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Resilience, posttraumatic stress and recovery:

Insights from brain network dynamics

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ABSTRACT

Resilience, posttraumatic stress and recovery:

Insights from brain network dynamics

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In our lifetimes we will encounter varying degrees of stress and traumatic experiences, and how we respond to them will strongly affect our well-being and mental health. While there is considerable literature on the neural correlates of stress-related mental disorders, there are only very few models including a perspective on the neural factors that render us vulnerable to stress and how therapeutic interventions can modulate brain activity to help us recover. This is in part due to the scarcity of longitudinal studies necessary to investigate both the trajectories of maladaptive changes that make us vulnerable as well as the positive changes that allow us to rebalance our brain back to healthy functioning. Moreover, only very few human neuroimaging studies have been able to incorporate a perspective on the temporal dimension of brain activation patterns. This is specially important given the highly dynamic nature of the stress response, and the current perspectives that propose fast shifts in large-scale brain networks as key in the production of an adaptive response to stress.

In this thesis, we started by investigating brain function in relation to stress and stress vulnerability using a standard method of static functional connectivity on a longitudinal dataset of healthy soldiers exposed to stress (Chapter 2). This allowed us to define a starting point comparable to most of the available fMRI studies on stress and revealed a broad network of differences between stress conditions. We then used a recently developed method (i.e., Leading Eigenvector Dynamic Analysis) to investigate the stability and temporal dominance of discrete and recurrent functional connectivity patterns (or states) showing that increased temporal stability of a frontoparietal functional connectivity state was associated with stress vulnerability (Chapter 3). Exploration of transitions between the detected functional connectivity states showed that the increase in temporal stability was furthermore accompanied by decreased transitions from the frontoparietal to the default mode state in vulnerable participants (Chapter 4). We then applied a second state-of-the-art method (i.e., Hidden Markov Models) to detect recurrent activation patterns in a dataset of participants with PTSD before and after cognitive therapy. We showed that PTSD is associated with a decrease in the temporal dominance of activation patterns related to the default mode network. Moreover, cognitive therapy was related to normalization of these default mode activation patterns back to a level similar to that of healthy participants (Chapter 5). In the final chapter, we integrate our findings into a brain dynamic perspective of stress vulnerability, PTSD and recovery.

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Chapter 1

INTRODUCTION

OVERALL AIM OF THIS THESIS

Stress, the main focus of this thesis, is something that all of us will experience in our lifetimes in varying degrees of severity – from the stresses of everyday life to major events such as a exposure to violence or the death of a loved one. Despite its potentially harmful nature, not all stress is detrimental for organisms and in fact stress has been shaped by evolution to increase the chances of survival of our species. However, its harmful potential and the way we respond to it when it appears extreme or is maintained over prolonged periods of time, rendering us vulnerable to the development of mental disorders, is the interest driving this thesis. In brief, my goal has been to study the brain's response to stress, incorporating a perspective on both resilience and vulnerability, and culminating with a study on the brain under conditions of post-traumatic stress disorder and its recovery through cognitive therapy. To do so, I have used state-of-the-art techniques developed at Oxford University allowing me to look at fast changes in brain activity, including the time component of time-varying network analysis in the study of the stress in humans, both during health and in disease.

RESEARCH MOTIVATION

Impact of stress on mental health

But first of all, why is it important to understand our responses to stress? Over the past half century, a large amount of scientific efforts and resources have been put into trying to gain a better understanding of the various conditions resulting to some extent from the experience of stress and trauma. Obvious examples of these disorders are acute stress disorder and posttraumatic stress disorder (PTSD), both characterised among other symptoms by the presence of flashbacks, nightmares, and low mood, and triggered by the experience of a traumatic event. Recent findings of a cohort study of UK personnel involved in the Iraq and Afghanistan conflicts reported a prevalence of

PTSD as high as 17.1% among ex-serving military personnel deployed in a combat role (Stevelink et al., 2018). However, these are not the only mental health disorders influenced by the experience of stress. The development of an episode of major depressive disorder (MDD) often follows a particularly stressful situation or period of life (Bandoli et al., 2017; Mall et al., 2018). Another condition that often follows traumatic experiences is psychosis (Freeman and Fowler, 2009; Neria et al., 2002). In all these cases, understanding how stress impacts the brain as well as how more resilient people confront it is essential for understanding the disorders, ways to prevent them, and strategies to facilitate recovery.

Impact of stress on other health conditions

We could even go one step further. In the last few decades, an ever growing amount of research shows that the effect of stress on our health might be much more pervasive than we used to think, suggesting that it might increase the risk for cognitive impairment in old age, Alzheimer's disease, Parkinson's disease, and even heart disease (Dimsdale, 2008; Hemmerle et al., 2014; Kikuchi et al., 2002; Nunomura et al., 2006; Praticò et al., 2002; Schiavone et al., 2013; Shan and Lin, 2006; Vitlic et al., 2014). In these cases, a better understanding of the individual neurobiological vulnerabilities to stress and how to attenuate them may also help prevention and, perhaps even, hold some clues about these disorders whose origins have traditionally been associated with other factors.

MDD and PTSD as a result of stress and trauma

The focus of this thesis, nevertheless, is on stress related psychopathology, in particular early manifestations of depression and posttraumatic stress. Even though according to the DSM-5 PTSD and MDD aggregate into different families, both disorders have environmental stressors and stress responses at the core of their pathogenesis. In fact, when looking at posttraumatic mental health disorders of combat origin, PTSD and MDD are found to be the most prevalent disorders (Bleich et al., 1997) and approximately 40-50% of people that suffer from PTSD also suffer from MDD (Breslau et al., 1997; Rytwinski et al., 2013).

Shared vulnerabilities for MDD and PTSD

Although the exact reason for this high comorbidity between the two disorders is currently under debate, a large body of empirical evidence supports common risk factors (i.e., stemming from a shared risk or vulnerability). On the one hand, epidemiological studies have shown that while trauma victims with PTSD have an increased risk for MDD this is not the case for trauma victims who have not developed PTSD (Breslau, 2002; Breslau et al., 2000). This relationship between PTSD and depression appears to be bidirectional, since recent reports provide evidence for MDD increasing the risk for developing PTSD by 603%, although in this sample the incidence of PTSD was remarkably low (0.08%) (Chang et al., 2017). Furthermore, many studies have shown the existence of specific shared vulnerabilities between the two disorders, both at the psychological and biological level. For example, a recent study showed that the personality trait neuroticism, having felt unable to escape from the threatening situation, and having been trapped for a longer time period, all increased the risk for both PTSD and depression (Chen et al., 2017). This work also highlighted disorder-specific vulnerabilities, such as having felt extreme panic or fear for PTSD, and introversion and psychoticism for depression. Furthermore, while socialization was a protective factor for both of them, extroversion was a protective factor only for depression (Chen et al., 2017). In terms of biological vulnerabilities, many studies have highlighted the influence of several neurochemical agents such as brain-derived neurotrophic factor and neuropeptide Y on the risk for *both* MDD and PTSD (Andero and Ressler, 2012; Autry and Monteggia, 2012; Husum et al., 2001; Morales-Medina et al., 2010; Schmeltzer et al., 2016; L. Zhang et al., 2016). Based on this evidence, it is reasonable to consider both MDD and PTSD as stress-related disorders with some shared vulnerabilities.

WHAT IS STRESS?

As the main focus of this thesis, I next define stress both from a psychological and a physiological perspective.

Psychological definition

In psychology, stress is defined as the emotional and physiological reactions experienced during a situation in which the demands upon the individual surpass the

individuals coping resources. According to the American Psychological Association, there are 3 different types of stress: acute stress (i.e., brief, everyday life), episodic acute stress (i.e., frequent acute stress), and chronic stress (e.g., living in a conflict or war zone). We typically also distinguish between positive (brief and mild), tolerable (serious but temporary and buffered by supportive relationships) and toxic stress (prolonged in the absence of supportive relationships).

Physiological definition

In a physiological context, the term stress was first used by the endocrinologist Hans Selye in 1936 to describe the *inadequate* physiological response of an organism, human or other animal, to any mental, emotional, or physical demands, whether actual or imagined (Selye, 1936). According to his views, stress could be described as an actual or anticipated disruption of homeostasis, term that refers to a dynamic and harmonious equilibrium. The process of achieving homeostasis or equilibrium through physiological or behavioural change is referred to as allostasis and this is a process that takes place in our everyday life in response to stressors or lifestyle factors such as diet, sleep-wake factors, and exercise. This process is mediated by hormones, such as cortisol and adrenaline/epinephrine, as well as other neurotransmitters which usually have an adaptive effect on the brain. However, when they are released in excess or for excessive periods of time (or when they are not released when required), their effect accumulates leading to wear and tear, which is often referred to as "allostatic load". Maintaining homeostasis is the responsibility of the autonomic nervous system (ANS) together with the endocrine system. The ANS is divided into the parasympathetic system, responsible for 'rest and digest' responses, and the sympathetic system, in charge of the fight or flight response.

The autonomic nervous system and the hypothalamus-pituitary-adrenal axis

The two systems have different response dynamics. The autonomic nervous system is responsible for providing a rapid response to stress through activation of its sympathetic and (inhibition of) its parasympathetic branches. The sympathetic activation is what is often referred to as the 'fight-or-flight' response: within seconds, noradrenaline is released from widely distributed synapses, amongst those the locus coeruleus, the brain's main source of noradrenaline. This is closely followed by an

increase in the circulating levels of adrenaline by the adrenal medulla. The release of catecholamines (i.e., adrenaline and noradrenaline) results in increased heart rate, peripheral vasoconstriction, and energy mobilization. Regarding the central nervous system, noradrenergic projections include the whole cerebral cortex, hypothalamus, thalamus and amygdala. These changes start almost immediately after the stressor is perceived and return to baseline within 30 to 60 minutes.

As the initial surge of adrenaline attenuates, the hypothalamus activates the long-term (hormonal) component of the stress response system, the hypothalamic-pituitary-adrenal (HPA) axis, in order to maintain the stress "gas pedal" pressed down. This carries the release of corticosteroids, which can be divided in two groups: glucocorticoids and mineralcorticoids. In brief, when the hypothalamus is activated by stress, it releases corticotropin releasing factor (CRF), which binds to its receptors in the pituitary gland. As a result, the adrenocorticotropic hormone (ACTH) is released and it binds to receptors on the adrenal cortex, in turn stimulating adrenal release of cortisol, the main human glucocorticoid. Cortisol works as a regulator of the amygdala, hippocampus and prefrontal cortex activity. Importantly, most body cells have glucocortisol receptors, and it therefore affects a very wide range of bodily functions, including immune response suppression, potentiation of sympathetic-nervous-system-mediated vasoconstriction, and suppression of reproductive function.

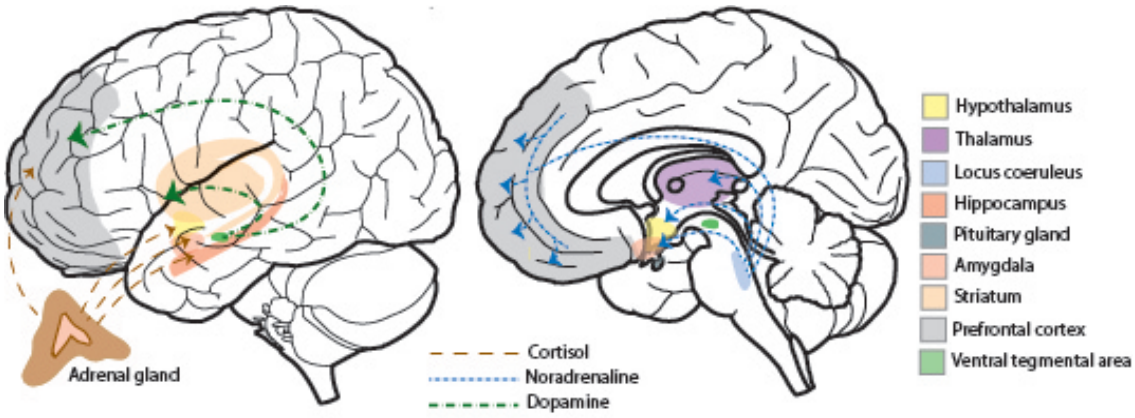


Figure 1.1 Acute stress: simplified representation of key structures, neurotransmitters, and hormones involved in the neurochemical response. *Three different systems are illustrated: the neuroendocrine system (brown), the autonomic nervous system (blue), and the dopaminergic system (green). Each of these systems is*

responsible for the release of a wave of neurochemicals following a specific temporal pattern, resulting in a time-dependent modulation of distinct neural circuits.

Cortisol peaks after 20 minutes and is usually released for several hours after encountering the stressor. Once a certain blood concentration of cortisol is reached, a negative feedback response signals to the brain to inhibit the release of CRF and ACTH, allowing the organisms to return to homeostasis. However, the effects of corticosteroids seem to develop both at short and long timescales, depending on the specific brain regions involved. For example, these effects are quickly reversed in the hippocampus but they take longer to normalise in the basolateral amygdala (Karst et al., 2010). This highlights the importance of spatiotemporal fluctuations and their study in the context of stress and trauma.

The dopaminergic system

A less well-known rapid effect of acute stress concerns the dopaminergic system, traditionally related to the reward system. However, it has been known for decades that stress increases extracellular levels of dopamine in the prefrontal cortex, nucleus accumbens and dorsal striatal regions (Abercrombie et al., 1989). More recent studies have shown that specific subpopulations of dopaminergic neurons in the dorsolateral parts of the midbrain in monkeys (Matsumoto and Hikosaka, 2009) and the nucleus accumbens and dorsal striatum in rats (Budygin et al., 2012) respond not only to reward but also to aversive events. Furthermore, the dopaminergic system interacts with the catecholaminergic system in stressful contexts. A positron emission tomography (PET) study using [^{11}c]raclopride, a dopamine D2 receptor radiotracer that decreases proportionally to increases in synaptic dopamine in the striatum (Endres et al., 1997), showed that its reduction after stress was significantly correlated with the magnitude of salivary cortisol, interpreting this association as a facilitating effect of cortisol on dopamine neuron firing (Pruessner, 2004). Furthermore, as mentioned above, cortisol has an important regulatory effect on the amygdala, site in which the dopaminergic innervation is especially responsive to stress (Inglis and Moghaddam, 1999).

TRANSITION FROM ACUTE TO CHRONIC STRESS

The effect of an accumulation of acute stress effects on the brain is a key phenomenon in the transition to chronic stress and stress-related psychopathology. A new model (Sousa, 2016), inspired by research in the field of pain and chronic pain, proposes that the stress-related progress to chronicity corresponds with a shift in the set of brain regions and patterns that are reliably activated by stress, referred to as a shift from a stress 'neurosensorial' to a 'chronic maladaptive' matrix. This theory posits that, each time a stressful stimulus is presented, the stress neurosensorial matrix is activated, with the nature of the stressor determining the specific sensorial pathway recruited (e.g., physical nature: brain stem nuclei, psychosocial nature: amygdala, hippocampus and/or frontal cortex). With time, the way the brain deals with stress, in terms of regions and patterns of activation, suffers a marked shift. The new chronic neuromatrix is then characterised by involvement of additional neurons with distinct projections, resulting in specific modifications of nodes and networks involved in stress control (Sousa, 2016).

A series of events (e.g., release of different hormones, synaptic remodelling of neurons) play a key role in the transition from acute to chronic stress, potentiating one neuronal pathway at the expense of another. In the model by Sousa (2016), these events are independent from each other but interact to some extent, since they are modulated by 4 main factors: 1) susceptibility 2) response and initial injury; 3) transition to chronicity and 4) maintenance of a 'stressed-brain'.

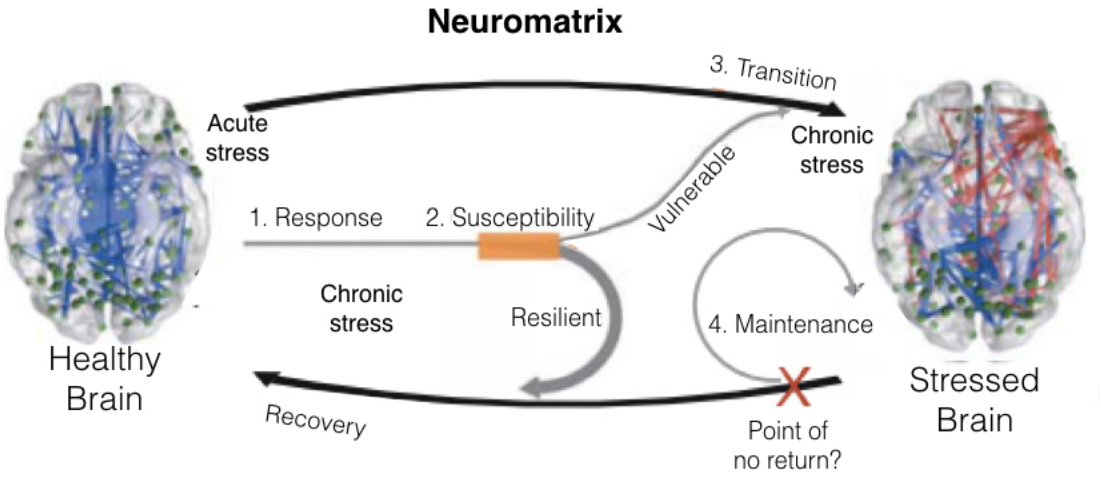


Figure 1.2. The stress neuromatrix model. *This model suggests a dynamic and more comprehensive approach to the functional neuroanatomy of stress. The model proposes four independent but interacting steps: 1) stress response 2) susceptibility 3) transition to chronicity and 4) maintenance of a 'stressed-brain'. Adapted from Sousa (2016).*

The starting point of this stress journey is proposed to be the hippocampus, which due to its richness in corticosteroid receptors is a target for stress and can therefore suffer changes at the dendritic and synaptic level as a result of repeated acute stress (fastest timescale, potentiation or inhibition depends on the specific subregion within the hippocampus) (Sousa, 2016). On a slower timescale, the changes discussed lead to loss of corticosteroid receptor-bearing hippocampal neurons, resulting from or in apoptosis, increased vulnerability to excitotoxicity, and oxidative stress after prolonged exposure to high levels of glucocorticoids (Sapolsky, 1985). This results in loss of feedback inhibition from the hippocampus over the HPA axis. Furthermore, much more common and rapid changes observed at the synaptic and dendritic level (Magarinos et al., 1997; Sousa et al., 2000) could be leading to more definite changes in neuronal structure and function when stress is sustained. In particular, it is suggested that, while the direct effects mediated by receptors are of relevance for the shift in network structure and function, the indirect effects resulting from glutamate release and the accompanying changes in the pattern of activity of the acutely activated nodes are the ones ultimately leading to cascading changes in the chronically affected nodes (Sousa, 2016). These effects on neuronal networks are proposed to justify the expansion of the disruptive effects to other locations and functions. An example given

by Sousa (2016) refers to the dysregulation of the medial prefrontal cortex which then progresses to the dorsomedial striatum impairing decision making (Dias-ferreira et al., 2009).

Importantly, if the context that triggered the stress response is modified or discontinued, any changes that have taken place might be largely reversible (Sousa, 2016). However, they also propose that in scenarios in which the stress response remains present for extended periods of time, a point of no return might be reached – whether this is the case, and if so, when does this happen is an open question whose exploration could bring us closer to a better understanding of disorders such as PTSD and depression. In the following sections some of the concepts related to Sousa's four steps and how these relate to this work will be discussed more in detail: in particular, concepts of resilience and vulnerability, models of maintenance of the stressed brain in the form of PTSD, and potential mechanisms for recovery through cognitive therapy for PTSD.

Stress Vulnerability and resilience

The field of human resilience is still very much in its infancy. Its science started with the observation that most people are able to maintain their mental health despite exposure to severe and sometimes prolonged psychological and/or physical adversity. The first studies in the field were conducted in the 1970's and ever since, many more studies have shown evidence supporting this idea of 'immunity' or resilience towards stress (Cicchetti and Rogosch, 1997; Masten et al., 1990). However, the exact mechanisms underlying this ability to protect oneself are not clear. To date, most of the insights on the neurobiology of resilience come from animal studies, with some of the findings having been replicated in human studies. In the following, we provide an overview of the role that the neuroendocrine, noradrenergic and dopaminergic systems, reviewed in regard to the stress response in previous sections, play in resilience and vulnerability. When possible, we discuss findings from human studies relating those systems to resilience and vulnerability.

Before digging into the literature of resilience it is important to clarify that there are two main types of resilience mechanisms: active and passive. Active resilience is understood as an adaptive coping mechanism that aids maintenance or quick reinstatement of normal behaviour after stress while passive resilience refers to a form

of resistance in which the effects of stress that would be observable in susceptible organisms do not manifest themselves at any point in time. In other words, active resilience involves a dynamic process of successful adaptation to adversity while passive resilience can be understood as the flip-side of vulnerability.

The neuroendocrine system

As mentioned, the HPA axis plays a key role in the stress response. However, its role in resilience and vulnerability, or even in fully developed psychiatric disorders, is not clear. Most studies have found elevated blood glucocorticoids in individuals with MDD, although there is a smaller subset with typically less severe symptoms that show reduced glucocorticoid levels (Stetler and Miller, 2011). In PTSD, however, hypocortisolemia seems to be the most consistent finding, although here, too, findings have been mixed (Meewisse et al., 2007). The opposed findings in two different disorders (i.e., MDD and PTSD) in which stress and adverse life events exposure are important risk factors, pose clear challenges to the understanding of the HPA axis in vulnerability or resilience to the development of stress-related disorders. A possible explanation for these inconsistencies might lie in the triggering process for glucorticoid release – it has been shown that CRF is often increased in adults who have reported a history of childhood abuse (Heim et al., 2008), which is one of the main risk factors for MDD and PTSD but does not always trigger the development of these disorders (Yehuda et al., 2005), and therefore some people with MDD and PTSD might show a different pattern of CRF.

Other hormones typically associated with resilience are testosterone and neuropeptide Y. Testosterone is strongly linked to risk-taking and competitive behaviours, and has been shown to increase after successful athletic competition (Oliveira et al., 2009) and decrease in highly stressful military survival training (Morgan et al., 2000a). A recent cross-sectional study also showed an association between being self-employed and abundant testosterone levels, suggesting that higher testosterone levels may lower reactivity to stress (Greene et al., 2014). However, this is a very new area of research in which very few longitudinal studies have been done, and therefore more research is needed before we can establish a causal link between resilience and testosterone. Furthermore, studies investigating the role of testosterone in PTSD have failed to show a clear relationship between the disorder and testosterone

levels (Karlović et al., 2012; Spivak et al., 2003). Neuropeptide Y is a peptide neurotransmitter that modulates stress response in animals and has also been shown to be associated with better performance under stress in a sample of Special Forces soldiers (Morgan et al., 2000b). A follow-up study showed that higher levels of neuropeptide Y predicted less psychological distress and dissociation following acute stress (Morgan et al., 2002), suggesting a protective role for neuropeptide Y in stressful situations.

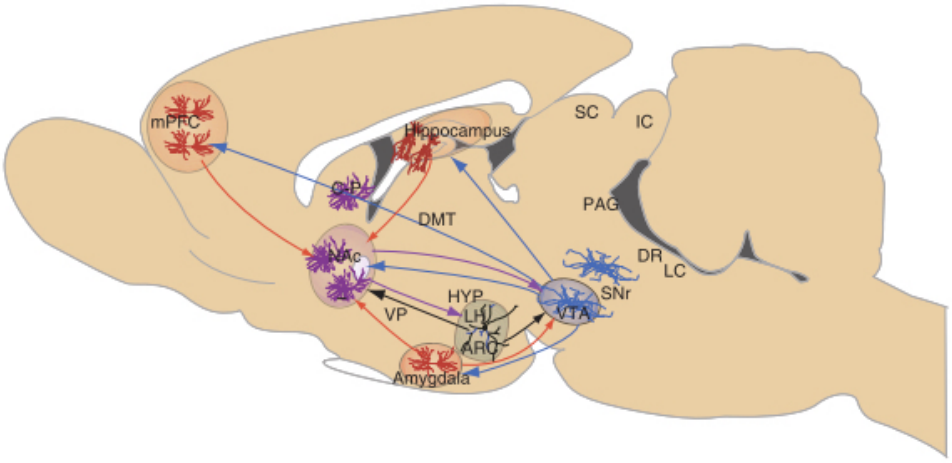


Figure 1.3. Brain circuitry implicated in resilience to depression and anxiety disorders in mice. *Main brain structures altered by stress in animal models of depression or implicated in human depression are outlined in different colours depending on the transmitter signal that they convey (red - glutamatergic neurons; purple - medium spiny neurons; black - hypothalamic peptidergic neurons; blue - ventral tegmental area dopaminergic neurons). Similarly, the colours of the arrows indicate afferent type (red - excitatory glutamatergic; purple - inhibitory; blue - dopaminergic; black - peptidergic). CP, caudate-putamen; DMT, dorsomedial thalamus; SC, superior colliculus; IC, inferior colliculus; VP, ventral pallidum; SNr, substantia nigra; PAG, periaqueductal gray; DR, dorsal raphe; LC, locus coeruleus. Reproduced from Russo et al., 2012.*

Noradrenergic system

As detailed in the previous section, stress also leads to release of noradrenaline from brainstem nuclei, mainly the locus coeruleus, stimulating brain regions involved in

emotional behaviours, such as the amygdala, the nucleus accumbens, the prefrontal cortex and the hippocampus. Excessive release of noradrenaline through the locus coeruleus has been associated with anxiety disorders (McCall et al., 2017; Tanaka et al., 2000) and PTSD (Geracioti et al., 2001; Southwick et al., 1997). Furthermore, pharmacotherapies that dampen noradrenaline transmission, such as the non-selective β-antagonist propranolol, have shown potential for enhancing extinction learning (Kindt et al., 2009; Kroes et al., 2016). Hence, while activation of the locus coeruleus and release of norepinephrine serves as an alarm system that enables survival in life-threatening situation, hyperresponsiveness of the locus coeruleus might be rendering individuals particularly vulnerable to stress.

The dopaminergic system

As mentioned in previous sections, relatively recent studies have also provided scientific support for the involvement of the dopaminergic system in processing of stress and aversive stimuli (Budygin et al., 2012; Matsumoto and Hikosaka, 2009; Pruessner, 2004). This increase in dopamine as a result of acute stress could hold one of the keys to vulnerability. Several studies have explored this option using a social defeat paradigm in mice, where the mouse is forced to intrude into the space territorialised by a larger mouse of a more aggressive strain, leading to an agonistic encounter that ultimately results in intruder subordination (Krishnan et al., 2007). After a period of social defeat encounters, approximately 40%-50% of mice can be labelled as susceptible based on their reduction on social interactions (avoidance behaviour). Those studies showed an increase in the firing rate of ventral tegmental area (VTA) dopamine neurons in susceptible but not resistant mice (Cao et al., 2010; Krishnan et al., 2007). A further study used optogenetics to provide direct (i.e., real time) evidence showing that induction of phasic firing of VTA dopamine neurons lead to avoidance behaviour in a social defeat paradigm. They further showed that it was the phasic activation of the VTA neurons projecting to the nucleus accumbens, but not to the medial prefrontal cortex, which were responsible for the induced susceptibility to social defeat (Chaudhury et al., 2013). Results were confirmed through optogenetic inhibition, when inhibition of the VTA-nucleus accumbens projections induced resilience, whereas inhibition of the VTA-medial prefrontal cortex projection promoted susceptibility (Chaudhury et al., 2013). In humans, a PET study found that

dopamine release in the ventral striatum in response to acute stress was present only in subjects reporting low parental care (Pruessner, 2004).

It is surprising that an increased firing rate of VTA dopamine neurons is associated with vulnerability, given that excitability of dopamine neurons is commonly seen as promoting reward and motivation, two processes involved in resilience (Feder et al., 2010). In particular, activation of VTA dopamine neurons is often considered an indicator of reward, as they the fire in response to food, sex or social interaction, and have also been shown to be inhibited by aversive stimuli or the absence of an expected reward (Hyman et al., 2006). In order to explain this paradox, it has been suggested that different subsets of VTA dopamine neurons may be activated by aversive and rewarding stimuli (Lammel et al., 2011).

The reward circuit

From a broader point of view, the nucleus accumbens and the ventral tegmental area's dopamine neurons are regarded as the core of the reward circuit, which extends further encompassing the entire ventral striatum, prefrontal and orbitofrontal cortices, amygdala, brainstem nuclei (i.e., pedunculopontine nucleus and the raphe nuclei), and lateral habenular nucleus (Berridge and Kringelbach, 2015; Haber and Knutson, 2010; Hikosaka et al., 2008; Schultz, 2000; Schultz et al., 2000). In short, the ventral striatum receives its main cortical input from the orbital cortex and the anterior cingulate cortex, together with a huge dopaminergic input from the midbrain. Then the ventral striatum projects to the central pallidum, the VTA and the substantia nigra, which then project back to the prefrontal cortex after passing through the medial dorsal nucleus of the thalamus.

Within this broader reward system, recent studies in animal affective neuroscience have identified a network responsible for generating hedonic enhancement of liking (i.e., core reactions to hedonic impact). This network, referred to as the 'hedonic hotspot' network, encompasses regions of the nucleus accumbens, connected ventral pallidum the brainstem parabranchial nucleus of the pons, prefrontal areas including orbitofrontal cortex, and insula (Castro and Berridge, 2014; Ho and Berridge, 2013; Pecina and Berridge, 2005; Smith and Berridge, 2005; Söderpalm and Berridge, 2000). The size of the hedonic hot spots identified so far is each about 1 cubic milimeter in

volume in rats, which could be extrapolated to a cubic centimetre in humans, if these were proportional to brain size (Berridge and Kringelbach, 2015). Thus, each structure containing an hedonic hot spot is, by comparison, much larger (e.g., nucleus accumbens is approximately 10 cubic milimeters in size), and so the majority of its neurons may lack the capacity to enhance 'liking' reactions or even be involved in the suppression of these reactions (i.e., cold spot).

Interestingly, this fits within an anatomical pattern of front-to-back valence organization in the shell of the nucleus accumbens that is thought to underlie the generation of additional emotions beyond 'liking', such as negative fearful behaviour (Faure et al., 2010; Ho and Berridge, 2014; Reynolds and Berridge, 2002; Richard et al., 2013). It has been proposed that, similarly to a musical keyboard that generates distinct notes, the nucleus accumbens shell contains a 'rostrocaudal affective keyboard' that generates multiple distinct qualities of appetitive versus fearful behaviours (Berridge and Kringelbach, 2015). Furthermore, the anatomical location of these keys is not fixed but rather amenable to emotional factors such as the valenced ambience of an environment (Reynolds and Berridge, 2008; Richard and Berridge, 2011). It has even been speculated that, some psychiatric disorders such as posttraumatic stress disorder, characterised by exaggerated fear reactions and strong anhedonia, might persistently retune nucleus accumbens generation in a fearful direction (Berridge and Kringelbach, 2015).

In any case, and given persistence of hedonic reactions throughout evolution, their underlying circuitry is likely to have been subjected to the same pressures that shaped evolution of other adaptive functions (i.e., threat processing and stress) (Anderson and Adolphs, 2014; LeDoux, 2012; Panksepp, 2011). Thus, they are likely to fulfil an adaptive role in threatening situations (Berridge and Kringelbach, 2015).

Cortico-basal ganglia loops

Moving further up at the systems level, the rewards circuit is one of the four cortico-basal ganglia loops. The striatum, together with the pallidum, substantia nigra, and subthalamic nucleus, form the basal ganglia, a set of structures strongly connected to the rest of the brain. Their widespread connectivity is collected in several loops or circuits (i.e., motor, oculomotor, reward and associative loops, *Figure 1.4*) (Alexander,

1986). Initially it was suggested that these circuits were organised in segregated parallel loops, but current views conceptualise the cortico-basal ganglia loops rather as a continuum in which cortical projections traditionally ascribed to different loops overlap in the striatum (Draganski et al., 2008; Haber and Knutson, 2010). Hence, the cortico-basal ganglia loop associated with reward is unlikely to work in isolation when promoting resilience. In fact, resilience relies in the development of adaptive behaviours, which require a combination of reward evaluation, associative learning and the ability to develop appropriate action plans over inappropriate behaviours, potentially implicating all four cortico-basal ganglia circuits (*Figure 1.4*) to different degrees (Haber and Knutson, 2010).

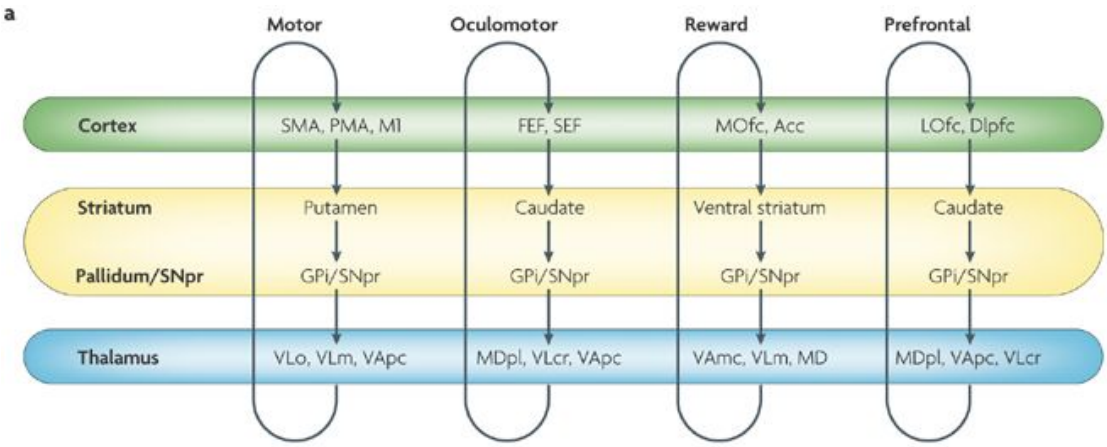


Figure 1.4. Stress dependence on balanced cortico-basal ganglia loops. *The cortico-basal ganglia loops, essential for adaptive behaviour, work as an integrative system, interacting through areas of projection overlap from the different circuits in the striatum. Dysregulation in one of the circuits is therefore likely to affect one or several of the other systems. Acc, anterior cingulate cortex; Dlpfc, dorsolateral prefrontal cortex; FEF, frontal eye fields; LOfc, lateral orbitofrontal cortex; M1, primary motor cortex; MDpl, mediodorsal nucleus of the thalamus, pars lateralis; MOfc, medial orbitofrontal cortex; PMA, premotor area; SEF, supplementary eye field; SMA, supplementary motor area; VAmc, ventral anterior nucleus of the thalamus, pars magnocellularis; VApc, ventral anterior nucleus of the thalamus, pars parvocellularis; VLcr, ventrolateral nucleus of the thalamus, pars caudalis, rostral division; VLm, ventrolateral nucleus of the thalamus, pars medialis; Reproduced from Kringelbach et al., 2007, adapted from Alexander et al., 1986.*

On the other side of the coin, a previous study by Dias-Ferreira and colleagues (2009) provides an illustrative example of how chronic stress in rats can lead to structural reorganization of corticostriatal circuits. Here, the authors conducted a series of experiments showing that chronic stress is associated with a shift towards habitual strategies, decreasing the ability of stressed animals to adapt their behaviour based on its consequences. This was accompanied by atrophy of the associative (prefrontal) cortico-striatal circuit and hypertrophy of the sensorimotor circuit. In other words, after chronic stress the sensorimotor cortico-striatal circuit takes advantage over the associative cortico-straital circuit leading to dysfunctions in decision-making (Dias-ferreira et al., 2009).

STUDYING STRESS VULNERABILITY AND RESILIENCE IN HUMANS

Although studies in animals have made considerable progress in advancing our understanding of the neurobiology of resilience and vulnerability, which can help the interpretation of human studies, translation from these results into applicable strategies for fostering human resilience remains scarce. In the meantime, the field of human resilience, which could inform strategies to address prevention of mental health disorders, remains limited due to several factors. In the following we provide an overview of the challenges that affect the study of resilience and vulnerability in humans.

Resilience as a dynamic process of adaptation

As mentioned in the previous section, we typically distinguish between passive and active resilience mechanisms, where active mechanisms refer to adaptive coping and passive mechanisms refer to the complete absence of negative consequences observable in vulnerable individuals after stress. While this distinction is very clear in animal studies, where stress induction and repeated or extended measurements with invasive techniques allow for the assessment of stress responses in detail, the differentiation between the two types of resilience in the stress response in human studies is less explicit. In order to facilitate progress coming from studies of human resilience, recent emphasis is being placed on the importance of closing the gap between resilience theory and empirical research (Kalisch et al., 2017). In this regard, most theorists agree that the avoidance of negative social, psychological and biological

consequences of extreme stress is achieved mainly through a dynamic process of adaptation (i.e., active resilience). According to this view, in order to understand (active) resilience in humans we need more longitudinal designs in which a stressor is present and data is acquired at different points in time, defining resilient individuals a posteriori. This implies that studies measuring resilience at a single point in time and therefore assuming that it is trait or stable personality profile, were actually looking into predispositions. These may contribute to positive adaptation in exactly the same way as some vulnerability factors promote mental health disorders. Under this umbrella, the effect of resilience factors on the stress response can be studied in cross-sectional studies, but a definition of resilient participants requires a longitudinal study design.

Approach to resilience in this thesis

All in all, the framework adopted in this thesis is grounded in the need to acknowledge the dynamic nature of resilience, not only at the behavioural but also at the neural level. The former can be addressed though the design of longitudinal studies acquiring repeated measures of mental health and global functioning, whereas the later can be addressed through the acquisition of repeated measures of brain function. In our study design, brain function has been measured using functional magnetic resonance imaging (fMRI) scans, which together with newly developed methods for the study of time-varying networks, allows us to investigate resilience and vulnerability mechanism at two different levels: 1) a slow changes happening at a timescale of minutes (fMRI scans) and 2) fast changes happening at a timescale of seconds (time-varying functional connectivity); see *LEiDA & HMMs overview*.

Previous fMRI studies on stress resilience

Previous studies have used several fMRI scans acquired along a stress induction paradigm to capture the different phases of the stress response and, potentially, the dynamic adaptive changes associated with resilience. Thanks to those studies, we know that those participants experiencing the highest subjective levels of stress show an associated increase in functional connectivity between the amygdala and the hippocampus as well as a decrease in functional connectivity between the amygdala and the ventral prefrontal cortex immediately after stress (Quaedflieg et al., 2015;

Vaisvaser et al., 2013). These studies also showed that the increased connectivity between the amygdala and hippocampus is still present 30 minutes after stress induction (Quaedflieg et al., 2015) and may last up to 2 hours (Vaisvaser et al., 2013). Given that effectively coping with stress requires not only a quick response but also its efficient termination once the threat is no longer present, this pattern of sustained functional connectivity could be related to stress susceptibility. However, very few studies have incorporated the perspective of time-varying functional connectivity able to capture fast changes happening at a timescale of seconds to the study of vulnerability and resilience in humans. By doing so, we can gain a better understanding of the processes that eventually lead to efficient termination of the stress response or, on the contrary, prolong brain activity characteristic of the stress response.

MANIFESTATION OF TRAUMA-RELATED DISORDERS

What is PTSD?

The second part of this thesis is a natural continuation of this research as, inevitably, some people will go on to develop PTSD after exposure to trauma. This disorder can result from the direct exposure or witnessing of a traumatic event such as a severe accident, natural disaster or exposure to violence. According to the (DSM-5, 2003) there are four clusters of symptoms, namely repeated and unwanted re-experiencing of the event, negative cognitions and mood, hyperarousal, and avoidance of stimuli related to the trauma. This widely used manual for the diagnosis of psychiatric disorders also recognizes a dissociative subtype of PTSD, in which the person, in addition to the aforementioned symptoms, experiences high levels of derealisation or depersonalization (i.e., detachment of oneself or the situation) in response to trauma-related stimuli. As discussed in a previous section, PTSD is highly comorbid with MDD, with approximately 40-50% of people that suffer from PTSD also suffering from MDD (Breslau et al., 1997; Rytwinski et al., 2013). Since the first recognition of PTSD by the *DSM-III* (1980) many psychological models have been formulated to explain this complex disorder, ranging from psychodynamic, cognitive and behavioural models all the way to neurocircuitry models of brain dysfunction. In the following, I will provide

a brief overview of these models and their contribution to the field, as well as discuss some of their limitations.

A psychodynamic approach to PTSD

The first models were psychodynamic. According to Horowitz (1983) there is a 'crying out' reaction after the trauma followed by a period of information overload in which the thoughts, memories and images of the trauma cannot be reconciled with current conceptualization of the self and the self in relation to others. This gives rise to the symptoms, which persist until the individual manages to integrate the traumatic material with long-term meaning representations. Although successful in the explanation of many of the symptoms, this theory paid very little attention to the individual's attributions and interpretations of the trauma and their potential effect on the outcome (Dalgleish, 2004). Nevertheless, it offered the first influential cognitive model of reactions to trauma, in spite of its roots in classical psychodynamic psychology.

Behavioural perspective on PTSD

From a behavioural perspective, several researchers have suggested that PTSD could be explained through principles of classical and instrumental conditioning (Becket et al., 1984; Keane et al., 1985; Kilpatrick et al., 1985). According to them, during the trauma, a previously neutral stimulus becomes associated with an unconditioned stimulus that innately evokes discomfort or fear, acquiring aversive properties and eliciting anxiety when this initially neutral stimulus is presented in future situations. This newly conditioned stimulus can be paired with other neutral stimuli, rendering them aversive in what is known as a process of generalization. The second stage of the disorder consists of the development of learned responses, such as avoidance or escape responses, which decrease or terminate the discomfort arising from the presence of the conditioned stimuli but maintain the association. Exposure-based therapies aim to break this association by repeated exposure to the traumatic event, either in the form of narrative details by the client or by direct exposure to trauma-related stimuli (for details see e.g., Foa & Rothbaum (1998)). While conditioning approaches provide a powerful explanation for many features observed in individuals with PTSD, they are

less able to provide an explanation for re-experiencing symptoms, effects on cognition (e.g., attention and declarative memory) and the influence of other emotions such as guilt or shame (Brewin, 2007). These limitations led to new perspectives incorporating the *meaning of the association* within the model.

Cognitive models of PTSD

The recognition of the above mentioned limitation gave way to cognitive theories incorporating the importance of the interpretation of the traumatic event and bringing back a stronger component of self, very present in earlier models but less prominent in the strictly behavioural accounts. Amongst them, the most influential and the one that serves as framework for the final chapter of this thesis is the one proposed by Ehlers & Clark in 2000. According to this model, the processing of a trauma in a way that leads to a sense of serious current threat is the key process responsible for the development of PTSD. This sense of serious, current threat can arise as the consequence of two key processes: 1) idiosyncratic negative appraisals (i.e., interpretations) of the trauma and/or its sequelae 2) The nature of the trauma memory and its link to other autobiographical memories. Once activated, the perception of current threat is accompanied by intrusions, re-experiencing of the trauma, symptoms of arousal and other negative emotional responses. It also motivates a series of behavioural and cognitive responses that are intended to reduce perceived threat and distress (e.g., avoidance of reminders), but also prevent the cognitive change necessary to overcome the disorder.

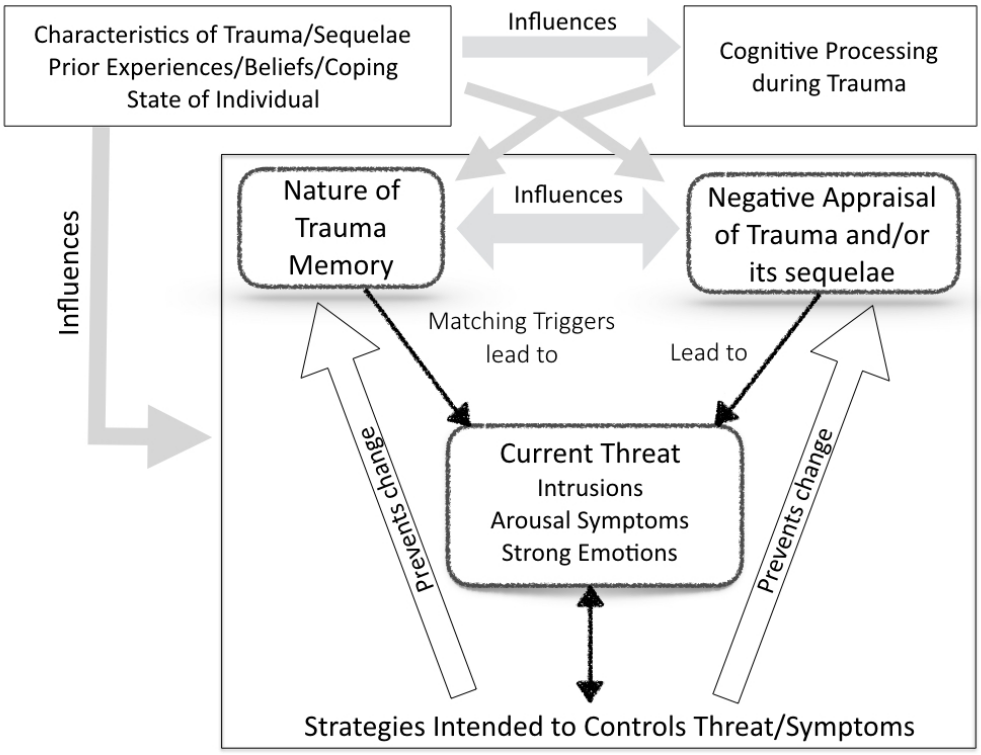


Figure 1.5 Cognitive model of PTSD proposed by Ehlers & Clark. PTSD is suggested to become persistent when individuals process the trauma in a way that leads to a sense of current threat. This results from 1) excessively negative appraisals of the trauma and/its sequelae 2) a disturbance in the autobiographical memory of the trauma characterised by poor elaboration and contextualisation, strong perceptual priming and strong associative memory. These two factors are in turn influenced by characteristics of the trauma and the type of cognitive processing, prior experiences and the state of the individual. Adapted from Ehlers & Clark (2000).

The nature of trauma memories in PTSD, in particular, presents an interesting phenomenon, since PTSD patients have problems in *intentionally* retrieving a complete memory of the traumatic event while reporting a high frequency of *involuntarily* triggered intrusive memories that forces them to re-experience the traumatic event in a very vivid and emotional way. This re-experiencing of the memory consists mainly of sensory impressions, predominantly visual (Ehlers & Steil, 1995; van der Kolk & Fisler, 1995), which are experienced as if they were happening 'right now' and come accompanied by the 'original' emotions of the traumatic event. Importantly, these intrusive memories are cue-driven due to their associative nature (Ehlers & Clark, 2000), meaning that they can be induced through the presentation of trauma-related stimuli in experimental paradigms (see Chapter 5).

Ehlers & Clark propose that the very specific way in which the trauma memory is encoded explains the apparent paradox that entails the coexistence of frequent but involuntary memories with difficulties in voluntary recall of the traumatic event. There are two routes for retrieval of autobiographical memories (see also Brewin et al., 1996). In particular, Ehlers & Clark propose a first route through higher-order meaning-based retrieval strategies, to which we recur intentionally, and a second route through data-driven (stimulus-triggered) recall, that occurs when stimuli associated with the event appear in the environment (e.g. particular smells or sounds). Generally, normal processing of autobiographical memories is responsible for reducing the ease with which memories of past experiences are unintentionally retrieved. This is facilitated by incorporation of autobiographical events in an autobiographical memory knowledge base organised by themes and personal time periods (Conway and Pleydell-Pearce, 2000), which enhances meaning-based retrieval while inhibiting data-driven retrieval (Conway, 1997a). This results in autobiographical recall of events with both specific information about the event and general information about the lifetime period in which it took place, as well as abstracted information about the general type of event (e.g. school days). Ehlers and Clark (2000) suggested that individuals with PTSD exhibit unorganised memories, at least in part, due to an inability to establish a self-referential perspective while experiencing the trauma, hindering its integration into the chronologic continuum of other autobiographical memories (see also Wheeler et al., 1997).

Ehlers & Clark further propose that the difficulties inhibiting data-driven recall are further exacerbated by strong 'stimulus-response' and 'stimulus-stimulus' associations, making triggering of memories of the event and the associated emotional responses even more likely (see also Conway, 1997b; Foa et al., 1989; Keane et al., 1985). Furthermore, perceptual priming for stimuli temporally associated with the traumatic event is suggested to be particularly strong due to reduced perceptual threshold for these stimuli.

In summary, the theory by Ehlers and Clark (2000) suggests that an appropriately contextualised autobiographical memory, sufficiently integrated in time and place, promotes meaning-based retrieval strategies while inhibiting sensory retrieval. Failure to do so results in unintentional recall due to a weak semantic route to retrieval, the here and now quality (i.e., no context in time) that leads to the perception of current

threat, the absence of links to subsequent information (e.g., "I did not die"), and the easy triggering by physically similar cues.

Neurophysiological models of PTSD

Early neurophysiological models of PTSD focused mainly on the fear conditioning and memory circuitries, probably because at the time, knowledge about the neural correlates of emotional numbing, avoidance behaviours or self-referential processes, also key in PTSD, was very limited. Furthermore, the majority of the neural mechanisms recruited during fear learning and expression are shared across species, facilitating translation from findings using animal models of PTSD. A brief overview of neuroscientific theories of PTSD, as well as the main principles driving them, will be provided in the next paragraphs, although an in-depth review of the matter is beyond the scope of this thesis' introduction.

Most of the studies in this field used classical (or Pavlovian) fear conditioning, in which an emotionally neutral stimulus (e.g., tone, conditioned stimulus) is presented before a naturally aversive or threatening one (e.g., electric shock, unconditioned stimulus), working as a predictor. Animals will then begin to associate the presentation of the previously neutral stimulus with the presentation of the threatening one, expressing as a result a number of the emotional responses (e.g., increased heart rate, perspiration) characteristic of the flight-or-fight response. Neurophysiological studies have shown that sensory information concerning both the initially neutral and the aversive stimuli converge in the amygdala, in particular the basolateral complex (Pape and Pare, 2010). Afterwards, the information is modulated thanks to the efferent projections from another amygdala nucleus, the central amygdala, to other brain systems. An example of this type of interaction would involve the projections from the amygdala to the ventral visual stream, relevant for modulating the representation of sensory stimuli (Vuilleumier, 2005). The involvement of the amygdala in the acquisition of conditioned fear has been confirmed by human lesion studies (Bechara et al., 1995; Phelps & LeDoux, 2005 for review). Early studies using non-invasive functional brain imaging, showed that a network involving areas beyond the amygdala and hippocampus, such as the sensory cortex, anterior cingulate cortex, insula, thalamus and prefrontal cortex are also implicated in human fear conditioning (LaBar and Cabeza, 2006; Sehlmeyer et al., 2009).

Another phenomenon often studied in the relation to PTSD is that of context conditioning, since sensory cues during fear conditioning are rarely restricted to one or two, but rather embedded in a wide set of environmental stimuli that are learned to predict the unconditioned stimulus. The dorsal hippocampus has been suggested to provide a substrate for binding the many features of a context into a unitary representation. In particular this is thought to happen by calling to mind a full representation of a feared context from partial information through the process of pattern recognition (O'Reilly and Jerry, 2001). Later research has expanded on this by showing that the hippocampus contains both time and space cells. The former fire at successive moments in temporally structured experiences and the latter when an animal occupies a particular location in an environment; together they are considered to play a key role in organising the elements of experience into coherent memories (Eichenbaum, 2014; Howard et al., 2014). A second phenomenon around fear conditioning that has been related to PTSD is fear extinction, that is, the process that leads to a reduction of the conditioned response by eliminating the predictive relationship between the conditioned and the unconditioned stimuli. Neurophysiological studies in this area have established a critical role of the ventromedial prefrontal cortex for extinction learning, in particular by exerting inhibitory control over the amygdala in order to reduce fear expression (Milad and Quirk, 2002). However, both the amygdala and the hippocampus play a role in extinction learning as well (Bouton et al., 2006; Kim and Richardson, 2008).

Centered on the functional neuroanatomy of amygdala and hippocampus together with the prefrontal cortex, the first neurocircuitry model of PTSD was proposed. The traditional 'fear' neurocircuitry model of PTSD posits that the amygdala is hyperactive and insufficiently modulated by the prefrontal cortex, which in normal circumstances would exhibit top down inhibition of the amygdala. This dysregulation could potentially explain symptoms of hyperarousal and the vividness of trauma recollections. Furthermore, the model proposed that abnormal functioning of the hippocampus would explain deficits in learning and memory (i.e., inability to extinguish a fear response) (Rauch et al., 2006) providing theoretical basis for the persistence of the symptomatology required for the diagnosis of PTSD.

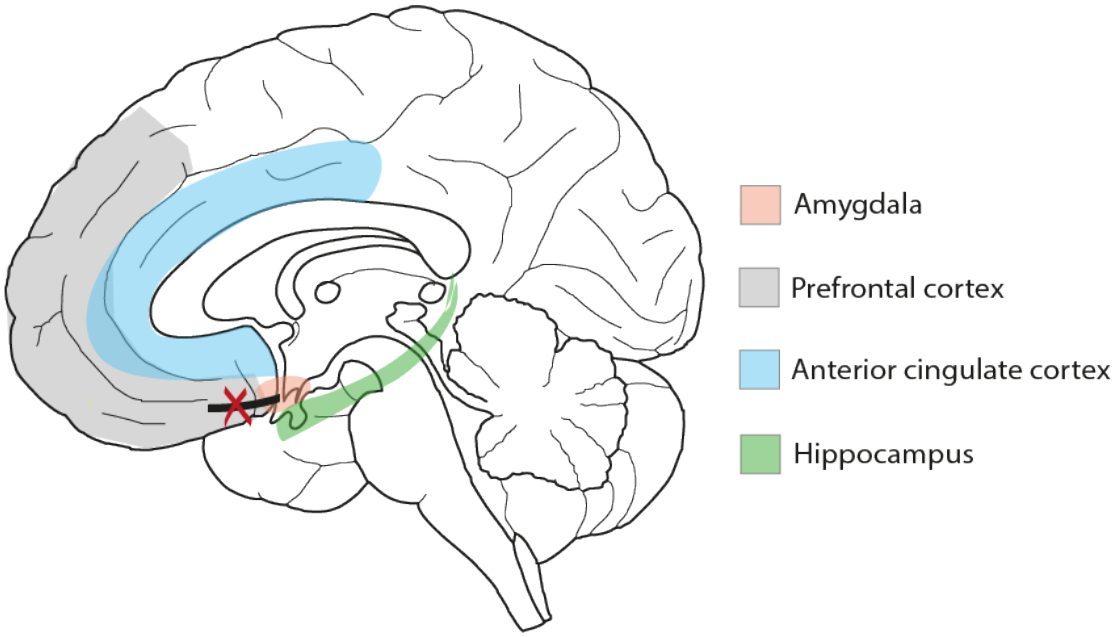


Figure 1.6 Fear neurocircuitry model of PTSD. *First neurocircuitry model of PTSD, according to which the prefrontal cortex is unable to modulate a hyperactive amygdala, which could explain symptoms of hyperarousal. The model further proposed that abnormal functioning of the hippocampus leads to deficits in learning and memory, explaining persistence of the symptoms. However, this model is not able to explain the myriad of symptoms characteristic of PTSD. Therefore, in our work we focus on the dynamics of large-scale brain networks, and how different temporal features of these networks might be related to the pathophysiology of PTSD.*

However, and although the 'fear' neurocircuitry model has been proven useful in advancing our understanding of PTSD, empirical and conceptual support for this model is not entirely satisfactory. In terms of empirical support, some studies report hyper- while other report hypo-activation of the rostral anterior cingulate cortex in PTSD in contrast to healthy controls (Bryant et al., 2005; Felmingham et al., 2009; Shin, 2001). Further inconsistencies have been found in relation to the role of the hippocampus (e.g., Bremner et al., 2003; Hayes et al., 2011). Furthermore, research has shown the involvement of other brain regions in PTSD that were not considered for the formulation of the model, such as the insula (Bruce et al., 2013; Y. Zhang et al., 2016b) the basal ganglia (Falconer et al., 2008; Linnman et al., 2011), the thalamus (Y. Zhang et al., 2016a) and the cerebellum (Frewen et al., 2012; Lanius et al., 2003).

To date, several meta-analysis have aimed to provide a synthesis of all the brain areas know to be involved in PTSD (Patel et al., 2012; Stark et al., 2015). During the first

months of my DPhil, I contributed to the most recent and largest meta-analysis of PTSD fMRI studies conducted to date. This work showed that, when reanalysing results from studies using whole-brain approaches, different activation in regions of the basal ganglia (putamen and globus pallidum) as well as fusiform gyrus were at the core of the differences between participants with PTSD and healthy participants exposed to trauma (healthy trauma-exposed). On the other hand, the contrast between participants with PTSD and healthy but trauma-naïve participants showed activation differences in anterior cingulate cortex, ventromedial prefrontal cortex, precuneus, insula and sensorimotor cortices. Differences in the activation of the amygdala for the trauma-exposed contrast, and the amygdala and hippocampus, for the trauma-naïve contrast, became significant only after inclusion of regions of interest studies (Stark et al., 2015). Hence, while meta-analyses should be considered carefully due to their limitations, most recent literature suggests that focusing on functioning of areas less traditionally associated with PTSD might hold some clues for the pathophysiology of PTSD.

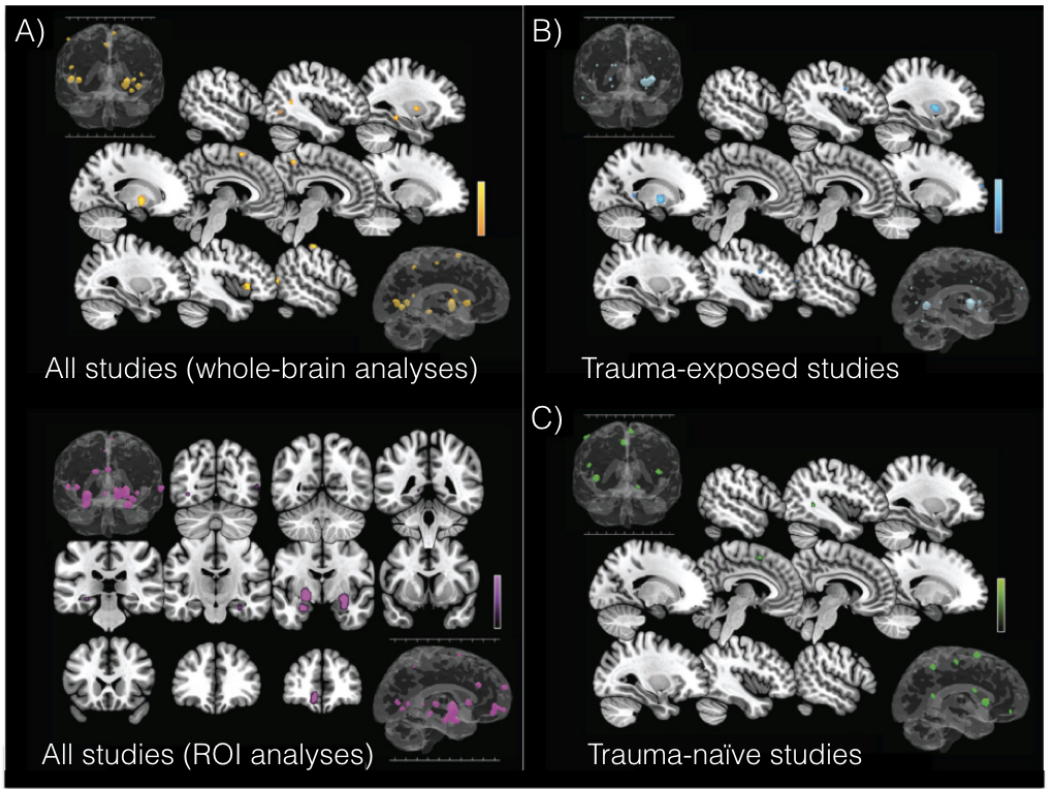


Figure 1.7. Meta-analysis of fMRI studies of PTSD. *In contrast to the previous studies, it was found that a) while the amygdala has been consistently associated with PTSD psychopathology, our results show that differences in this structure appear only when including region of interest studies. b) Whole-brain analyses comparing PTSD vs.*

trauma-exposed participants show that the differences concentrate in regions of the basal ganglia. c) Whole-brain analyses comparing PSTD vs. trauma-naïve participants shows differences in a more widespread pattern of regions. Results were considered significant at cluster level p<0.05. Reproduced from Stark et al., 2015.

Further points of concern have been raised regarding the conceptual support for the fear neurocircuitry model (Beckers et al., 2013). For example, if fear conditioning is really at the core of PTSD development, we would expect people susceptible to the development of the disorder to exhibit differences in fear conditioning in contrast to people who are not at risk. Although subtle differences have been found (Lonsdorf et al., 2010) other studies failed to find evidence for a clear link between individual vulnerability factors and excessive fear conditioning patterns (Baas et al., 2008; Grillon and Ameli, 2001). While this paradox could be due to suboptimal fear conditioning experimental designs (Beckers et al., 2013), it seems more plausible that the fear conditioning model might be too simplistic to account for the whole myriad of symptoms that are characteristic of PTSD. Indeed, even if the fear neurocircuitry might be a crucial part to the development of PTSD, and despite of fear reactions to imminent threats being to a large extent hard-wired responses, how these stimuli are perceived and interpreted involves many other systems that vary across individuals. In particular, and as suggested in previous sections, the cortico-basal ganglia loops are likely to play an important role in this context. Understanding how fear interacts with other emotional and cognitive systems to make sense of oneself and the world seems an important aspect of dealing with threatening situations while maintaining mental health.

The acknowledgement of insufficient explanation of PTSD symptomatology together with dysregulated activity in brain regions not included in the model brought about new models looking to integrate the newest findings. For example, (Liberzon and Abelson, 2016; Liberzon and Sripada, 2007) proposed a model still based on the conditioned-fear response but including other relevant processes highlighted in cognitive theories of PTSD, such as appraisal, reappraisal, emotional regulation and self-related and social emotional processing. Arguing that all these processes allow for the reassessment of the stimulus value, they proposed an overarching process of 'contextualization'. This 'contextualization' refers to the disambiguation of the most appropriate response for each particular situation. The process would rely on

subregions of the medial prefrontal cortex and contain three different categories: a) cognitive contextualization – judging the relevance of stimuli to the organisms memories and strategic goals (dorsolateral and dorsomedial prefrontal cortex) b) social contextualization –judging to which extent the stimuli are self-related (ventromedial prefrontal cortex) c) internal contextualization – overall homeostatic state of the internal milieu, including the state of drives, metabolic states and overall physiological load (rostral anterior insula and orbitofrontal cortex). This new model offered an explanation for *re-experiencing* symptoms through failures in discriminating contextual cues, *emotional numbing* as a failure to experience emotions linked to a particular context and difficulties in social interactions as exaggerated or inappropriate judgments that stimuli are self-related. Further, the inability to cognitively reappraise stimuli as safe or not threatening might be due to difficulties in emotion regulation.

Also aiming to complement the predominant fear-centered approach to PTSD, in this case by incorporating the reward system, (Stein and Paulus, 2009) proposed an imbalance theory according to which PTSD arises as the result of an attempt to establish a new homeostatic state after trauma has altered the approach-avoidance (i.e., reward-fear) system. The underlying mechanisms for the imbalance would stem from a dyregulation in the key modulatory neurotransmitters for the two systems, namely dopamine and noradrenaline. In support of their theory, they refer to the findings by (Milad et al., 2009), showing reduced extinction retention related to less activation in the hippocampus and bilateral ventromedial prefrontal cortex in participants with PTSD (dysregulation of the avoidance system). Further support comes from the findings by (Elman et al., 2009) showing reduced striatal activation to monetary gains in contrast to losses in PTSD together with more self-reported motivational and social deficit (dysregulation of the approach system). The reconceptualization of PTSD as an alteration in the approach/avoidance system allowed for a tentative explanation of 1) *emotional numbing and anhedonia* (downregulation of approach); 2) *hyperarousal and irritability* (upregulation of avoidance) 3) associated substance use problems (upregulation of approach); 4) *behavioural avoidance* (upregulation of avoidance) (Stein and Paulus, 2009). However, this model, which was broadly conceptualised as a model for anxiety disorders, would leave re-experiencing symptoms specific to PTSD unexplained.

Lanius et al., 2010 proposed another dual-system approach in which they suggest two pathways to emotion-dysregulation, one that results from *fear-conditioning* and a second one resulting from *distal vulnerability factors* exacerbating fear and other emotion regulation problems. In more detail, the *first* pathway refers to the concept of sensitization as a result of the fear reactions experienced after the trauma and the repeated re-experiencing of the traumatic memory. This sensitization would then result in the gradual augmentation and kindling of the reactivity of the individual. Kindling in this context refers to the expansion of symptoms over time due to the activation of the neural circuitry associated with the emotional memory becoming increasingly strengthened and spreading into neighbouring neural circuits (McFarlane, 2010; McFarlane et al., 2002; Post et al., 1997). Involved in this sensitization process would be a number of neurotransmitters, neurohormones, and neuropeptides that affect a network of brain regions including the ventromedial prefrontal cortex, amygdala and hippocampus (Heim and Nemeroff, 2009). The sensitization of the fear system would on its own result in dysregulation of other emotional systems leading to generalized emotion dysregulation and feelings of anger, anhedonia/numbing, and socially related panic. The individuals following the first pathway would most commonly be adults developing PTSD as a result of adult-onset traumas. The second pathway, however, extends to include the environment in which the individual was immersed before the traumatic event, constituting a classical stress-diasthesis (i.e., stress-vulnerability) model. Individuals with a history of childhood maltreatment and abuse would go on to experience dysregulation in emotional and arousal regulatory systems. The latter would lead to an inability to regulate physiological arousal after exposure to threatening and traumatic experiences later in life, leading to further exacerbation of emotion dysregulation and PTSD.

In a second paper published in the same year, Lanius and colleagues also tried to reconcile the contradictory findings regarding interactions between the amygdala and the ventromedial prefrontal cortex by suggesting two subtypes of PTSD: 1) emotional under-modulation (lack of control over or disinhibition of emotional responding) as occurs during re-experiencing/hyperarousal reactivity and 2) over-modulation (overcontrol of emotional states) in an attempt to restrict unwanted emotional experiences, as occurs during states of dissociation, numbing and analgesia (Frewen and Lanius, 2006; van der Kolk and Fisler, 1995). In terms of the neural correlates, Lanius et al., (2010b) suggest that emotional undermodulation might be mediated by

failure of prefrontal inhibition of limbic regions, whereas emotional overmodulation might be mediated by prefrontal inhibition of the same regions.

Large scale brain networks in PTSD (and depression)

As will be exposed more in more detail in a later section of the introduction, the realisation that brain activity is organised into replicable large-scale functional networks of coherent signal (Beckmann et al., 2005; Biswal et al., 1995; Fox et al., 2005) opened the door to new perspectives in many areas of neuroscience, amongst them, in the study of neuropsychiatric disorders. These newly discovered large-scale functional networks are nowadays usually referred to as intrinsic connectivity networks or resting-state networks. The first theory attributing PTSD to a dysregulation in the interaction between large-scale functional networks was proposed by Sripada and colleagues in 2012. In particular, they argued that PTSD is due, in part, to a disrupted equilibrium between two large-scale functional networks, the salience network (SN) and the default mode network (DMN) (Sripada et al., 2012). While the functional roles of the large-scale functional networks are not fully established yet, the SN is thought to be mainly involved with detection and orienting to salient external stimuli, while the DMN is known for its role in self-referential processing. The perspective suggested by Sripada et al., (2012) is based upon the triple network theory of psychopathology proposed by (Menon, 2011), which further involved the central executive network (CEN). The CEN is anchored in dorsolateral prefrontal cortex and posterior parietal cortex (Menon, 2011), but consists of three different subsystems characterised by specially strong within-subsystem connectivity. There are the above mentioned (anchor) frontoparietal network (FPN), specially important for highly adaptive processes, the cingulo-opercular system, involved in time-extended control processes, and the dorsal attention network, key for the coordination of attention to external stimuli (Corbetta and Shulman, 2002; Dosenbach et al., 2007). According to Menon's theory the SN arbitrates between the DMN and the CEN, acquiring a regulatory role that allows to balance externally oriented focus by the CEN and internally oriented processing by the DMN.

Further elaboration on this idea has been proposed recently by Akiki et al., (2017). They suggest that PTSD may be indeed characterised by an overactive and hyperconnected SN that does not manage to efficiently modulate default mode-central

executive interactions. The overactive SN is due to heightened threat detection in individuals with PTSD. This results in destabilization of the DMN and the CEN, which manifests as hypoconnectvity and hypoactivity of the two networks. They go on to hypothesize that an enhanced CEN to DMN connectivity, which has been observed to be stronger in participants responding to treatment, might be an acquired resilience mechanism by which individuals overcome dysregulation in a specific circuit or brain region resulting from trauma-exposure (Akiki et al., 2017).

Evolution of neurophysiological models

To summarise, over the last decades the field has evolved from threat-focused models to more comprehensive perspectives including concepts of emotion and reward dysregulation (Lanius et al., 2010b; Stein and Paulus, 2009). There have also been attempts to include the influence of early life traumatic experiences and vulnerabilities in the description of pathways leading to PTSD upon new exposure to trauma (Akiki et al., 2017; Lanius et al., 2010a). Methodologically, the focus on specific areas or small networks has shifted to an emphasis on the importance of investigating the behaviour of large-scale functional networks (Akiki et al., 2017; Sripada et al., 2012), which has lead to an accumulation of studies pointing towards dysfunctions in the DMN, SN and CEN/FPN in relation to PTSD. This evolution in the models and perspectives had as a goal to explain an increasing amount of symptoms of this very complex disorder from a neurobiological perspective. While much progress has been done in this regard, a model providing a comprehensive explanation of the whole myriad of symptoms is still missing.

For example, current models are unable to pinpoint the neural patterns of activity related to hyperarousal and dissociation, two seemingly contradictory symptoms that coexist in PTSD. So far, the closest approximation refers to dual-system models that propose temporal shifts in brain activity as a way to reconcile this contraction (Lanius et al., 2010a, 2010b; Stein and Paulus, 2009), giving rise to a subtype of PTSD or alternating patterns of symptoms. This would allow for alternation between predominant patterns of brain activity across time. Although the time-scale of these activation shifts is not specified in the available models, our understanding is that they refer to relatively slow timescales (e.g., hours to days). Nevertheless, the investigation of time-varying activity patterns at faster timescales might offer interesting insights on

how these slower shifts in brain activity patterns come together. Importantly, these theories touch upon an important limitation of most neuroimaging studies to date, and that is the assumption that patterns of neuronal activity remain constant over time. Recent development of sophisticated methods allow us to detect recurrent activation patterns of brain activity and with them multiplicity of scenarios related to the complex myriad of symptoms characteristic of PTSD. More details about the possibilities that these methods open up can be found under 'LEiDA & HMMs Overview'.

Mechanisms of Recovery through Psychotherapy – What do we know?

Despite of our limited understanding of the neural mechanisms underlying PTSD, it has been long recognised that psychological interventions are capable of reverting these symptoms in many cases, leading to full or at least partial recovery. At the psychological level, these interventions can modify not only the beliefs and ways of thinking of patients, but also their affective states and behaviours. However, the understanding we have about the neural mechanisms associated with those changes is even more limited than the understanding we have about the disorder. Here we argue that a better understanding of the neural changes associated with recovery could potentially bring important insights about the disorders and also contribute to the improvement of therapeutic interventions.

Potential mechanisms of psychotherapy

Nowadays, there are a number of evidence-based treatments for PTSD, which vary in the focus of their approach: while some treatments focus on imaginal exposure to the trauma memories and *in vivo* exposure to trauma reminders (Foa and Cahill, 2001), others have a more cognitive approach and focus on the reappraisal of the problematic meanings of the event and the contextual integration of the memory (Ehlers and Clark, 2000). However, currently available therapies seem to have some main elements in common, and it is most likely that associated changes in brain activity might be reflecting these general mechanisms regardless of the specificities of the therapeutic intervention. Some of these commonalities are emotion regulation, memory elaboration, cognitive processing, restructuring, and meaning making (Schnyder et al.,

2015). Accordingly, we would expect changes to appear across several networks involved in memory, emotion regulation and self-referential processing.

Thanks to previous studies we know that after successful therapy, participants show a reduction in amygdala activation (Aupperle et al., 2013; Felmingham et al., 2007; Van Rooij et al., 2016). However, this result was not always present at the whole-brain level (Kennis et al., 2016). Even more contradictory findings were obtained regarding other brain areas. In general, very few studies have been conducted to date evaluating brain activity changes before and after therapy, and those available have usually strong limitations due to small sample sizes and/or absence of control group (i.e., waiting list condition). Furthermore, all these studies also relied on the assumption that neuronal activity remains constant over time, disregarding the possibility of time-varying networks across time. In the following section, I provide a brief account of some relevant fMRI methods, including recently developed techniques that allow us to evaluate widespread changes incorporating the modulatory effect of time at high time-resolution.

STUDYING STRESS AND POSTTRAUMATIC STRESS IN VIVO IN HUMANS

In order to study these changes in the human brain we recur to a non-invasive *in vivo* neuroimaging technique known as functional magnetic resonance imaging (fMRI). This technique relies on measuring changes in blood flow as a way of inferring brain activity. The key component of an MRI scanner is a powerful electromagnet, commonly with a field of 3 tesla, although older scanners often had 1.5 tesla (see Methods in Chapter 5). This magnetic field affects the magnetic nuclei of atoms, which are normally randomly oriented, by aligning them with the direction of the field. When all the atomic nuclei are pointing in the same direction, they add up in a signal large enough to be measured. In particular, the magnetic signal from hydrogen nuclei in water (H_2O) can be detected and, depending on its surroundings, we can infer whether the signal comes from a region of grey matter, white matter or cerebrospinal fluid. Using reconstructions techniques we can also infer their spatial location and recreate a 3D image of the brain structure, which will be helpful for aligning the functional image to a common brain space in which the functional data from all participants can be compared. The creation of the functional image relies on oxygen being delivered to neurons by haemoglobin (blood). Haemoglobin is diamagnetic

when oxygenated but paramagnetic when deoxygenated, leading to differences in the MR signal of blood. Given that blood oxygenation varies according to the levels of neural activity these variations can be used to derive an estimate of brain activity — this is referred to as the blood oxygenation level dependent (BOLD) signal. In terms of how neural activity relates to blood oxygenation, we know that immediately after an increment of neural activity there is a momentary decrease in blood oxygenation (i.e., initial dip of the haemodynamic response), and this is followed by a period of blood flow increase surpassing the baseline need for oxygen. The blood flow peaks after approximately 6 seconds and then returns to baseline, often accompanied by a "post-stimulus undershoot". This is referred to as the haemodynamic function, whose temporal properties are critical to the interpretation of results from fMRI data.

Early fMRI analysis techniques

Over the last decades, analysis techniques have developed massively. In brief, first fMRI studies focused on *a priori* specified regions and compared the time-averaged signal across groups of participants using, for example, t-tests. This allowed for inferences regarding higher- or lower-activation of that particular structure disregarding activation patterns of the rest of the brain. More sophisticated approaches were developed into fMRI analysis tools in the 90's, in particular the general linear model (GLM) (mathematically identical to a multiple regression analysis) was introduced in the neuroimaging community (Friston et al., 1995b, 1995a). In brief, the GLM aims to explain the variation of a dependent variable (i.e., observed fMRI time course of a voxel) in terms of a linear combination of several reference functions (i.e., time courses of expected fMRI responses for different experimental conditions).

Static functional connectivity

Although initial neuroimaging work focused on individual brain regions, (e.g., the amygdala in stress research) it soon became clear that, since brain regions do not function in isolation, we should also investigate the relationship between them (i.e., networks). From this insight the term 'functional connectivity' (FC) was born to refer

to measures of statistical dependency between BOLD signals of two or more regions over the whole acquisition time, most commonly full correlations.

The first step towards this new approach was made through seed-based FC approaches, which involved *a priori* definition of a set of regions of interests to then investigate the statistical dependency of their signals most commonly through the use of correlations. As an example, in the field of stress and trauma, the most well researched relationships were those between the amygdala, hippocampus and prefrontal cortex. While early studies were looking at pairwise functional correlation (e.g., amygdala and prefrontal cortex), later approaches looked at the brain from a more holistic perspective. In those, a seed voxel (e.g., in the amygdala) would be specified *a priori* in order to then compute the FC between this voxel and all of the other voxels in the brain, the result being a whole-brain statistical map of FC with the seed voxel. While this approach was able to offer a much more comprehensive account of the relationship between the amygdala and the rest of the brain, it remained limited in offering a true whole-brain perspective, where the relationships between *all pairs* of regions are represented. In order to overcome these limitations, the dimensionality of the imaging data had to be reduced, from ~10.000 voxels to a handful of brain areas. To do so, the first brain parcellations were created (e.g., Automated Anatomical Labelling, AAL) allowing us to have one FC matrix per participant or brain scan (i.e., a whole-brain FC) by calculating measures of statistical dependency (e.g., full correlation or partial correlations, for more details see Chapter 2, Methods) between all brain areas. Importantly, the optimal brain parcellation (e.g., Shen, Tokoglu, Papademetris, & Constable, 2013; Tzourio-Mazoyer et al., 2002) is still a matter of current discussion, but this approach facilitated the incorporation of a more inclusive perspective on the study of stress and psychopathology whereby several neural systems could be investigated in a single study.

Intrinsic connectivity networks and stress

A different FC approach came from the realisation that brain activity is organised into replicable large-scale functional networks of coherent signal (Beckmann et al., 2005; Biswal et al., 1995; Fox et al., 2005), usually referred to as intrinsic connectivity networks or resting state networks. Importantly, these networks comprise sets of regions that are activated during specific cognitive tasks, such as visual processing or

detection of salient stimuli. Some of these networks seem particularly relevant for stress and stress-related psychopathology, being referred to as the 'core' networks of psychopathology (Menon, 2011): these are the DMN, the SN, and the CEN, specially its frontoparietal (FPN) subsystem. More detail about their function and neuroanatomy is provided under section 'Large-scale brain networks in PTSD (and depression)' and in the experimental chapters. The discovery of resting state networks was followed by an interest in developing methods that would allow their inference in a reliable and effective manner without having to define a region of interest a priori. This gave rise to the application of independent component analysis to fMRI data, allowing scientists to decompose the mixture of signals into several components that are maximally independent on either the spatial or the temporal dimension (Beckmann et al., 2005). Further work showed the high reproducibility across recording sessions and subjects of these networks as well as their correspondence with task-elicited networks (Damoiseaux et al., 2006; Smith et al., 2009).

Dynamic functional connectivity

Until recently, approaches to study brain activity and FC have assumed that these are temporally invariant phenomena in the sense, at least, that the mean and/or the variance of interregional coupling strengths does not change over time. Accordingly, assessing statistical dependencies between different brain regions over the duration of the whole scanning session (typically a minimum of 5-10 minutes) has been the predominant approach for the past decades, resulting in either a single time-averaged value of FC (pairwise approach) or matrix of values (whole-brain approach) per subject. While this approach greatly simplifies the analysis and illustration of results, there is no doubt that it is a considerable over-simplification of the brain's complexity (Deco et al., 2011; Hutchison et al., 2013) as evidenced by empirical studies (Bassett et al., 2011; Chang and Glover, 2010; Kiviniemi et al., 2011). Furthermore, when considering stress and stress-related disorders specifically, there is reason to believe that adopting a time-varying perspective of brain function could bring important insights to this field by incorporating a more accurate and detailed account of processes unfolding in the (healthy) stress response (Hermans et al., 2014; Schultz, 2007) and in the responses of people suffering from stress-related disorders (Lanius et al., 2010b).

In recent years there has been a marked effort to develop methods that allow us to characterise temporal changes both in resting state and task fMRI. First attempts started by computing measures of FC within a time window of a pre-specified duration – this window would then be slid by a number of data points to compute FC of a new, but usually partly overlapping, section of the scan. This process would carry on until the whole scanning period has been covered by the subsequent shifts of the sliding window, resulting in a sequence of FC measures aiming to capture the temporal evolution of the data. The main disadvantage of this dynamic FC approach lies in the inefficient use it makes of the data and in the difficulty in interpreting the noisy results that can be derived from it. This is related to the need to pre-specify the length of the time window. Short windows will result in less robust estimations due to inclusion of relatively few data points (noisy results), whereas longer windows will be less able to capture short-lived changes in FC (inefficient use of data). Optimising the size of the time window would require therefore consideration of many variables, such as signal-to-noise ratio of the data and time scales at which temporal variability is believed to exist.

Inevitably, time-varying FC approaches lead to an increase in information due to the addition of the temporal component. This is the reason why some studies started using additional statistics aimed to provide a summary of the degree of temporal variability, such as the standard deviation over the sequence of sliding window correlation coefficients (Chang and Glover, 2010). Others applied k-means clustering to the sliding window correlations in order to identify recurrent patterns of whole brain correlations (Allen et al., 2014). Another clustering approach was based on evidence showing that the pattern of activity corresponding to a particular RSN may be identified using only a few selected data points with high signal amplitude in a specific, within-network region (e.g., posterior cingulate cortex) (Tagliazucchi et al., 2012). Further research in this area confirmed the idea that specific RSN patterns can be accurately replicated with only a selected portion of the dataset and extended it further to show that a spatial similarity classification of the high amplitude frames could reveal spatial variations of resting-state networks (or co-activation patterns) with potential for functional relevance. The results offered support for the idea that interregional BOLD correlations, rather than the result from continuous, sustained interregional neuronal interactions, may actually find their origin in instantaneous co-

activations (or co-deactivations) of multiple brain regions at some critical time points (Liu and Duyn, 2013).

LEiDA & HMMs Overview

Following this idea of time-varying modes of co-activation, Cabral et al., 2017 proposed a method (Leading Eigenvector Dynamic Analysis, LEiDA) that would allow us to dispense with the window size limitation and detect patterns of BOLD phase coherence, i.e. of areas that co-vary together, either co-activating or co-deactivating together. In brief, the method is based on computing the BOLD phase coherence matrix at each time point, resulting in as many instantaneous FC matrices as data volumes. Subsequently, the first leading eigenvector for each instantaneous FC matrix is extracted and a k-means clustering approach applied in order to identify recurrent patterns of BOLD phase coherence instantaneous patterns across time (for more details, see Chapter 3, Methods). A second method based on Hidden Markov models (HMMs) aimed at detecting recurrent patterns of brain activity over time at the fastest possible timescales was developed by Vidaurre et al., (2017b, 2017a). One of the advantages of this method is that the recurrent patterns can be based on the covariance, and therefore be representative of FC patterns, or on the mean BOLD activation, representing in such case patterns of above or under average activation. They can also contain information from both covariance and mean BOLD activation, and variations in the predominance of one or the other type of information will place the recurrent patterns within an activation-interdependence gradient. Both methods, LEiDA and HMMs, have the advantage of not requiring the definition of a window length, overcoming limitations related to less robust estimations in case of inclusion of relatively few data points (short window) or inability to capture short-lived changes if there are many data points (relatively long windows). In exchange, both require the specification of the number of states to be used. More details about these two methods are provided in the specific chapters in which they we used, under the 'Methods' sections (LEiDA, Chapters 3 and 4; HMM, Chapter 5).

SUMMARY OF THE ARGUMENT

Psychiatric disorders such as depression and anxiety are among the leading causes of disability worldwide (Vos et al., 2014). These are to a large extent caused by widespread

life-threatening conditions around the globe together with inefficient prevention and treatment strategies. It has been suggested that one of the possible reasons for the limited prevention and treatment strategies is related to an incomplete understanding of the brain mechanisms underlying these disorders. In order to foster progress we need appropriate study designs and methodologies allowing us to get closer to the nature of the problem, in this case, the response of the brain to multiple and heterogeneous stressors unfolding across time. To achieve this goal, we have used two longitudinal fMRI datasets capturing: 1) pre-disease in a high-risk sample of elite soldiers 2) PTSD manifestation and PTSD recovery in a sample of civilians undergoing cognitive therapy for PTSD. The first dataset allowed the study of brain dynamics associated with resilience and vulnerability, while the second dataset was used to first characterise PTSD-related brain dynamics and then look at the changes associated with its remission. This work contributes to the research on stress and trauma-related disorders by approaching the relevant questions from a new angle in the field, namely, that of time-varying FC and activation. Although many theories in the field have pointed towards the importance of taking on a dynamic approach (Akiki et al., 2017; Cole et al., 2014; Hermans et al., 2014; Menon, 2011), only few fMRI studies in stress and PTSD have implemented a time-varying perspective to date.

The organisation of the rest of the thesis is as follows. *Chapter 2* explores response to acute stress from a traditional approach, looking at static FC of the whole-brain before, immediately after stress, and during stress recovery. This allows us to define a starting point to integrate findings from the time-varying decomposition of each of these phases in the following chapters. Subsequently, *Chapter 3* explores the temporal signatures (stability and dominance) of recurrent FC patterns in each of these stress phases. Results show significant correlations between vulnerability scores and the temporal signature of two networks, one characterised by decoupling between subcortical structures and the cerebral cortex, and another one characterised by decoupling between the bilateral frontoparietal network and the rest of the brain. *Chapter 4* continues the exploration of stress brain dynamics by looking at transitions, and establishes that precisely these two networks, together with a network related to the default mode system, drive the differences between stress phases and across the vulnerability-resilience gradient in in terms of transitioning from one time-varying FC network to another. *Chapter 5* moves away from the study of stress resilience to look at differences in brain dynamics of individuals with and without PTSD, as well as changes

associated with recovery after successful cognitive therapy. In this final study, I used a novel data-driven approach (Hidden Markov models, Vidaurre et al., (2017a, 2016)) to extract brain activation states based on specific (recurrent) patterns in the BOLD signal. The main result of this study showed that participants with PTSD spend less time in states engaging the medial temporal and the core subsystems of the default mode network, which are then upregulated after successful cognitive therapy. This thesis comes to an end with *Chapter 6,* where the implications of this work are discussed and integrated into a model of PTSD including a time-varying perspective. I conclude with a discussion on some of the steps that, in my opinion, could bring the field forward.

Chapter 2

CHARACTERISING THE RESILIENCE RESPONSE TO ACUTE STRESS IN STATIC FUNCTIONAL CONNECTIVITY

ABSTRACT

In this first chapter, we conduct standard analyses of static (i.e., temporally averaged) functional connectivity to characterise how the immediate brain response to stress evolves on to a stress recovery phase, and how this functional connectivity is modulated by stress vulnerability. Our results show an increase in striato-cortical functional connectivity during stress recovery in contrast to the immediate stress response, as well as before stress induction. Correlational analyses between functional connectivity and vulnerability scores reveal that participants with lower scores had stronger functional connectivity between bilateral angular gyrus and frontal cortex, both regions belonging to the default mode network, immediately after stress. Furthermore, there was a positive relationship between our vulnerability score and the strength of functional connectivity between limbic (i.e., amygdala and orbitofrontal cortex) and temporoparietal regions. In this first chapter, by using an approach similar to those applied in many other studies to date, we define a methodological baseline and enable ourselves to contrast these first results with those presented in the existing literature.

INTRODUCTION

Over the last decades, some important differences in the brain structure and function of those suffering from stress-related disorders with respect to healthy controls have been unveiled. Examples of these disorders are PTSD and MDD (Otte et al., 2016; Patel et al., 2012; Stark et al., 2015). However, despite all the efforts from the scientific community, the incidence of these disorders has not decreased. In fact, recent findings show that depression is the second leading cause of disability worldwide (Vos et al., 2014). These disorders can often be traced back to the influence of endogenous or exogenous stressors, such as, traumatic events, adverse life circumstances or physical diseases. In order to speed up progress that might bring us closer to prevention

strategies, several researchers have joined a call for stress resilience and vulnerability studies to use longitudinal designs able to capture the dynamic nature of resilience (Bonanno et al., 2015; Chmitorz et al., 2018; Kalisch et al., 2017, 2015). The idea behind this is to characterise divergent behavioural and neural trajectories across time, given that these might hold essential information on the differences between those who go on to develop a stress-related disorder versus those that remain healthy in the face of adversity.

Temporal evolution of the acute stress response

The timescale at which longitudinal measurements are obtained can offer highly complementary information regarding vulnerability and resilience. For example, studies acquiring several measurements across a time span of several hours upon induction of acute stress are complementary to those studying chronic stress across in a time period of weeks to months. In the context of the acute stress response, many fMRI studies have investigated the immediate response to stress but research on later stages of the acute stress response is more limited. However, it has been suggested that sustained activation of the stress-related brain circuits after stress may result in allostatic load (McEwen, 2007) and even be at the basis of stress-related psychopathology (Krystal and Neumeister, 2009; Morgan et al., 2003; Yehuda and LeDoux, 2007), which is why studies looking beyond the immediate or short-term stress reaction are particularly important.

In terms of neurochemical changes, the earliest responses to acute stressors begin almost immediately and normalize after 30 to 60 minutes. This fast stress response is modulated by neuropeptides, such as the CRH leading to release of cortisol, and catecholamines such as norepinephrine and dopamine. Somewhat slower is the HPA axis response, which leads to corticosteroid production reaching its peak 20 minutes after stressor onset and, even slower, is the response following corticosteroid binding. In particular, corticosteroids lead to altered gene transcription resulting in turn in changes in the levels of multiple proteins affecting neuronal function (Joels et al., 2012). These effects start about an hour after stress onset and can continue for at least several hours. Its effects have been studied in rats and shown to enhance prefrontal cortex function and working memory (Yuen et al., 2009) as well as dorsal hippocampus function (Karst and Joels, 2005).

In summary, the multiple waves of stress-related neurotransmitters and hormones identified in animal studies are thought to induce spatially and temporally specific modulation of neural networks. These are observable in human fMRI studies to some extent, and the link between animal and human studies has started to receive more attention in recent years (Hermans et al., 2014, 2011).

Longitudinal studies of acute stress using fMRI

Moving on to fMRI research, previous longitudinal studies investigating the acute stress response across time in healthy participants have shown an increase in the FC between the amygdala and the hippocampus and parahippocampal gyrus (Quaedflieg et al., 2015; Vaisvaser et al., 2013) immediately after stress. This increase came accompanied by a decrease in the FC between amygdala and centrolateral prefrontal cortex, ventral posterior cingulate cortex, cuneus, and culmen (Quaedflieg et al., 2015). Interestingly, both the increase in FC between the amygdala and the hippocampus as well as the decrease in FC between the amygdala and the ventral prefrontal cortex were positively correlated with subjective stress levels 20 minutes after stress induction (Quaedflieg et al., 2015; Vaisvaser et al., 2013). A similar study found increased FC between amygdala and dorsal anterior cingulate cortex, anterior insula, and a dorso-rostral pontine region, potentially corresponding with the locus coeruleus (van Marle et al., 2010) immediately after stress.

Given that the stress response has been shown to unfold across an extended period of time, several studies have investigated brain function at later stages of the stress response. Studies looking at resting state brain function 30 minutes or longer after stress induction have shown a prolonged period with increased connectivity between the amygdala and the hippocampus (Quaedflieg et al., 2015) which may last up to 2 hours (Vaisvaser et al., 2013). Furthermore, there have also been reports of increased FC between the amygdala and the dorsal anterior cingulate (Quaedflieg et al., 2015), and between the amygdala and three cortical midline structures, namely the posterior cingulate cortex and precuneus and the medial prefrontal cortex (Veer et al., 2011).

All these fMRI studies used a seed-based FC approach. While this allowed the detection of changes in the FC of a specific brain structure with a relatively high degree of sensitivity, they have the disadvantage of being blind to any changes beyond

the scope of the particular hypothesis that motivated that choice of region, and being biased by this choice. In this respect, a recent whole-brain study conducted on the same dataset that we are using in this chapter showed stronger thalamo-cortical connectivity and weaker cross-hemispherical parietotemporal connectivity immediately after stress (Maron-Katz et al., 2016). While the focus of both Maron-Katz et al.'s study our study is on the differences in FC strength between stress conditions, there are a couple of important differences that we should take into account. First, they used a more fine-grained parcellation that resulted in the identification of a large number of changes full correlations between stress conditions even after correcting across multiple comparisons. In order to extract the most prominent changes, they applied a second level-analysis using enrichment analysis (Huang et al., 2009; Ulitsky et al., 2010), which is often used in genetic studies to extract meaningful information out of a large volume of data. Our FC approach however started with a smaller number of parcels (i.e., 90 parcels, Tzourio-Mazoyer (2002)) and we applied two different measures of FC, one to look at extended networks of direct and indirect connections (i.e., full correlations) and a second one to estimate direct connections (i.e., partial correlations). Furthermore, while Maron-Katz et al.'s study focused only on the first two resting-state scans (one acquired before stress and one immediately after stress induction), we also investigated FC during the third (stress recovery) scan. Thus, the two studies are only partially comparable.

METHODS

Participants

Participants for this study consisted of military soldiers (n=60) who were all positioned in the same service unit, taking part in the same military course, and before operational employment.

Of the 60 individuals 6 were excluded due to missing data points and 6 we excluded due to signal artefacts and limited field of view in the scanner, resulting in a final sample of 48 elite unit soldiers. All soldiers were between 18 and 21 years old (mean=18.92; sd=0.986). Participants had no reported history of psychiatric or neurological disorders and no family history of major psychiatric disorders. They were screened for current use of psychoactive drugs. They reported not being exposed to

abuse during childhood and/or potentially traumatic events before entering the study. However, 5 of them had some type of learning disorder, namely (n=5 ADHD and n=1 ADHD/dyslexia). In addition, all participants had normal or corrected-to-normal vision. Written informed consent approved by Tel Aviv Sourasky Medical Center Ethics Committee and conformed to the Code of Ethics of the World Medical Association (Helsinki Declaration) was obtained for all participants.

Study design

The design of this study is longitudinal at two different levels. Measures of the participant's mental health and experiences were obtained at up to four times throughout a period of approximately 2 years while they we undertaking the military training and participating in their first missions *(longitudinal level 1, chronic stress)*. Approximately at the time of the third acquisition, participants took part in an fMRI experiment entailing acquisition of three resting-state scans along an acute social stress task (baseline, immediately after stress, and stress recovery) *(longitudinal level 2, acute social stress)*, described in detail in the next in the next section, *'Stress induction task' (*see also*, Figure 2.1)*. The first longitudinal level, meaning the different mental health measures obtained throughout two years, allowed for the definition of this sample as resilient given maintenance of high cognitive functioning despite of exposure to chronic stress. The second longitudinal level, meaning the three resting state-scans along an acute stress paradigm, allowed evaluation of their acute stress response across time as well as how this stress response is modulated by accumulated stress, rendering them more vulnerable. The stress induction paradigm is key to this study, as previous reports suggest that, often, physiological consequences of stress can only be observed after imposition of a new stressor, that is, after bringing to the surface the latent effects of previous exposures (Belda et al., 2015).

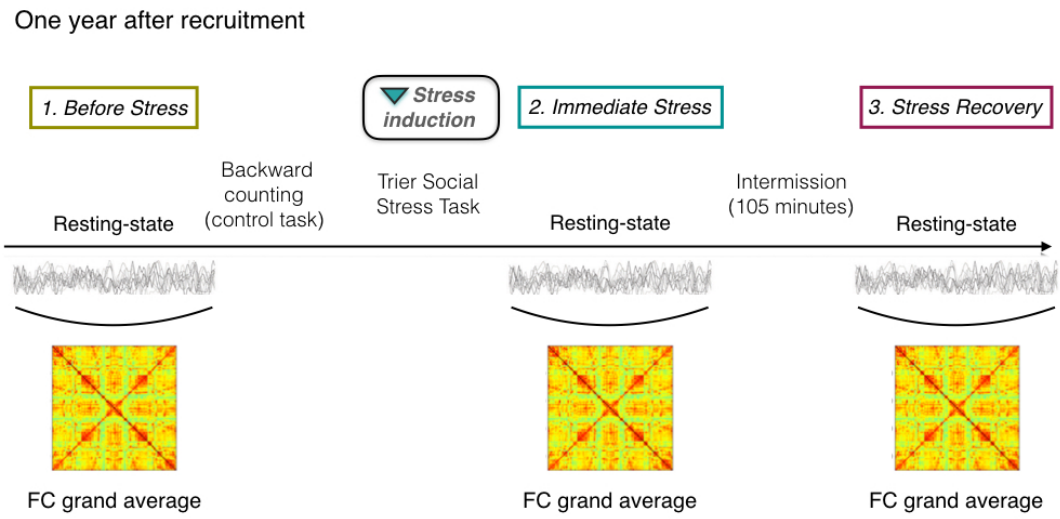


Figure 2.1. Longitudinal study design for neuroimaging of acute stress in elite soldiers. *Three resting-state scans were acquired for each participant: the first one before completion of any tasks, a second one 5 minutes after the stress induction task and a third and last one approximately 1 hour and 30 minutes after the stress task. We then evaluated pairwise differences in time invariant (i.e., time-averaged) functional connectivity between the different experimental conditions using full correlations and partial correlations (i.e., estimates of direct connectivity).*

Stress induction task

Social acute stress was induced via a component of the Trier Social Stress Test (Kirschbaum et al., 1993) adapted to an fMRI setting, in particular a subtraction arithmetic task (Wang et al., 2005). Participants were asked to continuously subtract 13 from 1022 for a period of 6 min, and communicate the results verbally to the experimenters. The experimenters were outside of the scanner, assessing performance of each participant and constantly demanding faster and more accurate performance. The participant could monitor time progress through a timer appearing at the top left corner of the screen. The stress task was preceded by a task involving backward counting from 1000 for a period of 6min, without external monitoring, (i.e., non-stressful control task). Stress induction was successful as suggested by the effects on behavioural and physiological measures (e.g., heart rate, cortisol) shown in previous publications from this data (Maron-Katz et al., 2016; Vaisvaser et al., 2013).

Behavioural measures

Participants were assessed using the Patient Health Questionnaire (PHQ-9) at 4 different visits: 1) within two weeks of recruitment (baseline) 2) four months after recruitment (pre-deployment) 3) one year after recruitment (post-combat) 4) two years after recruitment (post-combat follow-up). The PHQ9 is a 9 item self-report typically used as a screening and monitoring tool for depression severity in the general population (Kroenke et al., 2001). Here we use the PHQ9 as an indicator of a deviation from the resilient pathway towards incipient depression, as indexed by the typically mild to moderate scores of our participants. The main reason for using this scale over traditional resilience questionnaires (Connor and Davidson, 2003) was our emphasis on resilience as a dynamic process allowing the individual to avoid detrimental effects on their mental health after stress exposure. In contrast to the PHQ9, resilience questionnaires typically assess a combination of personality traits, coping skills and perceived social support and are therefore less likely to reflect fluctuation in mental health across time.

While we had data on other mental health variables, such as anxiety (State-Trait Anxiety Inventory) (Spielberger et al., 1983) and the PTSD (symptom) checklist (PCL) (Weathers et al., 1983) only the PHQ9 and the PCL were acquired at the 4 different visits. This longitudinal data included a 1-year follow-up assessment after the acquisition of the resting-state scans that we are analysing here. Making use of this data, we calculated correlations between the PCL and PHQ9 scores acquired at the time of the experiment and the PCL follow-up scores. This revealed that the PHQ9 at the time of the experiment was a better predictor of PTSD symptoms at one-year follow-up than the PCL. In particular, we run permutation analysis with the basis of a paired t-test and found that the PHQ9 significantly correlated with PCL scores one year later ($r = 0.634$, p-value = 0,0001), while the PCL acquired at the time of the experiment did not ($r = 0.52$, p-value = 0.358). Based on this, we concluded that the PHQ9 was better able to predict PTSD symptoms after one year of further combat exposure than the PCL. In the following, we refer to PHQ9 scores acquired at the time of our experiment as vulnerability scores.

It is also worth mentioning that the low specificity of most of the PHQ9 items makes it reasonable to regard scores >0 as potential indicators of both early PTSD and depression. From the 9 items, only one of them is specific to depression (i.e., poor

appetite or overeating). Two further items contain a depression-specific part, but are presented together with a statement that could refer to PTSD symptomatology (i.e., "Trouble falling stating asleep *(PTSD and/or depression)* or sleeping too much *(depression)"* and "Moving or speaking so slowly that other people could have noticed *(depression)* or the opposite - being so fidgety or restless that you have been moving around a lot more than usual" *(depression and/or PTSD*). The remaining items in this questionnaire may be indicative of depression, PTSD or just general health (e.g., sleep quality, appetite).

At post-combat, which is the measure that we used in our analysis, only 40 out of the 48 participants were assessed with the PHQ9. Out of those 40 participants, 21 did not reach the minimum cut off for mild symptoms, 11 of them could be considered to present mild symptoms, 5 moderate, and only 3 participants reached the threshold for moderately severe symptoms. The maximum score in our sample was 18 out of 27. When calculating the mean PHQ9 score for the different points in time, the highest mean score was 18.065 out of 27.

fMRI data acquisition and pre-processing

Brain scanning was performed on a 3T (GE, HDXt) MRI scanner with an 8-channel head coil. Functional imaging was acquired with gradient echo-planar imaging (EPI) sequence of T2*-weighted images (TR/TE/flip angle: 3000/35/90; FOV: 20× 20 cm; matrix size: 96×96) in 39 axial slices (thickness: 3mm; gap: 0mm) covering the whole cerebrum. Basic data preprocessing was performed using MELODIC (Beckmann and Smith, 2004) with the embedded tools MCFLIRT (Jenkinson et al., 2002) for motion correction and BET (Smith, 2002) for brain extraction, all part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The parameters used were default, including spatial smoothing using a Gaussian kernel of FWHM 5 mm, and highpass temporal filtering with sigma=50.0s.

Advanced data denoising was carried out using FMRIB's ICA-based Xnoiseifier (FIX) (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014). First, independent component analysis (ICA) components for 15 participants were hand-labelled according to 'signal', 'noise' or 'unknown' following the guidelines provided by Griffanti et al., (2017). The manual hand labelling on our own dataset allowed for an

optimised training of the FIX algorithm for this particular dataset. Subsequently, classification of the components for each participant's ICA decomposition was done according to a range of thresholds (5,10 and 15) to assess how much noise we could remove without affecting any signal components. Based on the results a conservative threshold of 10 was chosen for this dataset.

Once the data was denoised, linear registration to their structural scans was applied followed by nonlinear registration to MNI space. Mean BOLD time-series were then estimated on 90 brain areas of the Anatomical Automatic Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) by averaging the BOLD signal over all voxels belonging to each brain area. From these 90 brain regions, 4 were excluded due to signal artifacts in those regions, namely the left and right rectus (orbitofrontal cortex) and the left and right mid temporal pole. Matrices of full correlations and partial correlations (using standard L2 Tikhonov regularisation, with parameter rho=0.1) were computed using FSLnets, part of FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl.

Functional connectivity measures

First, full normalized correlations between every node timeseries and every other node timeseries were computed using the FSLnets toolbox (fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets), according to the following expression:

$$\boldsymbol{r} = \frac{\boldsymbol{cov_{xy}}}{\boldsymbol{s_x * s_y}} = \frac{\sum(\boldsymbol{x_i - X})(\boldsymbol{y_i - Y})}{(\boldsymbol{N-1})\, \boldsymbol{s_x}\, \boldsymbol{s_y}} \qquad \textit{Equation 2-1}$$

This is a straightforward approach but it has an important disadvantage, namely that full correlations are influenced by all the connections other than the direct node-to-node connection 'strength' *(Figure 2.2)*. Therefore, partial correlations, which are a true estimate of direct connectivity, were computed. This involves taking each pair of time series one by one and regressing out the others from each of the two timeseries of interest before estimating the correlation between them. In our case, in which we have 86 nodes, we regress N_{nodes} -2 out of the two nodes of interest. This will remove any spurious correlations due to correlation of any of the N_{nodes} -2 with the two nodes of interest. This is mathematically equivalent to invert the full correlation matrix.

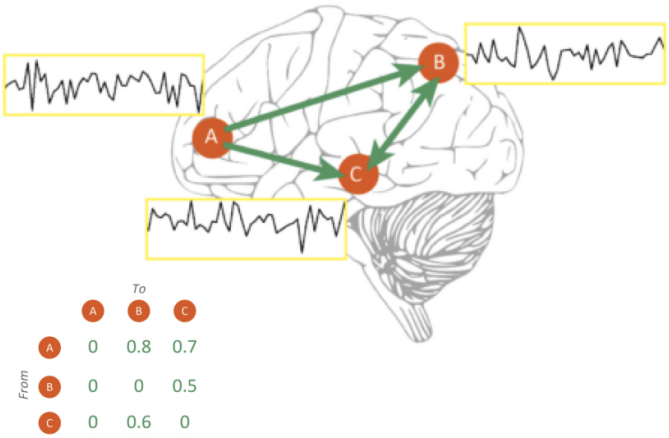


Figure 2.2. Functional connectivity and the estimation of direct connections. *In a three-node network A, B and C in which both A - B and B - C are connected, calculation of full correlations would incorrectly estimate a fully-connected network (including A - C connection). The use of partial correlations enables removal from the spurious connection A-C by regressing out the timeseries from the third node from the timeseries from the other two nodes before estimating their connection. Reproduced from Smith et al., (2013).*

Statistical analyses

Statistical analyses were carried out using Permutation analyses of Linear models (PALM) (Winkler et al., 2015, 2014). The GLM implemented in PALM is often used in the field of neuroimaging because it provides a framework under which most of the strategies for statistical analysis can be formulated. PALM is based on permutation testing, a class of non-parametric methods that bypasses a number of distributional assumptions in the data, which makes it particularly appropriate for neuroimaging data. Permutation testing typically makes use of a parametric test (see below) internally, yet the influence of the parametric test's assumptions is ameliorated by the permutation procedure.

Given the design of our study, we were interested in first characterising the differences in FC in *baseline vs. immediate stress* (i.e., stress-induced changes), *immediate stress vs. stress recovery* and *baseline vs. stress recovery*. To test these differences, we can use a set of paired t-tests. However, paired t-tests require careful consideration in the context of permutation testing, since one of the assumptions is that data are exchangeable under the null hypothesis. Repeated measures designs like

the one at hand, however, have a particular structure characterised by non-independence of some of the observations, which needs to be respected by redefining exchangeability. Hence, instead of assuming exchangeability across all observations of a given experiment, blocks of exchangeable units need to be defined. In our case, given that we had more than one measurement per subject and visit, we exchanged measurements within but not across visits. The number of permutations used was 10000.

Correction across multiple comparisons was carried out using false discovery rate (FDR) (Benjamini and Hochberg, 1995) across p-values resulting from the 3 condition contrasts. This allowed us to correct for both the multiple comparisons resulting from the 86x86 connections as well as the three contrasts performed. The process was done separately for full and partial correlations.

Once the differences between conditions were tested, we were interested in detecting any linear relationships between FC and vulnerability scores obtained through the PHQ9 within each of the conditions. Again, the number of permutations used was 10000. Three matrices of p-values resulted from the analysis on full correlations, one per stress condition (baseline, immediate stress and stress recovery). All three matrices were pooled together and FDR correction with a threshold of 0.01 was applied on all the p-values in order to correct across the three conditions. The same process was repeated on the partial correlations.

Visualisation of results

While whole-brain FC analyses are important for obtaining a comprehensive perspective of the differences between conditions, results can be difficult to interpret, specially when several measures of connectivity are being used. Here, we first test for differences in connectivity using full correlations. These analyses resulted often in a widespread network of significant differences, making difficult to summarise the main findings. In order to provide a summary statistic, we estimated the degree of (significantly different) connectedness for each node based on the obtained p-values. To achieve this purpose, we first, transformed each uncorrected p-value to its negative logarithm, which allowed us to focus on the range of the p-values that is most important (i.e. when they are close to zero) (Winkler et al., 2016). We then computed

the sum of p-values for each node and divided it by the number of nodes. Finally we took the exponential. This corresponds to the Fisher transform to combine p-values (Winkler et al. 2016).

This is in contrast to traditional approaches to evaluate differences in degree of connectedness. Traditional approaches start by establishing an arbitrary minimal threshold of connectivity strength. Then they compute the sum of connections above that threshold, obtaining the degree of connectedness, and run statistical analyses on this measure to establish any significant differences between subjects/conditions. This approach has the disadvantage of requiring the definition of an arbitrary threshold. Here, by first testing for differences in pairwise connectivity between stress conditions, and then logarithmically transforming the p-values, we obtain a measure of degree of connectedness weighted by the amount of significant differences between conditions. This allows us to circumvent the application of an arbitrary threshold. To visualise the results, we used BrainNet (Xia et al., 2013), representing significant differences in connectivity in the edges and the degree of connectedness in the size of the nodes.

After testing for differences in connectivity between stress conditions using full correlations, we run analyses on the partial correlations. For simplicity, we decided to show only significant differences in partial correlations (i.e., direct connections) when these were overlapping with significant differences in full correlations. We represent significant differences in partial correlations using a different colour for the relevant edge on the full correlation plots *(see figures 2.3 (right) and 2.6).*

RESULTS

Differences in functional connectivity between the stress conditions

When comparing before stress to immediately after stress, we observed that participants showed stronger FC between the medial orbital cortex and bilateral occipital cortex (mid and inferior) before stress in contrast to immediately after stress (*Figure 2.3*).

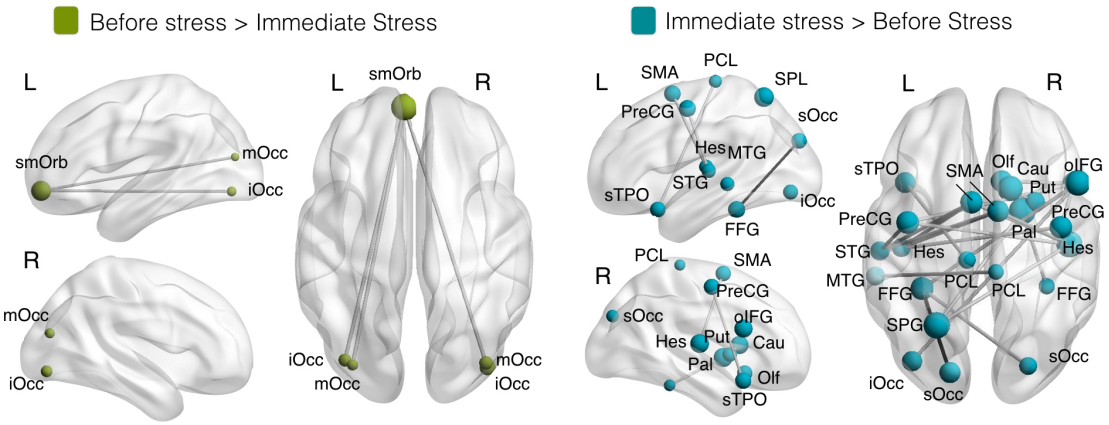


Figure 2.3. Acute stress provokes large whole-brain changes in functional connectivity*. Grey edges represent significant differences in full correlations, while black edges represent significant differences in partial correlations (i.e., direct connections). The sizes of the nodes represent degree of connectedness. Immediately after stress we observed an increase in the strength of functional connectivity among temporoparietal regions accompanied by a weakening of the connections between orbital and occipital regions. The level of significance is p<0.01 after correction for multiple comparisons taking into account the p-values of the three contrasts: before stress vs. immediate stress, immediate stress vs. stress recovery, before stress vs. immediate stress. The corresponding AAL labels for each abbreviation are provided in Supplementary Table 1.*

Immediately after stress they showed stronger FC in a network connecting the bilateral somatosensory cortices to their ipsilateral and contralateral Heschl Gyri, also known as the transverse temporal gyri *(Figure 2.3)*. The right supplementary motor area was also strongly connected to the left superior temporal gyrus, an area adjacent to the Heschl gyrus, which contains, in most people, Wernicke's area, responsible for language comprehension. The bilateral precentral gyrus was also part of this network of areas more strongly connected after stress than before stress – in particular, the left precentral gyrus is connected to the left superior temporal gyrus, right Heschl gyrus and right superior temporal pole, making the precentral gyrus one of the nodes with the highest degree of connectedness. The right precentral gyrus was also more strongly connected to the right temporal pole, and the right temporal pole was more strongly connected to the left precentral gyrus, and the left superior temporal gyrus. This formed a closed network of somatosensory and auditory areas that were more strongly connected after stress than before stress. The right temporal pole was more strongly connected to the olfactory cortex after stress than before stress, providing a stronger

connection to areas involved in affective processing. The right Heschl Gyrus also had stronger FC with the left superior parietal gyrus, the latter to the pallidum, and the pallidum to the right fusiform gyrus. The left fusiform gyrus was the other main hub of this network, due to its stronger connectivity with the inferior frontal gyrus (opercular) and the bilateral superior occipital cortex. Our partial correlation analysis showed that, from these links, only the connection between the left fusiform gyrus and the left superior occipital cortex was direct. We also observed stronger FC between isolated regions that did not apparently form part of larger networks: these stronger connections were between the mid left temporal gyrus and the right paracentral lobule, the left superior temporal gyrus and the left paracentral lobule, and between the right caudate and putamen.

To summarise, after stress induction, we observed stronger connectivity organised around two main hubs, the left precentral gyrus and the left fusiform gyrus. The left precentral gyrus was at the core of a network of somatosensory and auditory areas whereas the left fusiform was central in a network connecting to visual areas and inferior frontal gyrus. The only direct connection related to the left fusiform gyrus and the left superior occipital cortex. The right putamen, despite of only showing one significantly stronger link to the pallidum, had the third largest degree of connectedness value, which might indicate overall stronger connectivity with other areas that did not surpass the significance threshold set at $p<0.01$.

When comparing stress vs. recovery, we observed significantly stronger connectivity in a network encompassing regions of the temporal cortex, specially the right temporal cortex, medial frontal areas and medial occipital areas (*Figure 2.4*). In particular, the right Rolandic Operculum, together with the right Heschl gyrus, were the two main hubs of a network of significantly stronger connections in the early stage of stress in contrast to the late stage of stress. These two hubs appeared to have significantly stronger connections to medial superior frontal and orbitofrontal regions, and paracentral gyrus and precuneus, which then distributed these connections further to visual areas such as the cuneus, lingual gyrus, and superior and medial occipital cortices. There was also stronger interhemispheric connectivity between the Rolandic operculum and its adjacent areas of the contralateral hemisphere. The right Rolandic operculum and Heschl gyrus further dominated due to their significantly stronger connections to the bilateral supplementary motor area, paracentral lobule

and, right hippocampus. The hippocampus could also be considered a hub due to its connections to the parahippocampal gyrus and fusiform gyrus, bilateral supramarginal gyrus, bilateral Rolandic operculum, superior temporal gyrus, calcarine, and bilateral lingual cortex.

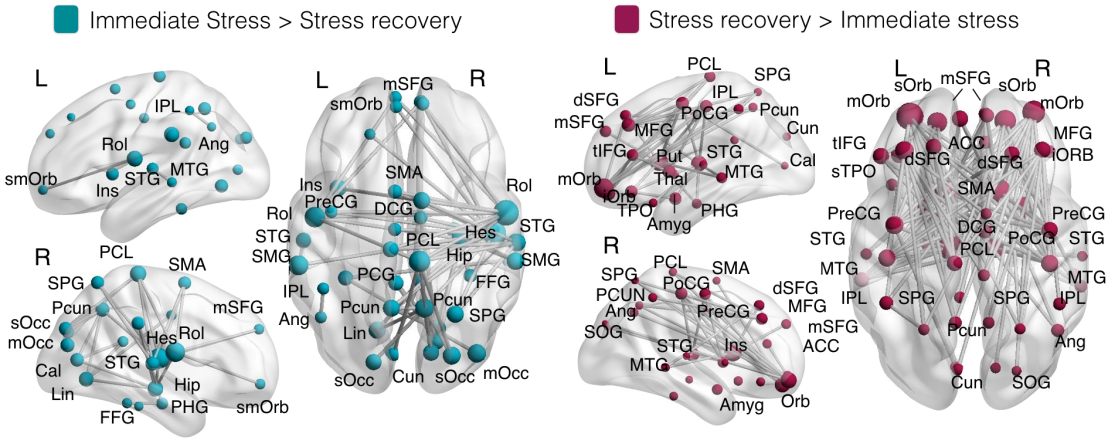


Figure 2.4. Stress recovery (compared to immediate stress) involves prefrontal and basal ganglia regulation. *The sizes of the nodes represent their degree of connectedness. During stress recovery we observed an increase in the functional connectivity of a widespread network with regions of the orbital cortex, caudate and thalamus as main hubs. This came accompanied by a decrease in the functional connectivity mainly among temporoparietal areas. The level of significance is p<0.01 after correction for multiple comparisons taking into account the p-values of the three contrasts: before stress vs. immediate stress, immediate stress vs. stress recovery, before stress vs. immediate stress. The corresponding AAL labels for each abbreviation are provided in Supplementary Table 1.*

When assessing stronger FC in recovery in contrast to immediate stress, we observed an increase in the strength of the connections among frontal, limbic and temporoparietal areas (*Figure 2.4*). In particular, the bilateral mid orbitofrontal nodes were amongst the nodes with the highest degrees, due to their widespread connectivity to regions such as the pre- and postcentral gyri, superior and inferior parietal cortices and precuneus. The other main hubs of this network were subcortical nodes, namely the bilateral caudate and the left thalamus. These structures appeared more strongly connected to superior frontal cortex, precentral gyrus, parahippocampal gyrus, medial temporal gyrus, and in the case of the thalamus, amygdala and pallidum

and superior temporal pole. In this contrast, there were no significant differences in direct connections.

In summary, as time passes after being exposed to a stressful event, participants seemed to increase their FC within a complex network of frontal, temporoparietal and, specially, limbic and subcortical areas such as the orbital cortex, caudate, putamen and thalamus.

When comparing before stress to recovery, we again observed stronger bilateral connectivity between the medial orbitofrontal cortex and occipital cortex before stress (*Figure 2.5*). We further observed stronger FC between medial occipital and the medial temporal gyrus as well as stronger FC between the paracentral and other occipital areas involved in visual processing, such as the cuneus, lingual, and calcarine, as well as the left precuneus gyrus. The left precuneus was also more strongly connected to the superior parietal cortex.

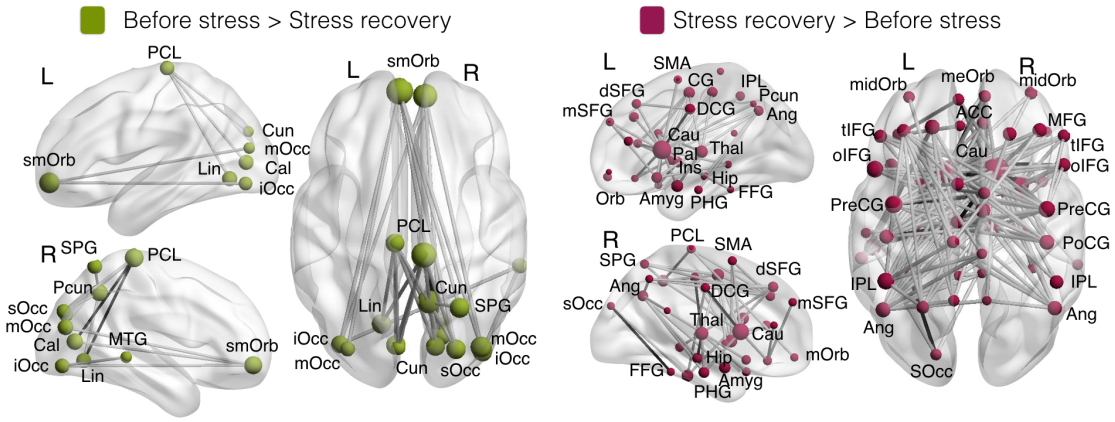


Figure 2.5. Stress recovery (compared to pre-stress) provokes significant prefrontal and basal ganglia regulation. *The sizes of the nodes represent their degree of connectedness. Grey edges represent significant differences in full correlations, while black edges represent significant differences in partial correlations (i.e., direct connections). When comparing stress recovery to the baseline (before stress) we found an increase in the functional connectivity around the caudate as main hub. This came accompanied by weakened functional connectivity between orbital and paracentral lobule and occipital regions. The level of significance was p<0.01 after correction for multiple comparisons taking into account the p-values from the three contrasts: before stress vs. immediate stress, immediate stress vs. stress recovery, before stress vs. immediate stress. The corresponding AAL labels for each abbreviation are provided in Supplementary Table 1.*

When considering the connections that become stronger during recovery in contrast to before stress, we observed a network of stronger FC around the bilateral caudate, thalamus and left putamen, in decreasing order of connectedness degree (*Figure 2.5*). The caudate exhibited significantly stronger connections to the inferior parietal lobe, supplementary motor area, pre- and post central gyrus, inferior orbital cortex, ipsilateral parahippocampal gyrus on the left and ipsilateral amygdala, medial temporal gyrus, medial and superior frontal gyrus on the right hemisphere. The thalamus was also connected to the pre-and postcentral gyri, hippocampus and parahippocampal gyrus on the left hemisphere, and amygdala in the right hemisphere.

Here partial correlations showed that before stress, there was a stronger direct connection between the right calcarine and lingual gyri and the paracentral lobule in contrast to stress recovery. In stress recovery, the following direct connections became

stronger: left fusiform gyrus and left superior occipital cortex, left postcentral gyrus and left caudate and putamen, bilateral caudate and mid cingulate gyrus, right temporal pole and right olfactory cortex, left hippocampus and thalamus, precentral gyrus and thalamus, and left caudate and left superomedial orbital cortex.

Characterising vulnerability and resilience in terms of functional connectivity

After testing for differences in between stress conditions, we explored how FC related to our measure of vulnerability based on a depression score within each of the stress conditions. Before stress, a few connections seemed to be positively associated with higher vulnerability scores. In particular, the interhemispheric connectivity between the supramarginal gyri became stronger as the vulnerability score increased *(Figure 2.6)*. The medial temporal gyrus and the inferior frontal gyrus (triangular pars) showed the same pattern. The dorsal cingulate gyrus and the posterior cingulate gyrus were more strongly connected to the medial frontal gyrus and medial occipital cortex respectively, in people with higher scores of depression. Furthermore, our partial correlation analysis showed that the increase in FC between the middle frontal gyrus and the dorsal cingulate cortex as the vulnerability score became higher was not due to correlations with other nodes. Our results did not show any negative associations between strength of connectivity and vulnerability scores before stress.

Immediately after stress, we observed an increase in FC between the bilateral olfactory regions and the bilateral Rolandic operculum, left Heschl gyrus, and left Supramarginal gyrus along with an increase in vulnerability scores *(Figure 2.6)*. The left olfactory cortex was also more strongly connected to the right supplementary motor area in participants vulnerable to stress. The other main hub was the left amygdala, which seemed more strongly connected to inferior frontal gyrus (opercular part), medial temporal gyrus, and superior parietal lobule in participants with higher vulnerability scores. Partial correlation analyses showed that the increase in FC between the amygdala and the opercular part of the inferior frontal gyrus was due to an increase in direct connectivity. On the contrary, participants with high vulnerability scores had weaker connections between the angular gyrus and frontal regions, such as the medial superior frontal cortex and the anterior cingulate cortex. They also had weaker connections between the left inferior parietal cortex and the insula as well as between the right thalamus and the left superior temporal gyrus.

Higher vulnerability scores were associated with a widespread network of stronger connections 1 hour and 30 minutes after stress. In particular, participants with higher scores exhibited stronger connectivity between frontal areas, such as inferior and superior frontal cortices and orbital regions, with parietal regions such as the angular gyrus and the inferior parietal cortex *(Figure 2.6)*. The main node of this network was again the left Heschl gyrus, which exhibited stronger connectivity with contralateral pre- and postcentral gyrus, inferior temporal gyrus and fusiform area. No negative associations were observed, indicating that people with higher vulnerability scores presented no decrease in FC.

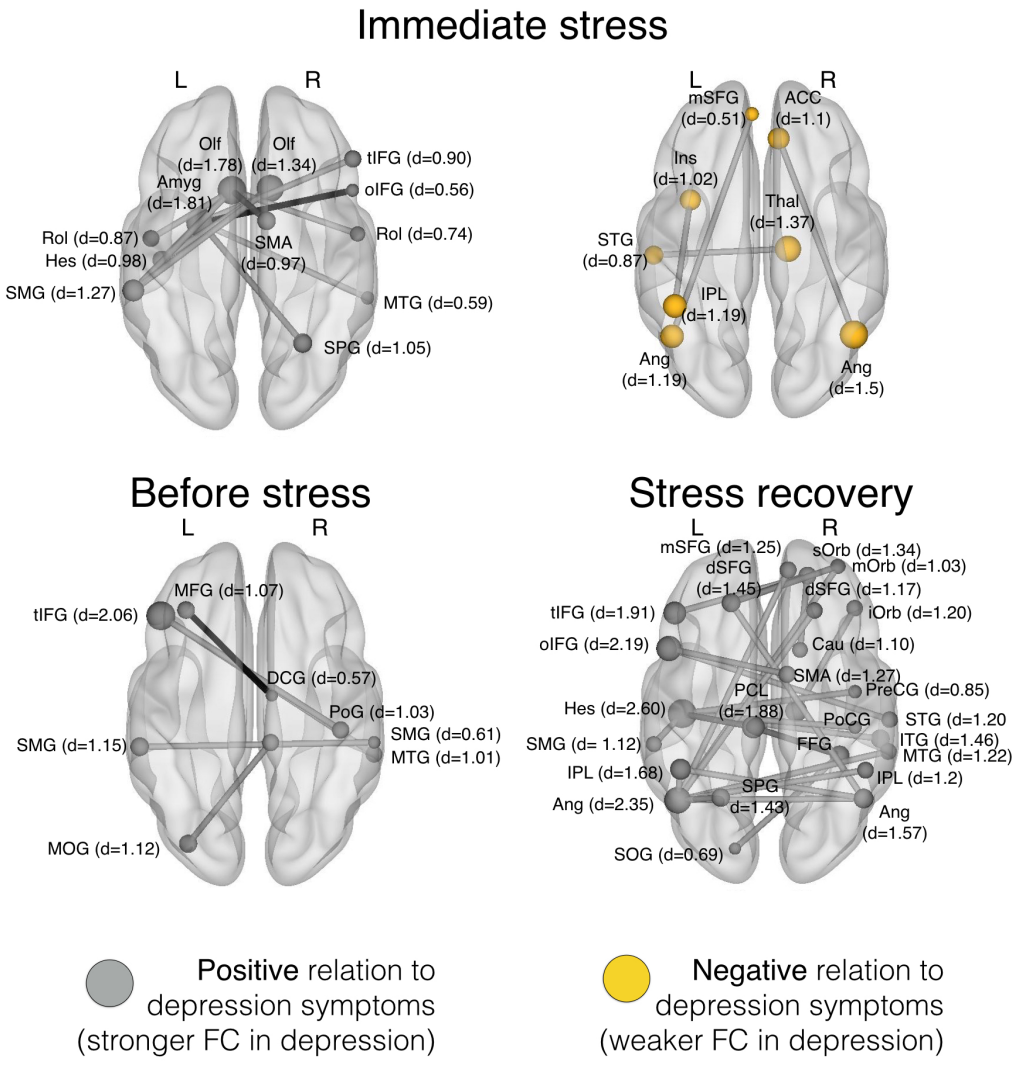


Figure 2.6. A subjective measure of resilience to acute stress is correlated with stronger functional connectivity between regions of the default mode network.

The sizes of the nodes represent their degree of connectedness. Grey edges represent significant differences in full correlations, while black edges represent significant differences in partial correlations (i.e., direct connections). Immediately after stress, resilience was associated with a pattern of stronger functional connectivity between bilateral angular gyrus and frontal areas, that is, between regions part of the DMN. This was presented together with stronger functional connectivity between limbic regions (amygdala and bilateral olfactory regions) and other brain regions in participants scoring higher in vulnerability. The level of significance was p<0.01 after correction for multiple comparisons taking into account the p-values of the three contrasts: before stress vs. immediate stress, immediate stress vs. stress recovery, before stress vs. immediate stress. The corresponding AAL labels for each abbreviation are provided in Supplementary Table 1.

DISCUSSION

Summary of results

In brief, during stress recovery the basal ganglia and thalamus became more strongly connected to frontal, temporal and parietal cortex. While this increase in connectivity was widely spread among frontal, temporal and parietal regions, the cortical regions with the highest increase in connectedness in stress recovery belonged the orbitofrontal cortex. The orbitofrontal increase in connectedness however appeared only when comparing stress recovery to the resting-state scan acquired before stress induction. Hence, overall, the results suggest that stress recovery is associated with an increase in connectivity between basal ganglia and widespread cortical regions, perhaps hinting at an increase in the activation of the cortico-basal ganglia loops in overcoming stressful situations. Specially marked was the increase in connectivity between basal ganglia and orbitofrontal cortex, main constituents of the reward cortico-basal ganglia loop.

Characterisation of functional connectivity immediately after stress

Overall, immediate stress as characterised in contrast to both before stress and stress recovery, shared the characteristic of stronger FC among temporoparietal regions and both frontal and parietal regions adjacent to the central gyrus. However, despite the observation of a shared network of differences that remained present independently of which of the other two conditions had been used for the contrast, it is important to

note some divergences from this shared 'immediate stress network'. In particular, when comparing *immediate stress* to *before stress*, we observed a hub in the right basal ganglia, with high degree of connectedness to temporal regions and to the primary motor cortex (precentral gyrus). Interestingly, the motor loop of the basal ganglia terminates in the primary motor area, supplementary motor area and premotor cortex. However, other stronger differences in FC were observed between cortical areas. The area labelled by the AAL as the Heschl gyrus, also known as transverse temporal gyrus, appeared also as a hub. This area belongs to the primary auditory cortex and is the first cortical structure to process auditory information. When looking at the brain regions more strongly connected immediately after stress than during stress recovery, the differences around the basal ganglia disappeared and the degree of connectedness between the right Heschl gyrus and adjacent areas (i.e., Rolandic Operculum and superior temporal gyrus) to the paracentral lobule and supplementary motor area gained prominence. Furthermore, the network of differences extended, showing increased FC to frontal and occipital areas.

Characterisation of functional connectivity in stress recovery

On the other hand, when comparing stress recovery to any of the other experimental conditions, we observed a very clear shift towards stronger connectivity between frontal areas and temporal and parietal areas predominantly. Interestingly, we also observed stronger connectivity among subcortical structures, in particular basal ganglia and thalamus, as well as with frontal and orbital areas and, again, cortical areas adjacent to the central sulcus (paracentral lobule, precentral gyrus (primary motor cortex) and postcentral gyrus (primary somatosensory cortex). Although the higher degree of connectedness of basal ganglia structures and the thalamus was present when comparing stress recovery to both immediate stress and before stress conditions, only the contrast with immediate stress showed a similar increase of connectedness in regions of the orbitofrontal cortex. On the contrary, the comparison of FC during stress recovery with the before stress condition had the bilateral caudate, dorsal part of the basal ganglia, as single hub, with all the other hubs showing significant differences in FC having lower and similar degrees of connectedness.

Integration of results with previous studies on this dataset

Despite of several FC studies having been conducted on this dataset (Maron-Katz et al., 2016; Vaisvaser et al., 2016, 2013), only one of them used a whole-brain approach (Maron-Katz et al., 2016). Although Maron-Katz et al.'s study analysed whole-brain FC by looking at each pair of regions like in this study, there are important differences between the two. First of all, the parcellation used by Maron-Katz et al. is more fine-grained than the AAL we applied in our analysis. Since their initial results were highlighting widespread connections across the brain, they opted for a two-level analysis to further characterise the strongest differences in FC. Their final network of differences was partly similar to the results we obtained using the AAL parcellation. However, they only assessed differences between immediate stress in contrast to before stress induction. As a consequence, the results regarding increased striato-cortical connectivity in stress recovery are unique to our study.

Cortico-basal ganglia loops in stress

When we consider the nature of the stress stimuli used in our paradigm, the concentration of differences in FC around cortical areas like the paracentral lobule, Rolandic operculum and superior temporal gyrus immediately after stress is not surprising. While acute and life-threatening stimuli lead to rapid stimulation of the hypothalamus and efferent visceral pathways, psychological stressors activate higher-order brain structures for further interpretations of perceived endangerment (Fuchs and Fluegge, 2003). As participants entered the stress recovery phase, the connectivity between the basal ganglia and cortex became more pronounced. The basal ganglia, which include the striatum, are a set of structures deeply rooted under the cerebral cortex, and their high degree of interconnectedness with stress-related structures such as the hippocampus, amygdala and prefrontal cortex is well documented (Haber and Knutson, 2010). The striatum, together with the thalamus, constitutes the subcortical core of several (partially) parallel thalamocortical loops projecting to different cortical areas. The function of each of these loops is still under debate, but they are traditionally related to motor, oculomotor, reward and associative functions. In particular, the reward circuit, whose main structures are strongly implicated in resilience to stress (Russo et al., 2012), might be specially relevant in this context. Remarkably, we observed an increase in FC between striatum, thalamus and

orbitofrontal areas, all part of the reward circuit. We also observed stronger functional connetivity between striatum, thalamus and dorsolateral prefrontal areas, related to the associative circuit, although in this case the increased strength of connectivity was less marked.

Despite the initial conceptualization of these loops as parallel and segregated from each other, more recent investigations support the hypothesis of a dual organization that permits both parallel and integrative processing central to both cognitive and motor processes (Belin and Everitt, 2008; Draganski et al., 2008; Haber et al., 2006, 2000) hence allowing for communication between different parts of the reward circuit and the associative circuit (Haber et al., 2003). Indeed, brain function during the immediate stress and stress recovery phases is likely to reflect adaptive behaviour, which in its early phases requires a combination of reward evaluation and associative learning, followed by development of appropriate action plans and inhibition of inappropriate choices based on the previous experience (Haber et al., 2003). A large amount of research exists highlighting the role of the striatum and connected regions of the prefrontal cortex during affective learning, key for goal-directed behaviours (Balleine et al., 2007).

Relationship between vulnerability and functional connectivity

When looking at the relationship between FC and vulnerability scores, we found that participants that are temporally vulnerable, as scoring higher in in a depression questionnaire at the time of the experiment, had stronger degree of connectedness between areas of emotional processing, such as the amygdala and orbitofrontal cortex, and the rest of the brain immediately after stress. Previous research has shown that vulnerability to stress is associated with modulation of stress brain activity by the amygdala (Charney, 2004; Feder et al., 2010; Franklin et al., 2012; Quaedflieg et al., 2015; Vaisvaser et al., 2013). Furthermore, we also observed that temporally vulnerable participants had weaker FC between regions belonging to the DMN, in particular frontal areas and bilateral angular gyri. Importantly, previous research has shown that disruption of the DMN is associated with psychiatric disorders such as depression and PTSD (Hamilton et al., 2011; King et al., 2016; Miller et al., 2017; Sheline et al., 2009; Wise et al., 2017).

Future steps

In the broader context of this thesis, the goal of this chapter was to approach the characterization of stress and stress vulnerability using similar methodology to previous studies, in order to establish a baseline comparable to the literature. In that regard, we can conclude that our results are coherent with previous studies. Furthermore, the whole-brain FC approach, in contrast to previous studies using an amygdala-centered seed-based FC, has highlighted the importance of the basal ganglia in relation to stress recovery. We also observed a relationship between vulnerability and FC of the DMN. In order to deepen our understanding of these and other networks in relation to stress beyond the insights that a static perspective can offer, we will explore their temporal behaviour in the next chapters. In particular, we will move from a pairwise static approach to a large-scale network, time-varying perspective. This will help us to get closer to a disambiguation of our static FC results, for example by investigating whether the observed differences are due to increased/decreased time visiting specific patterns of transient FC.

Chapter 3

Functional connectivity dynamics in stress resilience: Network dominance and temporal stability

Abstract

In this chapter, we used a time-varying functional connectivity approach to investigate the temporal dominance and stability of six states in relation to acute stress and resilience. Each of the states is characterised by two decoupled communities of brain regions showing coherence within each of the communities. Our results showed a decrease both in temporal dominance and stability of a state characterised by BOLD phase coherence among subcortical structures (basal ganglia and thalamus) in stress recovery in contrast to immediate stress. Furthermore, we found a positive relationship between temporal stability of a frontoparietal state and vulnerability scores immediately after stress, followed by a negative relationship between the same vulnerability scores and both temporal stability and dominance during stress recovery. Thus, we found that both temporal dominance and stability of specific transient networks change across stages of acute stress and are affected by stress vulnerability.

Introduction

In the previous chapter, we showed that the basal ganglia and the thalamus were at the core of a network more strongly connected to frontal, temporal and parietal cortex in stress recovery in contrast to immediately after stress. We also established a positive relationship between cortico-limbic strength of connectivity and vulnerability scores. However, we used a static approach, i.e., computed a single measure of functional connectivity over time, possibly neglecting relevant information contained in the temporal fluctuations. As mentioned in the introduction, in general brain function and, in particular, the acute stress response and the associated resilience mechanisms are intrinsically dynamic. In this context, animal research has highlighted the importance of different processes unfolding dynamically across time in response to

stress, some of them potentially related to resilience mechanism (Hermans et al., 2014; Krystal and Neumeister, 2009; McEwen, 2007). From a theoretical point of view, the model proposed by Sousa (2016) suggests that the transition from the acute to chronic stress occurs through repeated activation of stress patterns whenever a stressful stimulus is presented, eventually resulting in a shift in the pattern of activation. Thus, the response to stress once the transition to chronicity has taken place involves additional neurons and modified stress-control nodes and networks. The duration of the activation of the acute stress pattern might have potential implications in the transition to chronicity. In fact, efficient termination of the acute stress response has often been associated with resilience (Karatsoreos and McEwen, 2011). Therefore, investigating the time spent visiting particular activation patterns might potentially help to further our understanding of the stress response and why some individuals appear more vulnerable to the effects of stress than others. In summary, these ideas are indicative of the importance of considering the temporal dimension in order to understand the different stress mechanisms.

Investigating the stress response through resting-state networks

While in the previous chapter we used a FC approach based on computation of pairwise correlations, a more holistic approach to FC consists in the study of resting-state networks, i.e., large-scale functional networks of coherent signal among the regions that integrate them. Many of these networks are considered to underlie specific cognitive processes (e.g., Smith et al., 2009) required for responding adaptively to environmental conditions. A few previous studies have investigated differences in the large-scale networks in relation to stress. For example, a study by Soares et al., (2013a) found increased activation in the DMN, dorsal attention, ventral attention, sensorimotor and primary visual networks in a sample of students exposed to prolonged stress in contrast to a matched sample of non-stressed students. When assessing the same stressed students at a later point in time at which recovery had, at least to some extent, already taken place, a second study by the same research team found decreased FC in the DMN, ventral attention, and sensorimotor networks when comparing the 'recovery scans' to the 'prolonged stress scans'. Interestingly, the normalization of the networks after recovery was only partial, as they still showed stronger FC in dorsal attention, somatosensory and visual networks in comparison to non-stressed students (Soares et al., 2013b).

A later study, aiming to bridge the gap between our understanding of the stress response at the cellular and at the systems level investigated large-scale networks together with changes in neuronal dendritic morphology in rodents. In accordance with the results from the stressed students study by Soares et al., (2013a) the study on rodents found increased connectivity within somatosensory, visual and DMN following a paradigm of chronic stress (Henckens et al., 2015). This was accompanied by the expected stress-related changes at the cellular level, namely increase in dendritic length in the basolateral amygdala and decrease in the hippocampal CA3 pyramidal neurons.

Time-varying functional connectivity studies in stress

While few studies are available on the dynamics in large-scale brain networks in relation to arousal and stress, the field has recently started to recognise how temporal fluctuations in large-scale networks might be important for understanding adaptive behaviour. In a recent study, Young and colleagues used a very intuitive example to illustrate this: during a physical attack, it is natural for the brain to prioritize behaviours that are immediately relevant, such as identifying the location of the attacker, while other responses (e.g., processing of details about the attacker) are relegated to a second plane. The first behaviour, thought to rely on a different large-scale brain network, would have to be (transiently) prioritised. Thus, it has been suggested that changes in cognitive priorities are a product of shifts in interactions within and between networks (Hermans et al., 2014, 2011; McMenamin et al., 2014).

To investigate this, in the study by Young et al., (2017), they used a sliding window approach to estimate measures of within network cohesion for the SN, the CEN, and the DMN, the three main networks thought to be related to stress and stress-related psychopathology. Their results showed that SN within-network cohesion (i.e., all voxels within the SN network increase or decrease activity together) increases monotonically with arousal as measured through heart rate acquired continuously during an fMRI stress paradigm. They also found that between network cohesion (i.e., correlation of within network cohesion between two networks across time) of the SN-CEN pair holds an inverted-U relationship with arousal (Young et al., 2017).

A second study interested in the dynamics of the stress response used a 'threat of shock' paradigm to define early, intermediate and late phases. They found that following the cue for threat, there was an early transient phase in which signal in the SN and visual cortex increased and the signal in the DMN decreased (McMenamin et al., 2014).

Time-varying functional connectivity studies in major depression

In addition to gaining a better understanding of the different phases of the stress response, we are also interested in how the dynamics of the stress response are related to stress vulnerability. Here, as in Chapter 2, we use a measure of depression (PHQ9) obtained after a prolonged period of stress (approximately two years of military activity) as a proxy for vulnerability. Thus, in the following section, we discuss the few studies that have used a dynamic approach to the study of depression to provide some context for our study.

Most of the previous studies investigating time-varying FC have focused on variability of connectivity across time, in order to make inferences about temporal fluctuations in the connectivity between regions. For example, a previous study in depression showed decreased variability in the FC between regions of the parahippocampal gyrus and the medial prefrontal cortex together with increased variability in the FC between the medial prefrontal cortex and the insula. Furthermore, the increased variability in the FC between prefrontal cortex, part of the DMN, and the insula, part of the SN, correlated with a ruminative thinking style (Kaiser et al., 2015b). Two more recent studies looking at seed-based time-varying FC in major depression found increased dynamic FC between the medial prefrontal cortex and the posterior cingulate gyrus (Wise et al., 2017) and between the anterior insula and the right precuneus, temporal pole, and left dorsolateral prefrontal cortex (Pang et al., 2018). However, all these studies used a sliding window approach to investigate the dynamics, which has some limitations regarding inefficient use of the data. In brief, short windows will result in less robust estimations due to inclusion of relatively few data points (noisy results), whereas longer windows will be less able to capture short-lived changes in FC (inefficient use of data). Thus far, to our knowledge, only one study has used a whole-brain approach together with time-varying FC not based on a sliding window approach to investigate the dynamics of depression. This study based

its dynamic FC measure on instantaneous phase coherence and found decreased variability of FC between regions of the DMN and regions of the FPN in participants with major depressive disorder (Demirtaş et al., 2016).

Data-driven approaches for time-varying connectivity

Our approach, as in the study by Demirtas et al., (2016), is based on the coherence of the instantaneous phases of the BOLD signal. However, our analysis is different in that we compute the leading eigenvector for each matrix of instantaneous FC, reducing dimensionality and leading to a less noisy representation of the FC patterns whose temporal stability and dominance will be studied. As in previous publications using this method, these instantaneous FC patterns show similarity with known resting-state networks (Cabral et al., 2017; Lord et al., 2018). A resting-state network that rarely appears in the literature, specially in relation to stress, is the basal ganglia network (Robinson et al., 2009). A reason why the study of this network has remained scarce might be the difficulty to extract it using data-driven approaches such as ICA. This has been suggested to be related to a lower signal to noise ratio in subcortical structures (Robinson et al., 2009). Of note, the data-driven approach used in this chapter enabled us to detect a state resembling this basal ganglia network, allowing us to investigate its dynamics and, consequently, elaborate on the results obtained in Chapter 2 using a static FC approach.

METHODS

Participants

This study was conducted on the same dataset as Chapter 2, and therefore the participants, study design and stress induction task is the same. For more details, please see Chapter 2, *Methods*.

Behavioural measures

The Patient Health Questionnaire (PHQ9) is a 9 item self-report measure of depressive symptoms, which has been validated for use as a screening tool for depression in the

general population (Kroenke et al., 2001). The questions are based on the Diagnostic Statistical Manual IV criteria for a depressive disorder. For more details on the justification of its use, see Chapter 2, *Behavioural measures*.

fMRI data preprocessing

For details of data preprocessing of this dataset, please see Chapter 2, *Data preprocessing*, as we have followed here the same pipeline.

Time-varying Functional Connectivity

To characterise the time-varying FC we used Leading Eigenvector Dynamic Analysis (LEiDA) (Cabral et al., 2017). Briefly, this method makes a drastic reduction of dimensionality of the FC information. More explicitly, it reduces the instantaneous FC (i.e., FC at each point in time or TR) to its leading eigenvector and then clusters these vectors into recurrent spatial patterns across time that can be characterized in terms of probability of occurrence (i.e., fractional occupancy), duration (i.e., lifetime) and switching or transition profiles. To do so, first, the Hilbert transform is used to estimate the BOLD phases, θ(n,t), transforming the signal of each brain region in polar coordinates as *x(t)=A(t).*cos(θ(t)) (Figure 3.1a).* In other words, we obtain the angle of the BOLD signal for each brain region at each point in time. Subsequently, the phase coherence $FC_t(n,p,t)$ between brain areas *n* and *p* at time *t* is obtained using Equation 3.1:

$$\boldsymbol{FC_t(n,p,t) = cos\big(\theta(n,t) - \theta(p,t)\big)} \quad \textit{Equation 3-1}$$

Given that cos(0)=1, two areas n and p whose BOLD signals are temporarily aligned (i.e. similar angles) will result in a value for instantaneous FC close to 1. Time points in which the BOLD signals are orthogonal (e.g., one *increases* at 40° while the other one *decreases* at 40°) will show a value for instantaneous FC close to 0. Overall, we obtain a three-dimensional matrix with size *NxNxT,* where *N*=86 is the total number of brain areas and *T=99* is the total number of fMRI time points acquired. These $NxN\ FC_t(t)$

matrices will be symmetric across the diagonal given that phase coherence is undirected (*Figure3.1b, left*).

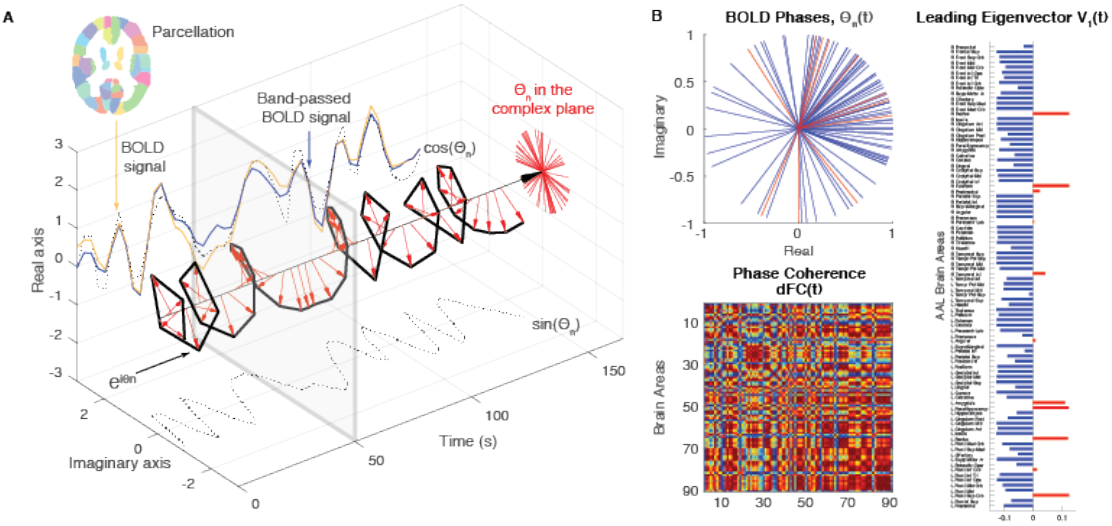


Figure 3.1. The leading eigenvector dynamic analysis (LEiDA) method allows detection of transient brain states based on phase coherence. *a) Illustration of the Hilbert transform on the BOLD signal of one brain region, allowing us to compute the phase through transformation into the polar coordinates. b) Representation of these coordinates as complex numbers (left, up) and transfer to matrix format (left, down). The leading eigenvector is then computed from the instantaneous functional connectivity matrix and shows the two communities (red, blue) and their association strength (magnitude)* (right).

Previous work has applied k-means clustering on similar time specific matrices to find the recurrent patterns of FC (Damaraju et al., 2014; Marusak et al., 2017). We, however, first computed the leading eigenvector V for each of the matrices *(Figure 3.1b, right)* capturing the dominant connectivity FCt(t) at time t, given by its outer product $V_1 V_1^T$. In doing so, we reduce dimensionality from N(N-1)/2 (upper triangular part of a symmetric matrix) to N while still taking into our analyses most of the variance in FC patterns. Most importantly, by doing this, our analysis becomes more robust against high-frequency noise, a disadvantage of all quasi-instantaneous measures of FC. This allows detection of the timepoints when the variance of the dynamic connectivity becomes dominated by a specific pattern without being concealed by the noise. A consequence of this is that the main patterns and their recurrences can be more easily

detected, potentially allowing the clustering algorithm to work more accurately (Cabral et al., 2017).

These eigenvectors of connectivity matrices have been previously used to find community structures in networks (Leicht and Newman, 2008; Newman, 2006), where network partition is performed according to the eigenvectors' elements signs (e.g. those regions with a positive sign belong to one community and those with a negative sign belong to the other). It is important to note that the sign is not identifiable; that is, the relative sign is well-defined, but it is arbitrary whether we obtain one solution or another with opposite sign for all regions. That means, that it is not possible to relate the positive sign to activation and the negative sign to deactivation. However, it allows us to find not only two different communities, the one with a negative sign in blue (or light grey) and the one with positive sign in yellow (or dark grey), but also their associated dominant connectivity patterns *(Supplementary figure 3)*. Please also note that, when all elements have the same sign it means that VV^T is non-negative, and the network has a mode of global covariance (State 1, coloured entirely in blue or light grey). Moreover, the 'strength' with which brain areas belong to either of the communities is indicated through the magnitude of eigenvector elements (Newman, 2006), coded as colour brightness in the rendered representation or sphere sizes in the schematic brain plots.

Following the calculation of a leading eigenvector for each time point (T=99) and each subject (n=48) at each experimental condition (before stress, immediate stress and stress recovery), a discrete number of FC patterns can be detected by using a k-means clustering algorithm. This algorithm was run for 19 partition models, with a *k* ranging from 2 to 20. For each clustering solution we obtained a *k* number of cluster centroids in the shape of *Nx1* vectors, which represent recurrent FC patterns, from now on also referred to as FC brain states. The FC patterns belonging to the clustering solutions ranging from *k*=2 to k=8 are presented in *Supplementary figure 1,* where we can observe that the FC patterns are similar across clustering solutions, increasing one at a time as we increase *k*.

Criteria for the number of states

To decide on the optimal number of FC states, we have two criteria: 1) inclusion of a FC state in which one of the two communities is dominated by regions of the basal ganglia 2) high compactness of the set of clusters according to the Dunn's Index *(Supplementary figure 2)*. The first criterion guarantees the possibility to study the role of the basal ganglia in relationship to stress from a time-varying FC perspective, complementing the results from Chapter 2. The second criterion optimises the number of states according to its compactness so that each centroid is made based on the contribution of FC states that are similar between them and different from those of other clusters. According to these two criteria, it seemed that the optimal compromise was estimated to be k=6 states, which has the second highest Dunn's index and contains a basal ganglia driven FC pattern. Despite of this one not being the clustering solution with the highest Dunn index, the algorithm was still able to reach the same index across different initialisations, showing stability in the FC patterns.

Temporal Dynamics statistics

After deciding the optimal number of states, we computed two temporal dynamics statistics: fractional occupancies and lifetimes. Here, fractional occupancy refers to the number of epochs assigned to a given cluster centroid or FC pattern divided by the total number of epochs in each experimental condition (i.e., 99 TR). Hence, the fractional occupancy represents the probability of occurrence of each FC state for each subject in each of the experimental conditions, and is therefore taken as a measure of temporal dominance for each FC pattern. Lifetimes refer to the average of consecutive TRs or timepoints during which a particular FC patterns was active, therefore representing their temporal stability.

Statistical analysis

Statistical analyses were carried out using Permutation Analysis of Linear Models (PALM) (Winkler et al., 2015, 2014). First, we investigated differences in the fractional occupancies and lifetimes of the states between conditions using permutation testing with the base of paired t-tests. We also used permutation testing with the base of one-sample t-tests to evaluate the relationship between fractional occupancies and

lifetimes and vulnerability scores. Correction for multiple comparisons was applied using FDR (Benjamini and Hochberg, 1995) across number of states.

RESULTS

Functional connectivity states

As discussed above, we chose k=6 as the most appropriate number of brain states for this particular study (see *Methods, Criteria for the number of states,* and *Supplementary figure 2).* From the six states, five resemble known resting-state networks, namely the default mode, sensorimotor, visual, frontoparietal, and subcortical networks, and a sixth state is characterised by global covariance (*Figure 3.2*). An illustration of each brain state together with its AAL labels is provided in *Supplementary figure 3*.

To assess the degree of overlap between the other five brain states and traditional resting state networks, we created spatial maps using the regions belonging to the yellow/red *(Figure 3.2)* or dark grey *(Supplementary figure 3)* communities and computed the percentage of voxels overlapping with the relevant resting-state network parcellations presented in (Yeo et al., 2011), a seminal paper that has had considerable influence in the field of resting-state networks neuroscience. We found an overlap of 47% between our second state and the default mode network, 81% between our third state and the sensorimotor network, 80% between our fourth state and the visual network, and a 59% between our fifth state and the frontoparietal network. The sixth state could not be compared to any of the Yeo et al., 2011 resting-state networks because, as most resting-state studies, the networks presented were containing mainly cortical regions.

We performed a second analysis in this case using Neurosynth, a platform containing results from over 14.000 fMRI studies (Yarkoni et al., 2011). This allowed us to conduct quantitative comparisons between our yellow/red community maps and meta-analysis maps related to our terms of interest, i.e., names of the resting-state networks that reached most overlap in the previous comparison. In particular, we used the keywords 'default mode' 'sensorimotor', 'visual', 'frontoparietal' and (additionally) 'basal ganglia' to generate Neurosynth spatial maps based on an automatic meta-analysis. We then computed the number of overlapping voxels with our own

yellow/red community spatial maps, in order to keep an approach comparable to the one used with the Yeo et al., (2011) cortical maps. The basal ganglia network reached a 40% of overlap when computing a meta-analysis based spatial map of this set of structures. The frontoparietal network reached only a 21% of overlap, but this could be related to the procedure Neurosynth uses to extract the coordinates. In particular, they automatically search for the coordinates in studies that have a significant amount of the term 'frontoparietal' in their text. While this might work quite well for more 'unitary' networks, like the somatosensory or visual network, the FPN has been shown to be particularly heterogenous, with several subsystems being part of an overarching control network that includes the frontoparietal network (Dixon et al., 2018). This can then result in an output map with coordinates of related subsystems, such as the dorsal attention network. Further comparisons showed that our default mode network had a 42% overlap, the sensorimotor a 60% and the visual a 63% of overlap with the respective Neurosynth spatial maps.

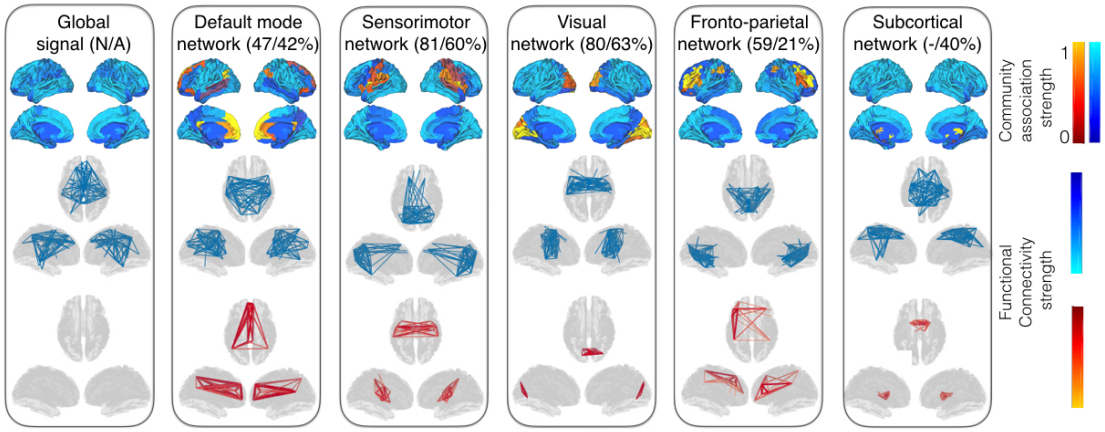


Figure 3.2. Comparing the six LEiDA states with known resting-state networks. a) Representation of each cluster in two different communities (blue/cold and yellow/hot) which are decoupled or in anti-phase with each other. Darker colours denote a stronger association of that particular brain region with its own community. b) Strongest functional connections (>97.5%) within the blue/cold community. c) Strongest functional connections (>97.5%) within the yellow/hot community.

In the following, we will refer to the states by the names or abbreviations of the resting-state networks they overlap with and consider this their primary community. It is however important to keep in mind that our states are characterised by decoupling between this one and a second community. The second community of the default

mode state, for example, shows most within-community strength in supramarginal gyrus, the Rolandic operculum and the precentral gyrus. The sensorimotor state has most within-community strength in the superior frontal cortex, posterior cingulate cortex, middle occipital cortex and precuneus, while the visual state has most within-community strength in the frontal and motor cortices, and the insula. The frontoparietal states' most strongly associated regions of the second community include the calcarine fissure, cuneus and lingual gyrus, as well as temporal regions such as the hippocampus, parahippocampus, and superior temporal lobe. Last, the second community of the basal ganglia state has strongest within-community strength in precuneus, precentral gyrus, and superior parietal cortex. Interestingly, in this state, the hippocampus and amygdala appear at the edge between the two communities, with the right hippocampus and amygdala leaning towards the first community and the left hippocampus and amygdala leaning towards the second one. In any case their magnitudes are very close to 0, meaning that these structures are neither strongly associated with the first, nor with the second community.

Comparison of temporal dominance and stability between experimental conditions

After the inspection of each FC state topology, we computed their fractional occupancies (i.e., probability of occurrence of a given state) and the mean lifetimes (i.e., number of consecutive epochs during which a given FC pattern or brain state dominates in variance). These two measures can give us some insight into the temporal dominance and temporal stability of each state, which in combination with the three different conditions allows us to better understand how the balance of the brain is perturbed and recovered after stress induction.

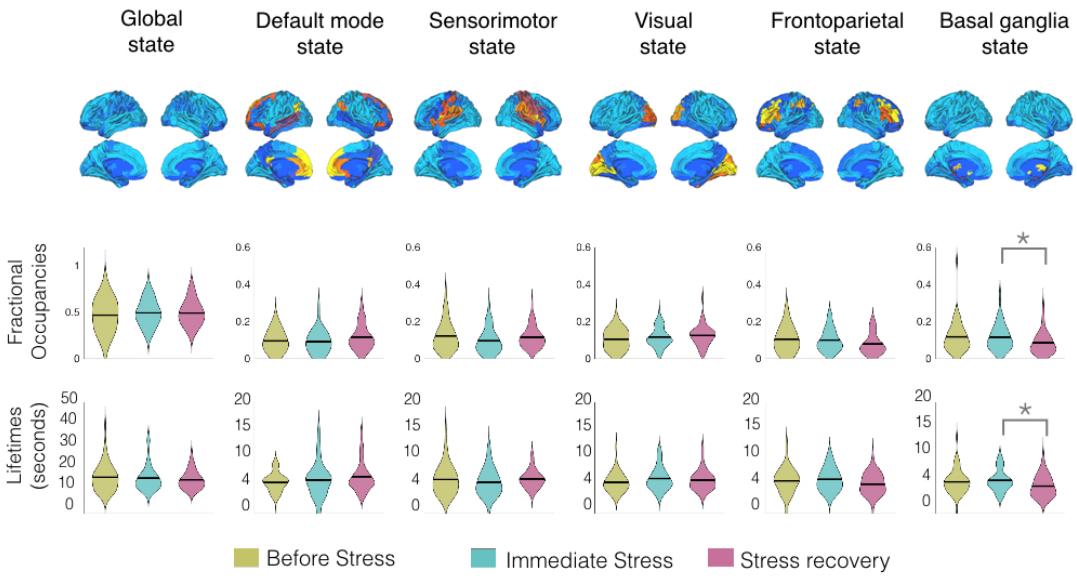


Figure 3.3. From the six states, a significant decrease in temporal stability and dominance of the basal ganglia state was found during stress recovery. *This state is characterised by coherence among the basal ganglia and decoupling from the cortex. Temporal dominance (fractional occupancies) and stability (mean lifetimes) are considered significant at * p< 0.05 after FDR correction.*

The basal ganglia state is the only one showing significant results after correction for multiple comparisons across number of states; in particular, we observed a significant decrease in both fractional occupancies (p = 0.0428) and lifetimes (p = 0.0202) during stress recovery in contrast to immediately after stress. None of the other states showed significant differences between stress conditions after correction for multiple comparisons, neither for the fractional occupancies nor for the lifetimes.

Vulnerability scores and their relationship to temporal dominance and stability

Following the comparison across conditions, we investigated the relationship between fractional occupancies and lifetimes of each state and the PHQ9 depression scores, used as a measure of vulnerability to the stress endured by our sample. Before stress, our results showed a significant positive relationship between lifetimes (i.e., temporal stability) of the basal ganglia network and vulnerability scores (p = 0.0504 after correction for multiple comparisons). This showed that, in anticipation of the experiment, participants that were showing the first signs of vulnerability spent more consecutive time in a network driven by basal ganglia (and thalamus) FC.

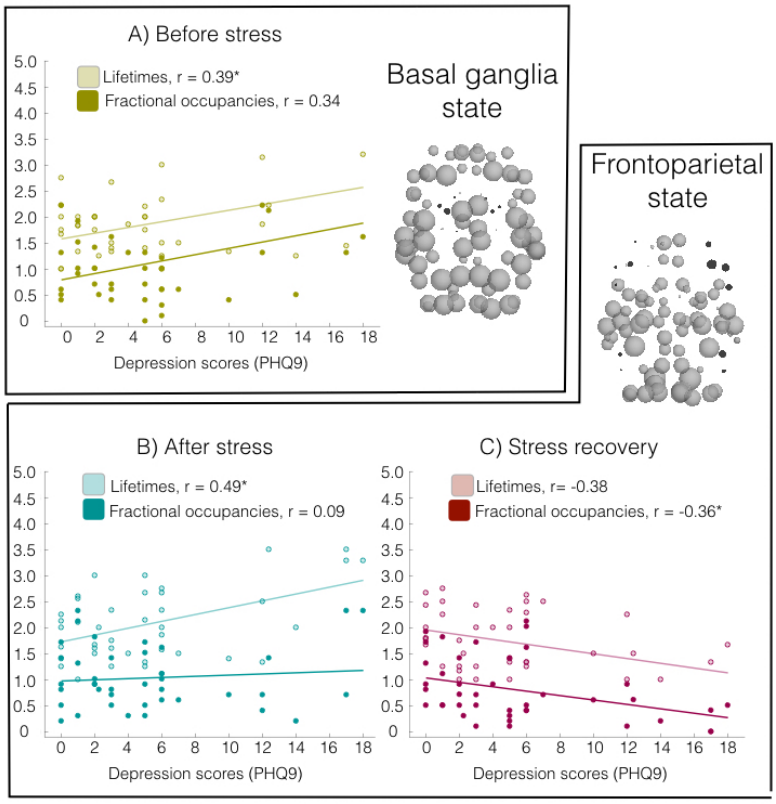


Figure 3.4. Vulnerability in relation to temporal dominance and stability. *a) Before stress, participants with higher vulnerability scores made longer visits (i.e., increased lifetimes or stability) to the basal ganglia state than those highly resilient. After stress, we observed a modulation in both fractional occupancies and lifetimes of the frontoparietal state in relation to vulnerability: b) Immediately after stress, there is an increase in the lifetimes of those scoring higher in vulnerability. c) During stress recovery, the relationship is inverted, showing that those with the highest scores in vulnerability have the shortest lifetimes and spend overall less time in the frontoparietal state.*

After stress, we observed a positive linear relationship between the lifetimes of the frontoparietal state and vulnerability scores (p=0.0084 after correction for multiple comparisons) (*Figure 3.4*). Interestingly, and despite the established increase in consecutive time spent on this network, the fractional occupancies or overall time spent on this network remained stable throughout the range of vulnerability scores. During stress recovery, however, there is a negative linear relationship between fractional occupancies and vulnerability scores (p = 0.0474 after correction for

multiple comparisons). The relationship between vulnerability and lifetimes, did not appear significant after correction for multiple comparisons ($p = 0.0732$). Hence, overall, we observe that participants scoring higher on the vulnerability scale spend more consecutive time on the basal ganglia state before stress and on the frontoparietal state after stress. Afterwards, during stress recovery, they are less able to engage the same frontoparietal state. No significant linear relationship between lifetimes or fractional occupancies and vulnerability scores was found for any of the other states (global covariance, default mode, sensorimotor and visual networks) (*Figure* 3.5).

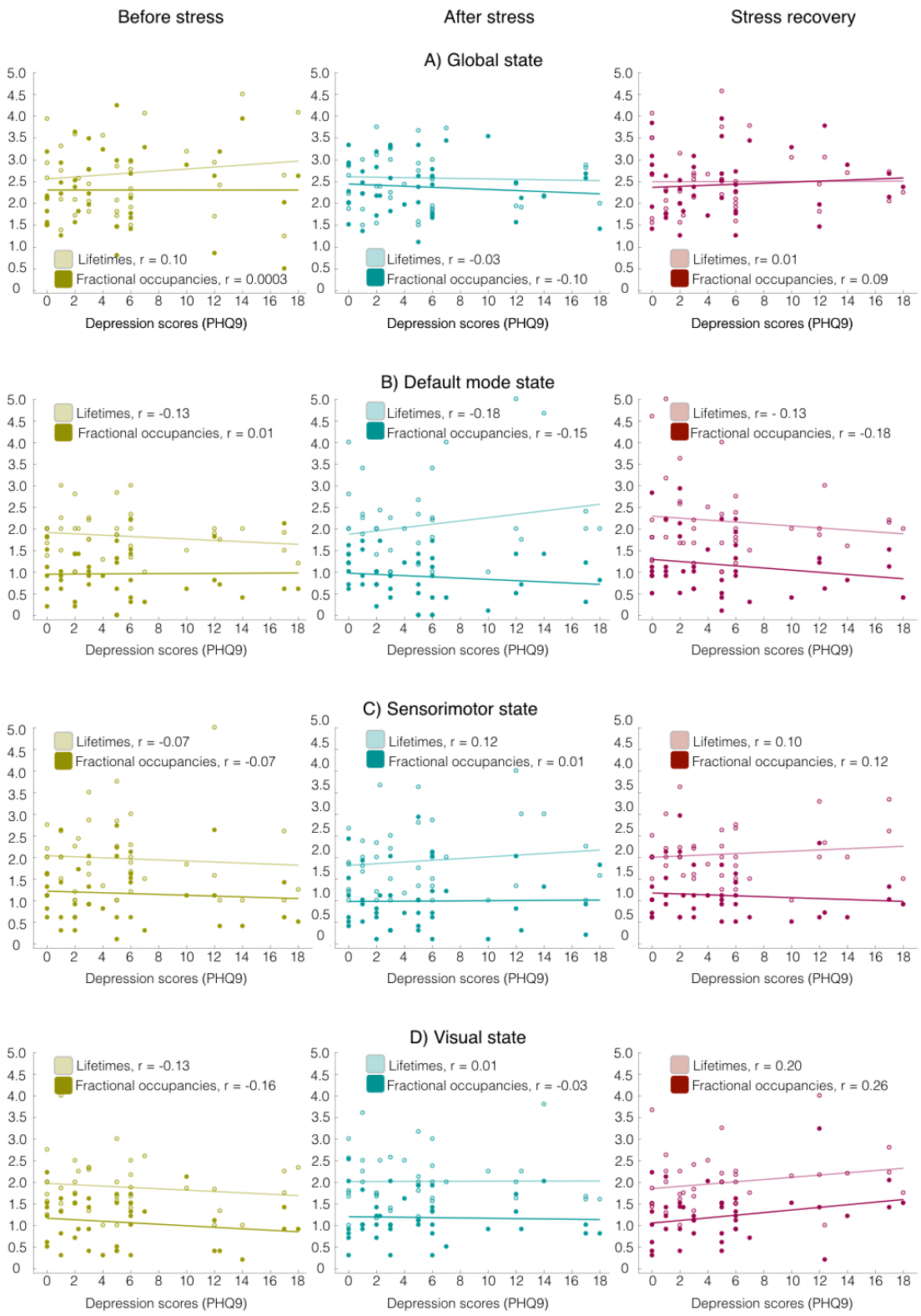


Figure 3.5. Vulnerability does not correlate with other transient functional connectivity states. *No other significant relationships between temporal stability (lifetimes) or dominance (fractional occupancies) were established at * p< 0.05 after FDR correction.*

Temporal dominance and stability of the basal ganglia state in stress

In this chapter, we have used LEiDA, a recently-proposed method (Cabral et al., 2017) to detect recurrent patterns of FC characterised by two communities of in-phase brain regions. These patterns resemble well-known resting-state networks, as confirmed though comparison with two sources: the resting-state networks in Yeo et al., (2011) and the spatial maps created through Neurosynth, a platform containing coordinates from over 14.000 published fMRI studies. In terms of their temporal properties, our results showed a decrease in both lifetimes and fractional occupancies of the state characterised by decoupling between the basal ganglia and the cerebral cortex during stress recovery in contrast to immediately after stress. This is coherent with the results from Chapter 2, in which we observed an increase in the static FC of the basal ganglia with frontal and parietal cortices in stress recovery in contrast to the other two stress conditions.

These differences between stress recovery and immediate stress seem to affect all participants equally, as no significant correlations were observed between the lifetimes and fractional occupancies of the basal ganglia state and vulnerability scores. In other words, the difference between conditions might be related to basic stress mechanisms, independent of subject-specific vulnerabilities. This is coherent with the evolutionary role of the basal ganglia and thalamus, part of the protoreptilian brain ('triune brain' theory, McLean (1990)), understood to represent the fundamental core crucial for survival in the face of threat. Together with the paleomammalian brain (i.e., limbic system), the protoreptilian brain is responsible for fast reactions to a stressor. In our experiment, as the environment becomes more familiar and the participant gets the time to recover from the induced stress, the time spent in this state decreased in favour of other states involving regions of the neocortex.

Only during the resting-state scan acquired before stress induction, we observe a positive relationship between the lifetimes and vulnerability scores. This could be reflecting different processes still belonging to the acclimation period. A reasonable speculation is that perhaps participants with higher vulnerability at the time of the experiment showed a more marked stress response in the unfamiliar environment that is an experimental setting.

Temporal features of the frontoparietal network are modulated by vulnerability symptoms

In regard to stress vulnerability, we also show that the higher the vulnerability score, the longer the lifetimes of the frontoparietal state immediately after stress. Despite of the increased duration of the states' lifetimes, the fractional occupancies were not affected. This is interesting, as the two measures follow quasi-parallel trajectories in all other states when assessing their relation to vulnerability scores (*Figures 3.4* and *3.5*). Furthermore, an hour and 30 minutes after stress, the relationship was inverted – the higher vulnerability scores, the shorter both the lifetimes and the fractional occupancies of the frontoparietal state.

Going back to the traditional resting-state networks, the FPN is considered to be part of the CEN, a system which can be decomposed into distinct cortical regions that are highly interconnected (Cole and Schneider, 2007; Yeo et al., 2011). These regions form different subsystems, characterised by high within subsystem connectivity (Cole et al., 2013; Power et al., 2011). The FPN is a subsystem considered to be especially involved in highly adaptive control process; it provides the anchor of the system with its hubs in the dorsolateral prefrontal cortex and the lateral posterior parietal cortex (Habas et al., 2009; Seeley et al., 2007). More specific roles involve active maintenance and manipulation of information in working memory, rule-based problem solving and decision making (Koechlin and Summerfield, 2007; Miller and Cohen, 2001; Müller and Knight, 2006; Petrides, 2005). The other two subsystems belonging to this overarching system of control are the cingulo-opercular and the dorsal attention network, involved in time-extended control processes and the coordination of attention to external stimuli, respectively (Corbetta and Shulman, 2002; Dosenbach et al., 2007).

Interestingly, cognitive control abilities and the control system responsible for those abilities have well-known limits (Mitchell and Cusack, 2008; Schmeichel et al., 2008). Furthermore, there are a variety of factors that reduce control capacity, and excessive stress is one of them (Sato et al., 2012). Under this circumstance, the increase in the lifetimes seen in more vulnerable participants could be reflecting a 'compensatory' mechanism trying to manage the overload of the frontoparietal system.

In more general terms, and given the FPN role in adaptive control processes, its appropriate functioning has been linked to maintenance of mental health (Cole et al.,

2014). If we consider its extensive connectivity and its flexibility (Cole et al., 2013) it is reasonable to think that suboptimal functioning of this network could have a widespread effect on the brain and on behaviour. Several theories have formulated explanations for the development of psychopathology based on the interaction between the FPN and, typically, the SN and the DMN. In general terms, the consequence of abnormalities in these interactions can translate into deficient context-dependent engagement and disengagement of cognitive systems. In the triple network theory, formulated by Menon (2011), the FPN has a relatively passive role – Menon suggests that the SN, and the anterior insula in particular, are crucial for initiating network switching leading to engagement of the CEN and disengagement of the DMN. Another theory, formulated by Cole et al., (2014), suggests that the way in which the control system achieves its flexibility is through implementation of feedback loops, further linking it to maintenance of homeostasis related to stress – in this case, the homeostasis refers to balance across distributed neural systems (Cole et al., 2014). When symptoms arise, the control system should be able to regulate the other systems in a goal-directed manner to overcome these symptoms, preventing the disorder. In the case of anxiety and depression, it has been suggested that the control system, rather than being primarily disrupted, is overloaded by the constant need to regulate symptoms, reducing its capacity for other cognitive demands (Anticevic et al., 2012).

Past, present and future

Previous studies using static FC approaches have shown evidence for disruption of the FPN in the context of depression (Etkin et al., 2009; Kaiser et al., 2015b, 2015a; Schultz et al., 2017). Although more studies are required in order to formulate more specific hypothesis of how this might be related to temporal fluctuations of brain function, our results offer some support towards temporal modulation of the frontoparietal state in relation to the first symptoms of depression or vulnerability to stress. In the next chapter, we will further explore the dynamics of brain function in the context of acute stress, in this case in the form of transitions from one state to another. In particular, we are interested in the observed increase in the lifetimes of the frontoparietal state immediately after stress and whether this might be further reflected in less frequent transitions from this to other networks in participants with higher depression scores. This would be consistent with theoretical frameworks regarding the frontoparietal and

related networks (e.g., central executive or control network) network as an adaptive hub fostering communication to other networks (Cole et al., 2014).

Chapter 4

Dynamic functional connectivity in stress resilience and vulnerability: brain state transitions

Abstract

Here, we continue to investigate the dynamics of stress and stress vulnerability by looking at the transitions between our states as detected through Leading Eigenvector Dynamic Analysis (LEiDA). Comparisons between conditions show that immediately after stress, participants switch more frequently between the basal ganglia, frontoparietal and visual states; during stress recovery, however, participants switch more often between visual, sensorimotor and default mode states. In relation to vulnerability scores, our results show that the increased lifetimes in the frontoparietal state in association with higher vulnerability scores observed immediately after stress were accompanied by less frequent transitions from this to other states, in particular, to the default mode network. Decreased transitions in relation to higher vulnerability scores were also found during stress recovery, in this case revealing a more widespread profile of decreased transitions among the frontoparietal, default mode and sensorimotor states. Results are discussed in light of theories proposing dynamic interactions between large-scale networks as essential for the maintenance of an internal equilibrium in the face of stressors.

Introduction

Summary of previous findings

In the previous chapter, we focused on the temporal features of each state individually, analysing their measures of temporal dominance and stability in the form of fractional occupancies and lifetimes. In this chapter we focus on the relationship between states in the form of transitions, and the directionality of those transitions. Of note, state transitions are related to lifetimes, because increased consecutive time visiting a particular state is necessarily associated with less switching to other states. However,

to which state the transition occurs and whether that transition is unidirectional or bidirectional might hold important information about the brain dynamics related to stress and stress vulnerability. We expect this analysis to add another dimension to the differences observed in the previous chapter, related to the basal ganglia and the frontoparietal states, as well as perhaps reveal meaningful patterns of transitions among the other states.

State transitions in stress and stress vulnerability

From the perspective of stress and stress-related disorders, not many theories include an explicit formulation about the transition patterns and their directionality. One of the few examples is the triple network theory proposed by Menon (2011), which suggests that the SN plays a crucial role in engaging the FPN, but its ability to do so is disrupted in disorders such as depression and PTSD. A second theory focuses on the role of the FPN as a control system integrated by flexible hubs that regulate other systems and whose disruption relates to mental disorders. In particular, an hypothesis suggested by this theory posits that the frontoparietal system is at the centre of a feedback loop involving a variety of other brain systems responsible for maintaining homeostatic balance via dynamic global connectivity (Cole et al., 2014). In particular it is suggested that, in the case of major depressive disorder, whose early symptomatology we have used to derive our vulnerability scores, the role of the FPN is disrupted by the need to constantly regulate symptoms, reducing its capacity for other cognitive demands. In Chapter 3, we observed an increase in the consecutive time spent on the frontoparietal state in more vulnerable individuals, in line with a compensatory upregulation of this system in a context of 'cognitive overload'. By investigating transitions from the frontoparietal state to other states we can explore the hypothesis of a (disrupted) feedback loop.

Exploring the relationship between states

The study of the fast dynamics of the BOLD activity is flourishing thanks to the methodological progress from recent years. (e.g., Cabral et al., 2017; Vidaurre, Smith, & Woolrich, 2017). There is however some early evidence, of a temporal order among some of the traditional resting-state networks. For example, Robinson et al., (2009)

found evidence suggesting that the basal ganglia network might temporally precede the DMN, to which it is anticorrelated. Precisely, most of the studies investigating the relationship between networks have looked at correlations between their BOLD timeseries, rather than quantifying the times in which a particular resting-sate network anticipates the other. In relation to that, the study by Robinson and colleagues also showed that the basal ganglia network was not only anti-correlated with the DMN but also positively correlated with both the right and left FPN.

Our approach to studying the relationship between states differs from the correlational approach in many ways. Here we basically assign one single state to each timepoint. That is, we first translate the BOLD timeseries into a states timecourse through the application of the LEiDA algorithm (see Chapter 3, *Methods*). According to this timecourse, only one state can be active at a specific timepoint. While this is a non-biological assumption required for this method, it practically facilitates the identification and quantification of state transitions. In particular, state transitions are computed as the probability for each FC state of transitioning to any of the other FC states. A few previous studies investigating transitions between resting state networks have used Granger causality, a statistical method to determine whether one time series is able to predict another (Devarajan et al., 2008; Goebel et al., 2003), but the validity of Granger causality remains unclear.

Here, we will be assessing the transitions between all six states detected through LEiDA in the previous chapter. This will, for the first time, offer a comprehensive overview of how transition patterns evolve along a stress induction paradigm and how these might be modulated by early depression symptomatology or vulnerability to stress.

METHODS

Participants

This study was conducted on the same dataset as Chapter 2, and therefore the participants, study design and stress induction task is the same. For more details, please see Chapter 2, *Methods*.

.

Transition matrices

Here, we computed the switching matrices for each of the experimental conditions (i.e., before stress, after stress and stress recovery) and for each participant. Each switching matrix represents the probability of, being in a given FC state, transitioning to any of the other FC states. Hence, they reflect not only how likely it is to transition between each pair of states but also the directionality of these transitions *(Figure 4.1).*

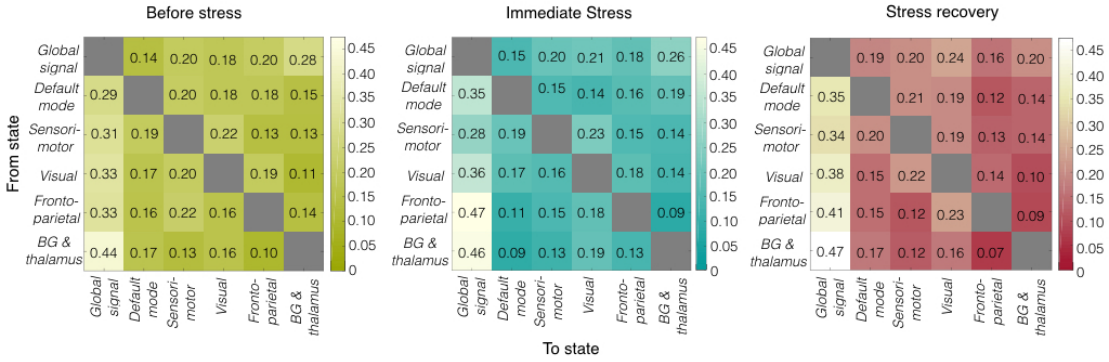


Figure 4.1. Changing between LEiDA states is given by the mean transition probability matrix, here shown for each experimental condition. *Each of them represent, on average, the probability of, being in a given FC state, transitioning to any of the other FC states. In this chapter, we first compare each pair of conditions to investigate how state transitions change across stress phases. Then, we investigate transition probabilities in relation to our vulnerability measure.*

Statistical analyses

Statistical analyses were carried out using a permutation-based approach (PALM, see Chapter 2, *Methods,* for details). First, differences in the transition probabilities between the different stress phases were assessed without including any covariates in the statistical design. Second, the same analysis was carried out using the vulnerability scores (PHQ9) as confounder, in order to investigate the differences between conditions after regressing out the influence of (preclinical) depression/vulnerability symptoms on the probabilities of transition *(Figure 4.3).* Third, we assessed any linear relationships between transition probabilities and vulnerability scores. Results are shown both uncorrected and after correction for multiple comparisons using FDR (Benjamini and Hochberg, 1995) (6x6 = 36 possible transitions).

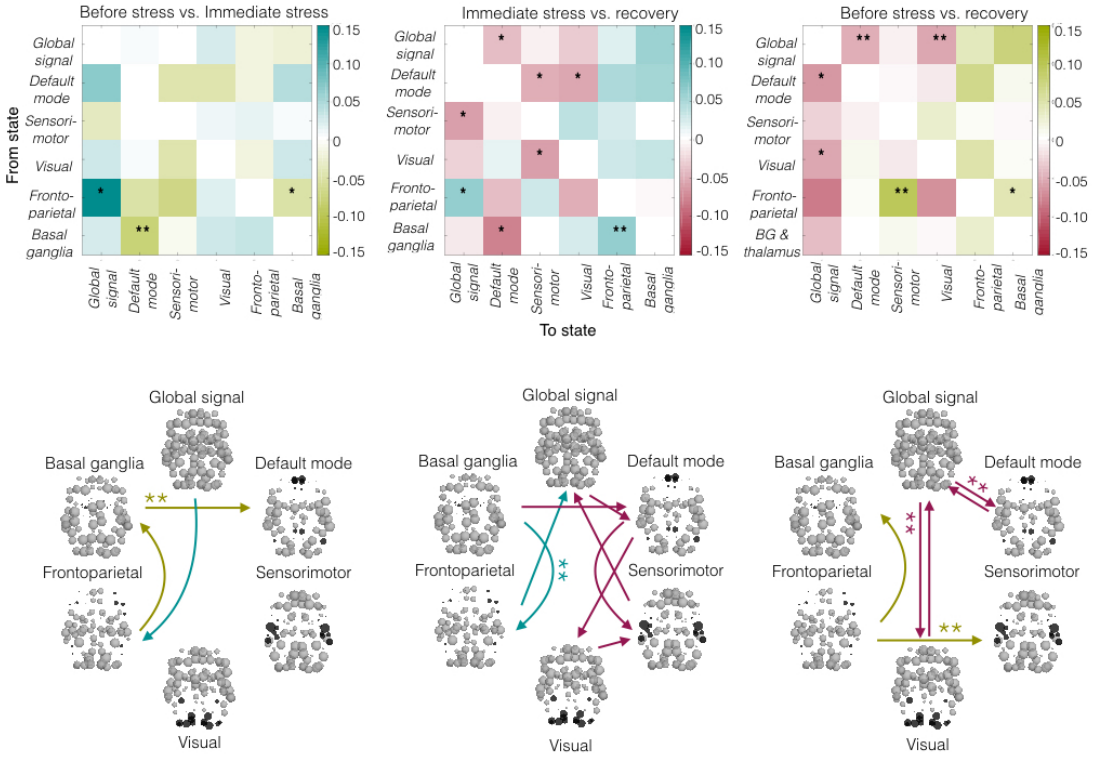


Figure 4.2. The brain dynamics found in conditions of immediate stress and stress recovery are characterised by patterns of different state transitions (shown in blue and red, respectively). *Top: Matrices representing the difference in transition probabilities for each pair of states between experimental conditions (*p < 0.05, ** p < 0.05 after FDR correction). Opacity represents the magnitude while tonality represents the direction of the difference. Bottom: Graphical representation of the largest differences in transition probabilities for each pair of experimental conditions. Significant differences after correction for multiple comparisons (p<0.05) are marked with a double asterisk, while mere trends are represented by arrows. See text for detailed description of the results.*

Differences between conditions

Results showed that before stress, participants switched more often from the basal ganglia to the default mode state (p= 0.0332 after correction for multiple comparisons) *(Figure 4.2).* We also observed more transitions from the frontoparietal to the basal ganglia state (p=0.0452), although this difference was not significant after correction for multiple comparisons. When looking at transition patterns taking place more often immediately after stress than before stress, we saw more transitions from the global to

the frontoparietal state (p = 0.0458), again only significant before correction for multiple comparisons.

When comparing immediate stress to recovery, results showed that immediately after stress, participants transition more often from the basal ganglia to the frontoparietal state (p = 0.0332 after correction for multiple comparisons) *(Figure 4.2)*. Furthermore, we also observed more common transitions from the frontoparietal state to the state of global coherence (p = 0.0284), although this difference did not survive correction for multiple comparisons. On the other hand, stress recovery appeared characterised by more frequent transitions from the global and the basal ganglia states to the default mode state (p = 0.0486 and p = 0.0364, respectively), and from this state to the sensorimotor state (p = 0.0326). From the default mode state more frequent transitions were also found to the visual state (p = 0.0354), and from the visual state to the sensorimotor state (p = 0.0326). However, none of these results remained significant after correction for multiple comparisons.

Finally, the comparison between before stress and recovery revealed more frequent transitions from the global to the visual (p = 0.0216 after correction for multiple comparisons) and the default mode states (p = 0.0451 after correction for multiple comparisons) *(Figure 4.2)*. Returning from these two states to the global state was also more frequent during recovery than before stress, p = 0.0256 and p = 0.0454 respectively, although this did not remain significant after multiple comparison correction. However, we observed more frequent transitions from the frontoparietal to the sensorimotor state (p = 0.0188) and from the frontoparietal state to the basal ganglia state (p = 0.0190) before stress in contrast to stress recovery.

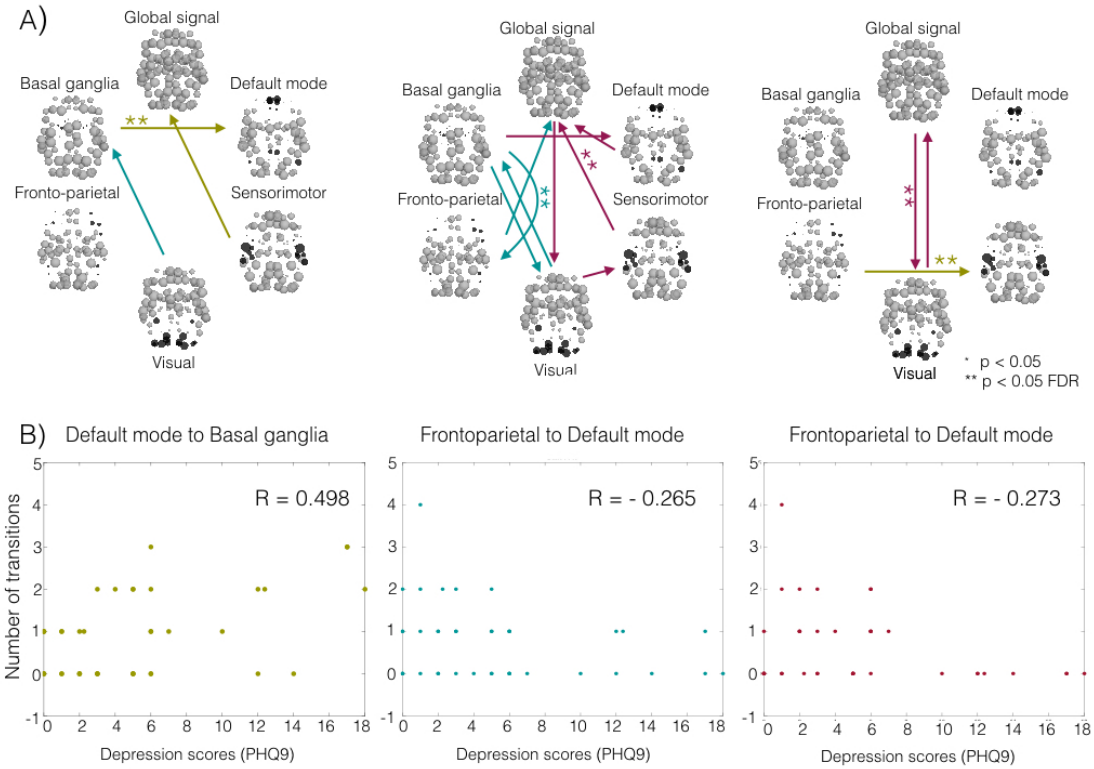


Figure 4.3. Significant differences in state transitions between stress phases not mediated by vulnerability scores. *a) Graphical illustration of differences in transition patterns between experimental conditions after controlling for the effect of depression/vulnerability scores. Both trends (arrows) and significant results after correction for multiple comparisons are represented (arrows with asterisks, p < 0.05 after FDR correction). b) Effect of vulnerability on three specific transition profiles; default mode to basal ganglia before stress, and frontoparietal to default mode both immediately after stress and during stress recovery. We show that the number of transitions between the estimated brain states is influenced by vulnerability scores.*

Differences between stress conditions controlling for vulnerability scores

A second analysis looking at the differences between conditions while controlling for the effect of vulnerability scores revealed that, indeed, most of the differences observed between conditions were related to the stress induction paradigm. In particular, we confirmed fewer transitions from the basal ganglia to the default mode state after stress induction (p = 0.0066 after correction) *(Figure 4.3)*. We were also able to confirm the more frequent transitions from the basal ganglia to the frontoparietal state immediately after stress in contrast to during stress recovery (p = 0.0494). We additionally observed that the more frequent transitions from the sensorimotor state

to the state of global coherence during stress recovery in contrast to immediately after stress, non-significant after FDR correction in the previous analysis, was significant once we controlled for the effect of vulnerability scores (p = 0.0321 after correction for multiple comparisons). Finally, the last contrast allowed us to confirm more frequent transitions from the state of global coherence to the visual network (p = 0.0022 after correction for multiple comparisons) during stress recovery in contrast to before stress induction. It also showed more frequent transitions from the frontoparietal to the sensorimotor state before stress in contrast to stress recovery (p = 0.0453 after correction for multiple comparisons).

Linear relationships between transitions and vulnerability scores

Results show that even before stress induction, participants with higher vulnerability scores, transition more frequently from the default mode to the basal ganglia state (p = 0.0270 after correction for multiple comparisons) *(Figure 4.4)*. While it was only nearly significant, we also observed more transitions in the opposite direction, meaning from the basal ganglia state to the default mode network (p = 0.0050 after correction). Furthermore, we found that during this phase of the experiment, the lower the scores in the depression/vulnerability scale, and therefore the more temporally resilient the participant, the more transitions from the global to the frontoparietal state (p = 0.0280)

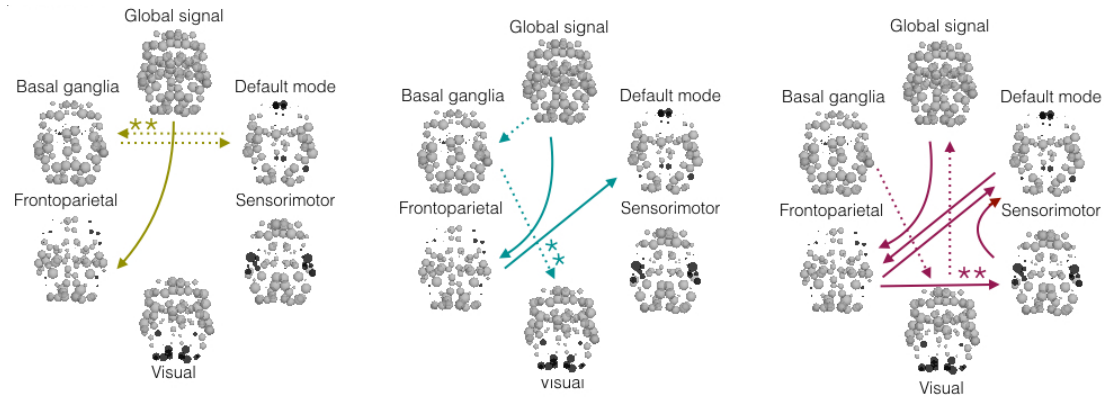


Figure 4.4. Resilience is associated with more transitions from the frontoparietal to other states. *Negative (arrow) and positive (dashed arrow) relationships between probability of transitions and vulnerability scores. Both trends*

(arrows, p< 0.05) and significant results after correction for multiple comparisons are represented (arrows with asterisks, p < 0.05 after FDR correction).

Immediately after stress, we observed that the higher the vulnerability scores, the more frequent participants switch from the global to the basal ganglia state (p = 0.0159) and from there to the visual state (p = 0.0120 after correction). On the other hand, the lower the vulnerability scores, the more transitions from the global coherence state to the frontoparietal state (p = 0.0418), as we observed even before stress induction, and from there to the default mode state (p = 0.0385). These were again only significant before correction for multiple comparisons *(Figure 4.4)*.

During stress recovery, we see the two different patterns of transitions carrying over *(Figure 4.4)*; on one hand, transitions from the basal ganglia to the visual state are more frequent in participants with higher vulnerability scores, on the other hand, transitions from (and to) the frontoparietal state, are more frequent in participants with lower vulnerability scores. In particular, we see that the higher the vulnerability scores, the more frequent transitions from the basal ganglia to the visual state (p = 0.0311) and from there to the state of global coherence (p= 0.0239). In terms of negative relationship with vulnerability, we see that participants scoring lower on vulnerability continue to transition more often from the global to the frontoparietal state (p = 0.0058) and from there to the default mode state (p = 0.0365). We see this pattern expand further, establishing a trend towards more frequent transitions from the default mode back to the frontoparietal state (p = 0.0340). From the frontoparietal state there is a second and less direct path of transitions back to the default mode, going first from the frontoparietal to the sensorimotor state (p = 0.0120 after correction for multiple comparisons) and from the sensorimotor network to the default mode state (p = 0.0113).

DISCUSSION

Differences between stress conditions

Our strongest results from the stress comparisons showed that immediately after stress, participants switched more often from the basal ganglia to the frontoparietal

state, possibly reflecting how a state of heightened arousal would engage the attentional system in order to protect oneself from possible threats. Furthermore, we observed less frequent transitions from the basal ganglia to the default mode state. As time passes and participants enter the recovery stage, we observed more frequent transitions among states involved with perception (i.e., sensorimotor and visual) and, again, the default mode state, perhaps reflecting a return to a more broadly exploratory state together with a resurface of self-referential processes once threat is placed in the past. In fact, we also observed a decrease in the transitions from the frontoparietal to the sensorimotor state, supporting the idea of a less alert state.

When considering results that did not remain significant after correction for multiple comparisons, we observed less frequent transitions from the frontoparietal to the basal ganglia state in both immediate stress and recovery in contrast to before stress induction *(Figure 4.2)*. This, together with the opposite pattern established immediately after stress induction (i.e., more frequent transitions *from* the basal ganglia *to* the fronto-parietal state) highlights the importance of considering not only the relationship between states but also the *directionality* of this relationship. This difference in the directionality in accordance to the stress phase could be reflecting a more bottom up processing style during stress contrasting with a more top-down processing once the stressor has passed.

Results after controlling for vulnerability scores show very similar differences in transitions patterns across conditions, but additionally highlights an increase in the transitions between basal ganglia and the visual state immediately after stress (*Figure 4.3)*. In this case, the transition pattern is bidirectional, at least when comparing immediate to stress recovery. This result supports the idea of an alerted state being reflected on the network transitions – in this case, an increase in arousal would increase activation and connectivity of the basal ganglia and prompt the engagement of a visually vigilant response. As discussed in Chapter 3, participants spend more time, both in the form of lifetimes and fractional occupancies, in the basal ganglia state immediately after stress than in stress recovery. Hence, it is not surprising that we also find a significant increase in the frequency with which they switch to and from the basal ganglia state during this stress condition.

Relationship between transitions and vulnerability scores

Following characterisation of the differences between stress conditions, both with and without controlling for the effect of preclinical depressive symptoms or vulnerability scores, we investigated the relationship between state transitions and vulnerability scores within each of the experimental conditions. On the one hand, participants scoring higher in vulnerability have higher probabilities to switch to other states from the basal ganglia state. In particular, we observed increased transitions to the default mode state before stress and to the visual state both immediately after stress and in the stress recovery condition. As discussed in the previous sections, transitions to and from the basal ganglia state are most frequent immediately after stress. However, while the most strongly established transition immediately after stress was from the basal ganglia to the frontoparietal state, once we focus on the relationship with vulnerability scores we see that higher scores are associated with more frequent transitions from the basal ganglia to the visual state. This suggests that the alerted visual state associated with more frequent interactions between basal ganglia and visual cortex is more pronounced in people showing the first signs of depression.

Although, to our knowledge, there are no previous studies looking at the dynamics of the basal ganglia network in relation to stress vulnerability and depression, some recent work has observed abnormalities in frontostriatal and limbic connectivity in major depression. In particular, a very recent study found four different biotypes of depression based on clustering of (static) FC patterns, two of which showed hyperconnectivity in thalamic and frontostriatal networks and were further associated with predominance of anhedonia and psychomotor retardation symptoms (Drysdale et al., 2016).

On the other hand, the lower the vulnerability score, the more frequent transitions from the state of global coherence to the frontoparietal state. This pattern carries over to transitions immediately after stress and during stress recovery. However, in after-stress conditions the pattern extends to include, first, more frequent transitions from the frontoparietal to the default mode state (immediate stress) and, second, from the default mode back to the frontoparietal state (stress recovery). In stress recovery we also observe a second closed loop from the frontoparietal to the default mode state passing though the sensorimotor state. The ubiquitous increase in transitions to the frontoparietal state as vulnerability scores decrease suggests that this might be a stable

trait of more resilient people, independently of stress condition. According to (Cole et al., 2014) the frontoparietal system might be at the centre of a feedback loop maintaining or reinstating homeostatic balance after stress via dynamic states interactions. They further propose that that this feedback loop's integrity is crucial for mental health, which is in line with our results showing increased transitions to the default mode state and the sensorimotor state in highly resilient participants after stress induction.

More specifically, it has been suggested that cooperation between the FPN and the DMN is crucial for the generation and maintenance of an internal train of thought (Smallwood et al., 2012). Specifically, the DMN would be providing autobiographical information and the FPN would be responsible for helping to sustain and buffer internal trains of though against disruption by the external world.

Integration with lifetimes and fractional occupancies results

It is interesting to consider, in a broader context, the differences in transitions, in particular those referring to the frontoparietal state, when, including the results regarding temporal stability and dominance. While in stress recovery the increase in transitions comes together with longer lifetimes and fractional occupancies in participants with the lowest vulnerability scores (i.e., resilient), this is not the case immediately after stress. Here, the increased transitions take place *despite of* the shorter lifetimes of the frontoparietal state in the most resilient participants. Hence, *briefly* engaging the frontoparietal state immediately after stress, potentially to aid transitions to the default mode state, might be a potential marker for resilience. For example, the frontoparietal-default mode transition pattern could be reflecting a capacity to quickly reinstate self-referential cognition or an internal train of thought through redirection of attentional processes once immediate threat has passed. Furthermore, it has been proposed that given the limited capacity of the top-down attentional system, internally and externally (i.e., threat) driven streams might come into conflict. This would perhaps result in an overload of the frontoparietal network, specially in those participants most vulnerable to stress, and get reflected in the positive relationship between vulnerability scores and lifetimes of the frontoparietal state immediately after stress (Chapter 3). In other words, the more vulnerable, the less able participants are to deny attentional amplification of the externally driven

stream, leading to an overload of the frontoparietal state. This might then be associated with the observed increased lifetimes and decreased dynamic interactions between the frontoparietal state and default mode state, reflecting an inability to establish an internal train of thought protected from demands of the external world (i.e., residual stress/threat). In support of this hypothesis, we observed that the decreased transitions between the frontoparietal and the default mode state in more vulnerable participants came accompanied by increased transitions from the basal ganglia to the visual state, pointing towards increased processing of perceptual information in vulnerable participants.

Limitations and future directions

It is important to keep in mind, however, that most of the results discussed in the previous paragraphs were only significant before correcting for the number of possible transitions between states (i.e., 6 x 6 = 36 possible transitions). Nevertheless, it is also important to mention that we only expected to observe a subtle effect given that this study used a social stress paradigm in a sample of highly resilient elite soldiers, able to endure very high levels of stress due to their training. Here, we observed some transitions that remained significant after correction across multiple comparisons, and these were further corroborated by a second analysis controlling for the effect of vulnerability scores, revealing a robust effect of stress on the transition patterns between FC states. Future studies could consider the inclusion of a control group, perhaps a group of civilians, to enable between groups comparisons.

Chapter 5

INSIGHTS INTO THE DYNAMICS OF PTSD SYMPTOMATOLOGY AND RECOVERY

ABSTRACT

In this final experimental chapter, we delve into how the temporal activation of the different brain networks differ between PTSD and healthy controls, and explore how these temporal fluctuations change after successful cognitive therapy for PTSD. To do so, we applied a data-driven approach —the Hidden Markov Model—, to extract seven states characterised by patterns of above- and below- average activation Our results revealed that two states related to the default mode system are engaged during less time in participants with PTSD than in those with remitted PTSD or healthy controls. These results were confirmed through correlational analyses, showing a negative relationship between time spent in these states and PTSD symptom severity. Given the potential implication of one of the default mode network subsystems in the contextualisation of autobiographical memories, which is thought to be compromised in PTSD and related to intrusive trauma memories, we evaluated the relationship between time spent in each of the states and severity of intrusive memories. This analysis revealed that the medial temporal DMN is indeed the main state associated with stronger intrusive memory qualities, together with the salience and one of the two visual states. Our results suggest that decreased temporal dominance is at least one of the factors driving the observed dysregulation of the DMN system in PTSD and that successful cognitive therapy for PTSD might help rebalance the temporal dominance of this system.

INTRODUCTION

As discussed in the general introduction, PTSD is a disorder that can result from the direct exposure or witnessing of a traumatic event such as a severe accident, natural disaster or exposure to violence. According to the DSM-5 (2013) there are four clusters of symptoms, namely repeated and unwanted re-experiencing of the event, negative cognitions and mood, hyperarousal, and avoidance of stimuli related to the trauma. Since the first recognition of PTSD by the DSM-III (1980) many psychological models have been proposed to explain this complex disorder, ranging from psychodynamic to

cognitive and behavioural models (see Introduction). In this work, we explore PTSD within the framework of one of the most recent and comprehensive psychological models (Ehlers and Clark, 2000). Remarkably, this model served as the framework for the development of cognitive therapy for PTSD and case conceptualisation (CT-PTSD; Ehlers et al., 2013, 2014; Ehlers, Clark, Hackmann, McManus, & Fennell, 2005), one of the first line treatments used worldwide (Benedek et al., 2009; e.g., Department of Veterans Affairs and Department of Defense, 2017; National Institute of Health and Care Excellence, 2018). This model suggests that the persistent form of the disorder occurs only if individuals process the traumatic event and/or its sequelae in a way that induces a sense of current threat. This can happen through two key processes that influence each other: 1) excessively negative appraisals (i.e., cognitive interpretations) about the trauma or its sequelae 2) a disturbance in the autobiographical memory of the traumatic event characterised by poor elaboration and contextualisation, strong perceptual priming and marked associative memory.

More specifically, memories of the traumatic event present a very interesting phenomenon in PTSD. On the one hand, individuals with PTSD have problems to retrieve an organised memory of the traumatic event *intentionally*. On the other hand, they report a high frequency of *involuntarily* triggered intrusive memories that force them to re-experience the traumatic event in a very vivid and emotional way. This re-experiencing of the memory consists mainly of sensory impressions, predominantly visual (Ehlers and Steil, 1995; van der Kolk and Fisler, 1995), which are experienced as if they were happening 'right now' and come accompanied by the 'original' emotions of the traumatic event. Importantly, these intrusive memories are cue-driven due to their associative nature (Ehlers and Clark, 2000) meaning that they can be induced through the presentation of trauma-related stimuli in experimental paradigms.

To explain the apparent paradox that entails the coexistence of frequent but involuntary memories with difficulties in voluntary recall of the traumatic event, Ehlers and Clark proposed a distinction between data-driven and conceptually-driven (i.e. meaning-based) processing during the trauma. These authors suggest that an appropriately contextualised autobiographical memory, sufficiently integrated in time, place, and in relation to other autobiographical memories, promotes meaning-based retrieval. This in turn helps to inhibit sensory retrieval of the traumatic event when associated stimuli appear in the environment (see also Conway and Pleydell-Pearce,

2000). Data-driven processing during the trauma involves predominantly processing perceptual information rather than meaning, and leads to a failure to contextualise the traumatic memory. The latter, together with the enhancement of perceptual priming, leads to easy triggering of unintentional recall by physically similar cues and the memory presents itself with a quality of 'here and now' (i.e., no context in time) and absence of links to subsequent information that is relevant for its current meaning (e.g., "I did not die"). This leads to the perception of current threat, which is considered to be at the core of the disorder.

A further style of cognitive processing that is thought to impede the contextualisation of trauma memories is an inability to establish a self-referential perspective while experiencing the trauma, hindering its integration into the chronologic continuum of other autobiographical memories (see Wheeler et al., 1997). Moreover, the difficulties inhibiting unintentional retrieval by perceptually similar cues are further exacerbated by strong 'stimulus-response' and 'stimulus-stimulus' associations, making triggering of memories of the event and the associated emotional responses even more likely (see also Conway, 1997b; Foa et al., 1989; Keane et al., 1985). Furthermore, perceptual priming for stimuli temporally associated with the traumatic event is suggested to be particularly strong due to reduced perceptual threshold for these stimuli.

Of note for any psychiatric disorders in which autobiographical memory is affected, our autobiographical memories and our sense of self hold a close bidirectional relationship. In particular, it has been suggested that autobiographical memory plays a fundamental role in the formation of self-identity whereas recollection of past personal memories is influenced by one's self-views, current beliefs and goals (Conway and Pleydell-Pearce, 2000; Klein and Lax, 2010). Furthermore, self-reference has been shown to affect the binding of memory to source (Cunningham et al., 2014; Sui and Zhu, 2005), the integration of parts into perceptual wholes (Sui and Humphreys, 2015), and the ability to switch from a prior association to a new association (Wang et al., 2016).

Several results in the literature illustrate how self-referential processing, in addition to autobiographical memory, is deeply affected in PTSD. For example, two prospective studies have shown that lack of self-referential processing during the trauma (as measured 2-weeks after the traumatic event) predicts PTSD six months later (Ehring et

al., 2008; Kleim et al., 2012). In regards to autobiographical memory, several studies have found that participants with PTSD or acute stress disorder show less specific autobiographical memory retrieval than trauma survivors without PTSD (Harvey et al., 1998; Mc Nally et al., 1995; Schönfeld et al., 2007; Schönfeld and Ehlers, 2006). This was further confirmed by a prospective study in which participants with PTSD symptomatology 2 weeks after the traumatic event showed decreased ability to retrieve as many specific autobiographical memories than those without PTSD symptoms. Moreover, retrieval of less specific autobiographical memories 2 weeks after trauma had additional predictive value over initial diagnoses and symptom severity for prediction of PTSD 6 months later (Kleim and Ehlers, 2008).

Two PTSD phenomena related to self-referential processing and autobiographical memories are 'mental defeat' and the feeling of being 'frozen in time'. The experience of mental defeat during the trauma (i.e., perceived loss of all psychological autonomy accompanied by a feeling of not being human any longer) correlates with chronic PTSD (Dunmore et al., 1999; Ehlers et al., 2000), and prospectively predicts PTSD severity in assault survivors (Dunmore et al., 2001; Kleim et al., 2007). Another characteristic phenomenon of PTSD that correlated with chronicity is the feeling of being 'frozen in time' years after the trauma (i.e., feeling disconnected from the former self) (Mc Nally et al., 1995).

In terms of self-referential processing and autobiographical memory, the DMN is deemed a particularly relevant resting-state network. The main regions integrating the DMN are the medial prefrontal cortex, medial parietal cortex and medial temporal lobes. Despite its initial description as a task-negative network, more recent work has shown that the activity of the DMN increases during goal-directed cognitive tasks as long as these tasks demand participants to engage in directed forms of self-generated thought; examples are retrieval of autobiographical, semantic or episodic information, thinking about or planning aspects of their personal future, imagining novel scenes, reasoning about moral dilemmas or other scenarios, inferring the mental states of other people, comprehending narratives, self-reflecting, referencing information to one-self, and appraising or reappraising emotional information (Andrews-Hanna, 2012; Andrews-Hanna et al., 2014; Buckner et al., 2008). All these cognitive processes are affected in people with PTSD and are central to most psychotherapies (Schnyder et al., 2015), even though cognitive therapies such as cognitive therapy for PTSD place the

strongest emphasis on integrating the trauma in the right autobiographical context and reconstructing a sense of self.

In coherence with the notion that self-generated thought comprises multiple component processes, it has been suggested that the DMN might comprise multiple interacting subsystems (Andrews-Hanna et al., 2010; Vidaurre et al., 2018a). Even though the exact number of subsystems and their regions are unclear at this point, at least 3 main subsystems have been proposed to coexist: 1) the midline core DMN, comprised by the anteromedial prefrontal cortex and the posterior cingulate cortex and involved in self-related processes and emotion evaluation 2) a medial temporal subsystem comprised by hippocampus, parahippocampal gyrus, retrosplenial cortex, posterior inferior parietal lobe and ventromedial prefrontal cortex that are involved mainly in autobiographical memory and contextual retrieval and 3) dorsal medial subsystem, comprised by dorsomedial prefrontal cortex, temporoparietal junction, lateral temporal cortex, and temporal pole, involved in mentalizing and social cognition (Andrews-Hanna et al., 2014).

Several studies have already pointed out abnormalities within the DMN in relation to PTSD, from hypo- and hyperactivity affecting different regions of this network (Patel et al., 2012), to decreased FC between the posterior cingulate cortex/precuneus and other brain regions (Bluhm et al., 2009; Sripada et al., 2012). One recent study looking into the different DMN subsystems found decreased FC between the posterior cingulate cortex, a region of the core DMN, and the hippocampus, a region of the medial temporal subsystem, in veterans with PTSD in contrast to trauma-exposed veterans without PTSD (Miller et al., 2017). However, this work did not find any group differences in connectivity between the regions of the core DMN and regions of the dorsolateral DMN, suggesting that PTSD selectively affects the medial temporal DMN.

Although these insights are informative, it is also important to consider that the vast majority of these studies used seed-based approaches for the study of neural activity and FC, which might be biasing the literature on PTSD. Indeed, several meta-analyses have shown that some brain regions that rarely receive attention in relation to PTSD, such as less prominent regions of the SN like the striatum or sensory processing areas like the fusiform gyrus, show significant differences in activation between PTSD and control groups (Patel et al., 2012; Stark et al., 2015). This calls for the use of data-

driven, whole brain approaches that allow the study of large-scale networks in order to obtain a more comprehensive understanding of the neural correlates of PTSD.

Furthermore, the vast majority of studies in the field of PTSD have used a static approach, averaging functional data over time and, therefore, possibly neglecting the information contained in the temporal fluctuations of brain function. Previous attempts looking at PTSD brain dynamics had a strong focus on classification of PTSD in contrast to healthy participants based on sliding window approaches applied to their resting-state activity (Jin et al., 2017; Li et al., 2014; Ou et al., 2014). However, as discussed in the introduction and previous chapters, dynamic approaches using sliding windows are limited due to the inefficient use of the data they make.

The aim of this chapter is to further our understanding of the neural correlates of PTSD by investigating temporal dominance of time-varying networks in relation to 1) PTSD symptomatology and remittance and 2) the qualities of intrusive memories induced by the fMRI task. To do so, we used a data-driven approach (Vidaurre et al., 2017b, 2016) that allowed us to decompose our data in their dominant large-scale networks of activity and study their temporal dynamics in two different conditions: presentation of neutral pictures and presentation of trauma-related pictures (i.e., symptom induction). This approach was applied to fMRI scans from 74 participants. The dataset consisted of healthy but trauma-exposed controls, PTSD participants before and/or after trauma-focused cognitive therapy for PTSD (CT-PTSD), and participants before and after being on a waiting list for 3 months. From the CT-PTSD group, some participants were scanned only before or after the therapy, while others underwent the fMRI scan both before and after CT. In contrast to sliding window approaches, this method has increased temporal resolution while providing statistical validity of any changes identified within session (i.e., by having a making more efficient use of the data, the estimation of differences becomes more accurate). Furthermore, it provides an interpretable model, compactly representing the information of the main networks through a reduced set of parameters, such that these networks and their temporal characteristics can be readily related to different behavioural measures later on. To our knowledge, this is the first study investigating time-varying networks in PTSD before and after successful cognitive therapy for PTSD, bringing us closer to a better understanding of the disorder and of the neural correlates associated with its healing with psychotherapeutic approaches.

METHODS

Participants

Participants were treatment-seeking assault and Road Traffic Accident (RTA) survivors with posttraumatic stress disorder as primary diagnosis according to DSM-IV-TR. Inclusion criteria were exposure to an assault or RTA and a number of standard fMRI-related safety criteria. They were recruited from an NHS outpatient clinic for anxiety disorders and trauma in London. A trauma-exposed healthy control group with the same inclusion criteria (i.e., exposure to assault or RTA and fulfilment of fMRI safety requirements) was recruited via flyers in the community or via recruitment from ongoing research studies at the Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK. Initial contact was established with 232 individuals, of which 58 were not interested in taking part in the study, 27 were screened out according to the fMRI safety standards and 58 met other exclusion criteria. Overall, eighty-nine participants were included and tree participants dropped out prior to assessment and treatment sessions. From the 86 remaining participants, 10 were excluded due to technical problems with their fMRI data acquisition. Furthermore, two participants who were only scanned after cognitive therapy were excluded because they did not respond to the therapy. The final sample consisted of 74 participants, of which 15 were controls, 43 had PTSD at the time of the initial assessment (31 before starting CT, and 12 before starting a waitlist), and 16 participants had remitted from PTSD following a course of CT. Of those with PTSD at initial assessment, 14 returned for a second scan after a course of CT and 8 returned for the second scan after a 3 months waiting list. Overall, the study included 26 RTA and 48 assault survivors. Average time after trauma was 4 years and 6.65 months, SD=6.45 years, and individuals mean age was 39.99 years, SD=12.25 years, see Supplementary table 2.

Procedure

This study was approved by the local ethical review board. Informed consent was obtained from each participant after complete description of the study. Participants were assessed and scanned individually either before and after a course of trauma-

focused cognitive therapy for PTSD (CT-PTSD), before and after waiting to be treated with CT (waiting list), or just at one timepoint (trauma-exposed control group without PTSD diagnosis, PTSD patients before or after treatment only (instead of at both timepoints)). This study design makes possible a large sample, cross-sectional study including all participants scanned before (n=43) and after CT (n=29), as well as a smaller sample study including only participants that attended both visits at the two different time points, either before and after CT (n=14) or before and after being on the waiting list (n=8). Two clinical psychologists, who were blind to the treatment conditions, conducted semi-structured interviews —the PTSD Symptom Scale Interview (PSS-I, Foa et al., (1993) before and after the treatment or wait list condition. Participants also completed self-report questionnaires to assess anxiety, depression and other variables.

Experimental task

During the fMRI scan, participants completed a cognitive task where they were shown blocks of 8 pictures and instructed to search for a neutral target (i.e., picture of a mushroom) within each of these series. They were told that some pictures may be trauma-related and that each series of pictures may contain between 0 and 2 neutral targets. They were asked to press a button box every time they detected a target (i.e., oddball paradigm). The tasks consisted of 40 blocks of pictures in total, 20 blocks of trauma and 20 blocks of neutral pictures. Order of conditions, i.e., trauma versus neutral, was pseudorandomised. Each picture was presented for 2000ms followed by a 0.4 seconds blank screen, with an 8 seconds interval between blocks during which a fixation cross was shown, resulting in a total task duration of 16 minutes.

Stimuli were photographs largely derived from the International Affective Picture System, IAPS (Lang et al., 1997), or from public websites, from various categories, i.e., trauma survivor, injury, trauma-related objects, pre-trauma events (e.g., person sneaking up from behind, a car overtaking in the rain), and pictures depicting actual traumatic events (RTA, assault). Pictures were matched in terms of luminance, number of persons, objects, etc. Parallel categories were introduced for the neutral pictures, i.e., person around the house, household- related objects, two persons around the house. Two task versions we constructed, one for assault survivors and one for RTA survivors, with trauma-specific pictures. Pictures across the task had been rated in

pilot studies and matched valence and arousal ratings in order to create two versions of each task for the two assessment times (pre- and post-therapy).

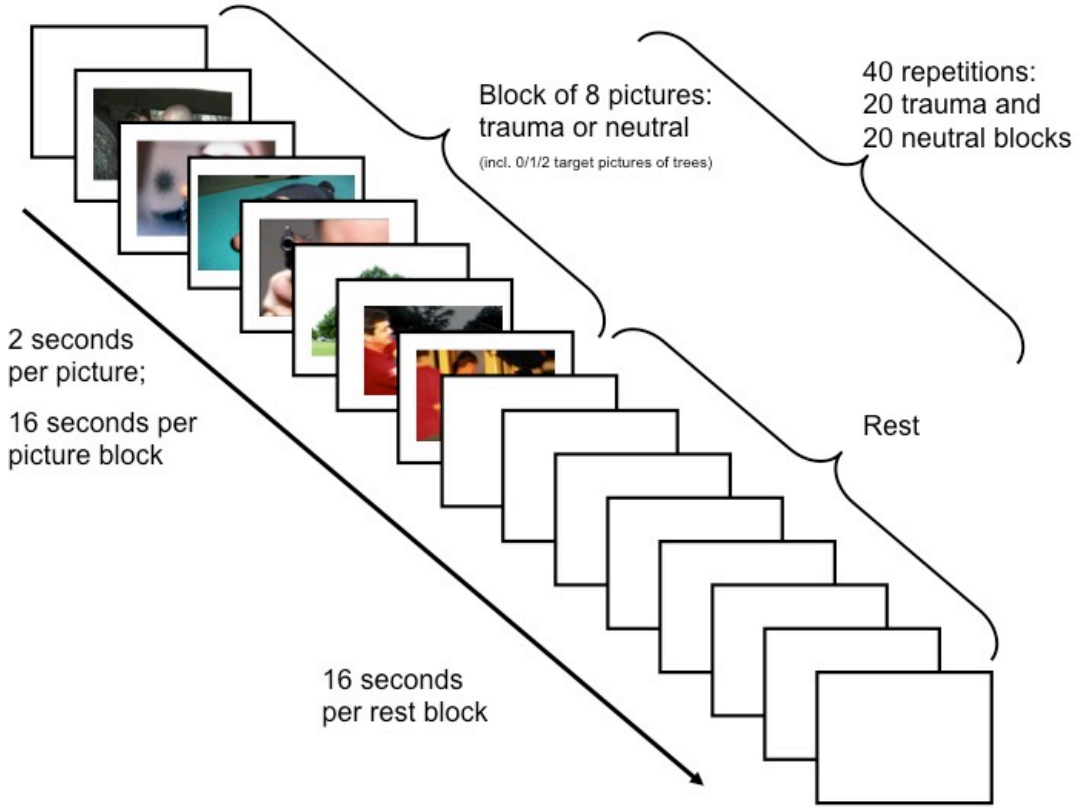


Figure 5.1. Illustration of the experimental trauma task completed during the fMRI scans. *The task containing blocks of trauma-related pictures and blocks of neutral pictures with a picture of a mushroom embedded in each of the blocks 0, 1 or 2 times. Each time the mushroom appears, the participants had to press a button.*

fMRI acquisition and preprocessing

Brain scanning was performed on a 1.5T Siemens Scanner using an echoplanar protocol (TR/TE/flip angle: 2400/40/80; FOV: 20× 20 cm; matrix size: 96×96) in 36 axial slices (thickness: 3mm; gap: 0.3mm). Data preprocessing was performed using FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) and included head motion correction (MCFLIRT), brain extraction, spatial smoothing with a 5 mm FWHM and highpass filtering. Advanced data denoising was carried out using FIX (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014). Denoised data was first linearly registered to their structural scans and then nonlinearly registered to MNI space. Mean BOLD time-series

were then estimated on 268 brain areas of the Shen functional atlas (Shen et al., 2013) by averaging the BOLD signal over all voxels belonging to each brain area. From these 268 brain regions, 32 were excluded due to signal drop out. The excluded timeseries belonged to regions of the orbitofrontal cortex, cerebellum and temporal lobe.

Hidden Markov Model analysis

Hidden Markov Modelling is an analysis technique to describe multivariate time series data (in this case extracted through the Shen functional atlas) as a sequence of visits to a finite number of states. Each of these states has a characteristic signature containing spatial and temporal (of when they activate) information. Different versions of the Hidden Markov Model (HMM) are possible. In the one used in this work, the spatial information corresponds to a spatial map representing, for each brain region, the level of activation relative to the average. Although information from FC can also be included in the model, we did not take this approach here due to challenges to estimate FC states. Those challenges are likely related to the characteristics of our fMRI data acquisition and preprocessing results. Mathematically, each state is represented by a multivariate Gaussian distribution, which is described by the mean, and, optionally, the covariance if we wished to model FC. In this case, we used only the mean to drive the states, so that each state has a specific average activation, but they all share a full covariance matrix in order to take into account the intrinsic relationship between brain regions. The state sequence is modulated by the probability of transition between all pairs of brain states, which is also estimated within the model; that is, the probability of a given state at a specific timepoint t depends on the state that was active at the previous timepoint $(t-1)$. Here we applied the HMM on all concatenated scanning sessions, that is including controls at visit one and PTSD participants at visit 1 and 2, such that we obtained an estimation of the states at the group level, instead of a subject specific state. This then allowed us to derive subject-specific information of when a state becomes active, which is referred to as the state timecourse) The fractional occupancies (FOs) are computed as the aggregations of the probabilities of being active at each timepoint for each subject. These are taken as an indicator of temporal dominance for each state. In this study, we derived the fractional occupancies independently for each task condition, that is, we obtained one measure of fractional occupancy for each state and for each participant for the trauma-related

blocks and a second measure for each state and for each participant for the neutral blocks. Each block could include presentation of a mushroom (0-2 per block), which was part of the oddball paradigm embedded in the block design of trauma and neutral pictures. However, this information was not used for the analysis. The HMM was inferred using the publicly available HMM-MAR toolbox, which provides estimates of the parameters of the state distributions, and the probabilities of each state to be active at each time point (Vidaurre et al., 2016, 2017a).

Statistical analysis

Statistical analyses were run using permutations testing, where we generated surrogate data by permuting the labels of the subjects. This allowed us to assess significance without further distributional assumptions. First, we looked at differences between the main groups (healthy controls, PTSD and remitted PTSD) in the entire sample, i.e., the PTSD and remitted PTSD groups contain both participants that completed both visits and participants that were only scanner before or after cognitive therapy. To assess these differences we used permutation testing with unpaired t-tests as the base statistic. Then we investigated differences between PTSD and remitted PTSD only on the subsample of participants that completed both fMRI scans, one before therapy and one after therapy. As a control for any therapy-unrelated changes that might occur with time we also evaluated differences between a subsample of participants that were scanned at two different points in time but without undergoing any therapy in-between scans. This was done using permutation testing with paired t-tests as the base statistic. Results are shown both after correcting across multiple comparisons, considering both number of states and of experimental conditions, and before correction for multiple comparisons. False Discovery Rate (FDR) (Benjamini and Hochberg, 1995; Storey, 2002) was the correction of choice to account for multiple comparisons.

A second set of statistics was run to test for linear relationships between fractional occupancies and PTSD severity variables. First, we tested for any linear relationships between the fractional occupancies of each state and PTSD severity as measured through three different symptom clusters. Similarly, we tested for any linear relationships between the fractional occupancies of each state and 1) number of intrusive memories elicited by the fMRI task, and 2) degree to which these intrusive

memories present flashback qualities (i.e., vividness, distress and feeling of 'here & now'). Here, in order to integrate different statistical tests into a single p-value, we made use of the non-parametric combination (NPC) algorithm (Vidaurre et al., 2018b; Winkler et al., 2016); which essentially integrates all hypotheses into a single one, such that we test that at least one alternative hypothesis is true. In particular, we integrated the p-values across variable of PTSD symptom severity. In other words, we obtained an aggregated p-value where the hypothesis is whether at least one of the symptoms clusters holds a significant linear relationship with the fractional occupancies of a specific state. Similarly, when testing for linear relationships between fractional occupancies for each state and the flashback qualities of intrusive memories, we integrated the p-values across number of intrusive memories and the three related variables related (i.e., vividness, distress and feeling of 'here& now') using the same procedure. The NPC algorithm to integrate the individual p-values involved a second-level permutation testing procedure; see Winkler et al, (2016) for further details. P-values were corrected across multiple comparisons using familywise error (FWE) correction (Thomas and Hayasaka, 2003).

RESULTS

Emerging states and their spatial features

A Hidden Markov model with seven different states was chosen to decompose the data and investigate specific differences in brain dynamics between healthy controls, PTSD and remitted participants. *Figure 5.2 (A)* shows a spatial map of the average activation for each of the states. Regions with values above 0 (orange/red) can be interpreted as having brain activity above average, whereas regions with values below 0 (blue/green) can be interpreted as having brain activity below average during the time in which these states are active. In order to quantitatively assess the similarity of these brain maps and specific well-established networks, we compared them to the spatial maps using the Neurosynth database and decoder (Gorgolewski et al., 2015; Yarkoni et al., 2011). The Neurosynth database contains the result coordinates from over 14.000 published fMRI studies and over 1300 terms extracted from those studies, providing the means for automated meta-analysis of fMRI data. This allowed us to upload our spatial maps to the platform and assess which key terms (e.g., default mode network)

were most often associated with similar maps in previous fMRI studies. Specifically, and given that all but one of our spatial maps were characterised by a pattern of activation above average (+) and a pattern of activation below average (-) we split these two sets of regions to obtain two different spatial maps per state. The sign of the map containing the regions below average was multiplied by -1 to invert the sign. Then we uploaded each spatial map to the Neurosynth Decoder, which compares each of the maps with patterns associated with cognitive and neuroanatomical terms in thousands of papers by running spatial correlation restricted by a grey matter mask (Gorgolewski et al., 2015). The output is a vector of correlations ordered in descending order and presented with their associated terms. We looked up to 25 terms for each state component (+ and -). When terms were redundant (e.g., parietal r=0.29, parietal cortex r = 0.27) we reported the highest correlation and its associated term. In the following we refer to the states based on the patterns of activation above the mean (+) for simplicity.

Of note, the Neurosynth platform includes both anatomical and cognitive terms, and the magnitude of the obtained correlations depends on the category, with anatomical terms usually obtaining higher correlation coefficients. The obtained correlation coefficients were moderate in absolute terms, but they were comparable to correlation coefficients obtained by other researchers (Gorgolewski et al., 2015). The relatively low correlations could be further related to the low spatial resolution of our data.

State 1 (Medial Temporal DMN +) shows above average activation in the medial prefrontal cortex (r=0.312), posterior cingulate (r=0.272), retrosplenial cortex (r=0.181) and precuneus (r=0.177), all regions belonging to the DMN. Indeed, an automatic meta-analysis using the term 'default' resulted in a spatial map that reached a correlation of r=0.257 with our spatial map. However, we did not observe activation in the supramarginal gyrus, which also belongs to the DMN, meaning that activation was restricted to midline areas of the DMN. Terms like 'autobiographical', 'autobiographical memory' and 'episodic' resulted in between maps correlations of r=0.223, r=0.182 and r=0.167, respectively. We also obtained correlations with concept-based meta-analysis maps of terms such as 'self' (r=0.212) and 'self-referential' (r=0.18). We observed above average activation in the visual cortex, hippocampus and parahippocampal gyrus, although and these neuroanatomical terms did not appear

among the first 25 maximally similar concepts, perhaps due to activation in these areas being weaker. The areas with activation below average were the parietal cortex (r=0.299), premotor (r=0.256) and motor (r=0.227) cortices, anterior insula (r=0.199) and intraparietal sulcus (r=0.196). This state is, on average, active during 5.3% of the time during trauma-relate picture presentation and 7.3% of the time during neutral picture presentation *(Figure 5.2)*

State 2 (Core DMN +) shows a similar pattern of activation, with spatial maps of the medial prefrontal cortex (r=0.297) and posterior cingulate cortex (r=0.262) reaching the highest correlations. The concept-based meta-analysis using the term 'default' produced a spatial map reaching a correlation of r=0.253 with this states' spatial map. Meta-analyses using the terms 'self' and 'self-referential' reached correlations of r = 0.194 and r= 0.177, respectively. While 'autobiographical' also appeared as a related term to this spatial map, the correlation was only r=0.179 and terms such as 'autobiographical memory' and 'episodic' did not appear among the first 25 maximally similar terms related to this state. We did nevertheless obtain correlations with other cognitive terms, such as 'Mind' (r=0.157) and 'Theory of mind' (r=0.156). In contrast to state 1 (medial temporal DMN), this state did not show above average activation in the hippocampus or the visual cortex. In fact, the visual cortex (r=0.375) was deactivated together with the fusiform gyrus (r=0.316), and a correlation of r=0.204 was obtained when comparing the below average activation map of this state to the meta-analysis map related to the 'ventral visual (stream)'. Thus, overall this map is characterised by above activation of DMN areas and below average activation of the visual ventral stream. This state is, on average, active during 2.7% of the time during trauma-related picture presentation and 3.5% of the time during neutral picture presentation *(Figure 5.2)*.

State 3 (PFC -) is characterised by below average activation of the prefrontal cortex (r=0.211), anterior cingulate cortex (r=0.139), anterior insula (r=0.178) and striatum (r=0.103). Terms such as 'memory' (r=0.109) and 'retrieval' (r=0.104) were associated with this network, which might indicate insufficient activation of these functions. This state is, on average, active during 0.8% of the time in each of the conditions *(Figure 5.2)*.

State 4 (Salience network +) shows above average activation in the insula (r=0.248), especially anterior insula (r=0.228), premotor cortex (r=0.236), supplementary motor

cortex (r=0.211), and somatosensory cortices (r=0.234). It also shows above activation in the cingulate cortex (r=0.21). The main term associated with this network was 'pain' (r=0.222), which could be referring both to emotional or physical pain. Below average activation within this spatial map was mainly in regions of the cerebellum, such as the crus (r=0.269) and lobule VI (r = 0.216), but also in the fusiform (r=0.19) and occipital lobe (r=0.19). This state is, on average, active during 20% of the time during trauma-related picture presentation and 15% of the time during neutral picture presentation *(Figure 5.2)*.

State 5 (Ventral visual stream +) above average activation was mainly in the visual cortex (r=0.436) and fusiform gyrus (r=0.423). Automatic meta-analyses using the terms 'objects' and 'face' gave rise to spatial maps that reached correlations of r=0.393 and 0.365 with our state's spatial map. This was accompanied by below average activation of the cingulate (r=0.258), both posterior cingulate (r=0.171) and anterior cingulate (r=0.166), prefrontal (r=0.186) and precuneus (r=0.136). Correlation between the below average activation map and the map corresponding to a concept-based meta-analysis using the keyword 'default mode' resulted in r=0.158. Overall, the spatial map of this state is characterised by activation of regions of the visual ventral stream together with below average activation of regions of the DMN. This state is, on average, active during 27% of the time during trauma-related picture presentation and 21% of the time during neutral picture presentation (*Figure 5.2)*.

State 6 (Auditory network +) shows above average activation in the temporal gyrus (r=0.091), superior temporal gyrus (r=0.105), and the areas of Broca and Wernicke. Terms such as 'listening' and 'sounds' reached correlations of r=0.096 and r=0.095, respectively. Below average activation was mainly in the visual cortex (r=0.425) and fusiform gyrus (r=0.397). Again, terms such as 'object' (r=0.36) and 'face' (r=0.328) were associated with this part of the spatial map, and the term 'ventral visual' (r=0.255) also resulted in a map that correlated with our spatial map. All in all, this state is characterised by above average activation in areas of sounds and language processing and below average activation in areas of visual processing. This state is, on average, active during 6.4% of the time during trauma-related picture presentation and 8.4% of the time during neutral picture presentation (*Figure 5.2)*.

State 7 (Visual ventral stream & dorsal attention network +) shows above average activation in the visual cortex (r=0.323) and fusiform gyrus (r=0.283), also showing a

correlation of r=0.187 with a spatial map resulting from a meta-analysis using the term 'ventral visual'. Accordingly, spatial maps resulting from concept-based meta-analyses using the terms 'face' and 'object' reached correlations of r = 0.237 and r = 0.251, respectively. This spatial map shows constrained below average activation of the medial prefrontal cortex (r=0.131) and anterior cingulate (r=0.116). Overall, this state could be involved with processing of the visual stimuli presented and is accompanied by below average activation of the pregenual anterior cingulate and paracingulate gyrus. This state is, on average, active during 38% of the time during trauma-related picture presentation and 44% of the time during neutral picture presentation *(Figure 5.2)*.

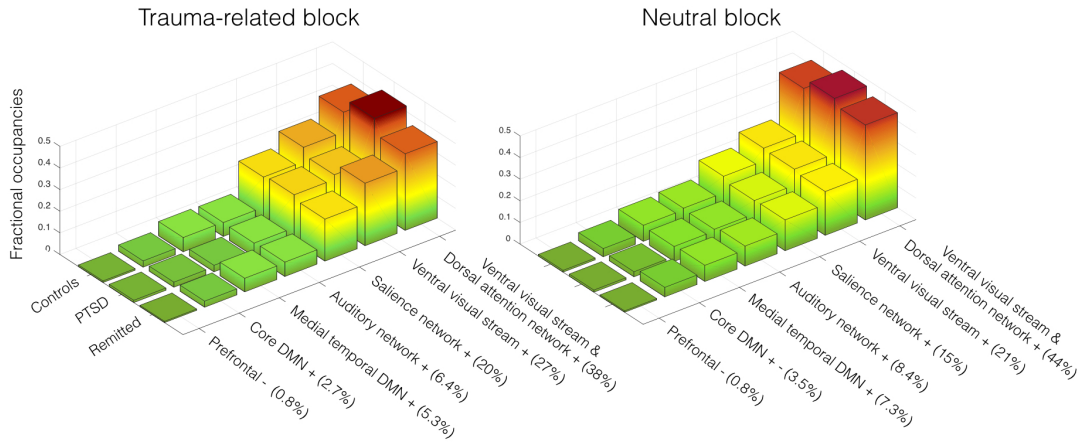


Figure 5.2. The brains of PTSD, remitted or control participants spend different amounts of time in different networks in the trauma-related and neutral blocks. *Here the brain states ordered in ascending order according to their fractional occupancies (trauma-related block and neutral block). We observe that the most time-dominant state is the Visual ventral stream & dorsal attention network (+), active during 38% and 44% of the time depending on the condition, and the least time-dominant state is the PFC(-), active only during 0.8% of the time independently of the task condition. We also observe that the 3 groups have more homogenous fractional occupancies during presentation of neutral pictures, while the trauma-related pictures induce more marked differences between groups in the 'Medial temporal DMN +', 'Visual Ventral stream +', and the 'Visual ventral stream & dorsal attention network'.*

Fractional Occupancies in healthy controls, PTSD and remitted PTSD participants

In order to investigate differences between groups, we extracted the fractional occupancy (i.e., time spent in each of these states) both during trauma-related and during neutral picture presentation for each participant. By extracting the fractional occupancies separately for each of the two conditions we were able to estimate how the different networks were affected during presentation of trauma reminders and during the subsequent neutral periods both before and after cognitive therapy.

Our results show that the 'Medial temporal DMN +' was active during less time in PTSD in contrast to both healthy controls ($p = 0.017$ after correction for multiple comparisons) and remitted PTSD ($p=0.013$ after correction for multiple comparisons) participants during presentation of trauma-related pictures *(Figure 5.3B)*. This suggests that PTSD is associated with down-regulation of this states' activation during processing of trauma-related pictures while subsequent successful CT-PTSD treatment is associated with activity upregulation, reaching levels similar to those observed in healthy control participants.

This spatial map from this state differs from the 'Core DMN +' in that, besides having the classical DMN regions (which have been related to self-referential processing), it includes areas involved with visual processing, necessary for our experimental paradigm, and medial temporal regions, involved with recall of autobiographical memories. Of note, there were no significant differences between groups during presentation of neutral pictures, where our results indicate that all groups spent a comparable amount of time on this state.

We also observed a significant difference in the time spent in the 'Core DMN +' between PTSD and remitted PTSD during presentation of neutral pictures ($p = 0.0017$ after correction for multiple comparisons) *(Figure 5.3B)*. However, in this case, healthy controls did not significantly differ from participants with PTSD, which might be due to a lack of power related to the smaller sample size of the control group. This seems to indicate that in the absence of trauma reminders, the core DMN is more often activated in healthy participants, especially those that have recovered from PTSD through cognitive therapy.

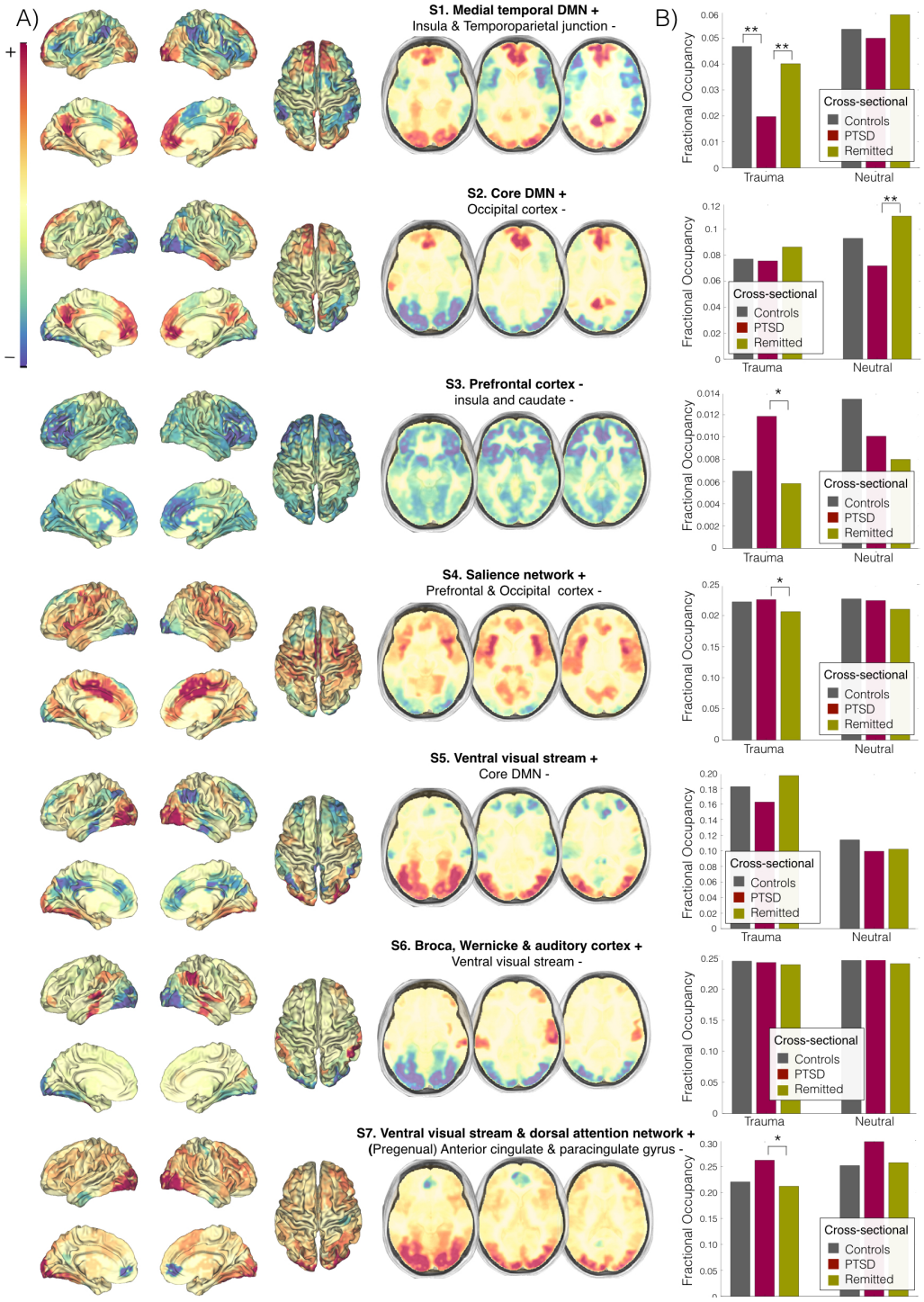


Figure 5.3. PTSD participants have significantly different brain responses to stimuli compared to remitted and control participants. *Here the activation states and cross-sectional analysis of their fractional occupancies. a) Spatial maps of the brain states showing areas with above average activation (red/orange) and areas with under average activation or deactivation (blue/green). b) Fractional occupancies (i.e., time spent in each of the states) for each of the groups (healthy controls, PTSD and remitted) for each of the task conditions (trauma-related pictures and neutral pictures). Strongest differences are found both in the medial temporal and core DMN. *p < 0.05, **FDR < 0.05 corrected across group comparisons, experimental condition and number of states.*

None of the fractional occupancy group comparisons related to the remaining states reached the significant level of $p<0.05$ after FDR correction for multiple comparisons considering all the combination of group contrasts (Controls vs. PTSD, PTSD vs. Remitted PTSD and Controls vs. Remitted PTSD), experimental condition (Trauma and Neutral pictures) and number of states (7 states). However, there was a certain trend towards decreased fractional occupancies of the 'Prefrontal – ' and the 'Salience network + ' in remitted PTSD in contrast to current PTSD ($p = 0.013$ and $p = 0.015$, respectively) during presentation of trauma-related stimuli. It is worth noting, however, that the 'Prefrontal - ' state's fractional occupancies were generally very low, with some participants, specially in the healthy control and remitted PTSD conditions, never engaging this state in the course of the experimental task. Analysis after exclusion of an outlier in the fractional occupancies of the 'Ventral visual stream & dorsal attention network + ' state also revealed less time spent in this network in remitted PTSD in contrast to current PTSD ($p = 0.018$) during trauma-related picture presentation, although this result was not significant after correction for multiple comparisons. During neutral picture presentation, no further differences between groups were observed.

Overall, our cross-sectional results suggest that successful cognitive therapy for PTSD results in upregulation of time spent in DMN-related networks. When the stimulus presented is neutral, only regions of the core DMN see this increase in activity, whereas when the stimulus is related to the traumatic experience of the participant, both the core and the medial temporal DMN states increase their activity. There is also a trend towards less time activating states related to saliency processing (salience +) and a state characterised by below average activation of the prefrontal cortex (PFC –).

Of note, these results refer to a sample of participants including longitudinal data (both pre- and post-cognitive therapy scans belong to the same participant) and cross-sectional data (participants that were able to undergo the scanning procedure only before or after the therapy). In the following section we present results from the same analysis conducted only on the longitudinal subsample, in which the two scans come from the same participant.

Differences between PTSD before and after CT and PTSD before and after waiting list

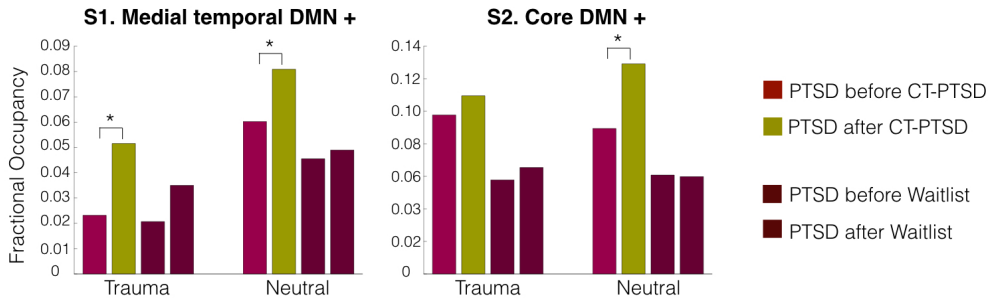


Figure 5.4. Longitudinal confirmation of main results of the chapter, showing the fractional occupancies for the two states with the most marked differences when comparing healthy controls, PTSD and remitted PTSD. *Results on a subsample of participants that completed both pre- and post- CT-PTSD scans confirm our previous analysis, showing an increase in the fractional occupancies of participants after CT-PTSD but not after being on the waiting list* (*p < 0.05).

In order to overcome any potential limitations from our mixed sample (i.e., containing both cross-sectional and longitudinal data), we evaluated the presence of differences before and after successful therapy in a subsample of participants that had completed both scans. We then assessed whether any differences were present in a subsample of participants that had also completed the two scans within a similar period of time but without receiving any cognitive therapy between visits (waiting list condition).

Longitudinal analyses on the subsample of participants confirmed that PTSD recovery though CT-PTSD reflects mainly in up-regulation of the time spent in DMN-related brain states. In particular, we observed increased fractional occupancies of the 'Medial temporal DMN +' state both during the presentation of trauma-related pictures (p = 0.008) and neutral pictures (p = 0.035) after CT-PTSD but not after being on the waiting list. Regarding the 'Core DMN +', we observed an increase in the fractional occupancy of the 'Core DMN +' in participants recovered from PTSD in contrast to these same participants before therapy, in line with our results from the mixed sample analysis. Importantly, this increase was not present after time on the waiting list *(Figure 5.4)*. Nevertheless, these results did not survive FDR correction across multiple comparisons and can therefore only be considered preliminary. The

same analysis on the fractional occupancies of the remaining brain states did not show any differences, neither between PTSD before and after treatment, nor between PTSD before and after being assigned to a waiting list conditions.

We then calculated the differences in fractional occupancies between PTSD before and after CT-PTSD as well as PTSD before and after being on the waiting list. The comparison between these did not show any significant differences. This could be due to smaller improvements taking place over time in spite of the absence of therapy and/or lack of statistical power due to the small sample size of this subsample.

Relationship between PTSD symptomatology and fractional occupancies

To further investigate the association between the temporal dominance of our states and PTSD symptomatology, we computed correlations between the fractional occupancies and the scores from the PTSD Symptom Scale Interview (PSS-I, Foa et al., (1993).

First, we computed the correlations between the fractional occupancies of our seven states and PSS-I scores. Based on this, we computed aggregated p-value to see which states fractional occupancies correlate significantly with one, a combination, or all the PSSI scores. As with previous analysis, we did this separately for the neutral and the trauma-related picture blocks. We found that the fractional occupancies of the 'Core DMN+' during neutral picture presentation are associated with PTSD symptomatology *(Figure 5.5a)*. Similar results were obtained with the fractional occupancies of the 'Medial temporal DMN+' and the 'Salience network +' during trauma-related picture presentation and PTSD symptomatology *(Figure 5.5b)*.

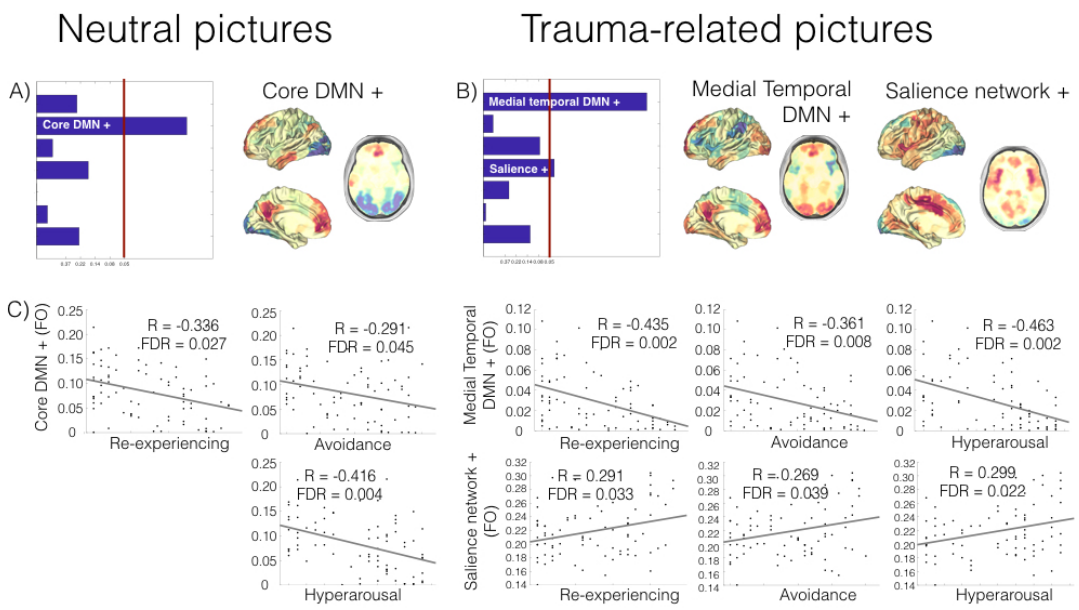


Figure 5.5 Correlational analysis with symptom severity confirms a negative relationship between fractional occupancies (FO) temporal dominance of the core and medial temporal default mode network. *Additionally, we find a positive relationship with the fractional occupancies of the salience state. a) Aggregated p-values for the correlations of all brain states' fractional occupancies across PTSD symptom clusters during presentation of neutral pictures, FWER corrected. b) Aggregated p-values for the correlations of all brain states' fractional occupancies across PTSD symptom clusters during presentation of trauma-related pictures, FWER corrected. c) Illustration of the three states fractional occupancies significantly correlating with all three symptom clusters.*

We then investigated whether this was due to strong correlation with a particular symptoms cluster, a combination, or all symptom clusters *(Figure 5.5c).* During presentation of neutral pictures, we obtained significant negative correlations between time spent in the 'Core DMN +' and all PTSD symptom clusters (Re-experiencing: FDR = 0.027, Avoidance: FDR = 0.045, Hyperarousal: FDR = 0.004), although the correlation was strongest for hyperarousal (r=0.42). During presentation of trauma-related pictures we also obtained negative correlations between time spent in the 'Medial Temporal DMN+' and all PTSD symptom clusters (Re-experiencing: FDR = 0.002, Avoidance: FDR = 0.008, Hyperarousal: FDR = 0.002), indicating that the more time this state is active, the less PTSD symptoms the participant has. Furthermore, we found a significant positive relationship between time spent in the 'Salience network +' state and severity of PTSD symptoms (Re-experiencing: FDR = 0.033, Avoidance: FDR = 0.039, Hyperarousal: FDR = 0.022). Finally, we found a significant positive

relationship between time spent in a state characterised by below average activation of the prefrontal cortex state (FDR = 0.024) and re-experiencing, as well as a state characterised by above average activation of the visual ventral stream and the dorsal attention network, and hyperarousal (FDR = 0.03). Time spent in the other brain states did not show any significant relationship with PTSD symptomatology after correcting across number of states and number of symptom clusters.

Relationship between flashback qualities of intrusive memories and fractional occupancies

In the following, we explore the relationship between fractional occupancies and different flashback qualities associated with intrusive memories in PTSD. In particular, we tested whether the fractional occupancies of these states were related to the amount of intrusive memories elicited by the experimental task and the flashback qualities of these intrusive memories (i.e., Vividness, Distress, and feeling of 'Here & Now'). To this end, we looked at correlation between fractional occupancies and number and flashback qualities of intrusive memories in any participant experiencing at least one intrusive memory, including those without PTSD, since a substantial proportion of trauma-exposed participants without PTSD also experienced intrusive memories during the task, both in our study and previous research (Michael et al., 2005). These correlations revealed that 'Medial temporal DMN+' fractional occupancies correlate *negatively* with distress (r=-0.38, p = 0.0008 FDR = 0.036) whereas the fractional occupancies of the 'Ventral visual stream & dorsal attention network' showed a *positive* correlation with this same variable although this was not significant after correction for multiple comparisons (r= 0.32; p=0.0072; FDR = 0.0607). In other words, when memories elicited by the images are particularly distressing, participants spent more time in a network involved with visual processing and top-down attention and less time in a network involved with contextualised memory recall. Furthermore, we also observed that the more time they spent in the salience state, the more they felt as if the trauma memory was currently happening (i.e., feeling of 'Here & Now'), although again this was not significant after FDR correction (R= 0.32; p = 0.0061; FDR = 0.0607) (*Figure 5. A, C* and *D*). When considering all p-values from correlations between memory variables and brain states' FO we observe that time spent in the 'Medial temporal DMN+' is related to at least one, but potentially a

combination of intrusive memory variables *(Figure 5.6b, left)*. Indeed, individual correlations reveal that additionally to the correlation with distress, the fractional occupancies of this network correlate with vividness and 'here & now' feeling, although the significance of these results does not survive correction for multiple comparisons (*Figure 5.6 a and c*).

Approaching this from the complementary perspective of obtaining aggregated p-values across the states for each of the memory variables, we observed that how distressing the memory is and how much it makes the participant feel as if it was happening again is reflected in (a combination) of the fractional occupancies of the estimated states *(Figure 5.6b, right)*.

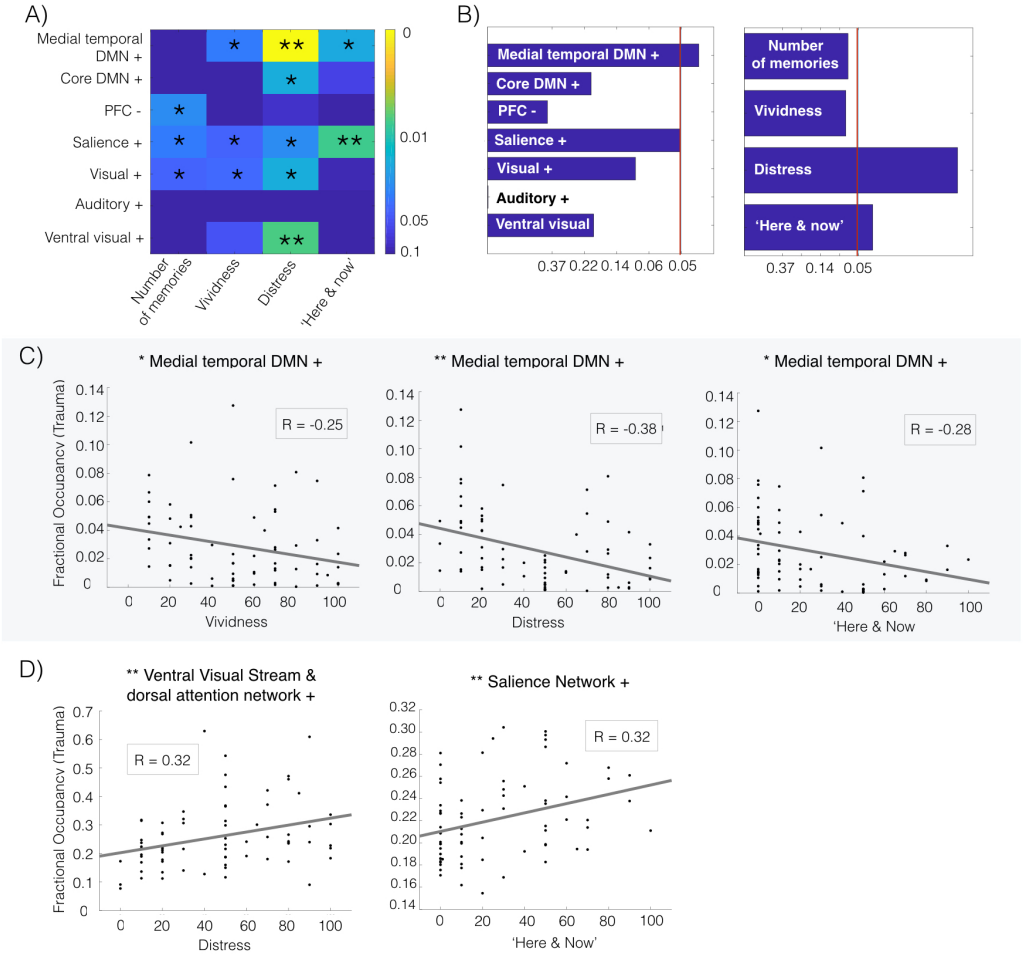


Figure 5.6. The fractional occupancy of some states are directly correlated with the qualities of intrusive memories. *a) Matrix of correlation p-values for each of the brain states fractional occupancies with each of the memory variables (*p < 0.05 **FWER < 0.05). b) Aggregated P-values for the correlations of brain states' FO across memory variables (left) and memory variables across brain states' FO (right), FWER corrected. c) Correlation between time spent in the 'Medial temporal DMN +', and*

quality of the intrusive memories. All variables related to quality of the memory are negatively correlated to time spent in this brain state, although only the relationship with how distressing the intrusive memories were remains significant after FWER correction. B) Significant correlations (FWER corrected) between brain states fractional occupancies and intrusive memory qualities. Time spent in the 'Ventral visual stream & dorsal attention network +' and 'Salience network' correlates positively with how distressing and how much the memory felt as if it was currently happening, respectively.

DISCUSSION

To date, research on the neural correlates of PTSD and the changes associated with remittance of the disorder have, at least to some extent, made inefficient use of the data by focusing only on few regions of interest and/or assuming temporal invariability in the patterns of brain activity across time. Most recent research and theories, however, suggests that the study of large-scale functional networks could bring interesting insights to the field due to their involvement in cognitive and emotional processing (Akiki et al., 2017; Menon, 2011; Sripada et al., 2012). Furthermore, recent developments in fMRI methodology allow now the study of temporal fluctuations in these large-scale functional brain networks, allowing us to reliably describe the fluctuations of neural activity over time. While there are a few previous studies applying a dynamic approach to fMRI data of participants with PTSD (Jin et al., 2017; Li et al., 2014; Ou et al., 2014), to our knowledge, there are no previous studies investigating how temporal fluctuations of large-scale functional networks can be modulated by PTSD treatment. Hence, this is the first study providing a compact model of how the main large-scale networks driving functional activity fluctuate in time and how this relates to both PTSD and PTSD remittance with cognitive therapy. Importantly, this expands our understanding of the disorder and provides interesting insights into how large-scale networks are rebalanced in association with recovery of mental health, which might in due time provide useful insights for the development of therapeutic approaches for the most resistant PTSD cases.

Here, we use a data-driven approach based on Hidden Markov Models that allows us to extract a predefined number of large-scale states (i.e., temporally recurrent activation of large-scale networks), representing regions of above average and under average activation. The model further allows us to estimate the amount of time during which these brain states are driving brain activity.

The medial temporal DMN and contextualisation of autobiographical memories

Results show that during the presentation of trauma-related pictures, a state related to the DMN, visual and medial temporal areas becomes activated for a longer period of time for both controls and remitted PTSD participants compared to participants with PTSD. A subsequent analysis focusing on a longitudinal subsample showed indeed that the time during which this brain state was active increased in PTSD participants after successful cognitive therapy but not after several months in the waiting list condition. Furthermore, we showed significant negative correlation between time spent in this DMN network and all three PTSD symptom clusters, meaning that the more severe the disorder, the less time participants engage this network during presentation of trauma-related stimuli. We further show negative correlations between time spent on this state and the distress accompanying intrusive memories prompted by the trauma-related pictures. Vividness of the memory (visual sensory impressions) and a feeling of 'here and now' were also negatively associated with time spent in this particular DMN state, although these associations were not significant after correction for multiple comparisons.

Based on its spatial map, we refer to this state as the medial temporal DMN. Our medial temporal DMN state is characterised by a pattern of above average activation in regions of the midline DMN, related to integration and retrieval of self-referential information in the context of one's own person (Northoff, 2011; Qin and Northoff, 2011) and hippocampus and parahippocampal gyrus, associated with contextualised recall of episodic and autobiographical memories (Eldridge et al., 2000). This is consistent with previous reports of a medial temporal DMN subsystem involved in episodic/contextual retrieval (Andrews-Hanna et al., 2014, 2010), in addition to mental simulation, including associative or constructive processes (Bar, 2009; Hassabis and Maguire, 2007). Furthermore, our medial temporal DMN state includes regions of the visual cortex, possibly engaged with visual processing of the presented images.

Other regions of this network, such as the posterior cingulate gyrus and the adjacent precuneus have been ascribed a role in integration of self-referential stimuli in the context of one's own person, as for example when subjects have to indicate whether a word or a statement is self-descriptive or not (Keyes, 2012; Northoff, 2011). The anterior part of the estimated spatial map, the anteromedial prefrontal cortex, is also strongly related to self-referential processing, such as when individuals reference

information to themselves, retrieve personal knowledge or autobiographical memories, create simulations for personal future events and consider their future goals or mental states (Andrews-Hanna, 2012; D'Argembeau, 2013; Denny et al., 2012; Moran et al., 2013; Spreng et al., 2009). Furthermore, the anteromedial prefrontal cortex holds a particularly advantageous position to integrate salient stimuli or information, whether external or internal, due to its widespread connectivity with mnemonic, limbic, autonomic, and semantic structures.

In addition to the strong activation of the midline DMN regions and visual cortex, we also observe above average activation of the hippocampus, parahippocampal cortex and anterior temporal cortex. The hippocampus, together with the retrosplenial cortex, precuneus and ventromedial prefrontal cortex, have been ascribed a role in supporting memory by retrieving information about the context of episodic events (Burgess et al., 2001) and can be activated both voluntarily and involuntarily (Hall et al., 2008). Specifically, since the hippocampus has both space and time cells (Howard et al., 2014; Kraus et al., 2013; MacDonald et al., 2011; Manns et al., 2007), the upregulation of a state might be related to organisation of spatiotemporal elements of the experience into coherent memories. Furthermore, the parahippocampal cortex contains the parahippocampal place area, which plays an important role in the recognition of environmental scenes (Aguirre et al., 1998; Epstein et al., 1999; Epstein and Kanwisher, 1998).

Of note, it is not surprising that the regions along the cortical midline exhibit the strongest activation, given that they have been shown to exhibit strong functional coherence with other DMN subsystems and have therefore been hypothesized to act as functional hubs that allow information transference between systems (Andrews-Hanna et al., 2014).

Overall, based on the function of the brain regions activated in this state and its negative relationship to PTSD symptomatology as well as the qualities of intrusive memories, its decreased engagement seems likely to be associated with the decontextualised recall of memories characteristic of the disorder (Ehlers and Clark, 2000). Furthermore, we have shown that successful cognitive therapy for PTSD increases the time spent in this network.

Core DMN as a self-referential hub

Further results from our study show that during the presentation of neutral pictures, remitted PTSD participants engage a network encompassing core regions of DMN during more time than participants with a current PTSD diagnosis. This result was also confirmed longitudinally, showing that the time during which this brain state was driving brain activity in the neutral picture condition was longer in PTSD participants after successful cognitive therapy but not after several months in the waiting list condition. Negative correlations with PTSD symptomatology provided further support for this result, and in this case, the association with the qualities of intrusive memories could not be shown. Overall, it seems that regions of the core DMN, which are associated mainly with self-referential processes (Northoff and Bermpohl, 2004; Qin and Northoff, 2011) but also emotion evaluation and mnemonic processes shared with the medial temporal DMN (Andrews-Hanna et al., 2014), are upregulated after recovery from PTSD, even when the pictures presented are not related to the traumatic event and have no emotional load. As mentioned, midline regions of the DMN have been suggested to work as functional hubs facilitating transference between the different subsystems (Andrews-Hanna et al., 2014). Therefore, the up-regulation of this state during presentation of neutral pictures might be representing a ubiquitous upregulation of the DMN that further facilitates the engagement of the medial temporal DMN when the emotional stability of the participant is probed by trauma reminders. In those cases, the stability might be maintained though activation of brain regions that anchor the participants in the (safe) present by facilitating the recall of the specific context of the traumatic experience, an aspect trained during cognitive therapy.

Previous studies of DMN dysregulation in PTSD

Our results appear in line with previous findings of spatial alterations in the DMN in PTSD (Bluhm et al., 2009; Patel et al., 2012; Qin et al., 2012; Sripada et al., 2012) and, in particular, in the medial temporal DMN (Miller et al., 2017). However, these previous findings were based on time-invariant FC, and are therefore fundamentally different from our findings. First, any decrease in static FC could be due to a decrease in either coupling strength or the amount of time during which this coupling is taking place. Second, our states are not driven by FC but by mean brain activity. While this

definition of states also results in large-scale networks, these networks are not defined by coupling or synchronisation among the regions that integrate them but by above or under average activation. Hence, we show that the alterations of the DMN associated with PTSD are specifically related to less time activating DMN-related networks. We also show that the time during which the core and the medial temporal DMN states are engaged can be upregulated through cognitive therapy, perhaps thanks to cognitive work on the contextualisation of the trauma memory. Despite the differences in the methodological approach, this appears in line with previous results showing increased FC of the posterior cingulate cortex with dorsolateral prefrontal cortex and anterior cingulate cortex following mindfulness-based exposure therapy (King et al., 2016). Furthermore, a recent systematic review showed that successful psychotherapeutic treatment was associated with decreased activity in limbic regions (amygdala) and increased activity in hippocampus, prefrontal cortex and ventral anterior cingulate cortex (Malejko et al., 2017).

Fractional occupancies of other states related to PTSD

The decreased time engaging DMN-related states in PTSD comes accompanied by increased time engaging 3 other states: the salience (+), prefrontal cortex (-), and the ventral visual stream and dorsal attention network (+) states. Importantly, the time engaging these networks is decreased after cognitive therapy. However, the difference between fractional occupancies in these networks before and after the therapy was not significant after correction across multiple comparisons taking into account group comparisons, experimental condition and number of states. Nevertheless, when looking at aggregated p-values of correlations between fractional occupancies and PTSD symptom severity, we observed that spending more time on the salience state correlates positively with severity of all three symptoms clusters.

Previous time-invariant studies have found increased connectivity between the insula, central region of the SN, and other SN regions (Birn et al., 2014; Cisler et al., 2015, 2013; Fonzo et al., 2010; Simmons et al., 2008; Sripada et al., 2012). In regard to treatment studies, prolonged exposure therapy in remitted participants with posttraumatic stress disorder has been found to be associated with decreased activation in the left anterior insula, together with increased connectivity between this region and the right cingulate cortex, both main regions of the SN (Simmons et al.,

2013). While the approach used in these studies is fundamentally different from ours - given their focus is on the strength of connectivity while we restrict ourselves to assessing the amount of time during which this network is active – the results are coherent in that the salience state gains predominance in PTSD and is rebalanced after treatment. The altered static connectivity within the SN has been explained through a dysregulation in the threat-sensitivity circuits. This is thought to underlie symptoms of hypervigilance and hyperarousal, based on studies pointing at increased anterior insula activation together with increased levels of awareness of bodily arousal during re-experiencing and hyperarousal symptoms (Hopper et al., 2014; Paulus and Stein, 2006; Shin and Liberzon, 2010).

With regard to our prefrontal (-) state, previous studies and perspectives on PTSD suggest decreased inhibition of limbic structures by the prefrontal cortex, which according to the traditional neurocircuitry model of PTSD is the main mechanism underlying the disorder (Liberzon and Sripada, 2007; Rauch et al., 2006). Later work suggested that the possible undermodulation resulting from decreased prefrontal cortex activation is characteristic of the hyperalertness subtype of PTSD (Ruth A. Lanius et al., 2010b). However, below average activation in this state is not restricted to the prefrontal cortex. As described in the results, we also observe below average activation of the insula and the striatum within this same sate. This is contradictory with results showing increased activation of the insula in PTSD. However, the model by Lanius et al., (2010b) suggests that there might be a dissociative subtype of PTSD characterised by decreased insula activation (or, rephrased in terms of our state, increased time visiting a state characterised by below average activation of the insula). To summarise, we observe coexistence of above average activation of the insula (in the salience (+) state), together with below average activation of the insula (in the prefrontal (-) state) at different times during our experimental paradigm. This would support Lanius et al.'s (2010b) hypothesis regarding a dynamic interplay between PTSD subtypes, the hyperalertness and the dissociative subtype. However, and despite of not being specified in their theory, that dynamic interplay Lanius and colleagues refer to is unlikely to occur at the timescales we are investigating here.

Lastly, our results also showed downregulation of time spent in a network of ventral visual stream activation, involved with visual object recognition, together with (pregenual) ACC deactivation, which plays a crucial role in the integration of inputs

for affect regulation and social interactions (Etkin et al., 2011). Furthermore, it has been suggested that visual attention can be directed by affective significance of visual stimuli before comprehensive processing of the stimuli (Rudrauf et al., 2008). The time spent in this network during the trauma-related condition correlates positively with hyperalertness, i.e., the more hyperalert our participants were, the more time they spent on affectively unregulated recognition of the trauma-related images. This state also shows above average activation of regions belonging to the dorsal attention network, involved with top-down attention (Fox et al., 2006), although this is closer to average activation. It seems plausible that the visual processing of the images would require some parallel recruitment of the dorsal attention network, which would allow maintenance of attention on the images and the object recognition involved.

Limitations

There are some limitations to our study, which are important to take into account and address in future research. First, and despite coherence between results from our mixed sample and our longitudinal subsample in the upregulation of DMN-related brain states after cognitive therapy it is important to note that the longitudinal subsample had a small sample size. Second, it has been shown that PTSD is a heterogenous disorder, with potential different phenotypes that were not taken into account in our analysis. Third, we could not incorporate information about FC in the states due to a suboptimal registration procedure, which made subjects too dissimilar in terms of their FC profiles. Overwhelmed by the size of the between-subject FC differences, the HMM was not able to capture any relevant within-subject changes. These large differences in FC between subjects are likely to be largely due to anatomical differences (Bijsterbosch et al., 2018).

Chapter 6

GENERAL DISCUSSION

Each year, more than half a billion people worldwide suffer from a stress-related mental disorder such as anxiety, PTSD or MDD. To some degree, these can be traced back to the influence of endogenous or exogenous stressors, such as, traumatic events, challenging life circumstances or physical illness. Despite the research efforts, statistics show that on average there has been no significant decrease in the number of cases over the last decades (Vos et al., 2015). Likely explanations include lack of prevention strategies, due to some extent to a limited understanding of vulnerability factors beyond socioeconomic aspects, and suboptimal treatments, especially for those presenting with the most severe cases. A better understanding of the neural correlates and mechanisms is important to improve both prevention and treatment approaches. The combination of animal and human studies in other areas of clinical neuroscience has enabled great progress in the last decades in comparison to neuropsychiatric research. There are two main features of neuropsychiatric disorders that make them particularly challenging. First, the relevant symptoms and behaviours that can be assessed in animal models of psychiatric disorders are often limited, sometimes excluding the key symptoms present in humans (e.g., re-experiencing in PTSD). Second, it seems that the neural signatures of psychiatric disorders are particularly heterogenous, and often, more subtle than those characteristic of, for example, neurodegenerative diseases. Fortunately, constant and steep improvement of human neuroimaging methods is enabling the study of human brain function in an ever more accurate and meaningful manner. This, in combination with carefully designed studies, may finally bring significant growth to the field of neuropsychiatry.

SUMMARY OF ORIGINAL CONTRIBUTIONS

This thesis contains two longitudinal fMRI studies. These were explored using standard FC analyses and two different recently proposed methods for the time-varying characterization of the BOLD signal. In the first experimental chapter, we applied a static FC approach on an experimental design in which three resting-state scans were acquired along a stress induction paradigm (Chapter 2). This allowed us to

study FC at different times during the stress response, including 1 hour and 30 minutes after the stressor (stress recovery), which is a phase often neglected in other studies. Our results showed an increase in striato-cortical FC in recovery in contrast to both immediately after stress and before stress. Correlational analysis also revealed interesting results for the characterisation of FC in regard to the first signs of mental disorder. In particular, we observed stronger FC of limbic areas (amygdala and orbitofrontal cortex) with temporoparietal cortex accompanied by weaker FC between areas of the DMN (bilateral angular gyrus and frontal regions) in participants with higher vulnerability scores. This first experimental chapter defined a methodological baseline, using an approach similar to those applied in many other studies to date. On top, we went on to build a more sophisticated and accurate characterisation of the stress dynamics.

We applied a state-of-the-art time-varying analysis in Chapters 3 and 4, where the data was characterised in terms of visits to a discrete number of states. We extracted several measures for each of our detected states, each representing a recurrent network. This approach allowed us to reveal an overall decrease in the temporal stability and dominance of a basal ganglia state in stress recovery in contrast to the other conditions *(Figure 3.3),* adding to the findings from Chapter 2 regarding static FC. In other words, we observed that the time-averaged increase in striato-cortical FC characteristic of stress recovery (Chapter 2) is probably related to less time visiting a brain cortico-basal ganglia state during that stress phase (i.e., decreased lifetimes). Furthermore, adding the time-varying perspective allowed for detection of other changes that were inaccessible in the static FC approach, specially related to the relationship of the states' temporal features and vulnerability scores. In particular, vulnerability scores were positively related to the lifetimes of the frontoparietal state immediately after stress and negatively related to both lifetimes and fractional occupancies in stress recovery *(Figure 3.4).* More vulnerable participants also presented difficulties to transition from the frontoparietal state to the default mode state immediately after stress, and to the default mode and sensorimotor state immediately after stress *(Figure 4.4).* Overall, Chapters 3 and 4 suggest that accumulation of stress as a result of continued exposure to military conflict reflects in increased lifetimes of a frontoparietal state and decreased transitions from the frontoparietal to other states, predominantly to the default mode state, after encountering a new stressor.

In Chapter 5, we moved from the study of healthy, and predominantly highly resilient participants, to the investigation of brain dynamics before and after successful cognitive therapy for PTSD. This allowed us to detect brain states affected in PTSD in contrast to healthy controls and evaluate whether the therapy is able to modulate these states back to a healthy 'activation behaviour'. In this situation of fully developed PTSD, the default mode system appears at the centre of differences. We observed two default mode states (i.e., core default mode and medial temporal default mode) being engaged less often in PTSD in contrast to remitted participants and, in the case of the medial temporal DMN, also in contrast to healthy controls *(Figure 5.3)*. Correlational analysis between time spent in these states and PTSD symptom severity provided further evidence in support of a dysregulation of the default mode system in PTSD *(Figure 5.4)*. Further analysis revealed that downregulation of the medial temporal DMN together with upregulation of the salience and visual states are linked to more marked intrusive qualities of trauma memories *(Figure 5.5)*. As pointed out in the experimental chapter, the application of this approach allows to separately evaluate one of the two main factors that could be responsible for the often observed dysregulation of the DMN in PTSD: strength of the synchronisation among DMN regions and total time during which the DMN is active as a network. In particular, we find that there is a decrease of the time during which the network is active, referred to as a decrease fractional occupancies or temporal dominance. To our knowledge, this is the first study investigating the temporal dynamics associated with successful psychological treatment of PTSD.

In the following section, we integrate our findings from Chapters 3, 4, and 5 into a dynamic perspective of PTSD in which we discuss a potential trajectory of how stress vulnerability modulates brain dynamics, how these dynamics are then further dysregulated in PTSD, and how they can be rebalanced through cognitive therapy for PTSD.

A DYNAMIC PERSPECTIVE ON PTSD

To date, several neurobiological models have proposed that the dynamic interaction between large-scale networks might play a prominent role in the cognitive and affective dysfunctions characteristic of PTSD and other stress-related disorders (Akiki et al., 2017; Menon, 2011). While these models emphasise the importance of deficits in

access, engagement, and disengagement of these networks, they are primarily based on static FC studies, which are limited in their ability to directly address these aspects.

Generally speaking, the inclusion of a time-varying perspective has the primary advantage of being more closely relatable to brain function, as we know that the brain is a dynamic system. More specifically, our approach allowed us to investigate one of the two aspects that appear indivisible in static FC studies, namely the time during which a set of regions synchronise. In static FC studies, this aspect appears together with the degree to which a set of regions synchronises, resulting in a single time-averaged measurement of the relationship between networks. This prevents assessment of specific hypotheses regarding when these networks are engaged, for how long, and whether specific patterns of transitions occur amongst them. Furthermore, by using a data-driven approach for the decomposition of the BOLD signal in several brain states we were able to evaluate all main networks driving brain activity at once, providing a compact model of how these large-scale networks fluctuate in time and how these fluctuation relate to both vulnerability and PTSD.

Our results showed that participants with higher vulnerability scores also had longer lifetimes in a frontoparietal state immediately after stress *(Figure 6.1a)*. The frontoparietal network is part of a control system that has been shown to have a limited capacity (Mitchell and Cusack, 2008; Schmeichel et al., 2008). A variety of factors have been found to reduce this control capacity, with excessive stress being one of them (Sato et al., 2012). Thus, we propose that when participants are exposed to stress, those that are most vulnerable to stress might have a higher overload of the system, which reflects in increased lifetimes of the frontoparietal state in order to cope with this stress. In this context, the increase in the lifetimes seen in more vulnerable participants could be related to a potential compensatory mechanism. A recent perspective investigating the dynamics of stress suggests that a strategic allocation of resources is vital for survival and that this is reflected in large-scale brain network shifts involved with specific cognitive functions (Hermans et al., 2014, 2011). One of these networks is the FPN, which is suggested to attract resources allocated to the SN immediately after stress once this phase subsides. As the FPN has a limited capacity, the increased allocation of resources in a relatively early stage might eventually become depleted, as the decreases in both temporal stability and dominance in the late stage of stress recovery seem to indicate. While we are not aware of any direct

evidence supporting the hypothesis of depleted resources in stress recovery, we believe it would be interesting for further investigation and the latest time-varying approaches might be very helpful tools in this endeavour.

Further results from this dataset showed that the increased lifetimes of the frontoparietal state in more vulnerable participants immediately after stress came accompanied by decreased transitions from this state to other states after stress, in particular the default mode and the sensorimotor states. It has been proposed that the frontoparietal system might be at the centre of a system maintaining or reinstating homeostatic balance after stress via feedback loops with other brain systems, such as the default mode and the sensorimotor networks (Cole et al., 2014). The integrity of these feedback loops is suggested to be key for mental health, which is in line with our results showing decreased transitions to the default mode state and the sensorimotor state in more vulnerable participants after stress induction. In particular, these authors suggest that when symptoms or discomfort arise, the FPN needs to regulate those symptoms, which reduces its capacity for other cognitive demands (Anticevic et al., 2012).

Another relevant theory anchored in the limited capacity of the FPN was proposed by Smallwood et al., (2012). More specifically, they suggest that cooperation between the FPN and the DMN is crucial for the generation and maintenance of an internal train of thought. Specifically, the DMN would be providing autobiographical information and the FPN would be responsible for helping to sustain and buffer internal trains of though against disruption from the external world. The content of such internal trains of thought is highly complex and heterogeneous, and could be adaptive (Smallwood et al., 2009, 2011; Smallwood, 2010; Spreng et al., 2010) or maladaptive (Broyd et al., 2009; McVay and Kane, 2010). The possibility of these internal thoughts being adaptive, perhaps in the sense of facilitating creative insight and possible solutions to ongoing problems, links back to our results, as we show that resilient participants transition more often back and forth from the frontoparietal to the DMN in both scans acquired after stress induction. More vulnerable participants, on the other hand, present a tendency to switch more often from the basal ganglia to the visual state immediately after stress and even during stress recovery, which might be related to a hyperalert state persisting over an excessively prolonged period of time. Specifically, amplification of perceptual information is suggested to disrupt and

compete with the establishment of an internal train of thought (Smallwood et al., 2012).

Although we were not able to specifically investigate evolution to chronicity in this thesis, we hypothesise that the decreased frontoparietal-default mode transitions after acute stress might be related to the evolution of chronic stress conditions. This might eventually lead to the default mode hypoactivation and hypoconnectivity established in numerous studies (King et al., 2016; Lanius et al., 2015; Sheline et al., 2009; Sripada et al., 2012; Wise et al., 2017).

Indeed, we evaluated differences in dynamics in participants with PTSD and the changes associated with their remittance resulting from undergoing cognitive therapy in Chapter 5. Here, we show that out of the seven states detected, the two related to the DMN show the strongest decrease in fractional occupancies when comparing PTSD to healthy controls and/or remitted PTSD participants *(Figure 6.1b)*. We further show a negative relationship between the temporal dominance of this state and both symptom severity and qualities of intrusive memories characteristic of PTSD, supporting the link between this disorder and disruption of the DMN shown in previous studies (King et al., 2016; Lanius et al., 2015; Sripada et al., 2012).

While the link between PTSD and DMN disruption has been acknowledged in previous models, its interpretation remains under debate. Akiki and colleagues (2017) propose that the decreased activity of the DMN is related to re-experiencing, dissociation, and avoidance, some of the symptom clusters defined in the DSM-5. However, there is not a clear and established correspondence between each symptoms cluster and the associated neural correlates, one of the main reasons for which the DSM-5 has been criticized (Stahl, 2013). We therefore refer to a well-established cognitive model for PTSD (Ehlers and Clark, 2000), which has served as the basis for the development of one of the first-line psychotherapeutic treatments for PTSD. We have focused on this model for the interpretation of our results, since it allows us, beyond symptom clusters, to discuss the cognitive mechanisms proposed to cause the observed symptomatology. Often these models are much richer than nosological classifications, which are meant to contain a simplification of the disorder that facilitates diagnosis but often fails to capture important phenomena related to the essence of the disorder (e.g., mental defeat during the traumatic event or a feeling of being frozen in time). Perhaps this is one of the reasons why previous

neurophysiological models of PTSD, strongly based on symptom clusters or fear conditioning principles, ignore to some extent important aspects in individual variability in the ability to integrate emotions, memories and perceptions about oneself and the surrounding world in a coherent framework, allowing oneself to 'make sense' of new and old experiences.

Fortunately, recent neuroscientific research has started to understand the neural correlates involved in the construction of this coherent framework of the surrounding world through self-referential thought, retrieval of memories and associations and creation of mental simulations. To interpret our findings related to decreased DMN activation in PTSD, we adopt the framework proposed by (Andrews-Hanna, 2012; Andrews-Hanna et al., 2014) according to which the DMN is comprised by at least three different subsystems. Each of them is proposed to have a specific set of roles related to internally directed cognition or self-referential processing. One of these components is the medial temporal DMN, active during autobiographical memory (McDermott et al., 2009; Schacter et al., 2008; Spreng et al., 2009; Svoboda et al., 2006) and retrieval of contextual associations (Bar, 2007). The HMM method applied in this thesis was able to detect a state overlapping with the topology of the medial temporal DMN subsystem. Interestingly, this state was less active in PTSD participants during presentation of trauma-related pictures and anticorrelated with the intrusive qualities of memories elicited by those images. This suggests that an inability to engage the medial temporal DMN in PTSD might be related to disrupted autobiographical memory and retrieval of contextual associations related to the trauma, which has been suggested to underlie intrusive memories and their associated vividness, feelings of the trauma reoccurring in the 'here & now,' and distress (Ehlers and Clark, 2000).

The HMM method applied here was also able to detect the occurrences of what is believed to be the core of the DMN, potentially functioning as a hub for the other subsystems. This component is considered crucial for the integration of salient information, either external or internal, with one's own current affective experience and episodic knowledge. While all DMN components are probably involved with self-referential processes, the core DMN is thought to have self-reflection and processing of personally-significant information as its main role (Andrews-Hanna, 2012). In this regard, we observed decreased ability in PTSD participants to engage a state related to this network during the presentation of neutral pictures, hinting at a decreased ability

in PTSD to integrate the salient information provided by the task, tailored to their traumatic experience, with either their affective experience or episodic knowledge. The fact that differences were only observed in the neutral experimental condition could be related to this condition being less demanding in terms of resource allocation than the more emotionally loaded trauma-related condition, and therefore allowing for the 'mental space' necessary for the integration of salient information.

The temporal dominance of both the medial temporal DMN and the core DMN were increased after cognitive therapy, suggesting that therapeutic mechanisms are associated with modulation of these two states. According to Ehlers and Clark (2000), cognitive therapy for PTSD works by helping to place the traumatic memory in the correct time and space, overcoming the mnemonic decontextualisation characteristic of PTSD. In this regard, the rebalancing of the medial temporal DMN during trauma-related picture presentation might be reflecting retrieval of recovered associations that had been masked by overwhelming sensory impressions at the time of encoding (i.e. traumatic experience). It has also been suggested that the unorganised and decontextualised memories typical of PTSD may to some extent result from an inability to establish a self-referential perspective while experiencing the trauma (Ehlers and Clark, 2000; Wheeler et al., 1997), an inability that would arise again during re-experiencing symptoms. The observed upregulation of the core DMN after therapy, with its prominent involvement in self-reflection, might therefore manifest to some extent in the integration of the traumatic memory with preceding and subsequent memories (i.e., 'I did not die') proposed to decrease the sense of current threat characteristic of PTSD (Ehlers & Clark, 2000).

A third component of the DMN suggested by Andrews-Hanna, the dorsomedial prefrontal DMN, was not detected by the method. This subsystem is suggested to play a key role in introspection about mental states of social agents, that is, when individuals appraise social information either in relation towards one's own mental states or the mental state of others (Frith and Frith, 2003; Lieberman, 2007; Van Overwalle, 2009). These cognitive functions would be affected by excessively negative appraisals of the traumatic event or its sequelae, which in PTSD often take the form of personal and/or interpersonal evaluations (e.g., "Others have not treated me fairly", "The event being was my fault") (Ehlers and Clark, 2000). The fact that we did not detect a state related to this DMN component might be related to the nature of the

fMRI task used in our study, oriented towards the elicitation of intrusive memories but lacking the social appraisal aspect associated with this DMN subsystem.

Of course, the dysregulation of the default mode system does not come in isolation. Our results also showed increased temporal dominance of the salience and the ventral visual stream states during presentation of trauma-related pictures in PTSD. The temporal dominance of these states was also positively correlated with the qualities of intrusive memories. This is coherent with previous studies (Fonzo et al., 2010; Simmons et al., 2008; Sripada et al., 2012) and with the strong saliency associated with these highly distressing memories that come with a very strong component of vividness, most often translated in visual sensory impressions.

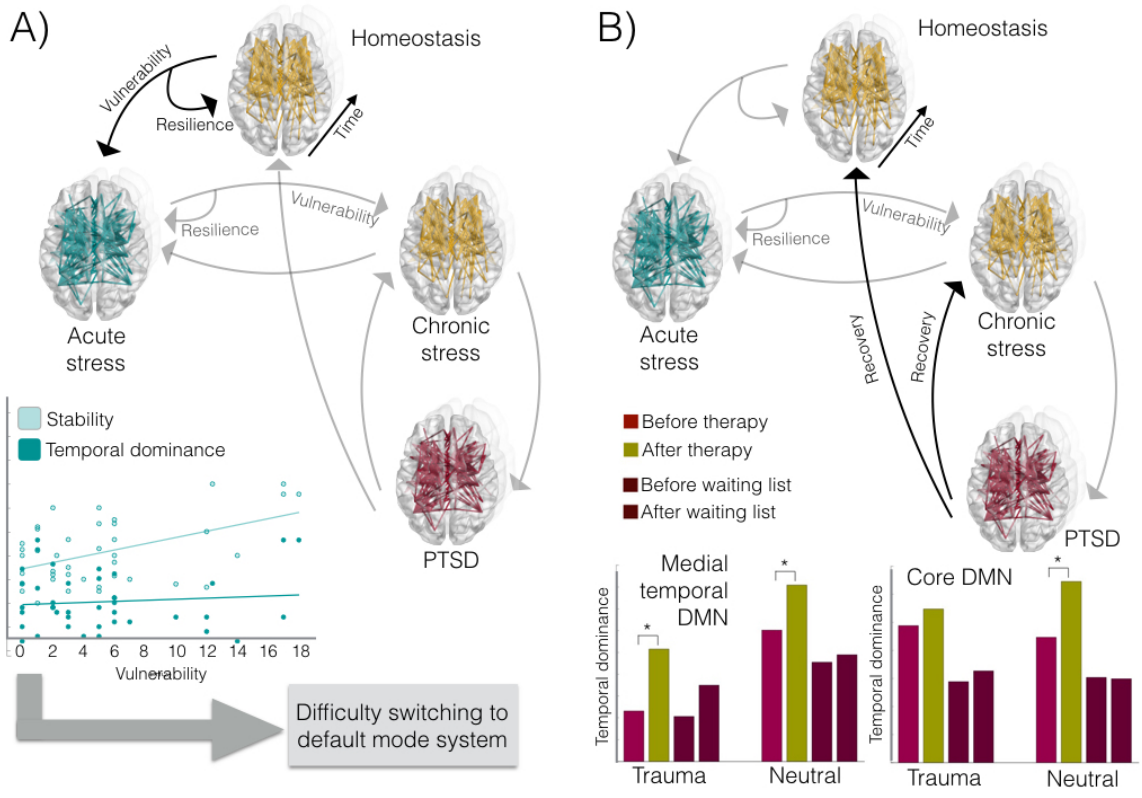


Figure 6.1. A time-varying approach reveals a potential trajectory from vulnerability to PSTD. *A) Acute stress is related to increased stability in the frontoparietal state together with difficulties switching to the default mode network in vulnerable individuals. B) This might be related to a marked decrease in the time visiting the default mode state, observed in PSTD individuals, which is amenable to rebalancing through successful cognitive therapy for PTSD.*

In brief, here we propose a model by which stress vulnerability in terms of the underlying brain dynamics is associated with increased lifetimes or temporal stability of the frontoparietal state as a way to cope with the overload of the system. The hyperalertness and increased perceptual information processing disrupts the system, impeding redirection of attention towards (adaptive) internally directed cognition though interactions with default mode states (Smallwood et al., 2012) or the reestablishment of feedback loops associated with homeostasis (Cole et al., 2014). Although not investigated in this thesis, the cumulative inability of the frontoparietal state to facilitate these transitions to other systems might eventually manifests itself as a downregulation of the default mode system in PTSD. Cognitive therapy is able to reverse this effect in treatment responsive participants, rebalancing the temporal dominance of at least two default mode components to levels close to those of healthy controls.

Future directions

While it is encouraging to see how cognitive therapy may be able to change brain activity back to dynamic configurations similar to those of healthy controls, it is important to keep in mind that not everyone benefits to the same extent from this type of treatment. If we want to improve current interventions, we need longitudinal studies comparing the trajectories of responders and non-responders, ideally using time-varying approaches. This would allow us to detect differences in one or more states, or perhaps even the relationship between specific states, guiding us further into the therapeutic interventions most likely to be successful. There are many possibilities regarding how the landscape of states could differ between treatment responders and non-responders. It could be for example that treatment resistant cases show more extreme dysregulation of the default mode states. It could alternatively be the case that while they show a dysregulation of the default mode state comparable to that of treatment responders, there is also widespread dysregulation in other networks, and they might therefore benefit from a specific combination of interventions targeting different elements of the PTSD symptoms.

Further work is also required to keep improving our understanding of resilience and vulnerability factors. This, in combination with extensive longitudinal studies in which the number and type of traumatic events are carefully assessed, might give us increased knowledge about the transition from (chronic) stress to PTSD. While this

area was not directly addressed in this thesis, understanding why and when people are most likely to develop stress-related disorders is key to the improvement of prevention strategies. One of the reasons why prevention strategies are often virtually inexistent might be the inability to direct them to the population that needs them most, making them extremely expensive. Furthermore, the insights from these studies acquiring and analysing extensive longitudinal data might be useful to further inform the design of these prevention strategies.

Another area that has the potential to lead scientific growth in the field is the investigation of the heterogeneity that characterises PTSD and other psychological disorders. A recent study in major depressive disorder has shown that predominance of a group of symptoms over another can be related to different neurophysiological subtypes of depression (Drysdale et al., 2016). While this study used a static connectivity approach, the application of dynamic methods could be very useful in this field, as some of the variability driving the heterogeneity might lie in time modulated network interactions. A better understanding of the likely PTSD subtypes may allow for more efficient treatments by placing a stronger emphasis on rebalancing the most strongly disrupted networks.

Furthermore, PTSD is a highly comorbid disorder, not only in regard other psychological disorders, but also with physical injuries. For example, in conflicts and wars, brain injury occurs in a context of extreme psychological stress, with the prevalence of PTSD in veterans returning from war zones who have a history of mild traumatic brain injury being as high as 34% (Tanielian and Jaycox, 2008). The comorbidity between these two conditions is not limited to war zone veterans, as civilian types of trauma such as motor vehicle accidents and interpersonal assault, the two trauma types suffered by the PTSD participants in Chapter 5, also involve often both mild traumatic brain injury (mTBI) and psychological trauma. The simultaneous presentation of the two disorders poses an additional difficulty for clinicians, as some posttraumatic stress symptoms overlap with mTBI symptoms (e.g., irritability, difficulty to concentrate). To date, much about the clinical consequences of suffering both disorders remains unknown, posing significant challenges for the assessment and treatment of patients with this type of comorbidity. Future studies need to address these questions in order to improve clinical management of complex cases with both psychological and physical injuries.

APPENDIX

LIST OF ACRONYMS

AAL	Automated Anatomical Labeling
ACTH	Adrenocorticotropic hormone
ANS	Autonomic nervous system
BOLD	Blood-Oxygen-Level Dependent
CEN	Central executive network
CRF	Corticotropin releasing factor
CT-PTSD	Cognitive therapy for PTSD
DMN	Default mode network
DSM	Diagnostic and Statistical Manual of Mental Disorders
FC	Functional connectivity
FDR	False Discovery Rate
FIX	FMRIB's ICA-based Xnoiseifier
fMRI	functional Magnetic Resonance Imaging
FPN	Frontoparietal network
FWE	Family Wise Error
GLM	General Linear Model
HMM	Hidden Markov Model
HPA	Hypothalamic-pituitary-adrenal
ICA	Independent component analysis
LEiDA	Leading Eigenvector Dynamic Analysis
MDD	Major depressive disorder
mTBI	Mild traumatic brain injury

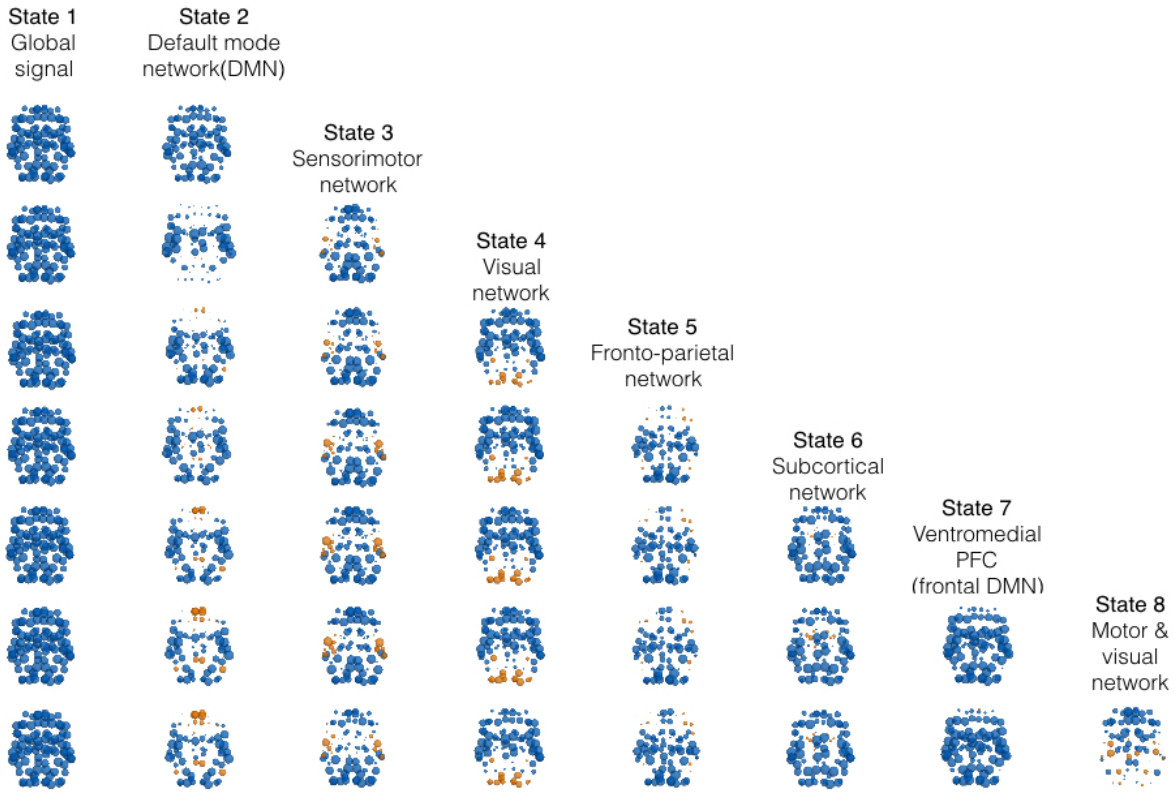
PET	Positron Emission Tomography
PTSD	Posttraumatic stress disorder
RTA	Road Traffic Accident
SN	Salience network
VTA	Ventral tegmental area

SUPPLEMENTARY FIGURES

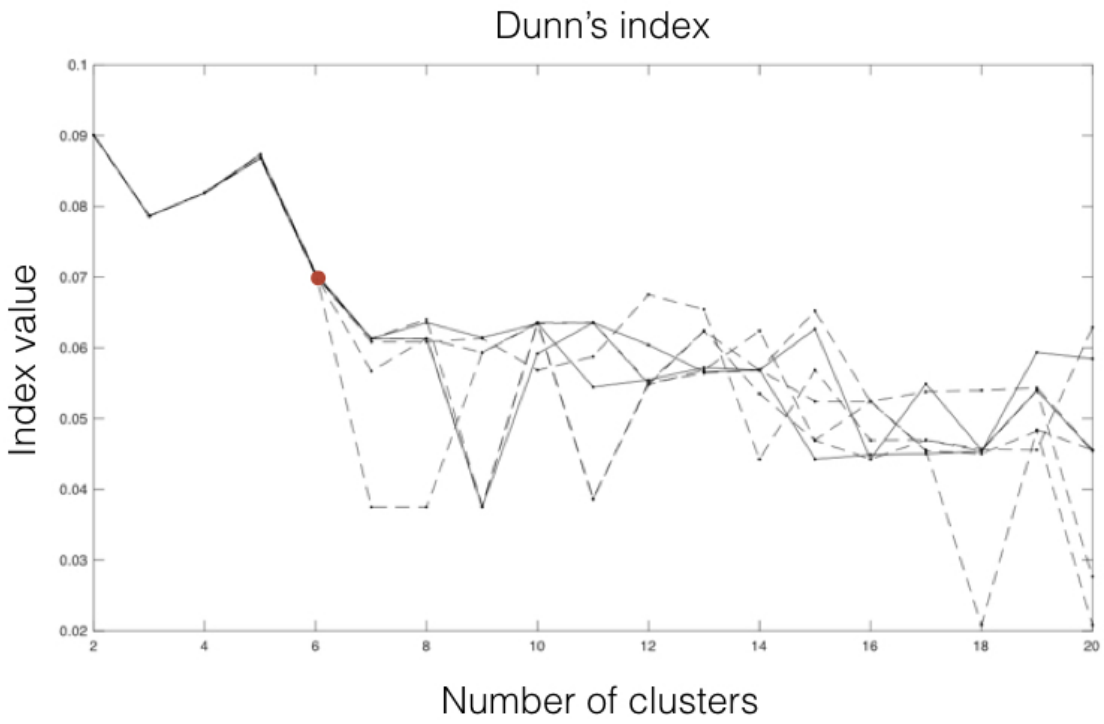
AAL region	**Abbreviation**
1. Precentral gyrus	PreCG
2. Superior frontal gyrus, dorsolateral	dSFG
3. Superior frontal gyrus, orbital part	sOrb
4. Middle frontal gyrus	MFG
5. Middle frontal gyrus, orbital part	midOrb
6. Inferior frontal gyrus, opercular part	oIFG
7. Inferior frontal gyrus, triangular part	tIFG
8. Inferior frontal gyrus, orbital part	iOrb
9. Rolandic operculum	Rol
10. Supplementary motor area	SMA
11. Olfactory cortex	Olf
12. Superior frontal gyrus, medial	mSFG
13. Superior frontal gyrus, medial orbital	smOrb
14. Gyrus rectus **	Rec
15. Insula	Ins
16. Anterior cingulate and paracingulate gyri	ACC
17. Middle cingulate and paracingulate gyri	DCC
18. Posterior cingulate gyrus	PCC

19. Hippocampus	Hip
20. Parahippocampal gyrus	PHG
21. Amygdala	Amyg
22. Calcarine fissure	Cal
23. Cuneus	Cun
24. Lingual gyrus	Ling
25. Superior occipital gyrus	sOcc
26. Middle occipital gyrus	mOcc
27. Inferior occipital gyrus	iOcc
28. Fusiform gyrus	FFG
29. Postcentral gyrus	PoCG
30. Superior parietal gyrus	SPG
31. Inferior parietal gyri	IPL
32. Supramarginal gyrus	SMG
33. Angular gyrus	Ang
34. Precuneus	Pcun
35. Paracentral lobule	PCL
36. Caudate	Cau
37. Putamen	Put
38. Pallidum	Pal
39. Thalamus	Thal
40. Heschl gyrus	Hes
41. Superior temporal	STG
42. Superior temporal pole**	sTPO
43. Middle Temporal	MTG
44. Middle temporal pole**	mTPO
45. Inferior Temporal	ITG

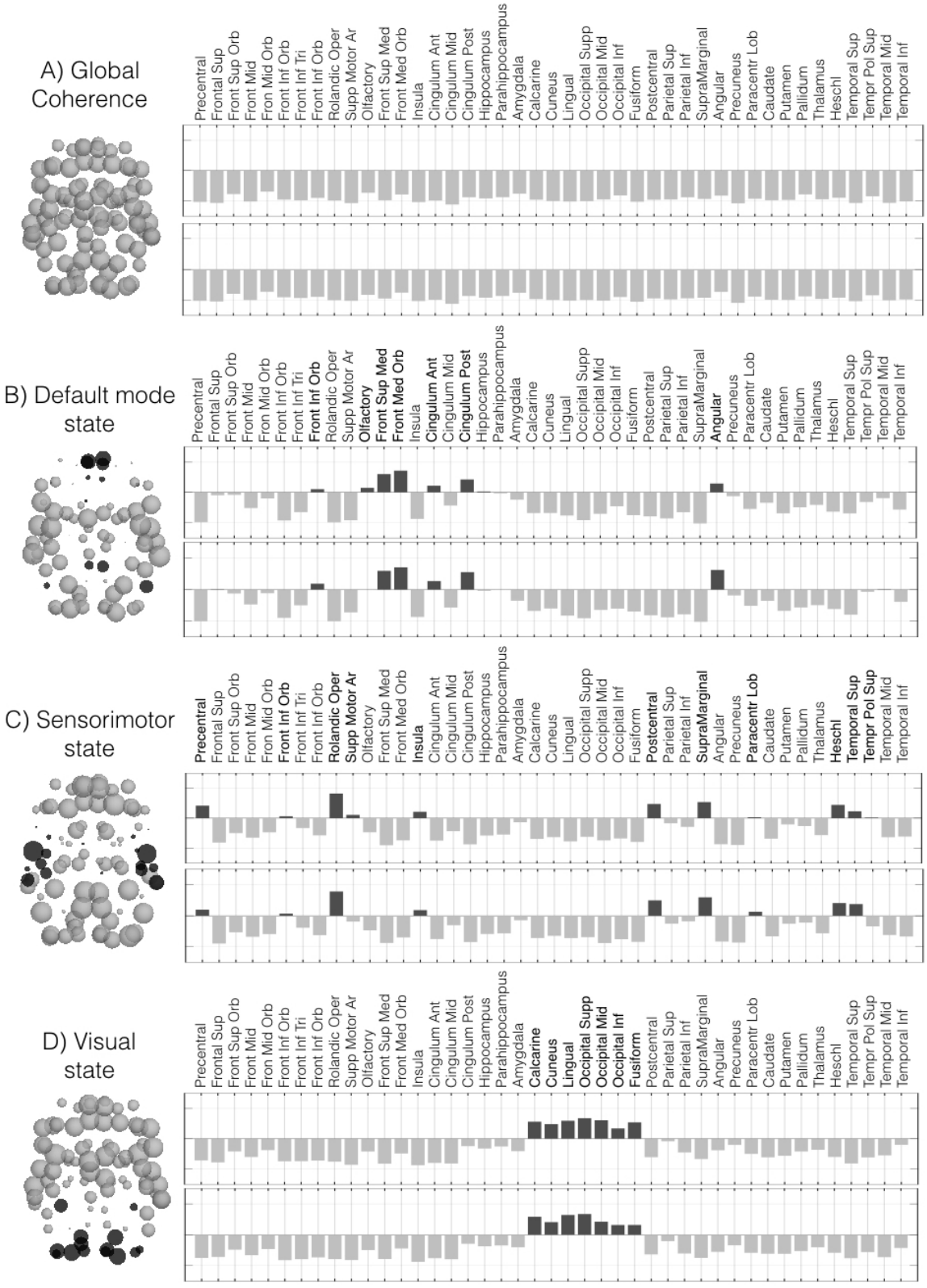
Supplementary table 1. Corresponding labels of AAL abbreviations used in figures 2.3 – 2.6. *** Denotes regions excluded due to signal dropout.*

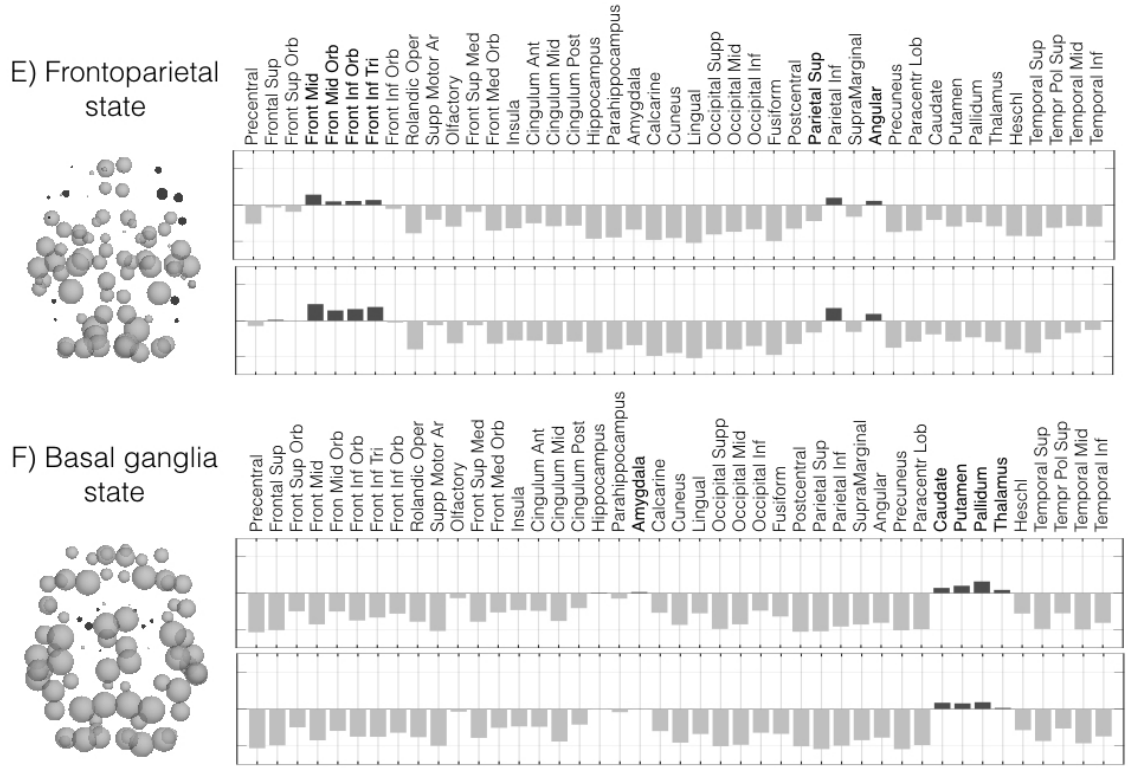


Supplementary figure 1. Representation of FC patterns arising from different clustering solution with a k ranging from 2 to 9. *The FC patterns appear robust across clustering solutions, with similar spatial maps resulting from different k, even if there is some variability in the magnitudes and community partitions. With each increase of k, a new FC state appears.*



Supplementary figure 2. Dunn's index computed for each clustering solution, ranging from k 2 to 20. *The highest Dunn's Index was obtained with k 5, but repeated initialisation of the algorithm (n = 8) showed that a stable and acceptable index was still reached with k6. The Dunn's index algorithm was initiated 8 times to assess stability, showing that despite of the highest index being reached with k 5, it remains stable until k 6.*


A) Global Coherence
B) Default mode state
C) Sensorimotor state
D) Visual state
Precentral
Frontal Sup
Front Sup Orb
Front Mid
Fron Mid Orb
Front Inf Orb
Front Inf Tri
Front Inf Orb
Rolandic Oper
Supp Motor Ar
Olfactory
Front Sup Med
Front Med Orb
Insula
Cingulum Ant
Cingulum Mid
Cingulum Post
Hippocampus
Parahippocampus
Amygdala
Calcarine
Cuneus
Lingual
Occipital Supp
Occipital Mid
Occipital Inf
Fusiform
Postcentral
Parietal Sup
Parietal Inf
SupraMarginal
Angular
Precuneus
Paracentr Lob
Caudate
Putamen
Pallidum
Thalamus
Heschl
Temporal Sup
Tempr Pol Sup
Temporal Mid
Temporal Inf



Supplementary figure 3. **Representation of the six states.** On the left, each brain region illustrated by a sphere; colour of the sphere codes the community to which it belongs while its size represents the strength with which it belongs to it. On the right, each brain regions is represented by a bar together with the corresponding AAL labels.

	Cross-sectional groups			Longitudinal groups			
	Controls (n=15)	PTSD (n=43)	Remitted (n=30)	preCT (n=14)	postCT (n=14)	preWAIT (n=8)	postWAIT (n=8)
Age at visit 1	39.67/12.32	40.26/12.62	39.73/21.83	39.93/14.43	N/A	44.12/13.94	N/A
Gender (M/F)	11/4	17/26	16/14	8/6	N/A	3/5	N/A
Years since trauma	4.48/6.18	4.21/6.87	3.35/4.12	4.33/4.85	N/A	6.59/9.42	N/A
Trauma type (RTA/Assault)	4/11	15/28	11/19	4/10	N/A	3/5	N/A

Supplementary table 2. **Demographics of the CT-PTSD dataset.**

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