2.89)
Mean FQ (S.D.)	21.05 (15.28)*	35.14 (20.51)	36.17 (18.30)*	8.77 (7.39)

Note: *p<0.05, **p<0.005.

significantly higher on MADRS and BAI relative to MDD and Anx (all p<0.05) and on FQ relative to MDD (p<0.05).
Categorization results and reaction times for each group are summarized in Table 4.2. No significant group effect was found for reaction time (negative words: F[3,255]=0.783, p=0.504, positive words: F[3,255]=1.945, p=0.123, neutral words: F[3,255]=0.515, p=0.672). A significant group effect was found for categorization of positive words (F[3,255]=3.104, p<0.05) and neutral words (F[3,255]=3.250, p<0.05), but not for negative words (F[3,255]=1.070, p=0.362). HC classified more words as being positive than MDD (p<0.05) patients. MDD patients judged more words as being neutral relative to HC (p<0.05).

4.3.2 Imaging data

Main effect of task

A robust main effect of task (words versus baseline) was found in left inferior frontal gyrus, left supplementary motor area (SMA) and bilateral occipital cortex activation across groups (Appendices, Table A.1).
Across all groups, emotional relative to neutral evaluated words did not elicited significantly increased activation (p_{FWE}<0.05). At a slightly more liberal threshold, uncorrected p<0.001, increased activation was observed in the left inferior parietal gyrus (-39, -36, 45, Z=4.45, cluster size=179 voxels), the left hippocampus (-30, -12, -15, Z=3.71, cluster size=14 voxels) and the right calcarine gyrus (15, -72, 6, Z=3.58, cluster size=11 voxels).

Group differences

A group effect by negative versus neutral words was found on right amygdala (SVC: 27, 3, -21; k=13, F=5.56, p_{FWE}=0.03; $p_{uncorr.}$=0.001). To positive versus neutral words a group effect was observed in ACC (6, 45, 18, k=21, F=8.04, p<0.001).
Anxiety patients showed increased right amygdala activation in response to negatively>neutral evaluated words, relative to HC (p_{FWE}=0.015, SVC, Figure 4.1). As can be seen in Figure 4.1 this difference in amygdala response to negative words (>neutral) was driven by the hypoactivation in HC during negatively evaluated words. Such an effect was not present in patients with MDD and DAC compared to HC. Relative to DAC patients, Anx patients showed increased right anterior cingulate cortex (ACC) activation to positively evaluated (>neutral) words Table 4.3. Further, no significant difference was observed between other patient groups.

Medication effect

Demographic and clinical data for medicated and unmedicated patients are provided in Appendices (Table A.2). No significant differences between medicated and unmedicated patients were found on age, years of education, MARDS, BAI and FQ scores (all p>0.05).
Unmedicated patients versus HC: Following exclusion of the medicated patients from Anx group, we observed increased right amygdala activation to negatively (>neutral) evaluated words compared to HC (p_{FWE}=0.007, SVC). Table 4.3 lists the brain areas showing differences in activation between unmedicated patients and HC. No significant differences were found between unmedicated MDD or DAC patients and HC to positively or negatively (>neutral) evaluated words.
Medicated patients versus HC: Interestingly, medicated

Table 4.2: Behavioral data for the evaluation of words. The number of words assigned to the three categories and the reaction time (RT) in seconds are given. Mean and standard deviation (S.D.) are given.

	MDD (n=65)	Anx (n=60)	DAC (n=77)	HC (n=57)
"Positive"	37.94 (9.48)	38.15 (8.51)	39.84 (10.51)	42.70 (9.51)
"Neutral"	43.92 (10.35)	42.27 (10.89)	40.18 (11.50)	38.32 (9.43)
"Negative"	38.06 (2.93)	39.53 (5.23)	39.18 (4.89)	38.54 (4.33)
RT "Positive"	1.47 (0.36)	1.42 (0.27)	1.48 (0.42)	1.35 (0.27)
RT "Neutral"	1.60 (0.38)	1.56 (0.33)	1.61 (0.42)	1.54 (0.37)
RT "Negative"	1.27 (0.28)	1.21 (0.23)	1.26 (0.32)	1.21 (0.31)

Table 4.3: Brain regions showing significant group differences in response to positive or negative (>neutral) words, for uncorrected p<0.001. L – left hemisphere, R – right hemisphere, k – cluster size. *Small volume correction, p_{FWE}<0.05.

Group	Region	Side	MNI–Coordinates x	y	z	*Z*-value	k	$p_{uncorrected}$ voxel-level	$p_{corrected}$ cluster-level
Anx>HC									
Negative>Neutral	Amygdala	R	27	3	-21	4.04	8	<0.001	0.001*
Anx>DAC									
Positive>Neutral	ACC	R	6	45	18	3.95	52	<0.001	0.034
unmed Anx > HC									
Negative>Neutral	Amygdala	R	27	3	-21	4.23	7	<0.001	0.001*
med Anx > HC									
Negative>Neutral	Amygdala	R	27	3	-18	3.69	4	<0.001	0.004*
unmed > med MDD									
Positive>Neutral	ACC	R	3	21	21	4.40	55	<0.001	0.028
Negative>Neutral	ACC	R	12	24	27	4.33	54	<0.001	0.030
	Posterior Cingulate	R	0	-24	33	3.82	69	<0.001	0.011
	Postcentral gyrus	R	27	-36	48	3.64	53	<0.001	0.032

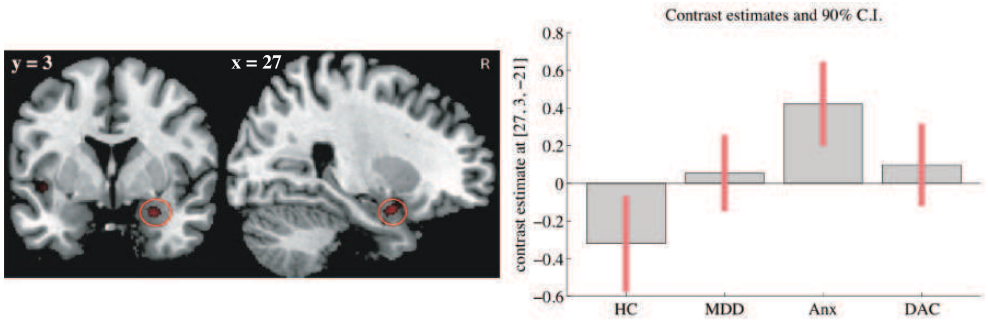

Figure 4.1: Right amygdala activation to negatively (>neutral) evaluated words in Anx patients relative to HC. Small volume correction, p_{FWE}<0.005.

Anx patients showed amygdala hyperactivation to negatively evaluated words (>neutral) compared to HC (Table 4.3, p_{FWE}=0.004, SVC).
Unmedicated versus medicated patients: Unmedicated MDD patients showed increased ACC activation in response to positively and negatively evaluated (>neutral) words, compared to medicated MDD patients (Table 4.3). No significant differences in the neural response to positively or negatively (>neutral) evaluated words were found between medicated versus unmedicated Anx or DAC patients.

Regression analysis of symptom severity and neural response to emotional words

A positive association between MADRS score and amygdala response to negative (>neutral) words was found in MDD patients (x=-18, y=0, z=-15; k=9, Z=3.60, p_{FWE}=0.006 SVC, Figure 4.2). No significant association between the neural response to emotional (>neutral) words and illness severity was found in DAC or Anx patients.
Secondly, regression analysis on symptom severity and neural response during emotionally evaluated words (>neutral) were conducted across all patients groups. This may help us to differentiate the effects of anxiety and depression. Interestingly, no effect of depression or state or trait anxiety symptoms on the neural response to emotional words was observed across all patients.

4.4 Discussion

The main hypothesis of the present study was that Anx and MDD patients would show amygdala hyperactivation in response to negatively evaluated words. We also investigated this issue in patients with depression and anxiety comorbidity. Our study provides evidence of amygdala hyperactivation in Anx patients, but not in MDD or DAC patients.
At a behavioral level, we expected that patients with depression, but also patients with depression–anxiety comorbidity, would judge more words as being negative than HC. In contrast, we found that MDD patients categorized significantly more words as neutral and less words as positive compared to HC. No significant difference regarding valence categorization of words was found between Anx or DAC patients compared to HC. Additionally, no significant differences in reaction times were found between MDD, Anx, DAC and HC.
Anxiety patients, irrespective of medication use, exhibited right amygdala hyperactivation in response to negatively, but not positively, evaluated words relative to HC. These results are in line with previous studies in patients with anxiety disorders reporting amygdala hyperactivation during anticipation of aversive stimuli [14], anticipation of public speaking [16] and viewing emotional images [15]. The present findings clearly show that amygdala hyperactivity in Anx patients is not restricted to complex visual stimuli such as emotional faces, but is also found during processing of emotional words.
Anx patients showed increased dorsal ACC activation (area 32) in response to positively evaluated words, relative to DAC patients. Lane et al. [33] hypothesized that this area is involved in selective attention to emotional stimuli. Following this line of argument, Anx patients were likely to be more attentive to emotional stimuli than DAC patients, which may imply that the presence of depressive symptoms reduces attentive vigilance.
In depression with or without anxiety, no significantly altered amygdala response to emotional (>neutral) words was present. The same was true after excluding the medicated patients from analysis, unmedicated MDD or DAC patients compared to HC showed no significant difference in the amygdala responses to negative (>neutral) words. In the present study, medicated and unmedicated MDD patients were indistinguishable in terms of demographic characteristics. Thus, medication use seems to not influence amygdala response to negative emotional words. However, a positive correlation between depression severity and left amygdala activation to negatively evaluated words was found in MDD patients, which suggests that increased depressive symptoms severity is associated with an enhanced emotional responsiveness to negatively evaluated words in MDD. We might conclude that amygdala hyperactivation to negatively evaluated words is associated with illness severity, rather than being a diagnosis effect.
With regarding to positive stimuli, MDD patients did not show decreased amygdala or ventral system activation, compared to HC. Although the MDD patients in our study classified fewer words as being positive compared to HC, this was not reflected in activation differences. In contrast to our findings, Epstein et al. [20] reported decreased ventral striatum activation to positive stimuli in ten unmedicated MDD compared to twelve HC. They suggested that depression is associated with inability to experience positive emotions [20]. Thus, the inconsistencies may be explained by task demands (emotional evaluation of the word versus reading the word) or sample size.
In addition to these findings, unmedicated MDD patients showed stronger dorsal ACC activation in response to positive and negatively evaluated words, relative to medicated patients; also, activation of the posterior cingulate cortex (PCC) was found in

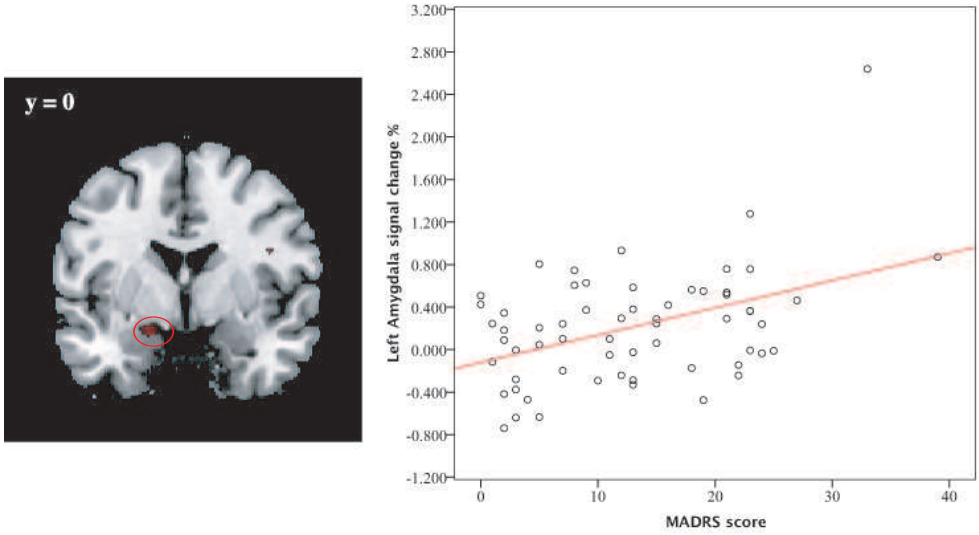

Figure 4.2: Positive correlation between left amygdala activation to negatively (>neutral) evaluated words and depression severity in MDD patients.

unmedicated compared to medicated MDD patients in response to negatively (>neutral) evaluated words. Fitzgerald et al. [34] in their meta-analysis concluded that ACC and PCC are part of the neural circuitry involved in the pathophysiology of major depression. Drevets and Raichle [35] suggested that dorsal ACC activation is associated with attention, and a recent study reported that stronger dorsal ACC activation to positive and negative emotion-evoking stimuli was associated with enhanced emotional awareness [36]. In line with these studies, we may conclude that Anx as well as unmedicated MDD patients have increased emotional awareness. PCC hyper–responsiveness has been previously reported in other studies that examined emotional processing of stimuli. The findings in these studies suggest that this region mediates the interaction of emotional and memory–related processes [9, 37, 35, 34]. Thus, we may infer that rating the valence of words by unmedicated MDD patients involved greater attention towards their own emotional experience. Furthermore, our results suggest a normalizing effect of antidepressants on ACC and PCC activation during emotional processing.

Contrary to our expectations, we failed to observe significant differences between DAC patients and HC. A possible explanation might be that correlations between symptom severity and neurophysiological responsiveness to emotional vs. neutral words are nonlinear, e.g. bell-shaped, as DAC patients had more severe pathology. However, post–hoc analyses showed that including a second-order term resulted in fits that were only marginally better than first-order (linear) models of amygdala response versus illness severity (data not shown). A similar finding was obtained for the DAC group during processing of pictures of faces (Demenescu et al., in preparation). Because the medicated DAC patients did not differ from the unmedicated DAC patients in other aspects, it is unlikely that the apparently normal amygdala response is a drug effect. Rather, the presence of depressive symptoms alongside anxious symptoms seems to counteract the amygdala response to negatively evaluated words. The mechanism for this is currently unknown, but adrenal corticosteroids may play a role because they can dampen the vigilance enhancing effects of monoamines.

A strength of the present study is the large number of participants included, so that we found very robust task related activation. Attribution of emotional valence to words (versus baseline) potently activated left prefrontal cortex (see Appendices) which is consistent with current literature on emotional evaluation of semantic stimuli. Another strength of this study is that patients were recruited through general practitioners and outpatient clinics using broad inclusion criteria and are highly representative for everyday psychiatric practice. A limitation of the study is the cross-sectional design, so that conclusions regarding

the effect of medication on amygdala activation need to be confirmed in a randomized controlled trial. Secondly, the words used herein were not selected based on their relevance for anxiety or mood diagnoses, but have a positive or negative connotation in general. Taken together, we may conclude that increased amygdala reactivity as seen during an emotional word evaluation task underlie anxiety disorders. In depression amygdala reactivity to emotional stimuli may be related to illness severity rather than a diagnosis effect, as suggested by the present findings.

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A Main effect of emotional attribution to words and the characteristics of medicated and unmedicated patients.

We attach here the supplementary material referred to in this chapter.

Table A.1: Main effect of all words and emotional words versus baseline across all groups and within each group (p_{FWE}<0.05)

Group	Region	Side	MNI–Coordinates x	y	z	*Z*-value	cluster size
Across all groups							
All words>baseline	Inferior Frontal gyrus	L	-48	21	-6	>8	568
	SMA	L	0	18	51	>8	170
	Inferior Occipital gyrus	L	-27	-90	-6	7.26	36
	Calcarine fissure	R	24	-93	0	6.88	29
HC							
All words>baseline	Inferior Frontal gyrus	L	-48	21	-6	>8	196
	SMA	L	0	15	51	5.28	24
MDD							
All words>baseline	Inferior Frontal gyrus	L	-48	21	-3	>8	367
	SMA	L	-3	21	48	6.25	44
	Inferior Occipital gyrus	R	27	-90	-3	6.09	15
Anx							
All words>baseline	Inferior Frontal gyrus	L	-45	30	-6	7.82	256
	SMA	L	-3	21	48	5.92	36
	Middle Occipital gyrus (BA 18)	L	-27	-90	0	5.90	9
DAC							
All words>baseline	Inferior Frontal gyrus	L	-42	24	-6	>8	366
	SMA	L	0	15	54	6.20	63
	Middle Occipital gyrus	R	27	-93	3	5.26	13

Words: positive+negative+neutral, emotional words: positive+negative words. HC – healthy controls, MDD – depressed patients, Anx – anxious patients, DAC – depression–anxiety comorbidity; SMA – supplementary motor area, BA – Brodmann area, ACC – anterior cingulate cortex; Side: L – left, R – right.

Table A.2: Characteristics of the unmedicated and medicated patients (n represents the number of participants). Mean and standard deviation (S.D.) are given.

	unmed MDD (n=44)	med MDD (n=21)	unmed Anx (n=40)	med And (n=20)	unmed DAC (n=42)	med DAC (n=34)
Mean age (SD)	35.34 (10.07)	38.29 (10.64)	33.92 (9.76)	38.20 (8.45)	36.10 (10.81)	37.94 (11.01)
Mean years of education (SD)	12.68 (2.43)	12.14 (3.60)	12.98 (3.29)	12.70 (3.40)	11.79 (3.38)	11.76 (3.10)
[1]MADRS Mean (SD)	11.48 (11.65)	14.57 (7.10)	8.25 (7.34)	12.00 (10.07)	19.95 (10.55)	18.06 (7.72)
[2]BAI Mean (SD)	6.08 (13.93)	8.71 (7.06)	11.45 (15.38)	13.00 (8.68)	17.12 (9.50)	17.85 (8.97)
[3]FQ Mean (SD)	19.39 (18.36)	18.10 (16.09)	25.80 (25.73)	33.10 (22.99)	27.83 (22.17)	31.32 (22.65)

MDD – major depressive disorder, Anx – anxiety disorders, DAC – depression and anxiety comorbidity, unmed – medication free, med – medication users, [1]Montgomery-Åsberg Depression Rating Scale, [2]Beck Anxiety Inventory, [3]Fear Questionnaire.

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Chapter 5

Neuroticism modulates amygdala – prefrontal connectivity in response to negative emotional facial expressions

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Abstract

Background: Neuroticism is associated with the experience of negative affect and the development of affective disorders. While evidence exists for a modulatory role of neuroticism on task induced brain activity, it is unknown how neuroticism affects brain connectivity, especially the crucial coupling between the amygdala and the prefrontal cortex. Here we investigate this relation between functional connectivity and personality in response to negative facial expressions.
Method: Sixty healthy control participants, from the Netherlands Study on Depression and Anxiety (NESDA), were scanned during an emotional faces gender decision task. Activity and functional amygdala connectivity (psychophysiological interaction [PPI]) related to faces of negative emotional valence (angry, fearful and sad) was compared to neutral facial expressions, while neuroticism scores were entered as a regressor.
Results: Activity for fearful compared to neutral faces in the dorsomedial prefrontal (dmPFC) cortex was positively correlated with neuroticism scores. PPI analyses revealed that right amygdala–dmPFC connectivity for angry and fearful compared to neutral faces was positively correlated with neuroticism scores. In contrast, left amygdala–anterior cingulate cortex (ACC) connectivity for angry, fearful and sad compared to neutral faces was negatively related to neuroticism levels.
Conclusions: DmPFC activity has frequently been associated with self-referential processing in social cognitive tasks. Our results therefore suggest that high neurotic participants display stronger self-referential processing in response to negative emotional faces. Second, in line with previous reports on ACC function, the negative correlation between amygdalaACC connectivity and neuroticism scores might indicate that those high in neuroticism display diminished control function of the ACC over the amygdala. These connectivity patterns might be associated with vulnerability to developing affective disorders such as depression and anxiety.

5.1 Introduction

Neuroticism is a widely recognized trait in various theoretical approaches to human personality [1, 2]. Characteristics of this trait include a tendency to worry and to be anxious [3], and is related to the experience of negative affect [1, 4, 5]. Neuroticism is also associated with affective disorders such as social anxiety disorder (SAD) and depression [6, 7, 8].
Functional magnetic resonance imaging (fMRI) studies have provided substantial evidence for the modulatory role of individual differences in neuroticism on neural activity related to emotion processing [9, 10]. Regions where activity is associated with neuroticism (and related personality traits) include the amygdala [11, 12, 13], the anterior cingulate cortex (ACC) [14, 12] and the medial prefrontal cortex [15, 11, 16]. However, these regions are functionally coupled, and such connectivity, especially between the amygdala and prefrontal regions, is crucial for the integration between emotion and cognition [17, 18]. To gain a better understanding of the neural basis of individual differences in emotion processing related to neuroticism, a focus on functional connectivity between limbic and prefrontal regions is therefore required.
Neuroticism is associated with alterations in cognitive–emotional functions such as affect regulation [19], self-consciousness [20] and self-regulation [21]. Thus, dysfunctional interactions between the amygdala and regions related to these functions, such as ventrolateral PFC (vlPFC), dorsolateral PFC (dlPFC) and ACC [22, 17] (cognitive control of emotion), and dorsomedial prefrontal cortex (dmPFC) [23, 24] (self-regulation and self-referential processing) are likely to be specifically associated with individual differences in neuroticism.
Only recently, fMRI studies have started to investigate personality–associated differences in functional connectivity during emotion processing. Whereas some of these studies focused on traits related to positive affect [25, 26], one study reported trait anxiety differences in amygdala–ACC coupling [27]. A mood induction study during positron emission tomography (PET) showed that neuroticism is associated with changes in subgenual cingulate coupling with prefrontal regions during mood induction, possibly reflecting a susceptibility marker for depression [28]. Despite these initial findings, to the best of our knowledge, no study has systematically addressed the question on how individual differences in neuroticism are associated with amygdala–prefrontal cortex connectivity for various negative emotional facial expressions.
To investigate the modulatory role of neuroticism on amygdala–prefrontal cortex connectivity during emotion processing, we applied a standardized face paradigm with different negative emotional facial expressions (angry, fearful and sad) in a large subject sample. This sample represented the healthy control subjects as part of the Netherlands Study on Depression and Anxiety (NESDA) [29]. We hypothesize that activity in the medial PFC, ACC and the amygdala is associated with neuroticism scores when processing negative as compared to neutral facial expressions. We also hypothesize that connectivity, between the amygdala on one hand and the lateral and medial prefrontal regions and the ACC on the other, should vary with individual differences in neuroticism.

5.2 Methods and Materials

5.2.1 Participants

Sixty healthy participants were selected from the general population (mean age=39.9, range: 2156, 37 females). Participants were recruited as healthy control participants in a large multi-center cohort study, the Netherlands Study of Depression and Anxiety (NESDA). Participants were tested at the Amsterdam Medical Center (AMC), Leiden University Medical Center (LUMC) and University Medical Center Groningen (UMCG). The exclusion criteria for these healthy participants were (1) a lifetime diagnosis of DSM axis I and/or axis II disorders, psychotic disorder or dementia, (2) current alcohol or substance abuse, (3) a history of seizure or head injury, (4) current use of beta-blockers medication, (5) hypertension (high blood pressure) 80/130mmHg, (6) more than 5 cigarettes smoked per day, (7) older than 57 years and (8) MRI incompatible implants or tattoos. During the preliminary analysis, 4 participants were excluded because of head movement artifacts. Written informed consent from each participant was obtained prior to the scanning session. The study was approved by the ethical review boards of each participating center.

5.2.2 Personality scores

To asses personality traits, all participants completed the NEO Five Factor Inventory [30]. This questionnaire consists of 60 items and measures five different personality traits: neuroticism, extraversion, openness, agreeableness and conscientiousness. Of these traits, neuroticism and extraversion are most closely related to emotion processing and alterations in neural activity [9]. Examples of the neuroticism questions include, I often feel nervous and tense, and Sometimes I feel

completely worthless.

5.2.3 Experimental design

Color photographs of faces depicting angry, fearful, sad, happy, and neutral facial expressions were presented together with scrambled faces in an event-related design. Photographs were selected from the Karolinska Directed Emotional Faces System [31] representing standardized facial expressions of emotions presented by amateur actors. Twenty-four faces were selected for each of the five facial expressions, comprising of 12 female and 12 male faces, and 80 scrambled faces. A total number of 200 photographs were presented pseudorandomly, such that there were maximally two faces presented before the presentation of a scrambled face, and there were no repetitions of the same emotional expressions. Each photograph was presented on the screen for 2.5 s, with an inter-stimulus interval (black screen) varied between 0.5 and 1.5 s (jitter). The total duration of the task was 747 s. The experimental paradigm was presented using E-prime software (Psychological Software Tools, Pittsburgh, PA, USA). Images were projected onto a translucent screen at the end of the scanner bed, visible via a mirror above the participant's head. Participants were instructed to indicate the gender by pressing one of two buttons of two magnet-compatible button boxes with the index finger of the left or right hand. During the presentation of scrambled faces, participants had to press left or right buttons in conformity with the instructions present on the screen, indicating either left or right by an arrow. Responses and reaction times were recorded. Participants were not aware that the implicit emotional variable was under study in the experiment.

5.2.4 Image acquisition

Images were acquired on a Philips Intera 3T MR-scanner. A sense-8 (UMCG and LUMC) and a sense-6 (AMC) channel head coil was used for radio frequency transmission and reception. For each subject a series of echo planar imaging (EPI) – sensitive to the blood oxygenation level dependent effect – volumes were obtained, entailing a T2*-weighted gradient echo sequence (repetition time [TR]=2300 ms, echo time [TE]=28.0 ms at UMCG and TE=30.0 ms at AMC and LUMC, flip angle 90 using axial whole-brain acquisition, with an interleaved slice acquisition order). The interslice gap was 0 mm and the plane thickness was 3 mm. The matrix sizes were: 64 x 64 voxels at UMCG and 96 x 96 voxels at AMC and LUMC. The EPIs were acquired at 39 slices at UMCG and 35 slices at AMC and LUMC. The inplane resolution was 3 x 3 mm at UMCG and 2.29 x 2.29 mm at AMC and LUMC. The axial images were acquired parallel to the anterior–posterior commissure plane. Functional data comprising 310 volumes were obtained per subject. A T1-weighted anatomical MRI was also acquired for each subject (TR=9 ms, TE=3.5 ms, matrix size 256 x 256).

5.2.5 Analysis

Preprocessing

Functional data were preprocessed and analyzed using the statistical parametric mapping software package (SPM5, http://www.fil.ion.ucl.ac.uk) implemented in Matlab 7.2 (The MathWorks Inc., http://www.mathworks.com).
The EPI volumes were reoriented in respect to the anterior commissure selected on the first volume. Time series were corrected for differences in slice acquisition times. The reference slice was 39 at UMCG and 2 at AMC and LUMC. After spatial realignment to the first image, a mean EPI was created. The movement parameters for each participant were inspected. If a participant moved more than 3 mm in any direction (anterior–posterior, right–left, inferior–superior) the data were excluded from further analysis. The anatomy scan was coregistered to the mean EPI image. Subsequently, T1, and with it EPI images, were spatially normalized to a standard stereotaxic space (Montreal Neurological Institute). During normalization, data were resampled into a 3 x 3 x 3 mm grid with 7th degree B-spline interpolation. The functional data were smoothed with a 3D isotropic Gaussian kernel of 8 mm full-width at half-maximum.

Imaging analysis

Low-frequency noise was removed by applying a high-pass filter (cut-off of 128 s) to the fMRI time series at each voxel. Significant hemodynamic changes for each condition were calculated using the general linear model [32], with respect to the event–related response convolved with canonical hemodynamic response function. To identify activity in regions related to face processing, we computed a t-contrast of all faces combined to the baseline, and tested this contrast at $p<0.05$ family wise error (FWE) corrected for multiple comparisons. To the test the hypotheses between the relation of neuroticism and negative affect, the analysis was subsequently restricted to the

negative emotional facial expressions. T-contrasts for angry>neutral, fearful>neutral, sad>neutral were calculated for each subject. Results of these weighted contrast (contrast images) were then entered in a second level random effect model. For each negative emotional facial expression (compared to neutral), neuroticism, extraversion, age and gender were entered as regressors.

A one-sample t-test was applied to test the positive and negative effect of the neuroticism scores regressor. Effectively, this analysis corresponds to detecting partial correlations between brain activity and neuroticism, when correcting for extraversion, age and gender. Since the amygdala, the ACC and the dmPFC all shown to have neuroticism dependent variation in activity when processing emotional stimuli they were defined as regions of interest (ROI). The amygdala and ACC volumes were based on the WFU pickatlas [33]. The dmPFC was defined as a 10 mm sphere around the peak voxel coordinates reported in a study on phobic proneness in relation to the processing of negative emotional faces [16]. We applied an initial significance threshold of $p<0.005$ (uncorrected) and a spatial extent of five voxels ($k>=5$), restricted to our a priori regions of interested (ROI): the amygdala, the ACC and the dmPFC. Furthermore we report activation outside our ROIs at $p<0.001$, $k>=10$ voxels uncorrected for multiple comparisons. Activations are reported in standard Montreal Neurological Institute (MNI) space.

Functional connectivity analysis: psycho–physiological interaction (PPI)

Psycho–physiological interaction (PPI) analyses were used to assess how activity in a brain region of interest covaries with a source region in response to the experimental condition [34]. Within each condition (different negative emotional faces compared to neutral faces), we separately examined functional connectivity from the left and right amygdala as a source region. To identify the amygdala activation for each participant we examined the contrast of all faces compared to the baseline at $p<0.05$ uncorrected. The deconvolved time series from a 5 mm radius sphere around the individually defined peak activated voxel within the amygdala (defined by the WFU pickatlas mask) was extracted (44 participants). The PPI was calculated as the element by element product of the left and the right amygdala time series (the first eigenvariate from all voxels' time series) and a vector coding for the effect of task (anger>neutral, fear>neutral, and sad>neutral). This product was subsequently reconvolved with the hemodynamic response function (HRF). This interaction term was then entered as a regressor in a first level model together with the time series of the amygdala and the vector coding for the task effect. The models were estimated and contrasts generated to test the effects of positive and negative PPIs. This analysis identified regions that display stronger functional connectivity with the amygdala for an emotional compared to a neutral facial expression, and for neutral compared to emotional facial expressions respectively.

The contrast images for the PPI effects were then entered in a second level analysis. In a similar manner to the conventional analysis, neuroticism, extraversion, age and gender were entered as regressors. Subsequently, the effects of neuroticism were tested, which identified brain regions that showed connectivity with the amygdala correlating positively or negatively with neuroticism scores, respectively. We applied an initial uncorrected threshold of $p<0.005$, $k>=5$, restricted to our a priori regions of interest, the ACC, the dmPFC, dlPFC and vlPFC. The ACC mask was based on the WFU pickatlas, while the dmPFC, dlPFC and vlPFC (lateral orbitofrontal cortex) masks were defined as a 15 mm sphere around the peak coordinates reported in a study on amygdala connectivity based on a large fMRI data set on processing angry and fearful faces [18]. Furthermore we report activation outside our ROIs at $p<0.001$, $k>=10$ voxels uncorrected for multiple comparisons.

5.3 Results

Because data were acquired at different sites, we conducted additional stepwise regression analyses to test whether this factor site would affect significance levels. When the model was extended with the factor site, no significant additional variance was explained compared to the model without the factor site (for each regression analysis $p>0.05$ for change in explained variance of the extended model, while neuroticism remained significant, $p< 0.005$ for each extended model).

5.3.1 Behavioral results

For the entire group, mean reaction times (RT) for the different emotional faces were: angry RT=825 ms, SD=158, fear RT=879 ms, SD=166, sad RT=874 ms, SD=163 and neutral RT=888 ms SD=155. There was a main effect of emotion on reaction time, driven by a faster RT for angry compared to neutral faces $t(55)=-7.6$, $p<0.05$. Accuracy overall was high: for

angry 98.3%, fear 98.5%, sad 96.1% and neutral 95.4% correct. There were no significant correlations between neuroticism (or extraversion) and the differences scores of each negative compared to neutral facial expression or for accuracy, (for each correlation $p>0.05$).

5.3.2 Personality scores

The sample scores for neuroticism were mean 24.3 (range: 13–36), SD=5.3. For extraversion these scores were mean 44.4 (range: 27–56), SD=6.6. There was a significant negative correlation between neuroticism and extraversion, $r=-0.49$, $p<0.05$. Since individual differences in extraversion also influence emotion processing, we aimed to exclude any possible effect by adding extraversion as a regressor in our model (see Passamonti et al. [26] for a comparable approach).

5.3.3 fMRI results

Main effects of emotional faces versus baseline

We compared all emotional faces together against the scrambled faces baseline to assess activity related to face processing. Main effects of the face processing were found in the bilateral fusiform gyrus (left, -42/-54/-24, z=6.1 k=25; right, 39/-45/-24, z=7.26, k=110), bilateral amygdala (left, -18/-6/-15, z=6.95, k=126; right, 21/-6/-15, z=7.13, k=82), and the right inferior frontal gyrus (51/27/21, z=6.37, k=174). All activations were $p<0.05$, whole brain FWE corrected.

Brain activity for emotional versus neutral faces and relation with neuroticism

In order to identify activity in brain regions that varied as a function of neuroticism scores, we tested the effect of neuroticism in a regression model. This resulted in the identification of activity in brain regions that was positively or negatively correlated with neuroticism in response to emotional facial expressions compared to neutral facial expressions. Table 5.1 shows activation clusters and peak coordinates. As one can see, of our a priori regions of interest, only the right dmPFC showed an effect of neuroticism. We found a positive relation between activity in this region for fearful compared to neutral emotional faces and neuroticism scores. The main effects of each contrast (regardless of individual differences) are provided in the supplementary material (Table A.1).

Functional connectivity: PPI analysis

In order to investigate how neuroticism is associated with functional connectivity of the amygdala and the prefrontal cortex, we tested the effect of neuroticism as a regressor in a model of connectivity with the left and right amygdala (separately) as source regions. This analysis resulted in the identification of brain regions showing connectivity with the amygdala that was either positively or negatively correlated with neuroticism scores when viewing emotional compared to neutral facial expressions.
Table 6.3 shows the modulatory effect of neuroticism on the connectivity of the left amygdala. For angry and fearful faces, connectivity of the left amygdala and right ACC was negatively associated with neuroticism scores. For sad compared to neutral faces, a similar relation between neuroticism and amygdala connectivity (with a more the dorsal part of the ACC) was observed. Both these findings indicate that the higher the neuroticism scores, the lower the functional coupling for negative emotional compared to neutral facial expressions between the left amygdala and the ACC. These effects are shown in Figure 5.1. For display purposes the partial correlation scores for neuroticism (the residuals after correcting neuroticism for extraversion, age and gender) were linear transformed (mean added and a scaled standard deviation) to approximate the original neuroticism scores.
Table 5.3 displays the positive and negative correlations between neuroticism and regions functionally coupled with the right amygdala for each of the negative emotional compared to neutral emotional facial expressions. A positive correlation was found between the amygdala connectivity with the right dorsomedial prefrontal (dmPFC) cortex for both angry and fearful compared to neutral facial expressions. This indicates that the higher the neuroticism scores, the stronger the coupling for fearful and angry compared to neutral faces between the right amygdala and the right dmPFC (see Figure 5.2).
It is important to note that within each of the neuroticism related connectivity effects in the ACC and dmPFC there were no significant main effects (irrespective of individual differences) of each contrast, even at a threshold of $p<0.05$ uncorrected. Within our other regions of interest we only found significant effects of a positive PPI (with the left amygdala) for the fear>neutral contrast in the ventral lateral prefrontal cortex (-36/33/-8, z=3.58, $p<0.05$ FWE, small volume corrected). No other contrasts showed a positive or negative PPI effect in our ROIs.

Table 5.1: Brain areas displaying a correlation between neuroticism scores and activity for angry, fearful and sad facial expressions.

Contrast	Region	Side	Voxels	Z values	p values	MNI–Coordinates x	y	z
Angry>Neutral	–	–	–	–	–	–	–	–
Fear>Neutral								
Positive	**dmPFC**	R	10	2.95	0.002	6	57	33
	Calcarine gyrus	L	17	3.71	<0.001	-3	-93	6
Sad>Neutral								
Positive	Posterior cingulate gyrus	R	10	3.65	<0.001	6	-48	30

Note: A priori regions of interest are shown in bold. dmPFC: dorsomedial prefrontal cortex. Other activations at a threshold of p<0.001, and minimal 10 contiguous voxels are also reported. No negative correlations were found.

Table 5.2: Association between neuroticism and functional connectivity with the left amygdala for angry, fearful and sad facial expressions.

Contrast	Region	Side	Voxels	Z values	p values	MNI–Coordinates x	y	z
Angry>Neutral								
Positive	Parahippocampal gyrus	R	12	4.39	<0.001	30	-39	-6
Negative	**ACC**	R	7	2.96	0.002	12	36	12
Fear>Neutral								
Negative	**ACC**	R	15	3.26	0.001	9	30	15
Sad>Neutral								
Negative	**dorsal ACC***	R	41	4.08	<0.001	12	12	30

Note: A priori regions of interest are shown in bold. *Small volume corrected, FWE p<0.05. ACC, anterior cingulate cortex. Other activations at a threshold of p<0.001, and minimal 10 contiguous voxels are also reported.

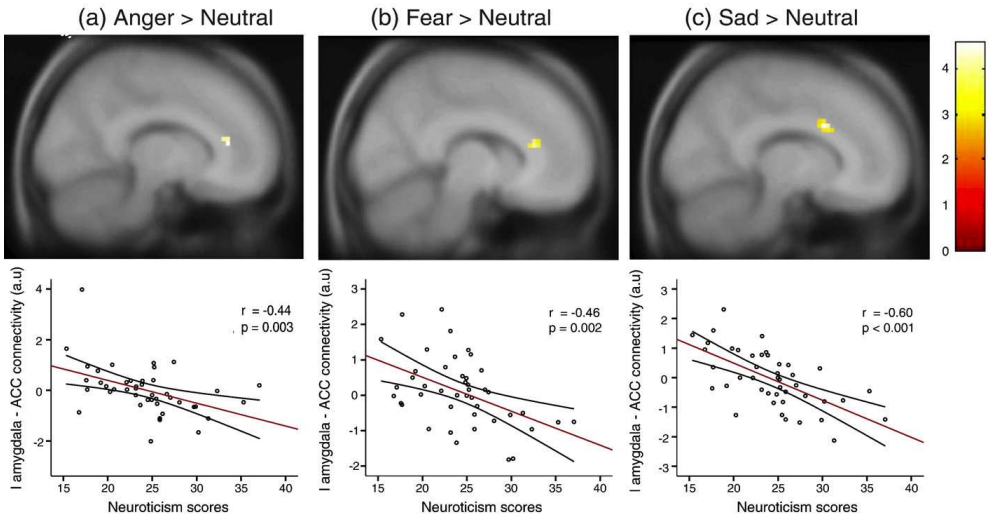

Figure 5.1: Brain regions displaying association between neuroticism and functional connectivity with the left amygdala for emotional compared to neutral faces. (a) Right anterior cingulate cortex for angry compared to neutral facial expressions. (b) Right anterior cingulate cortex for fearful compared to neutral facial expressions. (c) Right dorsal anterior cingulate cortex for sad compared to neutral facial expressions. The regression line and 95% confidence intervals are shown. The color bar represents the t-values.

Table 5.3: Association between neuroticism and functional connectivity with the right amygdala for angry, fearful and sad facial expressions.

Contrast	Region	Side	Voxels	Z values	p values	MNI–Coordinates		
						x	y	z
Angry>Neutral								
Positive	**dmPFC**	R	16	3.37	<0.001	21	42	36
Fear>Neutral								
Positive	**dmPFC***	R	49	3.22	0.001	21	42	36
	dmPFC*	L	15	3.26	0.001	-6	48	36
Sad>Neutral								
Positive	IFG	L	23	3.69	<0.001	-27	42	3

Note: A priori regions of interest are shown in bold. *Small volume corrected, FWE p<0.05. dmPFC, dorsomedial prefrontal cortex; IFG, inferior frontal gyrus. Other activation at a threshold of p<0.001, and minimal 10 contiguous voxels are also shown.

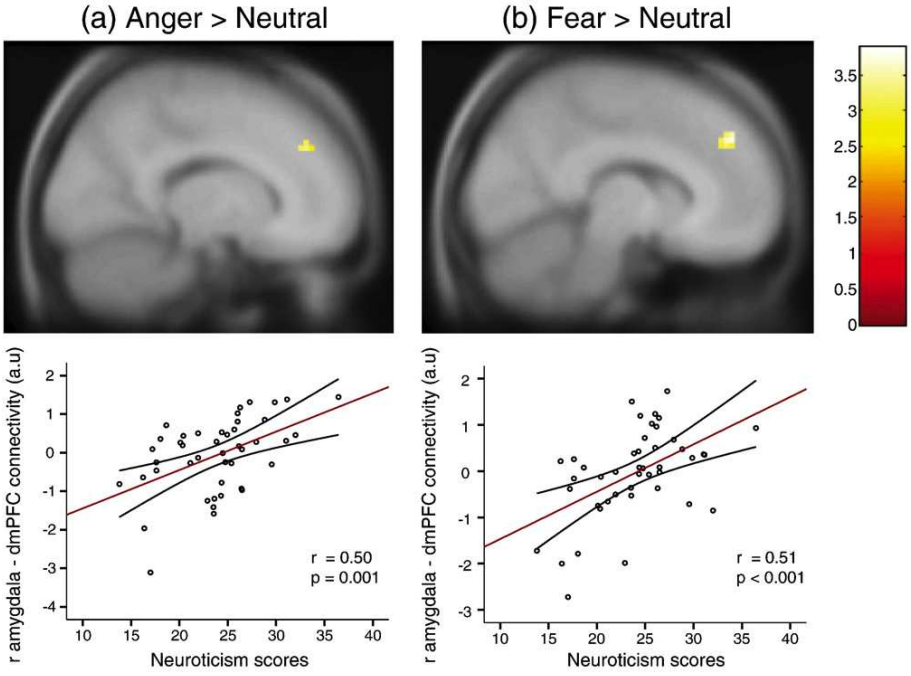

Figure 5.2: Brain regions displaying association between neuroticism and functional connectivity with the right amygdala for emotional compared to neutral faces. (a) Right dorsomedial prefrontal cortex for angry compared to neutral facial expressions. (b) Right dorsomedial prefrontal cortex for fearful compared to neutral facial expressions. The regression line and 95% confidence intervals are shown. The color bar represents the t-values.

5.4 Discussion

5.4.1 Neuroticism modulates brain activity in the dmPFC during the processing of fearful faces

In this study, we investigated the modulatory role of neuroticism on brain activity and functional connectivity while processing negative compared to neutral facial expressions. Our results showed that activation in the dmPFC varied as a function of neuroticism scores in response to fearful facial expressions. This finding is broadly in line with studies in which dmPFC activity was found to be related to neuroticism [35] and phobic proneness [16] during the processing of sad, respectively fearful and angry facial expressions. Both studies discuss these findings within a framework of self-referential processing, a construct strongly associated with this brain region [23, 36]. In accordance with these notions, neuroticism has been associated with an individual's self-schema, *i.e.* a "[...] constellation of self-referent information of one's own unique traits [...]" which "[...] serves to guide the processing of personally relevant information" [37]. Trapnell and Campbell [20] found that neuroticism related positively to ruminative self-consciousness (but not to reflective self-consciousness), which is associated with psychological distress [20]. Hence, our results may imply that higher levels of neuroticism are associated with a higher degree of self- referential negative appraisal during the processing of fearful expressions.

5.4.2 Neuroticism modulates amygdala–ACC and amygdala–dmPFC connectivity

We did not observe an effect of neuroticism on activity in the amygdala during processing of negative emotional expressions. However, and crucial to our hypothesis, connectivity analysis showed that neuroticism distinctively modulated connectivity between the left amygdala–right ACC and right amygdala–right dmPFC. We found no significant main effect for these regions, which is broadly in line with previous research, applying the same functional connectivity measure, that showed relatively small effects of functional coupling between the amygdala and these prefrontal regions when processing fearful compared to neutral faces [38]. Our results suggest that it is worthwhile to account for individual differences in neuroticism when studying functional connectivity related to the processing of negative emotional facial expressions.

We found that connectivity of the left amygdala with the ACC for angry and fearful, and dorsal ACC for sad facial expressions, correlated negatively with neuroticism scores. This indicates that subjects high in neuroticism, while processing negative compared to neutral emotional expression, displayed relatively less amygdala and ACC functional coupling. Numerous studies have shown the importance of amygdala–ACC functional connectivity in the context of emotion processing. For example, Etkin et al. [39] found that during high conflict trials in an emotional Stroop paradigm the amygdala and the rostral part of the ACC were negatively functionally coupled, suggesting an inhibitory role of the ACC over the amygdala. In line with our findings, a study on trait anxiety [27] showed a negative correlation of this trait with amygdala–ACC connectivity when viewing negative compared to neutral scenes. Moreover, Pezawas et al. [40], found that carriers of the short allele of the serotonin transporter gene (a polymorphism related to anxiety) showed relatively less functional coupling between the amygdala and ACC when processing angry and fearful faces. These findings suggest that persons high in neuroticism (or other individual differences related to anxiety) display less ACC related inhibitory control over the amygdala. It is of interest that the opposite pattern was found for a personality trait associated with positive affect and approach motivation; the behavioral activation system (BAS) [26]. These authors found that BAS positively predicted amygdala–ACC connectivity for angry compared to neutral faces. The studies mentioned demonstrate the relevance of amygdala–ACC connectivity in emotion processing and emotion regulation. These reports also suggest that individual differences in personality traits modulate amygdala–ACC functional connectivity, and that the direction of this correlation is different for traits related either to negative or positive affect.

In contrast to our results regarding left amygdala–ACC connectivity, we found a positive relation between neuroticism scores and right amygdala–right dmPFC connectivity. Participants with higher scores on neuroticism displayed relatively enhanced connectivity between the right amygdala and right dmPFC during the processing of angry and fearful compared to neutral faces. This finding relates to a study on functional connectivity in generalized social phobia (GSP) while processing self-referential praise and criticism [41]. Their results showed that patients (compared to healthy control participants) displayed stronger functional connectivity between amygdala and dmPFC for self-referential criticism. The authors argue that this

finding may "[...] reflect a negative attitude toward the self, particularly in response to social stimuli [...]", and that the mPFC may modulate amygdala engagement to initiate and maintain aspects of GSP [41]. In keeping with our finding of neuroticism related differences in dmPFC activity, our amygdala– dmPFC connectivity results also suggest that persons high in neuroticism might demonstrate stronger self-referential processing in response to negative emotional faces.

There is substantial evidence for the role of neuroticism in the development of, for example, anxiety disorders [6, 7]. The pattern we found in the relation between functional connectivity and neuroticism might provide insight in the neural basis of neuroticism- linked susceptibility to negative affect, and its associated vulnerability for the development of affective disorders. Taken together, our amygdala–ACC connectivity results indicate that high levels of neuroticism are associated with relatively less inhibitory control over negative facial expressions. Based on dmPFC activity and amygdala–dmPFC connectivity, we suggest that those high in neuroticism demonstrate stronger self-reference to negative facial expressions. Furthermore, it is of interest to note the apparent dissociation between neuroticism and connectivity from the left and the right amygdala. A recent meta-analysis on amygdala function showed evidence for a dissociation between the left and right amygdala regarding temporal dynamics [42], but did not find evidence for specific interactions between amygdala lateralization and valence or gender [42] in line with a previous meta-analysis [43]. Despite this knowledge on amygdala activity much less is known regarding lateralization of amygdala connectivity, and future research should therefore further explore possible lateralization in functional pathways from the amygdala.

Limitations

The interpretation of our connectivity analysis is restricted by the inherent limitations of functional connectivity measures. In our application of psycho–physiological interaction analysis, it is a measure of functional, but not effective connectivity [34]. The main difference between these concepts is that the former is a correlation method and its results do not imply a causal relation between regions involved. PPI analysis in and of itself, is therefore insufficient to assess the direction of effects (*i.e.,* reciprocal or unidirectional) between the amygdala and the dmPFC and ACC. This is an important limitation considering, for example, the argued regulatory role of the ACC over the amygdala. Nonetheless, other studies, applying different methodologies, have provided more direct evidence for a top– down regulatory role of the ACC over the amygdala. One tracing study, for example, showed that the ACC has more projections to the amygdala than vice versa [44]. Furthermore, deep brain stimulation experiments in depressed patients suggest that stimulating the ACC – through its connectivity pathways – affects several subcortical regions, including the amygdala [45, 46].

In this experiment, we found differences in amygdala–prefrontal connectivity associated with neuroticism during an emotion processing task, with only very mild demands on cognitive control over emotional functions. It is therefore very well possible that when engaged in more cognitively demanding tasks, additional amygdala–PFC connectivity pathways strongly involved in inhibitory control, would show associations related to individual differences in personality. For example, some studies have shown subcortical–prefrontal connectivity in relation to functions such as reappraisal [47, 48], extinction learning [49] and response conflict [39]. Future research on negative affect should therefore incorporate individual differences in neuroticism in functional connectivity on more challenging emotion regulation tasks. We would argue that in such paradigms individual differences are likely to be associated with, for example, amygdala–ventromedial or ventral lateral prefrontal connectivity.

Conclusion

Our present study indicates that individual differences in neuroticism are of importance in modulating functional connectivity of amygdala and prefrontal regions when processing negative emotional material. Neuroticism was negatively associated with amygdala–ACC, and positively related to amygdala–dmPFC connectivity when processing negative emotional facial expressions. These findings may provide insight into the neural mechanisms associated with susceptibility to negative emotional material, and may be relevant to the development of affective disorders.

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A Main effect of negative emotional expressions versus neutral faces

We attach here the supplementary material referred to in this chapter.

Table A.1: Main effects of each negative emotional compared to neutral facial expressions

Contrast	Region	Side	Voxels	Z values	p values	MNI–Coordinates		
						x	y	z
Angry>Neutral	MTG	R	19	3.85	<0.001	54	-42	6
	MTG	L	24	3.76	<0.001	-54	-60	0
Fear>Neutral	MTG	R	10	3.8	<0.001	51	-39	6
	MTG	L	14	3.75	<0.001	-57	-60	0
Sad>Neutral	Cerebellum	L	125	4.81	<0.001	-18	-57	-21
	Postcentral gyrus	R	81	4.6	<0.001	48	-24	54
	MTL	R	25	3.73	<0.001	57	-34	0
	IFG	R	15	3.66	<0.001	54	30	3
	Posterior cingulate cortex	R	10	3.54	<0.001	60	-36	30

Note: MTG: medial temporal gyrus, IFG: inferior frontal gyrus.

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Chapter 6

When amygdala neural response is not the whole story. A connectivity study of emotion perception

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in preparation

Abstract

Background: Patients with anxiety disorders display abnormal neural responses in amygdala and frontal regions during emotional processing. However, findings are inconsistent and the extent to which this abnormal neural response is specific to panic disorder and social phobia is unclear. The present study aimed to investigate brain activation and the psychophysiological interaction between amygdala and frontal regions during perception of emotional facial expressions in social phobia and panic disorder.
Methods: Functional magnetic resonance imaging was used in outpatients with social phobia and/or panic disorder and healthy participants, to measure the neural response to facial expressions of emotion. We further examined the interaction – modulated by emotional compared to neutral faces – between amygdala and frontal regions, using psychophysiological interaction in outpatients compared to healthy participants.
Results: No significant difference was found in the neural response to facial expressions (>scrambled) between social phobics and healthy participants. However, outpatients with comorbid social phobia and panic disorder showed less amygdala and fusiform gyrus activation to fearful and happy (>scrambled) faces compared to healthy participants. A negative left amygdala–middle frontal gyrus coupling during perception of fearful (>neutral) faces was observed in social phobics compared to healthy participants.
Conclusion: Results suggest that social phobics may have an impairment in regulating their negative emotions during perception of fearful faces. The comorbidity of social phobia and panic disorder may be associated with an impairment in perception of fearful and happy faces.

6.1 Introduction

Anxiety disorders are among the most prevalent mental disorders constituting 28.8% of the total psychiatric disorders. Within this percentage, social phobia (SP) has a 12.1% prevalence and panic disorder (PD) 4.7% [1]. Social phobia is characterized by extreme fear of negative evaluation and avoidance of social situations. Panic disorder is characterised by recurrent, spontaneous panic attacks, acute episodes of intense fear, accompanied by physical as well as cognitive symptoms [2].

Given the high share of anxiety disorders in the total psychiatric disorders and their common base – threat related stimuli –, an investigation of both their differences and common points in comparison with healthy controls, has the necessary prerequisites for identifying the specifics of their neural responses and psychophysiological interactions. The differences at both levels are prone to give an important insight into the mechanism of emotion perception in social phobia and panic disorder, a key factor in improving life quality and ease social burden.

The amygdala has been proposed as a central brain structure involved in processing threat-related stimuli [3] and therefore it is likely to play a role in the aforementioned anxiety disorders. Neuroimaging studies in subjects with SP using facial expressions reported amygdala hyperactivation in response to angry [4], fearful [5], happy [6] and neutral [7, 8] faces. Regarding PD, Kent and Rauch [9] in their review concluded that hyperactivation of the hippocampal–parahippocampal regions reported during symptom provocation are a "trait marker" for PD and hypothesised that amygdala may play an important role. In the last years neuroimaging studies reported abnormal amygdala activation during processing of facial expressions of emotion in PD patients. For example, amygdala hypoactivation was reported in response to fearful faces in PD patients compared to HC [10]. During perception of happy faces amygdala hyperactivation has been found in PD patients [11]. Thus, it seems that the amygdala might be the core of a shared mechanism involved in PD and SP, as suggested by Kent and Rauch [9].

In addition to studies reporting abnormal amygdala activation in anxiety disorders, abnormal neural responses were also found in frontal areas. For example, increased anterior cingulate cortex activation to disgusted faces has been reported in social phobia [12], whereas in panic disorder, decreased right prefrontal cortex activation in response to fearful faces has been reported [11]. From the abovementioned studies, we can conclude that not only amygdala plays an important role in anxiety disorders, but several regions including prefrontal cortical areas are part of the neural mechanism associated with anxiety disorders. However, it is unknown how amygdala coupling to frontal regions during emotional face perception is altered in SP and PD and whether possible changes in connectivity are common to these anxiety disorders.

Recently, functional neuroimaging studies have investigated the psychophysiological interactions during emotion perception and processing and the relation between anxiety disorders and brain connectivity. Cremers et al. [13] reported a positive association between right amygdala and dorsomedial prefrontal cortex (dmPFC) coupling and neuroticism and a negative association between neuroticism and left amygdala–anterior cingulate cortex (ACC) coupling. The authors suggested that these findings may provide an insight into the development of affective disorders, because neuroticism is associated with social anxiety disorder and depression. Additionally, Etkin et al. [14] reported increased dorsolateral prefrontal–amygdala functional connectivity during resting state in generalised anxiety disorder and showed that this connectivity was negatively correlated with anxiety measures. Furthermore, Liang et al. [15] suggested that greater amygdala–right frontal connectivity is associated with approach tendencies, whereas weaker amygdala–right frontal connectivity suggests withdrawal tendencies. It is therefore likely that anxious patients, who perceive the world as more threatening, have altered amygdala–frontal functional connectivity, specifically a weaker connectivity between cortex and amygdala.

To our knowledge, no study has investigated the amygdala interaction with other brain areas modulated by perception of facial expressions of emotions in patients with social phobia or panic disorder. The present study takes a step toward this goal examining the pattern of brain activation and the coupling of amygdala and frontal areas during emotional face perception in SP and PD. We hypothesised increased amygdala activation in response to angry, fearful, happy and neutral facial expressions in patients with SP. In PD increased amygdala activation was hypothesised in response to fearful faces. Patients with SP+PD comorbidity were included to examine the impact of comorbid PD and SP on the neural response during emotion perception. Based on previous findings mentioned above, we expected abnormal amygdala–frontal cortex coupling in SP and PD outpatients relative to healthy controls.

6.2 Methods

6.2.1 Participants

Participants were selected from the database of the Netherlands Study of Depression and Anxiety (NESDA, [16]). Three centers participated: University Medical Center Groningen (UMCG), Amsterdam Medical Center (AMC) and Leiden University Medical Center (LUMC). The Ethical Review Board of each center approved the study. Inclusion criteria required that all participants: 1) were between 18 and 56 years of age, 2) had no history of seizures or brain injury, 3) did not meet the criteria of any DSM axis I disorders for other disorders than SP, PD or GAD, 4) had no report of substance abuse and 5) had no physical limitations that prohibited them from undergoing an fMRI examination. All subjects were native Dutch speakers. After receiving written information, each participant gave written informed consent. The participants did not receive any compensation for their participation.
Twenty-three outpatients diagnosed with social phobia (SP), eighteen outpatients diagnosed with panic disorder (PD) and sixteen outpatients with a double diagnosis – social phobia and panic disorder – (SP+PD) were included in the present study. The diagnosis was established by trained clinical staff on the basis of the Composite International Diagnosis Interview (CIDI) - lifetime version 2.1 - (Andrews, 1998) in accordance with DSM-IV criteria (2001). All outpatients met the criteria for primary SP and/or PD, and some of them had a second diagnosis of general anxiety disorder (GAD). Twenty healthy controls (HC) were randomly selected from 56 HC scanned in the NESDA fMRI study, in order to match the sample sizes. Healthy controls did not meet the criteria for any current Axis I disorder and had no history of psychiatric disorders.
Six SP outpatients, four PD outpatients and eight SP+PD outpatients were using selective serotonin reuptake inhibitors (SSRIs) at the time of the study.
Before the scanning session, all participants were evaluated by means of a battery of standardised questionnaires and structured interviews. The Beck Anxiety Inventory (BAI, [17]), Fear Questionnaire (FQ, [18]) and Montgomery-Åsberg Depression Rating Scale (MADRS, [19]) were applied to all participants.

6.2.2 Faces paradigm

Color photographs of angry, fearful, sad, happy and neutral facial expressions and scrambled faces (control condition) were presented to all participants. The photographs were selected from the Karolinska Directed Emotional Faces System [20]. Twenty-four faces were selected for each of the five facial expressions, consisting of twelve female and twelve male faces, and 80 scrambled faces.
The experimental paradigm was presented using E-prime software (Psychological Software Tools, Pittsburgh, PA, USA). Stimuli were presented pseudo-randomly against a black background. Each stimulus was displayed on the screen for 2.5 seconds, with an interstimulus (black screen) interval varying between 0.5 and 1.5 seconds. The images were projected onto a translucent screen at the end of the scanner bed, visible via a mirror above the participant's head.
All participants were instructed to indicate the gender of each face by pressing one of two buttons of two magnet-compatible button boxes with the index finger of the left or right hand – left for male and right for female. During the presentation of scrambled faces, participants had to press left or right buttons in conformity with the instruction presented on the screen, *i.e.*, an arrow pointing to the left or to the right. The participants were not informed that the emotional expression was under study in the experiment.

6.2.3 MRI data acquisition

Images were acquired with a Philips 3T MR-scanner. A sense-8 (UMCG and LUMC) or a sense-6 (AMC) channel head coil was used for radio frequency transmission and reception. For each participant a series of echo planar imaging (EPI) volumes – sensitive to the blood oxygenation level dependent effect – were obtained, entailing a T2*-weighted gradient echo sequence (repetition time [TR]=2300 ms, echo time [TE]=28.0 ms at UMCG and TE=30.0 ms at AMC and LUMC) using axial whole-brain acquisition, with an interleaved slice acquisition order. The EPI volumes had 39 slices at UMCG and 35 slices at AMC and LUMC (0 mm gap, 3 mm thickness). The matrix sizes were: 64x64 voxels at UMCG and 96x96 voxels at AMC and LUMC. The in-plane resolution was 3x3 mm at UMCG and 2.29x2.29 mm at AMC and LUMC. The images were acquired parallel to the anterior-posterior commissure plane. A T1-weighted anatomical MRI was also acquired for each subject (TR=9 ms, TE=3.5 ms, matrix size 256x256, voxel size 1x1x1 mm).

6.2.4 Data analysis

The analyses on clinical and demographic data were performed with SPSS v.16.0 (SPSS Inc., Chicago,

IL, USA). In order to test for significant differences between groups an analysis of variance (ANOVA) was conducted. A post-hoc test was conducted using Bonferroni correction in case a significant effect was identified. Yates' chi-squared tests were conducted to test for gender and handedness effects. In order to control for multiple comparisons we applied Bonfferoni correction, thus the p-value 0.050 was divided by the number of tests (six Yates chi-squared tests were conducted for each variable) performed. A significant effect was identified if the p-value was smaller than 0.00833.

Functional imaging data were pre-processed and analysed using the statistical parametric mapping software package (SPM5) implemented in Matlab v.7.1.0 (The MathWorks Inc., MA, USA). The EPI volumes were corrected for slice time acquisition, realigned to the first volume, normalised to standard stereotaxic space defined by the Montreal Neurological Institute (MNI) template and spatially smoothed with a 8 mm full-width at half-maximum Gaussian kernel. Low-frequency noise was removed by applying a high-pass filter (cut-off of 128 s) to the fMRI time-series at each voxel.

Significant haemodynamic changes were identified using a general linear model, with respect to the event-related response convolved with the canonical haemodynamic response function (HRF, [21]). For each subject, a weighted contrast was computed for "angry> scrambled", "fearful>scrambled", "happy>scrambled", "neutral>scrambled" and "faces>scrambled".

A random-effects group analysis was conducted on weighted contrasts generated at single-subject level in four (facial expressions) by four (groups) repeated measures analysis of covariance (ANCOVA), with centers, age, years of education and medication (users/non-users) added as nuisance factors. The main effect of facial expressions is reported at a threshold of $p<0.050$ Family-Wise Error (FWE) corrected for multiple comparisons, whereas the group differences were explored at $p<0.001$ uncorrected and the clusters surviving corrected cluster-level $p<0.050$ are reported. Because the amygdala was of main interest, Small Volume Correction (SVC) was applied using the amygdala mask defined by the WFU pickatalas [22].

An additional analysis was conducted in the same way as mentioned above, testing for group differences in the neural response to emotional versus neutral faces including the following contrasts images: angry>neutral, fearful>neutral and happy>neutral.

Psychophysiological Interaction analysis

Psychophysiological interaction (PPI) is used to test the changes in one brain region in terms of an interaction with a psychological component (task) and activity in another brain area [23]. In other words, PPI analysis captures the modulation of activity in one brain region by activity in other brain areas dependent on specific active tasks.

In the present study, this interaction among neurophysiological measures and the experimental factor was examined for left and right amygdala defined as "seed" regions. Amygdala activation was identified at the subject level for the contrast "faces>baseline" inspected at $p<0.050$ uncorrected. The deconvolved time series was extracted from a sphere of 5 mm radius centered around the peak activated voxel within left and right amygdala (defined by WFU pickatlas) for each participant. This time-series reflects amygdala reactivity across all facial expressions, *i.e.*, we chose not to restrict the "seed" region to only those (amygdala) voxels that were activated in response to emotional faces. Choosing only voxels activated by the emotional faces could bias our results in finding a task related (emotion>neutral) connectivity pattern.

The PPI term (PPI regressor) was calculated as the element-by-element product of the amygdala time series and a vector coding for the task effect ("angry>neutral", "fearful>neutral" and "happy>neutral"). This product was subsequently re-convolved with the HRF. The interaction term was entered as a regressor in a first level model together with the amygdala time series (physiological variable) and a vector coding for the task effect (psychological variable). The effect of the interaction term was examined using the contrast [1 0 0] for a positive PPI effect and [-1 0 0] for a negative PPI effect, the first column representing the interaction term.

The individual contrast images were then entered into a second level analysis to identify the "target" regions showing changes in connectivity with the "seed" region depending on experimental context: angry, fearful or happy versus neutral faces perception between groups. It should be noted here that the causal direction of covariation cannot be determined using this method.

Ten PD, fifteen SP, nine SP+PD and seventeen HC were included in this analysis. A three (contrasts) x four (groups) repeated measure ANCOVA was performed on the contrast images for the PPI effects with center, age, education (years) and medication (users/non-user) as nuisance factors. An initial threshold of $p<0.001$ uncorrected was applied and clusters surviving the threshold of $p<0.050$ corrected are reported.

Within group analyses testing for the effect of anxiety symptoms severity on amygdala coupling were performed using nonparametric tests. Nonparametric tests were conducted because of the small number

of participants included in these analyses. One patients with SP and one with PD were excluded from this analysis because of missing scores on the BAI scale.

6.3 Results

6.3.1 Participant characteristics

No group difference was found on age, years of education, gender and handedness (all p>0.050). Table 6.1 presents the group characteristics. A main effect of group was found on BAI (F[3,72]=12.08, p<0.001), FQ (F[3,68]=16.17, p<0.001) and MADRS (F[3,71]=9.372, p<0.001) score. Post-hoc tests showed that patients groups scored higher on BAI, FQ and MADRS than HC (all p<0.050).

6.3.2 Imaging data

Main effect of task
Viewing facial expressions elicited activation in bilateral fusiform gyrus, bilateral amygdala and right inferior frontal gyrus (Table 6.2, Figure 6.1).
Secondly, we examined the neural response to emotional (angry, fearful and happy) versus neutral faces across all participants. Emotional facial expressions elicited more left middle occipital gyrus (x=-30, y=-90, z=-6) activation at p<0.050 FWE. There was no significant difference in amygdala response to emotional>neutral faces (uncorrected p<0.050: left amygdala - no effect, right amygdala: x=27, y=0, z=-18, Z=2.50, p=0.100 FWE, SVC).

Group differences
Table 6.2 displays the regions showing group differences in activation to facial expressions. No significant difference was found in amygdala response to fearful or any other facial expression (>scrambled) in PD compared to HC. In response to happy (>scrambled) faces greater left amygdala activation was found in HC compared to PD patients (Table 6.2, Figure 6.2). In response to angry, fearful or happy>neutral faces no significant differences in the neural response were observed between PD and HC.
HC showed greater left temporal pole extended to amygdala and fusiform gyrus activation to fearful (>scrambled) faces compared to SP+PD patients (Table 6.2, Figure 6.4). Relative to neutral faces, emotional faces did not elicit significant differences in amygdala activation in SP+PD patients compared to HC (Table 6.2).
No significant difference in the neural response to angry, fearful, happy or neutral (>scrambled) faces was found between SP and HC. Emotional faces compared to neutral faces did not elicit greater amygdala response in SP compared to HC (Table 6.2).
Relative to SP+PD outpatients, SP outpatients showed hyperactivation of left temporal pole extended to amygdala-hippocampus and fusiform gyrus in response to fearful faces (>scrambled).

PPI analysis
In the Appendix (Table A.1) are displayed the changes of the amygdala connectivity for emotional (angry, fearful or happy) versus neutral faces observed within each group.
SP outpatients compared to HC showed a negative coupling of left amygdala to posterior cingulate extended to precuneus during perception of angry (>neutral) faces. A negative coupling of left amygdala to right middle frontal gyrus during perception of fearful (>neutral) faces was observed in SP compared to HC (Table 6.3). PD and SP+PD outpatients showed no significant changes in connectivity between amygdala–frontal regions for emotions>neutral faces relative to HC.
Finally, we tested if amygdala–frontal areas coupling during perception of emotional facial expressions is modulated by anxiety severity. No significant effect of anxiety severity was found on the left or right amygdala connectivity during viewing emotional versus neutral faces.

6.4 Discussion

In the present study we investigated the neural mechanism associated with perception of facial expressions of emotions in patients with SP and/or PD, relative to HC. We expected amygdala hyperactivation to emotional and neutral facial expressions in SP and PD relative to HC.
We found that SP+PD outpatients showed amygdala hypoactivation in response to fearful and happy faces compared to HC. Amygdala hypoactivation in response to fearful faces has been previously reported in PD patients compared to healthy participants suggesting a diminished emotional response during perception of fearful facial expressions [10]. Amygdala hypoactivation was also reported during anticipatory anxiety in PD patients compared to HC suggesting that PD is associated with a functional impairment of the amygdala during anticipatory anxiety [24]. Interestingly, in the present study, outpatients with PD alone showed less amygdala activation during perception of happy faces compared to HC. Domschke et al. [11] reported amygdala hyperactivation in response to happy faces

Table 6.1: Demographic and clinical characteristics of the groups (n represents the number of participants).

Group	Age (SD)	Education years (SD)	Right-Handed (%)	Male (%)	SSRIs users (%)	BAI (SD)	FQ (SD)	MADRS (SD)
HC (n=20)	34.75 (9.81)	13.40 (2.68)	90.0	45.0	0	2.10 (2.27)	7.15 (7.89)	0.65 (1.31)
PD (n=18)	34.50 (10.36)	12.78 (3.47)	88.9	16.7	22.2	11.12 (6.59)	24.73 (21.82)	9.47 (7.67)
SP (n=23)	37.57 (10.02)	12.96 (3.65)	91.3	39.1	26.1	12.74 (9.47)	37.82 (18.95)	10.77 (10.13)
SP+PD (n=16)	34.50 (7.43)	12.38 (2.63)	93.8	12.5	50.0	18.38 (12.21)	41.87 (16.91)	12.56 (7.98)

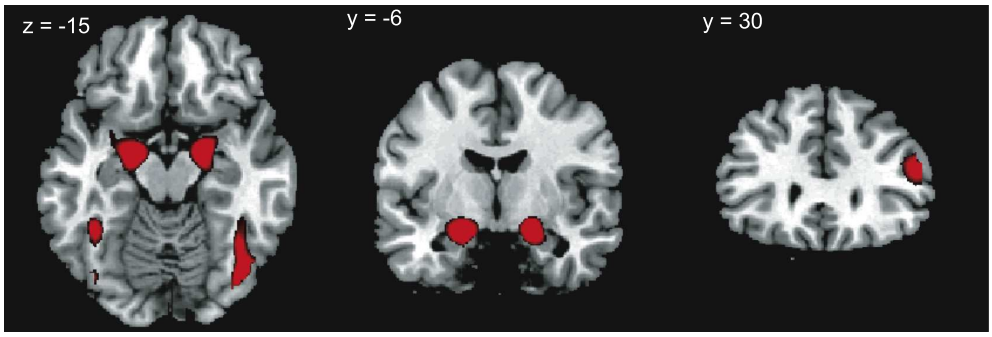

Figure 6.1: Main effect of task – viewing facial expressions versus scrambled faces at p<0.050 (FWE).

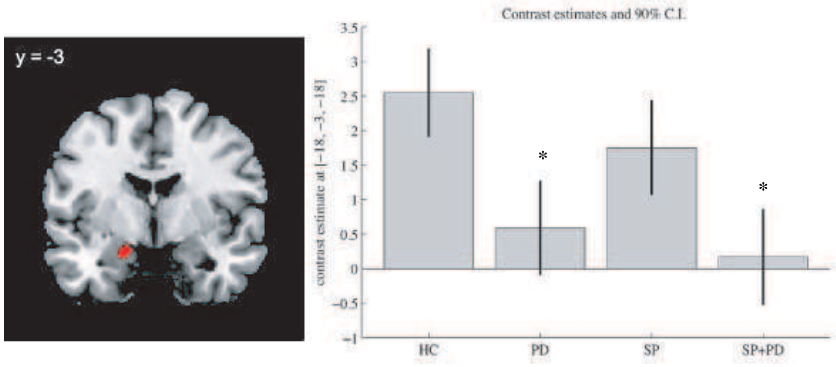

Figure 6.2: Left amygdala activation to happy (>scrambled) faces in HC compared to PD and compared to SP+PD. The bars indicate the % signal change and the 90 % confidence interval in the left amygdala within each group during perception of happy (>scrambled) faces. Stars indicate significant difference relative to HC.

Table 6.2: Neural response to facial expressions (>scrambled) – within and between groups. BA - Brodmann area. SP - social phobia, PD - panic disorder, SP+PD - social phobia and panic disorder comorbidity, HC - healthy controls. *Small Volume Correction.

	Region	Side	MNI x	MNI y	MNI z	Z-value	Cluster size	p-corrected cluster-level
Main effect of facial expressions > scrambled faces								
	fusiform gyrus	R	39	-51	-24	> 8	183	< 0.001
	fusiform gyrus	L	-42	-48	-21	> 8	100	< 0.001
	amygdala	R	21	-6	-15	> 8	134	< 0.001
	amygdala	L	-18	-6	-18	> 8	147	< 0.001
	inf. frontal gyrus	R	51	30	18	7.14	282	< 0.001
Group differences								
PD > HC								
Angry>Scrambled	sup. parietal lobule	L	-30	-75	33	4.40	64	0.032
HC > PD								
Happy>Scrambled	amygdala	L	-18	-3	-18	3.37	5	0.008*
HC > SP+PD								
Fearful>Scrambled	temporal pole (BA38)	L	-33	3	-21	4.14	58	0.045
	amygdala	L	-21	-6	-15	3.73		
	inf. occipital gyrus (BA37)	L	-51	-69	-3	4.35	78	0.015
	inf. temporal gyrus (BA37)	R	48	-45	-18	4.12	103	0.004
	fusiform gyrus	R	39	-48	-24	3.82		
Happy > Scrambled	amygdala	L	-21	0	-21	4.62	15	< 0.005*
SP+PD > HC								
Angry>Neutral	sup. frontal gyrus	R	15	21	48	4.07	62	0.031
SP > HC								
Angry>Neutral	calcarine gyrus	R	21	-72	9	4.50	81	0.011
	middle occipital gyrus	L	-21	-63	6	4.39	54	0.050
	sup. parietal gyrus	L	-21	-63	42	4.46	91	0.007
PD > SP+PD								
Fearful>Scrambled	inf. frontal gyrus	R	48	9	27	4.11	62	0.036
SP > SP+PD								
Fearful>Scrambled	temporal pole	L	-36	3	-21	4.91	74	0.019
	hippocampus	L	-24	-12	-12	3.90		
	amygdala	L	-21	3	-21	3.68		
	lingual gyrus	R	33	-81	-18	4.89	73	0.020
	fusiform gyrus	L	-39	-45	-21	4.36	58	0.045

Table 6.3: Group differences in amygdala – cortical regions coupling. BA - Brodmann Area. SP – social phobia, HC – healthy controls.

	Region	Side	MNI x	MNI y	MNI z	Z-value	Cluster size	p-corrected cluster-level
HC > SP: Left amygdala functional connectivity								
Angry>Neutral	cingulate gyrus (BA31)	L	-3	-51	42	4.32	177	<0.001
	cingulate gyrus (BA31)	R	6	-42	42	3.72		
	middle temporal gyrus	L	-39	-66	27	4.02	54	0.025
Fearful>Neutral	middle frontal gyrus (BA 8)	R	30	27	48	4.28	63	0.013

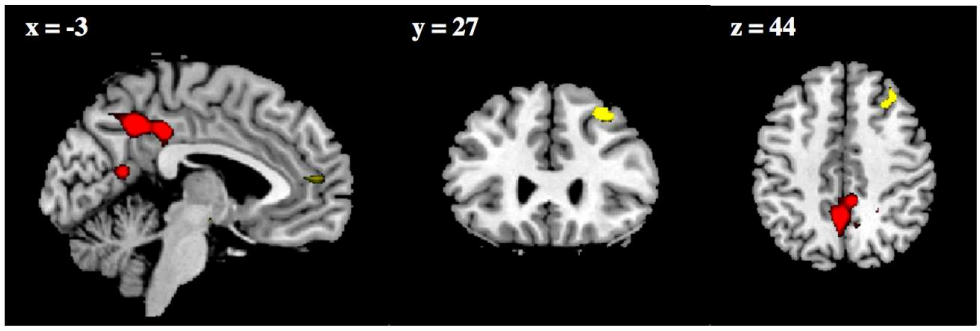

Figure 6.3: Abnormal amygdala–cortical regions coupling during perception of angry and fearful faces in SP relative to HC. Left amygdala – posterior cingulate gyrus coupling during angry>neutral faces (red) and left amygdala – right middle frontal gyrus coupling during fearful>neutral (yellow).

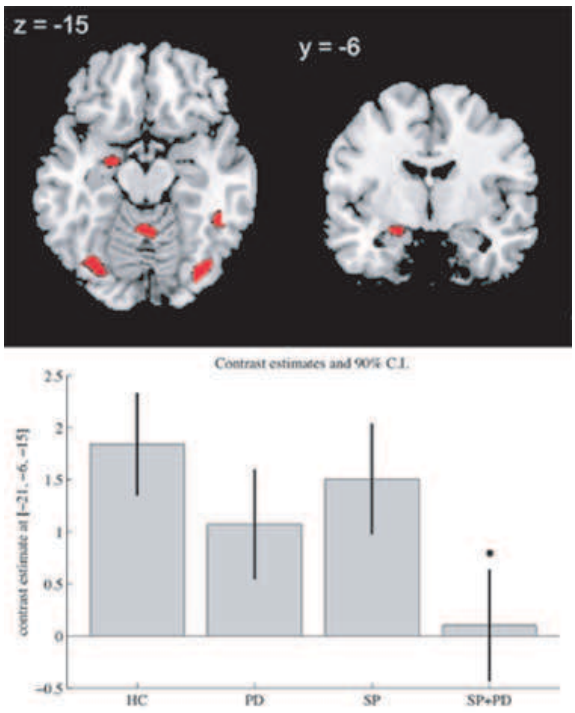

Figure 6.4: Left amygdala activation to fearful (>scrambled) faces in HC compared to SP+PD. The bars indicate the % signal change and the 90 % confidence interval in the left amygdala within each group during perception of fearful (>scrambled) faces. Stars indicate significant difference relative to HC.

in PD patients homozygous for the serotonin 5-HT$_{1A}$ -1019G allele compared to PD patients with 5-HT$_{1A}$ -1019C allele suggesting that serotonergic polymorphism 5-HT$_{1A}$ -1019G contribute to disrupted emotional processing. It is difficult, however to compare their study with the present study, because of the lack of healthy controls in their study. Thus, it is not clear if amygdala hyperactivation to happy faces reported by Domschke et al. [11] will differ significantly in PD patients compared to healthy participants. Hence, we may conclude that panic disorder may be associated with a weaker emotional response during positive emotions, as suggested by amygdala hypoactivation during perception of happy faces. The same holds true for comorbid SP and PD, which showed weaker emotional response not only to positive emotions, but also during perception of fearful facial expressions.

Furthermore, we found less fusiform gyrus activation during perception of fearful faces in SP+PD outpatients compared to HC and SP outpatients. Relative to PD outpatients, SP+PD outpatients showed right inferior frontal gyrus hypoactivation in response to fearful faces. These regions which were found to have lower activation in SP+PD have been reported to be involved in face perception and emotional response [25]. Taken together, we may conclude that outpatients with SP+PD comorbidity have a disturbed emotional processing of fearful faces. This mechanism was significantly different in SP+PD compared to SP or PD alone, thus we suggest that the comorbidity of PD and SP is not to be taken as a summation of the two.

In contrast to our hypothesis, we did not find significant amygdala activation during perception of angry, fearful, happy or neutral facial expressions in SP outpatients compared to HC. In addition we examined if emo-

tional (angry, fearful or happy) >neutral faces elicited significantly increased amygdala activation in anxiety patients compared to HC. No significant difference in amygdala response to emotional versus neutral faces was observed in SP patients (or in the other groups of anxiety patients) compared to HC. We may conclude that neutral faces elicited a similar amygdala activation as facial expressions of emotion in all participants, irrespective of the presence of an anxiety diagnosis.

The present findings are in line with some of the previous results reporting no difference in amygdala response to emotional faces in SP patients [12, 26]. However, other studies did report amygdala hyperactivation to negative [4, 5], positive [6] or neutral [8, 7] emotions in SP patients. Phan et al. [27] reported greater amygdala activation to harsh (angry, fearful and disgusted) compared to happy faces in generalised social phobia relative to healthy participants, whereas no significant differences in amygdala responses to happy or neutral faces compared to baseline (color photographs of radios) were found between groups. The authors suggested that the control condition may lead to inconsistencies in the results. Another explanation for the discrepancies between findings may be the task demands (gender discrimination, emotion recognition, valence rating), sample characteristics, *e.g.*, mood disorders comorbidity, illness severity – mild/moderate versus severe anxiety (BAI score between 8-15, [28]) or medication use.

In line with our hypothesis, abnormal amygdala–frontal areas connectivity was observed in SP outpatients compared to HC. However, this was not the case for PD or SP+PD comorbidity, which showed no significant changes in amygdala–frontal areas connectivity during perception of emotional facial expression (>neutral faces), compared to HC.

SP outpatients showed a negative left amygdala–right middle frontal gyrus connectivity during perception of fearful (>neutral) faces, compared to HC. The prefrontal cortex was reported to be involved in cognitive processes and emotion regulation [29] and amygdala–frontal areas connectivity predicts successful regulation of negative emotions [30]. Increased dorsolateral prefrontal cortex–amygdala connectivity during resting state was reported in generalised anxiety disorders (GAD) patients compared to HC and this connectivity was negatively correlated with anxiety measures [14]. The authors concluded that abnormalities in the amygdala–lateral prefrontal coupling characterised GAD and this coupling suggests the engagement of a compensatory mechanism [14]. Additionally, a recent meta-analysis on amygdala functional connectivity reported that amygdala has a complex network connection, including medial and inferior frontal gyri, and posterior and anterior cingulate, suggesting its implication in emotion-cognition interaction [31]. Given the previous findings, we may conclude that the negative connectivity of the amygdala–dorsal prefrontal cortex suggests an impairment of emotion regulation during perception of fearful faces in the SP outpatients.

Furthermore, SP outpatients showed stronger negative left amygdala–bilateral precuneus/PCC connectivity during angry (>neutral) faces, compared to HC. Precuneus has been associated with evaluation of one's own emotional state and self-focused evaluation, but also to be part of the default mode network [32]. Gentili et al. [33] reported more precuneus/PCC activation during face perception in social phobia compared to healthy participants suggesting that an impairment in the "default mode network" is associated with self-focused attention and "feelings of wariness of others' judgments". According to these findings, we conjecture that negative covariation between left amygdala and PCC/precuneus activation during perception of angry faces represents an inhibitory influence through this pathway. We may surmise that patients with SP have a stronger self-focused evaluation during perception of angry facial expressions than HC, because less decreased precuneus activation to angry faces was observed in SP than in HC (uncorr $p<0.050$).

Albeit the number of participants in this study is in the range of other published studies, we can always consider the sample size as a potential limitation. Moreover, some of the outpatients included herein were under medication at the time of scanning, which may have been a confound in the present findings because it has been reported previously that medication normalised amygdala response [34]. Thus, although we controlled for medication use, we cannot completely exclude effects of the type and dosage of medication. This can also be considered a limitation of this study.

In conclusion, this study provides evidence of an altered amygdala–frontal cortex coupling during perception of negative emotions in outpatients with SP compared to HC, which suggests an impairment of emotion regulation. PD may be associated with a lack of emotional response to positive emotions, as suggested by amygdala hypoactivation to happy faces, compared to HC. The same holds true for patients with SP and PD comorbidity which may be associated with an impairment of emotional processing not only of happy faces but also of fearful faces.

A Amygdala–cortical regions coupling modulated by the perception of emotional facial expressions.

We attach here the supplementary material referred to in this chapter.

Table A.1: Left and right amygdala functional connectivity. * Small Volume Correction, $p_{FWE}<0.050$, ACC – Anterior Cingulate Cortex, BA – Brodmann Area. PD – panic disorder, SP – social phobia, SP+PD – comorbid social phobia and panic disorder, HC – healthy controls.

	Region	Side	MNI			Z-value	Cluster	p-corrected
			x	y	z		size	cluster-level
SP+PD: Right amygdala – negative functional connectivity								
Happy>Neutral	dmPFC	R	21	39	30	3.65	26	0.020*
PD: Left amygdala – positive functional connectivity								
Angry>Neutral	ACC	L	-12	42	12	3.75	11	0.013*
SP: Left amygdala – negative functional connectivity								
Angry>Neutral	precuneus	R	6	-42	45	4.43	130	<0.001
	precuneus	L	0	-51	45	3.95	54	0.025
Fearful>Neutral	medial frontal gyrus	R	15	-6	54	4.89	140	<0.001
	middle frontal gyrus (BA9)	L	-39	21	39	4.85	53	0.027
	middle frontal gyrus (BA8)	R	33	30	48	4.42	89	0.002

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Epilogue

Overview – The subject of this thesis lies in the field of neuropsychology and it aimed to identify abnormalities in the neural correlates of emotional processing in community-based patients diagnosed with depression and/or anxiety. The importance of uncovering such abnormalities is in gaining a better understanding of the conditions, hopefully inspiring new treatment ultimately aiming at a faster reintegration of outpatients.
The studies presented in this thesis have made use of functional neuroimaging which, as pointed out also in the Introduction, allows for non-invasive identification of distributed patterns of human brain activity associated with perceptual, cognitive, emotional and behavioral processes. We used the fMRI method to measure the hemodynamic response, *i.e.*, changes in blood flow, related to neural activity in patients diagnosed with anxiety disorders and/or major depression at different stages of their illness.
Two experimental paradigms were employed to investigate the neural mechanism related to emotional processing in MDD and/or anxiety: implicit and explicit emotional processing. The two paradigms were meant to offer a multifaceted view on emotional processing, *i.e.*, implicit and explicit emotional processing, and hence a clearer picture of the neural basis involved in the aforementioned disorders. The implicit paradigm employed stimuli of facial expressions of emotion but participants were not explicitly instructed to pay attention to displayed emotions. The explicit emotional processing paradigm used word stimuli and involved additional cognitive processes, language processes (reading the words) and emotional memory retrieval (emotional attribution to words). With this we aimed to determine if the aberrant neural response is to be associated with perception of emotion or related to the interaction of different processes, *e.g.*, an emotion-cognition interaction.
First we reviewed emotion-discrimination accuracy in patients with affective disorders (see Chapter 2). In the first part of the experimental section of this thesis we studied the neural response to facial expressions of emotion and emotional words in MDD and/or anxiety patients compared with healthy controls (HC). In the second part, we examined the degree to which activation in frontal regions is explained by the interaction between amygdala activity and perception of emotional facial expressions - modulated by the neuroticism score in healthy participants. Finally, in the third and last part of the experiment section we investigated amygdala connectivity with frontal regions in patients with anxiety disorders by looking at group differences.

Emotion discrimination accuracy (see Chapter 2) – Facial expressions of emotion contain information about one's feelings and play an important role in social communication and interaction. Thus, an impairment of emotion recognition of facial expressions may be associated with poor social and interpersonal relations and, furthermore, can be connected with some of the symptoms the patients present. For example, a common occurrence in this respect is that patients with social phobia tend to avoid crowded public spaces for the psychological discomfort these places inflict. It is unclear though, to which extent this impairment is specific to depression and anxiety disorders. In our review on emotion-discrimination accuracy in affective disorders, we found that MDD and anxiety disorders are associated with overall emotion recognition impairment. This impairment was more pronounced in MDD patients, it was not apparent in children with anxiety disorders. Explanations for this deficit in depression may be determined by patients negative emotional experience, by the assessment of their internal mood state or by the cognitive impairments present in depression [1]. Because depression is characterized by negative cognitions (worthlessness, self-criticism, hopelessness) the patients

evaluation of external stimuli, including facial expressions, might be more negative than the evaluation done by healthy subjects [2, 3]. It remains unclear whether the impairment in emotion recognition is emotion specific.

Facial expressions of emotion – fMRI study (Chapter 3) – As concluded in Chapter 2, MDD and anxiety patients have difficulties in recognizing and labelling facial expressions of emotions. We investigated the underlying differences at the neural level between these two diagnostically distinct groups. Previous findings showed that inpatients with MDD and anxiety have an increase in the amygdala response to emotional facial expressions [4, 5, 6, 7]. However, as outpatients with MDD and/or anxiety have a variable and less pronounced symptom severity it is unclear whether or not the aberrant neural response to emotional facial expression is also present in them. We hypothesized that there would be differences in the neural response, especially in the amygdala response during the perception of facial expressions of emotion.
Furthermore, previous studies have shown that antidepressant medication normalized amygdala activation to emotional stimuli and also reduced symptom severity in patients with MDD or anxiety disorders [8, 9, 10, 11]. This was also under investigation.
Our findings show that emotional facial expressions elicited to a similar extent the same neural mechanism in patients with MDD and/or anxiety disorders as in HC. This response involved bilateral amygdala, bilateral fusiform gyrus and right inferior frontal gyrus, regions that are known to be responsible for face perception [12]. The groups seemed to apply similar neural strategies. These results show a default network with no gross abnormalities in activation for perception of facial expressions of emotions in outpatients. It is hence concluded that patient groups considered in this study have differences in activation that are statistically insignificant.
To further substantiate these unexpected findings we examined the amygdala response shape. Siegle et al. [13] reported sustained amygdala response to negative stimuli in seven depressed patients, whereas in fourteen patients with generalized social phobia a delayed amygdala response was found to angry, fearful and happy facial expressions [14]. Based on these studies, we expected that MDD patients exhibit a sustained amygdala response compared to HC. In anxiety patients, we expected a later amygdala response to facial expressions of emotions compared to HC. Strikingly, our data showed also no significant group differences in the amygdala response shape to facial expressions. Furthermore, after controlling for medication use, unmedicated outpatients compared to HC did not exhibit aberrant amygdala response to emotional facial expression.
One can consider different causes for these inconsistencies in findings such as different task characteristics (words versus picture stimuli), sample size and sample heterogeneity in terms of *e.g.*, illness severity. Nonetheless, our large sample size adds weight to our results. For outpatients and implicit emotional processing of facial expressions we believe our study constitutes a benchmark for further studies.

Emotional words – fMRI study – Visual emotional signals can be transmitted by mime or textually. However, to our knowledge, only few studies so far have examined emotional processing of textual verbal stimuli in depression or anxiety disorders. In Chapter 4 we aimed to delineate the neural mechanism responsible for emotional evaluation of word stimuli in outpatients with depression and/or anxiety. The findings showed that anxiety patients had a stronger emotional response to negatively evaluated words than healthy participants. In MDD patients a positive association was observed between amygdala response to negative evaluated words and MADRS score. This leads us to conclude that aberrant amygdala response is linked to illness severity rather than being a binary (*i.e.,* an all-or-nothing) diagnostic characteristic of MDD.

Differences between the two tasks – The word-evaluation task and the faces task induced different neural responses: perception of facial expressions of emotions induced no significant amygdala hyperactivation in outpatients with MDD and/or anxiety disorders, whereas significant amygdala hyperactivation was found in anxiety patients during emotional evaluation of words.
We could say that the word-evaluation task may be a more demanding task than the faces task, because it involves explicit emotional judgment and triggers emotional memories, *e.g.*, during emotional evaluation of negatively perceived words [15]. However, it remains an open question whether emotionally perceived words are more arousing than facial expressions. Within the experimental framework of this study we could not investigate this aspect.
Thus, being self-critical, if during perception of facial expressions of emotions no significant amygdala hyperactivation was observed in outpatients with MDD and/or anxiety disorders, whereas significant amygdala hyperactivation was found in anxiety patients during emotional evaluation of words, we can attribute the

contrast to the task differences, *e.g.,* task demand (gender discrimination versus emotional attribution to words) and stimulus type (faces versus words).
With regard to the comorbidity of depression and anxiety we suggest that it is not to be regarded as a simple summation of the two diagnoses: the brain activation patterns were very different from those in patients who were only depressed or only anxious. Further, even though the coexistence of depression and anxiety is associated with more severe clinical symptoms, surprisingly the amygdala response to emotional stimuli (facial expressions of emotion or words) in DAC patients was indistinguishable from that in HC (Chapter 3 and 4).

Amygdala connectivity – Connectivity modeling of brain functions associated with emotional and cognitive processes has become increasingly popular over the last years. In this framework, at the end of almost a decade of neuroimaging studies with different methods, the amygdala was confirmed by a meta-analysis to be part of a complex network of connections having direct or indirect connections with regions involved not only in emotional processing but also in cognitive processes [16, 17].
An exciting new field of the last years is functional connectivity of brain regions associated with emotional processing in conjunction with personality traits [18, 19]. For example, a mood induction study with positron emission tomography (PET) showed that neuroticism is associated with changes in subgenual cingulate coupling with prefrontal regions during mood induction, possibly reflecting a susceptibility marker for depression [19]. Furthermore, amygdala–ACC coupling was suggested to be modulated by trait anxiety [20]. In generalized anxiety disorder increased dorsolateral frontal cortex–amygdala functional connectivity was reported during resting state and this coupling was negatively correlated with anxiety [21].
Considering the above studies and the findings presented in the first chapters of this thesis, it was only natural for us to wonder about the existence of differences in connectivity that may be even more relevant than differences in neural activation. The main aim of the fifth and sixth chapters of this thesis was to examine how amygdala–frontal regions connectivity is influenced by neuroticism scores and, in addition, to look for diagnosis specific effects in patients with anxiety disorders. In other words, we aimed for a better understanding of the neural basis associated with vulnerability for the development of affective disorders. Furthermore, we continued the investigation of the BOLD effects in the emotional neural network in anxiety disorders, such as panic disorder (PD) and social phobia (SP).
We observed that individual differences in neuroticism corresponded with differences in functional connectivity of amygdala with prefrontal regions during processing of negatively perceived emotional material (Chapter 5). Persons high in neuroticism might have less ACC related inhibitory control over the amygdala during perception of negative facial expressions of emotions. The opposite was also observed: a positive association between neuroticism and amygdala– dmPFC coupling. This suggests that persons with high neuroticism may have more pronounced self-referential processing during processing of negative emotions. This connectivity pattern provides insight into the neural mechanism associated with vulnerability to affective disorders, such as depression and anxiety.
In Chapter 6 we aimed to examine the neural basis of emotional processing in PD and SP patients relative to HC looking not only at differences in amygdala–frontal cortex coupling but also at abnormal brain activation during perception of positive and negative facial expressions of emotion. We observed that during perception of happy facial expressions PD patients with or without SP had amygdala hypoactivation, relative to HC. Additionally, patients with SP+PD comorbidity showed amygdala hypoactivation to fearful faces, compared to HC. One explanation for this apparently paradoxical result (usually hyperactivation is found in anxiety disorders) may be that enhanced amygdala activation may also be present during the control condition (scrambled faces) so that no significant activation is seen when subtracting baseline from the fearful or happy faces conditions. Another explanation may be a lack of emotional response to positive emotions in PD with or without SP.
Interestingly, SP patients showed no significant differences compared to HC in the neural response to perception of facial expressions of emotions. However, a negative amygdala–frontal coupling was found during perception of fearful faces in SP compared to HC. The frontal cortex–amygdala circuit has been previously associated with emotion regulation processes [22, 23]. We may conclude that even though SP patients showed no gross abnormalities at the neural activation level, they did show a different functional connectivity within the brain network responsible for emotional perception, relative to HC.

The studies presented in Chapter 5 and 6 add to the existing literature on the presence of abnormal neural network activity associated with vulnerability to affective disorders and anxiety disorders. At first glance, the

findings do not seem to be entirely consistent. The level of neuroticism was found to correlate with connectivity between amygdala on the one hand and anterior and medial frontal regions (ACC and dmPFC) on the other during negative emotions.
However, in patients with (only) SP abnormal amygdala–middle frontal gyrus coupling was observed during perception of fearful facial expressions. The abnormal functional pattern of connectivity seen in high neuroticism is similar, but not identical to that in anxiety disorders patients. This suggests a potential vulnerability marker for affective disorders during negative emotional perception. A long-term approach may reveal markers of disease vulnerability. Hence, further studies are needed to support our hypothesis in anxiety disorders. Dynamic causal modeling may give a better insight in the neural mechanism of emotional processing in anxiety patients, such as the direction of these connections, *i.e.*, top-down or bottom-up regulation.

Remarks on the studies – The findings presented in this thesis are characteristic for community patients with MDD and/or anxiety disorders selected from primary care.
We observed that patients with depression and anxiety comorbidity are not to be considered as people with a summation of depression and anxiety. Depression and anxiety often arise sequentially in one patient [24] and because depression and anxiety comorbidity has a high prevalence and DAC patients are less likely to respond to treatment [25], it is important to further understand the neural mechanism - its development and manifestations - of this state.
In the last years there was some debate regarding the validity of a categorical distinction between depression and anxiety [26]. For the new DSM-V it has been proposed that the coexistence of depression and anxiety be considered as a separate diagnosis defined therein as Mixed Anxiety Depressive Disorder (MADD). Research criteria for such a diagnostic category were already included in the DSM-IV [27]. Reports based on large samples from primary care suggested the existence of this mixed condition (*e.g.*, Ormel et al. [28]). However, distinguishing MADD as a separate category has also been criticized on conceptual and empirical grounds [29]. Further studies are needed to confirm if MADD may be considered as a separate diagnosis, not only looking at symptoms severity, course and outcome, but also looking at the neural mechanism associated with emotional or cognitive processes. The same holds true for the comorbidity of anxiety disorders (social phobia and panic disorder) which does not seem to be a summation of SP and PD at the neural level. Thus, we suggest that future studies should control for the co-existence of a second diagnosis because – as observed in our study – the presence of comorbidity should be considered as a distinct diagnosis.
The neural mechanism associated with emotional processing may be studied using different paradigms. From our study the importance of the paradigm used to study emotional or cognitive processes, is more than apparent. For example, it is difficult to even directly compare the two studies presented in Chapter 3 and 4, because there are differences not only in the task demands (gender discrimination versus emotional attribution), but also in the stimuli used (faces versus words).
We observed no significant difference in illness severity between medicated and medication-free patients. This may lead us to conclude that aberrant amygdala response is associated with symptom severity rather than being diagnosis specific. This may not be the case for anxiety disorders when it comes to explicit emotional processing, where greater amygdala activation to negatively evaluated words was observed relative to controls. One explanation may be, as mentioned earlier, that amygdala is involved in earlier processing underlying emotional evaluation, as suggested by Lee et al. [15]. Another explanation may be that emotional evaluation of words trigger emotional memory leading to a stronger emotional response in anxiety patients and in depressed patients. However, given the structure of our experiment, it is not possible to conclude for the studies presented in Chapter 3 and 4 that words are perceived as more arousing or more emotional than facial expressions.
Finally, we observed that even if there are no gross abnormalities in the neural response in social phobia compared to HC, it might be that a distinct neural network is involved in emotional processing. Thus, further studies will need to test our hypothesis that outpatients with depression or anxiety disorders have an aberrant connectivity but no large differences in regional brain activation.

Novelty – The research presented in this thesis is a step forward in understanding the neural mechanisms associated with emotional processing in outpatients diagnosed with major depressive disorder (MDD) and/or anxiety disorders. Two features distinguish this thesis: the first one is that the study herein is based on large sample and the second one is that this study employs not only MDD and anxiety subjects, but it concurrently considers depression and anxiety as an extra group. Whereas the first aspect allows for a high degree of

confidence in the detected differences at statistically significant levels, the second feature may help to determine if the co-occurrence of depression and anxiety disorders can be conceived of as a summation of the two disorders or as a separate condition.
In terms of novelty, this thesis identifies the default network of emotion perception of community outpatients for two different types of emotional stimuli. We observed that perception of facial expressions of emotions in community-based outpatients relied to a large extent on the same neural mechanism as in healthy participants. It should be noted, however, that negative emotional evaluation of words elicited a stronger emotional response in anxiety outpatients relative to healthy volunteers. This indicates that evaluation of emotional words lead to an increased emotional response in patients with anxiety disorders.
Furthermore, this work shows that even if significant abnormalities in the neural response of certain regions are lacking, abnormalities related to their connectivity can occur. This shows that emotional perception is affected not only by the abnormalities in the neural response but also by abnormalities in connectivity. It is obvious that this unveils a much more intricate picture of emotional perception, one that is not necessarily restricted to outpatients. Indeed, our findings support the notion that psychiatric disorders are not due to dysfunction in single brain regions, but may be a consequence of disturbed interactions in large-scale networks. The precise dynamics thereof should be further determined in future research, *e.g.*, using advanced analysis methods such as dynamic causal modelling (DCM) or Granger causality mapping.

Conclusion – The work presented in this thesis contributes to the existent literature in that it offers an insight in the neuropsychopathology of MDD and anxiety in community outpatients. We suggest that co-existence of depression and anxiety disorders are not to be considered as summation of the two. The differential pattern of activation may be regarded as consistent with proposals of mixed anxiety depressive disorder as a distinct condition. MDD was not associated with trait-related functional brain abnormalities, as illness remission did not seem to be associated with changes in brain areas responding to emotional stimuli. Indeed, our findings suggest that functional abnormalities in brain activation are more related to illness severity – a state marker –, rather than being a trait marker. This hypothesis is also supported by antidepressant or anxiolytic medication studies, which showed that after treatment, there was reduced symptom severity and less aberrant neural activation [4, 30, 10]. We also found that explicit emotional processing triggers stronger amygdala response in patients with anxiety or depressions, which is consistent with prior findings in groups with more severe psychopathology. Whit regard to vulnerability for affective disorders, the present work showed that level of neuroticism predicts coupling of amygdala–fronto-medial regions and abnormalities of this network may be a marker for vulnerability. Lastly, we may conclude that even if there are no gross abnormalities at the level of regional brain activation, there may be an abnormal neural network connectivity associated with emotional or cognitive processes in patients compared to controls.

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