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The Stress Connection

Neuroimaging studies of emotion circuits in social stress, personality, and stress-related psychopathology

Ilya Milos Veer

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CHAPTER 1

General introduction

Chapter 1

The research described in this thesis revolves around the question of how stress impacts brain circuits involved in emotion perception and regulation, and how structural and functional changes in these circuits are implicated in the pathophysiology of stress-related neuropsychiatric disorders. This chapter serves as a brief overview and introduction of the main concepts and methods that are central to the research described in this thesis. First, the stress system and its main signaling agents are introduced, the effects of stress on cognition and emotion are discussed, and the intimate relation between stress and stress-related neuropsychiatric disorders is reviewed. The second part of this chapter offers an introduction to resting-state functional magnetic resonance imaging (fMRI), a neuroimaging method used in the majority of experiments described in this thesis, together with an overview of the most common data acquisition and analysis strategies. The introduction concludes with an outline of the experiments that were carried out for this thesis.

STRESS AND THE BRAIN

Every living organism is equipped with an innate system to adaptively cope with situations that threaten its bodily or psychological integrity, which are also known as stressors (McEwen, 2007; Selye, 1936). When facing a stressor, be it exogenous or endogenous, physical or psychogenic, the central nervous system orchestrates a cascade of (neuro)endocrine reactions that ensure an adequate response, thereby promoting survival of the organism (Joëls & Baram, 2009). The amygdala, located in the brain in the medial temporal lobe, just anterior to the hippocampus, is key in evoking stress responses (Ulrich-Lai & Herman, 2009). More specifically, sensory information is rapidly screened on importance by the amygdala, after which it will signal potential danger or, more generally, emotional salience of the incoming information to the rest of the brain (Hariri & Whalen, 2011; LeDoux, 2000; Phillips, Drevets, Rauch, & Lane, 2003a).

The prime function of the stress system is to activate the organism in order to undertake actions that are necessary to deal with the immediate threat. This phase

of the stress response is commonly known as the *fight-or-flight* response (Cannon, 1932), although *fright*, *freeze*, and *faint* nowadays are included in the spectrum of typical reactions to an acute stressor as well (Bracha, Ralston, Matsukawa, Williams, & Bracha, 2004). When facing stress, the amygdala activates the autonomic nervous system (ANS) through its neuronal projections to several brainstem nuclei. The ANS, in turn, promotes a rapid physical and behavioral response through the release of catecholamines, such as adrenaline (from the adrenal glands) and noradrenaline (from the locus coeruleus in the pons) (Ulrich-Lai & Herman, 2009). Typical autonomic stress effects mediated by the sympathetic arm of the ANS include the rise of heart rate and blood pressure, perspiration, dilation of the pupils, and an increase in overall arousal. The stress system is also equipped to adjust the initial autonomic phase of the stress response, so to enable the organism to return to a basal physical and behavioral state after the stressor has waned. This state is also known as *homeostasis*, and is mainly achieved by the parasympathetic arm of the ANS, and through activation of the hypothalamic–pituitary–adrenal (HPA) axis. Glucocorticoids, cortisol in humans, are the end product of the HPA-axis, and are secreted by the adrenal cortices (Sapolsky, Romero, & Munck, 2000; Ulrich-Lai & Herman, 2009). Whereas (nor)adrenaline exerts its effects in the order of tens of seconds, cortisol typically acts in the order of tens of minutes, and even longer, after perceiving a stressor (Joëls & Baram, 2009) [1].

Following its release, cortisol acts back on the HPA-axis in a negative feedback loop, so to attenuate the stress response and concurrent HPA-axis activity. This is mediated through corticosteroid receptors, of which two types can be discerned: mineralocorticoid (MR) and glucocorticoid (GR) receptors. The two receptors have differential binding properties, with MR's having a much (five- to tenfold) higher affinity for cortisol than GR's (Reul & de Kloet, 1985). Consequently, cortisol will mostly bind to GR's, which are ubiquitously distributed in the brain, either during

[1] (Nor)adrenaline and cortisol have been the two most studied stress agents. However, these are just a few among the many other agents involved in the stress response. As a comprehensive overview of these agents is beyond the scope of this thesis, the interested reader is referred to two excellent and detailed reviews on the neurobiology of stress (Joëls & Baram, 2009; Ulrich-Lai & Herman, 2009).

the peaks of diurnal cortisol secretion or during times of stress. MR's, in contrast, are found in more restricted brain areas, including the hippocampus, and will be bound even during the nadir of diurnal cortisol secretion (Reul & de Kloet, 1985; Sapolsky et al., 2000). Therefore, it is believed that MR's play an important role in fine-tuning normal fluctuations in activity of the HPA-axis (tonic regulation), whereas GR's are deemed crucial in regulating the stress system in response to a stressor (phasic regulation) [2].

Brain regions rich in corticosteroid receptors, such as the hippocampus, amygdala, and medial prefrontal cortex (mPFC), have been identified to mediate negative feedback of the HPA-axis, and the stress response in general (Herman, Ostrander, Mueller, & Figueiredo, 2005). Not surprisingly, these are the very same regions that fulfill a critical role in the cognitive processes related to stress perception and regulation, and have been shown sensitive to both anatomical and functional alterations in stress-related psychopathology. Hence, this will be the topic of the next two sections.

STRESS, COGNITION, AND EMOTION

Every organism needs a well functioning stress system to cope and interact with the complex and challenging environment it is exposed to in everyday life. To this end, adaptation to a stressful situation is achieved on multiple levels of the organism, from cell physiology to behavior. Whereas the previous section was more concerned with the neuroendocrine cascade following a stressor, this section will focus on how stress and stress hormones influence cognition and emotion, as well as the brain regions involved in these processes.

[2] Although cortisol does play a critical role in reaching homeostasis, its actions can differ markedly depending on the physiological endpoint of the action. In addition, cortisol causes immediate non-genomic, as well as slower genomic effects. This all contributes to a heterogeneous and rather complex picture of cortisol action, which can either be permissive, suppressive, or preparative (Sapolsky et al., 2000).

MEMORY

Without a doubt, memory has been studied most extensively in relation to stress over the past decades. It was the initial discovery that the hippocampus, a key structure in memory processes (Squire & Zola-Morgan, 1991), has a high affinity for glucocorticoids (McEwen, Weiss, & Schwartz, 1968), which has prompted this line of research. Nowadays, the effects of stress and glucocorticoids on memory are rather well mapped (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Wolf, 2009). Nonetheless, these effects are not always easy to understand given the (sometimes) paradoxical results, mostly depending on the specific memory process under scrutiny and timing with respect to stress exposure or administration of glucocorticoids.

Early studies demonstrated that increases in cortisol in response to psychosocial stress (Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996) or a pharmacological intervention (Newcomer, Craft, Hershey, Askins, & Bardgett, 1994) were related to reduced declarative memory performance. However, in a later stage it became apparent that this detrimental effect was mainly observed for retrieval of learned material (de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Kuhlmann, Piel, & Wolf, 2005; Wolf et al., 2001). In addition, lesser retrieval performance due to increased cortisol was demonstrated to be related to decreased hippocampal activity (de Quervain et al., 2003; Oei et al., 2007).

In contrast, (stress-induced) cortisol elevations seemed to be beneficial for memory encoding and consolidation. Several studies demonstrated increased memory performance when stress or cortisol was administered either before or after encoding of the material that had to be learned (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003; Maheu, Joober, Beaulieu, & Lupien, 2004). Nevertheless, this enhancing effect was often only found for emotionally arousing material (Buchanan & Lovallo, 2001; Cahill, Gorski, & Le, 2003; Kuhlmann & Wolf, 2006; Smeets, Otgaar, Candel, & Wolf, 2008). This observation motivated researchers to study the function of the amygdala in stress effects on memory, given its important role in saliency detection and the stress response in general. Indeed, the amygdala seems to facilitate memory consolidation of emotionally salient information through its in-

teractions with the hippocampus (McGaugh, 2004), which has been found to critically depend on the interplay between cortisol and noradrenaline in both structures (Roozendaal, McEwen, & Chattarji, 2009; Strange & Dolan, 2004; van Stegeren, Wolf, Everaerd, & Rombouts, 2008). Flashbulb memories, the vivid and detailed recollections of emotionally impacting events (Brown & Kulik, 1977), are, for example, likely to result from interactions between the amygdala and hippocampus.

Enhanced memory consolidation for emotionally salient information after a stressful experience seems beneficial, as it enables us to recognize and adapt to future challenges more easily. However, whether stress-induced impairment of memory retrieval serves an adaptive role is unclear (cf. exam stress), though it has been argued that this constitutes a mechanism to prevent a negative emotional overshoot in the face of acute stress, or might facilitate encoding of the current stressful situation without conflicting intrusions from previously stored information (Wolf, 2009) [3].

WORKING MEMORY

The second cognitive domain that throughout the years could count on considerable attention by stress researchers has been the domain of executive functions. Just as the hippocampus, the discovery of glucocorticoid receptors in the prefrontal cortex (PFC) inspired researchers to test the effects of stress and cortisol on this part of the brain in both rodents and humans (Cerqueira, Almeida, & Sousa, 2008; Kern et al., 2008; Wang et al., 2005), as well as on prefrontal-dependent cognitive processes,

[3] Cortisol seems to exert its effects in an inverted U-shape fashion. This means that the effects of cortisol on memory (or cognition in general) can be different, depending on the dose of administered cortisol, severity of stress, time of testing (given the normative diurnal pattern of cortisol secretion, with a peak in the morning and decreasing levels throughout the day), but also by basal cortisol differences related to age and gender. In addition, effects likely differ depending on whether the memorized material relates to the stressor or not (Lupien et al., 2007). Although study results from the past decades do indicate some level of consistency, more research is clearly needed to elucidate the precise mechanisms underlying glucocorticoid effects on memory.

such as working memory (Baddeley, 2003). Rather consistently, detrimental effects on working memory have been described after cortisol administration (Lupien, Gillin, & Hauger, 1999) or psychosocial stress (Elzinga & Roelofs, 2005; Luethi, Meier, & Sandi, 2008; Oei, Everaerd, Elzinga, van Well, & Bermond, 2006; Schoofs, Preuß, & Wolf, 2008).

Neuroimaging studies on the effects of stress on prefrontal-dependent cognition found impaired attentional control and reduced fronto-parietal coupling (Liston, McEwen, & Casey, 2009), while dorsolateral prefrontal cortex (dlPFC) activation during a working memory task was found reduced after stress (Qin, Hermans, van Marle, Luo, & Fernández, 2009), though in absence of an effect on performance. Other studies, in contrast, found increased dlPFC activation, either after physical stress (Porcelli et al., 2008), or even several hours after cortisol administration (Henckens, van Wingen, Joëls, & Fernández, 2011). This discrepancy in findings might, however, be explained by the type of stressor, differences in cortisol levels, but also the different types of tasks used to assess working memory. In sum, although the exact direction of the effects are not yet fully understood, stress and cortisol appear to have both immediate and prolonged effects on PFC-dependent working memory functioning.

EMOTION

Stressful situations will often lead to a specific emotion or influence the way we process emotional information, yet an emotion does not necessarily have to be accompanied by a stress response (Lupien et al., 2007). Nevertheless, although stress and emotion can be considered separate entities, the two are intimately linked. As was described previously, the amygdala is a crucial region for both salience detection and initiation of stress responses. In addition, the amygdala is a key binding site for both glucocorticoids and noradrenaline (Roozendaal et al., 2009), and is therefore a likely candidate to mediate stress effects on emotion processing.

It could be argued that the effects of stress on emotional memory consolidation, as were described previously, might be related to an increase in attention towards emotionally arousing information. Indeed, several studies have related stress to increased amygdala activity in response to emotionally salient stimuli, either by pharmacologically manipulating noradrenaline levels (Onur et al., 2009; van Stegeren et al., 2005), or after exposure to a stressful situation (van Marle, Hermans, Qin, & Fernández, 2009). Intuitively, such a mechanism seems quite adaptive, since rapid appraisal of a potentially threatening situation will likely increase our chance of survival. Effects of cortisol, on the other hand, appear to be reversed compared with noradrenaline. For example, reduced selective attention for emotionally arousing stimuli has been observed after cortisol administration (Putman & Berling, 2011; Putman, Hermans, & van Honk, 2010). Another study showed time-dependent effects of cortisol on emotion processing, indicating an acute effect reflected by reduced amygdala activity in response to emotional facial expressions irrespective of valence, while hours after cortisol administration suppressing effects were only found for positive faces (Henckens, van Wingen, Joëls, & Fernández, 2010).

To date, effects of stress and stress hormones on emotion regulation are, surprisingly enough, still rather sparse. Recently, it was shown that social stress could diminish the positive effects of an acquired emotion regulation strategy during fear conditioning (Raio, Orederu, Palazzolo, Shurick, & Phelps, 2013), while a study from our group, described in **chapter 2** of this thesis, demonstrated that social stress reduces the ability to inhibit emotionally salient distracting stimuli (Oei, Veer, Wolf, Rombouts, & Elzinga, 2012). However, within the stress group higher cortisol was related to better distracter inhibition, a finding that was replicated after cortisol administration (Oei, Tollenaar, Spinhoven, & Elzinga, 2009). These results were further substantiated in a more recent study, in which evidence was found for beneficial effects of moderate, but not high, cortisol levels on the inhibition of negative stimuli (Taylor, Ellenbogen, Washburn, & Joober, 2011). However, research on modulating effects of cortisol on emotion processing and regulation is still sparse and needs further attention in future studies.

STRESS AND PSYCHOPATHOLOGY

Our stress system seems to be specifically designed to adapt to short lived stressors. When we face a stressful situation, the cascade of actions that is initiated on the biological and behavioral level serves the purpose of removing the threat, and of subsequent recovery to homeostasis (Cannon, 1932). Hans Selye first described this cascade as the *general adaptation syndrome* in the early decades of the last century (Selye, 1936), though more recently the term *allostasis* has been introduced (McEwen, 1998; 2008). In contrast, long-term exposure to stress and severe acute stress have been related to prolonged activation of the stress system, conveying a higher probability of developing somatic disease and stress-related psychopathology (Brosschot, 2010). This prolonged state is also known as *allostatic load* (McEwen, 1998).

It is now widely acknowledged that the HPA-axis plays an important role in the pathophysiology of these disorders. For example, disturbed function of the HPA-axis has been reported for major depressive disorder (Belvederi Murri et al., 2014; Burke, Davis, Otte, & Mohr, 2005), though not always (Knorr, Vinberg, Kessing, & Wetterslev, 2010), and posttraumatic stress disorder (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007; Morris, Compas, & Garber, 2012), though this seemingly depended on the type of trauma, gender, and comparison group used. Imbalance in the noradrenergic system, on the other hand, has been linked to a wide range of anxiety disorders (Kalk, Nutt, & Lingford-Hughes, 2011), providing a clear link with the sympathetic symptoms that so often accompany these disorders.

Not surprisingly, key brain regions involved in regulation of stress-responses have been implicated in the pathophysiology of most stress-related psychiatric disorders as well, both on a functional and anatomical level (Drevets, Price, & Furey, 2008; Liberzon & Sripada, 2008; Mayberg, 1997; 2003; Phillips, Drevets, Rauch, & Lane, 2003b; Shin & Liberzon, 2010). Most studies in these disorders report increased amygdala activation to negatively arousing stimuli (Groenewold, Opmeer, de Jonge, Aleman, & Costafreda, 2013; Hamilton et al., 2012; Shin & Liberzon, 2010), a finding that closely mimics results from healthy controls obtained in the face of stress. Whether altered amygdala volumes accompany these functional changes, however,

is still a topic of debate (Hamilton, Siemer, & Gotlib, 2008; Shin & Liberzon, 2010; Woon & Hedges, 2009), and is therefore the topic of one the studies of this thesis.

Volume reductions of the hippocampus, in contrast, have been demonstrated repeatedly in major depression (Arnone, McIntosh, Ebmeier, Munafò, & Anderson, 2012; Koolschijn, van Haren, Lensvelt-Mulders, Hulshoff Pol, & Kahn, 2009) and posttraumatic stress disorder (Shin & Liberzon, 2010; Woon, Sood, & Hedges, 2010). These findings are in line with hippocampal atrophy related to long-term stress exposure (McEwen, 2008), again pointing at the close connection between the stress system and these disorders. On the functional level, hippocampus based memory function is often found compromised in depression (MacQueen & Frodl, 2011; Rock, Roiser, Riedel, & Blackwell, 2013), while mixed results have been observed in posttraumatic stress patients (Shin & Liberzon, 2010).

Central to the neurobiology of stress-related psychiatric disorders is the proposed failure of the prefrontal cortex in exerting top-down regulatory control over hyperresponsive ventral affective brain areas, including the amygdala (Phillips, Drevets, Rauch, & Lane, 2003b), which are key areas implicated in adaptive emotion regulation (Ochsner et al., 2004), and attenuation of stress responses (McEwen, 2008) as well. In depression, for example, hypo-activity of dorsal prefrontal areas has been observed, while the ventral subgenual anterior cingulate appears hyperactive (Drevets et al., 2008). In addition, abnormal interactions between the mPFC and amygdala were found in depressed patients during intentional emotion regulation (Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007). Findings of reduced volume in several regions of the lateral and medial PFC in depression might in fact underlie these functional differences (Koolschijn et al., 2009; van Tol et al., 2010). Similarly, abnormal structure and function of the prefrontal cortex has been linked to a range of anxiety disorders as well (Etkin & Wager, 2007; Shin & Liberzon, 2010; van Tol et al., 2010), while decreased feedback from the mPFC to the amygdala appears to underlie pathological anxiety (Kim et al., 2011b).

Lastly, it is important to note that the stress system can be targeted and disrupted at different stages in life, and that this might have different effects on brain structure and function. For example, the key regions of the stress system still develop

until late in adolescence and early adulthood. Consequently, severe acute or chronic stress might impede the normal neurodevelopmental trajectory, which could render the brain vulnerable for psychopathology later in life. Moreover, several lines of research indicate that different pathological conditions might arise depending on when in life someone is exposed to stress, as well as on the duration of exposure (Lupien, McEwen, Gunnar, & Heim, 2009).

INTERIM SUMMARY

Up to now, the key neuroendocrine responses and brain regions involved in initiating and regulating the stress response have been identified and discussed. In addition, effects of stress and stress hormones on cognition and emotion processing were reviewed, as well as the intimate relation between stress, emotion regulation, and neuropsychiatric disorders. Whereas increased attention towards, and prioritized processing of emotionally salient stimuli promotes swift action to remove the threat, it is equally important to disengage from this response when it is no longer needed. Furthermore, it seems pivotal to store these stressful situations in our memory, which might enable us to better predict and respond to similar challenges in the future.

Stress, emotion processing and regulation, and stress-related psychiatric disorders are complex concepts, often spanning a wide range of physical, cognitive, and behavioral aspects. As these cannot possibly emerge from (a breakdown of) any brain region in isolation, it should rather be the interplay between brain regions that generates these complex phenomena. After all, the brain is a network of interconnected neurons, and should perhaps best be studied as such. Over the past decade, the field of cognitive neuroimaging has slowly started to move from a localizationist to a connectionist point of view. One imaging technique that allows us to study functional connections between brain regions, and has caught the eye of many researchers in the field, is resting-state functional connectivity. As this technique has been employed in several studies of this thesis, the next sections will be dedicated to the history of resting-state fMRI, and resting-state data acquisition and analysis.

A BRIEF HISTORY OF RESTING-STATE FMRI

In 1995, dr. Bharat Biswal and colleagues published their more or less serendipitous finding that synchronized blood-oxygen-level dependent (BOLD) signal fluctuations of the left and right motor cortex could be observed even when participants were not actively engaged in a motor task (Biswal, Yetkin, Haughton, & Hyde, 1995). Although received with initial skepticism, in the years that would follow the field of (cognitive) neuroscience gradually picked up on the idea that brain activations measured in absence of an externally cued task might actually convey important information about the functional organization of the central nervous system.

Following the initial finding of synchronized motor cortex activity, the phenomenon, termed resting-state functional connectivity by its discoverers (Biswal et al., 1995), would also be demonstrated for a set of brain areas involved in language processing and speech production (i.e., Broca's and Wernicke's) (Hampson, Peterson, Skudlarski, Gatenby, & Gore, 2002), as well as for key regions of the visual stream (Hampson, Olson, Leung, Skudlarski, & Gore, 2004). These findings, together with the discovery in the early 2000's of a set of interconnected regions known as the *default mode network* (Greicius, Krasnow, Reiss, & Menon, 2003; Raichle et al., 2001), sparked the emergence of a new direction in neuroimaging research. Consequently, the last decade has witnessed a tremendous flight in studies on resting-state functional magnetic resonance imaging (RS-fMRI), which is illustrated in **Figure 1.1**: Since its conception in 1995 the number of articles published on resting-state functional connectivity or activity has grown exponentially, and will likely follow this trend in the foreseeable future.

WHY RESTING-STATE FMRI?

Whereas the study of resting-state MRI was predominantly the domain of MR physicists and methodologists at first, it steadily became popular among scientists from other disciplines, such as psychology and medical science, both from a fundamental

Figure 1.1 Exponential growth of the number of studies published on resting-state functional connectivity or activity since 1995, as identified with the following PubMed search query: *(resting [TIAB] OR resting state [TIAB] OR steady state [TIAB]) AND (functional connectivity [TIAB] OR (BOLD [TIAB] AND low frequency fluctuations [TIAB])) AND <year> [DP].*

and applied research perspective. From a medical point of view the emergence of resting-state fMRI in clinical research was considered nothing short of a blessing. That is, with this method medical researchers were finally able to acquire a measure of functional integrity of the brain in even the most cognitively disabled patient groups, as successful acquisition did not rely on the patient being able to meet task demands. However, it is oftentimes necessary to defend to psychologists why someone would want to study a participant during a "resting state". How could we ever draw conclusions on behavior and cognition without knowing what a participant does or thinks? First, it is important to note that this argument could, to some extent, also be raised for task fMRI studies, even though the participant's thoughts are more directed towards the cognitive process studied. Oftentimes we just do not know what kind of

task strategy a participant used, or whether there was some mind wandering going on during a low-level baseline task. Second, the term "resting-state" is somewhat misleading. Obviously, the brain does not shut down completely without external stimulation, so cognition and behavior do not begin or end with an externally cued task. It is equally interesting to study the brain while preparing (or expecting) a task, or when it is consolidating past experiences. Third, resting-state fMRI is especially useful for studying diffuse states of the brain, such as when sleeping, being under the influence of drugs, feeling stressed, or having a lowered mood.

In addition, resting-state functional connectivity networks have shown remarkable correspondence to patterns of task activation, which suggests that large-scale neural systems are configured rather consistently, even while "at rest" (Smith et al., 2009). Moreover, the same networks are found across participants, studies, and study groups (Biswal et al., 2010; Damoiseaux et al., 2006), and show good within-subject reproducibility (Shehzad et al., 2009).

Taken together, the success of resting-state fMRI in the field of cognitive neuroscience should likely be sought in the easy acquisition parameters, independence of elaborate task designs, and broad applicability in patient groups and cognitive states that are otherwise difficult to study in an MRI scanner. How resting-state fMRI data are typically acquired and analyzed will be the topic of the next section.

RESTING-STATE FMRI METHODS

Currently, broad consensus on how to analyze resting-state data is still lacking, and debates on resting-state analysis strategies are ongoing with the goal to achieve a gold standard for the field. As such, this section is rather intended to provide the interested reader an introduction to the techniques commonly used by resting-state researchers. Additionally, it serves as a broader introduction to the methods sections of the chapters in this thesis for which resting-state fMRI data were acquired and analyzed.

ACQUISITION

As became evident in the last section, it is relatively easy to acquire resting-state data, since one does not have to worry about elaborate task designs or compliance of participants with the task. However, this does not necessarily mean that other acquisition aspects are trivial. For example, at which point in the scan protocol should you acquire your resting-state data? The answer is: it depends. Oftentimes, data are acquired as a sort of bonus scan, and are therefore placed somewhere in between all the other scans as a filler. However, most people then tend to disregard the notion that a preceding task could influence the resting-state measure (Barnes, Bullmore, & Suckling, 2009; Pyka et al., 2009). To remove this potential confound, one could start with the resting-state scan, although this might in turn be confounded by scanner anxiety at the start of the protocol, or be influenced by situational factors directly before the participant entered the scanner. Another option would be to use the set of anatomical scans acquired in most experiments as a buffer between task and resting-state acquisition. Last, and from an experimental point of view probably best, one might design the experiment in a way that modulatory effects are actually welcomed.

Another choice pertains to the instructions the researcher gives. Let us look at a typical resting-state instruction, inherited from the early days: "Please lie still with your eyes closed, relax, and do not think of something in particular". The first question that arises is: why eyes closed? Likely, researchers wanted to stimulate introspective thought and mind wandering with this instruction, which has been a main interest of the field when the technique emerged. It seems that resting-state connectivity networks are quite similar when comparing eyes closed and open conditions, though the latter appears to give stronger correlations (Patriat et al., 2013; van Dijk et al., 2010). Whether participants fixate on a screen or just have their eyes open does not seem to differ. Importantly, eyes open acquisition will protect participants against feeling drowsy, or even falling asleep, states that both have been related to altered connectivity (Horovitz et al., 2008; Sämann et al., 2011).

Secondly, do we want participants to instruct to think of nothing in particular? A parallel is easily made with the classical instruction: "Do not think of a white

bear" (Wegner, Schneider, Carter, & White, 1987). Paradoxically, participants will think more of something they try to suppress. Yet above all, it is probably hard to define for a participant what *nothing in particular* would be anyway. Therefore, this part of the instruction can probably best be left out. Lastly, though not in the example, telling the participant how long the scan takes could lead to mental counting during acquisition, which might be an unwanted effect as well.

Almost without exception, T_2^*-weighted echo-planar imaging is used as the preferred scanner sequence, similar to what most researchers use for task fMRI. At this point, important choices have to be made related to the repetition time (TR), and to the number of volumes. Often, the TR is chosen equal to the TR of task acquisitions, which is typically between 2-3 seconds for whole brain coverage. Faster sampling, thus lower TR's, are generally always better, allowing richer characterization of the signal, and improved identification of higher frequency artifacts. However, this comes at the cost of lower spatial resolution, or one has to consider partial field of view acquisition. Though perhaps stating the obvious, the researcher is advised to use the same sequence for task and resting-state acquisition if the goal is to compare the two scans.

The length of acquisition (i.e., the number of volumes) should be chosen next. When resting-state data are acquired as a bonus, acquisition time is often chosen as short as possible due to the range of other scans acquired in the scan protocol, yet typical acquisition lengths are mostly kept between 5-10 minutes, which corresponds to 150-300 volumes with a rather standard TR of 2 seconds. Although it has been shown that connectivity strengths stabilize even at brief acquisition times of around 5 minutes (van Dijk et al., 2010), and that connectivity networks can be identified with acquisitions as short as 30 seconds (Jones et al., 2012), a recent study demonstrated that the reliability of connectivity measures, both within and between sessions, could be greatly improved when acquiring data for longer than 10 minutes (Birn et al., 2013). This might, as such, be of importance for longitudinal and multicenter studies especially.

ANALYSIS

The previous sections and studies described later in this thesis focus on measures of resting-state functional connectivity. It must, however, be acknowledged that resting-state data allows a richer description of the signal measured than covariation between brain regions alone. The main dichotomy that can be made is one of studying local or global resting-state characteristics. While functional connectivity is considered a *global* feature, one could, for example, also look at *local* changes in homogeneity of the resting-state signal between neighboring voxels (Zang, Jiang, Lu, He, & Tian, 2004), changes in signal amplitude (Zuo et al., 2010), or changes in fractal properties (Wink, Bullmore, Barnes, Bernard, & Suckling, 2008). Although local features do yield interesting information in their own right, the remainder of this section will be restricted to a review of functional connectivity methods.

After data acquisition, the first step is preprocessing of the raw data. In general, nothing fancy is done compared to standard task preprocessing: motion correction, slice timing correction, spatial smoothing, and temporal filtering. Nevertheless, some debate exists about the cut-off of the temporal filter. Early research into the frequency characteristics of resting-state signal has shown that the power of connectivity networks is predominantly found in the lower frequency range, below 0.1Hz (Cordes et al., 2001). Although a lower limit was never mentioned in this study, researchers typically choose to apply a band-pass temporal filter of 0.01-0.1Hz to their data. Whereas the high-pass filter is mainly used to remove scanner drift, which is sensible, the rationale behind using a low-pass filter is to remove high frequency artifacts from the data. Problem is that we can only characterize signal sources that are *at least* two times slower than our sampling rate (Nyquist rate). For a typical TR of 2 seconds (0.5Hz), this means that we can correctly characterize signal sources up to 0.25Hz (i.e., signal with a period not faster than 4 seconds). Any signal faster than this will cause aliasing into lower frequencies, and hence will not truly be removed by the low-pass filter. As physiological confounds are either close to (i.e., breathing; ≈ 0.2Hz) or far above this threshold (i.e., heart rate; ≈1Hz), it should be doubted whether using the standard filter setting of 0.1Hz, or a low-pass filter at all, makes

any sense. Moreover, it has been shown that power of connectivity networks resides in higher frequencies as well, and that we might actually be looking at a broadband phenomenon (Cole, Beckmann, & Smith, 2010; Niazy, Smith, & Beckmann, 2008; Smith, Niazy, Beckmann, & Miller, 2008).

After preprocessing, functional connectivity can be assessed, for which a researcher can choose from three main methods. The first method is a seed-based correlation analysis. The principle of this type of analysis is simple, intuitive, and highly hypothesis driven. First, a region of interest is chosen to serve as seed. This can be done based on anatomy, or guided by, for example, peak activity in task fMRI data. The mean signal (or first eigenvariate) is extracted from this seed region and used to correlate to all voxels of the brain, which is commonly done with the *general linear model,* using the seed's signal as a predictor. The resulting statistical map shows for which voxels the seed has the most predictive power (i.e., largest similarity in signal), thereby inferring functional connectivity. Individual connectivity maps can then be analyzed within and between groups to test for spatial (dis)similarities in functional connectivity of the seed of interest (Fox & Raichle, 2007).

Although connectivity patterns often resemble well-known resting-state networks, it is important to note that this method can only look at connectivity of the seed with each voxel, and not at connectivity between other constituents of the network. In addition, by using this method one is inherently limited to inferences about a small subset of all possible connections, thereby potentially missing out on valuable information. Lastly, there has been much debate about whether possible confounding signal sources (e.g., white matter, cerebrospinal fluid, motion, global signal) should be used as nuisance predictors in the general linear model, next to the seed signal, or how we can limit their influence otherwise. This debate is, however, beyond the scope of this introduction, but will be addressed in a bit more detail in the general discussion of this thesis.

The second popular method, independent component analysis (ICA), is in many options the counterpart of seed-based correlation analysis. That is, ICA is a multivariate data-driven technique that enables a researcher to look at whole brain connectivity networks without needing to have too many assumptions about specific

connections, and it can be run both within and across individuals. ICA decomposes the resting-state data into a set of spatially independent signal sources (i.e., maps), together with their associated time courses. These components can reflect interesting neuronal signal sources (i.e., resting-state networks), as well as noise elements in the data. Components of interest can then be identified, either by adopting a template matching procedure to components found in each individual separately (Greicius et al., 2007), or based on back-projecting group derived components to individual data space (Beckmann, Mackay, Filippini, & Smith, 2009). Finally, these can be tested within or between groups.

However, ICA is a stochastic method, which means that it can yield (slightly) different results (i.e., spatial distribution, or number of components) when it is run multiple times on the same data. This variability in results might be overcome by running the ICA multiple times and selecting only those components that are detected reliably in most runs (Himberg, Hyvärinen, & Esposito, 2004). Secondly, categorizing components as either noise or signal can sometimes appear a rather arbitrary process. Although researchers experienced in evaluating ICA components will do a pretty good job simply by visual inspection of components, one could use classifying algorithms to automatically carry out categorization (De Martino et al., 2007; Salimi-Khorshidi et al., 2014).

The third, and last method to be reviewed here is graph analysis, although this method has not been used for any of the studies in this thesis. This method can be used both in a hypothesis- and a data-driven manner, and is appealing in the sense that it treats the brain as one integrated system, which it undeniably is. The idea behind this analysis is that the brain can be parceled in any given number of meaningful functional *nodes* that might or might not interact with each other. If a connection between any two nodes is inferred, a line is drawn between those nodes, which is called an *edge*. Common parcellation schemes are, for example, based on the Automated Anatomical Labeling (AAL) atlas, or on components from high dimensional ICA, but even individual voxels can be treated as nodes. Next, bivariate correlations are calculated between all pairs of nodes, yielding an $N \times N$ correlation matrix, where N is the number of nodes. Subsequently, edges are defined based on identification of

meaningful correlations between nodes. For this, the matrix needs to be thresholded, which is typically done by applying an absolute correlation threshold, or choosing the *x* % highest correlations. The latter thresholding technique causes graphs to have the same number of edges in each individual, so maximizing comparability across participants. The resulting graph (i.e., connectivity network) can then be tested on a range of physical properties, such as, for example, efficiency of information flow, small-worldness, modularity, or hubness, each providing unique information on different aspects of information processing in the brain. The interested reader is referred to (Bullmore & Sporns, 2009) for an in-depth review of graph-based analysis of fMRI data.

THESIS OUTLINE

The remaining chapters are reports of the experimental studies carried out for this thesis. A brief overview of these chapters is offered below.

SECTION 1: SOCIAL STRESS

The first section is concerned with the results of an experimental study on the effects of social stress on brain activation and resting-state functional connectivity. In **Chapter 2** it was studied whether acute social stress could affect the ability to cope with emotionally salient distraction during a working memory paradigm, and the brain regions involved in this proces. The experiment served primarily to test whether the brain prioritizes processing of salient information over goal directed behavior under stress, but it also informs us on the neural mechanisms behind emotional intrusions, a key symptom in several stress-related disorders.

Chapter 3 describes the results of a study in which we looked at the relatively long-lasting effects of acute social stress on amygdala resting-state functional connectivity. As previous studies had concentrated on changes immediately following stress, it was unknown to what extent a stressful situation might have modulating effects on amygdala connectivity long after the stress has waned. Ultimately, the results of this

study could open new avenues for investigating adaptation to a stressor when immediate survival is no longer at stake.

Lastly, in **Chapter 4** it was explored whether interindividual differences in cortisol levels could be related to amygdala resting-state functional connectivity with areas known to be rich in glucocorticoid receptors, which are areas that are implicated in regulation of the stress-response as well. Findings from this study in healthy controls could further our knowledge on brain circuits through which adaptation to a stressor is achieved, and how cortisol might play a role in this adaptation.

SECTION 2: PSYCHOPATHOLOGY

The second section of this thesis is concerned with stress-related psychiatric disorders. In **Chapter 5** we studied whether resting-state functional connectivity networks differed between participants diagnosed with major depressive disorder and healthy controls. For this study, unmedicated patients without psychiatric comorbidity were included. In this well-controlled clinical sample, we looked whether large-scale functional connectivity networks related to depressive symptomatology showed differences between patients and controls, and whether these differences could be related to severity of depressive symptoms.

Chapter 6 reports on an experiment in which we compared hippocampus and amygdala volumes between female posttraumatic stress disorder patients with a history of childhood maltreatment and healthy controls without such a history. In addition, the shape of the surface was assessed for both subcortical structures, possibly revealing anatomical abnormalities in specific subnuclei, or subregions, associated with the disorder. The results of this study could shed more light on the impact of childhood trauma on the normal neurodevelopmental trajectory, and how this could relate to the development of posttraumatic stress disorder.

SECTION 3: PERSONALITY

In **Chapter 7** we explored whether individual differences in neuroticism and extraversion, two personality traits closely related to stress vulnerability and resilience, respectively, are associated with differential patterns of amygdala resting-state functional connectivity. The findings of this study could help identifying brain circuits that are potentially implicated in the pathogenesis of stress-related psychopathology.

Finally, **Chapter 8** provides a summary and discussion of the key findings of the experimental studies described in this thesis. In addition, limitations of the studies will be discussed, and recommendations for future research are offered.

SECTION 1

SOCIAL

STRESS

CHAPTER 2

Stress shifts brain activation towards ventral affective areas during emotional distraction

Oei, N. Y. L., **Veer, I. M.**, Wolf, O. T., Spinhoven, P., Rombouts, S. A. R. B., & Elzinga, B. M. (2012). *Social Cognitive & Affective Neuroscience*, *7*(4), 403-412.

ABSTRACT

Acute stress has been shown to impair working memory (WM), and to decrease prefrontal activation during WM in healthy humans. Stress also enhances amygdala responses towards emotional stimuli. Stress might thus be specifically detrimental to WM when one is distracted by emotional stimuli. Usually, emotional stimuli presented as distracters in a WM task slow down performance, while evoking more activation in ventral "affective" brain areas, and a relative deactivation in dorsal "executive" areas. We hypothesized that after acute social stress, this reciprocal dorsal–ventral pattern would be shifted towards greater increase of ventral "affective" activation during emotional distraction, while impairing WM performance. To investigate this, 34 healthy men, randomly assigned to a social stress or control condition, performed a Sternberg WM task with emotional and neutral distracters inside an MRI scanner. Results showed that WM performance after stress tended to be slower during emotional distraction. Brain activation during emotional distraction was enhanced in ventral affective areas, while dorsal executive areas tended to show less deactivation after stress. These results suggest that acute stress shifts priority towards processing of emotionally significant stimuli, at the cost of WM performance.

INTRODUCTION

Several studies in healthy humans showed that acute stress and stress hormones, catecholamines and glucocorticoids (GC), impair working memory (WM) (Arnsten, 2009; Luethi et al., 2008; Lupien et al., 1999; Oei et al., 2006; Ramos & Arnsten, 2007; Schoofs et al., 2008). WM is the ability to maintain relevant information in mind and to keep irrelevant information out of mind. Stress might be especially detrimental to WM by decreasing one's ability to keep irrelevant emotional information out of mind, because stress heightens the sensitivity towards potentially threatening stimuli (van Marle et al., 2009), while also compromising the efficiency of conscious effortful information processing by decreasing prefrontal activation during WM performance (Qin et al., 2009). The present study was, therefore, aimed at examining whether acute social stress enhances emotional distraction during WM, and at investigating the stress-induced changes in the underlying neural patterns, using functional magnetic resonance imaging (fMRI).

The preferential processing of emotional cues is considered adaptive, as these are likely to be important for our survival. Accordingly, healthy humans under stress-free circumstances attend to emotional stimuli, even when these are irrelevant to the WM task at hand, and consequently perform poorer at WM (e.g. Kensinger & Corkin, 2003). At the neural level, several studies found an antagonistic relationship between neural activations associated with emotional vs. executive processing, revealing that 'affective processing' is favored over 'executive processing' (Drevets & Raichle, 1998). When comparing neutral vs. emotional distracters in a WM task, ventral 'affective' brain areas, such as the inferior frontal gyrus (IFG) and amygdala show increased activation, along with a deactivation of more dorsal 'executive' brain areas, such as parietal regions and the right dorsolateral prefrontal cortex (DLPFC) (Anticevic, Repovs, & Barch, 2010; Dolcos & McCarthy, 2006; Mitchell et al., 2008; Morey et al., 2009; Perlstein, Elbert, & Stenger, 2002).

Attending to emotional stimuli becomes maladaptive when one is biased towards negative cues, and/or unable to disengage from negative information that is unrelated to the task, which is frequently observed in stress-related psychiatric disor-

ders such as post-traumatic stress disorder (PTSD). PTSD, which presumably is precipitated by acute traumatic stress, is associated with an over responsive amygdala and impaired prefrontal function (Elzinga & Bremner, 2002; Shin, Rauch, & Pitman, 2006). Recently, in a task combining emotional and executive processing (Morey et al., 2009) evidence for an imbalance in the interaction between ventral affective and dorsal executive brain areas was found in PTSD patients. PTSD patients showed higher activation in ventral affective brain regions, which was positively related to PTSD symptom severity, and, conversely, to higher activity in frontoparietal brain regions with lower PTSD symptom severity.

Although the acute stress response in healthy individuals is considered adaptive (de Kloet, Oitzl, & Joëls, 1999), its (temporary) effect on the brain shows similarities with PTSD, as even acute mild psychological stress impairs prefrontal cortex (PFC) function (Arnsten, 2009; Elzinga & Roelofs, 2005; Oei et al., 2006; Qin et al., 2009; Ramos & Arnsten, 2007; Schoofs et al., 2008), and heightens the sensitivity of the amygdala towards threatening stimuli (van Marle et al., 2009). We therefore expected that acute social stress would impair WM performance compared with a control condition, especially when distracters are emotional. We further hypothesized that the social stress would lead to an alteration in the reciprocal dorsal–ventral pattern during emotional distraction, with increased activations in ventral 'affective' brain areas compared with a non-stressful control condition. To examine our hypothesis, we analyzed behavioral performance and dorsal and ventral a priori selected regions of interest (ROIs) implicated in emotional distraction during WM (*dorsal system*: right DLPFC and bilateral parietal regions; *ventral system*: bilateral IFG and right amygdala) in previous studies (i.e., Dolcos & McCarthy, 2006; Dolcos, Kragel, Wang, & McCarthy, 2006; Mitchell et al., 2008). We also explored the role of GCs (salivary cortisol) in relation to behavioral performance and neural responses during distraction.

METHODS

PARTICIPANTS

Male volunteers from the general population were recruited by means of advertisements. Eligibility criteria were: no history of disease or chronic disease requiring medical attention, no dyslexia, no color blindness, no current use of prescribed medication or the use of remedies containing corticosteroids, no use of psychotropic drugs, no current or past psychiatric problems, determined by the Amsterdam Biographical interview (ABV; de Wilde, 1963). The Dutch version of the Symptom checklist (SCL-90) (Arrindell & Ettema, 1986) was used to assess psychoneuroticism (the cut-off score for exclusion was 145, following norm scores for a healthy population), the Dutch version of the Beck Depression Inventory, using a cut-off score for exclusion of > 10 (BDI; Bouman, Luteijn, Albersnagel, & Ploeg, 1985). Furthermore, a body mass index (BMI; kg/m^2) between 19 and 26, an age between 18 and 35 years, and right-handedness was required. Lastly, participants were required to have a total IQ score of > 90, determined by the relevant subtests of the Wechsler Adult intelligence Scale-III (WAIS-III; Wechsler, 1997).

Altogether, 40 healthy male participants were included in the present study and randomly assigned to an experimental and a control group in a randomized two-group design. From this sample two participants with IQs lower than 90 were excluded from analyses in the present study. Four other participants were excluded from the analyses: two participants were outliers because of extreme cortisol levels at baseline, probably reflecting saliva sample contamination or an acute infectious disease (one from stress group, 120 nmol/l; one from the control group, 36 nmol/l). Data from one participant from the stress group could not be collected because of a computer failure. One other participant from the control group was a multivariate outlier with regard to task performance. Each participant gave signed informed consent in which confidentiality, anonymity, and the opportunity to withdraw without penalty were assured.

The study was approved by the Medical Ethics Committee of the Leiden University Medical Center and carried out according to the standards of the Declaration of Helsinki (2000).

MATERIALS

To ascertain that no pre-stress differences between groups existed on intelligence and WM performance, the subscales Picture Completion, Arithmetic, Information, Block Design, of the WAIS-III (Wechsler, 1997) were used to estimate total IQ (TIQ), while Arithmetic, Digit span and Numbers and Letters were used to assess WM Index (WMI). Also state and trait anxiety (State-Trait Anxiety Inventory, STAI; Spielberger, 1983) was assessed.

Emotional Sternberg task

WM was measured using an adapted version of the Sternberg item-recognition task (Sternberg, 1966), developed and described by Oei et al. (2009). In the present version, the task consisted of a total of 180 trials, which lasted not langer than 25 min. Half of the trials were of low load (i.e., comparison load 4) and the other half of high load (comparison load 16). Comparison load was defined by the number of targets (1 or 4) to hold in WM, multiplied by the number of stimuli (4) in the item-recognition display. Comparison load 16 (4:4; target:recognition display) means that four targets (e.g., RZAS) have to be held in WM while there are four stimuli on the item-recognition display (e.g., CDMA), leading to 16 possible comparisons to perform before answering (i.e., RC-RD-RM-RA-ZC-ZD-ZM-ZA-SC- SD-SA-SM-AC-AD-AM-AA, etc.). Each trial started with a blue fixation cross (500 ms), followed by the target presentation (1000 ms), a distracter (1500 ms) and a recognition display (< 2000 ms) (see **Figure 2.1**). Random jitter in between trials ranged from 1500 to 4500 ms. Participants were instructed to ignore the distracter pictures, and to fixate their eyes on a red cross centered in each distracter. The target letter then had to be recognized from four letters in a recognition display. Participants pressed a 'yes' button indicating they had recognized a target, or a 'no' button, when no target letter was present. A target was present (present-target trials) in half of the trials, in the other half the target was absent (absent-target trials).

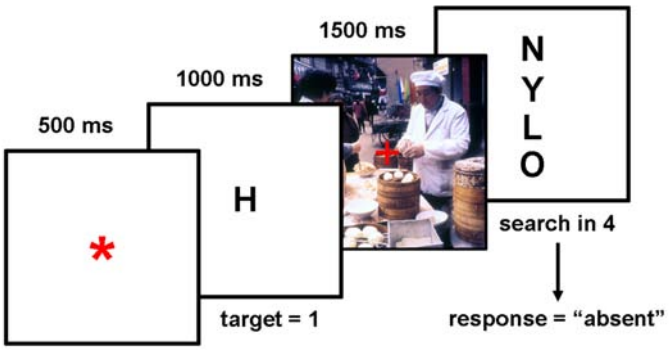

Figure 2.1 Example trial of the Sternberg task with distraction during the delay interval. In this example a low comparison load trial with a neutral distracter is depicted.

Distracters consisted of validated pictures selected from the International Affective Pictures System (IAPS; Lang, Bradley, & Cuthbert, 2008), of which 60 neutral pictures (rated on 9-points Likert scales; valence [1 very negative, 9 very positive]: 5.09 ± 0.54 [*M* ± *SD*]; arousal [1 not arousing at all, 9 highly arousing]: 3.21 ± 0.77 [*M* ± *SD*]) and 60 negatively arousing pictures (valence: 2.86 ± 0.93 [*M* ± *SD*]; arousal: 6.22 ± 0.52 [*M* ± *SD*]), that matched in background color and complexity, for example, amount of people or animals in the scene. A third category consisted of scrambled versions of both the neutral and emotional pictures (Dolcos & McCarthy, 2006). Trial order was pseudo-randomized using MATLAB, to optimize independence between regressors (the random generated order was confined by the rule that none of the categories would be presented more than three consecutive times). Task stimuli were back-projected on a screen located at the end of the scanner bore via an LCD projector located outside the scanner room. Subjects viewed stimuli on a screen through a mirror located on the head coil. Stimulus software (E-Prime, Psychology Software Tools) was used for stimulus presentation and recording of responses.

Subjective ratings

After the experiment participants rated all distracters on a 5-point Likert scale for distractibility (1 not distracting at all, 5 highly distracting), whereas arousal (1 not arousing at all, 5 highly arousing) and valence (1 very positive, 5 very negative) were assessed on 5-point Likert scales using the Self-Assessment Manikin (Bradley & Lang, 1994).

Stress induction

To induce stress, the Trier Social Stress Task (TSST) was employed (Kirschbaum, Pirke, & Hellhammer, 1993). The TSST protocol has consistently proven to raise cortisol levels (Kirschbaum & Hellhammer, 1994). This laboratory stressor consists of a 10-min period in anticipation of a 5-min free speech, and a 5-min arithmetic task (counting backwards from 1033 to zero, in steps of 13) in front of a selection committee of three psychologists. One committee member responded to incorrect answers by saying out loud "incorrect, please start over", while keeping up participant's performance by means of a clearly visible scoreboard. In the control condition, participants used the same anticipation period of 10 min to think of a movie to their liking, of which they were informed to having to answer open questions on paper for 5 min, in the same laboratory room, but without audience. Thereafter, they had 5 min to count backwards from 50 to 0 at a slow pace.

Physiological assessments

Salivary cortisol was assessed using Salivettes (Sarstedt, Germany). Saliva sampling is a stress-free method to assess unbound cortisol (Kirschbaum & Hellhammer, 1994). Saliva samples were stored at -20 °C until assayed at Professor Kirschbaum's laboratory (http://biopsychologie.tu-dresden.de). Cortisol concentrations in saliva were measured using a commercially available chemiluminescence-immuno-assay kit with high sensitivity (IBL, Hamburg, Germany). Inter- and intra-assay coefficients of variation were below 10 %. Systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), and heart rate (HR, bpm) were recorded using an automatic wrist blood pressure monitor (OMRON, R5-I).

Scan protocol

Imaging was carried out on a 3T Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands), using an 8-channel SENSE head coil for radiofrequency reception. For fMRI, T_2^*-weighted gradient-echo echo-planar images (GE-EPI) sensitive to BOLD contrast were obtained with the following acquisition parameters: repetition time (TR) = 2.2 s, echo time (TE) = 30 ms, flip angle = 80°, SENSE factor = 3, 38 axial slices, FOV = 220 × 220 mm, 2.75 mm isotropic voxels, 0.25 mm slice gap. A high-resolution anatomical image (T_1-weighted ultra-fast gradient-echo acquisition; TR = 9.75 ms, TE = 4.59 ms, flip angle = 8°, 140 axial slices, FOV = 224 × 224 mm, in-plane resolution 0.875 × 0.875 mm, slice thickness = 1.2 mm), and a high-resolution T_2^*-weighted gradient echo EPI scan (TR = 2.2 s, TE = 30 ms, flip angle = 80°, 84 axial slices, FOV = 220 × 220 mm, in-plane resolution 1.96 × 1.96 mm, slice thickness = 2 mm) were acquired for registration purposes. The scan procedure consisted of EPI during the emotional WM task (< 25 min), the T_1-weighted anatomical scan (6 min) and the high-resolution EPI (1 min). Furthermore, DTI and resting-state fMRI scans were acquired at the end of the procedure.

Procedure

Participants were invited on two occasions: The first time for further screening purposes (BDI, SCL-90, STAI, WAIS subtests), and the second time for the scan session. Participants were asked to refrain from caffeine or sugar containing drinks, and not to eat 2 h before arrival time. All participants arrived at either 8.30 a.m. or 10.30 a.m. Arrival time was balanced between and within groups, to keep morning cortisol levels as even as possible. After arrival, participants were given instructions regarding the protocol and the emotional WM task. Thirty minutes after arrival, the TSST protocol started. After the TSST, participant got into the scanner, where the emotional Sternberg task, the structural scan, high resolution EPI, DTI and resting-state scans were measured. Saliva was sampled at five time points: before ('baseline') and after the anticipation phase of the TSST ('pre-speech'), at the end of the TSST ('post-TSST'), after finishing the emotional WM task while still inside the scanner ('post-WM'), and after the scan procedure ('post-scan'). Blood pressure and heart rate were

sampled at all the same time points, except for those inside the scanner room. After scanning, participants were seated in front of a PC, to provide subjective ratings of the distracters on arousal, valence and distractibility. Hereafter, an exit-interview and a debriefing regarding the TSST followed. Participants were thanked and paid for their participation.

DATA PROCESSING AND ANALYSIS

Physiological data

Cortisol, BP, and HR were analyzed using repeated measures (RM) ANOVA, and unpaired *t*-tests.

Task data

Reaction times (RTs) were checked for errors, misses and outliers. Errors and misses were scored and removed. Univariate outliers were replaced by the mean per load by distracter type + 2 *SD*. Mahalanobis distance was calculated to check for multivariate outliers ($p[D^2] < .05$). RTs of correct trials were analyzed using RM ANOVAs, with as between-subjects factor Group (stress/control), and as within-subjects factors Target (present/absent), Load (high/low), and Distracter (emotional/neutral). Errors were analyzed similarly. Follow-up analysis of RM ANOVA effects, if relevant, was done with *t*-tests. Greenhouse–Geisser corrections were applied when the sphericity assumption was not met. SPSS Version 16.0 (SPSS Inc.) was used for the analyses.

FMRI data

FMRI data processing was carried out using FMRI Expert Analysis Tool (FEAT) Version 4.1, part of (FMRIBs Software Library [FSL], www.fmrib.ox.ac.uk/fsl; Smith et al., 2004). The following pre-statistics processing was applied: motion correction (Jenkinson, Bannister, Brady, & Smith, 2002); non-brain removal (Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 8mm; grand-mean intensity normalization of the entire 4D data set by a single multiplicative factor; high-

pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with σ = 50.0s). Time-series statistical analysis was carried out with local autocorrelation correction (Woolrich, Ripley, Brady, & Smith, 2001). FMRI EPI data were registered to the high resolution EPI scan of each participant, which was registered to the individual T_1-weighted structural scan, which was registered to the 2 mm MNI-152 standard space template (Jenkinson et al., 2002; Jenkinson & Smith, 2001). For each participant, eight explanatory variables (EVs) were included in the general linear model: Six EVs describing the period between target onset and distracter offset (total length 2.5 s), separate for Load (low/high) × Distracter type (Neu/Emo/Scr), on correct trials. Target-recognition periods on correct trials were modeled in one EV, independent of load or preceding distracter type, with variable durations depending on the response times of the participants. A last EV was included describing error trials, modeling the entire trial from target onset to target-recognition response.

Each EV was convolved with a double gamma hemodynamic response function to account for the hemodynamic response. The images of contrasts of parameter estimates and corresponding variances were then fed into a higher level mixed effects analysis, carried out with FMRIBs Local Analysis of Mixed Effects (FLAME) (Beckmann, Jenkinson, & Smith, 2003; Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004). The significance level of the *z*-statistic image of the contrast of interest (Emo > Neu) was set to $p < .001$ ($z > 3.1$, uncorrected). Before further analysis, the whole-brain activation map, consisting of all participants, was used to select ROIs, defined as clusters of significantly activated contiguous voxels in the four a priori chosen ROIs involved in coping with emotional distraction, that is, the right amygdala, bilateral IFG, right dorsolateral PFC, and bilateral parietal lobe (Dolcos et al., 2006; Dolcos & McCarthy, 2006; Mitchell et al., 2008). These activated clusters were further confined within boundaries of preselected atlas-based ROIs (from the anatomical Harvard–Oxford cortical probability atlas, with the exception of the right amygdala, which was confined by boundaries from the Harvard–Oxford subcortical probability atlas). Then, from these ROIs, parameter estimates (PE) were extracted (Emo and Neu at both Low and High Load) with zero determined by each individual's implicit baseline (Poldrack, 2007). Then, to examine whether stress modulated

the specific pattern of more activity in ventral areas, and less activity in dorsal areas during emotional distraction, and the differential (interaction) effects of Load and Distracter, a RM ANOVA was performed on the percentage change of the MR signal (PE/implicit baseline × 100) in the regions of interest, with as within-subjects factors neural system (dorsal/ventral), Load (low/high), Distracter type (neutral/emotional), and Group (stress/control) as between-subjects factor.

RESULTS

There were no significant differences in the remaining groups with regard to Age, BMI, BDI, SCL-90, Total IQ, WMI, and state anxiety, although trait anxiety showed a trend towards higher anxiety in the stress group (see **Table 2.1** for means and standard deviations).

Table 2.1 Means (*M*) and standard deviations (*SD*) of subject variables in stress and control group

	Control	Stress		
	M ± SD	*M ± SD*	*F* (1, 33)	*p*
Age	24.00 ± 2.62	24.47 ± 4.13	0.16	.69
BMI	22.70 ± 1.55	22.29 ± 2.56	0.32	.57
BDI	2.71 ± 3.53	3.53 ± 3.61	0.45	.51
SCL-90	103.24 ± 16.78	104.82 ± 11.51	0.10	.75
STAI-trait	29.82 ± 6.78	34.06 ± 7.45	3.01	.09
STAI-state	29.76 ± 6.24	32.47 ± 7.32	1.34	.26
TIQ	113.35 ± 14.66	114.00 ± 15.30	0.02	.90
WMI	114.47 ± 13.39	109.41 ± 10.13	1.54	.22

Note: BMI = body mass index; BDI = Beck Depression Inventory; SCL-90 = Symptom Checklist-90; STAI-trait= Trait version of the State-Trait anxiety index: TIQ = Total Intelligence Quotient: WMI = Working memory index.

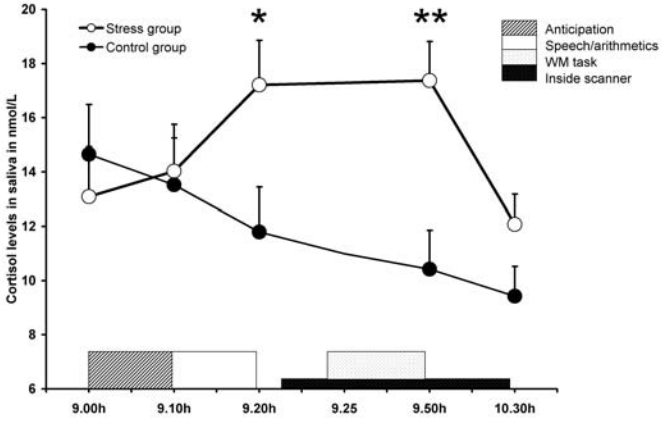

Figure 2.2 Mean levels of cortisol in saliva and standard errors in the stress and control group. Note: significant difference between groups, * $p < .05$, ** $p < .005$

Stress induction

As expected, the stress induction raised the cortisol levels in the stress group, as evidenced by a Group-by-Time interaction, $F(1.81, 57.83) = 6.95$, $p = .003$) (see **Figure 2.2)**. Follow-up t-tests showed that the groups did not differ at baseline, $t(32) = 0.59$, $p = .55$, while right after the stress induction, cortisol levels were significantly higher in the stress group compared with the control group $t(32) = -2.32$, $p = .027$. After the task, cortisol levels were still higher in the stress group, $t(32) = -3.42$, $p = .002$. The between-subjects factor Group was not significant, $F(1, 32) = 2.19$, $p = .15$.

Heart rate

There were no significant differences between groups in heart rate (all ps > .05).

Blood pressure

There were significant within-subjects effects of Time on systolic (SBP), $F(3, 96) = 9.11$, $p < .001$, and diastolic blood pressure (DBP), $F(3, 96) = 8.64$, $p < .001$, as well as of Condition-by-Time on SBP, $F(3, 96) = 12.52$, $p < .001$, and DBP, $F(3, 96) =$

8.00, $p < .001$. After the stress-induction SBP and DBP were significantly higher in the stress group than the control group, $t(32) = -3.09$, $p = .004$, and $t(32) = -4.70$, $p < .001$, respectively. There was also a significant between-groups effect of DBP, $F(1, 32) = 6.56$, $p < .02$, with a higher mean in the stress group ($M = 79.25$, $SE = 1.79$) than in the control group ($M = 72.75$, $SE = 1.79$).

Emotional WM performance

See means and standard deviations of RTs in **Table 2.2**. Within subjects, RTs were faster at low load compared with high load, at present vs. absent target trials, and when the distracter was neutral vs. emotional (all ps < .001). Overall, the stress group tended to be slower than the control group, $F(1, 32) = 3.66$, $p = .06$. Group, Target, and Distracter interacted at trend levels, $F(1, 32) = 3.61$, $p = .07$. Post hoc t-tests showed that during present-target trials, the stress group was slower than controls when distracters were emotional, $t(32) = -2.03$, $p = .05$, but not when they were neutral, $t(32) = -1.65$, $p = .11$ (**Figure 2.3**). In the control group, there was no significant difference in RTs between neutral and emotional trials. There were also no differences during absent-target trials.

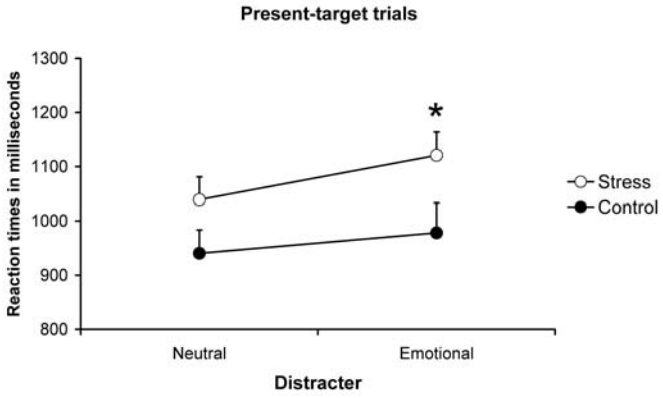

Figure 2.3 Present-target trials: Mean RTs (and *SE*'s) in emotional and neutral trials of the stress- and control group. * $p < .05$.

Table 2.2 Means (*M*) and standard deviations (*SD*) of reaction times and errors on the emotional Sternberg task in the stress and control group.

		Control		Stress	
	Target	Present	Absent	Present	Absent
		M ± SD	*M ± SD*	*M ± SD*	*M ± SD*
Load	Distracter	Reaction times			
Low	Emo	784.10 ± 180.74	794.50 ± 220.72	949.40 ± 202.67	943.00 ± 183.97
	Neu	736.53 ± 141.68	798.66 ± 222.85	849.29 ± 165.43	973.02 ± 206.98
High	Emo	1168.38 ± 302.61	1431.22 ± 415.09	1301.25 ± 194.71	1590.8 ± 281.41
	Neu	1138.61 ± 253.51	1357.21 ± 397.44	1240.20 ± 208.66	1537.74 ± 275.57
		Errors			
Low	Emo	1.12 ± 1.11	0.18 ± 0.39	0.64 ± 0.86	0.65 ± 0.86
	Neu	0.06 ± 0.68	0.35 ± 0.61	0.35 ± 0.61	0.47 ± 0.72
High	Emo	3.41 ± 2.48	0.65 ± 0.79	2.94 ± 1.98	1.18 ± 1.19
	Neu	2.82 ± 1.63	0.35 ± 0.99	3.11 ± 2.29	1.06 ± 1.30

WM errors

See **Table 2.2** for means and standard deviations of Errors. Within subjects analyses showed that more errors were made at high compared with low load, more during present-target trials vs. absent target trials, and also more errors were made when distracters were emotional compared with neutral, Fs(1, 32) > 5.99, ps < .002. There were no interactions with Group, Target, or Load, and there was no main effect of group, $F(1, 32) = 0.70$, $p = .41$.

Subjective ratings of neutral and emotional distracters

Participants were subjectively more distracted by emotional pictures ($M = 1.78$, $SD = 0.57$) than by neutral pictures ($M = 1.21$, $SD = 0.22$), $t(33) = 6.75$, $p < .001$, and rated

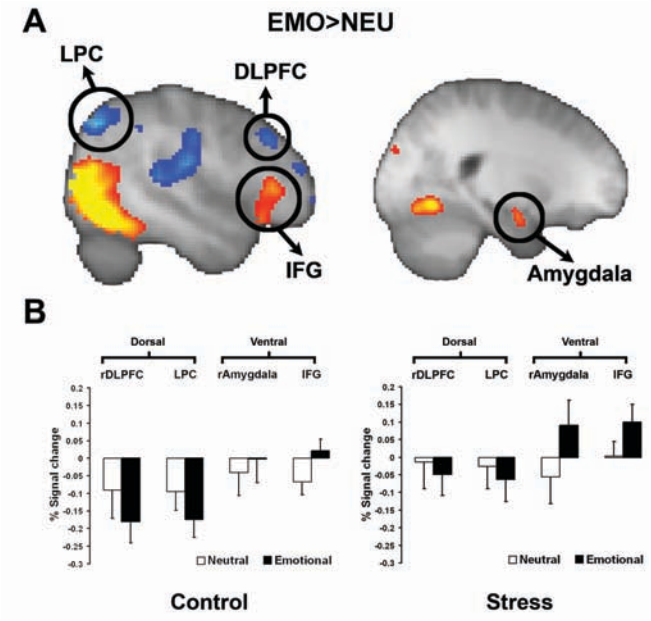

Figure 2.4 Brain activation during emotional compared with neutral distraction, and percent signal change in the ROI. (**A**) Combined group activation showing the typical pattern of dorsal deactivation and ventral activation in the presence of emotional distraction. LPC = lateral parietal cortex; DLPFC = dorsolateral prefrontal cortex; IFG = inferior frontal gyrus. (**B**) Graphs depict mean percent signal change and standard error in the four regions of interest in control (left) and stress group (right) as a function of distracter.

emotional distracters (M = 2.07, SD = 0.63) as more arousing than neutral distracters (M = 1.18, SD = 0.20), $t(33) = 9.99$, $p < .001$. The valence of emotional pictures was rated as more negative (M = 3.83, SD = 0.46) than the neutral pictures (M = 2.72, SD = 0.35), $t(33) = -15.99$, $p < .001$. There was no difference between the stress and control group in these ratings (all Fs < 2.34, and ps > .14).

FMRI analyses

The results from the Emo vs. Neu contrast in the whole-brain analysis of the combined groups are presented in **Table 2.3**. Consistent with previous reports (e.g., Dolcos & McCarthy, 2006), the typical pattern of dorsal 'executive' deactivations and ventral 'affective' activations was found (**Figure 2.4a**).

The four a priori ROIs (right DLPFC, bilateral LPC, right amygdala, bilateral IFG) were selected from these activations, discarding extended activation in voxels outside these regions (specifically in bilateral orbitofrontal regions) as determined by the probabilistic Harvard–Oxford cortical and subcortical atlases. Within the right DLPFC, the ROI was selected from the same region as reported by Dolcos and McCarthy (2006).

The RM ANOVA performed on the percentage change of the MR signal in the ROIs showed that there was a Group-by-Distracter interaction, $F(1, 32) = 5.06$, $p = .03$, which indicated more activation during emotional distraction in the stress group than in the control group, but not during neutral distraction. To specifically address our hypothesis that ventral activation would be enhanced, and dorsal activation decreased during emotional distraction, we further inspected this interaction in the dorsal and ventral ROIs. Separate ANOVAs revealed that the stress group compared to control group had a smaller deactivation in the dorsal system during emotional distraction at trend levels, $F(1, 33) = 3.09$, $p = .08$, and significantly greater activation of the ventral system, $F(1, 33) = 4.74$, $p = .04$ (see **Figure 2.4b** for mean signal change and standard error of the individual ROIs, as a function of group and distracter type). Finally, Neural system interacted with Load, $F(1, 32) = 15.05$, $p < .001$, with at low load, more activation in the ventral system than in the dorsal system, $t(33) = -3.29$, $p = .002$, and a tendency for less deactivation of the dorsal system at high compared with low load, $t(33) = -1.74$, $p = .09$.

Correlational analyses

Higher increases in cortisol levels at the time of task performance (mean pre- and post-WM minus baseline) were associated with less interference by emotional distraction (RTs emotional trials minus RTs neutral trials) at trend levels in the stress

Table 2.3 Peak voxels of significantly activated clusters in brain areas during distraction (Emotional vs Neutral distracters and vice versa), in the whole sample (*n* = 34).

	BA	voxels	L/R	MNI-Coordinates			*z*
Contrast				x	y	z	
Emo>neu							
Occipital fusiform gyrus	37	4544	R	42	-62	-12	7.24**
Inferior lateral occipital	19	3924	L	-52	-70	12	6.97**
Inferior orbitofrontal cortex		1766	L	-36	30	-2	5.20**
Inferior frontal gyrus		1182	R	52	30	4	4.58**
Amygdala		72	R	22	-4	-18	3.98
Temporal fusiform cortex		60	L	-30	-10	-36	4.11
Temporal pole	21	24	R	54	8	-32	3.65
Insular cortex		14	R	38	0	-16	3.48
Neu>Emo							
Superior temporal gyrus	22	3656	R	62	-4	-4	5.12**
Superior temporal gyrus	22	3391	L	-66	-28	8	5.14**
Precentral gyrus	3	1466	R	24	-26	70	4.76**
Pre-/postcentral gyrus		777	L	-24	-30	66	4.55**
Frontal pole		399	R	42	52	-10	4.23**
Precuneus		224		0	-70	22	3.95
Occipital pole		180	R	30	-94	-10	4.41
Middle frontal gyrus	6	125	L	-30	22	54	4.12
Superior frontal gyrus		115	R	24	38	46	4.34
Middle frontal gyrus	9	84	R	50	28	32	4.05
Lateral occipital cortex	39	79	L	-36	-60	38	3.54
Frontal pole (DLPFC)	46	62	R	46	44	16	3.79
Supramarginal gyrus		49	R	54	-38	52	3.55
Postcentral gyrus		29	R	52	-22	56	3.52
Pre/postcentral gyrus		25	R	36	-24	48	3.33
Middle frontal gyrus		16	L	-24	34	34	3.39
Supramarginal gyrus	40	13	R	46	-42	38	3.39

Note: *** = cluster-corrected ($z > 3.1$), $p < .05$. All other areas significant at $z = 3.1$, $p < .001$ (uncorrected). No small volume corrections were applied. BA = Brodmann area; L/R = left/right in the brain; voxel size is 2 mm isotropic.

group (r = -.37, p = .06), but not in the control group (ps > .13). In the stress group, the cortisol response was negatively correlated with neural response in the ventral system during emotional distraction (r = -.50, p = .04; amygdala: r = -.45, p = .07; IFG: r = -.30, p = .24). There was no significant relation between cortisol response and dorsal activation in stress or control group.

DISCUSSION

In the present study, healthy men were exposed to acute social stress before entering the MRI scanner. Inside the scanner, when cortisol levels were high, participants performed a Sternberg WM task with emotionally negative and neutral distracting pictures, shown during the delay phase of each trial. Emotional distracters evoked more ventral activation after acute social stress, and a tendency towards less deactivation (i.e., a smaller magnitude of below-implicit baseline BOLD signal) in dorsal areas compared to the control group. Furthermore, compared to the control group, WM performance tended to be impaired in the stress group during emotional distraction.

The present study is the first to use a validated stress procedure, the TSST, to test the stress effects on emotional distraction in WM. Our findings lend support to the recent accumulation of ideas on acute stress effects, that, although tackling different memory systems or processes, stress modulates the interaction between "higher executive" and "lower emotional" processes (Luethi et al., 2008; Schwabe & Wolf, 2009; van Marle et al., 2009). Intuitively, the idea that acute effects of stress on memory and cognition have survival value is attractive, as it seems adaptive to prioritize attending to dangerous instead of neutral stimuli, for later superior recall, and to be more ready to flee than ponder (Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006). For instance, Luethi et al. (2008) showed that stress enhanced implicit memory of negative emotional stimuli, while impairing explicit memory and WM. Stress also induced a shift from goal-directed behavior towards habits in instrumental stimulus–response processes (Schwabe & Wolf, 2009). Other recent imaging studies reported either enhanced ventral activation after stress (van Marle et al., 2009), or reduced

dorsal prefrontal activations during WM (Qin et al., 2009). We found comparable effects within one task design, which enhances the convergent validity of the idea that stress facilitates emotional processing at the cost of executive processing. Moreover, consistent with the idea that stress shifts brain activation towards ventral areas during emotional distraction, a recent study (Chuah et al., 2010) reported increased amygdala activation associated with increased emotional distraction during WM after 24 h sleep deprivation, which can be considered as an acute stressor (McEwen, 2006).

The present findings are also consistent with results from other studies showing that stress induces WM impairment (Oei et al., 2006; Schoofs et al., 2008). However, it remains unclear what the specific contribution of GCs is to these stress effects. On the one hand, GCs released during (Elzinga & Roelofs, 2005) and after stress (Oei et al., 2006; Schoofs et al., 2008) have been related to reduced WM performance. On the other hand, GC actions appear to be beneficial in dealing with emotional distraction (Oei et al., 2009; Putman, Hermans, Koppeschaar, van Schijndel, & van Honk, 2007). Here, individuals that responded to stress with high cortisol levels, showed less interference by emotional distraction and a smaller neural response to emotional distracters in the ventral ROIs, especially the amygdala. Although these effects were significant at trend levels, they are consistent with a previous study from our lab, showing that administration of 35 mg hydrocortisone significantly reduced emotional distraction using the same task (Oei et al., 2006). Hydrocortisone administration has also found to reduce selective attention for threat (Putman et al., 2007). Cortisol might act to suppress the first wave stress activity (e.g., noradrenergic [NA] activity) towards emotional stimuli. High NA activity has been shown to increase amygdala responses towards emotional stimuli (Onur et al., 2009), and is also associated with impaired WM performance and PFC function (Arnsten, Mathew, Ubriani, Taylor, & Li, 1999; Birnbaum, Gobeske, Auerbach, Taylor, & Arnsten, 1999; Mao, Arnsten, & Li, 1999; Ramos & Arnsten, 2007; Ramos et al., 2005). Moreover, blocking NA activity has shown to reduce interference by emotional distraction in the present task, which was partially mediated by individual cortisol levels (Oei, Tollenaar, Elzinga, & Spinhoven, 2010). Thus, future studies (e.g., using pharmacological manipulations) aimed at further disentangling the specific contributions and interactions of cortisol

and NA activity during stress on processing of emotional stimuli should monitor both cortisol and NA.

Given that WM is especially impaired after stress or GCs at high loads (Lupien et al., 1999; Oei et al., 2006), it could be expected that our stressed participants would be particularly distracted by emotional pictures at high load. This was, however, not confirmed. At high load, overall performance speed was quite low and only differentiated between emotional or neutral trials at the descriptive level. This might have been a drawback from having to perform the task inside the scanner, resulting in slightly altered behavioral response patterns compared with similar task data (Oei et al., 2009). At the neural level, more ventral activity was evoked when load was low than when load was high, which is consistent with other reports. Interference by similar emotionally negative distracting pictures was only observed under low- but not high load (Erthal et al., 2005), while amygdala responses to negative distracters under high load were shown to be reduced compared with low load, presumably because high load claims so much attention, that not enough attentional resources were left to be captured by emotional distracters (Pessoa, Padmala, & Morland, 2005). Furthermore, similar to Dolcos and McCarthy (2006) amygdala activity was higher when contrasting emotional vs. neutral distraction. In the control group, however, amygdala activity was not increased when comparing emotional distraction with baseline. As several studies have shown a higher sensitivity to threatening stimuli in women than in men (Canli, Desmond, Zhao, & Gabrieli, 2002a; Hamann, 2005), the fact that we only tested males, whereas Dolcos and McCarthy tested females, might explain why they found increased amygdala activation during emotional distraction compared to baseline.

Furthermore, only present-target trials appeared sensitive enough to detect effects of distraction in this paradigm, whereas absent-target trials did not differentiate between neutral and emotional distraction (Oei et al., 2009). Present- and absent-target trials usually produce different performances, probably because they elicit/evoke different search strategies (i.e., for present-target trials a self-terminating, and for absent-target trials an exhaustive search strategy) (Corbin & Marquer, 2008). Nonetheless, because neural activation during the delay of each trial preceded the

participants/knowledge of target presence or absence, we did not analyze the imaging data for present-targets only. Discarding half of the imaging data would also have greatly reduced the power to detect differences.

Together, the present results show greater activation in ventral "affective" areas after stress, and smaller deactivation in dorsal "executive" areas, during emotional distraction. This was related to slower WM performance during emotional distraction. These results might suggest that acute stress shifts priority towards processing of emotionally significant stimuli, at the cost of WM performance. Further research into the effects of stress on cognitive functioning and attention to (distracting) emotional stimuli in the environment should be aimed at elucidating the specific effects of cortisol and other stress hormones on neural and behavioral performance.

CHAPTER 3

Beyond acute social stress: Increased functional connectivity between amygdala and cortical midline structures

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ABSTRACT

Whereas we know a fair amount on the role of the amygdala in the acute stress response, virtually nothing is known about its role during the recovery period after the stress has waned. Functional connectivity analysis of the amygdala during this period might be useful in revealing brain circuits promoting adaptive recovery from a stressful event, as well as consolidation of emotionally relevant information in preparing for future challenges. Healthy participants were randomly assigned to either a psychosocial stress task (n = 18; stress group) or a comparable non-stressful control procedure (n = 20; controls). To study the prolonged effects of stress on amygdala functional connectivity, resting-state fMRI scans were acquired an hour after the stress task. Amygdala functional connectivity with other brain regions was assessed using seed-based correlations. The stress group exhibited a strong physiological and behavioral reaction to psychosocial stress exposure. Compared with controls the stress group showed increased amygdala functional connectivity with three cortical midline structures: the posterior cingulate cortex and precuneus (p < .05, corrected), and the medial prefrontal cortex (p < .05, small volume corrected). An hour after psychosocial stress, changes in amygdala functional connectivity were detected with cortical midline structures involved in the processing and regulation of emotions, as well as autobiographical memory. It is hypothesized that these effects could relate to top-down control of the amygdala and consolidation of self-relevant information after a stressful event. These results on functional connectivity in the recovery phase after stress might provide an important new vantage point in studying both sensitivity and resilience to stress.

INTRODUCTION

When we face a stressful situation, our brain initiates a stress response. The amygdala plays a key role in evoking this response, as it signals danger and, more generally, emotional salience of incoming sensory information to the rest of the brain to prepare ourselves for appropriate action (LeDoux, 2000; Phillips, Drevets, Rauch, & Lane, 2003a). Through its neuronal projections to several brainstem nuclei and the hypothalamus, the amygdala excites both the autonomic nervous system (ANS) and hypothalamic–pituitary–adrenal (HPA) axis. The ANS promotes a swift physical and behavioral response through the release of catecholamines, such as adrenaline and noradrenaline. In contrast, slower acting stress agents such as cortisol are secreted through activation of the HPA-axis to warrant homeostasis after the stressful event (Ulrich-Lai & Herman, 2009). A balanced integration of both pathways enables an adaptive modulation of both the physical and the behavioral stress response (Joëls & Baram, 2009).

To date, effects of stress on the amygdala have mostly been described during or directly after stress. For example, during psychosocial stress deactivation of limbic regions, including the amygdala, was found (Pruessner et al., 2008), whereas after psychosocial stress our group demonstrated increased amygdala responsivity towards negative stimuli during an emotional working memory task (Oei et al., 2012). Similar results were obtained by van Marle et al. (2009) after letting participants watch negatively arousing movie clips as a stressor. Using that same stress induction paradigm, these researchers also found increased functional connectivity (FC) between the amygdala and brain regions mediating autonomic activity, such as the dorsal anterior cingulate cortex (ACC) and brainstem. Thus, the effects found immediately following a stressor might possibly relate to activation of the acute autonomic stress response by the amygdala (van Marle, Hermans, Qin, & Fernández, 2010). In contrast, studying the recovery period after a stressful event is equally important, as prolonged activation during this period has been related to the development of psychopathology and somatic disease (Brosschot, Gerin, & Thayer, 2006). Nonetheless, relatively little is known about the role of the amygdala during this period when homeostasis rather

than immediate survival is being promoted, relating to processes such as the inhibition of autonomic responses evoked by the stressor, as well as emotion regulation and memory consolidation.

The amygdala receives modulatory input from cortical brain regions, which dampen its responsivity in the aftermath of negatively arousing events (LeDoux, 2000). Particularly regions in the medial prefrontal cortex (mPFC) have been found to be involved in modulating amygdala activity during emotional conflict and regulation of autonomic and affective responses, most notably the perigenual division of the anterior cingulate cortex (Egner, Etkin, Gale, & Hirsch, 2008; Etkin, Egner, Peraza, Kandel, & Hirsch, 2006; Gianaros et al., 2008; Pezawas et al., 2005; Wager et al., 2009), but also the ventro- and dorsomedial (vm/dm) portions of the PFC (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Urry et al., 2006). Interestingly, cortisol was found to strengthen FC between the amygdala and dmPFC more than four hours following its administration (Henckens et al., 2010). In addition, these same regions showed an increased inverse relation in glucose metabolism after psychosocial stress: Higher metabolism in the dmPFC was associated with lower metabolism in the amygdala (Kern et al., 2008). Moreover, steeper (i.e., more normative) decreases in diurnal cortisol were related to a stronger inverse coupling between the amygdala and the vmPFC during regulation of negative affect (Urry et al., 2006). These findings suggest an important role for an interaction between the mPFC and amygdala in achieving adaptive emotion regulation in the period following stress, potentially mediated by cortisol or stress in general.

Besides initiating the acute stress response, the amygdala is a key structure in promoting memory consolidation of emotionally salient information through its interactions with the hippocampus (McGaugh, 2004; McGaugh, Cahill, & Roozendaal, 1996). The amygdala seems to be essential in mediating the effects of stress hormones on learning and memory consolidation (Roozendaal et al., 2009). Therefore, increased interactions between the amygdala and hippocampus may underlie the enhancing effects of stress and/or cortisol and noradrenalin on emotional memory found in human studies (Buchanan & Lovallo, 2001; Cahill et al., 2003; Kuhlmann & Wolf, 2006; Strange & Dolan, 2004). The improved memory consolidation for emotionally rele-

vant and arousing information after a stressful experience is hypothesized to represent a mechanism that enables us to prepare for and adaptively face similar challenging situations in the future.

Resting-state (RS-)fMRI has become an important tool to study functional interactions in the human brain in the absence of overt behavior (Fox & Raichle, 2007). This makes the technique especially useful for studying diffuse states of the brain, such as stress, and may therefore provide valuable insights on how stress affects the neural circuitry underlying emotion regulation and memory consolidation when the acute phase of the stress has waned. Moreover, RS-fMRI has been found to provide reliable measures of amygdala FC that corroborate results of white matter tracing studies in non-human primates (Amaral & Price, 1984; Ghashghaei & Barbas, 2002): amygdala FC has been observed with several brain regions supporting the processing, regulation and consolidation of emotionally salient events, such as the mPFC, including the anterior cingulate cortex (ACC), dm/vmPFC and orbitofrontal cortex (OFC), as well as the insula, hippocampus and brainstem (Robinson, Laird, Glahn, Lovallo, & Fox, 2010; Roy et al., 2009; Stein et al., 2007a).

In the current study we investigated the long-term influence of psychosocial stress on resting-state FC (RSFC) of the amygdala stretching beyond the acute stress response, during the recovery phase. Healthy male participants were exposed to either social stress or a comparable non-stressful control condition before entering the MRI scanner. Amygdala RSFC was assessed one hour after stress exposure, when the acute stress response had already waned. We expected that stress would lead to increased RSFC between the amygdala and the mPFC, potentially pointing to top-down modulatory control over the amygdala. Secondly, we expected the amygdala to show increased interactions with brain areas involved in (emotional) memory formation and consolidation, such as (peri)hippocampal regions.

METHODS

PARTICIPANTS

Forty-seven male volunteers from the general population were recruited by means of advertisements. All participants were screened before inclusion. Eligibility criteria were: no history of disease or chronic disease requiring medical attention, no dyslexia, no color blindness, no current use of prescribed medication and/or use of remedies containing corticosteroids, no use of psychotropic drugs, no current or past psychiatric problems, as was determined by the Amsterdam Biographical interview (ABV) (de Wilde, 1963), the total score on the Dutch version of the Symptom checklist (SCL-90) (Arrindell & Ettema, 1986), the Dutch version of the Beck Depression Inventory (BDI) (Bouman et al., 1985), and the State-Trait Anxiety Inventory (STAI) (Spielberger, 1983). Furthermore, participants were required to have a Body Mass Index (BMI, in kg/m^2) between 19 and 26, to be between 18 and 30 years old, and to be right-handed. Forty participants were deemed eligible and included in the study. Participants were randomly assigned to either the experimental or control group in a randomized two-group design. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center, and written informed consent was given by all participants.

MATERIALS

Stress manipulation

To induce stress, the Trier Social Stress Test (TSST) was employed (Kirschbaum et al., 1993). The TSST protocol has consistently proven to raise cortisol levels (Kirschbaum & Hellhammer, 1994). This laboratory stressor consists of a ten-minute anticipation period, followed by a five-minute free speech that had to include one's positive and negative characteristics. After the anticipation period, the speech was given in front of a selection committee of three psychologists. Subsequently, participants had to perform a five-minute arithmetic task (counting backwards from 1033 to zero, in steps of 13) in front of the same committee. One of its members responded to

incorrect answers by saying out loud "incorrect, please start over", while keeping up the participant's performance by means of a clearly visible scoreboard. In the control condition, participants used the same anticipation period of ten minutes to think of a movie to their liking, about which they had to answer open questions on paper for five minutes in the same laboratory room, though without any audience. Thereafter, they were instructed to count backwards from 50 to zero at a slow pace, which lasted for another five minutes.

Physiological assessments

Salivary cortisol was assessed at multiple time points through- out the procedure (see procedure) using Salivettes (Sarstedt, Germany). Saliva sampling is a stress-free method to assess unbound cortisol (Kirschbaum & Hellhammer, 1994). Saliva samples were stored at –20 °C until assayed at Prof. Kirschbaum's laboratory (http://biopsychologie.tu-dresden.de). Cortisol concentrations in saliva (in nmol/L) were measured using a commercially available chemiluminescence-immuno-assay kit with high sensitivity (IBL, Hamburg, Germany). Inter- and intra-assay coefficients of variation were below 10 %. Systolic blood pressure (SBP, mm Hg), diastolic blood pressure (DBP, mm Hg), and heart rate (HR, bpm) were furthermore recorded outside the scanner room at multiple time points using an automatic wrist blood pressure monitor (OMRON, R5-I) to assess autonomic nervous system responsivity to the stressor. Furthermore, heart rate was monitored during RS acquisition using a pulse oximeter attached to the middle finger of the left hand. The average heart rate was logged every minute. In addition, the total number of respiratory peaks was counted, as was recorded by means of a respiratory belt around the chest. Repeated measures ANOVAs and post-hoc independent sample *t*-tests were carried out on the physiological data and VAS scale for each time point using SPSS Version 16.0 (SPSS Inc.).

FMRI data acquisition

Imaging data were acquired on a Philips 3T Achieva MRI scanner using an eight-channel SENSE head coil for radiofrequency reception (Philips Healthcare, Best, The Netherlands). Whole-brain RS-fMRI data were acquired using T_2^*-weight-

ed gradient-echo echo-planar imaging (EPI) with the following scan parameters: 160 volumes; 38 axial slices scanned in ascending order; repetition time (TR) = 2200 ms; echo time (TE) = 30 ms; flip angle = 80°; FOV = 220 × 220 mm; 2.75 mm isotropic voxels with a 0.25 mm slice gap. A high-resolution anatomical image (T_1-weighted ultra-fast gradient-echo acquisition; TR = 9.75 ms; TE = 4.59 ms; flip angle = 8°; 140 axial slices; FOV = 224 × 224 mm; in-plane resolution 0.875 × 0.875 mm; slice thickness = 1.2 mm), and a high-resolution T_2^*- weighted gradient echo EPI scan (TR = 2.2 s; TE = 30 ms; flip angle = 80°; 84 axial slices; FOV = 220 × 220 mm; in-plane resolution 1.96 × 1.96 mm, slice thickness = 2 mm) were acquired for registration to standard space.

FMRI data preprocessing

Prior to analysis, all resting-state fMRI data sets were submitted to a visual quality control check to ensure that no gross artifacts were present in the data. Next, data were analyzed using FSL Version 4.1.3 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). The following preprocessing steps were applied to the EPI data sets: motion correction, removal of non-brain tissue, spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum (FWHM), grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, and a high pass temporal filter of 100 s (i.e., ≥.01 Hz). The RS dataset was registered to the high resolution EPI image, the high resolution EPI image to the T_1-weighted image, and the T_1-weighted image to the 2 mm isotropic MNI-152 standard space image (T_1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada). The resulting transformation matrices were then combined to obtain a native to MNI space transformation matrix and its inverse (MNI to native space).

FMRI time course extraction and statistical analysis

For the current study, a seed based correlation approach (Fox & Raichle, 2007) was employed to reveal brain regions that are functionally connected to the amygdala during rest (e.g., Roy et al., 2009). To this end, binary masks of the bilateral amygdala

were created using the Harvard–Oxford Subcortical Atlas, as provided in MNI standard space within FSL: the center voxel was determined for the left and right amygdala, and spherical regions of interest (ROIs) were subsequently created around these voxels using a radius of 4 mm. Next, using the inverse transformation matrix, the amygdala masks were registered to each participant's RS-fMRI preprocessed dataset. The mean time course was subsequently extracted from the voxels falling within each amygdala mask in native space. These time courses were entered as a regressor in a general linear model (GLM), together with nine nuisance regressors, comprising the white matter signal, CSF signal, six motion parameters (rigid body: three translations and three rotations), and the global signal. The latter regressor was included to further reduce the influence of artifacts caused by physiological signal sources (i.e., cardiac and respiratory) on the results (Fox & Raichle, 2007). Each individual model was tested using FEAT version 5.98, part of FSL. The resulting individual parameter estimate (PE) maps, together with their corresponding within-subject variance maps, were then resliced into 2 mm isotropic MNI space and fed into a higher level between-groups mixed effects analysis (two-sample *t*-test). First, whole-brain *z*-statistic images were thresholded using clusters determined by an initial cluster-forming threshold of $z > 2.3$ and a (corrected) cluster significance threshold of $p < .05$ (Worsley, 2001). A small volume correction was applied for regions known to have functional and/or anatomical connections to the amygdala (Amaral & Price, 1984; Robinson et al., 2010; Roy et al., 2009; Stein et al., 2007a), and which were a priori hypothesized to be affected by stress in this study: the mPFC, including the pgACC, vm/dmPFC and OFC, as well as the hippocampus. Masks of these regions of interest were defined based on the Harvard–Oxford (sub)cortical probability atlases, as provided in FSL, and were then used to mask the raw statistical images. Subsequently, correction for multiple comparisons was carried out for only those voxels present in the ROI masks, using cluster based thresholding with the same parameter settings as for the whole-brain analysis ($z > 2.3$, $p < .05$).

Procedure

On the day of scanning participants arrived at either 8:30 or 10:30 a.m. The arrival time of the participants was balanced both between and within groups to keep morning cortisol levels as comparable as possible. Participants were asked to refrain from caffeine or sugar containing drinks, and not to eat two hours before arrival time to minimize unwanted effects on cortisol levels. After arrival, participants were seated in a quiet waiting room, where instructions were given about the protocol. Exactly 30 minutes after arrival, participants were given instructions belonging to either the control or stress condition. Both protocols started outside the scanner, where participants were either told to prepare a presentation, or to think about a movie to their liking. After preparation, they were brought to a quiet room in which the committee was seated (stress) or the movie questionnaire was handed out (control), and both protocols were continued. Each took 20 minutes to complete. Afterwards, the participant was brought to the scanner. The scanning protocol consisted of an emotional working memory task (Oei et al., 2012), several anatomical scans, and the RS scan which was acquired at the end of the scan protocol, 60 minutes after completion of the TSST. For the RS scan, participants were instructed to lie still with their eyes closed during the entire scan in the darkened scanner room. Saliva was sampled at five time points throughout the procedure: before ('baseline') and after the anticipation phase of the TSST or control condition ('pre TSST'), at the end of the TSST or control condition just before entering the scanner ('post TSST'), immediately after finishing the task scan ('post task') and immediately after the RS scan outside the scanner ('post RS'). At the exact same moments, a 10-point Likert scale was used to inquire about the subjectively perceived stress levels. Blood pressure and heart rate were sampled at the same time points, except when the participant was inside the scanner room due to MR-incompatibility of the equipment. An exit-interview, and, if applicable, a debriefing regarding the TSST followed at the end of the procedure. Subsequently, participants were thanked and paid for their participation in the study.

RESULTS

Two participants from the stress group were discarded in the analysis: one participant exhibited an extreme cortisol level at baseline (120 nmol/L), probably reflecting saliva sample contamination, while data from one participant could not be acquired due to scanner failure. The resulting analyses were therefore carried out on 20 control participants (mean age 23.95 ± 2.52 years) and 18 participants who were exposed to psychosocial stress (mean age 23.94 ± 3.12 years). The stress and control group did not differ in terms of age, BMI, STAI trait or state scores, and baseline heart rate, blood pressure or cortisol (all: $p > .1$).

Physiological and behavioral results

The stress group showed a strong physiological reaction to the stressor as measured by the salivary cortisol levels (see **Figure 3.1a**), which was confirmed by a Group-by-Time interaction, $F(1.69, 60.96) = 9.9$, $p < .001$. Post-hoc t-tests showed higher cortisol values in the stress group before ($p < .001$) and after ($p < .01$) the working memory task, and directly after the RS scan ($p < .05$) compared with controls. This effect was also reflected by the concurrent increase of the subjective stress ratings (see

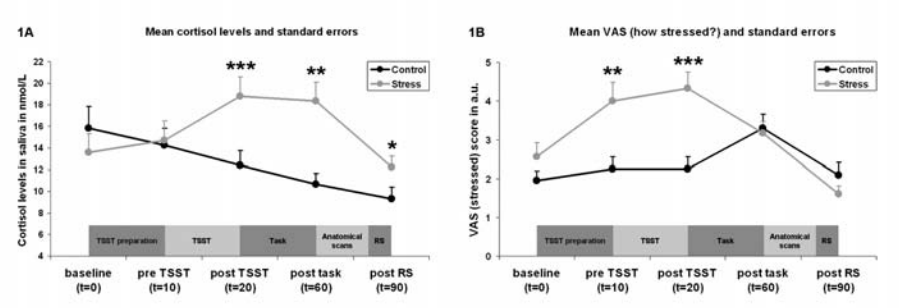

Figure 3.1 (**A**) Mean salivary cortisol levels and standard errors for both the stress and control group at the five time points of sampling (t = time in minutes from baseline). Note: *** $p < .001$, ** $p < .01$, * $p < .05$. (**B**) Mean subjective stress scores and standard errors for both the stress and control group at the five time points of sampling (t = time in minutes from baseline). Note: *** $p < .001$, ** $p < .01$, * $p < .05$.

Figure 3.1b), as was confirmed by the Group-by-Time interaction, $F(3.36, 120.98) = 19.21$, $p < .001$. Here, post-hoc tests showed higher ratings for the stress group before ($p < .01$) and after ($p < .001$) the TSST, but not after the RS scan. Lastly, both systolic and diastolic blood pressure (SBP/DPB) showed a Group-by-Time interaction, $F(3, 108) = 18.24$, $p < .001$ and $F(3, 108) = 6$, $p = .001$, respectively. While SBP showed a trend ($p = .088$) before the TSST, DBP was already increased in the stress group ($p < .014$). Both SBP and DBP were increased in the stress group after the TSST ($p < .001$) compared with the control group. We did not find a difference in heart rate and frequency of respiration during the RS scan, and in blood pressure directly after the RS scan between the two groups (all $p > .1$).

Functional connectivity results

Figure 3.2a shows the joint amygdala resting-state functional connectivity patterns for the two groups separately, as well as their overlap and differences. Within both groups the connectivity pattern largely overlapped with areas described to have functional and anatomical connections with the amygdala in previous studies (Amaral & Price, 1984; Robinson et al., 2010; Roy et al., 2009; Stein et al., 2007a). Areas involved included: brainstem, hippocampus, hypothalamus, subgenual cingulate cortex, dorsal cingulate cortex, posterior lateral orbitofrontal cortex, insula, temporal poles, and the primary visual cortex. The majority of these regions together form "the emotional brain", dedicated to the processing and regulation of emotion (Pessoa, 2008). A detailed description of the areas involved is provided in **Table 3.1**.

Compared to the control group, the stress group showed increased amygdala RSFC with the posterior cingulate cortex (PCC) and the adjacent precuneus ($p < .05$, corrected; see **Figure 3.2b**). In addition, when applying a small volume correction for our regions of interest, increased amygdala RSFC was demonstrated within the vmPFC in the stress group compared to the control group. However, changes in amygdala functional connectivity with the hippocampus were not found, which is contrary to our expectations. Post-hoc tests revealed that the effects were not driven by either the left or right amygdala alone. Lastly, no differences were observed for the opposite contrast control > stress.

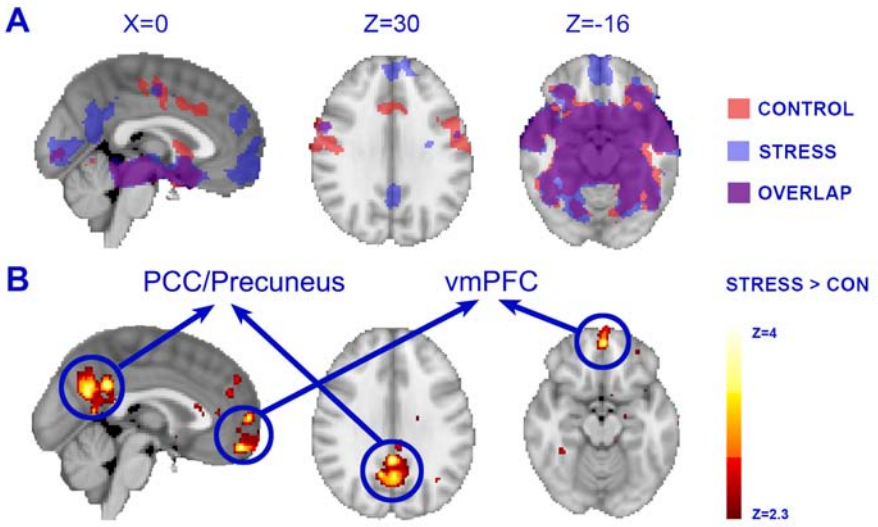

Figure 3.2 Group main (**A**) and between groups (**B**) effects of joint amygdala resting-state functional connectivity overlaid on the 2 mm MNI standard space template. Group main effects are cluster corrected at $p < .05$. Between group effects are shown uncorrected at $z > 2.3$ for illustration purposes. The left side of the brain corresponds to the right hemisphere and vice versa.

DISCUSSION

In the current study we investigated whether psychosocial stress modulates RSFC of the amygdala with other brain regions important for the processing, regulation and consolidation of emotionally salient events in healthy participants during the recovery phase, when the acute stress response has waned. It was expected that stress would increase amygdala RSFC with the mPFC, supporting regulatory feedback on the amygdala during recovery from the stressful event. In addition, increased amygdala RSFC was expected with regions facilitating (emotional) memory formation and consolidation, such as the hippocampus and its adjacent structures, indicating an increased propensity to store emotionally salient information in memory after stress.

The seed based correlation approach employed in this study generated whole brain RSFC patterns of the amygdala similar to those reported in previous studies (Robinson et al., 2010; Roy et al., 2009; Stein et al., 2007a). The comparison between the stress and control group yielded two major findings. Firstly, increased RSFC was found with the posterior cingulate cortex (PCC) and the adjacent precuneus. The

Table 3.1 Amygdala resting-state functional connectivity results

Region	Hemisphere	Cluster size 2mm voxels	Peak voxel coordinates (MNI)			*z*-value
			x	y	z	
Control						
Positive						
lateral orbitofrontal	R	35890	30	34	-18	5.09
cortex	L		-30	34	-16	5.29
hippocampus	R		28	-22	-16	6.03
	L		-26	-20	-16	6.19
putamen	R		30	-14	-4	6.39
	L		-30	-16	0	6.14
globus pallidus	R		24	-4	0	6.2
	L		-20	0	2	5.62
insula	R		42	-2	-8	5.55
	L		-40	-6	-8	4.81
hypothalamus	R		6	-4	-12	4.04
	L		-6	-2	-26	4.95
subcallosal cortex	R		8	10	-14	4.99
	L		-6	16	-14	4.54
temporal pole	R		46	10	-16	5.35
	L		-52	10	-16	5.36
superior temporal	R		54	-34	4	3.76
gyrus	R		48	-24	-4	3.57
	L		-54	-14	-8	4.54
	L		-52	-34	2	4.04
middle temporal	R		56	-12	-14	5
gyrus	L		-56	-14	-10	4.34
occipital cortex	R		14	-86	4	3.6
	L		-6	-92	4	4.52
brainstem	R		-2	-34	-16	6.13
dorsal anterior	R	7318	8	-8	40	4.27
cingulate cortex	L		-8	-8	44	4.23
postcentral gyrus	R		62	-16	38	4.82
	L		-46	-16	36	4.76
precentral gyrus	R		60	4	32	4.67
Negative						
posterior cingulate	R	12325	4	-36	26	4.41
cortex	L		-4	-36	26	4.3
precuneus	R		6	-66	30	3.13
	L		-8	-70	32	3.67

Table 3.1 Continued.

Region	Hemisphere	Cluster size 2mm voxels	Peak voxel coordinates (MNI)			*z*-value
			x	y	z	
Control						
Negative						
lateral frontal pole	R	4027	26	58	10	4.08
	L		-34	58	6	3.75
perigenual anterior cingulate cortex	R	1300	4	36	10	2.94
medial superior frontal gyrus			-2	26	50	3
Stress						
Positive						
lateral orbitofrontal cortex	R	41463	36	36	-12	4.25
	L		-38	32	-16	3.88
hippocampus	R		32	-14	-20	6.07
	L		-24	-30	-10	6.35
putamen	R		32	-16	0	5.47
	L		-30	-20	2	4.73
globus pallidus	R		24	-4	2	4.57
	L		-20	-4	0	4.98
insula	R		40	-10	-8	4.38
	L		-42	-4	-2	4.47
hypothalamus	R		4	-2	-14	5.1
	L		-6	-2	-14	5.39
subcallosal cortex	R		10	12	-18	5.81
	L		-6	14	-16	6.26
temporal pole	R		48	8	-24	5.23
	L		-42	10	-24	5.16
superior temporal gyrus	R		60	-12	-2	4
	R		52	-22	-4	4.04
	L		-62	-12	-8	4.86
	L		-52	-22	0	4.76
middle temporal gyrus	R		58	-12	-16	5.19
	L		-50	2	-22	4.82
occipital cortex	R		24	-94	0	3.81
	L		-8	-88	0	4.22
brainstem	R		-4	-34	-14	6.36
posterior cingulate cortex			0	-48	32	3.39
precuneus	R		2	-58	12	4.25
	L		-4	-58	8	4.31
dorsal anterior cingulate cortex	R	1556	8	-6	42	2.97
	L		-6	0	42	3.7
	L		-16	-40	56	3.2
precentral gyrus	R		40	-12	42	3.96
	L		-34	-16	44	4.4

Table 3.1 Continued.

Region	Hemisphere	Cluster size 2mm voxels	Peak voxel coordinates (MNI) x	y	z	*z*-value
Stress						
Positive						
ventromedial prefrontal cortex		2301	4	52	-14	5.31
dorsomedial prefrontal cortex			0	46	26	4.45
Negative						
lateral frontal pole	R	12371	30	60	4	3.64
	L		-28	60	18	3.76
medial superior frontal gyrus		84	0	22	50	3.35
Stress>Control						
posterior cingulate cortex	R	1260	2	-46	32	3.68
precuneus			0	-62	26	3.63
ventromedial prefrontal cortex		270	0	54	-16	3.68*
frontal pole			2	60	6	3.69*

Note: all *z*-values are corrected for multiple comparisons ($p < .05$), except for z-values with a * ($p < .05$, small volume corrected)

PCC/precuneus area is implicated in autobiographical memory processes (Buckner & Carroll, 2007; Cavanna & Trimble, 2006; Vann, Aggleton, & Maguire, 2009). Recently, evidence for a direct ascending anatomical connection between the basolateral nucleus and retrosplenial cortex, the most caudal part of the PCC, was found in the macaque brain (Buckwalter, Schumann, & Van Hoesen, 2007). The existence of such a connection seems to be supported by studies showing RSFC between the two regions in humans (Robinson et al., 2010; Stein et al., 2007a). In addition, a recent study found white matter pathways between these regions and the hippocampus (Greicius, Supekar, Menon, & Dougherty, 2009), a pivotal brain structure for storing and retrieving episodic information (Squire & Zola-Morgan, 1991). Further, the amygdala is richly and reciprocally connected to the hippocampus in primates (Amaral, 1986). We thus speculate that the current finding might reveal the cortico–limbic circuit through which stress enhances memory formation of emotionally salient events

(McGaugh, 2004; Roozendaal et al., 2009). While this could reflect a beneficial mechanism, served to adaptively face similar situations in the future, increased connectivity in this circuit may also turn maladaptive, thereby promoting disproportionate memory consolidation of negative experiences. This, in turn, may eventually form a basis for unwanted intrusive memories, a key symptom in posttraumatic stress disorder (Brohawn, Offringa, Pfaff, Hughes, & Shin, 2010), but also common to depression and anxiety. Nonetheless, in this study we did not explicitly test for memory of emotionally salient information. Therefore, future studies are warranted to investigate whether stress actually modulates emotional memory through increased FC between the amygdala and precuneus.

The second major finding in our study was that psychosocial stress, in line with our expectations, increased amygdala RSFC with the medial prefrontal cortex (mPFC). Especially the ventral part of the mPFC (vmPFC) has dense and reciprocal anatomical connections to the amygdala (Ghashghaei & Barbas, 2002; Ghashghaei, Hilgetag, & Barbas, 2007), which might drive the connectivity observed between these regions (Robinson et al., 2010; Roy et al., 2009; Stein et al., 2007a). Although the pgACC, acknowledged as part of the mPFC, has been described most extensively as a target region for top-down inhibitory control over the amygdala (Pessoa, 2008; Pezawas et al., 2005; Phillips, Drevets, Rauch, & Lane, 2003a), other studies report on yet another part of the mPFC, similar to the location we found in our study, that is implicated in regulating amygdala responses (Heinz et al., 2005; Johnstone et al., 2007; Urry et al., 2006). In addition, glucose metabolism in this region was shown to decrease with higher levels of cortisol, resulting from a comparable psychosocial stressor, and was inversely related to the metabolism of the hippocampus/amygdala (Kern et al., 2008). Further, a more normative diurnal cortisol pattern was found to relate to stronger functional coupling between the vmPFC and amygdala during downregulation of negative affect (Urry et al., 2006). Lastly, cortisol administration was shown to increase FC between the amygdala and mPFC (Henckens et al., 2010). Therefore, the current result might be in line with the notion that the amygdala receives modulatory control from the mPFC to regulate expression of emotions, or more specifically, to regulate the brain's response to stress. An overload in stress

may impact exactly this feedback circuit and thereby contribute to the pathogenesis of stress-related psychiatric disorders, such as depression, anxiety and posttraumatic stress disorder, as decoupling of these regions has been well-documented in relation to disturbed emotion regulation (Heinz et al., 2005; Johnstone et al., 2007; Phillips, Drevets, Rauch, & Lane, 2003b; Shin et al., 2006; Veer et al., 2010).

The midline brain regions, PCC/precuneus and mPFC, found in the current study are the core constituents of the default mode network (DMN) (Raichle et al., 2001). This network is proposed to be related to mind wandering (Mason et al., 2007), autobiographical memory processes (Buckner & Carroll, 2007), and self-referential thought (Gusnard, Akbudak, Shulman, & Raichle, 2001; Northoff & Bermpohl, 2004; Northoff et al., 2006; Raichle et al., 2001). Furthermore, in line with these functional accounts, the DMN is hypothesized to provide the infrastructure for integrating past, present and future events related to the self (Buckner & Carroll, 2007). This would enable us to reflect on and learn from past experiences, which is essential for adaptively coping with future challenges. Therefore, increased amygdala connectivity with these DMN regions could reflect stress-induced facilitation of self-evaluative processes under or after emotionally salient experiences. This might be particularly strong in our paradigm, because of the social evaluative component in the stressor we applied. Some support for this hypothesis, although taken tentatively, can be found in studies of social phobia showing increased activity in the precuneus/PCC and vmPFC when viewing emotional facial expressions (Gentili et al., 2009) and increased vmPFC within the DMN at rest (Liao et al., 2010). In addition, abnormally increased RSFC within the DMN has been described in other stress-related psychiatric disorders, such as major depression (Greicius et al., 2007), and posttraumatic stress disorder (Lanius et al., 2010). It is important to note that self-referential activity, as might be reflected by the enhanced connectivity with DMN regions, is compatible with both our previous accounts, being improved memory for emotionally salient events and downregulation of emotional states, as both processes are dependent on evaluation of the situation one encountered. Lastly, from a dynamical network perspective, it is highly plausible that separate resting-state connectivity networks engage or disengage in different configurations, depending on the circumstances to be dealt

with. Consequently, we might actually observe that the amygdala-centered connectivity network under scrutiny connects to the DMN to meet the demands set by a stressful situation.

We did not observe increased RSFC of the amygdala with the hippocampus itself and/or its adjacent areas after stress. However, the amygdala borders the hippocampus, which makes it hard to segment the two structures from one another, especially when dealing with the coarse resolution of functional MRI scans. When also taking into account the spatial smoothing applied during preprocessing, the time series derived from our amygdala seeds might have been 'contaminated' by signal from the hippocampus. Effects on our results could be twofold: subtle differences in connectivity between the amygdala and hippocampus may have been swamped through partial overlap in signal, as might be suggested by the very high correlation with the hippocampus in both groups. Secondly, the increased PCC/precuneus connectivity might actually be mediated by the hippocampus, which is supported by the strong white matter pathways between these regions (Greicius et al., 2009). Nonetheless, such a scenario would furthermore underscore that our results could relate to increased emotional memory formation after a stressful event.

Using the TSST, a real life psychosocial stress situation, we were successful in raising both physiological and subjective stress levels of participants in the stress group, as was reflected by substantial increases in the salivary cortisol response, blood pressure, and subjective stress ratings. The stress group demonstrated a cortisol response almost twice as high as their baseline levels, whereas the control group showed a steady decrease in their cortisol levels over the course of the experiment. Notably, the stress group still demonstrated higher cortisol levels than controls when the RS-fMRI scan was acquired, an hour after the TSST was completed. The group that was already stressed by the TSST rated their subjective experience of stress as higher before entering the scanner than the control group. However, stress-free controls showed an increase of subjective stress while inside the scanner, probably due to lying inside an MRI scanner, as all participants in the current study were scanner-naïve. Nonetheless, both groups were close to baseline directly after the RS scan. Therefore, it is likely that we were not able to show connectivity related to the immediate stress

response as shown by Van Marle et al. (2010). However, we do find robust differences that can only be attributed to the stressful experience our experimental group encountered. In our opinion, these differences could be interpreted to reflect processes promoting recovery and adaptation in the post-stressor period, either conscious or unconscious, to warrant homeostasis.

A limitation of the current study is the possible influence of physiological differences between the stress and control group on the functional connectivity effects we observed. Firstly, we have tried to minimize this by adding the global signal as a confound regressor, which has previously been shown to reduce effects of physiological fluctuations on the data (Fox & Raichle, 2007). Secondly, although heart rate and respiration were not measured comprehensively during resting-state data acquisition, our crude sampling method did not reveal any differences during RS data acquisition. Lastly, it is important to note that in a previous study a significant decrease in both heart rate and blood pressure was found within 10 min following the TSST, with heart rate already being returned to baseline levels (Oei et al., 2006). Therefore, we think it is unlikely that the differences in functional connectivity found one hour after stress exposure could be attributed to differences in physiological fluctuations between the two groups.

A second limitation of our study pertains to the possible influence that the emotional working memory task might have had on the amygdala functional connectivity patterns, although the RS scan was acquired 20 minutes post-task. Analysis of the task showed increased amygdala responsivity towards negative emotional stimuli after stress. Therefore, the differences in functional connectivity we observed might also be caused by a more thorough perception and processing of such stimuli under or after a stressful condition. This does, however, fit our hypothesis that the stress-induced increase in amygdala RSFC with the PCC/precuneus could reflect enhanced emotional memory. However, there was no association between amygdala responsivity on the task and the strength of the amygdala RSFC with the PCC/precuneus and the vmPFC. Though taken tentatively, this might speak against influence of the task on the current results.

On a final note, we should be cautious in relating our results to adaptive recovery from stress. Although we have measured amygdala RSFC in the recovery phase after stress, we cannot directly compare our effects to RSFC under acute stress or to a measure of recovery encompassing the hour after stress, which could have strengthened our hypothesis. However, we can compare our results to those obtained by Van Marle et al. (2010), albeit different stressors were used. The authors showed RSFC patterns pointing to autonomic activation in the direct aftermath of stress, whereas our results in absence of such activation better fit recovery processes such as regulatory feedback and preparation for future hardships. Nevertheless, we do recommend including a measure of recovery (rate) in future studies, so allowing a better characterization of amygdala RSFC in the post-stressor period.

In sum, here we show for the first time that psychosocial stress increases amygdala resting-state functional connectivity with the precuneus/PCC and vmPFC, areas known to be involved in memory, emotion regulation and social cognition. This result might be attributable to behavioral homeostasis after stress, which stretches beyond the initial stress response. Although our results are likely to reflect a healthy and adaptive response to a stressful situation, these may also provide a link to the pathogenesis of stress-related psychopathology and provide an important new vantage point in studying both sensitivity and resilience to stress in general.

CHAPTER 4

Endogenous cortisol is associated with functional connectivity between the amygdala and medial prefrontal cortex

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ABSTRACT

Whether glucocorticoids mediate medial prefrontal cortex (mPFC) regulation of the amygdala in humans remains unclear. In the current study we investigated whether cortisol levels under relatively stress-free circumstances are related to amygdala resting-state functional connectivity with the mPFC. Resting-state fMRI data were acquired from 20 healthy male participants. Salivary cortisol was sampled at multiple times throughout the experiment. The cortisol area under the curve increase (AUCi) was calculated as a measure of cortisol dynamics. Next, seed based correlations were employed on the resting-state fMRI data to reveal regions of amygdala functional connectivity related to variations in cortisol AUCi. The resulting statistical maps were corrected for multiple comparisons using cluster-based thresholding ($z > 2.3$, $p < .05$). Two regions in the mPFC showed decreasing negative functional connectivity with the amygdala when a lesser decrease in cortisol AUCi was observed: the perigenual anterior cingulate cortex and medial frontal pole (BA10). Although we initially showed a relation with cortisol AUCi, it seemed that the baseline cortisol levels were actually driving this effect: higher baseline cortisol levels related to stronger negative functional connectivity with the mPFC. Endogenous cortisol levels may modulate amygdala functional connectivity with specific regions in the mPFC, even under relatively stress-free circumstances. Our results corroborate previous findings from both animal and human studies, suggesting cortisol-mediated regulation of the amygdala by the mPFC. We propose that through this feedback mechanism the stress response might be adjusted, pointing to the putative role of cortisol in modulating stress- and, more generally, emotional responses.

INTRODUCTION

The release of glucocorticoids is one of the most prominent endocrine responses to a stressful situation. In humans, the glucocorticoid cortisol is secreted by the adrenal cortices after the hypothalamus-pituitary-adrenal (HPA) axis has been activated (Sapolsky et al., 2000; Ulrich-Lai & Herman, 2009). Whereas the autonomic nervous system supports a fast reaction to a stressful situation, cortisol typically reaches its peak plasma levels only after tens of minutes. Following its release, cortisol acts back on the HPA-axis in a negative feedback loop, thereby promoting inhibition of the stress response necessary to reach behavioral and physiological homeostasis (Herman et al., 2005; Ulrich-Lai & Herman, 2009).

Animal studies have provided ample evidence that the medial prefrontal cortex (mPFC) plays an important modulatory role within the stress circuitry (Cerqueira et al., 2008; Diorio, Viau, & Meaney, 1993; Sullivan & Gratton, 2002), either by stimulating or inhibiting HPA-axis activity, depending on which mPFC subdivision is involved (Radley, Arias, & Sawchenko, 2006; Ulrich-Lai & Herman, 2009). Whereas the ventral part of the mPFC has been attributed a more stimulatory role, the more dorsal part, in contrast, has rather been described as inhibiting HPA-axis activity. In addition, several studies suggest that this negative feedback circuit is mediated through the binding of cortisol to glucocorticoid receptors (GRs) in the mPFC (Boyle et al., 2005; Diorio et al., 1993; Furay, Bruestle, & Herman, 2008; Sánchez, Young, Plotsky, & Insel, 2000; Ulrich-Lai & Herman, 2009).

The amygdala, a key region in facilitating stress responses, is an important target of such inhibitory feedback by the mPFC (Herman et al., 2005). In humans, the mPFC was found to be involved in modulating amygdala activity during emotional conflict and regulation of autonomic and affective responses, most notably the perigenual division of the anterior cingulate cortex (Egner et al., 2008; Etkin et al., 2006; Gianaros et al., 2008; Pezawas et al., 2005; Wager et al., 2009), but also the ventro- and dorsomedial (vm/dm) portions of the PFC (Banks et al., 2007; Urry et al., 2006). Based on the animal research reviewed above, cortisol might act as an important mediator in adjusting amygdala responses through the mPFC.

This notion is supported by the abnormal interactions between the mPFC and amygdala that have been reported frequently in stress-related psychiatric disorders, such as depression and posttraumatic stress disorder (PTSD) (Drevets et al., 2008; Liberzon & Sripada, 2008; Phillips, Drevets, Rauch, & Lane, 2003b; Veer et al., 2010). Because of the concurrent HPA-axis dysregulation in these disorders (de Kloet et al., 2006; Pariante & Lightman, 2008), it is thought that prolonged exposure to abnormal cortisol levels is related to reduced top-down inhibition by the mPFC, thereby sustaining excessive amygdala activity (Liberzon et al., 2007).

So far, three studies in healthy humans have found support not only for a mediating role of cortisol in connectivity between the amygdala and mPFC, either after ingestion of hydrocortisone (Henckens et al., 2010), or after social stress (Kern et al., 2008), but also pertaining to individual differences in normal diurnal cortisol patterns (Urry et al., 2006). Except for the study of Kern et al., who used task-free positron emission tomography to assess glucose metabolism in the brain after social stress, these results were obtained with task paradigms in which emotionally salient stimuli were used.

Resting-state functional connectivity (RSFC) analysis of the amygdala-mPFC circuit, on the other hand, might provide more insight on whether cortisol levels are related to interactions between these regions in humans in absence of task-induced activation, potentially providing a more intrinsic measure of cortisol mediated brain networks. In a recent study of our group we found that social stress increased amygdala RSFC with the mPFC compared to controls (Veer, Oei, van Buchem, Elzinga, & Rombouts, 2011). However, this increased connectivity was not related to stress-induced cortisol levels, possibly due to a ceiling effect in the participants' cortisol responses or complex interactions with concurrent neuroendocrine responses to the stressor. Nonetheless, activation of the brain's stress circuitry was previously shown to be related even to subtle variations in stress-free cortisol fluctuations (Cunningham-Bussel et al., 2009; Urry et al., 2006). Therefore, we investigated whether such normal variations in endogenous cortisol also could be related to altered amygdala RSFC with the mPFC in a group of healthy young males under relatively stress-free circumstances.

METHODS

PARTICIPANTS

Twenty right-handed male volunteers (mean age 23.95 $\pm$ 2.52 years) from the general population were recruited by means of advertisements. All participants were screened before inclusion. Eligibility criteria were: no history of disease or chronic disease requiring medical attention, no dyslexia, no color blindness, no current use of prescribed medication and/or use of remedies containing corticosteroids, no use of psychotropic drugs, no current or past psychiatric problems, as was determined by the Amsterdam Biographical interview (ABV; de Wilde, 1963), and the Dutch version of the Symptom checklist (SCL-90; Arrindell & Ettema, 1986). Furthermore, participants were required to have a body mass index (BMI; kg/m^2) between 19 and 26, and to be between 18 and 30 years old. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center, and written informed consent was given by all participants.

MATERIALS

Physiological assessments

Salivary cortisol was assessed using Salivettes (Sarstedt, Germany). Saliva sampling is a stress-free method to assess unbound cortisol (Kirschbaum & Hellhammer, 1994). Saliva samples were stored at -20 $^{\circ}$ C until assayed at Prof. Kirschbaum's laboratory (http://biopsychologie.tu-dresden.de). Cortisol concentrations in saliva (in nmol/L) were measured using a commercially available chemiluminescence-immuno-assay kit with high sensitivity (IBL, Hamburg, Germany). Inter- and intra-assay coefficients of variation were below 10 %. The cortisol area under the curve increase (AUCi) was determined for each participant, providing a measure of cortisol changes over the course of the experiment (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Lastly, systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), and heart rate (HR, bpm) were recorded using an automatic wrist blood pressure monitor (OMRON, R5-I) to assess activity of the autonomic nervous sys-

tem. Repeated measures ANOVAs were carried out on the physiological data using SPSS Version 16.0 (SPSS Inc.).

FMRI data acquisition

Imaging data were acquired on a Philips 3T Achieva MRI scanner using an eight-channel SENSE head coil for radiofrequency reception (Philips Healthcare, Best, The Netherlands). Whole-brain RS-fMRI data were acquired using T_2^*-weighted gradient-echo echo-planar imaging with the following scan parameters: 160 volumes; 38 axial slices scanned in ascending order; repetition time (TR) = 2200 ms; echo time (TE) = 30 ms; flip angle = 80° ; FOV = 220 mm × 220 mm; 2.75 mm isotropic voxels with a 0.25 mm slice gap. A high-resolution anatomical image (T_1- weighted ultra-fast gradient-echo acquisition; TR = 9.75 ms; TE = 4.59 ms; flip angle = 8° ; 140 axial slices; FOV = 224 mm × 224 mm; in-plane resolution 0.875 mm × 0.875 mm; slice thickness = 1.2 mm), and a high-resolution T_2^*-weighted gradient-echo EPI scan (TR = 2.2 s; TE = 30 ms; flip angle = 80° ; 84 axial slices; FOV = 220 mm × 220 mm; in-plane resolution 1.96 mm × 1.96 mm, slice thickness = 2 mm) were acquired for registration and normalization to standard space.

FMRI data preprocessing

Prior to analysis, all resting-state fMRI data sets were submitted to a visual quality control check to ensure that no gross artifacts were present in the data. Next, data were analyzed using FSL Version 4.1.3 (FMRIB's Software Library, www.fmrib.ox-.ac.uk/fsl) (Smith et al., 2004). The following preprocessing steps were applied to the EPI data sets: motion correction (Jenkinson et al., 2002), removal of non-brain tissue (Smith, 2002), spatial smoothing using a Gaussian kernel of 6mm full width at half maximum (FWHM), grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, a highpass temporal filter of 100 s (i.e., ≥ 0.01 Hz). The RS dataset was registered to the high resolution EPI image, the high resolution EPI image to the T_1-weighted image, and the T_1-weighted image to the 2 mm isotropic MNI-152 standard space image (T_1-weighted standard brain averaged over 152 subjects; Montreal Neuro- logical Institute, Montreal, QC, Canada) (Jenkinson et

al., 2002; Jenkinson & Smith, 2001). The resulting transformation matrices were then combined to obtain a native to MNI space transformation matrix.

FMRI time course extraction and statistical analysis

A seed based correlation approach (Fox & Raichle, 2007) was employed to reveal brain regions that are functionally connected to the amygdala during rest (e.g., Veer et al., 2011). To this end, binary masks of the bilateral amygdala were created using the Harvard-Oxford Subcortical Atlas, as provided in MNI standard space within FSL: the center voxel was determined for the left and right amygdala, and spherical regions of interest (ROIs) were subsequently created around these voxels using a radius of 4 mm. Next, using the inverse transformation matrix, the amygdala masks were registered to each participant's RS-fMRI preprocessed dataset. The mean time course was subsequently extracted from the voxels falling within each amygdala mask in native space. These time courses were entered as a regressor in a general linear model (GLM), together with nine nuisance regressors comprising the white matter signal, CSF signal, six motion parameters (rigid body: three translations and three rotations), and the global signal. The latter regressor was included to further reduce the influence of artifacts caused by physiological signal sources (i.e., cardiac and respiratory) on the results (Fox & Raichle, 2007). Each individual model was tested using FEAT version 5.98, part of FSL. The resulting individual parameter estimate (PE) maps, together with their corresponding within-subject variance maps, were then resliced into 2 mm isotropic MNI space and fed into a higher level mixed effects regression analysis (one-sample *t*-test), using the demeaned AUCi cortisol values as regressor of interest. Whole-brain *z*-statistic images were thresholded using clusters determined by an initial cluster-forming threshold of $z > 2.3$ ($p < .01$, one-tailed), and a corrected cluster significance threshold of $p < .05$ (Worsley, 2001).

Procedure

The current article reports on results obtained within a larger study addressing the effects of social stress on an emotional working memory task (Sternberg paradigm, using negative and neutral distracters during the delay period between target and probe;

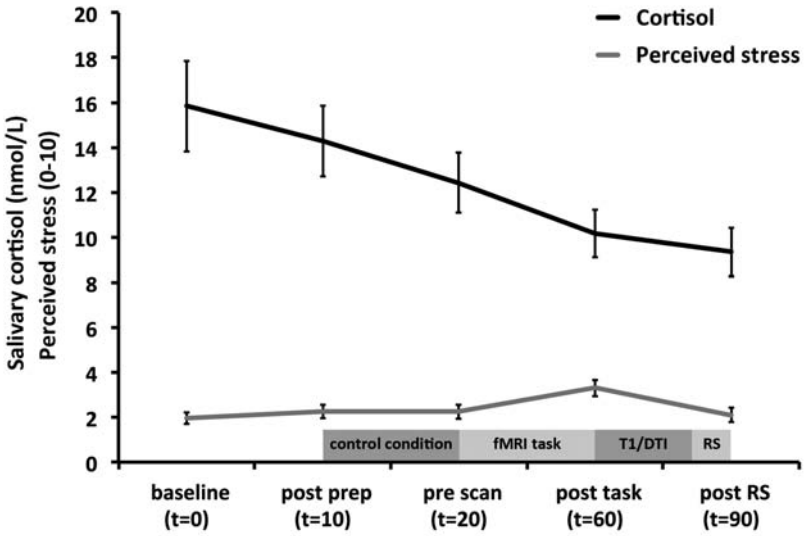

Figure 4.1 Mean salivary cortisol levels (nmol/L) and Likert scores (0-10) together with their standard error of the mean at each of the sampling time points (t = time in minutes from baseline). RS = resting-state scan, T_1/DTI = anatomical scans.

Oei et al., 2012) and resting-state functional connectivity (Veer et al., 2011). The results described here are based on the participants from the control group who were assigned to a non-stressful control condition (answering questions about a movie to their liking for five minutes, and counting backwards from 50 to zero) before entering the scanner. On the day of scanning participants arrived at either 8:30 or 10:30 AM, which was balanced within our participant group. Participants were asked to refrain from caffeine or sugar containing drinks, from smoking, and not to eat two hours before arrival time to minimize unwanted effects on cortisol levels. The scanning protocol consisted of the task scan, several anatomical scans, and the RS scan which was acquired at the end of the scan protocol, 50 minutes after entering the scanner and 20 minutes after completing the task scan. For the RS scan, participants were instructed to lie still with their eyes closed during the entire scan in the darkened scanner room.

Saliva was sampled at five time points throughout the procedure: before ('baseline', t = 0 min) and after preparation for the control condition ('post prep', t = 10 min), after completing the control condition just before entering the scanner ('pre scan', t = 20 min), immediately after finishing the emotional working memory task scan ('post task', t = 60 min), and immediately after the RS scan outside the scanner ('post RS', t = 90 min). At the exact same moments, a 10-point Likert scale was used to inquire about the subjectively perceived stress levels (see **Figure 4.1** for sampling time points and their relative timings). Blood pressure and heart rate were sampled at the same time points, except the fourth time point ('post task') when the participant was inside the scanner room, due to MR-incompatibility of the equipment. An exit-interview followed at the end of the procedure. Subsequently, participants were thanked and paid for their participation in the study.

RESULTS

PHYSIOLOGICAL AND BEHAVIORAL RESULTS

Cortisol

See **Figure 4.1** for average cortisol values at each sampling time point. A gradual decrease of endogenous cortisol levels over the course of the experiment was observed in our participants. This was confirmed by a main effect of Time, $F(1.38, 26.3) = 8.91$, $p = .003$, and a linear contrast post hoc, $F(1, 19) = 10.57$, $p = .004$. Nonetheless, a number of participants demonstrated only a minor decrease ($n = 9$) or even an increase ($n = 5$) in cortisol levels, as was reflected by the cortisol AUCi. Although the distribution of cortisol AUCi is skewed, no outliers were identified. No difference was found between the 'pre scan' and 'post RS' time points ($p > .1$).

Heart rate

Over the course of the experiment heart rate decreased, as expressed in a main effect of Time, $F(3, 57) = 3.25$, $p = .028$. No difference was found between the 'pre scan' and 'post RS' time points ($p > .1$).

Table 4.1 Resting-state functional connectivity results

Region	Hemisphere	Cluster size 2mm voxels	Peak voxel coordinates (MNI)			*z*-value
			x	y	z	
Amygdala						
Positive						
lateral orbitofrontal cortex	R	35890	30	34	-18	5.09
	L		-30	34	-16	5.29
hippocampus	R		28	-22	-16	6.03
	L		-26	-20	-16	6.19
putamen	R		30	-14	-4	6.39
	L		-30	-16	0	6.14
globus pallidus	R		24	-4	0	6.2
	L		-20	0	2	5.62
insula	R		42	-2	-8	5.55
	L		-40	-6	-8	4.81
hypothalamus	R		6	-4	-12	4.04
	L		-6	-2	-26	4.95
subcallosal cortex	R		8	10	-14	4.99
	L		-6	16	-14	4.54
temporal pole	R		46	10	-16	5.35
	L		-52	10	-16	5.36
superior temporal gyrus	R		54	-34	4	3.76
	R		48	-24	-4	3.57
	L		-54	-14	-8	4.54
	L		-52	-34	2	4.04
middle temporal gyrus	R		56	-12	-14	5
	L		-56	-14	-10	4.34
occipital cortex	R		14	-86	4	3.6
	L		-6	-92	4	4.52
brainstem	R		-2	-34	-16	6.13
dorsal anterior cingulate cortex	R	7318	8	-8	40	4.27
	L		-8	-8	44	4.23
postcentral gyrus	R		62	-16	38	4.82
	L		-46	-16	36	4.76
precentral gyrus	R		60	4	32	4.67
	L		-52	6	28	4.21
Negative						
posterior cingulate cortex	R	12325	4	-36	26	4.41
	L		-4	-36	26	4.3
precuneus	R		6	-66	30	3.13
	L		-8	-70	32	3.67
lateral frontal pole	R	4027	26	58	10	4.08
	L		-34	58	6	3.75
perigenual anterior cingulate cortex	R	1300	4	36	10	2.94
medial superior frontal gyrus			-2	26	50	3
Cortisol						
perigenual anterior cingulate cortex		584	-2	36	2	3.61
medial frontal pole (BA10)			-2	64	-4	3.2

Blood pressure

Blood pressure showed a different pattern in anticipation of scanning, participants had a decrease in both systolic (SBP) and diastolic (DBP) blood pressure, yet both were increased after scanning to values even above baseline (main effect of time: $F(3, 57) = 4.19$, $p = .009$, and $F(3, 57) = 15.78$, $p < .001$, SBP and DBP, respectively; 'post RS' larger than 'pre scan': $t(19) = 3.05$, $p = .007$ and $t(19) = 4.07$, $p < .001$, SBP and DBP, respectively). It must be noted, however, that the 'post RS' measurement took place directly after the scans, when participants were seated in another room. This could have increased blood pressure markedly because the participant suddenly had to stand upwards after a long period of lying still inside the scanner. Therefore, it is conceivable that this in fact is the cause of the increase in blood pressure.

Behavior

See **Figure 4.1** for the perceived stress scores. Subjective stress ratings demonstrated a main effect of time, $F(4, 76) = 10.26$, $p < .001$, with higher ratings 'post task' than 'pre scan', $t(19) = -3.8$, $p = .001$, but not at the 'post RS' measurement compared to 'post task' ($p > .1$).

Functional connectivity results

The pattern of amygdala functional connectivity within our participant group largely overlaps with previously described functional and anatomical connections of the amygdala (Robinson et al., 2010; Roy et al., 2009; Stein et al., 2007a). The areas involved include: brainstem, hippocampus, hypothalamus, subgenual cingulate cortex, dorsal cingulate cortex, posterior lateral orbitofrontal cortex, insula, temporal poles, and primary visual cortex (see **Table 4.1**). The majority of these regions together form the "emotional brain" circuitry, dedicated to the processing and regulation of emotion (Pessoa, 2008).

Figure 4.2 shows the two clusters of resting-state functional connectivity with the joint amygdala seeds that are positively correlated with cortisol AUCi ($p < .05$, cluster corrected): the perigenual anterior cingulate cortex (pgACC) and medial

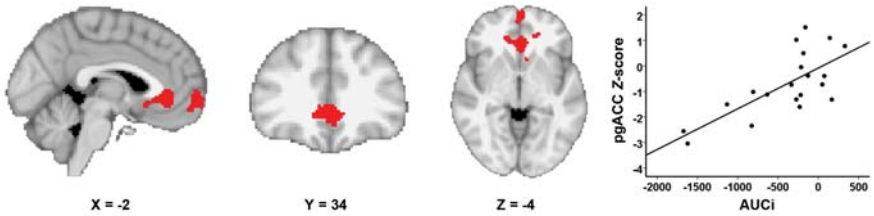

Figure 4.2 Results ($z > 2.3$, $p < .05$, cluster corrected for multiple comparisons) overlaid on the 2 mm MNI standard space template. The left side of the brain corresponds to the right hemisphere and vice versa. The scatter plot illustrates the correlation between cortisol AUCi and strength of amygdala RSFC with the pgACC.

frontal pole (BA10). That is, less decrease of cortisol levels over the course of the experiment is associated with less negative RSFC with the two mPFC regions. Moreover, mild cortisol AUCi increases appear to relate to an increase in positive amygdala RSFC with the pgACC and BA10. We did not observe an effect of cortisol AUCi looking at either left or right amygdala RSFC alone.

We furthermore tested whether cortisol levels at baseline were in fact driving the steepness of the AUCi slopes, and thereby possibly the effects on amygdala RSFC. That is, did higher cortisol levels at baseline relate to a larger cortisol decrease over the course of the experiment? This was indeed the case, as was illustrated by the negative correlation between baseline cortisol and AUCi ($r(20) = -0.87$, $p < .05$). In addition, when using the baseline values as predictor instead of cortisol AUCi, we found the exact same results, although being inverted. That is, higher baseline cortisol was associated with stronger negative amygdala RSFC with the two mPFC regions.

Lastly, to distinguish between delayed and more direct effects of cortisol, we averaged the absolute cortisol levels on time points 4 (post task) and 5 (post RS) and used these as a predictor of amygdala RSFC. However, no effect was observed.

DISCUSSION

Here we show that basal variations in endogenous cortisol in healthy young male participants are related to the strength of amygdala resting-state functional connectivity with two regions in the mPFC, specifically the pgACC and medial frontal pole (BA10). This result is in line with our hypothesis and the notion that cortisol impacts crosstalk between the mPFC and amygdala. Therefore, our findings potentially reflect a modulatory pathway within the human brain's stress and emotion circuitry that is mediated by cortisol.

Cortisol exerts its influence through both mineralocorticoid (MRs) and glucocorticoid receptors (GRs), which are differentially distributed throughout the brain (de Kloet, Joëls, & Holsboer, 2005; Joëls & Baram, 2009): Whereas MRs are predominantly found in the hippocampal formation, GRs are more ubiquitously located in the brain, though high concentrations of this receptor type have been located particularly in the medial prefrontal cortex (mPFC) (Diorio et al., 1993; Sánchez et al., 2000). Thus, the increase in RSFC between the amygdala and mPFC could very well be mediated by binding of cortisol to glucocorticoid receptors in this region.

The pgACC has been described extensively as an important region in exerting top-down inhibitory control over the amygdala (Pessoa, 2008; Pezawas et al., 2005; Phillips, Drevets, Rauch, & Lane, 2003a; Quirk & Beer, 2006), thereby contributing to adaptive emotion regulation. This is supported by the direct anatomical connections between the two regions (Ghashghaei et al., 2007; Ghashghaei & Barbas, 2002). As such, the pgACC also provides a good candidate for adjusting the stress response. Accordingly, studies in rodents ascribe this function to the dorsal prelimbic cortex, commonly considered a homologue of the human pgACC: lesions within this region have been found to cause diminished regulation and thereby disinhibition of the stress response (Boyle et al., 2005; Diorio et al., 1993; Furay et al., 2008; Ulrich-Lai & Herman, 2009). Additionally, in humans decoupling of the pgACC and amygdala has been well-documented in relation to disturbed emotion regulation in stress-related psychiatric disorders (Heinz et al., 2005; Johnstone et al., 2007; Phillips, Drevets, Rauch, & Lane, 2003b; Shin et al., 2006; Veer et al., 2010), a feature

that might also underlie the aberrant HPA-axis activity so often found to accompany these disorders (Liberzon et al., 2007; MacKenzie, Odontiadis, Le Mellédo, Prior, & Baker, 2007; McEwen, 2005). Moreover, recent studies indicate that glucocorticoid administration might be effective in treating posttraumatic stress disorder and phobias (de Quervain & Margraf, 2008), potentially impacting the pgACC. The putative role of stress agents in pgACC function is furthermore underscored in a recent study showing diminished decreased activity in the pgACC when viewing emotional faces after administration of vasopressin (Zink, Stein, Kempf, Hakimi, & Meyer-Lindenberg, 2010).

The association of cortisol with the connection between the amygdala and the medial frontal pole (BA10) does resemble one of the effects found in the group of participants that did receive stress (Veer et al., 2011). The current results thus suggest that participants who showed a lesser decrease or even a small increase in endogenous cortisol over the course of the experiment demonstrate a connectivity pattern similar to what is found in participants who had been exposed to stress. In the stress group, however, this effect was irrespective of the cortisol response to the stressor, possibly due to a ceiling effect in their physiological response or a more complex interaction between neuroendocrine responses to the stressor. On the other hand, using FDG-PET imaging Kern et al. (2008) did show that stress-induced cortisol was related to decreased glucose metabolism in BA10, albeit such a finding is often difficult to relate to RSFC measures as obtained with fMRI. Since BA10 is hypothesized to be involved in stimulus oriented behavior (Burgess, Dumontheil, & Gilbert, 2007a; Burgess, Gilbert, & Dumontheil, 2007b), the increased RSFC of BA10 with the amygdala found in our study might indicate that an increase in cortisol promotes more vigilance towards threatening stimuli in our surroundings.

We found that baseline cortisol showed a strong inverse association with AUCi dynamics. That is, higher cortisol levels at baseline were indicative of larger cortisol decreases over the course of the experiment, whereas participants with lower baseline cortisol levels tended to demonstrate either a flattened AUCi or a small increase. Urry et al. (2006) demonstrated that steeper (i.e., more normative) diurnal cortisol curves are related to higher vmPFC and lower amygdala activity and better

performance during affect regulation, which could pertain to the results found in the current study: Participants demonstrating large AUCi decreases also showed strong negative functional connectivity between the amygdala and mPFC. This might indicate how dynamical behavior of diurnal cortisol aids successful regulation of stress- and, more general, emotional responses.

In the current study setup, however, we cannot infer whether baseline cortisol alone, or its interaction with time, as is measured with the AUCi, is driving our effects. However, our analyses strongly suggest that baseline cortisol alone is predictive of functional coupling between the amygdala and mPFC. Baseline cortisol was measured almost 90 minutes before RS data acquisition, yet was still associated with the strength of functional coupling of the amygdala. This might be indicative of a slow acting effect of cortisol, which has previously been related to altered functional coupling between the amygdala and mPFC during an emotional task paradigm (Henckens et al., 2010), and homeostatic processes in the aftermath of stress in general (Sapolsky et al., 2000).

Since our effects are based on correlations, it must be noted that we cannot make any inference on causality. That is, effects could be interpreted as either bottom-up or top-down in the case of amygdala-mPFC connectivity, or either as cause or consequence in the case of cortisol levels. Nevertheless, our interpretation of mPFC mediated top-down regulation of the amygdala does seem plausible given the number of studies reporting such a causal relationship between the pgACC and amygdala (Pezawas et al., 2005; Quirk & Beer, 2006; Stein et al., 2007a). Furthermore, an mPFC dependent regulation of HPA-axis activity has been well established in animal research, pointing to a facilitating role of cortisol in this circuit (Boyle et al., 2005; Diorio et al., 1993; Furay et al., 2008; Radley et al., 2006; Ulrich-Lai & Herman, 2009). A second limitation of the method pertains to network specificity when studying cortisol. Although the amygdala and its connections are heavily implicated in the brain's stress circuitry, employing a seed-based connectivity analysis renders us blind to any effects of cortisol on other resting-state functional connectivity networks. Thirdly, our results might have been influenced by the emotional working memory task that preceded the resting-state scan. Although there was a 20-min interval in

between the two scans, we cannot rule out such an effect, especially since perceived stress was mildly elevated directly after the task. Nonetheless, we did not find a relation between perceived stress and the functional connectivity patterns observed, nor was there an association with cortisol either measured as AUCi, at baseline, or during resting-state acquisition.

Our participants were not exposed to a stress paradigm, so the nature of the difference in endogenous cortisol fluctuations remains speculative, though several explanations can be proposed: 1) although not intended, (the anticipation of) lying inside the MRI scanner might have induced stress in some of our participants. Mild increases in cortisol levels have been called "scanner-induced stress" recently (Muehlhan, Lueken, Wittchen, & Kirschbaum, 2011), a scenario that is especially plausible when including scanner-naïve participants, as was the case in our study. In addition, the increase in perceived stress inside the scanner argues in favor of such scanner-induced stress. 2) Related to the previous point, anticipation of the experiment might already have caused elevated cortisol levels in some participants prior to arrival, while tension could have decreased after intake and instructions. 3) A flattened cortisol curve, as was observed in several participants, also could have been related to stressful life circumstances rather than being induced by the experimental context (Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005). However, participants were specifically required to score low on psychoneuroticism, anxiety, and depressive symptoms to be included in this study, which renders it unlikely that a recent stressful life event would have caused flattening of the cortisol morning curve. 4) Another explanation could lie in the time of arrival, in spite of counterbalancing within the group, because subjects arriving early in the morning might demonstrate higher cortisol baseline levels and therefore a steeper decrease over the course of the experiment. However, no difference in either AUCi or baseline cortisol was found between the early and late arrival participants. 5) We did not, however, obtain information on the time participants woke up on the morning of the experiment. Therefore, we cannot exclude that some baseline cortisol levels were higher due to a shorter time frame between wakening and participation in the experiment. 6) Lastly, differences in genetic makeup (e.g., expression of cortisol receptors throughout the brain) potentially could explain the

individual differences in HPA-axis activity in our sample (Ouellet-Morin et al., 2008; Wüst, Federenko, Hellhammer, & Kirschbaum, 2000).

In sum, here we show that the strength of RSFC between the amygdala and mPFC can be related to individual differences in endogenous cortisol under relatively stress-free circumstances. Although tentative, this finding could be indicative of a cortisol-mediated regulatory circuit served to adaptively adjust stress- and, more generally, emotional responses. This hypothesis should be further tested, however, using a controlled manipulation of cortisol levels, for example by dose-response experiments in which several dosages of hydrocortisone are administered. Although the current analysis was carried out on a group of participants that was not intentionally exposed to stress, our results might explain how this feedback mechanism may cause cessation of a stress response, pointing to the putative role of glucocorticoids in reaching homeostasis after a stressful event (McEwen, 2005). The current results might also provide an important link to the pathophysiology of stress-related psychiatric disorders, in which such feedback seems to fail. For the first time in humans, our results show a link between endogenous cortisol and functional connectivity between the amygdala and pgACC, which might further establish the role of cortisol in adaptive emotion regulation.

SECTION 2

PSYCHOPATHOLOGY

CHAPTER 5

Whole brain resting-state analysis reveals decreased functional connectivity in major depression

Veer, I. M., Beckmann, C. F., van Tol, M. J., Ferrarini, L., Milles, J., Veltman, D. J., Aleman, A., van Buchem, M. A., van der Wee, N. J., & Rombouts, S. A. R. B. (2010). *Frontiers in Systems Neuroscience*, *4*, 41.

ABSTRACT

Recently, both increases and decreases in resting-state functional connectivity have been found in major depression. However, these studies only assessed functional connectivity within a specific network or between a few regions of interest, while comorbidity and use of medication was not always controlled for. Therefore, the aim of the current study was to investigate whole-brain functional connectivity, unbiased by *a priori* definition of regions or networks of interest, in medication-free depressive patients without comorbidity. We analyzed resting-state fMRI data of 19 medication-free patients with a recent diagnosis of major depression (within 6 months before inclusion) and no comorbidity, and 19 age- and gender-matched controls. Independent component analysis was employed on the concatenated data sets of all participants. Thirteen functionally relevant networks were identified, describing the entire study sample. Next, individual representations of the networks were created using a dual regression method. Statistical inference was subsequently done on these spatial maps using voxel-wise permutation tests. Abnormal functional connectivity was found within three resting-state networks in depression: 1) decreased bilateral amygdala and left anterior insula connectivity in an affective network, 2) reduced connectivity of the left frontal pole in a network associated with attention and working memory, and 3) decreased bilateral lingual gyrus connectivity within ventromedial visual regions. None of these effects were associated with symptom severity or gray matter density. We found abnormal resting-state functional connectivity not previously associated with major depression, which might relate to abnormal affect regulation and mild cognitive deficits, both associated with the symptomatology of the disorder.

INTRODUCTION

Patients suffering from a major depressive episode typically show pervasive depressed mood or anhedonia, accompanied by several cognitive and physical symptoms (APA, 1994). The apparent heterogeneity in depressive symptom domains (i.e., mood, cognition, motor, and vegetative) is unlikely to be explained by the (functional) breakdown of a single brain area (Davidson, Pizzagalli, Nitschke, & Putnam, 2002). It has thus been proposed that depressive symptoms are associated with dysregulation of a brain network encompassing large parts of the prefrontal cortex (PFC), limbic areas, and subcortical structures (Mayberg, 1997; 2003).

Based on data from blood flow and glucose metabolism SPECT and PET studies, and more recently task-related functional MRI (fMRI) studies, current models for depression postulate that ventral and dorsal subsystems of this brain network are differentially affected in this disease (Drevets et al., 2008; Mayberg, 2003). An imbalanced functional integration of these subsystems may lead to a heightened response to negative information in ventral regions (bottom–up) on the one hand, and a failure to regulate this response through dorsal regions (top–down) on the other hand (Phillips, Drevets, Rauch, & Lane, 2003b). For example, engagement of lateral PFC regions has been linked to efficient top–down regulation of affective responses (Dolcos & McCarthy, 2006; Pessoa, 2008), a mechanism that has been shown to fail in patients suffering depression (Johnstone et al., 2007).

Over the last decade, studying such functional interactions between brain regions or systems has become increasingly important for understanding the dynamic interactions between neural systems in both health and disease (Stephan, Riera, Deco, & Horwitz, 2008). In depression, several studies have shown abnormal functional connectivity (FC) during both cognitive and emotional task paradigms (Chen et al., 2008; Johnstone et al., 2007; Matthews, Strigo, Simmons, Yang, & Paulus, 2008; Urry et al., 2006), which have already provided valuable insights on how dysfunctional interactions between brain regions may relate to abnormal behavioral response patterns in depressed patients. However, it might also be beneficial to explore whether these connections are compromised in the absence of goal-directed (i.e., task-induced)

behavior. For example, resting-state (RS; i.e., without external task demands) FC may be able to predict how the brain responds to an externally cued task (Mennes et al., 2010). Studies employing RSFC have shown to be successful in mapping large-scale connectivity patterns in the brain (Biswal et al., 1995; Fox & Raichle, 2007; Lowe, Mock, & Sorenson, 1998). In addition, these so-called resting-state networks (RSNs) are found consistently across participants and over time (Damoiseaux et al., 2006; Shehzad et al., 2009) and show a remarkable overlap with patterns of task-induced activity (Smith et al., 2009).

RS-fMRI studies in major depression have recently reported on altered FC in several areas within the proposed network model of depression (Drevets et al., 2008; Mayberg, 1997). Decreased connectivity of the dorsal anterior cingulate cortex (ACC) with the medial thalamus and left pallidostriatum was found in patients suffering from depression, and a trend for decreased connectivity between the ACC and the amygdala (Anand, Li, Wang, Wu, Gao, Bukhari, Mathews, Kalnin, & Lowe, 2005b; 2005a). In another study, depressive patients were found to show increased connectivity of the subgenual ACC (cg25) and the thalamus within the default mode network (DMN) (Greicius et al., 2007), a canonical RSN (Greicius et al., 2003; Raichle et al., 2001). This finding was partially confirmed by a recent study showing unique cg25, but not thalamic, connectivity within the DMN in the depression group (Zhou et al., 2010). It must be noted, however, that for this effect only qualitative comparisons were carried out between the groups. Additionally, these researchers found increased intra-network connectivity in depression between regions of the DMN, and within the task positive network (TPN), associated with attention and working memory (Fox et al., 2005), together with increased anticorrelations between regions of the two networks (Zhou et al., 2010). A last study did not show any FC differences between major depressive disorder (MDD) patients and controls using conventional statistics (Craddock, Holtzheimer, Hu, & Mayberg, 2009). However, the authors were able to discriminate between patients and controls using support vector classification. In addition to the altered FC found in several task-related fMRI studies, these RS findings further support the idea of dysfunctional interactions as a core feature of depressive symptomatology.

To date, RS-fMRI studies focusing on depression examined connectivity in a limited number of predefined regions or networks of interest, thereby not fully exploring the data as acquired with RS-fMRI. That is, recent studies have identified several other networks of simultaneously oscillating brain regions (Beckmann, DeLuca, Devlin, & Smith, 2005; Damoiseaux et al., 2006), which may represent multiple functional domains. Furthermore, in some of the studies in MDD, comorbidity and use of medication could not be ruled out as potential confounders.

The aim of the present study was to investigate FC patterns using RS-fMRI in medication-free patients with MDD without comorbidity, and carefully matched healthy controls. Rather than focusing on predefined regions or networks of interest, we adopted an inclusive (exploratory) approach by investigating whole-brain RS-fMRI FC at the network level, ensuring the optimal use of the wealth of information present in the data. Based on the current neurobiological models for depression and the RS studies described above, we expected that altered connectivity would be observed in those RSNs that include areas known to be associated with affective (including ventral prefrontal cortex and limbic areas) and more cognitive (including lateral prefrontal and parietal areas) processing, as well as RSNs that show cortico–striatal connectivity.

METHODS

Participants

Participants were selected from the MRI study of the large-scale longitudinal multi-center Netherlands Study on Depression and Anxiety (NESDA; www.nesda.nl) (Penninx et al., 2008), which is designed to examine the long-term course and consequences of depression and anxiety disorders. Participants were recruited through general practitioners, primary care and specialized mental care institutions. For the current study, all participants were required to be fluent in Dutch and right-handed. Patients were included when they met the following criteria: 1) a recent diagnosis (i.e., within 6 months before inclusion) of MDD as indexed by the fourth edition of

Table 5.1 Demographic and clinical characteristics for the study sample.

	healthy controls (*n* = 19)	major depressive disorder (*n* = 19)
Age	36.11 ± 10.56 (21-53) y/o	36.21 ± 9.7 (20-57) y/o
Gender	8 male/11 female	8 male/11 female
Education *	14 ± 2.67 (9-18) years	12.21 ± 2.35 (9-18) years
MADRS **	0.63 ± 1.07 (0-3)	14.21 ± 9.62 (0-33)

Note: MADRS = Montgomery-Asberg depression rating scale. Except for sex, all values are mean ± *SD* (range). * $p < .05$, ** $p < .001$, using independent sample *t*-tests.

the diagnostic and statistical manual of mental disorders (DSM-IV) (APA, 1994), based on the Composite Interview Diagnostic Instrument (CIDI; lifetime version 2.1), administered by a trained clinical interviewer, 2) no current comorbidity with other DSM-IV axis-1 disorders, and 3) no use of psychotropic medication. Exclusion criterion for controls was a history of any DSM-IV axis-1 disorder based on the CIDI. Axis-2 disorders were not assessed in this study. Exclusion criteria for all participants were: 1) daily use of medication or other substances known to affect the central nervous system; 2) the presence or history of major internal or neurological disorders; 3) history of dependency on or recent abuse of alcohol and/or drugs (i.e., in the past year) as diagnosed with the CIDI; 4) hypertension; 5) general MRI-contraindications. None of the included patients underwent treatment for depression.

For the present study, imaging data were available from 23 MDD patients who fulfilled the aforementioned criteria. Two patients were removed from the sample due to excessive head motion during scan acquisition (> 3 mm in any of the acquired volumes). Two other patients were removed because no proper age-matched healthy control (HC) was available. For each of the remaining 19 MDD patients, we included in a pair-wise fashion an age- and sex-matched healthy control subject, although education was higher in controls (see **Table 5.1**). The mean Montgomery–Asberg depression rating scale (MADRS) (Montgomery & Asberg, 1979) symptom severity score for the MDD group was 14.21, *SD* 9.62, with five participants considered to be in remission (MADRS score < 10) at the time of the imaging study. Written informed

consent was obtained from all participants and none received compensation except for reimbursement of travel expenses. The study was approved by the Central Ethics Committees of the three participating medical centers (i.e., Leiden University Medical Center [LUMC], Amsterdam Medical Center [AMC], and University Medical Center Groningen [UMCG]).

MATERIALS

Data acquisition

Participants were scanned at one of the three participating centers within 8 weeks after completion of NESDA baseline interview (Penninx et al., 2008). RS-fMRI data were acquired at the end of the fixed imaging protocol: after completion of three task-related functional MRI runs (to be reported elsewhere), and the acquisition of an anatomical scan (scan sequence: Tower of London, word encoding, T_1-weighted scan, word recognition, perception of facial expression). In the darkened MR room participants were instructed to lie still with their eyes closed and not to fall asleep. Compliance to these instructions was verified as part of the exit interview.

Imaging data were acquired on a Philips 3T Achieva MRI scanner using a six- (Amsterdam) or eight-channel (Groningen and Leiden) SENSE head coil for radiofrequency reception (Philips Healthcare, Best, The Netherlands). RS-fMRI data were acquired using T_2^*-weighted gradient-echo echo-planar imaging with the following scan parameters in Amsterdam and Leiden: 200 whole-brain volumes; repetition time (TR) = 2300 ms; echo time (TE) = 30 ms; flip angle = 80° ; 35 axial slices; no slice gap; FOV = 220 × 220 mm; in plane voxel resolution = 2.3 mm × 2.3 mm; slice thickness = 3 mm; same in Groningen, except: TE = 28 ms; 39 axial slices; in plane voxel resolution = 3.45 mm × 3.45 mm. For registration purposes and analysis of gray matter density, a high-resolution T_1-weighted image was acquired with the following scan parameters: repetition time (TR) = 9 ms; echo time (TE) = 3.5 ms; flip angle = 80° ; 170 sagittal slices; no slice gap; FOV = 256 × 256 mm; in plane voxel resolution = 1 mm × 1 mm; slice thickness = 1 mm.

Data preprocessing

The preprocessing of RS-fMRI images was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.90, part of FSL (FMRIB's Software Library; www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). The following processing steps were applied: motion correction (Jenkinson et al., 2002), removal of non-brain tissue (Smith, 2002), spatial smoothing using a Gaussian kernel of 4-mm full width at half maximum, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 50 s (i.e., 0.01 Hz cut-off), and registration to the high resolution T_1 and MNI-152 standard space (T_1 standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) images (Jenkinson et al., 2002; Jenkinson & Smith, 2001). Normalized 4D data sets were subsequently resampled to 4 mm isotropic voxels to reduce computational burden in the following analysis steps.

Extracting resting-state networks

Standard group independent component analysis (ICA) was carried out using probabilistic ICA (PICA) (Beckmann & Smith, 2004), as implemented in FSL's MELODIC tool, Version 3.09. Default group PICA processing steps were applied to the individual preprocessed and normalized data sets: masking out non-brain voxels, voxel-wise de-meaning of the data, and normalization of the voxel-wise variance based on all data sets. Subsequently, data sets from both MDD patients and HCs were concatenated in time to create a single 4D data set, which was then projected into a 20-dimensional subspace using principal component analysis. Next, the data set was decomposed into 20 sets of independent vectors, which describe signal variation across the temporal (time-courses) and spatial (maps) domain by optimizing for non-Gaussian spatial source distributions using the FastICA algorithm (Hyvärinen, 1999). At this model order selection, it has been shown that most of the frequently observed large-scale RSNs can be discerned in the data when using this method (Abou Elseoud et al., 2010). The resulting estimated component maps were divided by the standard deviation of the residual noise and thresholded at a posterior probability threshold of $p > .5$ (i.e., an equal loss is placed on false positives and false negatives)

by fitting a Gaussian/Gamma mixture model to the histogram of intensity values (Beckmann & Smith, 2004).

Statistical analyses

Subject specific statistical maps were created to test for differences between the MDD and HC groups in the identified components. This was done adopting a dual regression procedure (as previously described in: Filippini et al., 2009). In short, multiple linear regression of the *z*-thresholded Group PICA maps against the preprocessed individual 4D resampled data sets yielded a subject specific time course for each of the group components. Next, multiple linear regression of these time courses was carried out against the preprocessed individual 4D data sets in the standard space resolution (i.e., 2 mm), thereby providing better spatial specificity. This resulted in subject specific *z*-maps for each of the 20 group components.

Prior to statistical inference 13 out of the 20 components were identified as anatomically and functionally relevant RSNs upon visual inspection, the seven others reflecting distinct artifacts resulting from head motion, fluctuations in cerebrospinal fluid, and physiological or scanner noise. Criteria for inclusion were: signal within the low frequency range of 0.1–0.01 Hz (Cordes et al., 2001; Lowe et al., 1998), connectivity patterns were mainly located in gray matter, and presence of coherent clusters of voxels (De Martino et al., 2007). Inference was carried out only on the subject specific *z*-maps of the 13 relevant RSNs. Statistical difference was assessed non-parametrically using FSL's Randomize tool, Version 2.1, incorporating threshold-free cluster enhancement (TFCE) (Smith & Nichols, 2009). Besides modeling regressors for each of the two groups, additional nuisance regressors describing scanner location and age were added to the model. Separate null distributions of *t*-values were derived for the contrasts reflecting the between and within group effects by performing 5000 random permutations and testing the difference between groups or against zero for each iteration (Nichols & Holmes, 2002). For each RSN, the resulting statistical maps were thresholded at $p < .05$ (TFCE-corrected for family-wise errors) for the group main effects. Between-group effects were thresholded controlling the local false discovery rate (FDR) (Efron, 2004; Filippini et al., 2009) at $q < .01$, and subsequently spatially

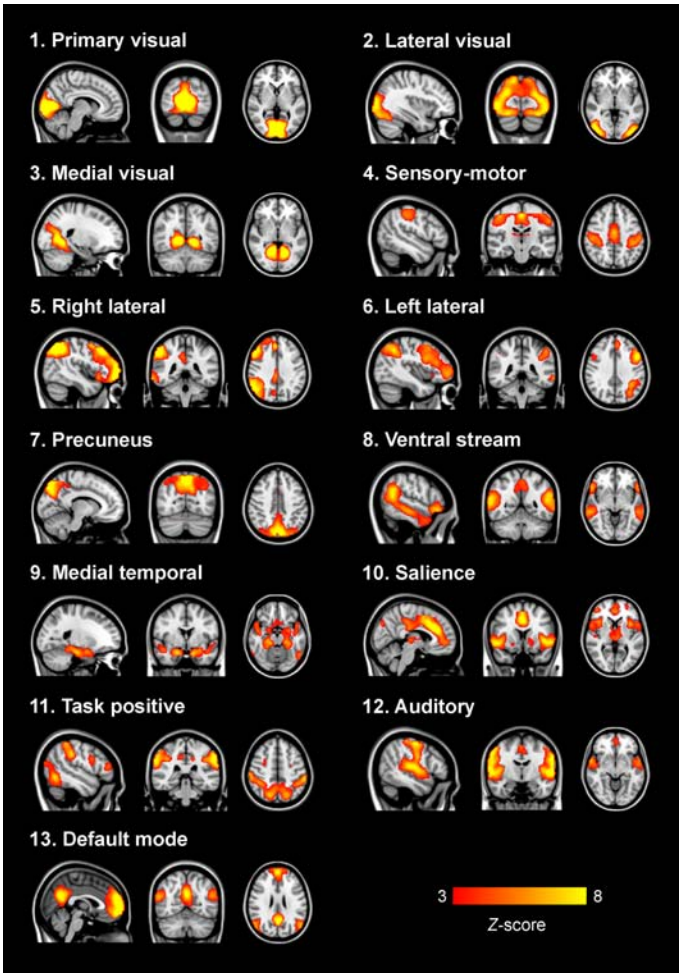

Figure 5.1 Depicted here are the 13 functionally relevant RSNs resulting from the group PICA step carried out on the concatenated data sets from both patients and controls. Most networks have previously been described (for example in: Beckmann et al., 2005; Damoiseaux et al., 2006) and show assemblies of regions associated with sensory processing, affective processing, and higher order cognitive processes. Images are *z*-statistics, ranging from 3 to 8, overlaid on the 2 mm MNI-152 standard brain. The left hemisphere of the brain corresponds to the right side in this image.

masked with a binary representation of the conjunction of the group main effects images. Note that we applied a more stringent FDR threshold than the more generally accepted $q \leq .05$, together with masking for the group main effects, to decrease susceptibility to Type I errors when testing multiple RSNs.

Gray matter morphology

Major depressive disorder-related gray matter (GM) abnormalities have been found previously in several regions of the brain, although not always consistently (Lorenzetti, Fornito, Allen, & Yücel, 2009; Sheline, 2003). To test whether altered FC in the present study might be explained by MRI-detectable loss of gray matter, a VBM style analysis was run on the acquired high-resolution T_1-weighted data sets (Ashburner & Friston, 2000; Good et al., 2001). Using FSL's VBM toolbox, all structural images were first brain extracted, then tissue-type segmented, normalized to MNI-152 standard space and non-linearly registered to each other (e.g., Douaud et al., 2007). Next, standard space binary masks were created from the voxels that covered each RSN (conjunction of the FWE-corrected HC > 0 and MDD > 0 contrast maps) as well as from voxels showing differences between the two groups within the separate networks (local FDR controlled HC > MDD and MDD > HC contrast maps). The binary masks were then used to extract mean gray matter intensity scores within these masks for each of the participants. To rule out the influence of any subtle GM density variations, we included the GM values, from both the difference masks and the RSN as a whole, as regressors in the statistical model (see, e.g., Damoiseaux et al., 2008). Additionally, using SPSS Version 16.0 (SPSS Inc.) between-group *t*-tests were carried out on the participants' mean intensity scores derived from each mask to test whether the two groups differed in GM density on average. Note that whole brain VBM results of a large sample (including MDD) from the NESDA study is reported elsewhere (van Tol et al., 2010).

RESULTS

Resting-state functional connectivity

Thirteen functionally relevant RSNs were found using the group PICA analysis (**Figure 5.1**). Most of these networks have been described in previous studies using similar methodology and were shown to be stable across participants and over time (Beckmann et al., 2005; Damoiseaux et al., 2006). The assemblies of brain areas shown in these networks covered the primary [1], lateral [2] and medial visual cortex [3], sensory-motor cortex [4], ventral stream [8] auditory cortex [12], the hippocampus–amygdala complex [9], precuneus [7] together with the DMN [13], a network associated with salience processing (Seeley, Menon, et al., 2007b) [10], and networks encompassing areas associated with higher order cognition such as attention [11] and working memory [5, 6].

The presence of all 13 networks found with PICA was confirmed in both the HC and MDD group by testing the main effects of group on the subject specific z-maps of these networks (all $p < .05$, TFCE and FWE-corrected). Between-group differences in the voxel-wise spatial distribution of the FC maps were subsequently revealed in three networks (local FDR-corrected at $q \leq .01$) (see **Figure 5.2** and **Tables 5.2-5.4**).

Within these networks nearly all differences indicated decreased FC in the MDD group. The first network showed an assembly of functionally connected regions in the auditory cortex (Heschl's gyrus) bilaterally, extending into the pre- and postcentral gyri, as well as more ventral areas known to be involved in affective processing, including the insula and temporal poles bilaterally, the medial PFC (BA 10), and bilateral amygdala. Whereas the amygdala and left insula showed connectivity with the rest of the network in HCs, these regions showed decreased FC in the depressed group.

In addition, increased FC in the MDD group was found in the right inferior frontal gyrus (IFG) within this RSN (**Figure 5.2a and 5.2b**, RSN 12). The second network mainly showed FC within the lateral parietal cortex, temporal–occipital junction, and precentral gyrus, which are areas involved in attention and working

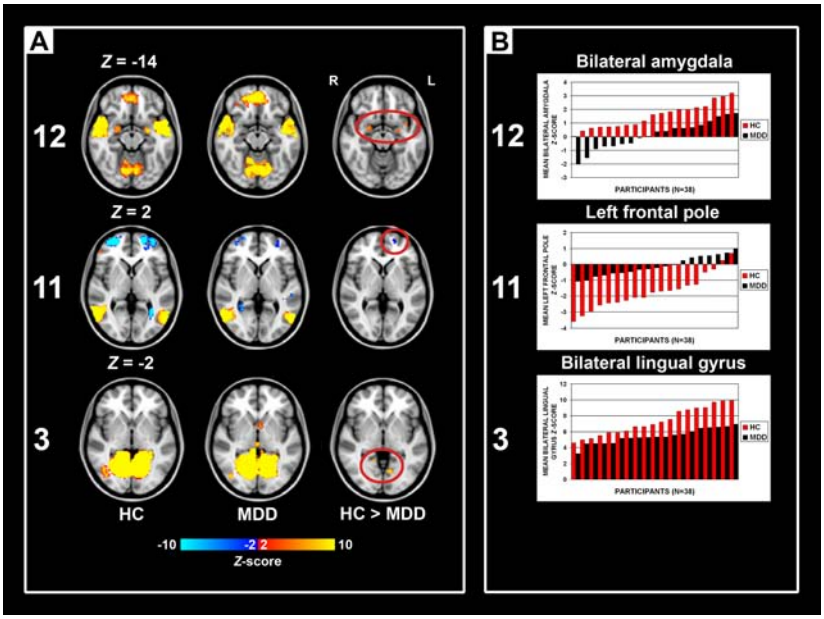

Figure 5.2 Group main effects and between-group effects. Numbering corresponds to the networks depicted in **Figure 5.1**. (**A**) Depicted here are the group main and between-group effects for three RSNs. Group main effects are corrected for family-wise errors ($p < .05$) and between-group effects are corrected according to a local false discovery rate of 1 %. RSN 12 shows an assembly of ventral affective regions, such as temporal poles, insula, medial prefrontal cortex, and amygdala, the latter two regions demonstrating decreased connectivity within the MDD group. RSN 11 shows brain regions linked to attention, of which the left frontal pole shows decreased connectivity in the MDD group. RSN 3 shows MDD-related decreased connectivity of the bilateral lingual gyrus with other medial visual areas. Images are *z*-statistics, ranging from 2 to 10, overlaid on the 2 mm MNI-152 standard brain. The left hemisphere of the brain corresponds to the right side in this image. HC, healthy controls; MDD, major depressive disorder. (**B**) Distribution of the mean individual z-scores within the bilateral amygdala (12), left frontal pole (11), and bilateral lingual gyrus (3). Depicted in red are the controls, in black the MDD group, both sorted from smallest to highest *z*-value.

memory. In addition, the frontal poles were found to be negatively associated with the time course of this network. Reduced FC of the left frontal pole was demonstrated in

Table 5.2 RSN 12 characteristics and statistics.

Region	coordinates (MNI space)			p_{FWE}		$p_{local\ FDR}$	$p_{local\ FDR}$
	x	y	z	HC	MDD	HC > MDD	MDD > HC
Positive							
left cerebellum	-16	-68	-22	.002	<.001	ns	ns
right cerebellum	18	-68	-22	<.001	<.001	ns	ns
left superior temporal	-44	0	-14	<.001	<.001	ns	ns
gyrus	-46	-30	6	<.001	<.001	ns	ns
right superior temporal	52	4	-14	<.001	<.001	<.001	ns
gyrus	58	-32	6	<.001	<.001	ns	ns
left amygdala	-24	-6	-14	.007	<.001	<.001	ns
right amygdala	24	-4	-16	.02	ns	<.001	ns
left/right medial							
prefrontal cortex	0	48	-14	.005	<.001	ns	ns
left insula	-40	-6	-2	<.001	<.001	ns	ns
	-36	4	-18	<.001	ns	<.001	ns
right insula	38	-6	6	<.001	<.001	ns	ns
right thalamus	12	-22	0	ns	.008	ns	ns
left/right anterior cingulate							
gyrus	0	2	38	<.001	<.001	ns	ns
left pre- and postcentral							
gyrus	-44	-20	44	<.001	<.001	ns	ns
right pre- and postcentral							
gyrus	48	-16	44	<.001	<.001	ns	ns
left/right postcentral gyrus	0	-26	50	.002	<.001	ns	ns
right inferior frontal gyrus	56	24	16	ns	<.001	ns	<.001
Negative							
left thalamus	-12	-6	12	ns	.039	ns	ns
left middle frontal gyrus	-28	32	36	.01	ns	ns	ns
left precentral gyrus	-28	6	48	ns	.026	ns	ns

Note: group main effects are FWE-corrected for multiple comparisons, between group contrasts are corrected for multiple comparisons using a local false discovery rate (FDR) of 1 %. HC = healthy controls, MDD = major depressive disorder, ns = not significant.

the MDD group (**Figure 5.2a and 5.2b,** RSN 11). The third network showed functionally integrated areas within the medial occipital cortex, mostly covering Brodmann area 19, involved in visual processing. Although both controls and depressed participants demonstrated this connectivity pattern, a consistent decrease in functional integration of the lingual gyrus was found bilaterally in the MDD group in this RSN (**Figure 5.2a and 5.2b,** RSN 3).

The wide range in MADRS scores in the patient group allowed us to examine the relation between current symptom severity and the strength of the function-

Table 5.3 RSN 11 characteristics and statistics.

Region	coordinates (MNI space)			p_{FWE}		$p_{local\ FDR}$	$p_{local\ FDR}$
	x	y	z	HC	MDD	HC > MDD	MDD > HC
Positive							
left inferior temporal gyrus	-48	-62	-12	<.001	<.001	ns	ns
right inferior temporal gyrus	54	-60	-8	<.001	<.001	ns	ns
left lateral occipital cortex	-40	-80	18	<.001	<.001	ns	ns
right lateral occipital cortex	44	-72	14	<.001	<.001	ns	ns
left supramarginal gyrus	-56	-28	24	<.001	<.001	ns	ns
	-46	-38	40	<.001	<.001	ns	ns
right supramarginal gyrus	58	-40	24	<.001	<.001	ns	ns
	40	-38	40	<.001	<.001	ns	ns
left posterior cingulate cortex	-10	-38	40	<.001	<.001	ns	ns
right posterior cingulate cortex	12	-38	42	<.001	<.001	ns	ns
left middle frontal gyrus	-46	36	12	.025	ns	ns	ns
right middle frontal gyrus	50	40	8	.028	ns	ns	ns
right precentral gyrus	48	8	26	.035	ns	ns	ns
left/right anterior cingulate gyrus	2	2	32	.037	ns	ns	ns
Negative							
left hippocampus	-28	-24	-16	.002	ns	ns	ns
left middle temporal gyrus	-58	-30	-10	.002	.002	ns	ns
right middle temporal gyrus	58	-20	-10	.003	ns	ns	ns
left frontal pole	-24	56	-4	<.001	ns	ns	<.001
right frontal pole	32	56	-2	<.001	<.001	ns	ns
left paracingulate gyrus	-8	32	36	.003	ns	ns	ns
right paracingulate gyrus	4	32	38	.003	.003	ns	ns
left middle frontal gyrus	-36	16	38	ns	<.001	ns	ns
left/right cuneus	2	-78	36	<.001	<.001	ns	ns

Note: group main effects are FWE-corrected for multiple comparisons, between group contrasts are corrected for multiple comparisons using a local false discovery rate (FDR) of 1 %. HC = healthy controls, MDD = major depressive disorder, ns = not significant.

al connections with the areas showing abnormal connectivity in this study. Within the depression group, Pearson correlation coefficients were calculated between the MADRS scores and the individual *z*-scores obtained from the affected areas within the corresponding individual component maps. However, no association was found between FC strength and symptom severity in any of these regions.

Table 5.4 RSN 3 characteristics and statistics.

Region	coordinates (MNI space)			p_{FWE}		$p_{local\ FDR}$	$p_{local\ FDR}$
	x	y	z	HC	MDD	HC > MDD	MDD > HC
Positive							
left lingual gyrus	-10	-68	-2	<.001	<.001	<.001	ns
right lingual gyrus	16	-68	-2	<.001	<.001	ns	ns
	16	-50	-2	<.001	<.001	<.001	ns
left lateral occipital cortex	-38	-76	22	<.001	<.001	ns	ns
right lateral occipital cortex	50	-72	16	.013	<.001	ns	ns
left cuneus	-14	-76	22	<.001	<.001	ns	ns
right cuneus	18	-76	22	<.001	<.001	ns	ns
right precentral gyrus	40	8	28	ns	.03	ns	ns
left caudate nucleus	-6	8	4	ns	.011	ns	ns
right caudate nucleus	8	8	4	ns	.016	ns	ns

Note: group main effects are FWE-corrected for multiple comparisons, between group contrasts are corrected for multiple comparisons using a local false discovery rate (FDR) of 1 %. HC = healthy controls, MDD = major depressive disorder, ns = not significant.

Gray matter results

No differences in mean gray matter were observed between controls and depressed participants in any of the three RSNs as a whole, or in the areas showing between-group differences within these RSNs, all $t(36) < 1$, $p > .3$. In addition, adding GM density values as covariates in the statistical model did not change the functional connectivity results as described in the previous section. This indicates that the altered FC within the three networks is unlikely to be related to macroscopic (i.e., MRI observable) gray matter abnormalities.

DISCUSSION

In the present study we set out to investigate differences in whole brain FC between medication-free MDD patients without comorbidity, and a group of age- and sex-matched healthy controls using RS-fMRI. It was expected that altered connectivity would be observed in those RSNs which contain regions previously described to

show altered RSFC in depression (Anand, Li, Wang, Wu, Gao, Bukhari, Mathews, Kalnin, & Lowe, 2005a; 2005b; Greicius et al., 2007; Zhou et al., 2010), as well as in other regions known to be involved in affective pathology (Chen et al., 2008; Johnstone et al., 2007; Matthews et al., 2008; Phillips, Drevets, Rauch, & Lane, 2003b; Urry et al., 2006). In this study we mainly found evidence for MDD-related *decreased* FC within three RSNs. These alterations have not been associated with major depression before.

First, altered FC was found in a network with regions known to be involved in emotional processing and affect regulation, such as the anterior insula, dorsal anterior cingulate cortex (dACC), ventromedial prefrontal cortex (vmPFC), temporal poles and amygdala (Pessoa, 2008). MDD patients showed strongly reduced connectivity with the amygdala within this RSN. Coupling between the vmPFC and amygdala has previously been found during downregulation of negative affect in healthy controls (Urry et al., 2006), as was reflected by decreasing amygdala activation with increasing vmPFC activation. In a similar study in depression, MDD patients showed altered coupling between these regions, potentially reflecting impaired top–down control over amygdala responses and inability to down-regulate negative affect (Johnstone et al., 2007). Involvement of the anterior insula along with dACC and somatosensory regions in this network may furthermore underscore its potential role in interoceptive awareness and emotional experience (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004). Besides regions showing decreased FC in this RSN, the depression group also demonstrated increased connectivity of the rIFG. This region has been implicated in coping with exertion of both cognitive (Aron, Robbins, & Poldrack, 2004) and emotional (Dolcos et al., 2006) control. Recently, IFG function was found compromised in MDD when executive control had to be exerted in minimizing emotional distraction (Wang et al., 2008). Abnormal recruitment of the rIFG within the current RSN may indicate a higher propensity towards inhibition of emotional responses in depression, although the neurocircuitry to successfully do this is compromised. Taken together, the observed decoupling of the amygdala, decreased left insula connectivity, and increased rIFG connectivity within this network may be related to the impaired regulation and integration of affective responses observed in

MDD patients.

Second, we found reduced involvement of the left lateral frontal pole in a network often referred to as the TPN (Fox et al., 2005), its constituent regions commonly found activated during tasks that require cognitive effort or attention (Corbetta & Shulman, 2002). The lateral frontal poles are thought to play a key role in executive function and stimulus oriented behavior (Burgess, Dumontheil, & Gilbert, 2007a; Burgess, Gilbert, & Dumontheil, 2007b), which would complement the proposed function of this RSN. Reduced FC of the left lateral frontal pole, as was found in depression within this network, may thus reflect a suboptimally integrated attention system or reduced externally oriented attention in MDD. This abnormal connectivity pattern may relate to the cognitive deficiencies often observed in depressed patients (Ebmeier, Rose, & Steele, 2006; Rogers et al., 2004), yet this relation should be assessed in task-related imaging studies designed to address this question more directly.

Finally, we demonstrated decreased FC of the bilateral lingual gyrus in MDD in a network including ventromedial occipito-temporal areas. Although both groups showed strong connectivity with the bilateral lingual gyrus within this network, MDD patients revealed a consistent decrease in connectivity strength. Abnormalities in the visual stream are not commonly reported in MDD, and the interpretation of this effect in the depressed patients in the current study must therefore remain speculative.

In the present study we did not find abnormalities in regions previously reported to show altered RSFC in MDD. For example, increased involvement of the subgenual ACC and thalamus in the DMN has been found in MDD (Greicius et al., 2007; Zhou et al., 2010), but was not observed in the current study. Previous work furthermore reported increased connectivity of multiple brain regions within the TPN (Zhou et al., 2010). In the present study, in contrast, we showed MDD-related *reduced* connectivity of the frontal poles, which is at variance with previously found increases in connectivity in this network. In addition, support for reduced coupling between the dorsal ACC and seeds from the pallidostriatum and thalamus in MDD was not found, as has been described in previous studies (Anand, Li, Wang, Wu, Gao, Bukhari, Mathews, Kalnin, & Lowe, 2005a; 2005b).

The discrepancy in results between these studies and ours could be ascribed to differences in patient samples and analysis methods. In contrast to other studies, we report on a sample of medication-free MDD patients without comorbidity and with carefully age- and gender-matched controls. Secondly, for the current study we employed ICA analysis at the group level to obtain whole brain patterns of FC. It is conceivable that this method yields different results compared to approaches using correlations with, or between *a priori* defined regions of interest, or even when using ICA on individual data sets, although little is at present known about cross-validity between the methods.

A limitation of the present study was that our patient sample was mildly depressed on average. In addition, some patients already showed a clinically significant decrease in symptom severity because of the delay between the diagnostic assessment and the time of scanning. While this may have decreased the overall sensitivity of the study, the method applied was still successful in detecting brain functional correlates of depression, even in a mildly affected patient sample. Moreover, the effects found here were shown not to be associated with the current state of symptom severity, indicating that the observed alterations in FC may not be specific to the active state of the disorder and may not cease to exist during the remitted state.

Another limitation of the current study was the possible influence of between-group differences in heart rate variability and breathing on the results. The sampling rate used in this study (2.2 seconds per volume) was too low to avoid aliasing of these physiological signals in the data acquired. Applying temporal filtering will, therefore, not remove signal variance associated with these signals. Since physiological activity was not monitored in the current study, it remains unclear if any difference between the two groups has influenced the results. However, it has been shown that ICA is capable of detecting signal sources associated with confounding physiological signals, and that it can successfully split these from the signals of interest (Beckmann et al., 2005). We therefore think that it is unlikely that any of the differences found in this study were introduced by these physiological signals.

Because MDD-related gray matter (GM) abnormalities have been reported elsewhere (Lorenzetti et al., 2009; Sheline, 2003), we investigated whether our

MDD sample showed regions of altered GM density, potentially biasing FC within the RSNs. However, no differences were observed in average GM density between controls and patients in either of the affected RSNs as a whole, nor in the regions showing altered FC within these RSNs. In addition, GM density variance did not contribute to the altered FC patterns observed. Therefore, it is unlikely that the differences in FC were related to global or focal changes in GM density within the current study sample.

Our MDD group furthermore consisted of both first episode and recurrent episode MDD patients. Recurrence of depressive episodes can be considered an aggravation of MDD, which might cause – or conversely be caused by – an exacerbation of abnormal FC patterns. However, the small size of both subgroups, as well as the cross-sectional nature of the current study, prevented us to address this question and compare the two groups in a meaningful way. Nevertheless, follow-up data are currently being collected as part of the NESDA study. Analysis of these data should allow us to shed more light on this matter and to test whether the RSFC at baseline may have a predictive value in determining which patients are more vulnerable to develop recurrent depressive episodes. To this end, support vector classification of individual RSFC maps could be employed (Craddock et al., 2009).

In conclusion, we showed that (a history of) major depression is associated with altered FC within multiple RSNs, which could reflect less integrated processing of affective information in ventral (limbic) areas and compromised cognitive functional pathways in dorsal (PFC) regions. The current findings thereby complement previous findings on both affective and cognitive abnormalities in depression and will further increase our knowledge about the pathophysiology of the disorder.

CHAPTER 6

Evidence for focal right amygdala volume reductions in posttraumatic stress disorder following childhood trauma

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ABSTRACT

Hippocampus and amygdala volumes in posttraumatic stress disorder (PTSD) related to childhood trauma are relatively understudied, albeit the potential importance to the disorder. Whereas a few studies reported hippocampal volume reductions, no evidence was found for abnormal amygdala volumes. Further reasearch is thus warranted. Here we investigated hippocampus and amygdala volumes and shapes in an adult sample of PTSD patients related to childhood trauma. T_1-weighted magnetic resonance images were acquired from 12 female PTSD patients with trauma related to physical, sexual, and/or emotional abuse before age 18, and 12 age- and education-matched healthy female controls. Automated segmentation of the hippocampus and amygdala was carried out, and volumes were calculated and corrected for total intracranial volume. Additionally, a shape analysis was done on the surface of the structures to explore abnormalities in specific subnuclei. Decreased right amygdala volumes were found in PTSD patients as compared with controls. Volume reductions appeared to be specifically located in the basolateral and superficial nuclei groups. Severity of sexual abuse during childhood was negatively correlated with the size of the amygdala. No difference in hippocampal volumes was found. Although our results are not conclusive, we hypothesize that traumatic events in childhood might impede normal development of the amygdala, which could render a person more vulnerable to develop PTSD later in life.

INTRODUCTION

Patients suffering posttraumatic stress disorder (PTSD) experience negatively arousing intrusions, often reliving the traumatic experience that shaped the disorder. Key roles in the neuropathology of PTSD and its symptomatology have been attributed to the amygdala and hippocampus (Pitman et al., 2012). At the functional level, abnormal hippocampus activity has mainly been associated with trauma-related memory (Astur et al., 2006; Bremner, Vythilingam, Vermetten, Southwick, McGlashan, Staib, et al., 2003b; Brohawn et al., 2010; Shin et al., 2004; Thomaes et al., 2009), while the amygdala often has been found hyperresponsive to trauma or threat-related stimuli in PTSD (Bryant et al., 2008; Protopopescu et al., 2005; Shin et al., 2005). Abnormal function of these subcortical brain structures might be explained by an underlying compromised anatomical integrity.

Indeed, the hippocampus has frequently been found to be smaller in PTSD patients or traumatized subjects without PTSD compared with healthy controls (Apfel et al., 2011; Bossini et al., 2008; Villarreal et al., 2002; Vythilingam et al., 2005; Wang et al., 2010; Wignall et al., 2004), though not always (Fennema-Notestine, Stein, Kennedy, Archibald, & Jernigan, 2002; Golier et al., 2005; Pederson et al., 2004). Nevertheless, bilateral hippocampus volume decreases appeared to be consistent in recent meta-analyses (Karl et al., 2006; Woon et al., 2010; Woon & Hedges, 2011). Volumetric studies of the amygdala, in contrast, mostly failed to show differences (Bonne et al., 2001; Bremner et al., 1997; Fennema-Notestine et al., 2002; Gilbertson et al., 2002; Gurvits et al., 1996; Lindauer et al., 2004; 2005; Wignall et al., 2004), though decreases have been reported (Matsuoka, Yamawaki, Inagaki, Akechi, & Uchitomi, 2003; Goran Pavlisa, Papa, Pavić, & Pavlisa, 2006). Nevertheless, recent meta-analyses do offer evidence for decreased right (Karl et al., 2006) and left amygdala volumes in the disorder (Karl et al., 2006; Woon & Hedges, 2009), though the effect sizes are low.

Whereas most studies have focused on patients that have been exposed to trauma in adulthood, volumetric data on the hippocampus and amygdala are still sparse in adult PTSD patient samples with a history of childhood maltreatment

(Bremner et al., 1997; Bremner, Vythilingam, Vermetten, Southwick, McGlashan, Nazeer, et al., 2003a; Pederson et al., 2004; Stein, Koverola, Hanna, Torchia, & McClarty, 1997). It seems especially relevant to study the detrimental effects of traumatic experiences during childhood, since these may cause a change in the normal developmental trajectory (i.e., increase in volume) of the hippocampus and amygdala throughout adolescence into adulthood (Giedd et al., 1999; Guo et al., 2007; Østby et al., 2009). Consequently, such abnormal trajectory could render the brain more vulnerable to develop affective psychopathology later in life.

A recent meta-analysis in PTSD patients with a history of childhood trauma (Woon & Hedges, 2008) indicated that bilateral hippocampal volume reductions actually might not become evident until the disorder manifests itself during adulthood, since studies investigating childhood PTSD did not report volumetric differences in this structure (Carrion et al., 2001; De Bellis, Hall, Boring, Frustaci, & Moritz, 2001; De Bellis et al., 1999; 2002). As such, this could be taken as evidence for a deviant neurodevelopmental trajectory of the hippocampus in the pathogenesis of adult PTSD. No differences in amygdala volumes were found between PTSD patients with a history of childhood maltreatment and controls, neither when studied in children or in adults (Woon & Hedges, 2008). Surprisingly, however, only one study investigated amygdala volumes in an adult sample with PTSD related to childhood trauma to date (Bremner et al., 1997). Replication of previous results and further investigation of this specific group of patients is thus warranted, especially given the current standard of higher field strength data acquisition and availability of more advanced segmentation algorithms.

To this end, we studied the volumes of the hippocampus and amygdala in a group of adult female PTSD patients who suffered childhood trauma and compared these to age and education matched healthy control females with no history of trauma. Additionally, we were interested whether potential volume increases or reductions could be observed in specific subnuclei of the hippocampus or amygdala, which would provide further specificity with respect to functional subdivisions of these structures in the disorder. Therefore, a shape analysis was employed on the segmented structures to determine local morphological changes. Given the previously reported studies in

PTSD following trauma experienced in either childhood or adulthood, we expected smaller hippocampus and amygdala volumes in our PTSD group compared with healthy controls.

METHODS

PARTICIPANTS

Twenty-four females participated in the current study, 12 patients diagnosed with PTSD (mean age 28.08 ± 7.2) and 12 healthy control participants (mean age 26.83 ± 6.55). Patients were recruited within primary mental health care institutions (de Voorde, Leiden and Trauma center, PsyQ, The Hague) in the vicinity of the Leiden University Medical Center, where this study was conducted. Control participants were recruited by means of advertisements, and were matched to the patients for age and years of education followed.

Inclusion criteria for the patient group were: 1) PTSD diagnosis according to the MINI-International Neuropsychiatric Interview (Sheehan, Lecrubier, & Sheehan, 1998), administered by a trained clinical research assistant; 2) Interpersonal trauma related to emotional abuse, emotional neglect, sexual, and/or physical abuse during childhood or adolescence (< 18 years old), as determined by the Traumatic Experiences Checklist (TEC) (Nijenhuis, Van der Hart, & Kruger, 2002). Exclusion criteria were: 1) Repetitive psychotic episodes; 2) Use of antipsychotic medication. However, other stable use of psychotropic medication was allowed (use of citalopram (n = 2), duloxetine (n = 1), fluoxetine (n = 1), venlafaxine (n = 1), and methylphenidate (n = 1). In addition, several patients fulfilled additional diagnostic criteria for comorbid major depression (n = 5), social anxiety disorder (n = 4), panic disorder (n = 2), and obsessive-compulsive disorder (n = 1). Of note, some patients fulfilled criteria for multiple comorbid disorders. In the current study, comorbid personality disorders were not assessed.

Healthy controls were screened for absence of current or past psychiatric disorders, as determined by the MINI. Additionally, controls had to score low (< 145,

Table 6.1 Study sample demographics and psychometrics.

	PTSD	Healthy Controls
Age	28.08 (±7.2)	26.83 (±6.55)
Education (years)	7.58 (±2.19)	8.25 (±1.91)
Harvard Trauma Questionnaire	73.08 (±11.56)**	32.83 (±2.92)
Traumatic Experiences Checklist	39.83 (±17.95)**	3.5 (±4.36)
Emotional neglect	5.33 (±4.27)*	0.67 (±1.23)
Emotional abuse	5 (±4.33)*	0.17 (±0.58)
Physical abuse	8.36 (±4.18)**	0.42 (±1)
Sexual abuse	7.25 (±5.38)**	0
Dissociative Experience Scale	27.86 (±13.65)**	8.36 (±8.29)
Beck Depression Inventory	32.17 (±11.32)**	2.17 (±2.76)
Symptom Check List 90	223.67 (±49.69)**	101.08 (±7.04)
State-Trait Anxiety Inventory (trait)	62.5 (±6.88)**	31.25 (±6.65)
State-Trait Anxiety Inventory (state)	45.5 (±10.79)**	29.25 (±4.96)

Note: values represent mean ± standard deviation; * PTSD > Healthy Controls ($p < .005$); ** PTSD > Healthy Controls ($p < .001$); all participants were female and right-handed.

according to norm scores of a healthy population) on the Symptom Checklist (SCL-90) (Arrindell & Ettema, 1986), assessing levels of psychoneuroticism. Exclusion criteria for all participants were: 1) Presence or history of a major internal or neurological illness; 2) MINI diagnosis of substance abuse and/or addiction (alcohol and drugs); 3) Pregnancy; 4) General MRI contraindications. Lastly, all participants were required to: 1) Be right-handed; 2) Understand and speak Dutch sufficiently to complete each element of the study; 3) Have a body mass index between 19 and 26 kg/m^2.

On the day of the scan session, all participants were assessed with the Harvard Trauma Questionnaire (Mollica et al., 1992), the Dutch version of the Beck Depression Inventory (Bouman et al., 1985), the Dissociative Experience Scale (Bernstein & Putnam, 1986), and the State-Trait Anxiety Inventory (Spielberger, 1983). All demographic and clinical details of the final study sample are provided in **Table 6.1**.

The Medical Ethical Committee of the Leiden University Medical Center approved the study, and all participants gave written informed consent.

MATERIALS

MRI data acquisition

Imaging data were acquired on a Philips 3T Achieva MRI scanner using an eight-channel SENSE head coil for radiofrequency reception (Philips Healthcare, Best, The Netherlands). A high-resolution anatomical image (3D T_1-weighted ultra-fast gradient-echo acquisition; TR = 9.75 ms; TE = 4.59 ms; flip angle = 8°; 140 axial slices; FOV = 224 × 224 mm; in-plane resolution 0.875 × 0.875 mm; slice thickness = 1.2 mm) was acquired for segmentation of the amygdala and hippocampus.

Demographic and psychometric data analysis

Demographic and psychometric data were all compared between groups using independent samples *t*-tests using SPSS Version 18.0 (IBM), with the significance threshold set at $p = .05$.

Segmentation of the amygdala and hippocampus

Prior to analysis, all T_1-weighted images were submitted to a visual quality control check to ensure that no gross artifacts were present in the data. Next, data were analyzed using FSL Version 4.1.3 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004) using the FIRST tool for automated model-based registration and segmentation of subcortical structures (Patenaude, Smith, Kennedy, & Jenkinson, 2011). The following processing steps were employed: 1) Affine registration of the T_1-weighted images to the MNI-152 1 mm isotropic standard space template (Montreal Neurological Institute, Montreal, QC, Canada). 2) Second stage affine registration using an MNI-152 subcortical mask to exclude voxels outside the subcortical regions. 3) Automated segmentation of the bilateral amygdala and hippocampus. The segmentation procedure is informed by shape and intensity information of anatomical models of these structures that were constructed from manually segmented images provided by the Center for Morphometric Analysis (CMA), MGH, Boston. 4) Boundary correction to ameliorate partial volume effects using tissue classification information based on FSL's FAST segmentation tool. For more information and a

detailed description of the method we refer to Patenaude et al. (2011). All registration and segmentation results were visually checked for errors by an experienced neuroscientist (I.V.).

Volume analysis

For the boundary corrected segmentations of the amygdala and hippocampus, left and right side separately, volumes in mm^3 were calculated using the FSL command line tool *fslstats*. Each volume was normalized for differences in total intracranial volume. Volume differences between groups were then analyzed using a multivariate analysis of variance (MANOVA) in SPPS statistics 18.0 (IBM), setting the significance threshold at $p = .05$. Effect sizes (ω^2) of the between-groups effects were calculated additionally. Additionally, paired *t*-tests were carried out within each group to test for effects of lateralization. Last, correlations were calculated between structures that differed in volume between the two groups and scores on the HTQ, the TEC total, and TEC subscales Emotional Neglect, Emotional Abuse, Physical Abuse, and Sexual Abuse. Taking the mutual correlation between the six (subscales of the) questionnaires into account (average $r = 0.4$), the Bonferroni corrected significance threshold is $p < .017$ (as calculated with SISA, an online statistics calculator; www.quantitativeskills.com/sisa/)

Shape analysis

We opted to investigate whether the amygdala and hippocampus differed between patients and controls in local shape and size to reveal a possible predisposition for subregions to show volume increases or reductions. To this end, surface meshes were created from the individual segmentations of both structures in native space. Each mesh is composed of a set of triangles. The apex of neighboring triangles is called a vertex. The number of vertices is fixed for each subcortical structure to ascertain comparability both across and between participants. Surface meshes from the FIRST models that were used to aid segmentation were used as a common template to which each individual surface mesh was aligned. For each of the four structures comparisons between the two groups were carried out using non-parametric permutation based

statistics (FSL Randomise tool), with the height of each of the vertices entered as dependent variables (Patenaude et al., 2011; Zarei et al., 2010). Per vertex a null distribution of *F*-values was derived for the between group contrast by performing 5000 random permutations (Nichols & Holmes, 2002). The resulting statistical maps were cluster corrected for multiple comparisons, using an initial cluster forming threshold of $F(1, 22) > 4.3$ ($p < .05$), and a corrected $p < .05$. Localization of effects was carried out using the Juelich histological atlas, provided in FSL's image viewer.

PROCEDURE

Upon arrival on the day of the scan session participants were first instructed about the proceedings of the day and then filled out several questionnaires (HTQ, DES, BDI, and STAI). Afterwards, participants were brought to the scanner. Before participants entered the scanner, and after the scanning protocol was completed, a 10-point Likert scale was used to inquire about the perceived levels of stress, anxiety, concentration, and intrusions. Before and after the scan protocol, outside the scanner, participants also rated the four items of the short Dissociation Tension Scale (Stiglmayr, Schmahl, Bremner, Bohus, & Ebner-Priemer, 2009). An exit-interview and extensive debriefing followed at the end of the experiment. Subsequently, participants were thanked and paid for their participation in the study.

RESULTS

Behavioral results

Patients and controls did not differ on age and years of education (both $p > .05$). As expected, patients scored higher ($p < .005$) on all clinical scales (see **Table 6.1** for means and standard deviations) compared to controls.

Table 6.2 Amygdala and hippocampus volumetry results.

	PTSD	Healthy Controls
Left amygdala	1498.52 (±353.34)	1567.91 (±322.91)
Right amygdala	1356.3 (±332.99)*	1667.55 (±264.45)
Left hippocampus	5095.85 (±931.51)	5275.52 (±617.11)
Right hippocampus	5197.27 (±474.75)	5347.99 (±420.98)

Note: values are in mm^3 and represent mean volumes ± standard deviation, normalized for intracranial volume; * PTSD < Healthy Controls (p = .019).

Volumetric results

Table 6.2 lists the volumes of the left and right amygdala and hippocampus. All four structures met the criteria of homogeneity of variance and normality to justify parametric statistics. The multivariate test revealed a trend for the independent variable Group, $F(4, 19) = 2.33$, $p = .093$, though with an observed power of .56. Subsequent univariate tests showed that right amygdala volumes were smaller in the PTSD patients (mean ± *SD*: 1365.3 ± 332.99) than in the healthy controls (mean ± *SD*: 1667.55 ± 264.45), $F(1, 22) = 6.43$, $p = .019$, $\omega^2 = .19$, reflecting a medium to strong effect size, and a moderate observed power of .68. No differences were found for the left amygdala or the left and right hippocampus (all $p > .25$). Paired t-tests did not reveal volumetric asymmetry between the left and right side of the amygdala and hippocampus within both groups ($p > .2$). Last, right amygdala volumes correlated negatively with the Traumatic Experiences Checklist subscale Sexual Abuse ($r = -.64$, $p = .013$, one-tailed).

Shape results

The vertex analysis revealed focal volume reduction in PTSD patients compared with healthy controls on the surface of the right amygdala (**Figure 6.1a**). The affected area showed good overlap with two main groups of subnuclei of the amygdala: the basolateral (red), and the superficial or cortical (light blue) group (Amunts et al., 2005). The effects encompassed 18.8 % and 14.6 % of the amygdala surface, respectively. Although volumes of the left amygdala and bilateral hippocampus did not differ between the two groups, it could still be possible that shape differences are observed

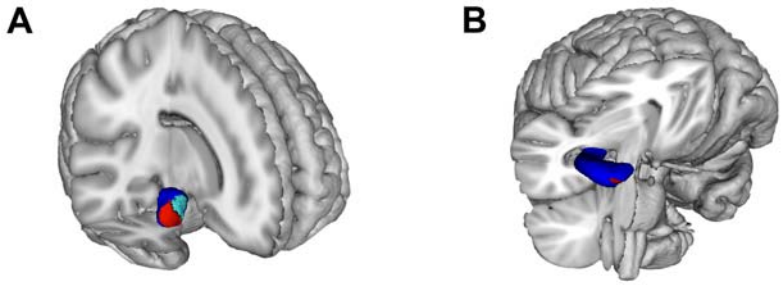


Figure 6.1 Shape analysis results, revealing loci of decreased volume in PTSD compared with controls on the surface of the amygdala and hippocampus (dark blue). (**A**) Volume reductions are found specifically in parts of the basolateral (red) and superficial (light blue) groups of the right amygdala ($p < .05$, corrected). (**B**) A small trend for volume decrease was found in the anterior subiculum of the right hippocampus ($p < .05$, uncorrected). All subgroups were identified using the Juelich Histological Atlas, incorporated in FSL.

in these structures (e.g., existence of focal increases as well as decreases, which on average yield volumes similar to the control group). However, at a lenient uncorrected threshold of $p < .05$ the vertex analysis only revealed a marginal decreased volume of the anterior subiculum of the right hippocampus in PTSD patients compared with healthy controls (**Figure 6.1b**).

DISCUSSION

Up to now, surprisingly little research has been done on amygdala volumes in PTSD, especially not in patients that have been exposure to childhood trauma. In this study we investigated whether volume and shape of the amygdala and hippocampus differed between adult female PTSD patients that have been exposed to childhood maltreatment, and a group of age and education matched healthy control women. Whereas no differences were observed in the volumes of the bilateral hippocampus and left amygdala, we did find smaller right amygdala volumes in the PTSD group compared to controls. Moreover, the difference was mainly located at the surface of the basolat-

eral and superficial nuclei groups. This is the first study to report on amygdala volume reductions in an adult sample with PTSD associated with childhood maltreatment, together with evidence for this reduction to occur in specific amygdala subregions. Moreover, volume reductions were associated with severity of sexual abuse during childhood. Our results provide new insights on how adverse events during childhood could render the brain vulnerable to develop PTSD later in life.

Increased dendritic branching and spine density of amygdala neurons has been reported in rodents after chronic restraint stress (Mitra, Ferguson, & Sapolsky, 2009; Roozendaal et al., 2009; Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002), as well as increased myelination after maternal separation (Ono et al., 2008), which was accompanied by higher levels of anxious behavior. Similarly, several human studies have shown that early life adversity, such as prolonged orphanage rearing or poor care due to maternal depression, is related to larger amygdala volumes in adolescence compared to their peers, as well as an increased risk to develop affective psychopathology (Lupien et al., 2011; Mehta et al., 2009; Tottenham et al., 2010). In adulthood, however, no evidence was found for a difference in amygdala volumes between PTSD patients who were exposed to childhood maltreatment and controls (Woon & Hedges, 2008). Nonetheless, *decreased* volumes have been reported in adult borderline patients with a history of childhood abuse (Driessen et al., 2000; Schmahl, Vermetten, Elzinga, & Douglas Bremner, 2003), which is in line with the current finding.

With respect to the apparent discrepancy in amygdala volume differences between childhood and adulthood samples, the following could be hypothesized: Severe adversity during childhood could increase the sensitivity of the amygdala through dendritic growth and synaptic connectivity (Roozendaal et al., 2009), resulting in a larger total volume. While this process could be beneficial to increase chances of survival in a hostile environment by amplification of threatening cues, it could eventually come with a cost: repetitive activation of the amygdala could ultimately result in wear and tear (cf. "neurotoxicity hypothesis") (Lupien et al., 2009; Sapolsky, Krey, & McEwen, 1986), which would be manifested as volume reductions in adulthood. Some support for this idea is lent by studies showing larger amygdala volumes in first

episode depression, which seem to normalize to the size of controls after recurrent depressive episodes (Frodl et al., 2003; Lange & Irle, 2004; Tottenham et al., 2010). On the other hand, the recent meta-analysis by Woon and Hedges (2008) did not find any evidence for altered amygdala volumes in children with maltreatment-related PTSD. Given that the amygdala continue to develop during adolescence (Giedd et al., 1999; Guo et al., 2007; Østby et al., 2009), alternatively it could be hypothesized that severe adversity puts a break on normal maturation of the amygdala. As such, a difference in volume would not become apparent until adulthood.

The volume reductions found in this study appeared to be localized in the basolateral and superficial (or cortical) nuclei groups of the amygdala, as was determined by the shape analysis. These two groups together form the ventral portion of the human amygdala and receive major input and feedback projections from sensory and prefrontal brain regions (Sah, Faber, Lopez De Armentia, & Power, 2003). The role of the basolateral group has been described extensively in the literature, assigning it a crucial role in promoting emotional memory formation (Roozendaal et al., 2009), as well as fear conditioning (LeDoux, 2000). In addition, it has been shown that stress hormones are important modulators within the basolateral amygdala in creating memory traces for emotionally salient events (McGaugh, 2004; Roozendaal et al., 2009). Moreover, induced stress may facilitate this process. As such, exposure to severe stress can lead to enhanced fear conditioning and traumatic memory formation, which lies at the heart of the symptomatology of PTSD. Reduced volumes of these specific groups of nuclei specifically may therefore reflect wear and tear due to repetitive activation of the traumatic memory traces and conditioned fear responses in PTSD.

A negative correlation was found between the sexual abuse subscale of the Traumatic Experience Checklist and right amygdala volume in the PTSD group, indicating smaller volumes when sexual abuse was more severe during childhood. While this could point at the particularly devastating effects of childhood sexual abuse, the association should be interpreted with caution: As small group sizes are especially prone to spurious correlations, replication in a larger group of patients is certainly warranted.

Irrespective of the type of trauma encountered, previous studies have reported hippocampal volume reductions rather consistently (Karl et al., 2006; Woon et al., 2010; Woon & Hedges, 2011). While our patient group seemed to have smaller hippocampus volumes on average, the difference failed to reach significance. A potential explanation for this null finding, however, could be the large standard deviations observed for this structure, in combination with the small sample size.

The current study suffers several limitations. First, our sample size is small (n = 12), yet comparable in size with the four studies discussed by Woon and Hedges (Woon & Hedges, 2008) on volumetric differences in adult PTSD associated with childhood maltreatment, which included 16.25 patients (SD = 4.57) on average. Nevertheless, even within our small group of patients, we found a significant reduction of right amygdala volume compared to controls, with a concurrent medium to strong effect size. The observed power, however, was moderate, indicating that for replication of our findings, future studies should use a larger sample size.

Second, in the current study we did not include a group of PTSD patients with trauma originating in adulthood, so we cannot infer whether the volume reduction of the right amygdala is specific to childhood trauma. Nonetheless, recent meta-analyses in adult PTSD samples predominantly related to adulthood trauma only showed a tendency towards smaller amygdala volumes or no differences at all between patients and controls (Karl et al., 2006; Woon & Hedges, 2009), possibly indicating that our findings might indeed be specific to childhood trauma. Clearly, longitudinal studies are needed to further elucidate the time course of amygdala volume changes in PTSD associated with childhood trauma to draw conclusions on the developmental trajectory of the amygdala following childhood trauma.

Third, most of the patients included in the current study suffered from comorbid psychopathology, which is typical for patients with PTSD, and half of the patients used psychotropic medication. We therefore cannot disentangle whether our findings reflect a PTSD endophenotype per se or are rather related to complex psychopathology, while it remains unclear to what extent medication might have influenced these volumetric differences.

Fourth, our PTSD sample comprised female patients only. Although the number of traumatic events encountered, irrespective of the type of event, is similar or even higher in males than in females, females approximately do have a twofold higher risk to develop PTSD (Breslau, Chilcoat, Kessler, Peterson, & Lucia, 1999; de Vries & Olff, 2009; Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995). In addition, a recent Dutch prevalence study reported that females are confronted with physical and sexual abuse, two of our inclusion criteria, more frequently in childhood than males (de Vries & Olff, 2009).

Conversely, our study has several strengths. All studies on volumetric differences of the hippocampus and amygdala were done on 1.5 Tesla data. In comparison, the 3T MR scanner used in the current study allows for an increase in the signal to noise ratio, which should facilitate easier and more precise segmentation of the structures under scrutiny. Second, the recent emergence of advanced imaging processing software permitted us to study shape differences alongside the volumetric measures. Here we show that such a tool might offer important information on which groups of subnuclei are affected specifically. Last, the scores of the clinical scales indicate that our patient group was severely affected, which was also reflected by the high comorbidity rate. Conceivably, the differences found in the current study might have emerged specifically due to the severely affected nature of the patient group.

In sum, we found smaller right amygdala volumes in PTSD patients compared with controls, whereas the left amygdala and bilateral hippocampus did not differ between the two groups. In addition, this volume reduction appeared to originate in the basolateral and centromedial nuclei groups of the right amygdala. Although our results are not conclusive, we hypothesize that traumatic events in childhood might impede normal development of the amygdala, rendering a person more vulnerable to develop PTSD, or psychopathology in general, later in life. Future longitudinal studies are needed, however, to test this hypothesis, and to shed more light on the detrimental effects of childhood trauma on both structure and function of the brain, and its relation to the pathogenesis of PTSD.

S CT N 3

P RS N L T

CHAPTER 7

Neuroticism and extraversion are associated with amygdala resting-state functional connectivity

Aghajani, M. *, **Veer, I. M.** *, van Tol, M. J., Aleman, A., van Buchem, M. A., Veltman, D. J., Rombouts, S. A. R. B., & van der Wee, N. J. (2014). *Cognitive Affective & Behavioral Neuroscience, 14*(2), 836-848. (* share first authorship)

ABSTRACT

The personality traits neuroticism and extraversion are differentially related to socioemotional functioning, and susceptibility to affective disorders. However, the neurobiology underlying this differential relationship is still poorly understood. This discrepancy could perhaps best be studied by adopting a brain connectivity approach. Whereas the amygdala has repeatedly been linked to neuroticism and extraversion, no study has yet focused on the intrinsic functional architecture of amygdala-centered networks in relation to both traits. To this end, seed-based correlation analysis was employed to reveal amygdala resting-state functional connectivity (RSFC), and its associations with neuroticism and extraversion, in 50 healthy participants. Higher neuroticism scores were associated with increased amygdala RSFC with the precuneus, and decreased amygdala RSFC with the temporal poles, insula, and superior temporal gyrus ($p < .05$, cluster corrected). Conversely, higher extraversion scores were associated with increased amygdala RSFC with the putamen, temporal pole, insula, and several regions of the occipital cortex ($p < .05$, cluster corrected). The shifts in amygdala RSFC associated with neuroticism may relate to the less-adaptive perception and processing of self-relevant and socioemotional information that is frequently seen in neurotic individuals, whereas the amygdala RSFC pattern associated with extraversion may relate to the heightened reward sensitivity and enhanced socioemotional functioning in extraverts. We hypothesize that the variability in amygdala RSFC observed in the present study could potentially link neuroticism and extraversion to the neurobiology underlying increased susceptibility or resilience to affective disorders.

INTRODUCTION

Human personality describes the distinctive and persistent patterns of thoughts, emotions, and actions that occur across contexts and over time (Mischel, 2004). The influential Big Five model of personality suggests that individual variations in behavior can be described along five trait dimensions: neuroticism, extraversion, agreeableness, conscientiousness, and openness (McCrae & Costa, 1991). Of these traits, neuroticism and extraversion are the most widely studied dimensions (Kennis, Rademaker, & Geuze, 2013; McCrae & Costa, 1991), both describing individual differences in socioemotional functioning and susceptibility to affective disorders.

Neuroticism is linked to vulnerability to depression and anxiety (Bienvenu et al., 2001; Clark, Watson, & Mineka, 1994; Durrett & Trull, 2005), less favorable treatment outcomes in general (Geerts & Bouhuys, 1998), and a higher risk for comorbid psychiatric disorders (Khan, Jacobson, Gardner, Prescott, & Kendler, 2005). These negative consequences are hypothesized to originate from neuroticism's relationship with maladaptive cognitive and emotional functioning. This includes being extremely sensitive to negative social cues in the environment (McCrae & Costa, 1991), interpreting ambiguous social cues as threatening or negative (Bolger & Zuckerman, 1995), experiencing difficulties in affect regulation (Tamir, 2005), and demonstrating a more negative self-referential information processing style (Trapnell & Campbell, 1999). Extraversion, in contrast, is linked to a higher propensity for experiencing positive emotional states (Larsen & Ketelaar, 1991), and decreased susceptibility to affective disorders (Kotov, Gamez, Schmidt, & Watson, 2010). This is thought to stem from extraversion's relationship with sensitivity to positive and rewarding cues in the environment (McCrae & Costa, 1991). Extraverts show a strong tendency to engage in rewarding social interactions, are enthusiastic and optimistic in general, and tend to be assertive and talkative in social situations. To this end, it is not surprising that neuroticism and extraversion are commonly found to be inversely correlated (McCrae & Costa, 1991).

Structural and functional properties of the amygdala, a subcortical brain region, are deemed to be fundamental with regard to both neuroticism and extra-

version (Canli, Sivers, Whitfield, Gotlib, & Gabrieli, 2002b; Cremers et al., 2010; 2011; Haas, Omura, Constable, & Canli, 2007; Kennis et al., 2013; Montag, Reuter, Jurkiewicz, Markett, & Panksepp, 2013; Reuter et al., 2004; Stein, Simmons, Feinstein, & Paulus, 2007b; Vaidya et al., 2007). Functional magnetic resonance imaging (fMRI) studies have suggested that the amygdala is involved in emotional learning (Canli, Zhao, Brewer, & Gabrieli, 2000), emotional arousal (Phelps & LeDoux, 2005), and modulation of vigilance in the face of threat (Mobbs et al., 2007; Whalen, 1998). Existing evidence from primate studies has indicated highly interconnected anatomical connections between the amygdala and the prefrontal cortex (PFC), anterior cingulate cortex (ACC), and hippocampus (Amaral, 1986; Ghashghaei & Barbas, 2002). Additionally, functional imaging studies of the human brain have indicated functional coupling of the amygdala with the PFC, ACC, and hippocampus (Phillips, Drevets, Rauch, & Lane, 2003a; Roy et al., 2009; Stein et al., 2007a).

The amygdala's anatomical and functional connections with the PFC, ACC, and hippocampus are thought to constitute an integrated neural circuit dedicated to various aspects of emotional processing and regulation. The amygdala, subgenual ACC, ventrolateral PFC, and orbitofrontal cortex (OFC) form a ventral system involved in the identification of the emotional significance of a stimulus and the production of an affective state in response to that stimulus (Phillips, Drevets, Rauch, & Lane, 2003a; Stein et al., 2007a). The supragenual ACC, dorsomedial PFC, dorsolateral PFC, and hippocampus, on the other hand, are implicated in a dorsal system that exerts cognitive control, regulates affective states, and provides contextual information (Pessoa, 2008; Phillips, Drevets, Rauch, & Lane, 2003a; Stein et al., 2007a). There is ample evidence for increased sensitivity of the ventral system and decreased regulatory ability of the dorsal system in affective disorders (Phillips, Ladouceur, & Drevets, 2008; Phillips, Drevets, Rauch, & Lane, 2003b; Price & Drevets, 2010), which is hypothesized to underlie the affective symptomatology. Given that neuroticism is a strong vulnerability factor for affective psychopathology, increased sensitivity of the ventral system and decreased regulatory control of the dorsal system could be expected in neurotic individuals. Such an imbalance between the dorsal and ventral systems seems to be less likely, or even reversed, in extraverts, since extraver-

sion typically serves as a protective factor against affective psychopathology. In addition, extraversion is likely to involve enhanced functional integrity of brain networks subserving reward and motivation. Compatible with this notion, extraverts typically show higher activity within the reward circuitry in response to rewarding stimuli (Canli et al., 2002; Cohen, Young, Baek, Kessler, & Ranganath, 2005; Deckersbach et al., 2006; Kumari, ffytche, Williams, & Gray, 2004).

The human brain is believed to comprise functionally integrated networks that serve complex behavioral phenotypes (Raichle, 2011). The activity within these functional networks can best be viewed as dimensional, ranging from underactive to normal to overactive (Sylvester et al., 2012), and thus providing a framework for describing both normal and abnormal behavior. For example, high trait anxiety and anxiety disorders involve overactivity in the cingulo-opercular and ventral attention networks, as well as underactivity in the frontoparietal and default mode networks (Sylvester et al., 2012). Relatedly, self-reported anxiety has been linked to stronger functional connectivity (FC) within the salience network (Markett et al., 2013; Seeley, Menon, et al., 2007b), which includes the insular, frontal, and cingulate cortices, as well as subcortical regions such as the amygdala. Of interest to the present study, FC analysis of amygdala-centered networks previously has revealed network disorganization in anxiety patients (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009). Specifically, decreased amygdala FC with the insular and cingulate regions, and increased amygdala FC with a compensatory frontoparietal executive control network were demonstrated. Equally relevant, a recent study has shown that amygdala FC with the anterior insula relates to state anxiety, whereas structural connectivity between these regions is related to trait anxiety (Baur, Hänggi, Langer, & Jäncke, 2013). Adopting a brain connectivity approach may thus prove useful for investigating the association between individual differences in neuroticism and extraversion and the functional architecture of amygdala-centered networks.

A recent fMRI study demonstrated decreased FC between the amygdala and the dorsal ACC in response to emotional stimuli in individuals with higher neuroticism scores (Cremers et al., 2010), which could reflect reduced inhibitory control over the amygdala. In addition, a resting-state functional connectivity (RSFC)

study suggested that neuroticism relates to the RSFC of brain regions implicated in self-evaluation and emotion regulation (e.g., PFC and precuneus), whereas extraversion relates to the RSFC of brain regions implicated in reward and motivation (e.g., striatum) (Adelstein et al., 2011). Although they are informative on the neurobiology underlying human personality, these studies are either limited by the complexity of their experimental designs and task performance (Cremers et al., 2010) or lack specific information on amygdala-centered networks (Adelstein et al., 2011). Therefore, the purpose of the present study was to examine in a healthy population whether neuroticism and extraversion are associated with amygdala RSFC.

On the basis of the established anatomical and functional connections of the amygdala, and of the studies reviewed above, we expected participants with higher neuroticism scores to demonstrate increased amygdala RSFC with regions of the ventral affective system, including the subgenual ACC, ventrolateral PFC, and OFC. Such a relationship could be indicative of a higher propensity to experience (negative) emotional arousal. In contrast, we expected participants with higher neuroticism scores to demonstrate decreased negative amygdala RSFC with regions of the dorsal control system, including the supragenual ACC, dorsomedial PFC, dorsolateral PFC, and hippocampus, potentially indicating less adaptive emotion regulation. In addition, we expected higher extraversion scores to be associated with increased amygdala RSFC with brain regions implicated in reward processing, such as the medial PFC and striatum, which could reflect the tight relationship between extraversion and reward sensitivity. Finally, considering the inverse correlation between neuroticism and extraversion, these traits could demonstrate opposing relationships within regions functionally connected to the amygdala.

METHODS

PARTICIPANTS

A group of 54 right-handed healthy participants were selected from the MRI study of the large-scale multicenter Netherlands Study of Depression and Anxiety (NESDA;

Penninx et al., 2008). Participants were scanned at one of the three participating centers: Academic Medical Center (AMC; n = 17) Amsterdam, Leiden University Medical Center (LUMC; n = 26), and University Medical Center Groningen (UMCG; n = 11). The exclusion criteria for the participants were: 1) a history of neurological disorders or head injury, 2) a lifetime diagnosis of DSM Axis I and/or Axis II disorders, 3) use of any medication affecting the cardiovascular and/or central nervous system, 4) current alcohol and/or substance abuse, 5) hypertension, 6) pregnancy, and 7) general MRI contra-indications. Four of the participants (one from AMC and three from LUMC) were excluded from the study due to large susceptibility artifacts in their resting-state (RS) data. Consequently, 50 healthy participants (32 female, 18 male, age: M = 40.51, SD = 9.45) were included in the imaging study. The study was approved by the medical ethics committees of the participating centers, and written informed consent was obtained from all participants prior to scanning.

MATERIALS

Personality assessment

The personality profile of the participants was assessed using the NEO Five-Factor Inventory (NEO-FFI; Costa & McCrae, 1992). This inventory consists of 60 items that measure five different personality dimensions (12 questions each): neuroticism, extraversion, openness, agreeableness, and conscientiousness. The items of this questionnaire are descriptive statements that can be rated on a 5-point Likert scale (0 = *strongly disagree* to 4 = *strongly agree*). Our sample's neuroticism (M = 12.02, SD = 4.46, range = 1–23) and extraversion (M = 32.4, SD = 6.69, range = 15–44) scores were within the lower and upper ranges, respectively, of a normal nonclinical reference population (Costa & McCrae, 1992). In line with previous reports (Costa & McCrae, 1992; Cremers et al., 2011; 2010), neuroticism and extraversion were negatively correlated (r = –.44, p < .01). Importantly, neuroticism and extraversion scores did not differ between the three scan sites, $F(2, 47) = 1.21$, p = .31, and $F(2, 47) = 0.76$, p = .47, for neuroticism and extraversion respectively.

Image acquisition

Participants were scanned at one of the three participating centers. RS-fMRI images were acquired while the participants were instructed to lie still with their eyes closed but not to fall asleep. RS-fMRI data were acquired at the end of a fixed imaging protocol, after completion of three task-related functional MRI runs and the acquisition of an anatomical scan (scan sequence: Tower of London, word encoding, T_1-weighted scan, word recognition, and perception of facial expression).

Philips 3T MRI scanners (Philips Healthcare, Best, The Netherlands) were used to acquire the imaging data, using a six-channel (AMC) or an eight-channel (LUMC and UMCG) SENSE (Sensitivity Encoding) head coil. For anatomical reference, a T_1-weighted anatomical scan was acquired for each participant with the following scan parameters: repetition time (TR) = 9 ms, echo time (TE) = 3.5 ms, 170 sagittal slices with an isotropic voxel resolution of 1.0 mm^3, no slice gap, and FOV = 256 × 256 mm. For the RS functional brain images, 200 T_2^*-weighted gradient-echo echo-planar imaging (EPI) volumes were acquired, using the following scan parameters at AMC and LUMC: TR = 2300 ms, TE = 30 ms, flip angle = 80° , 35 axial slices with an in-plane voxel resolution of 2.3 mm^2, 3.0 mm slice thickness, no slice gap, FOV = 220 × 220 mm, and interleaved slice acquisition. At UMCG the parameters were the same, except for the following: TE = 28 ms, 39 axial slices with an in-plane voxel resolution of 3.45 mm^2. The total RS acquisition time was 7 min 40 s.

Image preprocessing

The RS-fMRI data of all participants were preprocessed and analyzed using FEAT (FMRI Expert Analysis Tool) version 5.9, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The preprocessing consisted of: 1) non-brain tissue removal, 2) motion correction, 3) grand-mean intensity normalization of the entire 4D data set by a single scaling factor, 4) spatial smoothing with a 6-mm full width at half maximum Gaussian kernel, 5) high-pass temporal filtering using Gaussian-weighted least-squares straight line fitting with a 0.01-Hz cutoff to remove low-frequency artifacts, and 6) registration of the RS data to the T_1-weighted anatomical image (rigid body transformation), as well as normalization of the T_1 image to the 2-mm

Montreal Neurological Institute (MNI) standard space image (linear affine transformation). Both registration matrices were combined into a single matrix describing the transformation from the RS data to MNI standard space, and its inverse matrix was calculated. The maximum allowable displacement due to excessive head motion was set at 3 mm translation or 3° rotation in any direction.

Functional connectivity analysis

The functional connectivity analysis was conducted employing a seed-based correlation approach (e.g., Fox & Raichle, 2007). Using the probabilistic Harvard–Oxford subcortical atlas (MNI standard space) included in FSL, we defined regions of interest (ROIs) in the left and right amygdala: In the center of a group of voxels having a probability of at least 80 % to represent the amygdala, a spherical mask with a radius of 4 mm was created for both the left and right amygdala (Veer et al., 2011; Veer, Oei, van Buchem, Elzinga, & Rombouts, 2012). For each participant, both amygdala masks were registered to the RS data set. Mean time series of each individual participant's ROIs were then extracted and used as predictors in a general linear model (GLM). Signal from the deep white matter and cerebrospinal fluid, as well as six motion parameters, and the global signal were added to this model as covariates of no interest. Contrasts were created for the left and right amygdala separately, and both amygdala combined, to identify voxels that demonstrated either positive or negative temporal correlations with these ROIs. This resulted in individual RSFC maps of the left and right amygdala, both separately and combined, which were then fed into a higher-level mixed effects multiple linear regression analysis, again using the GLM. In order to examine the association between neuroticism, extraversion, and amygdala RSFC, NEO-FFI neuroticism and extraversion scores were included in the model as predictors, together with sex, age, and scan site as covariates of no interest. Both traits were entered in the same higher-level model to take into account possible shared variance, given the theoretical and statistical (i.e., the anticorrelations commonly found) relations between the two traits. Separate contrasts were defined for neuroticism and extraversion, which, in the context of the GLM, should reveal amygdala RSFC uniquely associated with each of the two traits. A cluster-corrected threshold of $p <$

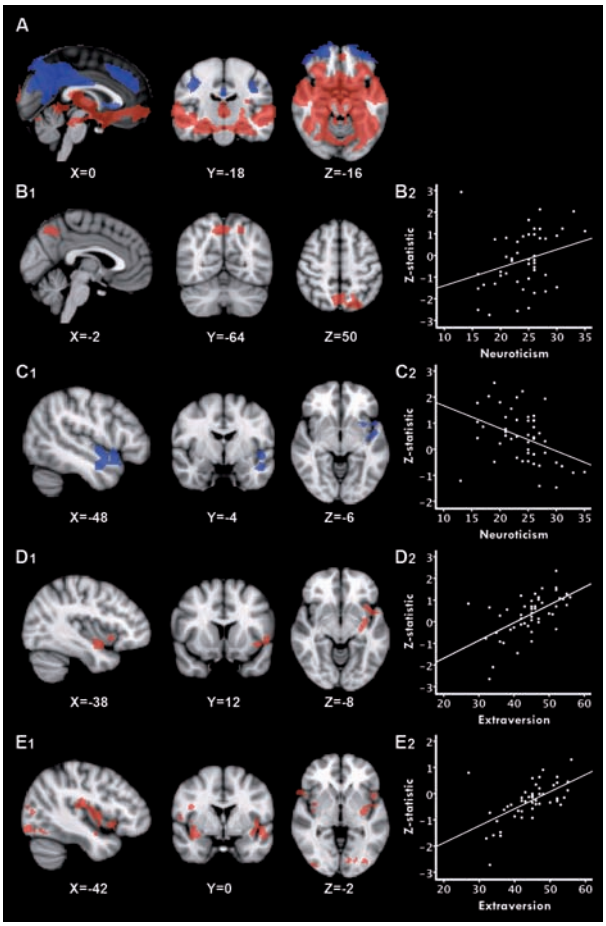


Figure 7.1 (**A**) Resting-state functional connectivity (RSFC) of the bilateral amygdala. Red indicates positive, blue indicates negative amygdala RSFC. (**B1**) Association between neuroticism and left amygdala RSFC with the precuneus. (**C1**) Negative association between neuroticism and left amygdala RSFC with the temporal pole, insula, and superior temporal gyrus (STG). (**D1**) Positive association between extraversion and right amygdala RSFC with the insula and putamen. (**E1**) Positive association between extraversion and left amygdala RSFC with the temporal pole, insula, and occipital cortex. Associations for each effect are plotted in (**B2-E2**). *Z*-statistical maps are corrected for multiple comparisons at the cluster level ($z > 2.3$, $p < .05$), and superimposed on the 2 mm MNI-152 T_1 standard brain. The right side of the images corresponds to the left side of the brain, and vice versa.

.05 with an initial cluster-forming threshold of $z > 2.3$ was used for multiple-comparisons correction. This yielded group-level RSFC maps for the left and the right amygdala separately, as well as for both amygdala combined, and their associations with the neuroticism and extraversion scores. Given our a priori expected associations between neuroticism and/or extraversion, and amygdala FC with regions in the medial and lateral PFC, hippocampus, and striatum, a combined pre-threshold mask of these regions (number of voxels [2 mm MNI] = 95,746) was created to reduce the number of multiple comparisons. Again, a cluster-corrected significance threshold of $p < .05$, with an initial cluster-forming threshold of $z > 2.3$, was used within this mask.

RESULTS

Whole-brain analysis of amygdala RSFC revealed connectivity patterns largely consistent with those from previous RS-fMRI studies (Roy et al., 2009; Stein et al., 2007a; Veer et al., 2011) (see **Figure 7.1a**). The amygdala showed positive RSFC with brain regions implicated in the identification of the emotional significance of a stimulus and in the production of an affective state in response to that stimulus (e.g., subgenual ACC, ventrolateral PFC). On the other hand, negative amygdala RSFC was found with brain regions that are assumed to exert cognitive control and regulate affective states (e.g., dorsomedial PFC, supragenual ACC). **Supplemental Table 7.1** provides clusters and peak coordinates of the amygdala RSFC.

Neuroticism was positively associated with RSFC of the left amygdala with the precuneus (see **Figure 7.1b** and **Table 7.1**). That is, the negative RSFC between the left amygdala and the precuneus observed in our sample, and reported by previous RS-fMRI studies (Roy et al., 2009; Stein et al., 2007a; Veer et al., 2011), was preserved in participants with lower neuroticism scores, yet this connectivity increased to positive in participants with higher neuroticism scores. In contrast, neuroticism was negatively associated with RSFC of the left amygdala with the left temporal pole, insula, and superior temporal gyrus (STG; see **Figure 7.1c** and **Table 7.1**). Specifically, the positive left amygdala RSFC with the temporal pole, insula, and STG ob-

Table 7.1 Clusters and coordinates of the association between amygdala RSFC and neuroticism.

Region	Hemisphere	Voxels	*z*-value	Peak voxel MNI coordinates		
				x	y	z
left amygdala RSFC						
postive						
precuneus		890	5.17	4	-66	50
lateral occipital cortex	L		3.33	-22	-68	50
negative						
middle temporal gyrus	L	858	4.28	-48	-4	-22
planum polare	L		3.72	-50	-2	-6
temporal pole	L		3.76	-50	12	-12
insula	L		3.51	-40	-12	12
insula	L		2.89	-36	4	-14

Note: all *z*-values are corrected for multiple comparisons at the cluster-level ($z > 2.3$; $p < .05$).

served in our sample was preserved in participants with lower neuroticism scores, but this connectivity diminished, and even became negative, in participants with higher neuroticism scores. No relation between neuroticism and right amygdala RSFC was observed at the set threshold.

Extraversion was positively associated with right amygdala RSFC with the insula and putamen (see **Figure 7.1d** and **Table 7.2**) and with left amygdala RSFC with the temporal pole, insula, putamen, and several regions in the occipital cortex (see **Figure 7.1e** and **Table 7.2**). That is, increased amygdala RSFC with these regions was observed in participants with higher extraversion scores, whereas this connectivity decreased to negative in participants with lower extraversion scores.

Because a contrast defined on only one of the regressors, as was done here for neuroticism and extraversion separately, explains variance uniquely associated with that regressor, orthogonalizing the one trait with respect to the other should not change the results. Nevertheless, to check this assumption we repeated the analysis while orthogonalizing neuroticism with respect to extraversion, and vice versa. As we expected, the results remained as described above.

The analysis restricted to the voxels of the pre-threshold mask did not reveal connectivity associated with either neuroticism or extraversion within our a-pri-

Table 7.2 Clusters and coordinates of the association between amygdala RSFC and extraversion.

Region	Hemisphere	Voxels	*z*-value	Peak voxel MNI coordinates		
				x	y	z
right amygdala RSFC						
postive						
insula	L	451	3.95	-38	14	-8
putamen	L		3.23	-32	-12	-8
left amygdala RSFC						
postive						
precuneus	R	1761	3.94	18	-60	18
lateral occipital cortex	R		3.94	42	-62	18
intracalcarine cortex	R		3.09	12	-68	4
brain stem			3.38	4	-36	-14
putamen	L	1314	4.06	-32	-10	-4
temporal pole	L		3.17	-64	14	-10
insula	L		2.87	-44	10	-4
insula	R	1129	4.29	38	0	-12
inferior frontal gyrus	R		3.85	48	34	14
lateral occipital cortex	L	778	3.51	-36	-78	6
lateral occipital cortex	R	500	3.94	42	-62	18

Note: all *z*-values are corrected for multiple comparisons at the cluster-level ($z > 2.3$; $p < .05$).

ori-defined ROIs. To aid ROI selection in future studies, rather than for inference in the present study, we additionally report the connectivity maps at an uncorrected threshold of $z > 2.3$ in the **Supplemental Material**.

DISCUSSION

In the present study, we examined whether individual differences in neuroticism and extraversion are associated with alterations in RSFC of the amygdala. Although we did not find an association between neuroticism and amygdala RSFC with our a priori hypothesized regions, we did demonstrate that individual differences in neuroticism are associated with altered RSFC of the amygdala with the precuneus, temporal poles, insula, and STG. Extraversion scores were associated with RSFC of the

amygdala with the putamen, as was hypothesized, the temporal poles, bilateral insula, and several regions within the occipital cortex. Lastly, neuroticism and extraversion showed contrasting amygdala RSFC with the temporal pole and insula. This is the first study to demonstrate such associations between individual differences in both neuroticism and extraversion and functional connectivity of the amygdala at rest.

Amygdala RSFC and neuroticism

Our analysis showed that neuroticism was associated with increased left amygdala RSFC with the precuneus. The precuneus plays a pivotal role in self-referential information processing (Buckner & Carroll, 2007; Cavanna & Trimble, 2006). For example, perception and processing of personality trait adjectives that are self-descriptive, and thus that closely reflect our own personality, are related to increased activity in this region (Kircher et al., 2002), whereas activity appears to decrease as processed information becomes less self-relevant (Lou et al., 2004). Furthermore, the precuneus is thought to mediate the retrieval of remote, but context-rich, autobiographical memories (Buckner & Carroll, 2007; Gilboa, Winocur, Grady, Hevenor, & Moscovitch, 2004). Bearing in mind the role of the amygdala in (negative) emotional arousal (Phelps & LeDoux, 2005), our finding may thus relate to disproportionate emotional coloring of self-referential or autobiographical information processing. This adds to the notion of increased self-conscious rumination and aberrant self-referential information processing frequently seen in neurotic individuals (Lam, Smith, Checkley, Rijsdijk, & Sham, 2003; Stöber, 2003; Trapnell & Campbell, 1999). Moreover, our finding may also provide some clues as to neuroticism's relationship with affective disorders. Aberrant self-referential information processing, perhaps partly driven by increased amygdala-precuneus RSFC, may increase the propensity for psychosocial stress and negative emotions, and thus promote affective psychopathology. Consistent with this notion, psychosocial stress has been shown to induce increased amygdala–precuneus FC (Veer et al., 2011), whereas augmented functional interactions between these regions have been implicated in social anxiety and panic disorder (Liao et al., 2010; Pannekoek et al., 2013).

Our analysis also revealed decreased left amygdala RSFC with the left tem-

poral pole, insula, and STG in participants with higher neuroticism scores. The temporal pole and insula have strong reciprocal connections with the amygdala, and both play crucial roles in socioemotional behavior such as recognizing and understanding others' intentions, desires, and emotions (Olson, Plotzker, & Ezzyat, 2007; Singer, 2006; Singer, Critchley, & Preuschoff, 2009). The STG is deemed a key component of a neural circuit dedicated to the perception and processing of facial information (Adolphs, 2002), and STG–amygdala FC in particular is considered vital to facial emotion recognition (Adolphs, 2002; Hennenlotter & Schroeder, 2006). Decreased RSFC between the amygdala and these regions may thus hinder the process of recognizing social cues and recruiting emotional mechanisms to interpret these cues, a process crucial to adaptive socioemotional functioning (Hughes & Dunn, 1998; Singer, 2006). Consistent with this notion, socioemotional impairments are frequently seen in neurotic individuals. These impairments include being extremely sensitive to negative social cues (McCrae & Costa, 1991) and misinterpreting ambiguous social cues as being threatening or negative (Bolger & Zuckerman, 1995; Schmidt & Riniolo, 1999). Our finding of decreased left amygdala RSFC with the temporal poles, insula, and STG may also hint at a complex neural circuitry that links neuroticism to vulnerability to affective disorders by impairing adaptive socioemotional functioning. In line with this hypothesis, impairments in socioemotional functioning are frequently reported in depressed patients (Kerr, Dunbar, & Bentall, 2003; Zobel et al., 2010), which tend to persist during remission (Inoue, Tonooka, Yamada, & Kanba, 2004).

Within this framework, aberrant amygdala–insula FC may be of particular importance in negative emotionality and the susceptibility to affective disorders, because recent data have suggested a central role for a salience network (Seeley, Menon, et al., 2007b) that has the insula as one of its key nodes. While decreased amygdala–insula FC may impede emotional awareness and identification of emotional cues (Craig, 2009; 2010), abnormally increased anterior insular FC with the dorsal ACC and dorsolateral PFC is thought to interfere with salience processing (Seeley, Menon, et al., 2007b). As such, diminished amygdala-insula coupling is reported in anxiety and depression (Etkin et al., 2009; Perlman et al., 2012; Veer et al., 2010; Zeng et al., 2012), whereas increased insula coupling with the dorsal ACC and dorsolateral PFC

strongly relates to state and trait anxiety in healthy participants (Markett et al., 2013; Seeley, Menon, et al., 2007b). The present report, therefore, further supports the idea that abnormal FC with regions of the salience network relates to negative affect and susceptibility to affective psychopathology.

Amygdala RSFC and extraversion

Extraversion was associated with increased RSFC of the right amygdala with the insula and putamen, and of the left amygdala with the putamen, temporal pole, insula, and several regions within the occipital cortex. The putamen, together with the amygdala, is part of an integrated neural circuitry dedicated to various aspects of reward processing (Haber & Knutson, 2010). Striatal regions, including the putamen, respond to the anticipated magnitude, probability, and immediacy of rewards (Ballard & Knutson, 2009; Knutson, Taylor, Kaufman, Peterson, & Glover, 2005; Yacubian et al., 2006), whereas the amygdala is mainly involved in stimulus–reward association learning (Murray, 2007). The increased amygdala RSFC with the putamen in participants with higher extraversion scores may thus suggest an enhanced functional integration of the reward circuitry in extraverts. In keeping with this notion, it was found that high levels of extraversion predict RSFC of brain regions that have been implicated in reward and motivation in a previous study (Adelstein et al., 2011). Our results may thus suggest a mechanism for the protective effects of extraversion against affective psychopathology: Heightened reward sensitivity, as reflected by enhanced functional integration of the reward circuitry, could increase the propensity to experience positive emotions, and promote psychological well-being. Conversely, diminished reward sensitivity on both the behavioral and neuronal level is frequently reported in affective disorders (DeVido et al., 2009; Henriques & Davidson, 2000), which is thought to relate to some of the affective symptoms.

Our group recently showed positive associations between extraversion and right amygdala volume (Cremers et al., 2011). In the present analyses, we therefore controlled for volumetric variation in a post-hoc analysis, but the results remained the same. This suggests that morphological differences of the amygdala are unlikely to underlie the connectivity effects found in the present study.

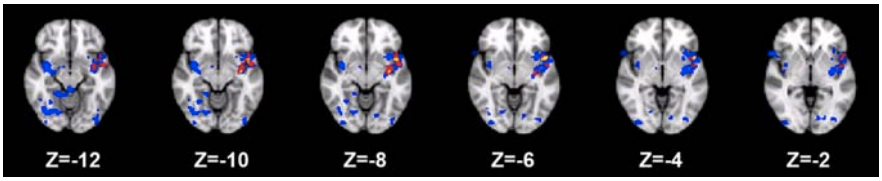


Figure 7.2 Overlap of the associations between neuroticism/extraversion and amygdala RSFC with the temporal cortex and insula. Blue denotes regions where an association was found between extraversion and left amygdala RSFC (see **Figure 7.1e**). Red denotes either an overlap of the association between extraversion and right amygdala RSFC and the association between neuroticism and right amygdala RSFC (see **Figure 7.1c and 7.1d**), an overlap of the association between extraversion and left amygdala RSFC and the association between neuroticism and right amygdala RSFC (see **Figure 7.1c and 7.1e**), or an overlap of the association between extraversion and left and right amygdala RSFC (see **Figure 7.1d and 7.1e**). Yellow denotes the voxels where all three effects overlap. The results are overlaid on the 2 mm MNI-152 T_1 standard brain. The right side of the images corresponds to the left side of the brain, and vice versa.

Our results further revealed increased amygdala RSFC with the temporal pole and insula in extraverts. This clearly contrasts our finding of decreased, and even negative, amygdala RSFC with the temporal pole and insula in the more neurotic individuals, which is in agreement with the inverse correlation between the two traits found in the present study, as well as in previous studies. As we stated earlier, amygdala FC with the temporal pole and insula may be particularly important in recognizing social cues and recruiting emotional mechanisms to interpret these cues. Thus, whereas decreased FC between the amygdala and these regions may hinder adaptive socioemotional functioning, and consequently promote psychopathology, preserved amygdala FC with these regions may curb this susceptibility.

Functional specificity within the insula

In this study, we found associations between neuroticism/extraversion and amygdala functional connectivity in both the anterior and posterior parts of the insula. As a recent meta-analysis has illustrated, the insula can be roughly subdivided into four regions that are each associated with a general functional domain: sensorimotor (dorsal mid and dorsal posterior), cognitive (dorsal anterior), chemical sensory (ventral mid),

and socioemotional (ventral anterior) (Kurth, Zilles, Fox, Laird, & Eickhoff, 2010). However, specific functions, such as empathy, interoception, and pain, were found to be associated with both the anterior and posterior insula. Unfortunately, it is always problematic to assign functional significance to RS results, as is the case in our study, so we cannot state exactly what function our findings may relate to. However, the overarching function of the insula seems the monitoring of saliency in both the internal and external environment, which naturally complements the role of the amygdala as general salience detector. Hence, we argue that reduced amygdala-insula RSFC in more neurotic individuals could be reminiscent of less well-integrated salience monitoring and detection, which may in turn be associated with vulnerability to psychopathology, whereas the opposite could be the case for high extraverts.

When exploring the overlap in amygdala-insula RSFC between neuroticism and extraversion, this seems to be most evident in the dorsal anterior insula, though this can also be observed in the more posterior portion (see **Figure 7.2**). It has been suggested that the dorsal anterior insula is a site for functional integration of the different functional domains represented in the insula (Kurth et al., 2010). As such, we hypothesize that this area could be a suitable candidate to mediate the differential effects of neuroticism and extraversion on affective networks.

Neuroticism, extraversion, and amygdala RSFC with the PFC

In the present study, we did not find the expected association between neuroticism or extraversion, and amygdala RSFC with regions of the ventral (subgenual ACC, ventrolateral PFC, and OFC), and dorsal (supragenual ACC, dorsomedial PFC, and dorsolateral PFC) systems. We offer two possible explanations for these null findings. First, given that studies on the relationship between amygdala RSFC and both traits are lacking, our hypotheses were primarily based on previous task-dependent fMRI findings. Although FC patterns during rest and task performance show similarities (Smith et al., 2009), it is conceivable that specific functional networks might be more context-dependent and could only be mapped by using specific tasks (e.g., threat-related stimuli). This might to some extent account for the inconsistencies between our and the previous task-dependent findings. Second, our sample did not include partic-

ipants with neuroticism scores in the clinical range, and neither did our participants have very low extraversion scores. A relation to the aberrant amygdala FC with regions of the ventral and dorsal PFC, which has been demonstrated in affective disorders (Pezawas et al., 2005; Phillips, Drevets, Rauch, & Lane, 2003b), might have been found if we were to include a group of highly neurotic individuals more susceptible to affective disorders, or of their low-extravert counterparts. Nonetheless, inspection of the uncorrected connectivity maps does reveal preliminary evidence that both traits might be associated with these target regions in the PFC. These findings could guide ROI selection in future studies, and thereby facilitate the mapping of amygdala-PFC circuits in relation to personality traits associated with either sensitivity or resilience to psychiatric disorders.

Limitations and future directions

The present study has several limitations that should be noted. First, the mean neuroticism and extraversion scores obtained from our sample were below and above average, respectively, as compared to the norm scores of the healthy population. This was to be expected, given that the participants included were originally recruited to serve as controls for anxiety and depression patients. As such, the exclusion criteria for controls in the NESDA study might have biased our sample toward lower than average neuroticism, but higher extraversion scores. Nonetheless, we do report a shift in amygdala functional connectivity with higher neuroticism and extraversion scores, which closely follows the altered amygdala connectivity that has been found in previous studies of stress and depression from our lab (Veer et al., 2010; 2011).

Second, physiological fluctuations (of heart rate and respiration) were not recorded during the RS data acquisition, although this may have been a source of noise influencing our data. However, we chose to include the global signal in our model as a nuisance regressor in order to minimize the effect of physiological fluctuations on our fMRI data (Fox & Raichle, 2007). Although global signal regression is believed to remove global sources of noise and minimize the influence of physiological fluctuations, some studies have suggested that it may also introduce artifactual anticorrelations (e.g., Murphy, Birn, Handwerker, Jones, & Bandettini, 2009). Recent data,

however, have shown that global signal regression suppresses false correlations and improves connection specificity, and more importantly, evidence for anticorrelations can be seen even without global signal regression (Fox, Zhang, Snyder, & Raichle, 2009; Weissenbacher et al., 2009). Further analysis of our data without global signal regression confirmed our findings. Although the results remained largely the same, some effects did not pass statistical significance, which was probably caused by higher residual noise in the data.

Third, by defining the most certain amygdala voxels as our seed region, alterations in FC of specific amygdalar nuclei may have gone unobserved. Whereas the amygdala is composed of functionally distinct nuclei (Balleine & Killcross, 2006), we still lack a well-established method for parcellating these subnuclei, due to their small size and homogeneous appearance. Although manually defining amygdalar nuclei in native space is susceptible to human error, using probability-based masks of amygdalar nuclei in standard space is susceptible to registration errors and disregards individual variations in neuroanatomy (Bach, Behrens, Garrido, Weiskopf, & Dolan, 2011; Saygin, Osher, Augustinack, Fischl, & Gabrieli, 2011). In light of the limitations pertaining to amygdala parcellation, we opted to examine connectivity of the most certain amygdala voxels rather than connectivity of the amygdalar nuclei separately.

Fourth, the potential influence of an emotional-task paradigm that preceded the RS data acquisition should be noted. Although this may have had negligible confounding effects on the data, it might also reveal the prolonged effects of emotional processing on amygdala RSFC. In that case, the increased amygdala-precuneus RSFC reported here is in line with findings from a recent study that examined the prolonged effects of social stress on amygdala RSFC (Veer et al., 2011).

Fifth, data acquisition was conducted at three different sites. Although the same scanner type was used, differences in scan quality might still have existed. Additionally, one of the sites scanned using slightly different imaging parameters. To reduce the possible effect of scan site in our analysis, we included this as a confound variable in our higher-level model. Moreover, it seems unlikely that differences between scan sites drove our results, since neuroticism and extraversion scores were distributed equally within each of the three scan sites.

Sixth, adding two correlated traits to the same linear regression model has the advantage that when a contrast is specified for one of the traits only, any variance that is shared with the other trait will be removed from this contrast. Consequently, only variance (in this context, RSFC) will be shown that is uniquely explained by the corresponding trait, which means variance over and above what can be explained by the other trait. This implies that the other trait could still account for variance in the same regions to some extent, though this would not show up in the results. Therefore, our results are limited to connectivity patterns uniquely associated to either one of the traits, and they do not necessarily describe the full range of amygdala RSFC associated with each trait.

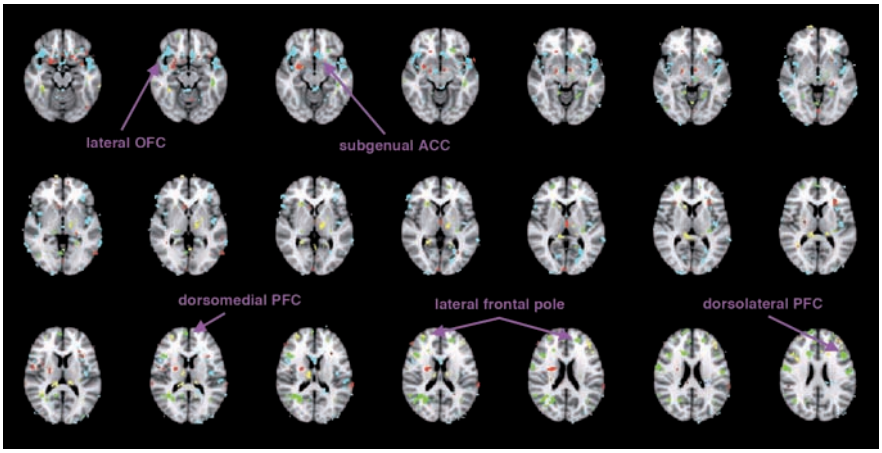
Finally, the scope of our findings is limited to the Big Five model of personality used in this study, since it is just one of many classifications for describing human personality. Nevertheless, the Big Five model has proven extremely useful in studying both normal and abnormal behavior and is currently the most widely used taxonomy of personality (Deyoung et al., 2010). Moreover, the Big Five traits are strongly heritable (Riemann, Angleitner, & Strelau, 1997), with a genetic factor structure that is invariant across cultures (Yamagata et al., 2006), rendering the traits particularly suitable for studying the neural substrates of personality. Yet, for a deeper understanding of personality, it would be both important and interesting to examine whether the present findings could be replicated using different but closely related classification schemes.

Future studies are warranted to investigate whether the altered amygdala FC reported here actually affects self-relevant, socioemotional, and reward-related processing. To this end, both RS and task-dependent fMRI could be employed in conjunction, given that these techniques provide complementary information on brain functioning. Moreover, to improve our comprehension of the mechanisms that link neuroticism to psychopathology, our findings need to be extended to healthy participants whose neuroticism scores would be extending toward those of a clinical population.

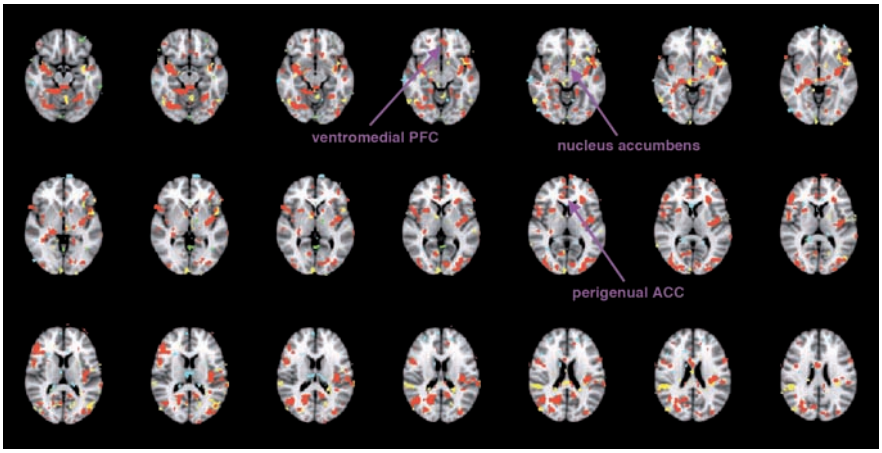
Conclusion

In sum, the results of the present study have revealed trait-specific amygdala RSFC patterns that may partly underlie functional differences between neuroticism and extraversion. Neuroticism was associated with increased amygdala RSFC with the precuneus and decreased amygdala RSFC with the temporal pole, insula, and STG. This may relate to less adaptive perception and processing of self-relevant and socioemotional information in neurotic individuals. Conversely, extraversion was associated with increased amygdala RSFC with the putamen, temporal pole, and insula, which could relate to the heightened reward sensitivity and enhanced socioemotional functioning in extraverts. We hypothesize that these trait-specific RSFC patterns could potentially link neuroticism and extraversion to the neurobiology underlying increased susceptibility or resilience to affective disorders.

SUPPLEMENTAL MATERIAL



Supplemental Figures Amygdala resting-state functional connectivity associated with neuroticism (above) and extraversion (below) at an uncorrected threshold of $z > 2.3$. Red and yellow denote a positive association with functional connectivity of the left and right amygdala, respectively. Blue and green denote a negative association with functional connectivity of the left and right amygdala, respectively. The results are superimposed on the 2 mm MNI-152 standard brain. The right side of the images corresponds to the left side of the brain and vice-versa.



Supplemental Table 7.1 Joint amygdala resting-state functional connectivity results.

Region	Hemisphere	Voxels	*z*-value	Peak voxel MNI coordinates		
				x	y	z
postive						
temporal pole	R	34848	6.47	-52	12	-24
	L		6.40	-54	12	-12
middle temporal gyrus	R		6.43	60	-4	-18
	L		5.55	-60	-6	-18
hippocampus	R		6.20	22	-18	-18
	L		6.28	-24	-26	-16
orbitofrontal cortex	R		6.12	20	12	-20
	L		6.82	-16	12	-22
hypothalamus	R		5.12	6	-4	-12
	L		5.30	-6	-4	-16
subcallosal cortex	R		5.10	2	22	-12
	L		5.01	-2	-24	-12
superior temporal gyrus	R		5.04	56	0	-12
	L		4.51	-56	0	-10
putamen	R		4.84	28	4	-4
	L		4.43	-28	0	-4
brainstem			4.82	8	-36	-22
insula	R		4.58	40	-2	-8
	L		4.74	-38	4	-10
dorsal anterior cingulate	R		4.73	2	34	-8
cortex	L		3.55	-2	44	-8
negative						
paracingulate gyrus	R	45148	5.35	4	34	36
	L		5.23	-2	32	36
posterior cingulate cortex	R		5.16	2	-42	32
	L		5.70	-2	-34	32
precuneus	R		4.73	2	-64	38
	L		4.80	-2	-66	38
lateral frontal pole	R		4.70	40	56	2
	L		3.87	-40	54	0
middle frontal gyrus	R		4.44	44	30	36
	L		4.45	-46	26	36

Note: all *z*-values are corrected for multiple comparisons at the cluster-level ($z > 2.3$; $p < .05$).

CHAPTER 8

General discussion

Chapter 8

SUMMARY OF FINDINGS

Social stress and emotional working memory

In **Chapter 2** the effects of acute social stress on distracter inhibition during working memory were studied. Participants had to keep in mind a set of letters for one and a half second, during which a neutral or emotionally negative picture was shown that had to be ignored. Subsequently, presence of the remembered letters (targets) had to be verified in a second set of letters (probe). Working memory performance, as measured by reaction times to the probes, was slower for negative than for neutral distraction in stressed participants compared with non-stressed controls, together with greater activation in ventral "affective" areas and, reduced deactivation in dorsal "executive" areas during distraction. In addition, smaller distracter interference and reduced activity of the ventral "affective" areas were both associated with higher cortisol levels in the stress group. Together, these results suggest that the brain prioritizes processing of salient information at the cost of cognitive performance in the aftermath of acute stress, while cortisol might play a modulatory role.

Social stress and resting-state functional connectivity

Chapter 3 described the prolonged effects of social stress on amygdala resting-state functional connectivity. Compared with non-stressed controls, increased connectivity was found with the precuneus, posterior cingulate cortex, and ventromedial prefrontal cortex in stressed participants. These midline structures are key nodes of the *default mode network*, and have been implicated in memory, emotion regulation, and social cognition. Differences in cortisol response to the stressor, however, were not associated with the strength of this connection. Although speculative, the stress effects on amygdala connectivity might be reminiscent of the process of reaching (behavioral) homeostasis after stress, which could linger long beyond the initial stress response.

Cortisol and resting-state functional connectivity

In **Chapter 4** it was tested whether amygdala resting-state functional connectivity might be related to individual differences in endogenous cortisol fluctuations under

relatively stress-free circumstances. Steeper cortisol decreases over the course of the experiment were associated with stronger *negative* amygdala functional connectivity with the medial prefrontal cortex, most notably the perigenual anterior cingulate cortex. It is hypothesized that this finding could be indicative of a cortisol-mediated regulatory network, served to adaptively adjust stress- and, more generally, emotional responses.

Resting-state functional connectivity in major depression

Differences in whole brain resting-state connectivity networks were assessed between unmedicated patients with *major depressive disorder* and matched healthy controls in **Chapter 5**. Within a ventral network, comprising key affective regions, depression was associated with reduced functional connectivity with the bilateral amygdala. In addition, reduced *negative* connectivity with the left frontal pole was found in the dorsal *task-positive network* in depressed patients compared with controls, as well as weaker connectivity with the lingual gyrus in a medial visual network. None of the effects were associated with symptom severity, suggesting these to be trait rather than state differences. Overall, these findings could reflect maladaptive emotional processing in ventral affective areas and compromised cognitive processing in dorsal regions, corroborating the current neural network models of depression.

PTSD and medial temporal lobe volumes

In **Chapter 6** differences in volumes of the hippocampus and amygdala were assessed between female *posttraumatic stress disorder* patients with a history of childhood maltreatment and matched healthy controls. Smaller right amygdala volumes were found in patients compared with controls, whereas the left amygdala and bilateral hippocampus did not differ between the two groups. In addition, this volume reduction appeared to be specific to the basolateral and centromedial nuclei groups of the right amygdala. Smaller amygdala volumes were furthermore associated with more severe sexual abuse during childhood. It is hypothesized that traumatic events in childhood might impede normal development of the amygdala, which could render someone more vulnerable to develop psychopathology later in life.

Personality and resting-state functional connectivity

Finally, in **Chapter 7** it was tested to what extent amygdala resting-state functional connectivity relates to interindividual differences in neuroticism and extraversion, personality traits that are associated with vulnerability and resilience, respectively, to affective disorders. Higher neuroticism was related to increased amygdala connectivity with the precuneus, and decreased amygdala connectivity with the temporal pole, insula, and superior temporal gyrus, which could be indicative of less adaptive perception and processing of self-relevant and socio-emotional information in neurotic individuals. Extraversion, on the other hand, was associated with increased amygdala connectivity with the putamen, temporal pole, and insula, which could relate to the heightened reward sensitivity and enhanced socio-emotional functioning observed in extraverts. These trait-specific functional connectivity patterns could potentially provide insights into the neurobiology underlying increased susceptibility or resilience to affective disorders.

INTEGRATION OF FINDINGS

The aim of this thesis was to provide more insight in how stress impacts emotion processing and regulation, how affective brain networks are modulated in the aftermath of a stressful situation, and how changes in functional connectivity within these networks can be related to stress-related psychopathology. Given its important role in the orchestration of stress responses (Ulrich-Lai & Herman, 2009) and (abnormal) emotion processing (Hariri & Whalen, 2011; Phillips, Drevets, Rauch, & Lane, 2003a; 2003b), the majority of the research described in this thesis revolved around the amygdala.

Consistent with the hypothesis that the brain prioritizes processing of salient information under stress, we found that ventral "affective" regions, most notably the amygdala, increased their response to negative pictures that had to be ignored, while dorsal "cognitive control" areas demonstrated relatively decreased activity (Oei et al., 2012). Although this study was designed to assess the effects of stress on inhibition

of distracters rather than on working memory per se, we did find an indication for slower, but not worse, performance for the stress group, yet only as a function of distracter type. This corroborates findings from previous studies in which reduced working memory performance could be measured after psychosocial stress or cortisol administration (Elzinga & Roelofs, 2005; Lupien et al., 1999; Oei et al., 2006; Schoofs et al., 2008), though an absence of behavioral differences (Porcelli et al., 2008; Qin et al., 2009), and even increased performance (Henckens et al., 2011), have been observed as well. Of note, larger cortisol responses were related to better performance and less amygdala activity in our study. On the one hand, these results are at odds with the study of Lupien et al. (1999), but corroborate the beneficial effects of cortisol on working memory (Henckens et al., 2011) and distracter inhibition (Oei et al., 2009). However, the stress-induced cortisol levels sampled in our study were relatively low compared with both other studies in which cortisol was administered, while concurrent stress-induced increases in noradrenaline might further obscure a direct comparison between experiments.

Our finding of increased amygdala activity in response to negatively arousing stimuli after stress is in keeping with the results from a previous study (van Marle et al., 2009). It can be appreciated that the shift from cognitive processing to vigilance towards salient, and potentially threatening, information under stress benefits immediate survival from an evolutionary perspective. However, as observed in our non-stressed participants, we are in general quite capable to actively inhibit intrusive information that could keep us from engaging in goal-directed behavior, which helps us to achieve our aims and objectives in everyday life.

Although adaptive in the short run, this regulatory mechanism might fail in more chronic stress states, and could, as such, form the basis for the pathological anxiety (Kim et al., 2011b), rumination (Siegle, Steinhauer, Thase, Stenger, & Carter, 2002), or trauma-related intrusions (Shin & Liberzon, 2010), symptoms observed in a range of affective disorders. To test this hypothesis directly, we compared posttraumatic stress patients and healthy control participants on the same distracter inhibition task used before, though considering the disorder as a chronic stress condition, given the HPA-axis dysregulation that is typical for PTSD, instead of temporarily inducing

psychosocial stress. In line with our expectations, patients showed increased amygdala responses to emotionally salient pictures that had to be ignored, similar to the effects in healthy controls after stress. This might indicate a chronic state of increased attention towards threatening stimuli and reduced ability to dampen this response in posttraumatic stress disorder (Veer et al., in preparation).

To date, surprisingly few studies have been carried out on the effects of stress on connectivity within affective brain circuits in healthy participants. A recent study tested the whether changes in amygdala functional connectivity could be observed immediately following stress, which was induced by viewing negatively arousing video clips (van Marle et al., 2010). The authors reported increased connectivity with areas of the *salience network*, such as the dorsal anterior cingulate cortex (dACC), insula, and brainstem, which have been found to coactivate in response to a wide variety of both internally and externally generated salient signals (Seeley, Keller, et al., 2007a). Thus, increased connectivity between the amygdala and this network after stress could reflect the neural trajectory through which heightened monitoring and evaluation of information is achieved in the face of a stressful event. This is further substantiated by an earlier finding of increased blood flow within regions of the salience network *during* stress (Wang et al., 2005). Moreover, connectivity of the dACC with either other regions of the salience network (Seeley, Keller, et al., 2007a), or the amygdala (Kim, Gee, Loucks, Davis, & Whalen, 2011a), was found to be stronger when reported state anxiety was higher. Although this provides a potential link to stress-related disorders in which vigilance and autonomic tone is sustained, such relation has yet to be established.

In contrast to the immediate effects described above, we studied whether a stressful event modulates amygdala functional connectivity even long, in our case an hour, after the stress has been terminated (Veer et al., 2011). Instead of expecting connectivity changes related to the acute stress response, it was expected to find altered functional connectivity with regions more associated with regulation of stress responses, and (emotional) memory formation and consolidation. In this study we found increased connectivity with core regions of the *default mode network* (DMN), the posterior cingulate cortex (PCC) and precuneus, and medial prefrontal cortex

(mPFC), which have been implicated in mind wandering (Mason et al., 2007), autobiographical memory processes (Buckner & Carroll, 2007), and self-referential thought (Gusnard et al., 2001; Northoff et al., 2006; Raichle et al., 2001). As such, the network is hypothesized to provide the infrastructure for integrating past, present and future events that are related to the self (Buckner & Carroll, 2007). This would enable us to reflect on and learn from past experiences, which is essential to adaptively cope with future challenges. Given the dense connections between the hippocampus and both the PCC and amygdala (Amaral, 1986; Greicius et al., 2009), the increased amygdala connectivity with the DMN found here could potentially underlie stress-induced increased encoding and consolidation of emotionally salient events (Wolf, 2009).

In this study in healthy young males we did not find an association between the strength of amygdala connectivity and stress-induced cortisol levels. However, it is important to note that stress effects on memory do seem to depend on an interplay between cortisol and noradrenaline (Roozendaal et al., 2009; Strange & Dolan, 2004; van Stegeren et al., 2008), which was not assessed in our study. Perhaps surprisingly, we did find a relation between interindividual differences in endogenous cortisol and amygdala connectivity in our non-stressed controls (Veer et al., 2012). Higher cortisol levels at the start of the experiment, and subsequent steeper cortisol decreases over the course of the experiment, were associated with stronger negative amygdala connectivity with the perigenual ACC (pgACC). Lesions in the dorsal prelimbic cortex, which is considered a homologue of the human pgACC, causes disinhibition of stress responses in rodents (Boyle et al., 2005; Diorio et al., 1993; Furay et al., 2008). Given the hypothesized role of the pgACC in emotional conflict and regulation of autonomic and affective responses in humans (Etkin et al., 2006; Gianaros et al., 2008; Wager et al., 2009), a regulatory pathway between this area and the amygdala might be crucial for the negative feedback of cortisol in terminating stress responses. However, another recent study found diminished negative connectivity between the amygdala and a more dorsomedial portion of the PFC after hydrocortisone intake (Henckens, van Wingen, Joëls, & Fernández, 2012). Future studies are thus warranted to elucidate the effects of cortisol on amygdala-mPFC connectivity, and its relation

to regulation of stress responses, taking into account both the tonic and phasic effects of cortisol.

Similar to our findings after stress, we found an increase in amygdala connectivity with the precuneus in participants who scored higher on the personality dimension neuroticism (Aghajani et al., 2013). Neuroticism has been intimately linked to self-evaluative and ruminative behavior (Trapnell & Campbell, 1999), as well as to increased vulnerability for developing affective disorders (Bienvenu et al., 2001). Therefore, whereas self-evaluation could be an important regulatory feature in the aftermath of stress, especially when the stressful situation encountered was social in nature, higher neurotic individuals could be more susceptible to get stuck in a "ruminative loop". It is this susceptibility that has been proposed to be a major feature underlying depressive symptoms (Holtzheimer & Mayberg, 2011), while perseverative rumination has been linked to prolonged autonomic signs of stress (Brosschot, 2010). In addition, increased activity within cortical midline structures has been reported in a recent study when participants had more worry-related thoughts in response to worry-inducing sentences (Servaas, Riese, Ormel, & Aleman, 2014).

Although depression-related abnormalities in DMN connectivity have been described in literature (Greicius et al., 2007; Sambataro, Wolf, & Vasic, 2013a; Sambataro, Wolf, Pennuto, Vasic, & Wolf, 2013b; Sheline, Price, Yan, & Mintun, 2010; Zhou et al., 2010), we did not observe any differences within this specific network between our sample of depressed patients and healthy controls (Veer et al., 2010). However, we suffered the limitation of having only mildly depressed participants in our sample, of which several were already in remission at the time of scanning. Nevertheless, we did observe altered connectivity within three other networks. Patients showed reduced functional connectivity with the amygdala in a network comprising a set of other regions involved in emotion processing and regulation, such as the mPFC, temporal poles, and insula, which might mediate the affective symptoms of the disorder. We demonstrated a similar decrease in amygdala functional connectivity with the insula and temporal poles in higher neurotic individuals, while the opposite pattern was found for the more extravert participants (Aghajani et al., 2013). Again, this might reveal a neural pathway that underlies the increased susceptibility to develop

affective psychopathology for higher neurotic individuals, whereas at the same time it might be considered a neurobiological marker for extraversion-related resilience to develop these disorders.

Only one study described in this thesis focused on the anatomical integrity of the amygdala, which was assessed in posttraumatic stress disorder patients with a history of childhood maltreatment (Veer et al., submitted). Here we found a smaller volume of the right amygdala compared with healthy controls, specifically in the centromedial and basolateral complex. The centromedial nucleus of the amygdala plays a major role in the stress response, as it initiates and regulates autonomic responses (Ulrich-Lai & Herman, 2009). The basolateral nucleus, on the other hand, has been implicated in responses to psychogenic stressors, regulation of the HPA-axis, as well as emotional memory (Roozendaal et al., 2009; Ulrich-Lai & Herman, 2009). Thus, the smaller right amygdala volume that was found in our study might relate to several hallmark symptoms of posttraumatic stress, including hyperarousal and intrusions of trauma-related memories (APA, 1994; Shin & Liberzon, 2010). A recent study reported *decreased* right amygdala grey matter for risk allele carriers of the brain-derived neurotrophic factor Val66Met polymorphism, associated with increased susceptibility for affective disorders (Montag, Weber, Fliessbach, Elger, & Reuter, 2009), while another study reported an association between *greater* right amygdala grey matter density and higher extraversion scores (Cremers et al., 2011), which could again be hypothesized to be a neurobiological marker for resilience to these disorders.

So far, resting-state functional connectivity studies of the amygdala are relatively sparse in posttraumatic stress disorder, and mostly carried out in male combat veterans, whereas our sample comprised female patients with a history of childhood maltreatment. Increased connectivity has been reported between the basolateral amygdala and the dorsal ACC and dorsomedial PFC (Brown et al., 2014), while another study showed decreased negative amygdala connectivity with the same region, as well as decreased connectivity with the hippocampus (Sripada et al., 2012). Studying the same female PTSD sample as used for assessment of medial temporal lobe volumes, we found results that point in the same direction (Veer et al., in preparation). Of major relevance to our specific patient sample with a history of childhood trauma,

reduced grey matter density in this exact dorsomedial PFC region has been described in participants that reported childhood emotional maltreatment (van Harmelen et al., 2010).

LIMITATIONS

The studies that were carried out for this thesis have several limitations. First, in our stress induction experiment we used the Trier Social Stress Test as stressor (TSST), which has social evaluative threat as its main stress-inducing component (Kirschbaum et al., 1993). However, other forms of stress-induction have been used in literature as well, including cold pressor stress (Cahill et al., 2003), and negatively arousing video clips (Hermans et al., 2011), which might all probe different aspects of the stress response. For example, a meta-analysis of stress-induction studies has shown that negative social evaluation in combination with uncontrollability of the situation, which both are aspects of the TSST, causes the highest increase in cortisol levels by far (Dickerson & Kemeny, 2004). Therefore, when elevation of cortisol levels is the main objective of stress-induction, it could be advised to use the TSST, or other forms of social evaluative threat. However, whereas social stress might be highly commendable in relation to, for example, social anxiety disorder and emotional abuse, videos of violence might be more suited to study similarities with trauma related to sexual or physical abuse and combat experience.

Second, timing of measurements with respect to stress-induction is pivotal. Here, we studied effects directly after social stress (task), and one hour after induction (resting-state). In both cases our results are limited to effects of the stress response that happen on that specific point of time, which renders us blind to effects during other stages of the response. Although challenging to design and carry out, experiments that probe different stages of the stress response (Vaisvaser et al., 2013), or time-dependent effects of stress hormones (Henckens et al., 2010; 2011) are most likely to provide us a more comprehensive picture of the neurobiological sequelae of stress.

Third, not only is it well established that the stress response differs between males and females, but it also does within females, depending on the menstrual cycle (Kajantie & Phillips, 2006). Additionally, these differences are reflected by distinctive neural activity in stress-related brain regions as well (Goldstein, Jerram, Abbs, Whitfield-Gabrieli, & Makris, 2010; Wang et al., 2007). To this end, we decided to only include male participants in our social stress study. Our findings and conclusions with respect to the effects of stress are therefore limited to the male population. Conversely, we only assessed female posttraumatic stress patients, given the difficulty we encountered in finding male patients with a history of childhood maltreatment. To illustrate, physical and sexual abuse, which were two of our criteria, are more prevalent during childhood in females than in males (de Vries & Olff, 2009).

Fourth, in three of the four resting-state functional connectivity studies we employed a seed-based connectivity analysis, choosing the amygdala as seed. Although this type of analysis is well suited to address hypothesis-driven questions, as was the case in these studies, results are inherently limited to the connections of the seeds that are chosen a priori. This means that differences between our groups in neural circuits not associated with the amygdala seeds might have gone unobserved. In contrast, the more data-driven independent component analysis has the potential to explore the breadth of connectivity changes that might occur anywhere in the brain, which was used on our depression data. However, it has been shown that group differences might or might not become evident depending on the model order (i.e., number of components) that was chosen (Abou Elseoud et al., 2011). This suggests that it might even be feasible to run the analysis at a range of model orders, although this could, of course, easily lead to chance capitalization. A similar argument can be made for the number of components tested within a certain model order. Whereas with seed-based analyses one only has to correct for the number of voxels tested, a correction should additionally be carried out for the number of components tested. However, doing this for a typical number of networks, say ten, dramatically lowers the significance threshold to a point that we can be quite confident to have protected ourselves to false positive findings, at the cost of becoming highly susceptible to not finding true effects (i.e., false negatives). A possible solution to this problem has re-

cently been suggested by Abou Elseoud et al. (2014).

Fifth, as was already alluded to in the introduction, the use of global signal regression in seed-based connectivity analyses has become a matter of debate in recent years. Initially, this step was intended to correct for global confounding signal sources in the fMRI data, such as physiological noise. Although global signal regression has been praised for its potential to increase connectivity specificity (Weissenbacher et al., 2009), it has been shown that this analysis step necessarily also introduces negative correlations (assumed negative connectivity) to arise in the data (Murphy et al., 2009), and could potentially even cause spurious effects between groups (Saad et al., 2012). Although elegant techniques exist to correct for physiological confounds, such as RETROICOR (Chang & Glover, 2009; Glover, Li, & Ress, 2000), these typically depend on proper acquisition of the physiological signals (e.g., heart rate and respiration). In our seed-based connectivity studies, however, these data were incomplete, or not available at all, which led us to use global signal regression to try to account for these confounding factors. Importantly, after reanalyzing the data from our neuroticism and extraversion study without global signal regression, the results were highly similar. Nevertheless, future studies should best refrain from using global signal regression, as alternative correction strategies have become widely available in recent years. One such solution is ICA-based denoising of the data, as ICA has the potential to separate apparent neural signal sources from non-neuronal noise (Salimi-Khorshidi et al., 2014). In addition, new acquisition techniques, such as multiplexed fMRI acquisition, can substantially accelerate repetition times (TR < 1 s) between volumes, yielding better temporal specificity and better characterization of higher frequency artifact signal sources in the data (Feinberg et al., 2010; Uğurbil et al., 2013).

Sixth, we cannot infer causality from our connectivity measures, as these are merely correlational in nature. Any conclusions on the directionality of the effects are therefore highly speculative. Nevertheless, tract tracing and in vivo intervention studies in primates and rodents do inform us on the information flow within certain pathways or brain circuits, which can lead us to formulate causal hypotheses based on the connectivity effects measured in humans. Excitingly, recent research has suggested that high-resolution fMRI data acquired on a high field MR system could potentially

reveal causal connectivity patterns between regions in the visual cortex, making use of information from distinct cortical layers (Polimeni, Witzel, Fischl, Greve, & Wald, 2010). The authors describe a correlation in BOLD signal between the output layer of V1 and the input layer of area MT, thus suggesting information flow from the former to the latter region.

Seventh, another limitation pertains to multicollinearity issues in the *general linear model* (GLM), which was used for our seed-based connectivity studies. Estimation of parameter estimates (i.e., betas) of each individual predictor critically depends on which other predictors have been added to the model, and to what extent these predictors correlate among each other. It is this correlation that can influence the estimation of the parameter estimates, and even can cause otherwise uncorrelated variables to show an association (Andrade, Paradis, Rouquette, & Poline, 1999; Kraha, Turner, Nimon, Zientek, & Henson, 2012). In fact, the effects of global signal regression on the data described previously are the consequence of multicollinearity issues, given that the global signal will always correlate with any given voxel to some extent. However, typically a range of other "nuisance" variables are added to the regression model, including regressors for motion, white matter, and cerebrospinal fluid, which through collinearity may all alter the parameter estimate of the seed of interest in their own respect. Therefore, reporting parameter estimates only, as is commonly (though not exclusively; Courville & Thompson, 2001) done in imaging studies, does not reveal the complete picture of relations between the different regression variables. Although several additional metrics have been proposed to better understand and interpret regression results in the face of multicollinearity (Kraha et al., 2012), these have yet to be implemented in fMRI analysis suites. Nonetheless, regression results are statistically valid, but should always be interpreted with respect to the other predictors in the model.

A final limitation relates to the small sample sizes used in most of the studies described in this thesis, especially the posttraumatic stress study. It has been argued that small sample sizes not only could lead to an increase in false negatives due to low power, but will also overestimate effect sizes of the effects that do pass the stringent correction for multiple comparisons (Button et al., 2013; Cremers, 2013; Yarkoni,

2009). As such, small sample sizes also hamper reproducibility of findings across studies. Unfortunately, however, it is not always possible to achieve large sample sizes due to, for example, patients that are hard to find, complicated and extensive research designs, financial limitations, or just lack of time. Detailed overviews of the issues related to reliability and replication of findings in cognitive and affective neuroimaging studies, as well as possible solutions, are offered in a recent special issue of *Cognitive, Affective, & Behavioral Neuroscience* (volume 13, issue 4, 2013).

FUTURE RESEARCH

This thesis concludes with some recommendations for future research. First, as was already argued in the limitations section, when designing a stress experiment the method of stress-induction should be chosen according to the specific research question, depending on, for example, which aspect of the neuroendocrine response is of interest, or to which disorders the type of stress should compare.

Second, the modulating effects of cortisol depend greatly on the timing of cortisol secretion or administration with respect to the stressful situation or the cognitive process to be studied, as well as the height of cortisol levels (Lupien et al., 2007; Sapolsky et al., 2000). Oftentimes, stress-induction methods, achieved cortisol responses, and the time of testing differ widely between studies, which makes it difficult to determine the exact effects of the hormone. Whereas this is difficult, if not impossible, to control for in stress-induction studies, experiments in which cortisol is administered should employ comparable doses. In addition, dose-response studies are warranted to determine level-dependent effects of cortisol on brain and cognition more accurately.

Third, all too often resting-state acquisition is still a byproduct of a larger imaging protocol. If one is truly interested in the unique information that resting-state fMRI has to offer, experiments should rather be designed to target task-independent neural activity specifically. Moreover, although simple group comparisons of resting-state data could inform us, for example, which brain circuits might be involved

in the pathophysiology of a disorder, future studies should strive to manipulate resting-state activity to be able to attach functional significance to these circuits.

Fourth, although symptomatic for the entire field of neuroimaging, more effort should be put into replicating resting-state findings, especially given the power issues related to smaller sample sizes described earlier. In addition, consensus on preprocessing and analysis standards would further improve comparability of findings between studies. Importantly, large-scale data sharing initiatives have emerged in recent years (e.g., the *1000 functional connectomes project*: www.nitrc.org/projects/fcon_1000), which already have resulted in the description of consistencies and discrepancies in resting-state derived metrics over a large collection of data acquired in different labs from all over the world (Biswal et al., 2010).

Fifth, traditionally resting-state activity and connectivity mostly have been studied as a static phenomenon over the period of acquisition. As the BOLD response is already a gross underestimation of the underlying neural dynamics, it is quite unrealistic to assume that functional connections do not change over the course of minutes, or even seconds. In recent years, attempts have been made to capture these dynamic changes over time, which are expected to give a deeper understanding of how connections between brain regions are related to information processing and behavior (Smith et al., 2009). The interested reader is referred to an excellent review providing an in-depth discussion of the concept, current methods, and limitations of time-varying functional connectivity (Hutchison et al., 2013).

CONCLUSION

In sum, in this thesis I have provided an introduction to the effects of stress on cognition, brain structure and function, and the relation to stress-related psychopathology. In addition, the studies that were carried out in the context of this thesis demonstrate how stress can influence information processing and even cause changes in functional connectivity up to an hour after the stress has waned. Moreover, it was shown through which circuit cortisol might modulate stress responses, and how personality dimen-

sions related to vulnerability and resilience to affective disorders can be associated with changes in brain circuits involved in the processing and regulation of emotions. Lastly, volume reductions were reported in specific subnuclei of the amygdala, which might relate to specific symptoms of posttraumatic stress disorder, and reduced integrity of large-scale connectivity networks was described in depression. Taken together, these findings strengthen our knowledge on the effects of stress and stress hormones on the brain, at the same time opening important new avenues for future research.

CHAPTER 9

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DUTCH SUMMARY

Ieder organisme is uitgerust met een aangeboren systeem dat adaptief om moet kunnen gaan met situaties die onze fysieke en psychologische gesteldheid bedreigen. Zulke situaties worden ook wel stressoren genoemd. Wanneer we geconfronteerd worden met een stressor, zet ons brein een reeks neuro-endocriene reacties in gang die zowel lichaam als geest in staat stellen een gepaste reactie op de stressvolle situatie te geven. Overleving van het organisme staat hierbij steeds centraal. Nadat het hoofd is geboden aan de stressor is het echter ook van belang weer terug te keren naar een rusttoestand, ook wel bekend als *homeostase*. Een flexibele interactie tussen het activeren en het remmen van het stresssysteem is onontbeerlijk voor onze fysieke en geestelijke gezondheid.

De amygdala, een kleine en evolutionair oude hersenkern die in beide hersenhelften verborgen ligt onder de neocortex, is van groot belang voor het initiëren van stressresponsen. De kernfunctie van de amygdala is dan ook het brein te alarmeren wanneer de omgeving ons saillante informatie verschaft. Dat wil zeggen, informatie die ons helpt onze overlevingskansen in algemene zin te vergroten, bijvoorbeeld in het geval van dreigend gevaar, maar ook bij potentiële beloningen. De amygdala heeft sterke verbindingen met kernen in de hersenstam die het autonome zenuwstelsel aansturen, die op hun beurt weer basale functies als ademhaling en hartslag beïnvloeden. Via deze route wordt het organisme fysiek en geestelijk in staat gesteld snel op een stressor te reageren. Deze eerste reactie wordt met name gemedieerd door het hormoon (nor)adrenaline.

Tegelijkertijd wordt de trager opererende hypothalamus-hypofyse-bijnierschors (Engelse afkorting: HPA) as geactiveerd, met als belangrijkste hormonale eindproduct cortisol. Waar (nor)adrenaline een nagenoeg direct effect heeft, piekt cortisol typisch pas 10 tot 20 minuten na aanvang van de stressor. Een van de functies van dit hormoon is dan ook het ondersteunen van het bereiken van homeostase. Terwijl cortisol in het lichaam onder andere de energiehuishouding reguleert, zorgt het hormoon in het brein voor een belangrijke terugkoppeling op de HPA-as. Hiermee wordt het beëindigen van de stressrespons gefaciliteerd en de verdere aanmaak van

cortisol gestopt.

De studies beschreven in dit proefschrift hadden tot doel om de neurale mechanismen te identificeren die een persoon in staat stellen om adequaat op een stressor te reageren en daarvan te herstellen, en om na te gaan welke rol cortisol hierin speelt. Ook werd onderzocht hoe deze regulerende circuits in het brein onder druk staan bij mensen met een verhoogde kwetsbaarheid voor een stress-gerelateerde psychische stoornis en bij mensen met een depressie of posttraumatische stress. Hierbij is gebruik gemaakt van magnetische resonantie imaging (MRI), waarmee zowel structuur als functie van het brein gemeten kan worden. In de meeste studies is een specifieke MRI methode toegepast, waarmee bekeken kon worden hoe verschillende hersengebieden met elkaar communiceren (ook wel functionele connectiviteit genoemd) bij het initieren en weer afremmen van een stressrespons.

In **hoofdstuk 2** worden de effecten van acute sociale stress beschreven op het vermogen irrelevante afleidende stimuli te negeren tijdens het uitvoeren van een werkgeheugentaak. Gezonde deelnemers moesten gedurende anderhalve seconde een aantal letters onthouden, waarbij op hetzelfde moment een neutraal of emotioneel negatief plaatje werd getoond. Dit plaatje was irrelevant voor het correct uitvoeren van de taak en moest dan ook genegeerd worden. Vervolgens kregen de deelnemers een reeks letters te zien en moesten zij aangeven een van de onthouden letters voorkwam in deze reeks. De werkgeheugen prestatie, gemeten aan de hand van de reactietijden op de tweede reeks letters, was langzamer wanneer negatieve plaatjes werden getoond dan wanneer neutrale plaatjes werden getoond, met name voor deelnemers die van tevoren een praatje hadden moeten geven voor een beoordelingscommissie bestaande uit drie voor de proefpersoon onbekende leden (sociale stress) in vergelijking met een controlegroep zonder stress. In het brein werd een zelfde patroon gezien: ventrale hersengebieden betrokken bij verwerking van emotionele stimuli (zoals de amygdala) waren actiever bij proefpersonen na sociale stress, terwijl activatie in dorsale gebieden belangrijk voor het uitvoeren van een cognitieve taak (zoals de dorsolaterale prefrontale cortex) juist verminderd was wanneer de afleidende plaatjes werden getoond. Tot slot bleek dat minder interferentie van de afleidende plaatjes en een verminderde activiteit van de ventrale hersengebieden beide gerelateerd waren aan een hogere cor-

tisolrespons in de stress groep. Deze resultaten lijken erop te wijzen dat het brein de verwerking van belangrijke informatie uit de omgeving voorrang geeft ten koste van een verminderde cognitieve prestatie in nasleep van acute stress, waarbij cortisol mogelijk een modulerende rol speelt.

Hoofdstuk 3 beschrijft de late effecten van sociale stress op functionele connectiviteit van de amygdala tijdens een scan waarbij de proefpersoon niet bezig is met het uitvoeren van een specifieke taak (*resting-state*). Een uur na de stress werd in de stressgroep, vergeleken met de controlegroep, sterkere connectiviteit gevonden met de precuneus, posterieure cingulaire cortex, en de ventromediale prefrontale cortex. Deze gebieden die in de mediale lengteas van het brein liggen en behoren tot de kerncentra van het default mode network, spelen een belangrijke rol in geheugen, emotie regulatie en sociale cognitie. In tegenstelling tot de gevonden relatie bij de werkgeheugentaak, waren verschillen in cortisolrespons niet gerelateerd aan de sterkte van de connectiviteit in de stressgroep. De gevonden stresseffecten op functionele connectiviteit van de amygdala zouden wel eens, ook al is het voorlopig speculatief, gerelateerd kunnen zijn aan het bereiken van (gedragsmatige) homeostase na stress, wat langdurig kan aanhouden na de initiële stressrespons.

In **hoofdstuk 4** werd bekeken in hoeverre functionele connectiviteit van de amygdala geassocieerd is met individuele verschillen in endogene cortisol fluctuaties, ditmaal bij proefpersonen die de stressmanipulatie niet hadden ondergaan. Het bleek dat een sterkere cortisol afname gedurende het experiment samenhing met een sterkere negatieve connectiviteit van de amygdala met de mediale prefrontale cortex, met name het gedeelte dat de perigenuale anterieure cingulaire cortex wordt genoemd. Deze resultaten zouden indicatief kunnen zijn voor een door cortisol gemedieerd regulerend netwerk dat zorgt voor een adaptieve regulering van stress- en, in meer algemeen zin, emotionele reactiviteit.

Verschillen in functionele connectiviteit tussen proefpersonen met depressie en gezonde controles staan centraal in **hoofdstuk 5**. Hiertoe werden verscheidene hersennetwerken bekeken tijdens een resting-state scan. Een ventraal netwerk, bestaande uit hersengebieden die van belang zijn voor de verwerking van emotionele stimuli, liet verminderde integratie van de bilaterale amygdala zien in de depressie-

groep vergeleken met gezonde controle proefpersonen. Ook werd verminderde negatieve connectiviteit met de linker frontale pool gevonden in het taak-positieve netwerk (geassocieerd met aandachtsprocessen en uitvoering van diverse cognitieve taken), en zwakkere connectiviteit met de linguale gyrus in een primair visueel netwerk. Geen van de gevonden verschillen was gerelateerd aan de ernst van de depressie, wat suggereert dat deze verschillen meer een algemeen kenmerk van het ziektebeeld zijn dan een afspiegeling van de huidige toestand van de depressie. Deze bevindingen kunnen wijzen op een minder adaptieve verwerking van emotionele informatie in ventrale affectieve hersengebieden en een verstoorde werkzaamheid van dorsale cognitieve gebieden, twee processen die de kern vormen van huidige netwerkmodellen van depressie.

Hoofdstuk 6 beschrijft een studie naar hippocampus- en amygdala (mediale temporale kwab) volumes van vrouwen met posttraumatische stress stoornis (PTSS) en een geschiedenis van interpersoonlijk trauma gedurende hun jeugd. Een kleiner volume van de rechter amygdala werd gevonden in de PTSS groep vergeleken met een groep vrouwen zonder stoornis. De linker amygdala en bilaterale hippocampus verschilden niet tussen de twee groepen. De volumevermindering bleek specifiek voor de basolaterale en centromediale nuclei groepen van de rechter amygdala. Tot slot was een kleinere rechter amygdala volume geassocieerd met een zwaardere geschiedenis van seksueel misbruik in de jeugd. Deze resultaten kunnen wijzen op een verstoring van het normale ontwikkelingstraject van de amygdala door een sterk traumatiserende ervaring, waardoor iemand kwetsbaarder wordt voor het ontwikkelen van een affectieve stoornis later in het leven.

Tot slot beschrijft **hoofdstuk 7** in hoeverre functionele connectiviteit van de amygdala geassocieerd is met individuele verschillen in neuroticisme en extraversie, persoonlijkheidsfactoren die in verband worden gebracht met respectievelijk kwetsbaarheid voor en weerbaarheid tegen affectieve stoornissen. Een hogere mate van neuroticisme was geassocieerd met sterkere amygdala connectiviteit met de precuneus en verminderde amygdala connectiviteit met de temporale pool, insula, en superieure temporale gyrus. Deze resultaten kunnen wijzen op een minder adaptieve perceptie en verwerking van zelfrelevante en sociaal-emotionele informatie in meer

neurotische personen. Extraversie, aan de andere kant, was geassocieerd met een sterkere amygdala connectiviteit met de putamen, temporale pool, en insula. Mogelijk weerspiegelt deze bevinding de verhoogde gevoeligheid voor beloningen en een beter sociaal-emotioneel functioneren, wat vaak wordt gevonden bij meer extraverte mensen. De voor deze persoonlijkheidsfactoren specifieke connectiviteitspatronen bieden mogelijk inzichten over de neurale processen die ten grondslag liggen aan een verhoogde kwetsbaarheid voor, of juist weerbaarheid tegen affectieve stoornissen.

Samenvattend, is in dit proefschrift een reeks studies beschreven, waarvan de resultaten laten zien hoe stress informatieverwerking kan beïnvloeden en veranderingen kan veroorzaken in de communicatie tussen hersengebieden, ook nadat de stressvolle gebeurtenis al lang voorbij is. Verder is een hersencircuit gevonden waarmee cortisol mogelijk stressresponsen moduleert, en zijn persoonlijkheidsfactoren die geassocieerd zijn met kwetsbaarheid voor of weerbaarheid tegen affectieve stoornissen in verband gebracht met veranderingen in hersennetwerken die betrokken zijn bij het verwerken en reguleren van emoties. Tot slot zijn kleinere volumes van specifieke subkernen van de amygdala gerapporteerd, welke een verband kunnen hebben met specifieke symptomen van posttraumatische stress, en is verminderde integriteit van affectieve en regulerende hersennetwerken gevonden in depressie. De resultaten uit dit proefschrift vergroten onze kennis over de effecten van stress en stresshormonen op het brein en bieden belangrijke nieuwe aanknopingspunten voor toekomstig onderzoek.

Chapter 9

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Ilya Veer
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Acknowledgments

CURRICULUM VITAE

Ilya Veer, born in Amsterdam on June 15th 1981, attended high school at the *Barlaeus Gymnasium* in Amsterdam from 1993 till 1999. In 2005 he received his MSc in clinical neuropsychology and biological psychology from the *University of Amsterdam* (cum laude). Ilya started his PhD at the *Leiden Institute for Brain and Cognition* (LIBC) and the *Leiden University Medical Center* (LUMC) in October 2006, supervised by prof. dr. Serge Rombouts and prof. dr. Mark van Buchem. Between June 2011 and April 2013 he worked as a postdoctoral researcher at the LIBC, during which he was involved in setting up the research-dedicated LIBC scanner facility, and provided training and scientific support for the institute's researchers. As of April 2013, Ilya works as a postdoctoral researcher at the department of Psychiatry and Psychotherapy of the *Charité University Hospital* (Berlin, Germany). In the research group of prof. dr. Henrik Walter, he continues to study the effects of stress on emotion regulation and the brain.

Chapter 9

LIST OF PUBLICATIONS

IN PRESS

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