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

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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

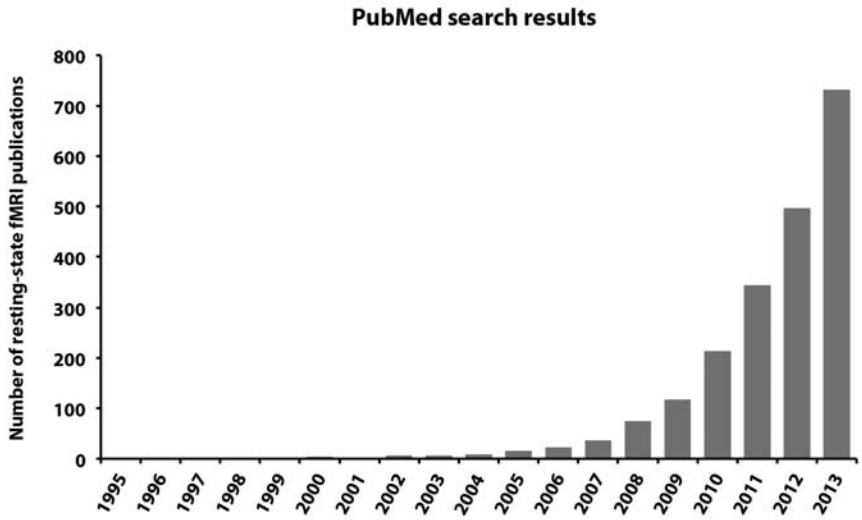

Figure 1.1 Exponential growth of the number of studies published on resting-state functional connectivity or activity since 1995, as identified with the following PubMed search query: *(resting [TIAB] OR resting state [TIAB] OR steady state [TIAB]) AND (functional connectivity [TIAB] OR (BOLD [TIAB]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