Universidade do Minho
Escola de Ciências da Saúde

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A multimodal neuroimaging approach to the interplay between stress and aging

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To my family

"Learn from yesterday, live for today, hope for tomorrow. The important thing is to not stop questioning."

Albert Einstein

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Abstract

The new trend in modern societies includes changes in the aging and stress exposure profiles. As individuals get older, either healthy or pathologically, several changes occur in body systems, namely in the brain structure and function. This is obviously a complex process characterised by high inter- and intra-individual variability; such variability in the aging process may arise from the interaction of multiple factors, including exposure to stressful experiences across the lifespan. In the present work, using a multimodal neuroimaging and neurocognitive approaches, we characterized the effect of stress on the active and "resting" human brain and its interplay with mood during healthy aging. We explored the impact of stress on decision-making processes, on the activation/deactivation patterns of Resting State Networks (RSNs) and its interplay with mood, on brain structure and function during the aging process.

Data shows that chronic stress biases decision-making strategies in humans towards habits, causing an imbalanced activation of the networks that govern decision processes, shifting the activation from the associative to the sensorimotor circuits. These functional changes are paralleled by atrophy of the medial prefrontal cortex and the caudate, and by an increase in the volume of the putamina. Importantly, we demonstrate that after a stress-free period, both the structural and functional changes triggered by stress are reversible and decisions become again goal-directed. Stress triggers also an increased functional connectivity of the default mode (DMN), dorsal attention (DAN), ventral attention (VAN), sensorimotor (SMN), and primary visual (VN) networks, paralleled by impairments in the deactivation patterns of the RSNs and by a constriction of the DMN volume. After recovering from stress, the individuals display a decreased resting functional connectivity in the DMN, VAN and SMN; however, this functional plastic recovery was only partial, as there were still areas of increased connectivity in DAN, SMN and primary VN. Additionally, these subjects display increased deactivations in the DMN, SMN, and auditory network (AN), showing a normalization of the deactivation pattern in RSNs after recovery from stress. Finally, we dissect the critical influence of stress and mood in brain structure and function, with these variables interacting with each other and acting as mediators across the lifespan. This interplay is most evident at combined high stress and depressive mood levels, accelerating the typical age-induced decline or alternatively reducing or even reversing the normal aging impact.

In this thesis we reveal the effects of stress on the active and "resting" human brain and its interplay with mood during the healthy aging process. We conclude that stress biases decision-making strategies accompanied by brain structural and functional reorganizations, impacting on selective activation/deactivation patterns of RSNs with concomitant volumetric atrophies. Along with mood, stress has also a critical influence, in brain structure and function during the lifespan. The present study contributes to clarify combined stress and mood effects during the aging decline, paving the way for interventional therapies that empower stress coping mechanisms in different phases of life.

Resumo

Alterações nos perfis de envelhecimento e de exposição ao stress constituem, nas sociedades modernas, novas tendências. Com o envelhecimento, tanto saudável como patológico, ocorrem diversas alterações no organismo, nomeadamente na estrutura e função cerebral. Este é, evidentemente, um processo complexo caracterizado por uma grande variabilidade inter- e intra-individual, a qual pode resultar da interacção de múltiplos factores, entre eles a exposição a situações de stress ao longo da vida. No presente estudo, recorrendo a uma análise multimodal de neuroimagem e abordagens neurocognitivas, caracterizamos o efeito do stress e da sua interação com o humor, no cérebro ativo e em "repouso", durante o processo de envelhecimento saudável. Exploramos ainda o impacto do stress em processos de tomada de decisão, nos padrões de ativação/desativação das *Resting State Networks* (RSNs), e a sua interação com o humor na estrutura e função cerebral durante o processo de envelhecimento.

Os dados mostram que o stress crónico introduz um viés nas estratégias de tomada de decisão nos humanos, no sentido de comportamentos regulados por hábitos, provocando uma desregulação dos padrões de ativação nas redes que guiam os processos de decisão, bem como na alteração da ativação do circuito associativo para o sensoriomotor. Estas alterações funcionais são acompanhadas por uma atrofia do córtex pré-frontal medial e do caudado, e por um aumento no volume do putamina. Após um período sem exposição ao stress, demonstramos que tanto as alterações estruturais como funcionais desencadeadas por esta exposição são reversíveis, e as decisões tornam-se novamente orientadas por objectivos. O stress despoleta também um aumento na conectividade funcional da *default mode* (DMN), *dorsal attention* (DAN), *ventral attention* (VAN), *sensorimotor* (SMN), e *primary visual* (VN) *networks*, acompanhada por alterações nos padrões de desativação das RSNs e por uma redução no volume da DMN. Após a recuperação do impacto do stress, os indivíduos revelam uma diminuição na conectividade funcional da DMN, VAN e SMN; no entanto, esta recuperação funcional plástica é apenas parcial, uma vez que ainda existiam áreas com aumento na conectividade da DAN, SMN e *primary* VN. Adicionalmente, estes indivíduos apresentam um aumento na desativação da DMN, SMN e *auditory network* (AN), revelando a normalização do padrão de desativação nas RSNs depois de recuperados do efeito do stress. Finalmente, dissecamos a influência crítica do stress e do humor na estrutura e função do cérebro, sendo que estas variáveis interagem entre si e atuam como mediadores no decurso da vida. Esta interação é especialmente evidente quando conjugados níveis elevados de stress e estados

depressivos, acelerando o declínio típico provocado pelo envelhecimento, ou alternativamente reduzindo ou mesmo revertendo o impacto do processo de envelhecimento.

Nesta tese, demonstramos os efeitos do stress no cérebro humano ativo e em "repouso" e da sua interação com o humor, durante o processo de envelhecimento saudável. Concluímos que os vieses introduzidos pelo stress, nos processos de tomada de decisão, acompanhados por reorganizações estruturais e funcionais no cérebro, têm um impacto nos padrões de ativação/desativação concomitantes com atrofias volumétricas. Em conjunto com o humor, o stress tem uma influência crítica na estrutura e função do cérebro durante a vida. O presente estudo contribui para clarificar os efeitos conjuntos do stress e do humor durante o declínio provocado pelo envelhecimento, permitindo que mecanismos de superação do stress se possam estabelecer como terapias interventivas em diferentes fases da vida.

Abbreviation List

AD Axial Diffusivity

AN Auditory Network

BOLD Blood-Oxygen-Level-Dependent

CNS Central Nervous System

CSF CerebroSpinal Fluid

CT Computed Tomography

DAN Dorsal Attention Network

DMN Default Mode Network

DTI Diffusion Tensor Imaging

EEG ElectroEncephaloGraphy

FA Fractional Anisotropy

fMRI functional Magnetic Resonance Imaging

GM Gray Matter

HAROLD Hemispheric Asymmetry Reduction in OLDer adults

HPA Hypothalamus-Pituitary-Adrenal

HVN High Visual Network

MD Mean Diffusivity

MEG MagnEtoencephaloGraphy

mPFC medial PreFrontal Cortex

MRI Magnetic Resonance Imaging

MRS Magnetic Resonance Spectroscopy

PASA Posterior-Anterior Shift with Aging

PCu PreCuneus

PET Positron Emission Tomography

pCC posterior Cingulate Cortex

PVN Primary Visual Network

RD Radial Diffusivity

RSN Resting State Network

SAM Sympathetic Adrenomedullary System

SMN SensoriMotor Network

SN Salience Network

STAC Scaffolding Theory of Aging and Cognition

VAN Ventral Attention Network

VBM Voxel Based Morphometry

VN Visual Network

WHO World Health Organization

WM White Matter

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Chapter 1

Introduction

1 - Introduction

1.1 Introduction: Cross-sectional interaction between stress and brain structure and function during lifespan

Stress is a major burden in modern societies and may trigger several disorders, namely in the Central Nervous System (CNS). It is predicted by the World Health Organization (WHO) that mental disease, including stress-related disorders (e.g. major depressive disorder, posttraumatic stress disorder), will be one of the major leading causes of disabilities in 2020, leading to a large economic impact. Stress is not a stable and constant factor as it can occur with variable frequency, intensity, predicatibily and controlability (Lucassen et al., 2014). However, acute (or better eustress) and the chronic maladaptive (or better distress) stress can be distinguished. For many years it has been recognized that acute stress is a state of increased vigilance and alertness and to get the organism ready to take action before the impact of dangers (Arthur, 1987). Under brief stressful conditions, the ability to perceive changes in the surrounding environment becomes critical to mount an appropriate response. On the other hand, it is well established that prolonged and repetitive stress (maladaptive) has deleterious impact in multiple biological systems, including the CNS. Stress triggers mainly two pathways of response, one fast-reacting neural path, also referred to as the Sympathetic Adrenomedullary System (SAM) and another slower neuroendocrine pathway, involving the Hypothalamus-Pituitary-Adrenal (HPA) axis. The HPA axis is regulated via excitatory and inhibitory loops of limbic and prefrontal structures that are involved in stress processing and emotion, such as the prefrontal and anterior cingulate cortex, amygdala, insula and hippocampus (de Kloet et al., 2005). Cortisol, as the major stress hormone in humans is involved in the fine-balanced regulation of stress-induced alterations and basal cortical secretion, being fundamental for the maintenance of the homeostasis (Dedovic et al., 2009).

The ability to mount an appropriate response to stress is vital for the survival of every living organism. However, when the homeostatic mechanisms to cope with stressful stimuli are disrupted, either because the individual has a particular vulnerability or because the response system is exhausted by a continuous activation (through prolonged stress exposure), maladaptive responses take place and

predispose to cognitive impairment and even to pathological conditions (Sapolsky, 1996;Lupien et al., 2009;McEwen and Gianaros, 2011;Sousa and Almeida, 2012). Maladaptive stress affects cognitive behavior through sequential structural modulation of brain networks, mainly as a consequence of the release of corticosteroids (Sousa et al., 2008;Liston et al., 2009). In fact, several studies have revealed stress-induced deficits in spatial reference, working-memory and behavioral flexibility. These behavioral changes are attributed to synaptic/dendritic reorganization in both the hippocampus and the medial prefrontal cortex (Sousa et al., 2000;Cerqueira et al., 2007;Ossewaarde et al., 2011). Stress is also known to impact brain structures that are functionally abnormal in mood disorders. In fact, evidence seems to indicate that stress is the most common risk factor for the development of mood disorders, such as major depressive disorder (Pittenger and Duman, 2008;Risch et al., 2009).

As described, the brain is the key organ involved in the stress response, and it changes both structurally and functionally with aging as a result of stressful experiences. In fact, in modern societies, with increasing human competitive attitude, ever changing life styles and extreme workloads, stress has triggered unparalleled health damages (Lucassen et al., 2014). Consistent with this, studies have been shown that stress (e.g. psychological), or even its anticipation, may accelerate the rate of biological aging by increasing the speed of cellular aging processes (Ahola et al., 2012;O'Donovan et al., 2012) being, as a consequence, associated with high incidence of mental and physical illnesses.

Maladaptive stress responses contribute to a wide range of health diseases, being also associated with an increased risk for age-related disorders, namely Alzheimer's disease (Sotiropoulos et al., 2011). As so, stress may act as an important precipitator of aging and age-related diseases, which is important to address when studying aging population.

Presently, the proportion of people aged over 60 years is growing faster than any other age group. It has doubled since 1980 and it is forecasted to reach 2 billion by 2050, as a result of both longer life expectancy and declining birth rates. This population shift, presents both challenges and opportunities in a near future for modern societies. People are living longer and generally healthier lives, which evidences the success of health, science and medical progressions and economic development over disease and injury. Furthermore, older individuals make important contributions to society as family members, active workers and volunteers, which in parallel with their rich life experience makes them a

vital social resource of knowledge. This scenario, however, challenges society to adapt, in order to maximize the health, functional capacity and well-being of older people, including preventing and managing age-associated chronic diseases such as dementias, and designing sustainable policies on long-term and palliative care (National Institute on Aging, 2007;World Health Organization, 2014). Finally, given the association between cognitive status and quality of life, particularly in aging, successful aging is ultimately dependent on how societies understand neurocognitive aging, finding solutions and strategies to add healthy life years to our population.

At the brain level, normal brain aging is a continuous process characterized by a pattern of selective loss and preservation of structures and functions. It is known, for example, that the number of dopaminergic receptors declines with age, or that many brain structures show volumetric decrease due to neuronal atrophy and, in parallel white matter (WM) becomes less dense and loses integrity, decreasing the complexity of neuronal connections. The brain tends to respond to these neural changes by engaging in continuous functional and structural reorganizations - brain plasticity – that preserve its function, namely cognition (Park and Reuter-Lorenz, 2009;McEwen and Gianaros, 2011). Crucially, however, maladaptive stress affects cognitive behavior through sequential structural and functional modulation of brain networks throughout aging.

So far most of the studies have been designed to study different regions of the brain separately, and with single technical approaches, thus precluding the global view that is essential to understand the human brain across the lifespan. Despite the fast and continuous advances in neuroimaging, the relationship between different brain parameters in aging and stress is still very limited. Thus, multimodal approaches, with cross sectional and longitudinal analysis, are a need in the field. Integration of neuroimaging techniques, such as structural magnetic resonance imaging (MRI), functional imaging (fMRI) and diffusion tensor imaging (DTI), is crucial to achieve a better understanding of brain structure and function. In this introductory chapter we will start with an overall overview of the impact of stress in the structure and function of the brain, followed by a brief description of the brain-related changes in aging. Finally, we will look for the cross-sectional interaction between stress and brain structure and function during lifespan. Indeed, whereas the neuroimaging methods allow unravelling the patterns of morphological and functional alterations within the brain, some methodological considerations will also be explored.

1.2 Stress: Cognitive, Structural and Functional Brain Changes

It is well established that prolonged stress has deleterious impact in multiple biological systems, including the CNS. In fact, prolonged stress exposure impairs spatial working memory, perceptual attention, behavioral flexibility and decision-making both in rodents and in humans (Joels et al., 2004;Cerqueira et al., 2005;Dias-Ferreira et al., 2009;Yuen et al., 2012). Decision-making tasks are one of the most frequent cognitive processes on a daily basis and, therefore, during lifespan. Interestingly, along with language, decision-making is known to be relatively preserved during healthy aging (Sanfey and Hastie, 2000;Kovalchik et al., 2005).

Structural and functional changes of several brain regions have been associated with these stress-targeting cognitive effects. In fact, consistent stress-induced alterations have been found in the stress and emotion circuitry. Most of the studies point to reduced volumes in stressed subjects (under different stress factors and conditions) in the structures associated with the control of the HPA axis, namely, prefrontal and anterior cingulate cortex, hippocampus and amygdala (Lucassen et al., 2014). Smaller prefrontal and anterior cingulate cortex volumes have been observed in patients with major depressive disorder (Frodl et al., 2008), in maltreated children (De Brito et al., 2013) and in individuals with long-term occupational stress (Blix et al., 2013). Reduced volumes in the hippocampus have also been reported in depressed subjects, patients with major depressive disorders and in women who were maltreated during childhood. In line with these findings, increased hippocampal volumes were noted in patients with post-traumatic stress disorder under pharmacological treatments (Kempton et al., 2011;van der Werff et al., 2013). Amygdala alterations were also observed in studies reporting larger volumes related with early stress exposure and with hemispheric shift (larger right versus left amygdala) (Pruessner et al., 2010). On the other hand, volume decreases in amygdala have also been associated with early and late-onset depressed subjects (Burke et al., 2011;van Uden et al., 2011). In contrast, some structural studies also point to possible increased volumes in the hippocampus, the ventral medial prefrontal and anterior cingulate cortex (van der Werff et al., 2013). Curiously, there is very limited information investigating the stress-effects on structural aspects other than on the gray matter (GM); as a result, we know very little about the WM volume and integrity.

In addition, although the investigation of the functional brain changes associated to stress has been providing consistent findings related with structural brain data, research conducted in humans is still at its beginning. Functional studies involving fMRI and positron emission tomography (PET) techniques have mainly associated stress-induced activity alterations with the prefrontal cortex, limbic system and basal ganglia. Despite some inconsistent results, the tendency shows that stress is linked with decreased activity in the orbitofrontal cortex and the hippocampus, while the dorsolateral prefrontal, the anterior cingulate cortex, the basal ganglia and the ventral striatum increase their activity under stress conditions. Both increased and decreased activity has also been described in the amygdala, insula and thalamus (Starcke and Brand, 2012). Specifically, decreased medial prefrontal cortex activity was found in subjects showing more uncontrollable and overwhelming stress responses; decreased prefrontal cortex activity was associated with increased cortisol secretion in response to a physiological stress task (for review see Dedovic et al., 2009); early life stress in depressed patients decreased local connectivity of the ventrolateral prefrontal cortex and correlated negatively with global connectivity of dorsolateral prefrontal cortex (Cisler et al., 2013). Finally, studies comparing patients with exposure to trauma reported an increased activity of the ventral medial prefrontal and rostral anterior cingulate cortex, and a relatively lower activity of the amygdala and the dorsal anterior cingulate cortex (for review see (for review see van der Werff et al., 2013).

Another cryptic area relates to the effect of stress in the functional architecture of the resting state networks (RSNs), both during task performance or resting state conditions. Although neuropsychiatric diseases (e.g. bipolar disorders, schizophrenia) have been associated with abnormal patterns of RSNs deactivation, which may be related with difficulties in task-focusing and cognitive resources allocation, studies performed during prolonged stress, an established risk factor for neuropsychiatric disorders, are absent. The very few specific functional connectivity studies on this topic have reported these main findings: reduced functional connectivity strength in patients with major depressive disorder and a history of childhood maltreatment within the prefrontal-limbic-thalamic-cerebellar circuitry (Wang et al., 2013); increased connectivity in stressed subjects between the amygdala and the medial prefrontal cortex and between the amygdala and the posterior cingulate cortex/precuneus (Veer et al., 2011); and positive correlation between the connectivity of the posterior cingulate cortex/precuneus with the

perigenual anterior cingulate cortex and the right amygdala with posttraumatic stress disorder symptomatology (van der Werff et al., 2013).

As exposed above, chronic or repeated stress has an impact on brain structure and function (task associated or during rest) and therefore in cognitive functioning and mental/physical health. This exposure may affect different brain structures according the timing of exposure. Namely, chronic stress impact has been associated with different effects during lifespan (prenatal, perinatal, adolescence, adulthood or aging - (Lupien et al., 2009)). In fact, it has been reported that aging is associated with higher levels of cortisol and that elevated plasma glucocorticoid levels over years in older adults is negatively associated with performance in memory tasks and hippocampal volume (Raskind et al., 1994;Lupien et al., 1998). Nevertheless, several structural and functional changes have been associated with aging, independently of these maladaptive stress effects. We will now address the age-associated brain changes followed by an overview of the impact of stress during lifespan.

1.3 Aging: Cognitive, Structural and Functional Brain Changes

Research on normal aging has shown that cognitive performance over the lifespan is very heterogeneous. Age-related structural and physiological changes in the brain may have implications for the decline, as well as for the preservation, of cognitive function (information processing, attention, memory and executive control) in older subjects. Indeed, research on aging has outlined distinct patterns of decline and stability in cognition over the lifespan. Cross-sectional and longitudinal studies focused on cognitive performance through the lifespan find robust declines in abilities such as encoding new memories of episodes or facts, working memory and processing speed. By contrast, short term-memory, autobiographical memory, semantic knowledge and emotional processing remain relatively stable – suggesting that aging has distinctive effects on the neural system that underlies various abilities (Hedden and Gabrieli, 2004;Dennis and Cabeza, 2008;McDowd et al., 2011). Understanding these age-related changes will probably allow to decode the neurobiological aspects of pathological versus normal aging.

The brain tends to respond to all these neural changes by engaging in continuous reorganizations and repairs to keep its homeostatic control and support cognitive functions. Aging quality varies according

to space (brain regions), time (lifespan phase) and external influences. Understanding and characterizing the relationship between structural and functional brain changes across lifespan, in combination with life experience became one of the most prominent challenges in the comprehension of the cognitive function in middle/late ages. Therefore, preserved cognitive performance is assumed as a vital feature of successful aging.

Normal brain aging is an inevitable, complex and heterogeneous process, characterized by a selective pattern of structural and functional changes. With age, the whole brain itself and many of its specific structures present volumetric reductions, the WM becomes less dense and loses integrity and even highly cognitive functioning brains are usually characterized by destructive neurofibrillary plaques and tangles. Which structures change, at what rates, when do they start aging and how does integrity changes, are some of the questions that can be approached with *in vivo* MRI. Within the last 30 years, neuroimaging of aging research has naturally correlated behavioral data with structural and functional (stimulated and resting-state) neuroimaging measures, through the use of several techniques, including MRI and PET. During healthy aging, many domains become less efficient, including executive and speed processing, working memory ability, long-term memory and attention. At the same time, other cognitive aspects such as knowledge storage, implicit memory (Park et al., 2002) and language comprehension are more resistant to age-induced alterations (Meunier et al., 2014).

Healthy brain aging is consistently characterized by an overall atrophy associated with decrease of the GM and WM volumes and expansion of the cerebrospinal fluid (CSF) spaces. Brain aging is characterized as a developmental process with inherent associated dynamics that can be investigated via trajectories presenting growth/decline and acceleration/preservation rates of change. Alterations in whole brain volume as a function of aging are not linear, but they do increase in old age. While some brain areas decline from early life, others continue to increase before beginning eventually to deteriorate (Raz et al., 2005;Walhovd et al., 2009;Lemaitre et al., 2012;Fjell et al., 2013).

The cortex is known to increase in size into early adulthood, presenting declines per year at a rate of 0.12% in younger adults and 0.35% in adults over 52 years of age, and ventricles expand 0.43% in younger adults and at a rate of 4.25% after the age of 70 (Dennis and Cabeza, 2008). Indeed, total brain volume is more correlated with age after 60 years old and the total surface area presents global

reductions of 3.68 cm^2 per year (Lemaitre et al., 2012;Fjell et al., 2013). The GM volume rises in early periods of life, decreasing later at different rates across the brain while WM volume exhibits weak initial age correlations that increase significantly beyond adolescence into middle age. Regarding matter losses, the GM may begin earlier and progress gradually, frequently associated with neuronal cell death (Gilmore et al., 2007;Lemaitre et al., 2012), whereas the WM may start later and progress more abruptly accompanying the myelin sheath deteriorating after around the age of 40 (Wozniak and Lim, 2006;Gunning-Dixon et al., 2009).

The GM loss, presenting a global volume reduction of 1.89 cm^3 and average thickness of 0.004 mm per year, appears to be somewhat greater in the cortex than in subcortical structures. Age-related GM volumetric reductions, revealed by MRI, have shown to have the highest effects in the frontal lobe, including the consistently reported vulnerability of the prefrontal region (also associated with pronounced age-related decline in several cognitive processes), the middle frontal gyrus, the superior frontal gyrus and the frontal pole (decline from 0.9 % to 1.5 % per year) (Raz et al., 2005;Abe et al., 2008;Dennis and Cabeza, 2008;Ziegler et al., 2012). These volumetric reductions in frontal regions are paralleled with higher cortical thickness (superior to 0.0055 mm/year) and surface area (superior to 0.28 % per year) reductions (Lemaitre et al., 2012). Despite some inconsistent results, probably due to different methodologies implemented, the second higher atrophy rate seems to be in the parietal lobe (0.34 % to 0.90 % per year). The occipital lobe shows small or non-significant age-related atrophy and temporal lobes have specific individual structure rates of decline (Resnick et al., 2003;Raz et al., 2005;Smith et al., 2007;Dennis and Cabeza, 2008). These patterns of brain atrophy have been associated with the “last in, first out” hypothesis, supporting that the brain regions that are the last to mature or develop are the first to be affected by aging (Raz, 2001;Raz et al., 2005;Lemaitre et al., 2012). In terms of subcortical structures, the most consistent and largest annual percentage of reduction has been reported in the caudate (0.8 - 1 % per year). Other regions reported with high annual reduction percentages are the nucleus accumbens, the putamen, the thalamus, the cerebellum, the hippocampus and the amygdala. On the other hand, it is well established across studies that the CSF compartments increase in volume during aging (Raz et al., 2005;Dennis and Cabeza, 2008;Walhovd et al., 2011;Tamnes et al., 2013). Interestingly, linear aging trajectories have been described for the amygdala, thalamus, nucleus accumbens, putamen and the cerebellar cortex,

whereas stability followed by decline has been reported in the hippocampus and the brain stem (Abe et al., 2008;Walhovd et al., 2011;Fjell et al., 2013).

Regarding cortical thinning patterns, occipital and parietal regions present higher thinning rates from infancy to young adulthood, whereas in older adulthood, frontal and temporal regions show higher thickness decreases. Specifically, aging-induced thickness reductions are associated with the inferior, middle and superior frontal gyri, as well as the precentral, paracentral, precuneus, and posterior cingulate gyri (Fjell et al., 2009;Tamnes et al., 2010;Lemaitre et al., 2012;van Soelen et al., 2012).

Different from the GM volume decline, literature indicates that the WM aging follows a nonlinear course, based on a quadratic, inverted U-shaped trajectory. WM volumes increase until middle age, fifth decade, followed by a rapid decline, even faster than GM losses. WM volumetric data reveals a development of frontal, parietal, and deep WM until the volume peaks around early fifties, whereas the occipital and the cingulate WM remain relatively stable before the declining. Volume decreases appear to be more significant in cortical than in subcortical regions with stronger aging-induced decreases in superior and medial frontal and prefrontal and anterior cingulate regions and lower effects in temporal and parieto-occipital regions (Salat et al., 2009;Voineskos et al., 2010). Most of the regional WM structures peaked in their volumes in the fifth to sixth decade of life with the exception of corpus callosum with maximum volume in the thirties. Comparing with the GM, the age effects in the WM volumes are associated to a greater spatial extent (Salat et al., 2009;Raz et al., 2010;Westlye et al., 2010;Sala et al., 2012).

Age-related volume decline appears to be associated with additional factors like WM integrity decline, changes in synaptic density and shrinkage of neurons. While WM volumetric data points to a two-phase progress with initial growth until middle-age followed by rapid decline, DTI data supports a three-phase development process with accelerating alterations in the earliest and latest part of life with an intermediate slow decline. The DTI peaks are estimated to be in the early thirties (earlier than WM volume peaks). Global fractional anisotropy (FA) peaks between 24 and 33 years of age, followed by a small linear decrease until approximately 65 years with a subsequent accelerating decline. Additionally, mean diffusivity (MD) and radial diffusivity (RD) present opposite behavior, supporting that the DTI metrics are sensitive to microstructural integrity changes, even before tissue loss (Raz et al.,

2010;Westlye et al., 2010). Age-related decline in diffusivity and anisotropy is generally more pronounced in anterior regions compared to posterior, evidenced by all cortical lobes and corpus callosum (Gunning-Dixon et al., 2009). Specifically, most of the WM tracts present age-related FA decreases, MD and RD increases and axial diffusivity (AD) tract specific alterations. Negative linear correlation with age is associated with the left cingulate and fornix volumes, while volume loss of corpus callosum and right inferior fronto-occipital fasciculus and FA and MD values of the corpus callosum, fornix, cingulum, bilateral association and corticospinal tracts are associated with an inverted U-shaped lifespan trajectory (Sala et al., 2012). WM alterations are often associated with age-related slowing of behavior, as WM decreased integrity points to a less efficient and slower system for information transmission with age.

One of the most remarkable and at the same time challenging advances of neuroimaging is to provide a method to link brain and behavior by using functional neuroimaging. In fact, together with the study of age-related structural changes, it is crucial to assess the effects of aging at rest and during cognitive tasks. The first functional studies focused on verbal working memory and long-term memory, showed that older adults present across different cognitive domains a higher symmetric activation pattern when compared to young people (less lateralized patterns), evidenced for example in the prefrontal areas (Cabeza et al., 1997;Reuter-Lorenz et al., 2000). This model has been referred as Hemispheric Asymmetry Reduction in OLDer adults (HAROLD) (Cabeza, 2002). It has also been shown a lower functional activity in posterior regions and higher in anterior regions in older individuals; this shift from posterior to anterior involvement is known as Posterior-Anterior Shift with Aging (PASA) (Dennis and Cabeza, 2008). Another consistent finding among several functional studies is that the brain regions that present specialized responses in specific cognitive processes in young individuals tend to become less specialized in older adults, presenting more similar patterns across a wide range of cognitive processes, the so-called functional dedifferentiation (Park et al., 2004). More recently, and based on the evidences that while age-related declines in cognitive function and brain structure are observed, the functional brain activity increases with age, particularly in the frontal cortex, Park and Reuter-Lorenz proposed the Scaffolding Theory of Aging and Cognition (STAC) (Park and Reuter-Lorenz, 2009). The STAC theory posits the continuous engagement of a compensatory scaffolding mechanism - the patterns of brain activation that include both declining networks and the associated compensatory

circuitry recruited to meet the lifespan demanding tasks. Older age has also been associated with reduced and also greater regional brain activations in response to different challenge tasks (Dennis and Cabeza, 2008;Turner et al., 2012). Interestingly, it has been indicated that increasing brain responsiveness is associated with better cognitive performance, particularly in frontal cortex (Eyler et al., 2011). This finding underlines the existence of continuous functional plasticity, which brings optimism about the possibility of improving cognitive function in old age.

Aging has also a key influence in functional connectivity analyses, both in resting-state and task induced deactivations. In resting-state, most of the studies point to a general decrease in functional connectivity with increasing age, however this global pattern presents some inconsistencies in specific networks and under certain conditions (Biswal et al., 2010;Mowinckel et al., 2012;Chou et al., 2013;Ferreira and Busatto, 2013). The default mode network (DMN) has been by far the most investigated RSN to date and by consequence the one presenting the most consistent age-induced alterations. Healthy aging is associated with decreased functional connectivity within DMN system, especially pronounced over 60 years of age (Damoiseaux et al., 2008;Mevel et al., 2011;Mowinckel et al., 2012;Tomasi and Volkow, 2012). While some studies report a global decreased connectivity in the DMN pattern, others report more specific decreases in its subsystems (Campbell et al., 2013). This age-related disconnectivity within the DMN has been corroborated by different methodologies, including independent component analysis, seed-based methods and graph analysis (Ferreira and Busatto, 2013). Additionally, recent studies have showed that these DMN functional connectivity declines are more pronounced in long-range connections, with an estimated connectivity decline of 6 ± 1% per decade of life (Tomasi and Volkow, 2012;Mevel et al., 2013).

Although the fact that aging effects on other networks than DMN are still largely unexplored, some reliable results have been observed. The central trend regarding age-induced alterations in resting-state functional connectivity is a widespread reduction throughout the brain during lifespan. Several recent investigations have supported this trend, reporting decreased connectivity in older adults in both attention networks, sensorimotor network (SMN), salience network (SN), auditory network (AN) and visual networks (Allen et al., 2011;Mowinckel et al., 2012;Tomasi and Volkow, 2012). Importantly, recent studies have described that long-range connections may be more vulnerable to the aging effects (Tomasi and Volkow, 2012;Mevel et al., 2013). However, age-related increases in connectivity have

also been observed in attention networks and SMN, whereas visual networks presented some preserved connectivity along healthy aging (Biswal et al., 2010;Filippini et al., 2012;Mowinckel et al., 2012). Interestingly, certain inter-network connectivities, such as salience to auditory and default mode to visual, are also apparently decreased with aging (Onoda et al., 2012).

Apart from age-induced changes in functional connectivity during rest, task-based approaches to study the RSNs have also been performed. In fact, one of the hallmarks of a healthy brain is its ability to shift between adaptive patterns of task-activity and rest-periods. Task-induced deactivation studies report decreased DMN deactivation in the elderly, especially in the posterior cingulate and parietal cortex (Lustig et al., 2003;Grady et al., 2006). Specifically, deactivation may correspond to a deviation in DMN towards a tuning down task-focused behavior that requires attention focus and other demanding cognitive processes, suggesting that there is an age-related reduction in the ability to suspend DMN activity when the experimental condition requires focused attention. Moreover, task-induced DMN deactivation has been related with the performance in several cognitive tasks, with lower task performers presenting decreased functional connectivity (Sambataro et al., 2010;Mevel et al., 2013). Interestingly, the interplay between the DMN and the attention networks (anti-correlated networks) is weaker in older individuals, pointing to abnormal activations during task performance (Wu et al., 2011). In addition to the DMN, other networks have been associated with task-induced deactivations (Harrison et al., 2011). Functional connectivity patterns may be related with the specificity of the stimulation task and their performance level, therefore assessing the age-effects on functional connectivity during tasks is much more complex to interpret.

Importantly, it has been described that brain networks are adaptively re-organized (plasticity effect) in response to age-related declines in neuronal, functional and also structural integrity. The high level of functional connectivity within RSNs points to direct neuroanatomical connections between these regions to facilitate the continuing neuronal communication (van den Heuvel et al., 2009). Indeed, age-induced alterations in measures of functional connectivity may also be modulated by the integrity of the connecting WM fiber pathways, which change significantly with age. Li and colleagues suggested a link between asymmetric connectivity reduction and HAROLD theory, based on the neural aging alterations (Li et al., 2009). They reported that neural connectivity is generally reduced in the aging brain, namely in prefrontal–parietal resting functional connectivity in the left hemisphere. Interestingly, laterality

change in functional activity was negatively correlated with resting state connectivity and positively correlated with structural connectivity. In a different study, a positive partial correlation among the medial prefrontal cortex and the anterior cingulate (two of the DMN nodes) between the functional time courses and fiber densities was also found, except for children (Supekar et al., 2010). Theoretically, when structural and functional connectivity become disrupted, brain plasticity may induce increased connectivity between other regions as a possible compensatory mechanism. Curiously, there is still a lack of studies addressing how these two systems interact with each other, how the alterations of the structural network correlate with alterations in the functional networks.

1.4 Interaction between stress and brain structure and function in aging

For a long time, popular sayings such as “worrying will give you wrinkles” or as Ralph Waldo Emerson quoted “all diseases run into one, old age”, point to an interplay between stress and aging. Indeed, one of the hallmarks of healthy aging is its variability, based on a wide range of preservation, adaptation and losses in structure, function, and performance across several cognitive domains. As a consequence of cumulative effects over the lifespan, different micro- and macro-structural and functional architectures in the brains of elderly people (even at the same age) have been observed. Investigating these differences in healthy and pathological forms of age-related change is a major challenge for neuroimaging studies and became one of the most promising challenges for the comprehension of the cognitive function in later ages.

Investigating stress and stressful experiences has posed both conceptual and methodological challenges to the researcher’s understanding on how an individual’s health condition can be affected by such complexes over the life course. These challenges have been overwhelmed with recent advances in translational animal and human studies, pointing that this interplay between stress and aging begins in embryo state and continues until death. Several studies have reported predominantly prejudicial effects of stress on the aging process. Behavioral stress affects, with possible reversibility, both structure and function of the prefrontal cortex, a region where neurons become less efficient with aging (McEwen and Morrison, 2013), with age moderating the stress-induced effects on declarative memory (Hidalgo et al., 2014). Studies have also shown that chronic stress exposure and antidepressant treatment at the end of the developmental period can have a significant and long-lasting

impact, highly relevant for healthy aging (Scharf et al., 2013) and that animals exposed to a stressful environment during their adolescence, showed a maladapted phenotype in adulthood (McCormick et al., 2008). Moreover, chronic exposure to certain interlinked biochemical pathways that mediate stress-related dysregulation, along with depression, may contribute to accelerated aging, cell damage and certain disease stages (Wolkowitz et al., 2010).

In sum, several studies point to the interplay between stress and aging, with potential stress contribution to premature brain aging. However this knowledge is still very limited and more research is needed on the exact brain mechanisms by which stress may contribute permanently or reversibly to biological aging.

1.5 Objectives

The main research question addressed in this thesis is how aging, stress and their repeated interplay impact on the active and "resting" human brain. For that, we designed a multimodal neuroimaging and neurocognitive approach specifically aimed to:

1- Investigate the brain dynamics regarding the stress influence on decision making process (Chapter 3.1);

2- Study the stress impact on functional resting brain (Chapter 3.2);

3- Examine the effects of chronic stress exposure on the resting state networks following recovery (Chapter 3.3);

4- Characterize the influence of stress and mood and their interplay on brain structure and function during healthy aging (Chapters 3.4 and 3.5).

1.6 References

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Chapter 2

Methodological considerations in neuroimaging techniques

2 - Methodological considerations in neuroimaging techniques

2.1 General introduction

Since the time of Galileo Galilei, imaging has been identified as “the eyes of science”. Numerous questions that we can ask about human structure and function are reliant on the sophistication and advances of imaging technology. Modern imaging technologies allow the investigation of multi-dimensional, multi-parameter and multi-modality data. Imaging methods are being increasingly used from microscopic to organism level to measure physical parameters, tissue properties, dynamics and temporal variations on biological functions. Nowadays, available imaging tools include, among others, Microscopy methods, Ultrasounds, Computed Tomography (CT), MRI, Magnetoencephalography (MEG) and PET.

In medicine, MRI is mainly used to produce structural images of organs (including the CNS), but it can also provide information about the physico-chemical properties of tissues, their perfusion and vascularization. Furthermore, the remarkable developments of MRI in the last decades have provided sensitive tools to achieve non-invasive, *in vivo*, unprecedented characterizations of the human brain. The rapid progression and availability of neuroimaging methodologies had a huge impact in neuroscience research. The brain is by far the most complex, intriguing and unknown organ of the human body, with the fantastic, unique and sole ability to study itself. Exploring the brain organization and architecture, is based on the quality of data regarding the locations, dynamics, variations, magnitudes, and types of functional and structural changes. Indeed, in the neuroimaging domain we can identify two extensive categories, structural and functional neuroimaging (despite structure and function being often inextricably intertwined in the brain), comprising techniques such as fMRI, DTI and magnetic resonance spectroscopy (MRS).

2.2 Structural Neuroimaging

When neuroimaging tools became available, the application of different MR pulse sequences, segmentation techniques, voxel and surface based processing, and diffusion processes empowered the acquisition of a multiplicity of structural brain markers. One of the first approaches used to study the brain involved structural MRI techniques to observe brain morphology, namely size and shape of specific structures and tissue types. Structural MRI analyses rely on calculations of total GM and WM of major lobes, specific cortical regions and subcortical structures, with high sensitivity, economy of time and no rater bias.

Structural MRI techniques include manual-outlined and/or automatic volumetric studies (Buckner et al., 2004;Keller and Roberts, 2009), cortical thickness analyses (Ducharme et al., 2013), voxel-based morphometry (VBM) (Ashburner and Friston, 2000;Coutinho et al., 2013) and shape analyses studies (Scher et al., 2011) (Figure 1). Using these techniques, we now know that the brain structure changes faster than previously thought, due to the influence of a variety of factors. These factors, that might induce structural changes within weeks, months or years, include normal development (Park and Reuter-Lorenz, 2009), neuropsychiatric disorders (Dashjamts et al., 2012;Braskie and Thompson, 2013), learning, stressful events and environmental factors (Kuhn and Gallinat, 2013).

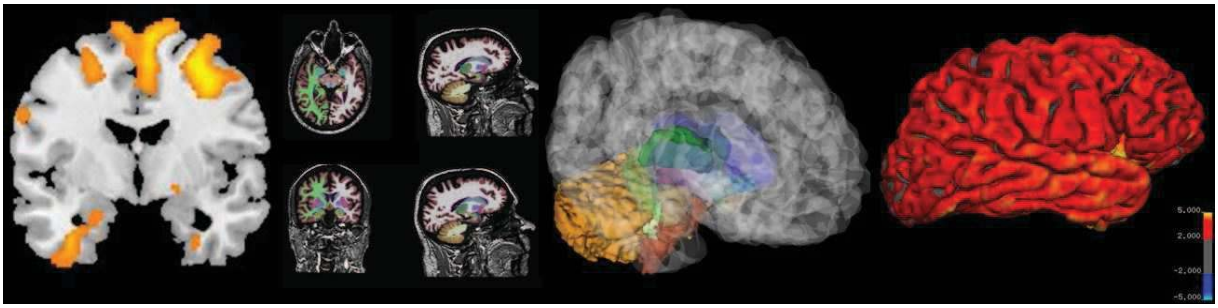

Figure 1: Structural MRI techniques. Voxel-based morphometry, automatic segmentations, shape analysis, cortical thickness analysis (from left to right).

DTI (Basser et al., 1994b;a;Pierpaoli et al., 1996) is a variant of conventional MRI, based on the tissue water diffusion rate. It is a non-invasive method, with unparalleled sensitivity to water movements within the architecture of the tissues. With DTI analysis it is possible to infer, in each voxel, properties such as the molecular diffusion rate, MD and the directional preference of diffusion, FA. Diffusion in the WM is less restricted along the axon and tends to be anisotropic (directionally-dependent) whereas,

in the GM is usually less anisotropic and in the CSF is unrestricted in all directions (isotropic) (Pierpaoli et al., 1996;Hagmann et al., 2006). Another family of parameters that can be extrapolated from DTI is the three-dimensional representation of WM pathways or fiber bundles, the so-called WM tractography (Jones et al., 1999;Mori et al., 1999). These fiber tracts are comprised of neuronal axons that enable the transmission of neural signs, connecting different brain regions, and are commonly referred as structural connectivity (Rykhlevskaia et al., 2008) (Figure 2).

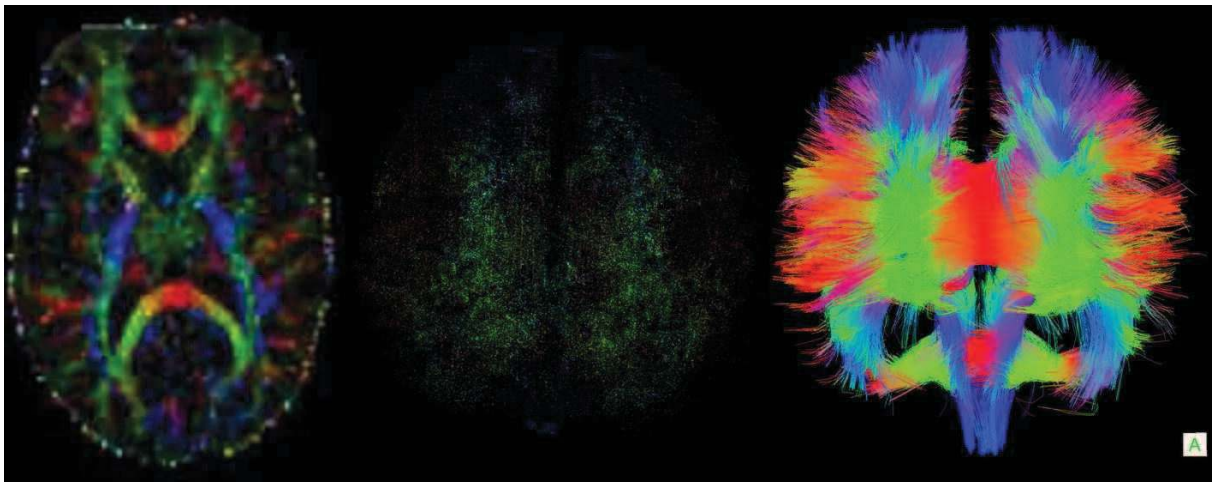

Figure 2: DTI techniques. Colored Fractional Anisotropy maps, glyph visualization and tractography (from left to right).

DTI is sensitive to microstructural tissue properties and, thus, it can be used in WM research and clinical work to explore WM anatomy and structure *in vivo*. In fact, this sensitivity, providing diffusion summary measures and tissue fiber orientation, has made DTI widely used as a clinical tool, especially in conditions where abnormalities in WM are expected (Sundgren et al., 2004;Mori and Zhang, 2006). DTI has been successfully implemented to study patients with acute stroke or brain tumors; neurodegenerative and neuropsychiatric disorders as well as changes in the WM microstructure during neurodevelopment and aging (Hygino da Cruz Jr et al., 2011;Voineskos et al., 2012). DTI variables (e.g. FA) are usually related with alterations in structure (possibly due to particular conditions/disease) pointing to specific myelination levels and axonal injury (Gupta et al., 2012;Soares et al., 2013).

2.3 Functional neuroimaging

In the last 20 years, fMRI has grown and gained widespread acceptance as a powerful tool for mapping brain function. Indeed, fMRI had a huge impact in the neuroscience domain, overtaking other

modalities (e.g. Electroencephalography (EEG) and PET) as the predominant means for measuring changes in brain activity (Bandettini, 2007;Aue et al., 2009;Van Horn and Poldrack, 2009;Ferreira and Busatto, 2013). FMRI has become popular because of its non-invasiveness, excellent spatial resolution, and essentially, signal reliability. Briefly, when a response is solicited to a specific brain region, the metabolism in that area increases, leading to an increased blood flow and influx of more oxygenated hemoglobin. As a consequence, the supply of oxygenated hemoglobin exceeds the metabolic demand and the balance between oxygenated and deoxygenated hemoglobin is altered triggering a change in image contrast, the blood-oxygen-level-dependent (BOLD) effect (Ogawa and Lee, 1990;Ogawa et al., 1990).

Since the first studies in fMRI until recently, the research efforts concentrated on identifying differential responses of brain regions to various stimuli or task challenges. FMRI studies typically involved comparing periods of brain activation during a task against periods of a matched baseline task or a "rest" condition. Rest has been a common baseline for fMRI paradigms since it was believed that the resting brain's neural circuits were apparently quiescent without an external stimulus. However, task activation, the traditional focus of fMRI and PET research, is in fact, only the tip of the iceberg of brain activity. More recently, the interest in fMRI research has shifted towards a wider perspective targeting the understanding of how brain regions interact with each other and how this leads to behavioral phenomena. The technical advances in neuroimaging methods have provided advanced tools that instigated new insights to interpret the brain as a network of interacting regions. Based on this assumption, the concept of functional connectivity emerged, describing the neural activity of brain regions that are functionally co-activated even when they are anatomically separated (Rykhlevskaia et al., 2008;van den Heuvel and Hulshoff Pol, 2010).

It is well established that the brain is organized into multiple spatially distributed large-scale networks; this is not only evidenced by task-based fMRI studies (Thomason et al., 2008;Anticevic et al., 2010), but also by resting state fMRI studies (Biswal et al., 1995;Damoiseaux et al., 2006;Fox and Raichle, 2007;Mantini et al., 2007). The latter, also known as resting state networks (RSNs), a growing field in fMRI, are acquired during resting periods when the subject are not performing any attention-demanding task and are instructed to remain still and keep their eyes closed or open while fixating a cross sign (i.e. '+'). In fact, RSNs are networks throughout the brain which present coherent low

frequency (<0.1 Hz) signal fluctuations, when the participants are not focused on any external demand. Interestingly, RSNs can also be investigated during "resting" periods of functional paradigms, in the so-called, deactivation process (Thomason et al., 2008;Anticevic et al., 2010;Duan et al., 2012).

RSNs were initially discovered for the motor system (Biswal et al., 1995), nevertheless their study remained unexplored until some years later when DMN was revealed through resting-state functional connectivity analyses (Raichle et al., 2001;Greicius et al., 2003a). In addition to the DMN, there are a number of other consistent identifiable RSNs, including attention networks, the dorsal attention network (DAN) and the ventral attention network (VAN), SN, SMN, AN and visual networks (VN), primary (PVN) and high (HVN) (Figure 3).

Importantly, the RSNs offer a completely new window into the neural basis of spontaneous human thoughts and introspections.

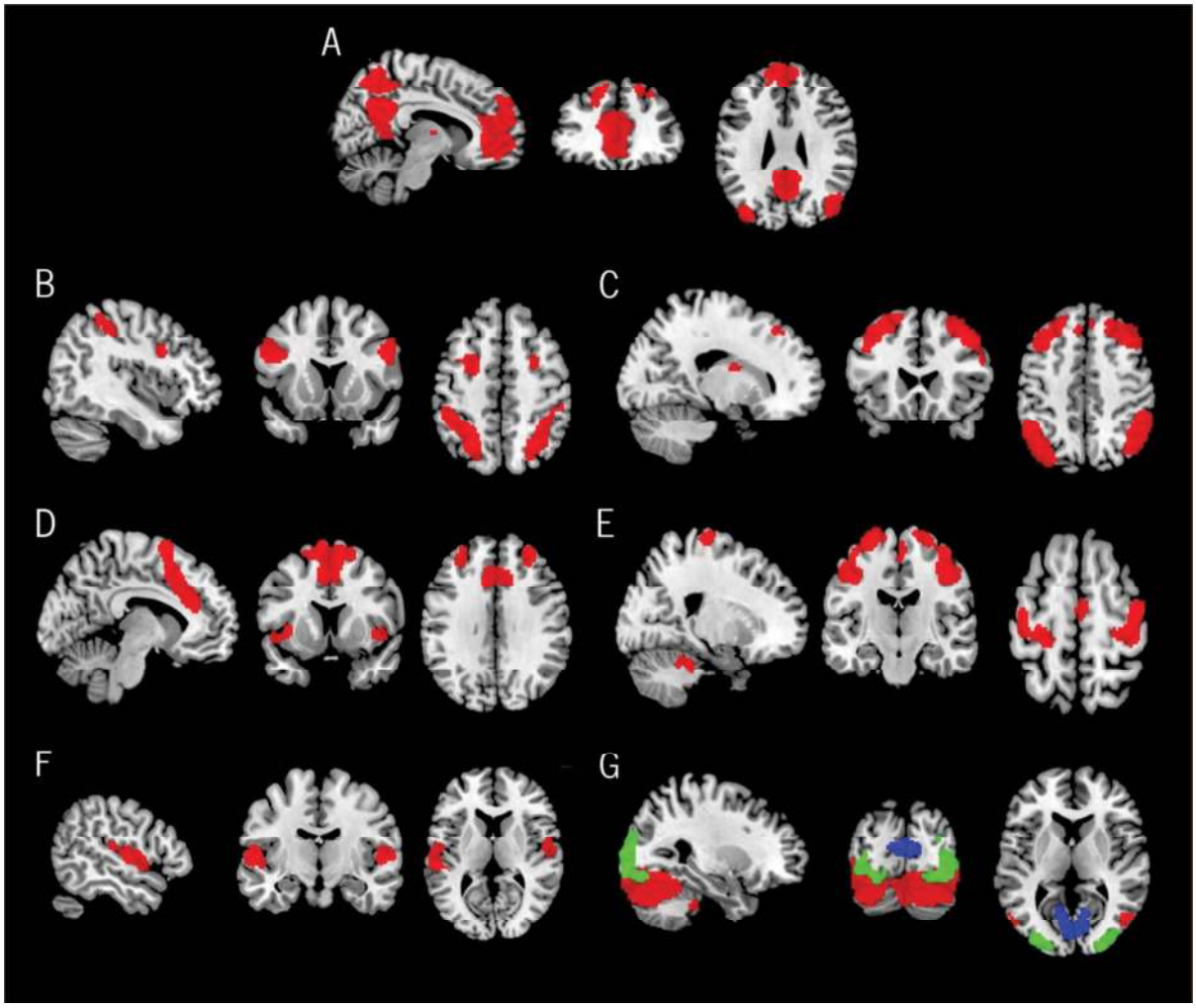

Figure 3: Resting state networks. A – Default Mode Network; B – Dorsal Attention Network; C – Ventral Attention Network; D – Salience Network; E – Sensorimotor Network; F – Auditory Network; G – Visual Networks.

Probably the most fundamental RSN, and by far the most studied, the DMN was firstly identified from PET data (Raichle et al., 2001). Typically, the DMN comprises areas of the posterior cingulate cortex (pCC) and adjacent precuneus (PCu), the medial prefrontal cortex (mPFC), medial, lateral and inferior parietal cortex, and medial and inferior temporal cortex (Raichle et al., 2001;Buckner et al., 2008). The DMN is thought to serve important cognitive functions such as supporting internal mental activity detached from the external world, based on personal introspection, autobiographical memories, and thoughts of the future, but also in connecting internal and external attention in monitoring the world around us (Greicius et al., 2003b;Mason et al., 2007;Buckner et al., 2008). Alterations in this normal pattern of DMN activation have been associated with several mental conditions, disease states and

neuropsychiatric disorders (Zhang and Raichle, 2010;Meda et al., 2012;Madre et al., 2013;Sampaio et al., 2013).

Although the DMN typically refers to a constellation of brain regions functionally more intensely activated during rest, there is also evidence about its deactivation during goal-oriented or attention-demanding tasks (Fox et al., 2005;Harrison et al., 2011;Arbabshirani et al., 2012). Based on this dampening of activation during such demanding tasks, the DMN is also called the "task negative network". Specifically, deactivation may correspond to a deviation in the default-mode towards a tuning down task-focused behavior that requires attention focus and other demanding cognitive processes. Interestingly, task-induced DMN deactivation was related with performance in several cognitive tasks, the more DMN regions are supressed, the better people perform (Mayer et al., 2010), and as a task becomes more difficult, the DMN suppression increases (Singh and Fawcett, 2008). Additionally, failure of deactivation has been associated with neuropsychiatric disorders (Pomarol-Clotet et al., 2008;Guerrero-Pedraza et al., 2012).

In addition to the typical DMN, two largely segregated canonical networks in their spatial distribution have also been consistently observed during the brain's spontaneous resting state (Fox et al., 2006) and related with attention-demanding tasks (Duan et al., 2012;Majerus et al., 2012): a bilateral DAN and the VAN. The DAN includes the bilateral dorsal frontal and parietal cortices (intraparietal sulcus) and has been associated with goal-directed, voluntary top-down attention processes as inhibitory control, working memory and decision process (Corbetta and Shulman, 2002;Kim, 2010). This network presents increased activity in the preparation and selection for stimuli and responses, such as after presentation of cues indicating where, when, or to what participants should direct their attention (Corbetta and Shulman, 2002;Astafiev et al., 2003;Shulman et al., 2003). The VAN, largely right-lateralized, includes the ventral frontal and parietal cortices (temporo-parietal junction), the insular cortex and subcortical regions. It appears to be involved with salience processing and mediates exogenous stimulus-driven, bottom-up attention processes. This system shows increased activity when detecting unexpected salient targets, after abrupt changes in sensory stimuli, whether perceptual, emotional or homeostatic and with increasing familiarity strength (Corbetta and Shulman, 2002;Seeley et al., 2007).

While the DMN shows deactivation during cognitively demanding tasks, activation in attentional networks typically increases. These two attention networks have been employed as a model in task-related studies suggesting that different attentional processes are carried out by the interaction and cooperation between the two systems. Neither DAN nor VAN controls attentional processes isolated, and the flexible interaction between both enables the dynamic regulator of attention processes in relation to goal-directed top-down and bottom-up sensory stimulation (Corbetta and Shulman, 2002;Fox et al., 2006;Vossel et al., 2013). It has been also suggested that the brain may oscillate between two counterbalanced and anticorrelated modes: the DMN, focused on personal introspection and the DAN, also called task positive network, focused on the processing of external stimuli (Fox et al., 2005;Uddin et al., 2009).

The bilateral insula and the anterior cingulate cortex constitute the SN, which is associated with the integration of sensory data from multiple modalities to segregate the most relevant among internal and external stimuli in order to guide behavior and also in decision-making processes (Seeley et al., 2007;Menon and Uddin, 2010;Ferreira and Busatto, 2013).

The SMN comprises the precentral, postcentral gyrus, cerebellum and portion of the frontal gyrus. This network is characterized by the regions that subserve sensorial and motor tasks (Fox and Raichle, 2007;Habas et al., 2009). This organized pattern may occur in the absence of any overt motor activity, although increased activations are strongly related to the action–execution and perception–somesthesis paradigms. Indeed, several studies reported specific changes in SMN after motor learning (Vahdat et al., 2011).

The AN, including the superior temporal and inferior frontal gyrus, is known to be responsible for the auditory processing and language comprehension. Increased activation of the AN is correlated with auditory processing and language comprehension, stimulated by, cognition–language–speech, perception–audition paradigms and action–execution–speech (Smith et al., 2009;Rosazza and Minati, 2011;Husain and Schmidt, 2014).

Finally, in the literature, up to three distinct components have been associated with the visual system, based on the independent component analysis decomposition strategy. Basically, the three visual networks correspond to medial (HVN), occipital pole (usually called PVN) and lateral visual areas. The

explicitly visual behavior and mental imagery lead to an increased activation in these networks. Specifically, cognition-language-orthography paradigms are associated to higher connectivity in the occipital network and cognition-space paradigms are tend to recruit the lateral visual network (Ganis et al., 2004;Smith et al., 2009). Interestingly, the previous exposure to visual stimuli can modulate the subsequent functional connectivity in visual networks, pointing to its possible role in memory consolidation (Mantini et al., 2007;Hasson et al., 2009).

Importantly, alterations in the normal pattern of these RSNs have been associated with several disease states and neuropsychiatric disorders, presenting enormous potentialities for future clinical diagnosis and treatments (Fox and Greicius, 2010;Zhang and Raichle, 2010;Lee et al., 2013;Sampaio et al., 2013). Despite the fact that RSNs change over the course of development and aging and present short-term and learning-induced changes in functional connectivity, these RSNs have been shown to be robust and reliable. In fact, the functional connectivity is consistently observed across several behavioral states, including different resting conditions, task demands and performances, and persists in different consciousness states such as sleep and anesthesia (Heine et al., 2012) and across different species (Becerra et al., 2011;Rosazza and Minati, 2011).

2.4 Multimodal neuroimaging

Despite the tremendous advances in neuroimaging techniques and analyses approaches, unimodal studies are still the predominant way to investigate and assess brain alterations. A better understanding of the brain structure–function interactions requires the combination of multiple imaging techniques. Collecting multimodal brain data from the same individual using different neuroimaging methods will be certainly a trend for the future, contributing for a better understanding of the complex brain anatomical, functional and physiological interplay in normal development/aging and disease states. As such, the future of healthy brain aging and stress research, among others, will benefit from the constant and progressive advances in neuroimaging technologies and methodologies and its integration with other domains (e.g. neurophysiology, microscopy).

In multimodal neuroimaging studies, one of the most popular approaches implemented is to study brain connectivity with the combination of microstructural organization (DTI) and functional activation

patterns using resting state or task related functional MRI (Le Bihan, 2012) (Figure 4). The RSN regions display high levels of functional connectivity, pointing to the existence of structural pathways and connections (anatomical WM tracts) that may facilitate the constant neuronal communication. There is also an increasing consensus that changes in this intrinsic spontaneous brain activity under rest conditions can thus reflect functional, physiological, or structural alterations due to disease conditions (Brier et al., 2012;Shumskaya et al., 2012). Several studies have investigated this relationship between RSNs, such as DMN, attention, visual and motor networks, and direct anatomical pathways architecture using DTI tractography (van den Heuvel et al., 2009;Cabral et al., 2012). Interestingly, one of the main obstacles to the spread of these studies is related with its technical and methodological demands; to mitigate this obstacle, we developed a new intuitive and automatized tool, the BrainCAT (Marques et al., 2013).

Using such approaches will allow for the study of the plastic changes in the brain. Recent neuroimaging studies have pointed to an extraordinary degree of brain plasticity, by adapting its structure and function under different circumstances, including aging (Park and Reuter-Lorenz, 2009;Lovden et al., 2013) and stress states (McEwen and Gianaros, 2011). The understanding of these adaptation and compensation brain mechanisms is an essential keystone for future improvements in life-quality in health and disease and has been one of the key targets of the present thesis.

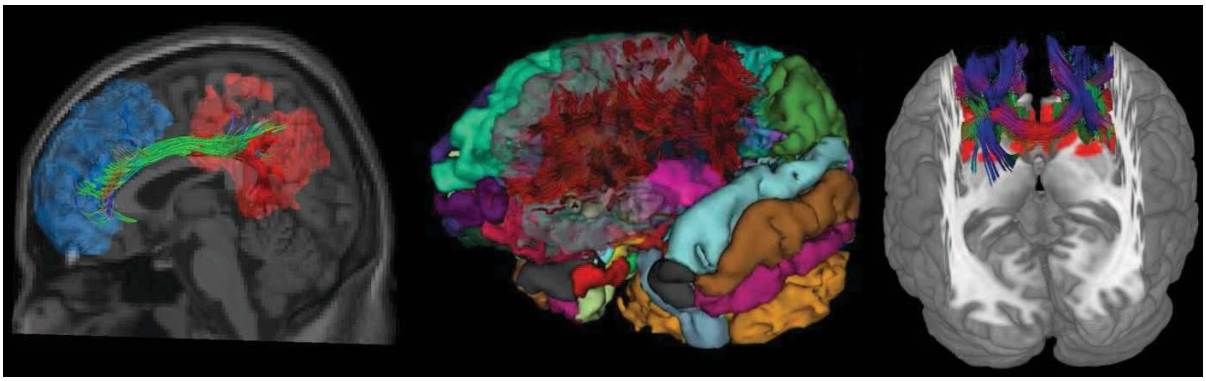

Figure 4: Multimodal neuroimaging analyses.

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Chapter 3

Experimental Work

Chapter 3.1

Soares, J.M., Sampaio, A., Ferreira, L.M., Santos, N.C., Marques, F., Palha, J.A., Cerqueira, J.J., and Sousa, N. (2012)

Stress-induced changes in human decision-making are reversible

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Stress-induced changes in human decision-making are reversible

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Appropriate decision-making relies on the ability to shift between different behavioral strategies according to the context in which decisions are made. A cohort of subjects exposed to prolonged stress, and respective gender- and age-matched controls, performed an instrumental behavioral task to assess their decision-making strategies. The stressed cohort was reevaluated after a 6-week stress-free period. The behavioral analysis was complemented by a functional magnetic resonance imaging (fMRI) study to detect the patterns of activation in corticostriatal networks ruling goal-directed and habitual actions. Using structural MRI, the volumes of the main cortical and subcortical regions implicated in instrumental behavior were determined. Here we show that chronic stress biases decision-making strategies in humans toward habits, as choices of stressed subjects become insensitive to changes in outcome value. Using functional imaging techniques, we demonstrate that prolonged exposure to stress in humans causes an imbalanced activation of the networks that govern decision processes, shifting activation from the associative to the sensorimotor circuits. These functional changes are paralleled by atrophy of the medial prefrontal cortex and the caudate, and by an increase in the volume of the putamina. Importantly, a longitudinal assessment of the stressed individuals showed that both the structural and functional changes triggered by stress are reversible and that decisions become again goal-directed.
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Introduction

The ability to mount an appropriate response to stress is vital for the survival of every living organism. However, when the homeostatic mechanisms to cope with stressful stimuli are disrupted, either because the individual has a particular vulnerability or because the response system is exhausted by a continuous activation, maladaptive responses take place and predispose to cognitive impairment and even to pathological conditions.[1–3] Maladaptive stress affects cognitive behavior through sequential structural modulation of brain networks, mainly as a consequence of the release of corticosteroids.[4,5] In fact, several studies have revealed stress-induced deficits in spatial reference- and working-memory and behavioral flexibility;[6,7] these behavioral changes are attributed to synaptic/dendritic reorganization in both the hippocampus[6] and the medial prefrontal cortex.[7,8] Recently, we showed, in rodents, that chronic stress triggers changes in the frontostriatal networks that govern instrumental behavior decisions.[9] Briefly, different corticostriatal circuits are thought to control competing behavioral strategies during choice situations: whereas the medial prefrontal cortex and the caudate nuclei (associative striatum) have been implicated in goal-directed actions, the putamen (sensorimotor striatum) has been implicated in habitual behavior.[10,11] In that study, we showed that chronic stressed rats display an atrophy of the associative network (medial prefrontal cortex and dorsomedial striatum), in parallel with a hypertrophy of the dorsolateral (sensorimotor) striatum and the most lateral portions of the orbitofrontal cortex. In addition, the structural changes were associated with a bias in decision-making strategies, as behaviors in stressed rats rapidly shifted from goal-directed actions to habits.[9]

This automatization of recurring decision processes into stereotypic behaviors or habits caused by exposure to stress can be viewed as 'advantageous', as it increases behavioral efficiency by releasing cognitive resources for more demanding tasks.[10] Typically, habitual responses do not require the evaluation of their consequences and can be elicited by particular situations or stimuli.[10,11] However, to adapt to ever-changing life conditions, the ability to select the appropriate actions to obtain specific outcomes based on their consequences is of utmost importance. The capacity to shift between habit-based and goal-directed actions is a condition for appropriate decision-making.[12] Importantly, this flexibility includes the ability to inhibit automatisms (habits) in order to use the more demanding goal-directed strategies, which was shown to be impaired in stressed rodents.[9]

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Materials and methods

Subjects, psychological tests and cortisol measurements. Two cohorts of medical students participated in this experiment: one was under their normal academic activities (controls, $n=12$; 6 females, 6 males; mean age, 23.6 ± 2.11), whereas the other included subjects that had just finished their long period of preparation for the medical residence selection exam (chronic psychosocial stress; stress group, $n=12$, 6 females, 6 males; mean age, 23.9 ± 0.70). For the longitudinal assessment, stressed individuals ($n=12$) were reassessed in similar conditions 6–7 weeks after the end of the exposure to stress. To control for the influence of test–re-test, a smaller cohort of stress-recovered individuals ($n=4$; 2 females, 2 males; mean age, 24.25 ± 0.96) naïve to the first experiment were also included. In addition, we also reassessed a smaller cohort of control subjects ($n=4$; 2 females, 2 males; mean age, 24.33 ± 1.15) 6 weeks after the first assessment. After arrival, the subjects responded to a laterality test and to a self-administered questionnaire regarding stress assessment (perceived stress questionnaire).[13] The perceived stress questionnaire is a reliable and validated instrument to assess chronic psychosocial stress in both healthy and clinical adult samples.[5,13,14] It measures four scales (worries, tension, joy, demands); the first three scales represent internal stress reactions, whereas the scale 'demands' relates to perceived external stressors. Participants were further assessed with the Hamilton anxiety scale[15] and the Hamilton depression scale[16] by a certified psychologist. The Hamilton scales provide a broad assessment and are widely used, including healthy populations;[17] here, the clinical cut-scores for anxiety and depression were not used, but rather a continuous approach to the variables to compare absolute scores was utilized. Upon filling of the questionnaires, and immediately before the instrumental task, subjects collected saliva samples using Salivette (Sarstedt, Germany) collection devices. Collection took place between 0900 h and 0500 h in all subjects (the variation in cortisol levels in this time period is relatively small;[18] furthermore, subjects from the control and the stressed group were mixed in order to have a similar distribution of the collection time for cortisol, allowing to minimize the impact of the variation in collection time). Samples were stored at $-20\,^{\circ}C$ until the biologically active, free fraction of the stress hormone cortisol was analyzed using an immunoassay (IBL, Hamburg, Germany). Subjects were preassessed to exclude those with a previous history of neurological or psychiatric illness; none indicated a history of eating disorders. The study was conducted in accordance with the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of Hospital de S. Marcos (Braga, Portugal). The study goals and tests were explained to all participants and all gave informed written consent.

Instrumental task. The task was adapted from a validated protocol.[19] Subjects were asked to fast for at least 12 h before their scheduled arrival time at the laboratory, but were permitted to drink water. Before starting the instrumental task, hunger level and pleasantness of the liquid foods were checked for each subject. The liquid-food rewards were chocolate milk and tomato juice. Alternatively, apple or strawberry juices were given to subjects that did not find pleasant either chocolate or tomato juice. These liquid foods were selected as they can be administered in liquid form, are palatable at room temperature, and their flavor and texture are distinguishable; in this way, sensory-specific satiety effects were kept and the likelihood of the subjects developing a generalized satiety to all liquid foods was minimized. The food rewards were delivered by means of separate syringe pumps (one for each liquid) positioned in the scanner room; subjects received the food through polyethylene plastic tubes (straw-like) although they lay supine in the scanner. The task, with an event-related jittered design, consisted of different sessions: sessions of valued (VAL) actions with reward deliver followed by sessions of devalued (DEV) actions with the outcome devaluation and extinction. Between the two sessions there was a 30-min break, during which subjects were fed to satiety with one of the two liquid foods, outside of the scanner. Each session consisted of 150 trials (50 trials per condition: chocolate, tomato and neutral) subdivided in five blocks of 30 trials each. In each trial, the decision time was 1.5 s; after each decision, the choice appeared highlighted during 4 s; this was followed by the reward delivery time, a black screen with a red fixation with 2 s duration, and the jittered interstimulus interval with 4 s mean duration (Supplementary Figure 1). Before the experiment, subjects were informed about the pairs of fractal patterns that would appear on each trial and were instructed to select one of the possible actions on each trial. They were informed that according to their choices they would receive 0.75 ml of liquid food (valued outcome), the same quantity of a neutral solution (water) or nothing; although there was no information about which action was associated with which particular outcome, subjects were told that one of each pair of actions was associated with a higher probability of obtaining an outcome than the other. During the first session, subjects were instructed to learn to choose the actions that led to high probabilities of pleasant liquid foods, including chocolate and tomato juice. Choosing this option led to a chance of obtaining chocolate milk ($P=0.4$) or orange juice ($P=0.3$) in the chocolate condition, and tomato juice ($P=0.4$) or orange juice ($P=0.3$) in the tomato condition. After this session, in which subjects learned to preferentially choose the options that gave them the best chance of obtaining a juice reward, they were then removed from the scanner and invited to eat to satiety (selective satiation), until they did not want to eat any more, and the pleasantness rating for that food had decreased (devaluation), as checked by a reassessment similar to the one used before session 1. This selective outcome devaluation procedure served to devalue one of the outcomes associated with a particular instrumental action, leaving the value of the outcome associated with the other action intact. To test the effects of the devaluation procedure, subjects underwent a second session, in which they were presented with the same trial types involving the same actions and once again had to select whichever action they preferred. The chosen stimulus increased in brightness as it did during the first session but, in this session, the outcome was no longer presented (that is, the subjects were tested in extinction for these outcomes). That is, the devalued and

non-devalued outcomes were never presented again to the subjects during the test. Yet, to maintain responses on both the actions subjects still received the non-devalued orange juice outcome so that the overall outcome was now available with equal probability on the two available actions ($P=0.3$ each). The total acquisition time was between 2.5–3 h. Using this design and by comparing the different patterns of activation between the first (first 30 trials) and the last block (last 30 trials), one can appreciate the different brain areas associated with distinct behaviors during the instrumental task.

Data acquisition. The different MRI acquisition sequences of the brain were conducted in different sessions on the same day, using a clinically approved Siemens Magnetom Avanto 1.5. T (Erlangen, Germany). The detailed description of data acquisition is provided in Supplementary Materials and methods.

Image processing. The detailed description of fMRI and volumetric data analysis[20–24] is provided in Supplementary Materials and methods.

Statistical analysis. Results of the psychological scales, cortisol levels, behavioral performance and regional volumes were analyzed in the IBM SPSS Statistics software, v.19. (IBM, New York, USA). The detailed description of the statistical analysis is provided in Supplementary Materials/subjects and Methods.

Results

Stress insensitivity to outcome devaluation is reversible. In order to investigate whether our previous findings in rodents translate into humans, we designed two experiments. In the first, two cohorts of medical students were recruited: one was under their normal academic activities (controls, $n=12$), whereas the other included subjects that had just finished their long period of preparation for the medical residence selection exam (stress group, $n=12$). Stressed individuals displayed increased scores in the stress-perceived questionnaire (Figure 1a; $t_{22}=3.429$, $P=0.002$) and in the Hamilton anxiety ($t_{22}=2.202$, $P=0.042$) and depression scores ($t_{22}=3.698$, $P=0.001$) when compared with controls; a trend was also found for increased salivary cortisol levels in stressed subjects ($t_{22}=2.077$, $P=0.05$). In the second experiment, we performed a longitudinal study in the same stressed individuals (stress recovered, $n=12$) by reassessing their psychological and behavioral performance 6–7

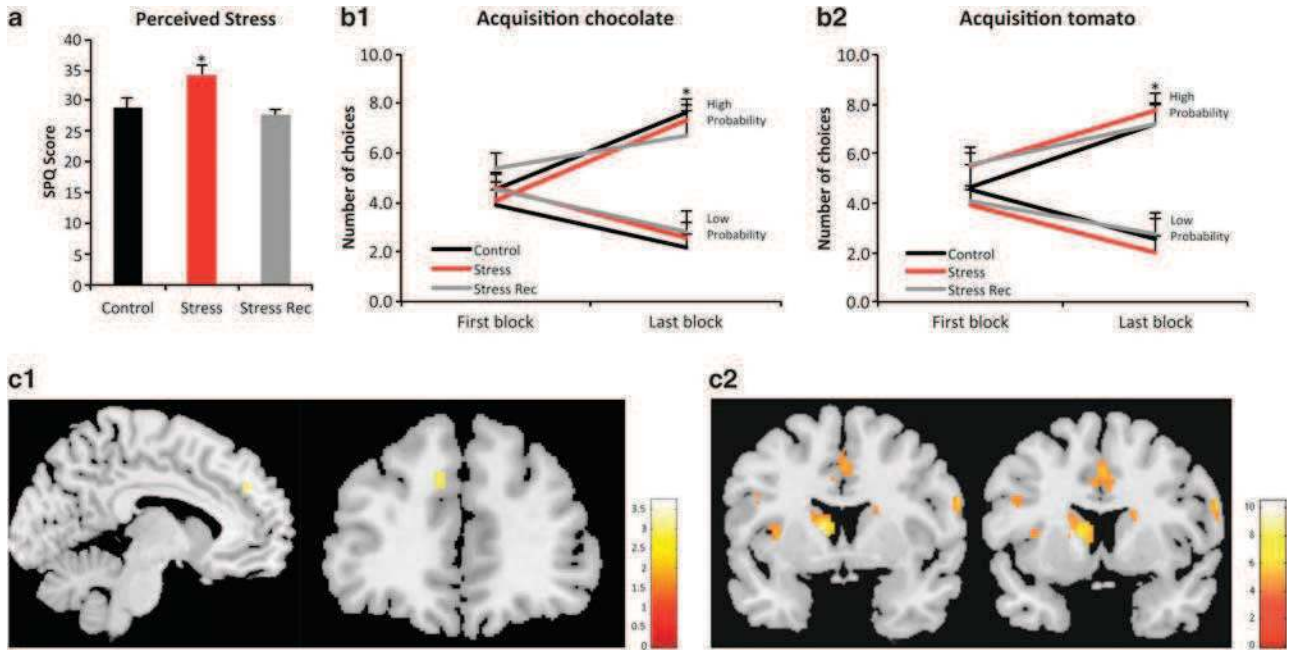

Figure 1 Exposure to chronic stress does not influence the acquisition of instrumental tasks and activates the associative fronto-striatal network. (**a**) Mean score of the stress perceived questionnaire (control vs stress $t_{22}=3.429$, $P=0.002$; stress vs stress recovered $t_{11}=3.663$, $P=0.004$). (**b**) Response rate during the acquisition of the task, for the valued rewards ((**b1**) chocolate, (**b2**) tomato) in both the high (chocolate: control $t_{11}=2.568$, $P=0.026$; stress $t_{11}=3.806$, $P=0.003$; stress recovered $t_{11}=2.615$, $P=0.024$; tomato: control $t_{11}=3.144$, $P=0.009$; stress $t_{11}=2.556$, $P=0.027$; stress recovered $t_{11}=2.828$, $P=0.016$) and the low probability options (chocolate: control $t_{11}=1.321$, $P=0.213$; stress $t_{11}=2.152$, $P=0.054$; stress recovered $t_{11}=3.120$, $P=0.010$; tomato: control $t_{11}=2.677$, $P=0.022$; stress $t_{11}=2.335$, $P=0.039$; stress recovered $t_{11}=2.187$, $P=0.051$). No significant differences were found between groups. (**c1**), Pattern of activation when deciding between high- vs low-value choices during the learning phase of the task (that is, contrast between the last and first block of the first session). The activation in the medial prefrontal cortex (left medial superior gyrus; $x=-10$, $y=44$, $z=32$; Z score $=2.81$; $P<0.002$, uncorrected) demonstrates the engagement of this brain region during the acquisition of the decision task. No other brain region showed effects at this significance in this contrast. (**c2**), Pattern of brain activation in controls throughout the learning phase of the task. There is activation of components of the associative network, namely the medial prefrontal cortex (anterior cingulate: $x=0$, $y=10$, $z=42$; Z score $=4.13$; $P<0.05$, corrected for small volume for family wise error (FWE)) and the caudate nucleus (left: $x=-12$, $y=6$, $z=10$; Z score $=4.49$; $P<0.05$, corrected for small volume for FWE and right $x=18$, $y=10$, $z=18$; Z score $=3.67$; $P<0.05$, corrected for small volume for FWE).

weeks after the end of the exposure to stress, which allowed to infer on the (ir)reversibility of the stress-induced changes. The results clearly showed that a stress-free period of a few weeks is sufficient to normalize all the psychological changes (Figure 1a; stress perception: $t_{11}=3.663$, $P=0.004$; anxiety score: $t_{11}=2.766$, $P=0.018$; depression score: $t_{11}=4.551$, $P=0.001$); salivary cortisol levels were partially restored ($t_{11}=1.835$, $P=0.094$). To control for the influence of test–re-test, a smaller cohort of stress-recovered individuals ($n=4$) naïve to the first experiment were also included; no differences were obtained in any parameter under study between stress-recovered non-naïve and naïve groups (data not shown). In addition, a smaller cohort of control subjects ($n=4$) was also reassessed 6 weeks after the first assessment; no significant differences were found between the two assessment moments in any of the parameters analyzed (data not shown).

Following the psychological and analytical determinations, we tested whether chronic stress affected the ability of the individuals from all groups to perform actions based on the consequences of their behavior, using an operant instrumental task adapted from Valentin *et al*.[19] All subjects were under 12 h fasting. After receiving instructions on the task, subjects learned how to associate an action to an outcome (valued: chocolate and tomato juice; neutral: water). All groups increased their choices of options with a high probability of reward (chocolate and tomato juice) (tomato: control $t_{11}=3.144$, $P=0.009$; stress $t_{11}=2.556$, $P=0.027$; stress recovered $t_{11}=2.828$, $P=0.016$; chocolate: control $t_{11}=2.568$, $P=0.026$; stress $t_{11}=3.806$, $P=0.003$; stress recovered $t_{11}=2.615$, $P=0.024$) in detriment of low-probability options, indicating that both control and stressed subjects had no difficulty in associating the particular action they were performing with the specific outcome obtained (Figure 1b). Of note, there was no preference for high or low probability options when the reward was neutral (all comparisons nonsignificant, data not shown). This operant task was performed with the subject inside a scanner, during the acquisition of fMRI, which allowed determining the patterns of activity of fronto-striatal networks during the decision-making processes.[25] The response rate during the acquisition phase (Figure 1b) was observed to be similar and goal-directed in all experimental groups. Although distinction between high- and low-value outcomes triggers a specific activation of the medial frontal gyrus (Figure 1c1), a region of the brain associated with high-level executive function and decision-making,[26] the learning of the instrumental task in goal-directed actions activated highly the cingulate gyrus and the caudate nuclei (Figure 1c2), both key components of the associative network.

In a second session, we tested whether subjects were sensitive to outcome devaluation, by providing free access to one of the rewards (for example, chocolate), until they referred satiety before starting the task. In accordance with our rodent studies, controls adapted their choices in response to sensory-specific satiety, whereas stressed subjects were insensitive to the expected value of the outcome, as indicated by the lack of a devaluation effect (Figure 2a; control: $t_{11}=3.767$, $P=0.003$; stress: $t_{11}=1.464$, $P=0.171$); importantly, group comparisons proved that the stress group significantly differs from controls in the number of devalued choices (Figure 2a; $t_{22}=-2.143$, $P=0.043$), but not in valued options ($t_{22}=0.410$, $P=0.686$). These data suggest that individuals without stress exposure perform actions because of the consequences of their behavior, whereas stressed subjects rapidly develop habitual behaviors and do not adjust their actions to their current needs. Importantly, during the devaluation phase of the task stressed subjects activate significantly more the left putamen than controls (Figure 2b2), whereas controls display a greater activation of the right caudate than stress subjects (Figure 2b1); these findings correlate with an impairment in devaluation observed in stressed subjects and support the view that habit-based decisions are linked to an overactivation of components of the sensorimotor corticostriatal network. Interestingly, after a period of recovery from stress, these subjects regain the ability to orient their action by goal-directed decisions (Figure 2a;

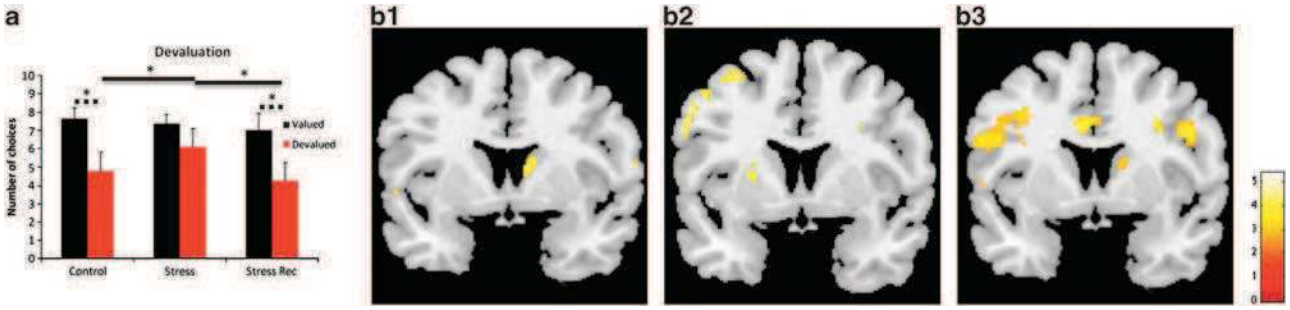

Figure 2 The stress insensitivity to outcome devaluation is reversible and associated with variations of the activation of the corticostriatal networks. (**a**) Response rate for the high probability option of the devalued reward before (last block of the first scanning session) and after (first block of the second scanning session) devaluation. Controls significantly reduced their preference (control: $t_{11}=3.767$, $P=0.003$), whereas stressed subjects were insensitive to the decrease in the value of the outcome (stress: $t_{11}=1.464$, $P=0.171$), but regained a goal-directed behavior after a stress-free period (stress recovered: $t_{11}=3.336$, $P=0.007$). Group comparisons showed that the stress group significantly differs in the number of devalued choices from both controls ($t_{22}=-2.143$, $P=0.043$) and stress-recovered subjects ($t_{11}=2.918$, $P=0.014$). (**b**) Pattern of activation during devaluation phase of the task. Controls display a higher activation in the right caudate nuclei ($x=8$, $y=6$, $z=12$; Z score $=3.45$; $P<0.05$ corrected for small volume for FWE) than stressed subjects (**b1**), whereas stressed subjects display a greater activation of the left putamen ($x=-26$, $y=0$, $z=16$; Z score $=3.35$; $P<0.05$ corrected for small volume for FWE) than controls (**b2**); after a period of recovery from stress, a higher activation of the right caudate ($x=20$, $y=-4$, $z=22$; $Z=3.39$; $P<0.005$, uncorrected) is observed when compared with activation immediately after stress (**b3**). *$P<0.05$; line: within group comparisons; dashed line: between groups comparisons.

stress recovered: $t_{11}=3.336$, $P=0.007$; stress vs stress recovered: valued $t_{11}=0.338$, $P=0.742$ devalued $t_{11}=2.918$, $P=0.014$) and display a greater activation of the right caudate during devaluation than subjects immediately after stress exposure (Figure 2b), in a pattern of activation very similar to the one found in controls.

Structural plasticity in the stressed brain. The impairment in decision-making and shift in behavioral strategies observed in stressed individuals evoke the effects observed after manipulations of the associative or sensorimotor corticostriatal circuits.[9,27,28] Therefore, using neuroimaging morphological techniques, we first investigated the effects of chronic exposure to stress on the structure of striatal and cortical circuits known to be required for goal-directed actions and habits; in addition, we also assessed the recovery from stress in the same parameters. The present data reveals opposing effects of chronic stress in the caudate and in the putamen: whereas we found an atrophy of the caudate (relative volumes) that was only significant on the right (Figure 3a; left: $t_{22}=2.067$, $P=0.051$; right: $t_{22}=2.676$, $P=0.014$), the putamen revealed a significantly increased relative volume in both hemispheres (Figure 3a; left: $t_{22}=2.617$, $P=0.016$; right: $t_{22}=3.132$, $P=0.005$). As a consequence, the caudate-to-putamen ratio was increased in controls relative to stressed individuals (left: 0.72 vs 0.61, $t_{22}=3.565$, $P=0.002$; right: 0.76 vs 0.64, $t_{22}=4.190$, $P<0.001$, respectively), which suggests a bidirectional modulation of neuronal connectivity in the dorsal striatum expressed by a global hypertrophy of the sensorimotor striatum, and a shrinkage of the associative striatum. In addition, the orbitofrontal cortex, which is also a target of stress[5,9] and has been implicated in decision-making,[29,30] showed a different pattern of change, with the most medial portions of the orbitofrontal cortex displaying a structural atrophy that reached statistical significance in the left hemisphere (Figure 3a; left: $t_{22}=3.764$, $P=0.001$; right: $t_{22}=1.494$, $P=0.149$), whereas nonsignificant increases were found in the lateral components of this cortical region (left: $t_{22}=30.319$, $P=0.075$; right: $t_{22}=1.355$, $P=0.189$). No differences were found in the motor or somatosensory cortices (Figure 3a; motor: left: $t_{22}=1.450$, $P=0.161$; right: $t_{22}=0.459$, $P=0.651$; sensory: left: $t_{22}=1.272$, $P=0.217$; right: $t_{22}=0.543$, $P=0.593$) or in total intracranial volumes ($t_{22}=0.033$, $P=0.974$). Noticeably, most structural changes found in stressed subjects were transient. Indeed, data from the second neuroimagiological assessment revealed a complete recovery of the caudate (Figure 3b; left: $t_{11}=2.590$, $P=0.025$; right: $t_{11}=2.494$, $P=0.030$), right putamen (Figure 3b; $t_{11}=2.246$, $P=0.046$) and left medial portions of the orbitofrontal cortex volumes (Figure 3b; $t_{11}=2.914$, $P=0.014$) and a trend for restoration in the volume of the left putamen (Figure 3b; $t_{11}=1.495$, $P=0.163$). Importantly, these results demonstrate that stress-induced changes are not permanent and after a short period of recovery from stress (6 weeks) young adults display an impressive plasticity in fronto-striatal networks.

Discussion

The burden of chronic stress exposure is increasing in our modern society. Although stress response is vital for the survival of every living organism, maladaptive responses to stress can produce changes in the brain and affect cognitive processes, attention and executive functions,[1–3] such as decision-making. The selection of the appropriate actions in particular situations is an extremely dynamic process. Actions can be selected based on their consequences (for example, when we first select the best route to drive from home to work). This goal-directed behavior is crucial to face the ever-changing environment but demands an effortful control and monitoring of the response. To increase the efficiency, one can automatize recurring decision processes as habits (or rules). Habitual responses no longer need the evaluation of their consequences and can be elicited by particular situations or stimuli (for example, after driving to work for some time in the established route, we automatically, when entering the car, go that way). The ability to shift back and forth between these two types of strategies is necessary for appropriate decision-making in everyday life. For example, in a novel situation, it may be crucial to be able to inhibit a habit and use a

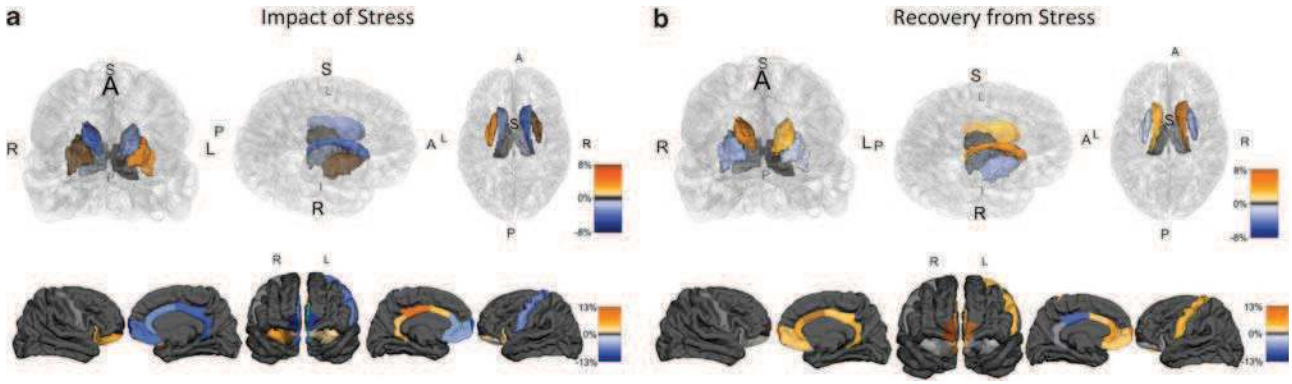

Figure 3 Volumetric changes in the brain after stress exposure (**a**) and after recovery from stress (**b**). Upper panels represent changes in subcortical regions, whereas the lower panels represent volumetric variations in cortical regions. (**a**) The impact of stress in the structure of corticostriatal loop. The color changes illustrate variations in volumes of stressed subjects in contrast to controls. (**b**) The amount of recovery from the impact of stress in the structure of cortico-basal ganglia loop. The color changes illustrate variations in volumes in stressed subjects after recovery from stress.

goal-directed strategy (for example, if we need to go to another place first, besides work, it is most likely inappropriate to use our habitual route to work).

In this study, we show that humans exposed to chronic stress rapidly shift toward habitual strategies (in other words, following the above example, stressed individuals are more likely to choose the habitual route, even when the right choice would be to go a different way). More specifically, our findings demonstrate that prolonged exposure to stress triggers a reorganization of corticostriatal circuits that determine decisions under instrumental tasks (instrumental behavior is determined by the association between an action and an outcome, as tested in this study; in this form of responses, actions can be either 'goal-directed' or 'habitual'. By testing the subjects from the two distinct conditions—control vs stressed—in a paradigm in which they work for a reward, the pattern of their instrumental responses can be discriminated). An atrophy of the associative corticostriatal circuit that rules goal-directed actions, in parallel with a hypertrophy of the sensorimotor corticostrial network, was found in young subjects displaying signs and symptoms of stress; these structural changes were associated with a decreased activation of this circuit in instrumental tasks. Most importantly, stressed individuals had a bias to habits in their decision-making processes. Interestingly, we also demonstrate the remarkable plasticity of these neuronal circuits, by showing that after a stress-free period, both the structural and the functional changes were reverted and the pattern of decision in previously stressed subjects was again biased, which became again goal-directed. Of note, other studies, in distinct experimental conditions, have shown volumetric variations in a similar (or even shorter) time frame.[31,32] In the stress field, studies have also demonstrated rapid structural changes triggered by stress exposure.[6,33] These changes typically occur at the dendritic level and are likely to represent alterations in synaptic connectivity between different brain regions. Alterations in several molecules, including trophic factors and adhesion molecules, are assumed to underlie such structural changes, which occur in opposite directions in distinct brain regions. Importantly, these changes are associated with functional impairments at specific neural circuit level.

Our results confirm a divergent structural reorganization of corticostriatal circuits in humans exposed to prolonged stress, with hypertrophy/overactivation of the sensorimotor and atrophy/deactivation of the associative corticostriatal circuits. This frontostriatal reorganization is accompanied by a shift toward habitual strategies, affecting the ability of stressed individuals to perform actions based on their consequences. These results expand previous studies showing that acute stress can modulate decision-making processes in humans.[34] For the first time it is now demonstrated that such behavioral changes are linked to alterations in the frontostriatal networks in humans, thus providing insights into the neural circuits underlying the shift between goal-directed actions and habitual behavior, and that can lead to dysfunctional decision-making upon exposure to stress.

Noticeable, this stress-decision bias was found to be reversible after the end of the stress exposure, with signs of plasticity both at the structural and at the activational levels. This is in accordance with previous data in rodents and primates showing that stress-induced changes in the structure of the prefrontal cortex are reversible, at least in young subjects.[7] As a consequence of the structural/activational reorganization, we found a behavioral restoration of decision-making strategies in subjects that have been exposed to stress. This novel finding is of paramount importance inasmuch as optimization of decision-making processes confers an important advantage in response to a constantly changing environment. Indeed, under conditions of maladaptative stress, there is a reduced ability to shift from habitual strategies to goal-directed behaviors, even when conditions would recommend that shift. However, it is also true that the fronto-striatal networks, even after prolonged stress, preserve the plastic properties that allow for a functional recovery once the stressful stimuli are gone. Therefore, these results are not only of relevance to understand the mechanisms through which stress is modulating decision-making in both physiological and pathological conditions, but they certainly also pave the way for interventional therapies that empower stress-coping mechanisms.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)

Supplementary Information (SI)

Materials / subjects and Methods

Instrumental task

An adapted validated protocol was used,[1] where: in each trial, subjects were faced with the choice between two possible actions. Each trial type had unique pairs of images representing those actions. On the chocolate and tomato trials, one action delivered the respective reward with a probability of p=0.4. In addition, both actions delivered a common outcome of orange juice with an overall probability of p=0.3 with the constraint that the orange juice and the chocolate or tomato rewards could not be delivered on the same trial. Therefore, the overall probability of a food outcome was p=0.7 for the high-probability action, but p=0.3 for the low-probability actions for each trial type. To provide a control condition against which to assess the effects of the rewards on neural activity, the neutral solution was delivered with the probabilities of p=0.7 and p=0.3 after the two actions on neutral trials. In addition, Valentin et al[1] showed that in the absence of generalized effect on the value of the foods has been clearly demonstrated.

Data acquisition

The different MRI acquisition sequences of the brain were conducted in different sessions in the same day, on a clinical approved Siemens Magnetom Avanto 1.5 T on Hospital São Marcos, Braga. For all the subjects a T1 high-resolution anatomical sequence, and gradient echo T2* weighted echo-planar images (EPIs) were acquired, using the Siemens Auto Align scout protocol in each session to minimize variations in head positioning. For anatomical reference, a sagittal 3D MPRAGE (magnetization prepared rapid gradient echo) sequence was performed with the following imaging parameters: repetition time (TR) = 2.4 s, echo time (TE) = 3.62 ms, 160 saggital slices with no gap, field-of-view (FoV) = 234 mm, flip angle (FA) = 8º, in-plane resolution = 1.2 x 1.2 mm^2 and thickness = 1.2 mm. The functional task imaging with BOLD (blood oxygenation level dependent) contrast was conducted with 900 axial volumes (30 minutes) in each session. The functional imaging parameters were: TR = 2 s, TE = 20 ms, FA = 90º, in-plane resolution and slice thickness 3.3 mm, 38 ascending interleaved axial slices with no gap and FoV = 212 mm. The paradigm was presented using the fully integrated fMRI system IFIS-SA.

Image processing

Before further processing and analysis of the different image sequences, all the images were inspected and confirmed that they were not affected by critical head motion and that participants had no brain lesions.

fMRI analysis: in the analysis of functional data the first 5 volumes were discarded to allow signal stabilization. The fMRI data preprocessing was performed using Statistical Parametric Mapping version 8 (SPM8) analysis software (http://www.fil.ion.ucl.ac.uk/spm). Images were corrected for slice-timing, motion with realignment to the mean image of the sequence, spatially transformed to the standard MNI (Montreal Neurologic Institute) coordinates, resampled to 2x2x2 mm^3, smoothed with a 8-mm full-width half-maximum Gaussian kernel and high pass temporal filtered (filter width of 128s). The fMRI paradigm analyses were performed using the general linear model (GLM) approach. In order to minimize the motion effects in the most critical sequences, the Artifact Detection Tool (ART, http://www.nitrc.org/projects/artifact_detect/) was used to extract the motion parameters and the outliers, to be inputted in the GLM design matrix. The GLM analysis was performed in SPM8 software. The fMRI paradigm was analyzed by creating a set of regressors at the time of each decision-making, which were convolved with the hemodynamic response function. Eight different regressors were created for each participant, high and low valued (H-VAL and L-VAL), devalued (H-DEV and L-DEV) and neutral (H-NEU and L-NEU) probability actions and the differences between different probability actions (H-VAL – L-VAL and H-DEV – L-DEV) to test for the effects of goal-directed learning. The description of statistics and coordinates for the different activation areas in the distinct conditions tested are given in Supplementary Table 1.

Volumetric data analysis: Segmentation of brain structures from T1-weighted 3D structural MRI data and estimation of specific structure volumes was performed using the freely available Freesurfer toolkit version 5.0 (http://surfer.nmr.mgh.harvard.edu). Freesurfer was the toolkit used since it is a free set of software tools for the study of cortical and subcortical anatomy and its pipeline is optimized. The Freesurfer pipeline uses a probabilistic brain atlas estimated from a manual labeled training set proposed in 2002[2] and has undergone several improvements over the years.[3,4] The technique has been shown to be comparable in accuracy to manual labeling and reliable and robust across sessions, scanner platforms, updates and field strengths.[4,5] All the data files initially in DICOM format were firstly converted to the Freesurfer standard format MGZ (compressed Massachusetts General Hospital file) and then processed by a 30-step procedure by three main reconstruction command. Standard semi-automated

workflow procedures employing both surface-based and volume-based pipelines were used in this multimodal neuroimaging study. Briefly, the pipeline comprises the following steps: pre-processing of MRI images, non-parametric, and non-uniform intensity normalization; normalization to the standard Talairach space using a twelve degrees of freedom affine transformation; intensity normalization with corrections of fluctuations in scan intensity to achieve a mean white matter intensity of 110; skull strip, leaving only the brain and overlying pial surface; registration using a transform matrix to align the patient volume with the Fressurfer atlas when applying segmentation labels; reconstruction of cortical and pial surfaces with a sub millimeter precision; inflation of each tessellated cortical surface representing GM-WM boundary to normalize the individual differences in the depth of gyri-sulci. Manual adjustments and visual inspection were needed in the normalization procedure, skull strip, segmentations and pial surface boundary. Trained researchers controlled the reconstructions quality and accuracy, and visually inspected brain segmentations/labels. Estimated intracranial volume (ICV) validated by Buckner and colleagues[6] was used to correct the volumetric data. The detailed description of the variations in the volumes of the different regions analyzed are given in Supplementary Table 2.

Statistical analysis

The design of the study is based on two experiments: one contrasting the effects of stress (control vs stress) and the other contrasting the effect of recovery from stress (stress vs stress recovery). Results of the psychological scales, cortisol levels, behavioral performance and regional volumes were analyzed in the IBM SPSS Statistics software, v.19 (IBM, New York). Comparisons between the control and stress groups were done with two-tailed independent-samples t-test. All within group comparisons, including the acquisition of the task and comparisons in the stressed group between the first and the second (recovery) moment were performed with a two-tailed paired-samples t-test. For all these comparisons significance level was set at 0.05. Values are presented as mean ± standard error of the mean. Group analyses of the fMRI data were performed using the second level random effect analyses in SPM8. Within and between group analyses were implemented with one-sample and two-sample t-tests, respectively, using directional t-contrasts. The comparison between different blocks in the same group was also done using two-sample directional t-tests. T-map threshold was defined at $p < 0.005$ uncorrected with an extent threshold at five voxels for whole brain analysis. Small volume correction analyses on *a priori* regions of

interest in areas related with food reward and decision making were performed. Definition of *a priori* small volumes was based on activation peaks reported in previous fMRI studies with similar paradigms[1,7,8] as centroids to define 10 mm diameter spheres.

Supplementary references

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Supplementary tables

Supplementary Table 1 Descriptive statistics and coordinates of activation areas in different conditions. For all conditions, activated regions, peak MNI coordinates, cluster size, and Z scores are presented. Peaks within the regions were considered significant at $p < 0.005$, uncorrected for multiple comparisons with extent threshold at 5 voxels.

Condition	Regions	Peak MNI coordinates	Cluster size (voxels)	Maximum Z score
Contrast between high and low probability	Frontal superior medial (left)	-10, 44, 32	17	2.81
Acquisition [+]	Caudate (left) *	-12, 6, 10	109	4.49
	Postcentral (right)	62, 2, 20		4.49
	Precentral (right)	62, 8, 24	141	3.85
	Frontal inferior pars opercularis (right)	58, 16, 26		3.84
	Anterior cingulate *	0, 10, 42	240	4.13
	Insula (right)	34, -18, 8	23	3.80
	Insula (left)	-36, 4, 6	48	3.78
	Caudate (right) *	18, 10, 18	17	3.67
	Frontal inferior pars opercularis (left)	-46, 8, 26	10	3.24
Devaluation in control subjects (Control > Stress)	Insula (right)	36, -2, 18	457	3.72
	Caudate (right)*	8, 6, 12	29	3.45
	Caudate (left) *	-10, -2, 18	21	3.11
	Insula (left)	-44, -4, 0	141	3.10
	Postcentral (right)	62, 14, 18	44	2.95
Devaluation in stressed subjects (Stress > Control)	Insula (left)	-34, -6, 18	357	3.65
	Putamen (left)*	-26, 0, 16		3.35
	Precentral (left)	-48, -6, 48		3.63
	Postcentral (left)	-46, -20, 36	1204	3.21
	Precentral (left)	-38, 0, 38		3.17
	Frontal inferior pars opercularis (left)	-38, 12, 16		3.15
	Frontal middle (right)	40, 32, 48	118	3.57
	Anterior cingulate (right)	6, -6, 44	91	3.40
	Frontal superior (left)	-14, 22, 52	52	3.16
	Frontal inferior pars opercularis (right)	30, 12, 30	31	3.05
Devaluation in stressed subjects after recovery (StressRec > Stress)	Postcentral (right)	58, -8, 24	879	4.12
	Precentral (right)	50, -2, 26		3.73
	Anterior Cingulate (left) *	-8, 4, 32	254	3.67
	Postcentral (left)	-60, -2, 26		3.65
	Frontal inferior pars triangularis (left)	-44, 16, 26	1461	3.46
	Frontal middle (left)	-32, 10, 40		3.40
	Frontal inferior pars opercularis (right)	30, 6, 34	37	3.53
	Caudate (right)	20, -4, 22	26	3.39
	Anterior cingulate (right)	2, -36, 32	71	3.39
	Frontal Superior (left)	-22, 64, 8	29	3.11
	Frontal Superior (right)	16, 30, 36	24	2.83
	Frontal inferior pars triangularis (right)	38, 36, 12	19	2.79
	Frontal middle (right)	40, 34, 32	30	2.79

(*) Region of interest that survives $p < 0.05$ small volume correction for FWE

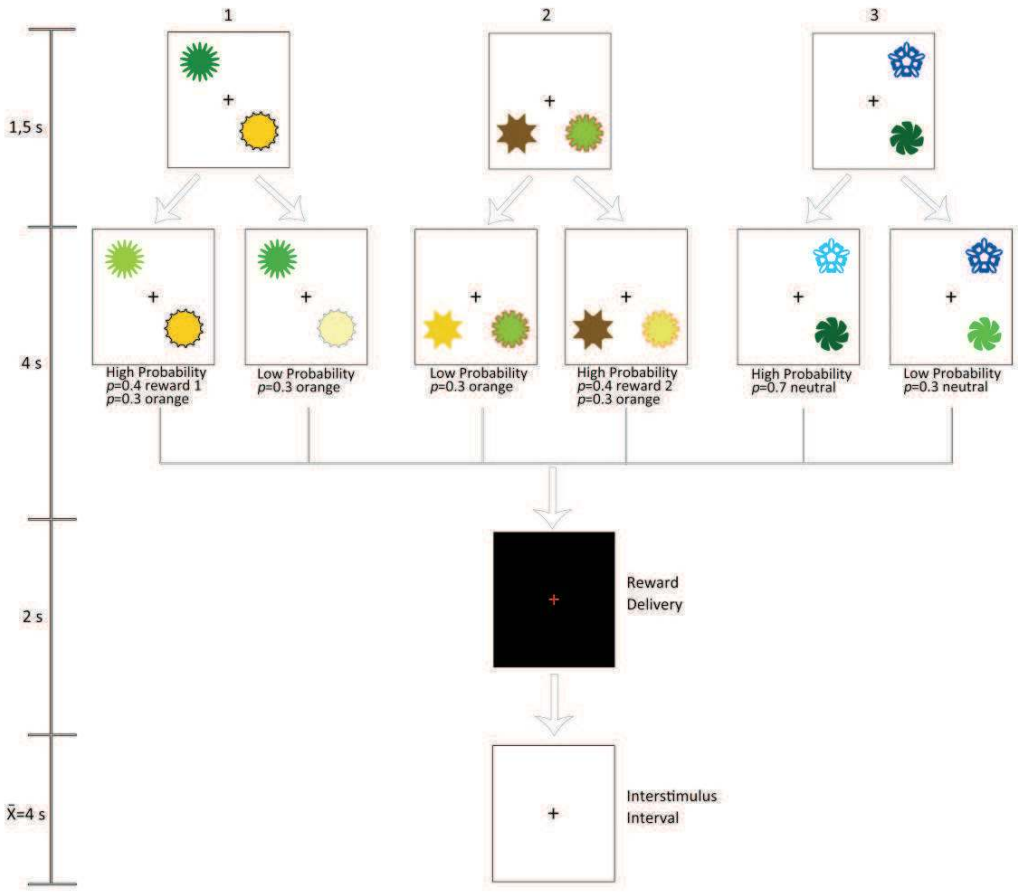
([+]) $p < 0.001$ uncorrected for multiple comparisons

Supplementary Table 2 Volumes (% relative to total intracranial volume) of cortical and subcortical regions (ilustrated in figure 2) and results of the statistical comparisons.

		Control	Stress	Stress-Rec	Control vs Stress	Stress vs Stress-Rec
mOFC (‰)	left	3.56±0.0793	3.18±0.0646	3.44±0.0827	t_{22}=3.764, P=0.001*	t_{11}=2.914, P=0.014*
	right	3.32±0.0737	3.17±0.0660	3.13±0.0940	t_{22}=1.494, P=0.149	t_{11}=0.390, P=0.704
lOFC (‰)	left	4.94±0.110	4.99±0.122	4.87±0.101	t_{22}=0.319, P=0.753	t_{11}=0.833, P=0.423
	right	4.70±0.103	4.88±0.0837	4.78±0.108	t_{22}=1.355, P=0.189	t_{11}=0.692, P=0.504
sFC (‰)	left	14.6±0.429	14.6±0.385	14.8±0.720	t_{22}=0.061, P=0.952	t_{11}=0.258, P=0.801
	right	14.1±0.411	14.0±0.429	14.0±0.523	t_{22}=0.168, P=0.868	t_{11}=0.028, P=0.978
cmFC (‰)	left	4.55±0.224	4.24±0.128	4.22±0.155	t_{22}=1.183, P=0.249	t_{11}=0.101, P=0.921
	right	4.34±0.231	3.79±0.0834	3.79±0.123	t_{22}=2.242, P=0.035*	t_{11}=0.038, P=0.971
rmFC (‰)	left	10.8±0.565	10.1±0.367	10.1±0.435	t_{22}=0.968, P=0.344	t_{11}=0.115, P=0.910
	right	10.1±0.353	9.94±0.370	10.0±0.417	t_{22}=0.321, P=0.751	t_{11}=0.106, P=0.917
poiFC (‰)	left	3.45±0.177	2.98±0.124	2.94±0.122	t_{22}=2.162, P=0.042*	t_{11}=0.228, P=0.824
	right	2.82±0.0870	2.57±0.110	2.55±0.141	t_{22}=1.835, P=0.080	t_{11}=0.142, P=0.890
ptiFC (‰)	left	2.36±0.0922	2.18±0.103	2.13±0.0960	t_{22}=1.272, P=0.217	t_{11}=0.300, P=0.770
	right	2.85±0.164	2.68±0.109	2.77±0.136	t_{22}=0.899, P=0.378	t_{11}=0.704, P=0.496
orbiFC (‰)	left	1.42±0.0512	1.47±0.0452	1.42±0.0536	t_{22}=0.710, P=0.485	t_{11}=0.716, P=0.489
	right	1.71±0.0720	1.82±0.0682	1.76±0.100	t_{22}=1.051, P=0.305	t_{11}=0.632, P=0.540
FP (‰)	left	0.514±0.0326	0.483±0.0333	0.496±0.0344	t_{22}=0.660, P=0.516	t_{11}=0.441, P=0.668
	right	0.670±0.0330	0.587±0.0227	0.639±0.0239	t_{22}=2.089, P=0.049*	t_{11}=1.802, P=0.099
istCg (‰)	left	1.76±0.113	1.62±0.0730	1.67±0.0802	t_{22}=1.010, P=0.323	t_{11}=0.543, P=0.598
	right	1.69±0.844	1.46±0.0660	1.54±0.0929	t_{22}=2.161, P=0.042*	t_{11}=0.617, P=0.550
pCg (‰)	left	2.20±0.102	1.93±0.0807	2.06±0.0539	t_{22}=2.065, P=0.051	t_{11}=0.193, P=0.850
	right	2.20±0.127	2.03±0.0541	2.05±0.0787	t_{22}=1.228, P=0.232	t_{11}=1.263, P=0.233
caCg (‰)	left	1.29±0.0734	1.20±0.0745	1.25±0.0879	t_{22}=0.939, P=0.358	t_{11}=0.500, P=0.627
	right	1.35±0.112	1.42±0.0903	1.48±0.116	t_{22}=0.440, P=0.664	t_{11}=0.612, P=0.553
raCg (‰)	left	1.88±0.0773	1.84±0.0700	1.91±0.0902	t_{22}=0.362, P=0.721	t_{11}=0.605, P=0.558
	right	1.40±0.0719	1.44±0.0744	1.45±0.103	t_{22}=0.433, P=0.669	t_{11}=0.042, P=0.967
PMC (‰)	left	8.16±0.148	8.55±0.231	8.53±0.246	t_{22}=0.319, P=0.753	t_{11}=0.087, P=0.932
	right	8.16±0.204	8.38±0.218	8.37±0.190	t_{22}=0.459, P=0.651	t_{11}=0.042, P=0.967
PSC (‰)	left	5.54±0.117	5.66±0.190	5.52±0.183	t_{22}=1.272, P=0.217	t_{11}=0.297, P=0.772
	right	5.54±0.117	5.66±0.190	5.52±0.183	t_{22}=0.543, P=0.593	t_{11}=0.540, P=0.600
Putamen	left	3.45±0.569	3.74±0.0960	3.67±0.118	t_{22}=2.617, P=0.016*	t_{11}=1.495, P=0.163
(‰)	right	3.27±0.0830	3.62±0.0757	3.50±0.105	t_{22}=3.132, P=0.005*	t_{11}=2.246, P=0.046*
Caudate	left	2.45±0.0556	2.28±0.0597	2.39±0.0714	t_{22}=2.067, P=0.051	t_{11}=2.590, P=0.025*
(‰)	right	2.48±0.0391	2.29±0.0576	2.42±0.0703	t_{22}=2.676, P=0.014*	t_{11}=2.494, P=0.030*
Thalamus	left	4.54±0.0619	4.50±0.0736	4.51±0.0761	t_{22}=0.426, P=0.675	t_{11}=0.112, P=0.913
(‰)	right	4.61±0.0768	4.57±0.0830	4.54±0.0846	t_{22}=0.405, P=0.689	t_{11}=0.272, P=0.791
Total intracranial volume (cm^3)		1676±50.0	1674±37.9	1672±43.19	t_{22}=0.033, P=0.974	t_{11}=0.478, P=0.642

Supplementary legend

Supplementary Figure 1. Schematic representation of the functional paradigm. Subjects were instructed to learn to choose the actions that led to high probabilities of pleasant liquid foods, including chocolate and tomato juice. Choosing this option led to a chance of obtaining chocolate milk (p=0.4) or orange juice (p=0.3) in condition 1,tomato juice (p=0.4) or orange juice (p=0.3) in condition 2 or water (p=0.7 or p=0.3) in condition 3.



Chapter 3.2

Soares, J.M., Sampaio, A., Ferreira, L.M., Santos, N.C., Marques, P., Marques, F., Palha, J.A., Cerqueira, J.J., and Sousa, N. (2013)

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Stress Impact on Resting State Brain Networks

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Abstract

Resting state brain networks (RSNs) are spatially distributed large-scale networks, evidenced by resting state functional magnetic resonance imaging (fMRI) studies. Importantly, RSNs are implicated in several relevant brain functions and present abnormal functional patterns in many neuropsychiatric disorders, for which stress exposure is an established risk factor. Yet, so far, little is known about the effect of stress in the architecture of RSNs, both in resting state conditions or during shift to task performance. Herein we assessed the architecture of the RSNs using functional magnetic resonance imaging (fMRI) in a cohort of participants exposed to prolonged stress (participants that had just finished their long period of preparation for the medical residence selection exam), and respective gender- and age-matched controls (medical students under normal academic activities). Analysis focused on the pattern of activity in resting state conditions and after deactivation. A volumetric estimation of the RSNs was also performed. Data shows that stressed participants displayed greater activation of the default mode (DMN), dorsal attention (DAN), ventral attention (VAN), sensorimotor (SMN), and primary visual (VN) networks than controls. Importantly, stressed participants also evidenced impairments in the deactivation of resting state-networks when compared to controls. These functional changes are paralleled by a constriction of the DMN that is in line with the pattern of brain atrophy observed after stress exposure. These results reveal that stress impacts on activation-deactivation pattern of RSNs, a finding that may underlie stress-induced changes in several dimensions of brain activity.

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Introduction

For many years it has been recognized that acute stress is state of increased vigilance and alertness and to get the organism ready to take action before the impact of dangers [1]. Under brief stressful conditions, the ability to perceive changes in the surrounding environment becomes critical to mount an appropriate response. However, when the homeostatic mechanisms are disrupted, namely through prolonged stress exposure, maladaptive responses take place and trigger inappropriate functional responses with behavioral consequences, including deficits in attention control [2–5]. Recently, we showed, both in humans and rodents, that chronic stress triggers long-lasting, but reversible, changes in the frontostriatal networks that govern instrumental behavior decisions with impairments in decision-making processes [6,7].

It is well established that the brain is organized into multiple spatially distributed large-scale networks; this is evidenced by task-based functional magnetic resonance imaging (fMRI) studies [8–10] but also by resting state fMRI studies [11–13]. The latter, also known as resting state networks (RSNs), include the default mode (DMN), attention (dorsal and ventral), sensorimotor (SMN), visual (VN), auditory (AN), language and memory networks. The DMN is a network of brain cortical areas that present high metabolic activity when the brain is "at rest" and the individual is not focused on any external demand. This network displays a high degree of functional connectivity between various interacting brain areas. Typically, the DMN comprises areas of the posterior cingulate cortex (pCC) and adjacent precuneus (PCu), the medial prefrontal cortex (mPFC), medial, lateral and inferior parietal cortex, and medial and inferior temporal cortex [14,15]. The DMN is thought to serve important cognitive functions such as supporting internal mental activity detached from the external world, but also in connecting internal and external attention in monitoring the world around us [16,17]. There is also evidence that task-induced deactivations of the DMN have been functionally associated with goal-directed behavior [18]. Specifically, deactivation may correspond to a deviation in the default-mode towards a tuning down task-focused behavior that requires attention focus and other demanding cognitive processes. Moreover, task-induced DMN deactivation was related with performance in several cognitive tasks (e.g. [19]), whereas failure of deactivation has been associated with neuropsychiatric diseases (e.g. [20,21]). While the DMN shows deactivation during cognitively demanding tasks [19,22], activation in attentional

networks (dorsal attention and ventral attention networks) typically increases [23,24]. Specifically, in addition to the typical DMN, two largely segregated canonical networks in their spatial distribution have also been consistently observed during the brain's resting state and related with attention-demanding tasks: a bilateral dorsal attention network (DAN), which includes the dorsal frontal and parietal cortices (intraparietal sulcus), and the ventral attention network (VAN), largely right-lateralized, which includes the ventral frontal and parietal cortices (temporo-parietal junction), the insular cortex and subcortical regions [24,25]. While the DAN has been associated with goal-directed, top-down attention processes as inhibitory control, working memory and response selection, the VAN is related with salience processing and mediates stimulus-driven, bottom-up attention processes [24–26]. Moreover, it is relevant to note that dorsal and ventral systems appear to interact not only during cognitive tasks [27,28] but also during spontaneous activity [25].

In addition to the typical DMN, VAN and DAN, other networks have also been consistently observed during the brain's resting state, including: the VN involving the occipital and bilateral temporal regions which is linked to the visual processing network and mental imagery [13,29]; the AN including the superior temporal and inferior frontal gyrus, known for being responsible in auditory processing and language comprehension; the SMN involving the precentral, postcentral gyrus, cerebellum, portion of the frontal gyrus that subserves sensorial and motor tasks [12,25,30,31] and the self-referential network including the medial prefrontal, the anterior cingulate cortex and the hypothalamus [13]. Importantly, all these RSNs were also shown to present abnormal functional patterns in many neuropsychiatric disorders [32–36] For example, in autism, RSNs are much more loosely connected [32,33]; increased functional connectivity was found in social anxiety disorder patients between the right posterior inferior temporal gyrus and the left inferior occipital gyrus, and between the right parahippocampal/hippocampal gyrus and the left middle temporal gyrus [34]; patients with borderline personality disorder showed an increase in functional connectivity in the left frontopolar cortex and the left insula, whereas decreased connectivity was found in the left cuneus [35]; patients with major depressive disorder exhibited increased functional connectivity in the anterior medial cortex regions and decreased functional connectivity in the posterior medial cortex regions compared with controls [36].

Notably, the effect of stress in the functional architecture of RSNs, both during task performance or resting state conditions, is largely unknown. Moreover, although neuropsychiatric diseases (e.g. bipolar disorders, schizophrenia) have been associated with abnormal patterns of RSNs deactivation, which may be related with difficulties in task-focusing and cognitive resources allocation, studies performed during prolonged stress, an established risk factor for neuropsychiatric disorders, are absent. Thus, the main goal of this study was to test if the functional connectivity of RSNs might be aberrant in chronic stress conditions. To achieve this goal, we assessed the architectural differences of RSNs using fMRI independent component analysis (ICA) on resting state data and task induced deactivation analysis, and region-of-interest surface assessments.

Materials and Methods

Participants, Psychological Tests and Cortisol Measurements

The participants included in this study were 8 controls (2 males, 6 females; mean age, 24.25±1.98) and 8 stress (2 males, 6 females; mean age, 23.86±0.35) participants submitted to prolonged psychological stress exposure. Control participants included a cohort of medical students under their normal academic activities, whereas the stress group included participants that had just finished their long period of preparation for the medical residence selection exam. Participants responded to a laterality test and to a self-administered questionnaire regarding stress assessment (Perceived Stress Scale – PSS - [37]. Participants were further assessed with the Hamilton anxiety scale [38] and the Hamilton depression scale [39] by a certified psychologist. Upon filling of the questionnaires, and immediately before the MRI and fMRI acquisitions, participants collected saliva samples with the help of Salivette (Sarstedt, Germany) collection devices. Collection took place between 9am and 5pm in all participants. Samples were stored at -20°C until the biologically active, free fraction of the stress hormone cortisol was analyzed using an immunoassay (IBL, Hamburg).

Ethics Statement

The study was conducted in accordance with the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of Hospital de Braga (Portugal). The study goals and tests were explained to all participants and all gave informed written consent.

Data Acquisition

Participants were scanned on a clinical approved Siemens Magnetom Avanto 1.5 T (Siemens Medical Solutions, Erlangen, Germany) on Hospital de Braga using the Siemens 12-channel receive-only head coil. The different imaging sessions were conducted in the same day and the Siemens Auto Align scout protocol was used to minimize variations in head positioning. For structural analysis and registration to standard space, a T1 high-resolution anatomical sequence, 3D MPRAGE (magnetization prepared rapid gradient echo) was performed with the following scan parameters: repetition time (TR) = 2.4 s, echo time (TE) = 3.62 ms, 160 sagittal slices with no gap, field-of-view (FoV) = 234 mm, flip angle (FA) = 8°, in-plane resolution = 1.2×1.2 mm^2 and slice thickness = 1.2 mm. During RS-fMRI acquisition, using gradient echo T2* weighted echo-planar images (EPIs), participants were instructed to keep the eyes closed and to think in nothing particular. The imaging parameters were: 100 volumes, TR = 3 s, TE = 50 ms, FA = 90°, in-plane resolution = 3.4×3.4 mm^2, 30 interleaved slices, slice thickness = 5 mm, imaging matrix 64×64 and FoV = 220 mm. fMRI paradigm acquisition was acquired using: TR = 2 s, TE = 20 ms, FA = 90°, in-plane resolution and slice thickness 3.3 mm, 38 ascending interleaved axial slices with no gap and FoV = 212 mm. The functional paradigm is described in [7] and was presented using the fully integrated fMRI system IFIS-SA.

Image Pre-processing

Before any data processing and analysis, all the different acquisitions were inspected and confirmed that they were not affected by critical head motion and that participants had no brain lesions.

To achieve signal stabilization and allow participants to adjust to the scanner noise, the first 5 volumes (15 seconds) were discarded. Data preprocessing was performed using SPM8 (Statistical Parametrical Mapping, version 8, http://www.fil.ion.ucl.ac.uk) analysis software. Images were firstly corrected for slice timing using first slice as reference and SPM8's Fourier phase shift interpolation. To correct for head motion, images were realigned to the mean image with a six-parameter rigid-body spatial

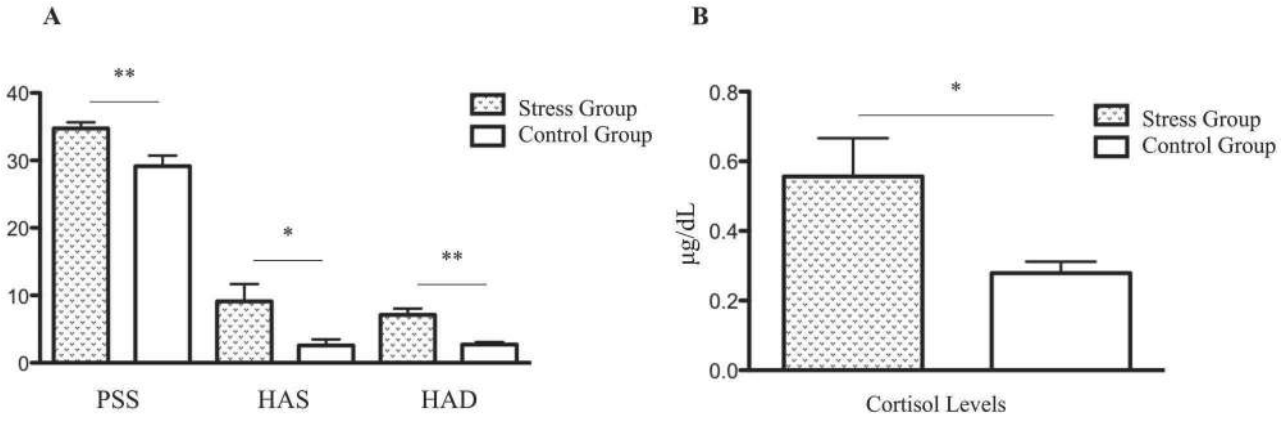

Figure 1. Clinical characteristics of the cohort. (A): Perceived Stress Scale (PSS), Hamilton Anxiety Scale (HAS); Hamilton Depression Scale (HAD) and (B): Salivary Cortisol levels of the stressed and control groups. **P<0.01; *P<0.05.
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transformation and estimation was performed at 0.9 quality, 4 mm separation, 5 mm FWHM smoothing kernel using 2nd degree B-Spline interpolation. No participants exceed head motion higher than 2 mm in translation or 1° in rotation. Images were then spatially normalized to the MNI (Montreal Neurological Institute) standard coordinate system using SPM8 EPI template and trilinear interpolation. Data were then re-sampled to $3\times3\times3$ mm^3 using sinc interpolation, smoothed to decrease spatial noise with a 8 mm, full-width, half-maximum, Gaussian kernel, temporally band-pass filtered (0.01–0.08 Hz) and the linear trend was removed. The pre-processing of fMRI paradigm images was previously described [7].

Independent Component Analysis and Identification of RSN

Spatial independent component analysis was conducted for all participants using the Group ICA 2.0d of fMRI Toolbox (GIFT, http://www.icatb.sourceforge.net) [40,41]. Concisely, ICA analysis consists in extracting the individual spatial independent maps and their related time courses. The reduction of dimensionality of the functional data and computational load was performed with Principal Component Analysis (PCA). 20 components were estimated for each subject and ICA calculation was then performed using the iterative Infomax algorithm. The ICASSO tool was used to assess the ICA reliability, and 20 computational runs were performed on the dataset, during which the components were being recomputed and compared across runs and the robustness of the results was ensured [42]. The independent components were obtained and each voxel of the spatial map was expressed as a t-statistic map, which was finally converted to a z-statistic. Z-statistic describes the voxels that contributed more intensely to a specific independent component, providing a degree of functional connectivity within the network [43,44]. The final components were visually inspected, sorted and spatially correlated with resting state functional networks from [45]. The best-fit components of each individual (z-maps) were used to perform group statistical analyses.

RSN Deactivation during fMRI Task Analysis

fMRI paradigm was analyzed by creating a set of regressors at rest and at the time of making a decision, which were convolved with the hemodynamic response function. In order to reliably map task-induced deactivations, we combined all the resting periods (resting baseline condition) and all the decision periods using a protocol previously described [7] (decision condition), given that decision periods were equally demanding. The contrast used to assess task-induced deactivations was the resting baseline condition *minus* decision condition. Resulting functional patterns were masked with the previously described RSNs templates.

Structural Analysis

Structural analysis based on segmentation of brain structures from T1 high-resolution anatomical data was performed using the freely available Freesurfer toolkit version 5.0 (http://surfer.nmr.mgh.harvard.edu). Intracranial volume (ICV) was used to correct the volumes and the processing pipeline was the same as previously described [7]. DMN was defined by the summed volume of the angular gyrus of inferior parietal lobe, the posterior cingulate, the precuneus and the frontopolar region [14,15]. The summed volume of the middle frontal gyrus (dorsolateral and prefrontal region) and the posterior parietal region constituted the DAN [46,47]. VAN was constituted by the sum of the temporal-parietal junction and the ventral frontal cortex volumes [25]. SMN was defined by the summed volume of the paracentral, precentral postcentral and the cerebellum [45]. The summed volume of the cuneus, pericalcarine and the lingual region constituted the primary VN [45].

Statistical Analyses

Results of the psychological scales, cortisol levels, and regional volumes were analyzed in the IBM SPSS Statistics software, v.19 (IBM, New York). Comparisons between the control and stress groups were done with two-tailed independent-samples t-test. For all these comparisons significance level was set at 0.05. Values are presented as mean ± standard error of the mean.

Group analysis of the fMRI resting state and task induced deactivations were performed using the second level random effect analyses in SPM8. Initially, within group analyses were performed only to confirm the activation of the RS networks in the different groups, using one-sample t-tests. Therefore, between group analyses were implemented with directional two-sample t-tests, to directly compare the groups based on the two experiments designed. Functional results for all RSNs were considered significant at a corrected for multiple comparisons $p<0.05$ threshold (based on the combination of height threshold with a minimum cluster size), determined by Monte Carlo simulation program (AlphaSim). Anatomical labeling was defined by a

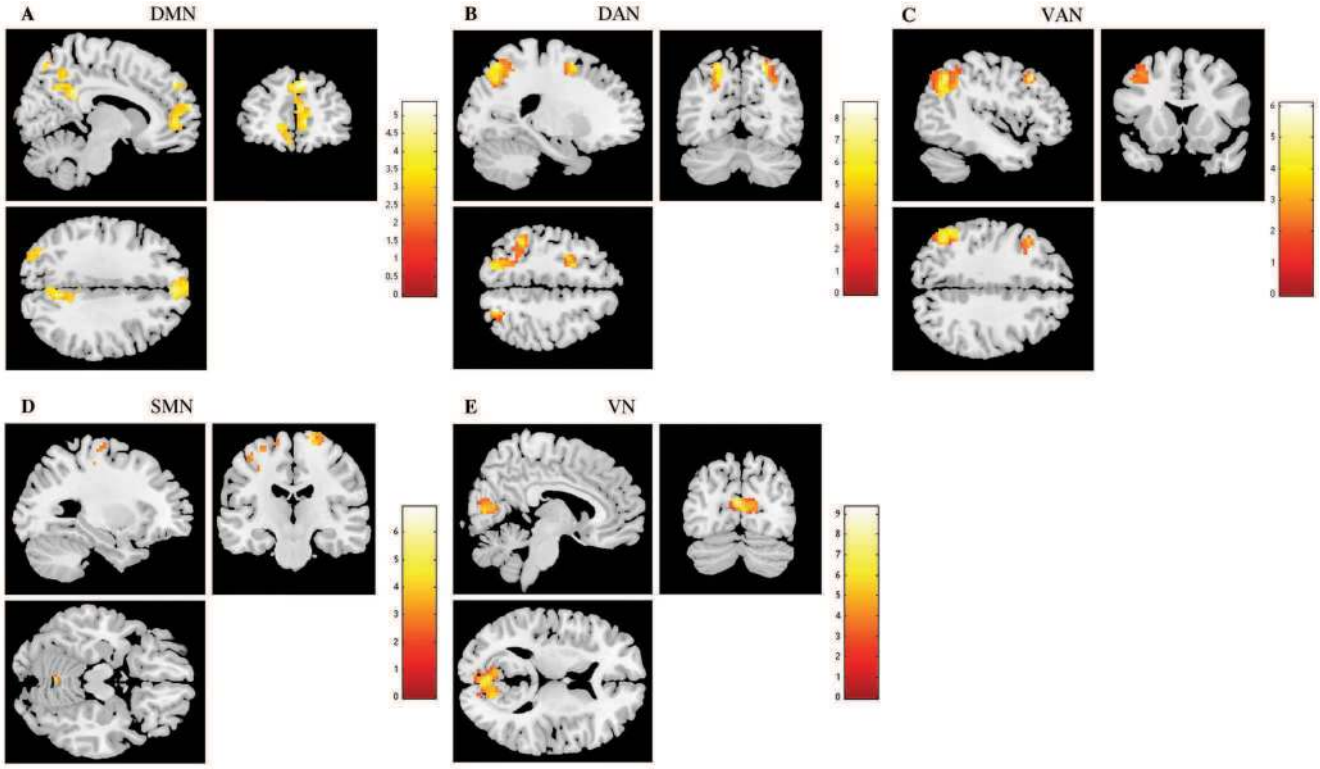

Figure 2. The impact of stress in Resting State Networks (RSNs) at rest. The images depict areas in which stress participants display greater activity than controls in the default mode network (DMN) (A), dorsal attention network (DAN) (B), ventral attention network (VAN) (C), sensorimotor network (SMN) (D) and visual network (VN) (E), extracted by independent component analysis and using two-sample t-tests, with results considered significant at a corrected for multiple comparisons $p<0.05$ threshold. Noticeable, there were no areas of increased activation of these RSNs in controls than in stress individuals.
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combination of visual inspection and Anatomical Automatic Labeling atlas (AAL) [48].

Results

Physiological and Behavioral Results

Stress impact was confirmed in several dimensions: in the Perceived Stress Scale (PSS, Figure 1A; $P<0.007$) and in the Hamilton anxiety (HAS, Figure 1A; $P<0.042$) and depression scores (HAD, Figure 1A; $P<0.001$); finally, we found a significant increase in salivary cortisol levels in stressed participants (Figure 1B; $P<0.042$).

Functional Connectivity Results

The one-sample t-tests revealed a typically spatial pattern of activation (connectivity) and deactivation in DMN, DAN, VAN, SMN, VN and AN in both experimental groups (results not shown). Increased resting functional connectivity was identified in DMN, both attention networks, SMN and VN in the stress group when compared to controls (Figure 2 and Table 1). In contrast, in the comparison control>stress, there was no significant increase of connectivity in any of the studied RSNs. More specifically, in what regards DMN activity, stress increased functional connectivity in the medial prefrontal cortex, medial orbitofrontal cortex, pCC and the precuneus (pCUN) (Table 1). In DAN, increased functional connectivity was found in the superior parietal, right middle occipital and left medial and superior frontal in stress group (Table 1). Increased functional activation was found in the left angular, superior parietal and middle frontal in stressed participants in VAN (Table 1). In SMN, stress increased functional connectivity in the left paracentral lobule, precentral, right postcentral and the left cerebellum (Table 1). Finally, increased functional activation was found in the calcarine in stressed participants in VN (Table 1).

In task-induced deactivations, increased deactivations in DMN, both attention networks, SMN and VN were found in controls compared to stressed participants (Figure 3 and Table 2). More specifically, increased deactivations in the left middle occipital, angular and in the pCUN, middle occipital and temporal and parahippocampal right was found in DMN of controls when compared to stress participants (Table 2). In DAN, controls presented higher functional deactivation in the inferior temporal and superior parietal (Table 2). Controls showed an increased deactivation of the VAN, specifically in the left angular and inferior parietal and temporal, compared to stressed participants (Table 2). In SMN, controls presented higher functional deactivation in the cerebellum and in the left precentral (Table 2). Left calcarine was highly deactivated in controls compared to stress participants in the VN (Table 2). No significant region was found to display greater deactivation in stressed participants than in controls in any of the studied RSNs.

Expansion/Contraction Maps of the RSNs

Whole brain analysis for relative intracranial volumes did not differ between experimental groups. However, a significant reduction ($p<0.014$) in total DMN volume (corrected ICV) was seen in stressed participants compared to controls (Figure 4). Specific areas of contraction were observed in the left pCC

Table 1. Group Differences (Stress>Control) at rest, in brain regions of the DMN, DAN, VAN, SMN and VN maps (two sample t-tests, corrected for multiple comparisons, $p<0.05$).

Condition	Regions	Peak MNI coordinates	Cluster size (voxels)	Maximum Z score
Default Mode Network (Stress>Controls)	Frontal superior medial (left)	0, 63, 9	499	3.82
	Frontal medial orbitofrontal (left)	−12, 54, −3		3.64
	Precuneus (right)	3, −63, 39	253	3.78
	Cingulum posterior (right)	9, −45, 30		3.47
	Precuneus (left)	0, −63, 60		3.39
	Occipital middle (left)	−33, −81, 27	61	3.41
Dorsal Attention Network (Stress>Controls)	Parietal superior (right)	21, −66, 51	64	5.03
	Occipital middle (right)	30, −63, 39		4.20
	Frontal superior (left)	−21, 3, 57	71	4.62
	Frontal middle (left)	−30, −3, 51		3.99
	Parietal superior (left)	−18, −72, 48	332	4.61
Ventral Attention Network (Stress>Controls)	Angular (left)	−51, −54, 33	260	4.18
	Parietal superior (left)	−33, −60, 51		2.09
	Frontal middle (left)	−45, 21, 45	70	3.75
Sensorimotor Network (Stress>Controls)	Paracentral Lobule (left)	−15, −27, 69	114	4.49
	Precentral (left)	−18, −12, 78		4.24
	Precentral (right)	18, −27, 69	62	4.11
	Postcentral (right)	33, −30, 54		2.86
	Cerebellum (left)	−3, −60, −18	22	3.42
	Precentral (left)	−42, −12, 51	21	3.27
Visual Network (Stress>Controls)	Calcarine (left)	−3, −72, 15	214	5.18
	Calcarine (right)	15, −72, 12		4.26

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($p<0.025$) and the left and right parietal inferior ($p<0.024$ and $p<0.016$, respectively). No significant areas of constriction or expansion were found in the dorsal and ventral attention in the SMN and primary VN ($p = 0.86$, $p = 0.55$, $p = 0.87$ and $p = 0.67$, respectively) between experimental groups.

Discussion

Herein, we showed for the first time that stress increases the activation of the DMN at rest in the ventral mPFC, pCC, adjacent pCUN and inferior parietal cortex. Based on previous studies highlighting the role of the DMN at rest [16,17,49,50], our results suggest an augment in self-reflective thoughts but also an increased dynamic interaction between emotional processing (i.e., ventral regions) and cognitive functions (i.e., dorsal regions) in stressed participants, as a result of increased activity in the anterior components of the DMN. The increases in the posterior regions of the DMN observed in stressed participants, particularly the pCC and the inferolateral parietal lobes, are likely associated with longer processing of emotionally salient stimuli and episodic memory retrieval [51,52]. Notably, this increase in functional connectivity was associated with a contraction of the connectivity map of the DMN, with specific reductions in the left pCC and left and right parietal inferior regions. These are likely to reflect the stress-induced atrophic effects in cortical regions, observed in several previous reports [5,7], although the alternative explanation of a reduction of the number of neurons recruited cannot be completely excluded at this moment.

The characterization of changes in functional connectivity between brain networks subserving distinct psychophysiological functions is of relevance to understand the symptoms triggered by stress, namely mood and anxiety changes [53]. Indeed, stress is well-known to be a precipitating factor for mood changes, a finding confirmed in the present study by the increased scoring in a validated scale of depression. Interestingly, in depressed participants, an increased fMRI connectivity pattern between the "dorsal nexus" (a bilateral dorsal medial prefrontal cortex region) and the DMN has been reported [54]; importantly, this hyperconnectivity has recently been shown to be reduced by antidepressants [55–57]. Strikingly, the present study reveals an increased functional connectivity between mPFC (part of dorsal nexus) and pCC in stressed participants. Finally, the finding of increased activation in resting states of the anterior cingulate cortex is also of relevance for the affective processing of negative information, known to be altered in depressed patients [36,58,59] but may additionally be related to a higher vigilance and alertness in stressed participants.

Indeed, another behavioral dimension targeted by stress is emotional hyperactivity; again, herein we confirmed that stressed individuals score higher in the Hamilton anxiety scale. The finding of increased connectivity between the superior parietal, right middle occipital and left medial and superior frontal in the stress group suggests that brain regions belonging to DAN could play a role in emotional regulation and in the higher state of vigilance and awareness, which is typical of stressed-induced hyperemotionality. Moreover, the present findings of increased functional connectivity in the pCC in stressed participants than in controls,

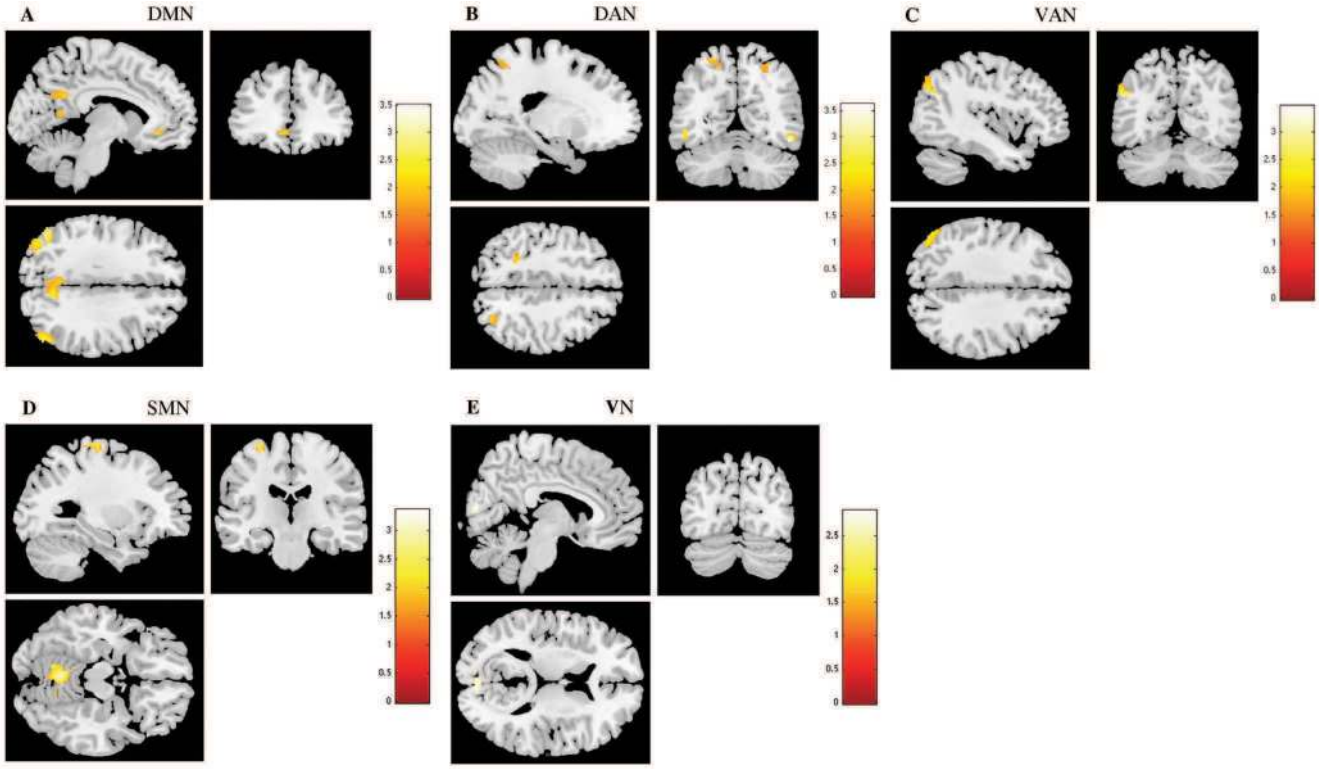

Figure 3. The impact of stress in Resting State Networks (RSNs) during task-induced deactivations. The images illustrate areas of increased deactivation in controls when compared to stressed participants in the default mode network (DMN) (A), dorsal attention network (DAN) (B), ventral attention network (VAN) (C), sensorimotor network (SMN) (D) and visual network (VN) (E), extracted by general linear model analysis and using two-sample t-tests, with results considered significant at a corrected for multiple comparisons $p<0.05$ threshold. Importantly, no areas of increased deactivation of these RSNs were found in stressed individuals when compared to controls.
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Table 2. Group Differences (Stress<Control) in brain regions of the DMN, DAN and VAN maps in task-induced deactivation (two sample t-tests, corrected for multiple comparisons, $p<0.05$).

Condition	Regions	Peak MNI coordinates	Cluster size (voxels)	Maximum Z score
Default Mode Network (Stress<Controls)	Occipital middle (left)	−44, −74, 26	282	3.28
	Precuneus (right)	6, −50, 12	747	3.23
	Angular (left)	−50, −68, 30	74	3.01
	Occipital middle (right)	42, −74, 22	351	2.80
	Temporal middle (right)	48, −74, 36		2.59
	Angular (right)	54, −66, 28		2.52
	Parahippocampal (right)	30, −26, −18	86	2.18
Dorsal Attention Network (Stress<Controls)	Temporal inferior (right)	48, −60, −14	60	3.37
	Temporal inferior (left)	−48, −60, −4	61	2.53
	Parietal superior (right)	28, −72, 50	163	2.36
	Parietal superior (left)	−24, −62, 62	115	2.13
Ventral Attention Network (Stress<Controls)	Angular (left)	−48, −68, 30	270	3.24
	Parietal inferior (left)	−28, −80, 44		2.38
	Temporal inferior (left)	−54, −56, −10	73	2.27
Sensorimotor Network (Stress>Controls)	Cerebellum (left)	−6, −56, −12	521	3.16
	Cerebellum (right)	12, −56, −10		2.85
	Precentral (left)	−30, −22, 70	72	2.19
Visual Network (Stress>Controls	Calcarine (left)	4, −86, 14	83	2.74

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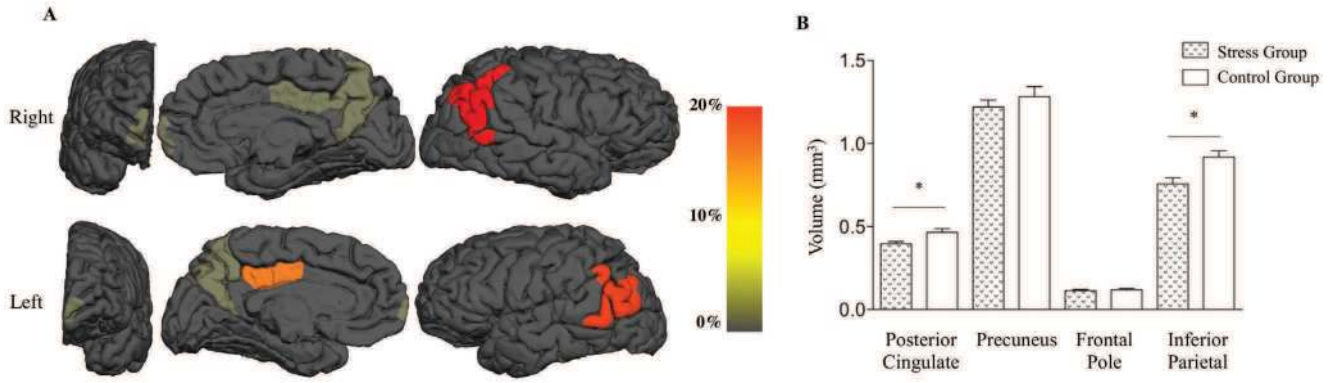

Figure 4. Volumetric Changes in the default mode network (DMN) after stress exposure. (A) Schematic representation of global DMN volumetric changes. (B) Regional volumetric differences in the DMN between Stress and Control groups. *P<0.05.
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along with a decreased deactivation in pCUN is of notice. The pCC and the pCUN are sometime referred to as a pivotal hub of the DMN in social cognition and in theory of mind [60]. Our results are also in line with previous fMRI studies revealing a lower deactivation in the pCUN on anxious patients [60,61]. As a matter of fact, our findings sustain the hypothesis that pCUN would be able to suspend functional connectivity within the DMN, being related to perception of socially relevant emotional state and self-related mental representations [15].

Finally, in accordance with these data evincing emotional hyperactivity as increased anxiety states and related vigilance and alertness, we observed a greater activation of the sensorimotor (SMN), and primary visual (VN) networks in stressed participants. This suggests an hyperactivation of cortical and subcortical attention areas oriented to perception–action, brain systems required to stress-related fight or flight responses. Also, this is in line with disrupted sensorimotor gating mechanisms (necessary to mediate threat selective attention to the most salient signals and ignore other non relevant signals that emerge simultaneously) found in stress conditions [62]. Therefore, our results suggest that stress induces an increase in the general level of alertness and motor response in the stress participants, as suggested by others [63].

Interestingly, the present study reveals not only differences in the pattern of activation of RSNs, but also relevant differences in deactivation of these networks. The VAN was found to be associated with task control function [64–66] and to be implicated in "salience" processing [47]. Importantly, the greater functional connectivity found in the VAN during resting state fMRI in stressed participants is likely to be of relevance to understand the decreased functional deactivation of RSNs during task-focused behavior, suggesting a difficulty in moving from more oriented, self-related processes towards a tuning down task-focused behaviour that requires allocation of attention and other cognitive. In fact, it has been shown that the VAN has an important role in cognitive control related to switching between the DMN and task-related networks [46], even thought, the increased rest activity of the DMN in stressed participants might simply require an increased effort for its deactivation during the transition from rest to task-focused activity, which might impact on functional performance. This is consistent with previous studies showing that failure of RSNs deactivation was already evidenced in several neuropsychiatric diseases such as schizophrenia, first-episode psychosis, mild cognitive impairment and mild Alzheimer's disease (e.g. [20,21,67]).

Although the RSNs studied herein provide a valuable framework through which alterations of functional connectivity driven by chronic stress exposure can be assessed, they do not cover the whole cortex and thus do not provide a complete description of brain functional architecture. Another limitation of this study relates to the impossibility to provide information on the functional connectivity of RSNs with several regions of the limbic system. In addition, one must still be cautious about the neurophysiological relevance of RSNs, namely on the functional significance of these task-networks when dynamically assembled and modulated during different behavioral states.

In conclusion, while there is substantial evidence for an association between RSNs activation/deactivation abnormalities and psychiatric disorders [68–73], this is to the best of our knowledge the first study exploring the functional significance of RSNs patterns after sustained stress exposure. The similarities of present findings with those evidenced by depressed and anxious patients clearly suggest that these patterns of abnormal activity of RSNs in stress participants may represent a neurobiological marker for the stress-induced increased emotionality. The present data, however, also reveals a deficit in the deactivation of the RSNs that reflects an impaired turning off of the un-activated state. Future studies might permit to clarify specific relationship between specific RSNs abnormalities and core phenomena of stress-related disorders as well as whether plastic phenomena also operate after the end of the stress exposure.

Author Contributions

Conceived and designed the experiments: JMS AS JAP JJC NS. Performed the experiments: JMS AS LMF NCS PM FM. Analyzed the data: JMS AS LMF NCS PM JAP JJC NS. Wrote the paper: JMS AS JAP JJC NS.

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Chapter 3.3

Soares, J.M., Sampaio, A., Marques, P., Ferreira, L.M., Santos, N.C., Marques, F., Palha, J.A., Cerqueira, J.J., and Sousa, N. (2013)

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Plasticity of resting state brain networks in recovery from stress

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Chronic stress has been widely reported to have deleterious impact in multiple biological systems. Specifically, structural and functional remodeling of several brain regions following prolonged stress exposure have been described; importantly, some of these changes are eventually reversible. Recently, we showed the impact of stress on resting state networks (RSNs), but nothing is known about the plasticity of RSNs after recovery from stress. Herein, we examined the "plasticity" of RSNs, both at functional and structural levels, by comparing the same individuals before and after recovery from the exposure to chronic stress; results were also contrasted with a control group. Here we show that the stressed individuals after recovery displayed a decreased resting functional connectivity in the default mode network (DMN), ventral attention network (VAN), and sensorimotor network (SMN) when compared to themselves immediately after stress; however, this functional plastic recovery was only partial as when compared with the control group, as there were still areas of increased connectivity in dorsal attention network (DAN), SMN and primary visual network (VN) in participants recovered from stress. Data also shows that participants after recovery from stress displayed increased deactivations in DMN, SMN, and auditory network (AN), to levels similar to those of controls, showing a normalization of the deactivation pattern in RSNs after recovery from stress. In contrast, structural changes (volumetry) of the brain areas involving these networks are absent after the recovery period. These results reveal plastic phenomena in specific RSNs and a functional remodeling of the activation-deactivation pattern following recovery from chronic-stress, which is not accompanied by significant structural plasticity.

Keywords: resting state networks, functional connectivity, deactivation, recovery from stress, plasticity

INTRODUCTION

When the homeostatic mechanisms are disrupted, namely through prolonged stress exposure, maladaptive responses take place and trigger inappropriate functional responses. It is well-established that prolonged stress has deleterious impact in multiple biological systems, including the central nervous system. In fact, prolonged stress exposure impairs spatial working memory, perceptual attention, behavioral flexibility, and decision making both in rodents and in humans (Joels et al., 2004; Cerqueira et al., 2005; Dias-Ferreira et al., 2009; Soares et al., 2012; Yuen et al., 2012), which has been associated with structural and functional changes of several brain regions. Importantly, some of these maladaptive structural and functional responses to increased chronic stress were shown to be reversible (Sousa et al., 1998; Heine et al., 2004; Cerqueira et al., 2005; Goldwater et al., 2009; Bian et al., 2012; Soares et al., 2012), including evidence showing that as trait positive affect may potentiate recovery and adaptive response (Papousek et al., 2010). However, certain stress effects and specific structural and functional changes may endure after this recovery period (Joels et al., 2004; Gourley et al., 2013). Of notice, most stress recovery studies were performed in rodent models.

A growing field of functional magnetic resonance imaging (fMRI) has provided new insights into the functional connectivity across different brain regions. Indeed, resting state fMRI is being widely used to assess brain regional interactions that comprise the resting state networks (RSNs) (De Luca et al., 2006; Fox and Raichle, 2007), both during resting periods and task-induced deactivations. Moreover, alterations in the normal patterns of RSNs have been associated with several disease states and neuropsychiatric disorders (Zhang and Raichle, 2010; Meda et al., 2012; Sripada et al., 2012), including stress exposure (Soares et al., 2013). Indeed, we previously reported that stressed participants had an hyperactivation pattern of the default mode (DMN), dorsal attention (DAN), ventral attention (VAN), sensorimotor (SMN), and primary visual (VN) networks, paralleled by structural constriction of the DMN brain regions (Soares et al., 2013).

The existence of plastic events in the RSNs after recovery from chronic stress is, however, largely unknown. Indeed, Vaisvaser

et al. (2013), using an acute social stress model, examined stress-induced responses in the RSNs and cortisol levels before stress, immediately after the acute stress exposure and 2 h later. The authors found a "recovery" pattern of the DMN connectivity after stress exposure in two of the central hubs of the DMN (seed ROIs at the posterior cingulate cortex and hippocampus), but not in the amygdala-hippocampal disconnectivity that was sustained at 2 h post-stress. Moreover, this increased connectivity was inversely correlated with cortisol levels (Vaisvaser et al., 2013). These results suggest that even acute psychosocial stressors are associated with a prolonged post-stress DMN connectivity response in specific brain regions. This study used only an acute stress model and studies addressing how RSNs respond to chronic stress and identifying specific networks that are associated to an efficient recovery are absent. Therefore, the present study examined the effects of chronic stress on the RSNs following recovery and investigated region-specific changes during successful recovery from chronic stress exposure.

MATERIALS AND METHODS

PARTICIPANTS, PSYCHOLOGICAL TESTS, AND CORTISOL MEASUREMENTS

The participants included in this study were 6 stress participants submitted to prolonged psychological stress exposure (3 males, 3 females; mean age, 23.83 ± 0.37), the same 6 stress participants, 6 weeks after the end of the exposure to stress and 6 controls (3 males, 3 females; mean age, 24.33 ± 1.24). Control participants included a cohort of medical students under their normal academic activities, whereas the stress group included participants that had just finished their long period of preparation for the medical residence selection exam (stress group). Participants responded to a laterality test and to a self-administered questionnaire regarding stress assessment (Perceived Stress Scale—PSS) (Cohen et al., 1983). Participants were further assessed with the Hamilton anxiety scale—HAS (Hamilton, 1959) and the Hamilton depression scale—HDS (Hamilton, 1967) by a certified psychologist. Upon filling of the questionnaires, and immediately before the imaging acquisitions, participants collected saliva samples with the help of Salivette (Sarstedt, Germany) collection devices. Collection took place between 9 and 5 p.m. in all participants. Samples were stored at −20°C until the biologically active, free fraction of the stress hormone cortisol was analyzed using an immunoassay (IBL, Hamburg).

ETHICS STATEMENT

The study was conducted in accordance with the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of Hospital de Braga (Portugal). The study goals and tests were explained to all participants and all gave informed written consent.

DATA ACQUISITION

Participants were scanned on a clinical approved Siemens Magnetom Avanto 1.5 T (Siemens Medical Solutions, Erlangen, Germany) on Hospital de Braga using the Siemens 12-channel receive-only head coil. The imaging sessions, including one structural T1, one resting state functional, and two task related functional acquisitions, were conducted in the same day and the Siemens Auto Align scout protocol was used to minimize variations in head positioning. For structural analysis, a T1 high-resolution anatomical sequence, 3D MPRAGE (magnetization prepared rapid gradient echo) was performed with the following scan parameters: repetition time (TR) = 2.4 s, echo time (TE) = 3.62 ms, 160 sagittal slices with no gap, field-of-view (FoV) = 234 mm, flip angle (FA) = 8°, in-plane resolution = $1.2 \times 1.2\,mm^2$ and slice thickness = 1.2 mm. During resting-state fMRI acquisition, using gradient echo T2* weighted echo-planar images (EPIs), participants were instructed to keep the eyes closed and to think about nothing in particular. The imaging parameters were: 100 volumes, TR = 3 s, TE = 50 ms, FA = 90°, in-plane resolution = $3.4 \times 3.4\,mm^2$, 30 interleaved slices, slice thickness = 5 mm, imaging matrix 64 × 64 and FoV = 220 mm. fMRI paradigm acquisition was acquired using: TR = 2 s, TE = 20 ms, FA = 90°, in-plane resolution and slice thickness 3.3 mm, 38 ascending interleaved axial slices with no gap and FoV = 212 mm. The functional paradigm acquisitions were previously described (Soares et al., 2012) and the paradigm was presented using the fully integrated fMRI system IFIS-SA.

IMAGE PRE-PROCESSING

Before any data processing and analysis, all acquisitions were visually inspected and confirmed that they were not affected by critical head motion and that participants had no brain lesions.

To achieve signal stabilization and allow participants to adjust to the scanner noise, the first 5 resting state fMRI volumes (15 s) were discarded. Data preprocessing was performed using SPM8 (Statistical Parametrical Mapping, version 8, http://www.fil.ion.ucl.ac.uk) analysis software. Images were firstly corrected for slice timing using first slice as reference and SPM8's Fourier phase shift interpolation. To correct for head motion, images were realigned to the mean image with a six-parameter rigid-body spatial transformation and estimation was performed at 0.9 quality, 4 mm separation, 5 mm FWHM smoothing kernel using 2nd degree B-Spline interpolation. No participants exceed head motion higher than 2 mm in translation or 1° in rotation. Images were then spatially normalized to the MNI (Montreal Neurological Institute) standard coordinate system using SPM8 EPI template and trilinear interpolation. Data were then re-sampled to 3 × 3 × 3 mm3 using sinc interpolation, smoothed to decrease spatial noise with a 8 mm, full-width at half-maximum (FWHM), Gaussian kernel, temporally band-pass filtered (0.01–0.08 Hz) and the linear trend was removed. The pre-processing of fMRI paradigm images was previously described (Soares et al., 2012).

INDEPENDENT COMPONENT ANALYSIS AND IDENTIFICATION OF RSN

Spatial independent component analysis was conducted for using the Group ICA 2.0d of fMRI Toolbox (GIFT, http://www.icatb.sourceforge.net) (Calhoun et al., 2001; Correa et al., 2005). Concisely, spatial ICA analysis is a fully data-driven approach that consists in extracting the non-overlapping spatial maps with temporally coherent time courses that maximize independence. The methodology employed by GIFT can be summarized in three main stages: dimensionality reduction, estimation of the group

independent components, and back-reconstruction of each subject's corresponding independent components. The reduction of dimensionality of the functional data and computational load was performed with Principal Component Analysis (PCA) in the concatenated dataset over all subjects, independently of the groups. Then, 20 independent components were estimated, based on a good trade-off (clustering/splitting) between preserving the information in the data while reducing its size (Beckmann et al., 2005; Zuo et al., 2010), using the iterative Infomax algorithm. The ICASSO tool was used to assess the ICA reliability, and 20 computational runs were performed on the dataset, during which the components were being recomputed and compared across runs and the robustness of the results was ensured (Himberg et al., 2004). The previous steps result in the estimation of a mixing matrix with partitions, unique to each subject. The individual independent components were then back-reconstructed from the group-level components. This back-reconstruction step is accomplished by projecting each subject's data onto the inverse of the partition of the calculated matrix corresponding to that subject. The obtained independent components were expressed in t-statistic maps, which were finally converted to a z-statistic. Z-statistic describes the voxels that contributed more intensely to a specific independent component, providing a degree of functional connectivity within the network (Bartels and Zeki, 2005; Beckmann et al., 2005). The final components were visually inspected, sorted, and spatially correlated with resting state functional networks from (Shirer et al., 2012). Each subject's map corresponding to the best-fit component of each RSN was used to perform group statistical analyses.

RSN DEACTIVATION DURING fMRI TASK ANALYSIS

The fMRI decision-making paradigm analyzed to investigate the task-induced deactivations consisted of two different event-related jittered design sessions. First session of valued actions with reward delivery and, after 30 min break, the second session consisted of the devalued actions with the outcome devaluation and extinction. Both sessions had 150 trials, each with 1.5 s for decision, 4 s with the choice highlighted, and 2 s for reward delivery, followed by the interstimulus interval with mean duration of 4 s [please see Soares et al. (2012), for further details].

fMRI paradigm was analyzed by creating a set of regressors at resting and decision making periods, which were convolved with the hemodynamic response function. In order to reliably map task-induced deactivations, we combined all the resting periods (resting baseline condition) and all the decision periods (decision condition), given that decision periods were equally demanding. The contrast used to assess task-induced deactivations was the resting baseline condition minus decision condition. Resulting functional patterns were masked with the previously described RSNs masks (Shirer et al., 2012).

STRUCTURAL ANALYSIS

Structural analysis based on segmentation of brain structures from T1 high-resolution anatomical data was performed using the freely available Freesurfer toolkit version 5.0 (http://surfer.nmr.mgh.harvard.edu). Intracranial volume (ICV) was used to correct the volumes and the processing pipeline was the same as previously described (Soares et al., 2012). DMN was defined by the summed volume of the angular gyrus of inferior parietal lobe, the posterior cingulate, the precuneus, and the frontopolar region (Raichle et al., 2001; Buckner et al., 2008). The summed volume of the middle frontal gyrus (dorsolateral and prefrontal region) and the posterior parietal region constituted the DAN (Seeley et al., 2007; Sridharan et al., 2008). VAN was constituted by the sum of the temporal-parietal junction and the ventral frontal cortex volumes (Fox et al., 2006). SMN was defined by the summed volume of the paracentral, precentral postcentral, and the cerebellum (Shirer et al., 2012). The summed volume of the cuneus, pericalcarine, and the lingual region constituted the primary VN (Shirer et al., 2012). Auditory network (AN) was defined by the summed volume of the temporal transverse and the temporal superior (Shirer et al., 2012).

STATISTICAL ANALYSES

Results of the psychological scales, cortisol levels, and regional volumes were analyzed in the IBM SPSS Statistics software, v.19 (IBM, New York). Comparisons between the stress recovered and stress were done with paired samples *t*-test and between stress recovered and control with two-tailed independent-samples *t*-test. For all these comparisons significance level was set at 0.05. Values are presented as mean ± standard error of the mean.

Group analysis of the resting state fMRI and task induced deactivations were performed using the second level random effect analyses in SPM8. Initially, within group analyses were performed only to confirm the functional connectivity of the RSNs in the different groups, using one-sample *t*-tests. Therefore, between group analyses were implemented with directional two-sample *t*-tests. Functional results for all RSNs were considered significant at $p < 0.05$ corrected for multiple comparisons using a combination of an uncorrected height threshold of $p < 0.025$ with a minimum cluster size. The cluster size was determined over 1000 Monte Carlo simulations using AlphaSim program distributed with REST software tool (http://resting-fmri.sourceforge.net/). AlphaSim input parameters were the following: individual voxel probability threshold = 0.025, cluster connection radius = 3 mm, gaussian filter width (FWHM) = 8 mm, number of Monte Carlo simulations = 1000 and mask was set to the corresponding RSN template mask. Anatomical labeling was defined by a combination of visual inspection and Anatomical Automatic Labeling atlas (AAL) (Tzourio-Mazoyer et al., 2002).

RESULTS

PHYSIOLOGICAL AND BEHAVIORAL RESULTS

Stress impact was confirmed in several parameters: PSS [**Figure 1A**; $t_{(10)} = 2.52$, $P < 0.05$; Stress Group $M = 35.50$; $SD = 2.59$; Control Group $M = 30.17$; $SD = 4.49$] and in the HAS [**Figure 1A**; $t_{(10)} = 2.37$, $P < 0.05$ Stress Group $M = 11.00$; $SD = 7.95$; Control Group $M = 3.00$; $SD = 2.28$], and depression scores [HAD, **Figure 1A**; $t_{(10)} = 3.65$, $P < 0.01$; Stress Group $M = 7.50$; $SD = 2.59$; Control Group $M = 3.17$; $SD = 1.33$], but only by a trend when it regards to salivary cortisol levels [**Figure 1B**; $t_{(10)} = 1.69$, $P = 0.12$; Stress Group $M = 0.44$; $SD = 0.29$; Control Group $M = 0.23$; $SD = 0.10$]. After a stress-free period of 6 weeks after the end of the stress exposure,

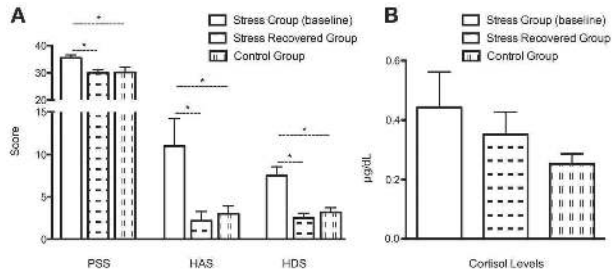

FIGURE 1 | Clinical characteristics of the cohort. (A) Perceived Stress Scale (PSS), Hamilton Anxiety Scale (HAS); Hamilton Depression Scale (HAD) and **(B)** Salivary Cortisol levels in the two groups (stress, stress recovered, and control group); $^*P < 0.05$.

we observed that all the psychological changes were restored [**Figure 1A**; SPP: $t_{(5)} = 3.72$, $P < 0.05$; Stress Group $M = 35.50$; $SD = 2.59$; Stress-Recovered Group $M = 30.00$; $SD = 3.03$; anxiety score: $t_{(5)} = 2.86$, $P < 0.05$, Stress Group $M = 11.00$; $SD = 7.95$; Stress-Recovered Group $M = 2.17$; $SD = 2.71$]; depression score: HAD: [$t_{(4)} = 4.84$, $P < 0.01$; Stress Group $M = 7.50$; $SD = 2.59$; Stress-Recovered Group $M = 2.5$; $SD = 0.35$], except salivary cortisol levels [$t_{(5)} = 0.67$, $P = 0.53$; Stress Group $M = 0.44$; $SD = 0.29$; Stress-Recovered Group $M = 1.38$; $SD = 0.8$]. Importantly, stress-recovered group did not differ with the control group in all psychological and salivary cortisol measures [PSS: $t_{(10)} = -0.08$, $P = 0.94$; HAS: $t_{(10)} = -0.58$, $P = 0.58$; HAD: $t_{(10)} = -0.85$, $P = 0.41$; (**Figure 1B**) Cortisol: $t_{(10)} = 1.42$, $P = 0.19$].

FUNCTIONAL CONNECTIVITY RESULTS

The ICA analysis revealed the typical spatial pattern of functional connectivity and deactivation in DMN, DAN, VAN, SMN, VN, and AN in all experimental conditions (results not shown).

RSNs in stress and stress—recovered groups

Increased resting functional connectivity was identified in DMN, VAN, and SMN and decreased connectivity in DAN and AN in the stress group when compared to stress recovered participants (**Figure 2** and **Table 1**).

Regarding DMN, stress group displayed increased functional connectivity mainly in the left cingulum, frontal medial orbitofrontal, right precuneus, and in the left lingual (**Table 1**). Increased functional connectivity was also found in VAN in stress group in the left parietal inferior and superior, right middle and superior frontal regions (**Table 1**) whereas in the SMN, increased functional connectivity was found in the left cerebellum (**Table 1**). In contrast, decreased functional connectivity was found in stress group in DAN, namely in the right parietal inferior, supramarginal, frontal inferior opercularis, and precentral regions (**Table 1**) as well as in the AN (left superior temporal region) (**Table 1**).

RSNs in stress—recovered and control groups

Regarding the functional connectivity comparison between stress-recovered and controls, we found that the former presented an increased functional connectivity in the DAN, SMN, and VN. Increased connectivity in the left superior occipital, bilateral superior parietal, right postcentral, left middle and superior frontal, bilateral inferior frontal opercularis and bilateral precentral was found in the DAN of stress-recovered compared to control group (**Table 2**). A differential pattern of functional connectivity was observed for the VAN that is, while the stress-recovered group presented higher connectivity in the left inferior parietal and bilateral angular, they presented decreased functional connectivity in the bilateral inferior parietal, left angular, bilateral middle frontal and left inferior frontal triangularis. Additionally, stress-recovered group showed decreased connectivity in the DMN in the right anterior cingulate, in the SMN in the bilateral precentral, left paracentral, right postcentral, and bilateral cerebellum and in the VN in the bilateral calcarine (**Table 2**) when compared to controls (**Figure 3**).

Task-induced deactivations in stress and stress—recovered groups

In task-induced deactivations, decreased deactivations in DMN, SMN, and AN were found in stress group when compared to stress-recovered participants (**Figure 4** and **Table 3**). More specifically, decreased deactivations in the left medial frontal orbitofrontal and superior medial frontal were found in DMN of stress group (**Table 3**). In SMN, stress group presented lower functional deactivation in the left cerebellum (**Table 3**). The left superior temporal and rolandic operculum in AN were less deactivated in stress group compared to stress-recovered participants (**Table 3**). No significant region was found to display greater deactivation in stressed participants than in stress recovered in any of the studied RSNs.

Task-induced deactivations in stress—recovered and control groups

To test for the degree of plasticity in RSNs, we compared deactivation between stress-recovered participants and controls. In this comparison, we found decreased deactivations in DMN, both attention networks, and AN (**Figure 5** and **Table 4**) in stress-recovered group. In DMN, stress-recovered group showed decreased deactivations in the left cuneus, anterior cingulate, right medial frontal orbitofrontal, fusiform and middle temporal and in the left inferior parietal in DAN (**Table 4**). In VAN, stress-recovered group showed lower deactivation in the left superior parietal and in AN in the bilateral superior temporal (**Table 4**). No significant region was found to display greater deactivation in stress recovered than in control participants in any of the studied RSNs.

EXPANSION/CONTRACTION MAPS OF THE RSNs

Whole brain analysis for relative ICVs did not differ between experimental groups. We showed in a previous study (Soares et al., 2013) that exposure to stress triggered a significant reduction in total DMN volume (corrected for ICV) with specific contraction in the left pCC, and bilateral parietal inferior brain regions. Herein, however, we did not find any significant differences in the volume of any of the RSNs between stress participants before and after recovery from stress. No significant areas of expansion or constriction were found in the dorsal and

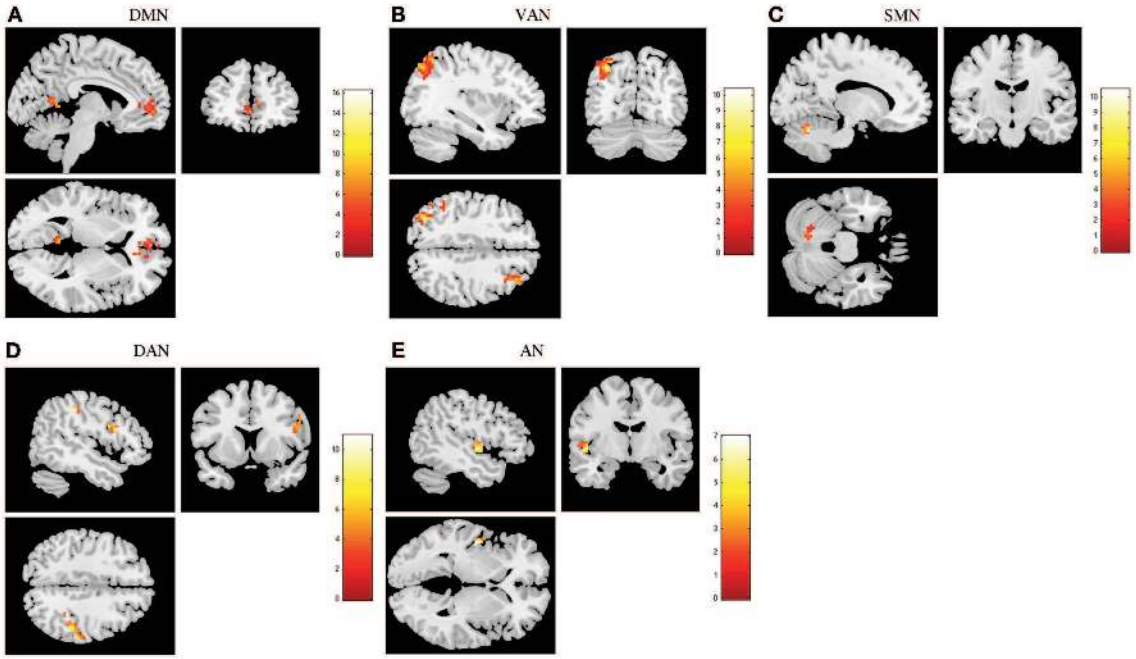

FIGURE 2 | The recovery from stress in resting state networks (RSNs) at rest. The images depict areas in which stress participants display greater functional connectivity than stress recovered in the default mode network (DMN) **(A)**, ventral attention network (VAN) **(B)** and sensorimotor network (SMN) **(C)** and lower functional connectivity in the dorsal attention network (DAN) **(D)** and auditory network (AN) **(E)**. Results were extracted by independent component analysis and using paired *t*-tests, with results considered significant at a corrected for multiple comparisons $p < 0.05$ threshold.

Table 1 | Group differences (Stress vs. Stress recovered) at rest, in brain regions of the DMN, VAN, SMN, DAN, and AN maps (paired *t*-tests, corrected for multiple comparisons, $p < 0.05$).

	Condition	Regions	Peak MNI coordinates	Cluster size (voxels)	Maximum *Z* score
Stress > Stress recovered	Default mode network	Cingulum anterior (left)	0, 36, 3	141	4.31
		Frontal medial orbitofrontal (left)	−6, 54, −3		3.32
		Precuneus (right)	9, −63, 27	137	4.17
		Lingual (left)	−9, −48, 3		3.60
	Ventral attention network	Parietal inferior (left)	−33, −69, 45	225	3.80
		Parietal superior (left)	−33, −60, 48		3.64
		Frontal middle (right)	33, 36, 39	108	3.43
		Frontal superior (right)	30, 9, 63		2.97
	Sensorimotor network	Cerebellum (left)	−15, −63, −24	49	3.82
Stress < Stress recovered	Dorsal attention network	Parietal inferior (right)	45, −36, 48	81	3.87
		Supramarginal (right)	51, −30, 42		3.84
		Frontal inferior opercularis (right)	51, 15, 33	58	3.45
		Precentral (right)	51, 6, 27		3.22
	Auditory network	Temporal superior (left)	−48, −9, 0	52	3.32

ventral attention networks, SMN, AN, and primary VN between stress and stress-recovered participants ($p = 0.99$, $p = 0.98$, $p = 0.87$, $p = 0.84$, and $p = 0.99$, respectively) and between stress-recovered and control groups ($p = 0.89$, $p = 0.54$, $p = 0.18$, $p = 0.47$, and $p = 0.87$, respectively).

DISCUSSION

In this study, we analzsed how the RSNs respond and change following recovery after chronic stress exposure. Our hypothesis was of a continuous recovery effect, in which the connectivity would be decreasing from stress toward the control group.

Table 2 | Group differences (Stress recovered vs. Controls) at rest, in brain regions of the DAN, VAN, SMN, VN, and DMNN maps (two sample *t*-tests, corrected for multiple comparisons, $p < 0.05$).

	Condition	Regions	Peak MNI coordinates	Cluster size (voxels)	Maximum *Z* score
Stress recovered > Controls	Dorsal attention network	Occipital superior (left)	−24, −75, 42	504	5.53
		Parietal superior (left)	−24, −66, 54		5.21
		Parietal superior (right)	21, −66, 57	322	5.05
		Postcentral (right)	57, −21, 48		4.54
		Frontal superior (left)	−27, −6, 63	65	4.15
		Frontal middle (left)	−30, −3, 54		3.96
		Frontal inferior opercularis (right)	55, 15, 33	93	3.93
		Precentral (right)	51, 6, 24		3.85
		Precentral (left)	−51, 6, 27	92	3.15
		Frontal inferior opercularis (left)	−39, 3, 27		2.74
	Ventral attention network	Parietal inferior (left)	−54, −48, 39	136	3.35
		Angular (left)	−48, −66, 33		3.14
		Angular (right)	45, −60, 36	57	2.73
	Sensorimotor network	Precentral (left)	−27, −21, 78	333	5.17
		Paracentral (left)	−15, −27, 72		4.46
		Precentral (right)	15, −18, 75	237	4.33
		Postcentral (right)	30, −30, 60		3.80
		Cerebellum (right)	12, −51, 21	72	3.77
		Cerebellum (left)	−9, −48, −15		2.74
	Visual network	Calcarine (right)	6, −78, 12	331	4.77
		Calcarine (left)	−12, −66, 12		3.93
Stress recovered < Controls	Default mode network	Cingulum anterior (right)	6, 36, 18	147	3.47
	Ventral attention network	Parietal inferior (left)	−33, −74, 51	101	4.21
		Angular (left)	−36, −69, 45		2.78
		Frontal middle (right)	33, 27, 39	75	3.77
		Frontal inferior triangularis (left)	−48, 42, 0	59	3.08
		Frontal middle (left)	−36, 45, 0		2.64
		Parietal inferior (right)	51, −48, 48	58	3.04

Indeed, we observed a decreased resting functional connectivity in the DMN, VAN, and SMN after stress recovery. Additionally, decreased functional connectivity was also observed in the DAN, SMN, and VN networks in controls, when compared with stress-recovered group. However, only a specific brain region of the DMN (the right anterior cingulate cortex—ACC) showed increased functional connectivity in controls when compared with stress-recovered participants. Results of increased functional connectivity of the DMN at rest after chronic stress exposure are consistent with those previously reported (Soares et al., 2013). More recently, Vaisvaser et al. (2013) evidenced similar results using an acute social stress model.

In the current study, we explored further the plasticity of the RSNs after recovery from the impact of chronic stress-induced changes and showed for the first time that all RSNs, with the exception of the DAN and AN, displayed a functional recovery after the cessation of the exposure to stress. Notably, the comparison with controls allowed us to observe a return to the initial levels the functional connectivity of the DMN, VAN, and AN, but still a sustained pattern of increased functional connectivity of the DAN, SMN, and VN networks.

These results suggest that DAN, SMN, and VN are less plastic when recovering from the impact of stress exposure. The DAN network has been associated with top-down attention processes as inhibitory control, working memory, and response selection. These cognitive processes depend upon the prefrontal integrity (dorsal frontal regions), which are brain regions vulnerable to the effects of stress (Cerqueira et al., 2007). Indeed, animal studies evidenced stress-related prefrontal remodeling (e.g., selective atrophy of the prefrontal cortex, elimination of dendritic spines) after chronic stress exposure (Cerqueira et al., 2007; Gourley et al., 2013). This stress-related prefrontal structural reorganization has been associated with impaired perceptual attention, behavioral flexibility, and decision making in rodents and humans (Cerqueira et al., 2005; Dias-Ferreira et al., 2009; Soares et al., 2012; Yuen et al., 2012). Interestingly, studies analysing the recovery of posttraumatic stress disorder reported that an increased thickness of the dorsolateral prefrontal cortex was associated with greater symptomatic alleviation (Lyoo et al., 2011). The concomitant SMN and VN sustained increased functional connectivity are possibly associated with a motor and visual readiness state that is required for the stress response. This

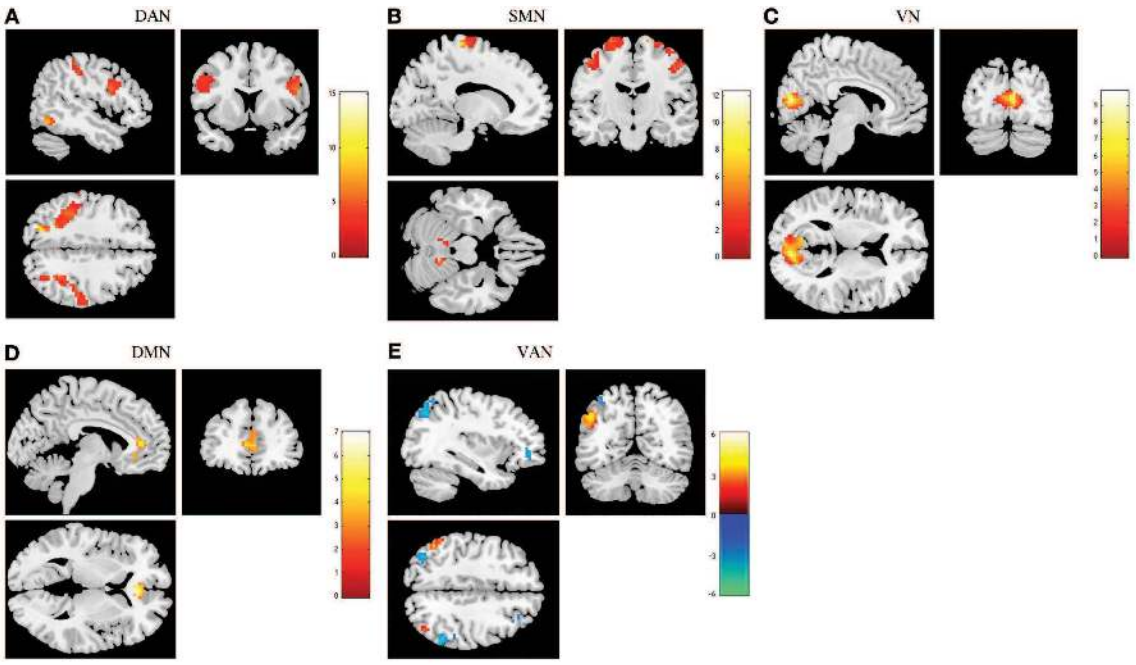

FIGURE 3 | Comparison between stress recovered participants and controls in resting state networks (RSNs). The images show areas in which stress recovered participants display greater functional connectivity than controls in the dorsal attention network (DAN) **(A)**, sensorimotor network (SMN) **(B)**, and primary visual network (VN) **(C)**. Lower functional connectivity was found in the default mode network (DMN) **(D)**. Ventral attention network (VAN) **(E)** displays increased functional connectivity in different regions both in stress recovered (orange) and in control (blue) participants. Results were extracted by independent component analysis and using two-sample *t*-tests, with results considered significant at a corrected for multiple comparisons $p < 0.05$ threshold.

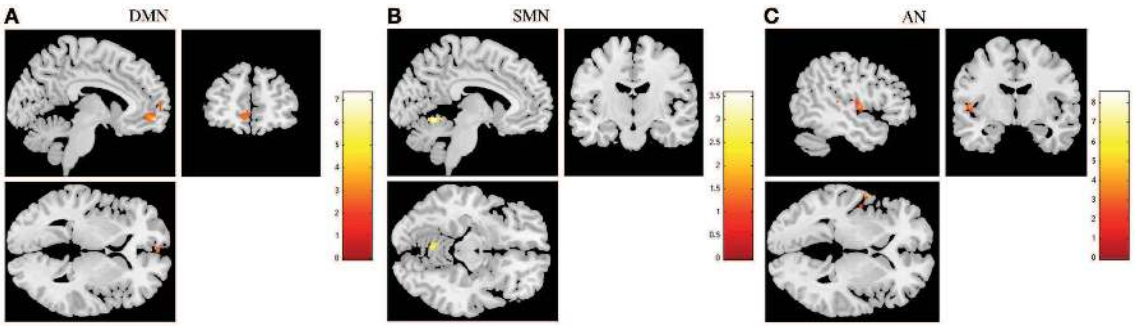

FIGURE 4 | The recovery from stress in resting state networks (RSNs) during task-induced deactivations. The images illustrate areas of decreased deactivation in stress group when compared to stressed recovered participants in the default mode network (DMN) **(A)**, sensorimotor network (SMN) **(B)**, and auditory network (AN) **(C)**, extracted by general linear model analysis and using paired *t*-tests, with results considered significant at a corrected for multiple comparisons $p < 0.05$ threshold. Importantly, no areas of increased deactivation of these RSNs were found in stressed individuals when compared to stress recovered.

specific pattern of plasticity suggests that some RSNs may be a tool for monitoring effective anti-stress interventions, similar to that proposed to verify the effect of the treatments in several neuropsychiatric diseases (Achard and Bullmore, 2007).

Besides the functional plastic recovery in the connectivity of the RSNs at rest, we also observed a continuum in the pattern of deactivation—that is, there was an increased deactivation from stress toward the control group in all the RSNs. DMN deactivation has been associated with reallocation of attentional resources to cognitively demanding tasks (Hu et al., 2013). Moreover, task-induced RSNs deactivation is correlated with behavioral performance: for example, stronger DMN deactivation in a working memory task predicts better performance (Uddin et al., 2009; Mayer et al., 2010). Increased deactivation observed in our control group and in our stress-recovered participants (comparing with stress). Additionally, abnormal patterns of RSNs deactivation have been associated with several neuropsychiatric diseases (Pomarol-Clotet et al., 2008; Guerrero-Pedraza et al., 2012).

Table 3 | Group differences (Stress < Stress recovered) in brain regions of the DMN, SMN, and AD maps in task-induced deactivation (paired *t*-tests, corrected for multiple comparisons, $p < 0.05$).

	Condition	Regions	Peak MNI coordinates	Cluster size (voxels)	Maximum *Z* score
Stress < Stress recovered	Default mode network	Frontal medial orbitofrontal (left)	−6, 56, −8	173	3.11
		Frontal superior medial (left)	−4, 64, 6		2.86
	Sensorimotor network	Cerebellum (left)	−4, −58, −8	57	2.27
	Auditory network	Temporal superior (left)	−58, 0, 2	82	3.28
		Rolandic operculum (left)	−50, −6, 4		2.48

Table 4 | Group differences (Stress recovered < Controls) in brain regions of the DMN, DAN, VAN, and AN maps in task-induced deactivation (two sample *t*-tests, corrected for multiple comparisons, $p < 0.05$).

	Condition	Regions	Peak MNI coordinates	Cluster size (voxels)	Maximum *Z* score
Stress recovered < Controls	Default mode network	Cuneus (left)	−12, −58, 24	74	2.58
		Cingulum anterior (left)	−4, 34, −8	260	2.56
		Frontal medial orbitofrontal (right)	6, 52, −12		2.29
		Fusiform (right)	26, −36, −16	52	2.46
		Temporal middle (right)	42, −66, 22	157	2.44
	Dorsal attention network	Parietal inferior (left)	−26, −44, 48	41	2.23
	Ventral attention network	Parietal superior (left)	−28, −80, 50	57	2.52
	Auditory network	Temporal superior (left)	−62, −8, 6	129	3.00
		Temporal superior (right)	64, −12, 6	31	2.65

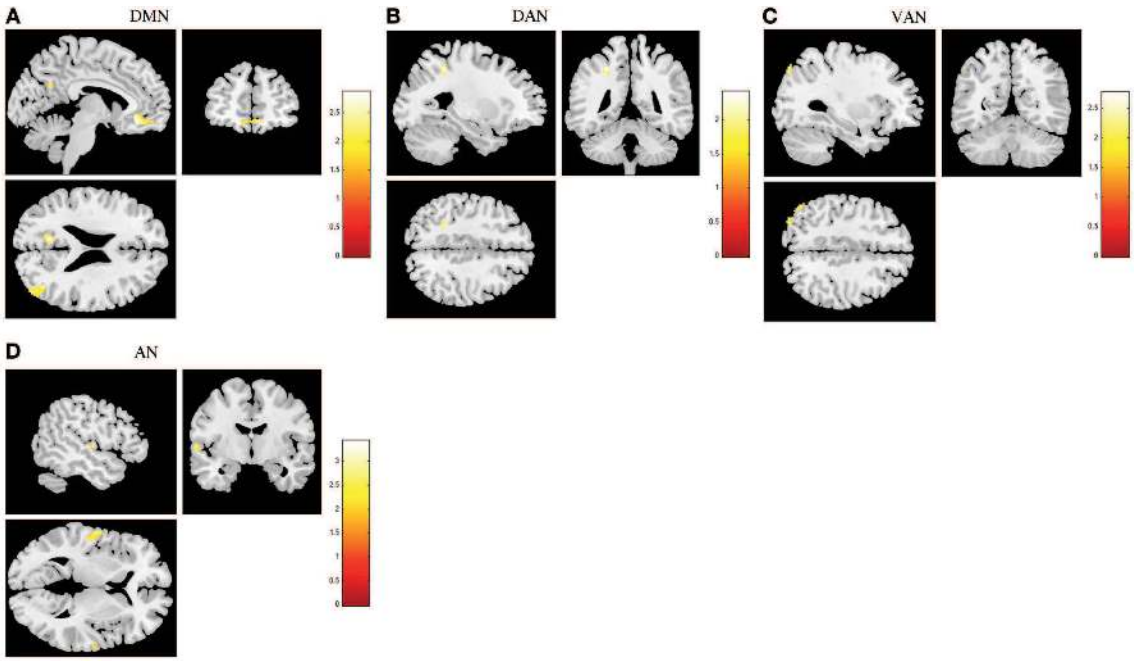

FIGURE 5 | Comparison between stress recovered participants and controls in resting state networks (RSNs) during task-induced deactivations. The images demonstrate areas of decreased deactivation in stress-recovered participants when compared to controls in the default mode network (DMN) **(A)**, dorsal attention network (DAN) **(B)**, ventral attention network (VAN) **(C)**, and auditory network (AN) **(D)**, extracted by general linear model analysis and using two-sample *t*-tests, with results considered significant at a corrected for multiple comparisons $p < 0.05$ threshold. Importantly, no areas of increased deactivation of these RSNs were found in stress recovered when compared to controls participants.

This study shows that while the functional remodeling of RSNs endures, the structural changes (volumetry) of the brain areas involving these networks is still absent after this period of recovery, as no significant areas of expansion or constriction were found in the networks between stress and stress recovered participants; however, in contrast to the difference previously reported in the volumetry of the DMN after stress exposure (Soares et al., 2013) we also did not find significant differences between stress recovered and controls. Difference in results may be related with the limited sample size; the fact that our groups did not differ in physiological cortisol levels and finally, because no direct comparisons were made between stress and control groups.

In summary, the present study contributes to better understand the plastic phenomena that occur in RSNs after the cessation of stress exposure. While we have previously shown the existence of stress-related impairments in the activation-deactivation of RSNs (Soares et al., 2013), here we demonstrate that a functional remodeling of the activation-deactivation pattern of the RSNs takes place following chronic-stress recovery. Although promising, our results should be interpreted with caution mainly due to the reduced size of our sample; therefore, future studies should try to replicate these observations in a larger sample, ideally using exactly the same participants in all conditions, as controls, stressed, and after recovery.

AUTHOR CONTRIBUTIONS

José M. Soares and Adriana Sampaio contributed in literature search, figures, study design, data collection, data analysis, data interpretation, and writing. Paulo Marques and Luís M. Ferreira as contributed in data collection and data analysis. Nadine C. Santos, Fernanda Marques, Joana A. Palha, João J. Cerqueira, and Nuno Sousa contributed in study design, data interpretation, and writing.

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Chapter 3.4

Soares, J.M., Marques, P., Magalhães, R., Santos, N.C., and Sousa, N.

Brain structure across the lifespan: the influence of stress and mood

Manuscript submitted

Brain structure across the lifespan: the influence of stress and mood

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Abstract

Normal brain aging is an inevitable and heterogeneous process characterized by a selective pattern of structural changes. Such heterogeneity arises as a consequence of cumulative effects over the lifespan, including stress and mood effects, which drive different micro- and macro-structural alterations in the brain. Investigating these differences in healthy age-related changes is a major challenge for the comprehension of the cognitive status. Herein we addressed the impact of normal aging, stress, mood and their interplay in the brain gray and white matter structure. We showed the critical impact of age in the white matter volume and how stress and mood influence brain volumetry across the lifespan. Moreover, we found a more profound effect of the interaction of aging/stress/mood on structures located in the left hemisphere. These findings help to clarify some divergent results associated with the aging decline and to enlighten the association between abnormal volumetric alterations and several states that may lead to psychiatric disorders.

Keywords: Aging, stress, mood, volumetry, gray matter, white matter

1. Introduction

Normal brain aging is an inevitable, complex and heterogeneous process, characterized by a selective pattern of structural and functional changes. With age, the whole brain itself and many of its specific structures present volumetric alterations, mostly reductions, white matter (WM) becomes less dense and loses integrity (Walhovd et al., 2009;Fjell et al., 2013). Specifically, "normal" brain aging has been consistently characterized by a noted overall atrophy associated with a decrease in brain volume and expansion of the cerebrospinal fluid (CSF) spaces (Tamnes et al., 2013). Although total brain volume is more correlated with age after 60 years old, gray matter (GM) volume decline may begin earlier and progress gradually, frequently associated with neuronal cell death, whereas WM may start later and progress more abruptly accompanying the myelin sheath deteriorating after the fourth decade of life (Dennis and Cabeza, 2008;Gunning-Dixon et al., 2009;Lemaitre et al., 2012;Fjell et al., 2013;Tamnes et al., 2013). Specifically, Both GM and WM volumetric reductions seem to be greater in the cortex than in subcortical structures, associated to a greater spatial extent in WM, with the highest effects in GM frontal lobe and in the WM superior and medial frontal and anterior cingulate regions (Raz et al., 2005;Dennis and Cabeza, 2008;Gunning-Dixon et al., 2009;Salat et al., 2009b;Voineskos et al., 2010;Sala et al., 2012).

Notably, not only such changes occur even in highly cognitive functioning individuals (Tamnes et al., 2013;Meunier et al., 2014), but during healthy aging, many domains become also less efficient, and the brain tends to respond to all these neural changes by engaging in continuous reorganizations to keep its homeostatic control and support cognitive functions, the so-termed "brain plasticity" (Park and Reuter-Lorenz, 2009;Lovden et al., 2013). Aging quality varies according to space (brain region), time (lifespan phase), subject (individual parameters) and external influences. Understanding and characterizing the structural brain changes across lifespan using Magnetic Resonance Imaging (MRI), taking into account the complex combination of distinct life experience, amongst which the exposure to stressful experience and variations in mood are major factors, became one of the most prominent challenges in the comprehension of the cognitive function in middle/late ages.

Despite of the limited information on the stress and mood-induced structural alterations, most studies point to reduced volumes in stressed participants in the anterior cingulate cortex, hippocampus and

amygdala (van der Werff et al., 2013;Lucassen et al., 2014). Smaller prefrontal and anterior cingulate cortex volumes have also been observed in patients with major depressive disorder (Frodl et al., 2008) and in participants with prolonged stress (Blix et al., 2013;De Brito et al., 2013). Reduced volumes in the hippocampus, caudate and putamen have also been reported in depressed subjects (Koolschijn et al., 2009;Kempton et al., 2011;van der Werff et al., 2013). Amygdala alterations were also observed in studies reporting larger volumes related with early stress exposure, including with hemispheric differences (Pruessner et al., 2010;van der Werff et al., 2013). On the other hand, volume decreases in amygdala have also been associated with early and late-onset depressed subjects (Burke et al., 2011;van Uden et al., 2011). Importantly, we previously found in young subjects that stress triggers an atrophy of the caudate and the orbitofrontal cortex, but a hypertrophy of the putamen, changes that were shown to be reversible and accompanied by a functional reorganization after a stress-free period (Soares et al., 2012). Curiously, there is a very limited amount of information regarding the stress and mood-effects on structural aspects on the WM.

Herein we addressed the impact of normal aging, stress, mood and their interplay in the brain GM and WM structure.

2. Materials and Methods

Ethics Statement

The present study was conducted in accordance with the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of Hospital de Braga (Portugal). The study goals and tests were explained to all participants and all gave informed written consent.

Participants, psychological tests and cortisol measurements

This study assessed a sample of 104 participants (52 males and 52 females, mean age 65.20 ± 8.07, minimum age 51 and maximum 82, 5.43 ± 3.84 mean years of education and mean of 26.66 ± 3.30 Mini-Mental State Examination (MMSE) (Folstein et al., 1975)) selected from a representative sample of the Portuguese population in terms of age, gender and education, of the SWITCHBOX Consortium project (Santos et al., 2013). Participants responded to a laterality test and to a questionnaire regarding perceived stress (Perceived Stress Scale – PSS) (mean 21.49 ± 8.18) (Cohen et al., 1983).

Participants were further assessed with the Geriatric Depression Scale (GDS, long version) (mean 10.91 ± 6.70) (Yesavage et al., 1982) by a certified psychologist.

Data acquisition and processing

Participants were scanned on a clinical approved Siemens Magnetom Avanto 1.5 T (Siemens Medical Solutions, Erlangen, Germany) at Hospital de Braga using the Siemens 12-channel receive-only head coil. The imaging session included one structural T1 high-resolution anatomical sequence, 3D MPRAGE (magnetization prepared rapid gradient echo). This protocol was performed with the following scan parameters: repetition time (TR) = 2.730 s, echo time (TE) = 3.48 ms, 176 sagittal slices with no gap, flip angle (FA) = 7°, in-plane resolution = 1.0 x 1.0 mm^2 and slice thickness = 1.0 mm.

Before any data processing and analysis, all acquisitions were visually inspected by a certified neuroradiologist and confirmed that participants had no brain lesions and the acquisitions were not affected by critical head motion. 7 participants were excluded from the analysis based on the head motion and/or brain lesions.

Structural analysis based on segmentation of brain cortical and subcortical structures from T1 high-resolution anatomical data was performed using the Freesurfer toolkit version 5.1 (http://surfer.nmr.mgh.harvard.edu) running on an Ubuntu 12.04 LTS system. This software package implements a semi-automated segmentation workflow including processing stages such as spatial registration to Talairach standard space, skull removal, normalization of white matter intensity, tesselation of GM-WM segmentation, among others. For the cortical parcellation, two atlases are available: one gyral based atlas resulting in 68 structures (Desikan et al., 2006) and another considering the giral and sulcal parts as separate regions resulting in 148 different brain areas (Destrieux et al., 2010). For the present study the subcortical, white matter and gyral-based cortical segmentations were considered. The employed workflow has suffered several improvements in the past years (Fischl et al., 2002;Fischl et al., 2004), is considered reliable across sessions, scanner platforms, updates and field strengths (Han and Fischl, 2007;Jovicich et al., 2009) and was already validated against manual segmentation procedures (Fischl et al., 2002).

Statistical Analyses

Statistical analyses (using the IBM SPSS Statistics software, v.22 (IBM, New York)) were performed with multiple regression models considering each volume as the dependent variable and age, gender, Intracranial Volume (ICV), PSS, GDS and age*PSS, age*GDS and age*PSS*GDS interactions as independent variables. For each positive or negative correlation, the results were controlled for the other covariates. The key assumptions for multivariate linear regression analysis were met and the covariates were mean-centered to avoid multicollinearity issues (Aiken and West, 1991;Frazier et al., 2004).

Dissection of the two-way interactions was performed centering the PSS or GDS scores one standard deviation (SD) bellow the mean, on the mean and one SD above the mean and assessing the age effect on brain volumetry in each model. In order to investigate the significant three-way interactions, the age effect was assessed in four different models: (1) centering both PSS and GDS scores one SD below the mean, (2) with the PSS scores centered one SD above the mean and GDS scores one SD below the mean, (3) PSS scores centered one SD below the mean and GDS one SD above the mean and (4) both variables centered one SD above the mean. Results were considered significant corrected for multiple comparisons using a False Discovery Rate (FDR) threshold of 0.05.

3. Results

Effect of age, stress and mood on brain volumetry

Volumetric analyses revealed that increased age was positively correlated with the volume of the choroid plexus (both sides), lateral ventricles and third ventricle and WM hypointensities. Most of the age correlations found were negative, including the total GM, cortical WM on both hemispheres, supratentorial volume, left accumbens and both hippocampi (Table 1). The WM presented more alterations, but only negative correlations were observed with increasing age (Table 2). More specifically, significant volumetric decreases in the WM volume with increasing age were found in the orbitofrontal cortex, superior frontal, inferior and middle temporal, parahippocampal, posterior cingulate and other frontal, parietal and temporal regions (Table 2).

The separate impact of stress (PSS score) and mood (GDS score) on brain volumetry did not reveal any significant changes, except for a decreased in WM volumetry in the right frontal pole with lower PSS scores (Table 2).

Interactions of age with stress and mood on brain volumetry

Tests for the two-way interaction between age and PSS revealed a significant interaction with the volume of the left frontal pole ($p = 0.0024$). Further analysis revealed that for lower PSS scores, decreased volumes were observed with increasing aging; however, as PSS scores get higher the slope of increase with age also increases. In the left temporal pole ($p = 0.0061$), for low GDS scores, its volume increases with age, while for medium to high GDS scores there is a reduction that gets more pronounced as the scores get higher (Table 2 and Figure 1 A).

In the WM regional volumetry, only negative age*GDS interactions were found (Table 4 and Figure 1 B). In the left frontal ($p = 0.0018$) and temporal pole ($p = 0.0066$), for low GDS scores, the WM volume increases with the age while for medium to high scores the volume decreases and the rate of decrease gets more prominent as the GDS scores get higher. In the left superior parietal ($p = 0.0083$) the WM volume decreases with age and this decrease gets more pronounced as GDS scores get higher.

The WM volume of the left paracentral ($p = 0.0092$) and the left superior frontal ($p = 0.0093$) regions showed three-way interactions, with both regions evidencing negative correlations between volume and age (Table 4 and Figure 1 C). Specifically, for low PSS scores, as GDS scores change from low to high the rate of volume decrease with age becomes more pronounced while for high PSS scores, the rate of volume decrease with age becomes less marked as GDS scores change from low to high.

4. Discussion

Several studies have consistently described the critical impact of the aging process, stress and mood on brain volumetry. Nevertheless, most of the neuroimaging studies focused on the effect of individual elements, precluding the critical influence of the complex interplay among various processes. In this study, we dissected the influence of life events on brain GM and WM volumetry, namely stress and mood, throughout aging, and how they interplay and impact on brain structure.

With aging, we found a global, as well as a regional, pattern of volumetric GM and WM decrease, accompanied by an expansion of the ventricles, choroid plexus and CSF, reflecting an atrophy of brain parenchyma. Total and subcortical GM was decreased with age, including the left accumbens, hippocampi and total cortical WM, frontal, temporal, occipital and parietal WM regions also presented significant volumetric decreases with age. Similar findings have been consistently reported in the literature, both in cross-sectional and longitudinal studies (Raz et al., 2005;Smith et al., 2007;Abe et al., 2008;Fjell et al., 2009;Walhovd et al., 2011;Tamnes et al., 2013). Specifically, in our sample we observed a higher decrease in global WM than GM, confirming a striker WM deterioration after the fifth decade (Gunning-Dixon et al., 2009;Lemaitre et al., 2012). This global WM volume decline, more pronounced in frontal regions (orbitofrontal, superior frontal and rostral middle frontal) was paralleled by an increase with age of the WM hypointensities volume, a measure of lesion burden (Leritz et al., 2014).

It is well known that the stress impact is diverse on different life phases (Sousa and Almeida, 2012). Additionally, stress and mood are states known to be intrinsically connected and that interplay over the lifespan (Calabrese et al., 2009). Here, in the volume of the left frontal pole GM, there was an inversion from decreases at low stress levels to increases at high levels with age. This result shows the critical stress impact in the frontal regions, especially at higher stress levels, leading to inversions from age-induced reductions to volume increases during aging (Cerqueira et al., 2007;Lupien et al., 2009). Importantly, behavioral stress affects, with possible reversibility, both structure and function of the prefrontal cortex, a region where neurons become less efficient with aging (McEwen and Morrison, 2013). On the other hand, on the left frontal pole WM, there is an increase in the volume reduction with age for higher depressive mood levels, in line with several previous findings (Konarski et al., 2008;Kong et al., 2014), showing the high susceptibility and variability of this region. Several studies have reported volumetric reductions in temporal regions associated with mood disorders (Drevets et al., 2008;Son et al., 2013) and herein we observed the depressive mood impact, especially at higher levels, in the increased volumetric reduction of the WM and GM in the left temporal pole. In this study we also found an increased volumetric reduction with age at high depressive mood levels in the left superior parietal WM, in line with the literature pointing to WM decreases in older patients with major depressive disorder (Zeng et al., 2012). Such lateralization effect, with more pronounced atrophy in

structures localized in the left hemisphere, is in good line with previous studies on this topic (see for review (Cerqueira et al., 2008)).

The most affected WM regions during aging by the interplay stress and mood are the left paracentral and the left superior frontal regions. Indeed, the superior frontal WM volume is known to present an accelerated decline with increasing age (Salat et al., 2009a). The volume of these regions decrease for all stress and mood level combinations, however the decrease is much more pronounced for high stress and low mood levels. This indicates that for better mood (i.e. less depressive), the effect of stress is negative since it increases the negative relation between age and volume; this negative impact of stress does not seem to operate so obviously for subjects with higher depressive mood. These higher volumetric reductions with age, especially under high levels of stress, in WM of these regions, may be associated with the lower predisposition to action and extraception observed with increasing age. Importantly, the impact of stress and mood during the lifespan seems to be higher in the WM compared to the GM volumetry, in line with the described faster WM deterioration after the fifth decade of life (Gunning-Dixon et al., 2009).

This study presents also some important limitations. The analyses of stress and mood states were based only in psychological scales without any biological marker and indicator. Also, our sample included only middle aged to older adults and was a cross-sectional design, precluding a complete lifespan assessment, from childhood to elderly ages and the evaluation of both individual differences and cohort effects. However, to the best of our knowledge there are no prior reports that evaluate the interplay between stress and mood on brain volumetry across the lifespan. In this study we have shown the critical influence of stress and mood, especially at higher levels, in brain volumetry. High levels of stress and/or mood may accelerate the typical age-induced decline or alternatively reduce the aging impact. We showed also that for the effects of stress and mood in brain volumetry, timing is crucial. Indeed, the clarification of the stress and mood interplay during aging may help to explain some divergent results associated with the aging decline. Moreover, we expect also to enlighten the association between abnormal volumetric alterations and several states that may lead to psychiatric disorders (Drevets et al., 2008;Konarski et al., 2008;Kempton et al., 2011;Kroes et al., 2011;Durkee et al., 2013;Kuhn and Gallinat, 2013).

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tables

Table 1

Effect of age on brain volumetry (corrected for multiple comparisons FDR 0.05).

Effect	Correlation	Region	T	P
Age	Positive	Choroid plexus (right)	5.8224	9.28E-08
		Lateral ventricle (left)	5,4376	4.78E-07
		White matter hypointensities	4.8414	5.47E-06
		Lateral ventricle (right)	4.6083	1.36E-05
		Choroid plexus (left)	3.7589	0.0003
		3ª Ventricle	3.2458	0.0017
	Negative	Cortical white matter (right)	-7.2174	1.78E-10
		Cortical white matter	-6.9783	5.35E-10
		Cortical white matter (left)	-6.5820	3.24E-09
		Supratentorial	-5.1779	1.41E-06
		Accumbens (left)	-4.6633	1.10E-05
		Hippocampus (left)	-4.0750	0.0001
		Hippocampus (right)	-3.8995	0.0001
		Total gray matter	-3.5415	0.0006

Table 2

Effect of age and stress on brain white matter regional volumetry (corrected for multiple comparisons FDR 0.05).

Effect	Correlation	Region	T	P
Age	Negative	Lateral orbitofrontal (right)	-5.4714	4.15E-07
		Lateral orbitofrontal (left)	-5.7956	1.04E-07
		Superior frontal (left)	-5.6346	2.08E-07
		Inferior temporal (left)	-5.4901	3.83E-07
		Cerebellum (right)	-4.8172	6.02E-06
		Posterior cingulate (right)	-4.5550	1.68E-05
		Superior frontal (right)	-4.5026	2.05E-05
		Inferior temporal (right)	-4.2957	4.47E-05
		Paracentral (left)	-4.2858	4.65E-05
		Paracentral (right)	-4.2858	0.00090
		Medial orbitofrontal (right)	-4.2247	5.82E-05
		Parahippocampal (right)	-4.1981	6.57E-05
		Superior parietal (right)	-4.0156	0.00020
		Postcentral (left)	-3.9499	0.00015
		Entorhinal (left)	-3.9136	0.00018
		Middle temporal (right)	-3.9029	0.00001
		Superior parietal (left)	-3.8834	0.00020
		Pars triangularis (right)	-3.8736	0.00021
		Middle temporal (left)	-3.8476	0.00022
		Pars orbitalis (right)	-3.7961	0.00027
		Precentral (left)	-3.7331	0.00033
		Fusiform (left)	-3.7016	0.00037
		Postcentral (right)	-3.5270	0.00067
		Lateral occipital (right)	-3.4985	0.00074
		Cerebellum (left)	-3.4613	0.0008
		Inferior parietal (right)	-3.4307	0.00092
		Posterior cingulate (left)	-3.4172	0.00096
		Rostral middle frontal (left)	-3.3386	0.00012
		Rostral middle frontal (right)	-3.3753	0.00110
		Superior temporal (right)	-3.3266	0.00128
		Parahippocampal (left)	-3.2781	0.00150
		Pars opercularis (right)	-3.2039	0.00189
		Insula (right)	-3.0532	0.00300

		Lingual (right)	-3.0464	0.00305
		Pars triangularis (left)	-3.0312	0.00320
		Fusiform (right)	-2.9598	0.00037
		Frontal pole (right)	-2.9472	0.00410
		Lingual (left)	-2.9103	0.00457
		Supramarginal (left)	-2.8668	0.00525
		Supramarginal (right)	-2.8627	0.00525
		Inferior parietal (left)	-2.8547	0.0092
PSS	Negative	Frontal pole (right)	-3.3396	1.23E-03

Table 3

Effect of the interplay between stress and aging and stress and mood on brain volumetry (corrected for multiple comparisons FDR 0.05). M - Mean; SD - Standard Deviation.

Interaction	Correlation	Region	M-SD/M/M+SD	T	P
Age*PSS	Positive	Frontal pole (left)	-2.6821/3.5549/9.7919	3.1282	0.0024
Age*GDS	Negative	Temporal pole (left)	10.2711/-1.7243/-13.7198	-2.8121	0.0061

Table 4

Effect of the interplay between aging, stress and mood on brain white matter regional volumetry (corrected for multiple comparisons FDR 0.05): M - Mean; SD - Standard Deviation; LPSS - Low Perceived Stress Scale scores; HPSS - High Perceived Stress Scale scores; LGDS – Low Geriatric Depression Scale scores; HGDS – High Geriatric Depression Scale scores.

Interaction	Correlation	Region	M-SD/M/M+SD	T	P
Age*GDS	Negative	Frontal pole (left)	1.1835/-0.9989/-3.1813	-3.2240	0.0018
		Temporal pole (left)	1.8836/-2.0358/-5.9551	-2.7813	0.0066
		Superior parietal (left)	-15.6389/-62.1391/-108.6391	-2.7026	0.0083
			LPSS_LGDS/LPSS_HGDS/HPSS_LGDS /HPSS_HGDS		
Age*PSS*GDS	Positive	Paracentral (left)	-5.0132/-37.1670/-52.2815/-9.5234	2.6634	0.0092
		Superior frontal (left)	-46.3864/-200.5990/-156.4589/-66.0731	2.6541	0.0093

Table S1

Standardized regression coefficient of the significant Age*PSS*GDS interactions in WM volumetry.

Factor	Regions	
	Paracentral (left)	Superior frontal (left)
Age	-0.3866	-0.3789
PSS	0.1076	-0.0630
GDS	-0.0658	-0.0738
Age*PSS	-0.0730	0.0197
Age*GDS	0.0394	-0.0515
Age*PSS*GDS	0.2785	0.1974

Figure Legends

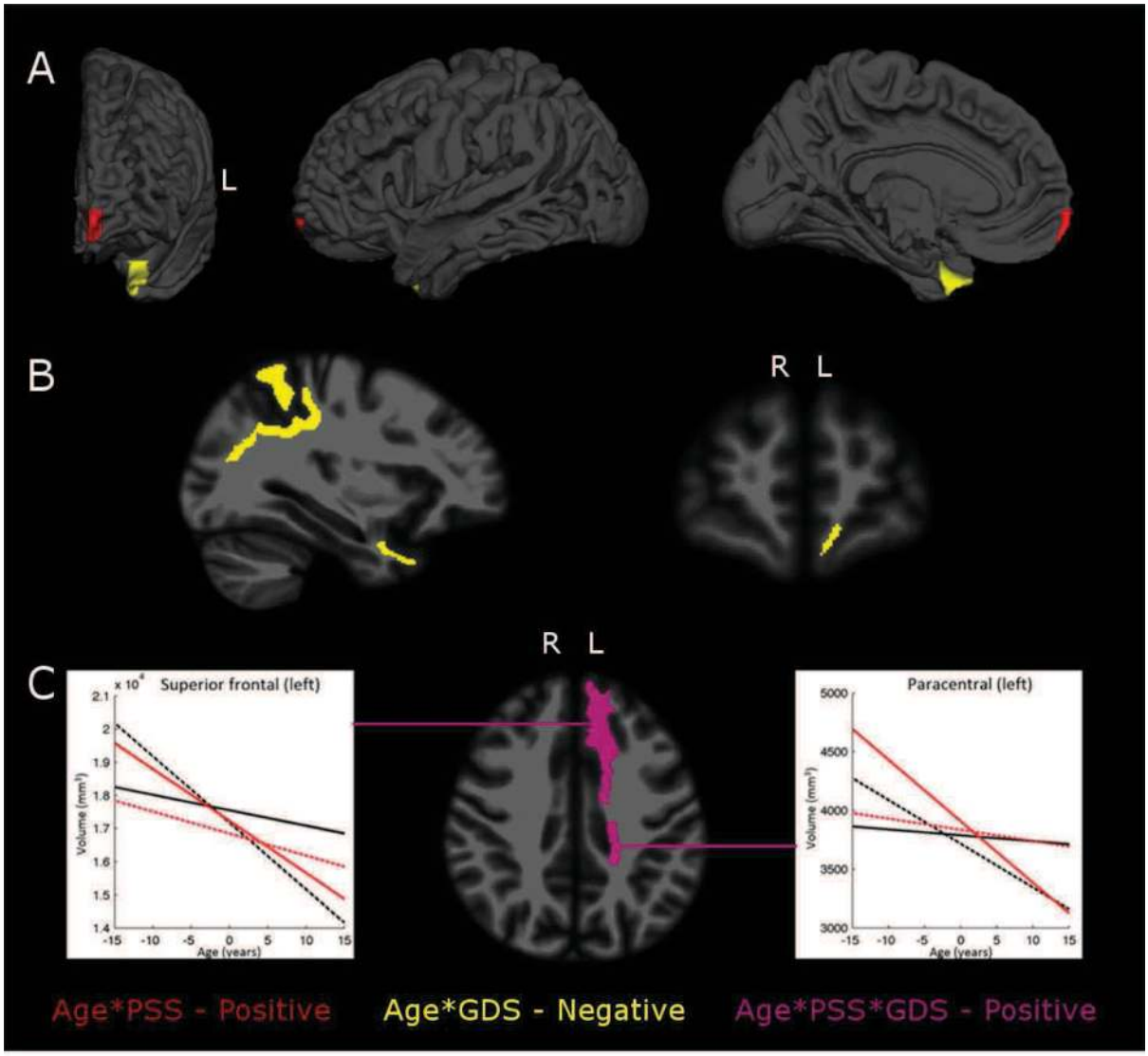

Figure 1. The interplay between stress or mood and aging in brain regional gray matter volumes (A), white matter regions (B) and the Age*PSS*GDS interactions (C). The images depict areas with significant interaction effects of age*PSS positive (in red), age*GDS negative (in yellow) and age*PSS*GDS positive (in violet). In (C) each graphic line is the regression line between age and regional volume for: Low PSS values (LPSS, black lines), combined with Low GDS (LPSS-LGDS, solid line) or with High GDS (LPSS-HGDS, dotted line); and High PSS value (HPSS, red lines), combined with Low GDS (HPSS-LGDS, solid line) or High GDS (HPSS-HGDS, dotted line).

Chapter 3.5

Soares, J.M., Marques, P., Magalhães, R., Santos, N.C., and Sousa, N.

The influence of Stress and Mood across the lifespan on Resting State Brain Networks

Manuscript submitted

The influence of Stress and Mood across the lifespan on Resting State Brain Networks

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Abstract

Aging, namely of the brain structure and function, is a complex process characterised by high inter- and intra-individual variability. Such variability may arise from the interaction of multiple factors, including exposure to stressful experience and mood variation, across the lifespan. Using a multimodal neuroimaging and neurocognitive approaches, we investigated the impact of stress, mood and their interplay, in the structure and function of Resting State Networks (RSNs) in aging. Data reveals a critical influence of stress and mood levels in different RSNs, with these variables interacting with each other and acting as mediators across the lifespan. This interplay is most significant at combined high stress and depressive mood levels, reverting in some specific networks the natural aging pattern. These results contribute to characterize the spectrum of functional connectivity and deactivation patterns and highlight the crucial impact of stress and mood levels during the aging process.

Keywords: Aging, stress, mood, resting state networks, functional connectivity

1. Introduction

Mental and physical health are critically dependent upon appropriate reception, processing and integration of internal and external signals, providing the ability to perform adaptive responses during lifetime. Indeed, one of the hallmarks of healthy aging is its variability, based on a wide range of preservation, adaptation and losses in structure, function, and performance across several domains, namely cognition. Such variability may arise from the experience of multiple factors across the lifespan, amongst which the exposure to stressful experience and variations in mood are major factors. To understand these changes, a key question is, how adverse experiences over the lifespan interact with the aging process in brain structure and function, namely in the resting state networks (RSNs).

Magnetic resonance imaging studies (MRI), including volumetric, diffusion and functional analysis, have provided new insights into the structural and functional modulation of brain regions and networks across the lifespan. Among them, resting state functional MRI is being widely used to assess brain regional interactions that comprise the resting state networks (RSNs), including the default mode network (DMN), dorsal and ventral attention networks (DAN and VAN), salience network (SN), sensorimotor network (SMN), auditory network (AN), and primary and higher visual networks (PVN and HVN), both during resting periods and in task-induced deactivations (De Luca et al., 2006;Fox and Raichle, 2007;Harrison et al., 2011).

Normal brain aging is a continuous process characterized by a pattern of selective loss and preservation of structures and functions, including volumetric decreases, white matter density and integrity loss, decrease of neuronal connection complexity and efficiency loss in several cognitive domains (Dennis and Cabeza, 2008;Meunier et al., 2014). Aging has also a key influence in RSNs, with the most consistent results showing decreased functional connectivity within the DMN system, especially pronounced over 60 years of age (Damoiseaux et al., 2008;Mowinckel et al., 2012). The central trend regarding age-induced alterations in resting-state functional connectivity is a widespread reduction throughout the brain during lifespan. Several recent investigations have supported this trend, reporting decreased connectivity in older adults in attention, SMN, SN, AN and visual networks (Allen et al., 2011;Mowinckel et al., 2012;Ferreira and Busatto, 2013). Additionally task-induced deactivation studies have also reported decreased DMN and other RSNs' deactivations in older adults (Grady et al.,

2006;Harrison et al., 2011).

Despite a much more limited information on stress- and (depressive) mood-induced brain alterations, there are some consistent reports on changes in brain regions implicated in the stress response and mood status, a large number of which are coincident. In fact, prolonged stress exposure impairs spatial working memory, perceptual attention, behavioral flexibility and decision making both in rodents and in humans, which has been associated with structural and functional changes of several brain regions (Dias-Ferreira et al., 2009;Soares et al., 2012;Yuen et al., 2012). Importantly, most of these maladaptive structural and functional responses to increased chronic stress were reported in young and mid-aged subjects and were in part shown to be reversible (Sousa et al., 2000;Bian et al., 2012;Soares et al., 2012). Moreover, we have recently shown that stressed subjects present increased activation of the DMN, DAN, VAN, SMN and PVN, associated with impairments in the deactivation patterns and paralleled by a DMN atrophy (Soares et al., 2013a). Of notice, we also demonstrated that after recovering from a stress period, young individuals regained the normal connectivity patterns in the DMN, VAN and SMN whereas there were still some areas of altered connectivity in the DAN, SMN and PVN. Moreover, a normalization of the deactivation patterns of the RSNs was also observed (Soares et al., 2013b). Stress also impacts in brain structures that are functionally abnormal in mood disorders. Evidence seems to indicate that stress is the most common risk factor for the development of mood disorders, such as major depression (Pittenger and Duman, 2008;Risch et al., 2009). Emerging findings from resting state major depression studies have pointed to altered connectivity and abnormal deactivation patterns. Despite the diverse RSN results described, the tendency suggests an increased functional connectivity in depressed subjects in DMN anterior regions, specially in young subjects (Zhu et al., 2012;Kerestes et al., 2013). However, several studies have also reported decreased connectivity in DMN and other RSNs in the elderly (Veer et al., 2010;Andreescu et al., 2011;Wu et al., 2011), failure in DMN deactivation during task performance (Sheline et al., 2009) and even no differences between controls and depressed subjects (Sexton et al., 2012).

Understanding the association between structural and functional brain alterations, in combination with life experiences, across lifespan is a critical challenge for the comprehension of the cognitive status. Herein we addressed this interplay using a multimodal neuroimaging and neurocognitive approaches to assess the effect of normal aging, stress, mood and their interplay in brain structural and functional

RSNs, both during rest and task-induced deactivation.

2. Materials and Methods

Ethics Statement

The study was conducted in accordance with the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of Hospital de Braga (Portugal). The study goals and tests were explained to all participants and all gave informed written consent.

Participants, psychological tests and cortisol measurements

This study assessed a sample of 104 participants (52 males and 52 females, mean age 65.20 ± 8.07, minimum age 51 and maximum 82, 5.43 ± 3.84 mean years of education and mean of 26.66 ± 3.30 Mini-Mental State Examination (MMSE) (Folstein et al., 1975) selected from a representative sample of the Portuguese population in terms of age, gender and education, all participating in the SWITCHBOX Consortium project (Santos et al., 2013). Participants responded to a laterality test and to a questionnaire regarding perceived stress (Perceived Stress Scale – PSS) (mean 21.49 ± 8.18) (Cohen et al., 1983). All subjects were further assessed with the Geriatric Depression Scale (GDS, long version) (mean 10.91 ± 6.70) (Yesavage et al., 1982) by a certified psychologist.

Data acquisition

Participants were scanned on a clinical approved Siemens Magnetom Avanto 1.5 T (Siemens Medical Solutions, Erlangen, Germany) MRI scanner on Hospital de Braga using a Siemens 12-channel receive-only head coil. The imaging sessions included one structural T1 - weighted and two functional T2* - weighted acquisitions (one resting state and one task related), conducted in the same day. For structural analysis, a T1 high-resolution anatomical sequence, 3D MPRAGE (magnetization prepared rapid gradient echo) was performed with the following scan parameters: repetition time (TR) = 2.730 s, echo time (TE) = 3.48 ms, 176 sagittal slices with no gap, flip angle (FA) = 7°, in-plane resolution = 1.0 x 1.0 mm^2 and slice thickness = 1.0 mm. During the resting-state fMRI acquisition, using gradient echo weighted echo-planar images (EPIs), the participants were instructed to keep their eyes closed and to think about nothing in particular. The imaging parameters were: 180 volumes, TR = 2 s, TE =

30 ms, FA =90°, in-plane resolution = 3.5 x 3.5 mm^2, 30 interleaved slices, slice thickness = 4 mm, imaging matrix 64 x 64 and FoV = 224 mm. The N-Back paradigm acquisition was acquired using the same parameters as the resting state protocol, except the number of volumes, 456 in this case. The functional paradigm was presented using the fully integrated and synchronized fMRI system IFIS-SA.

Image pre-processing

Before any data processing and analysis, all acquisitions were visually inspected by a certified neuroradiologist and confirmed that they were not affected by critical head motion and that participants had no brain lesions.

To achieve signal stabilization and allow participants to adjust to the scanner noise, the first 5 fMRI volumes (10 seconds) were discarded. For both resting state and task analysis, data preprocessing was performed using SPM8 (Statistical Parametrical Mapping, version 8, http://www.fil.ion.ucl.ac.uk) analysis software. Images were firstly corrected for slice timing using the first slice as reference and SPM8's Fourier phase shift interpolation. To correct for head motion, images were realigned to the mean image with a six-parameter rigid-body spatial transformation and estimation was performed at 0.9 quality, 4 mm separation, 5 mm full-width at half-maximum (FWHM) smoothing kernel and using 2nd degree B-Spline interpolation. For resting state analysis, 7 subjects were excluded once they exceed head motion higher than 2 mm in translation or 1° in rotation. Images were then spatially normalized with a non-linear transformation to the MNI (Montreal Neurological Institute) standard coordinate system using SPM8 EPI template and trilinear interpolation. Data were then re-sampled to 3x3x3 mm3 using sinc interpolation, smoothed to decrease spatial noise with a 8 mm, FWHM, Gaussian kernel. Resting state images were then temporally band-pass filtered (0.01-0.08 Hz) and the linear trend was removed, and fMRI task images were high pass temporal filtered (filter width of 128s).

Structural analysis

The structural analysis based on segmentation of brain cortical and subcortical structures from T1 high-resolution anatomical data was performed using the Freesurfer toolkit version 5.1 (http://surfer.nmr.mgh.harvard.edu). Intracranial volume (ICV) was used to correct the regional volumes and the processing pipeline was the same as previously described (Soares et al., 2012). DMN was defined by the summed volume of the angular gyrus of the inferior parietal lobe, the posterior

cingulate, the precuneus and the frontopolar region (Raichle et al., 2001;Buckner et al., 2008). The summed volume of the middle frontal gyrus (dorsolateral and prefrontal region) and the posterior parietal region constituted the DAN (Seeley et al., 2007;Sridharan et al., 2008). VAN was constituted by the sum of the temporal-parietal junction and the ventral frontal cortex volumes (Fox et al., 2006). SN was established summing the volumes of the insula and the caudal anterior cingulate cortex, and SMN by the summed volume of the paracentral, precentral, postcentral and the cerebellum regions (Shirer et al., 2012). The summed volume of the cuneus, pericalcarine and the lingual region constituted the primary VN, while the higher VN was defined by the lateral occipital volume (Shirer et al., 2012). Auditory network (AN) was defined by the summed volume of the temporal transverse and the temporal superior (Shirer et al., 2012).

Independent Component Analysis and identification of RSN

Independent component analysis was conducted using the Group ICA 2.0d of fMRI Toolbox (GIFT, http://www.icatb.sourceforge.net) (Calhoun et al., 2001;Correa et al., 2005). Briefly, spatial ICA analysis is a fully data-driven approach that consists in extracting the non-overlapping spatial maps with temporally coherent time courses that maximize independence. The GIFT workflow can be summarized in three main stages: dimensionality reduction, estimation of the group independent components and back-reconstruction of each subject's corresponding independent components. The reduction of dimensionality of the functional data and computational load was performed with Principal Component Analysis (PCA). Then, 20 independent components were estimated, based on a good trade-off (clustering/splitting) between preserving the information in the data while reducing its size (Beckmann et al., 2005;Zuo et al., 2010), using the iterative Infomax algorithm. The ICASSO tool was used to assess the ICA reliability, and 20 computational runs were performed on the dataset, during which the components were being recomputed and compared across runs (Himberg et al., 2004). The previous steps resulted in the estimation of a mixing matrix with partitions, unique to each subject. The individual independent components were then back-reconstructed from the group-level components. This back-reconstruction step is accomplished by projecting each subject's data onto the inverse of the partition of the calculated matrix corresponding to that subject. The obtained independent components were expressed in t-statistic maps, which were finally converted to a z-statistic. Z-statistic describes the voxels that contributed more intensely to a specific independent component, providing a degree of

functional connectivity within the network (Bartels and Zeki, 2005;Beckmann et al., 2005). The final components were visually inspected, sorted and spatially correlated with resting state functional networks from (Shirer et al., 2012). Each subject's map corresponding to the best-fit component of each RSN was used to perform second-level statistical analyses.

RSN deactivation during fMRI task analysis

The fMRI paradigm analyzed to investigate the task-induced deactivations was an n-back task, a standard executive working memory measure in cognitive neuroscience. Concisely, the task with a block design comprised 4 block categories, 0-back, 1-back, 2-back and rest. The block sequence was randomized; each block included 6 s for instruction, 40 s of letters sequence (16 letters; 4 correct responses; 0.5 s for each letter presentation and 1.5 s for response; letters included were b/c/d/p/t/w) and 10 s of rest for task blocks and the same structure for resting periods except the letters sequence, only x letter presented (and participants instructed to rest); each block was presented 4 times. The paradigm was analyzed by creating a set of regressors at resting and task periods, which were convolved with the hemodynamic response function. In order to reliably map task-induced deactivations, we combined all the resting periods (resting baseline condition) and all the n-back periods (task condition). The contrast used to assess task-induced deactivations was the resting baseline condition minus n-back condition. Resulting functional patterns were masked with the previously described RSNs masks (Shirer et al., 2012). For the analysis, 35 participants with more than 50 % of correct responses and less than 50 % of incorrect responses were included.

Statistical Analyses

For both structural (using the IBM SPSS Statistics software, v.22 (IBM, New York)) and functional analysis (using the SPM8 software), multiple regressions were performed modelling the effects of age, gender, PSS and GDS as well as the age*PSS, age*GDS and age*PSS*GDS interactions. In the structural analysis, ICV was also included to control for different head sizes while in the functional analyses we included gray matter volume as a regressor, once older individuals present consistently higher gray matter atrophy, with potential impact on decreased brain activity (Damoiseaux et al., 2008;Mowinckel et al., 2012). For each positive or negative correlation, the results were controlled for the other covariates. The key assumptions for multivariate linear regression analysis were met and the

covariates were mean-centered to avoid multicollinearity issues (Aiken and West, 1991;Frazier et al., 2004).

RSNs volumetric analyses were considered significant at $p < 0.05$. Functional analyses were performed using the second-level random effect analyses in SPM8. Initially, analyses were performed only to confirm the functional connectivity of the RSNs, using one-sample t-tests. Thereafter, multiple regression analysis was performed and results were considered significant at $p < 0.05$ corrected for multiple comparisons using a combination of a peak threshold of $p < 0.025$ with a minimum cluster size. The cluster size was determined over 1000 Monte Carlo simulations using AlphaSim program distributed with REST software tool (http://resting-fmri.sourceforge.net/) with the following input parameters: individual voxel probability threshold = 0.025, cluster connection radius = 3 mm, gaussian filter width (FWHM) = 8 mm, number of Monte Carlo simulations = 1000 and mask was set to the corresponding RSN template mask. Anatomical labeling was defined by a combination of visual inspection and Anatomical Automatic Labeling atlas (AAL) (Tzourio-Mazoyer et al., 2002).

The significant two-way interactions were further investigated fitting three additional models in which the PSS scores or GDS scores were centered: (1) one standard deviation (SD) below their mean, (2) at the mean and (3) one SD deviation above the mean while maintaining the remaining regressors mean centered. This enabled the investigation of how different PSS or GDS scores modulate the aging effect on brain functional patterns/volumetry. Similarly, for the three-way interactions, the age effect on RSNs functional patterns and volumetry was assessed by simultaneously centring the PSS and GDS scores: (1) both one SD deviation bellow their means; (2) the first variable one SD below and the second one SD above the respective means; (3) the first one SD above and the second one SD below its mean; and (4) both one SD above the respective means.

3. Results

Isolate effect of age, stress and mood on RSNs

At rest, increased functional connectivity associated with increased age was found in the VAN, specifically, bilaterally in the frontal superior region. On the contrary, increased age was significantly correlated with less connectivity in specific areas of the DAN (in the left parietal inferior), DMN (in the

right anterior cingulate, frontal medial orbitofrontal and precuneus), PVN (in the bilateral calcarine), SMN (in the bilateral cerebellum) and in the SN (in the left frontal superior and frontal superior medial and in the superior motor area) (Table 1). During task-induced deactivations, a negative correlation between age and functional connectivity was found in the left fusiform of the DMN (Table 2). In the volumetric analysis of the RSNs, a negative correlation with aging was observed in the total SMN volume ($T = -2.7062$, $P = 0.008$).

Regarding PSS scores, a positive correlation with functional connectivity during rest was found in the AN (in the left insula and temporal superior), DMN (in the left frontal superior and medial orbitofrontal, middle cingulate, occipital middle regions and in the right frontal middle, posterior cingulate and precuneus), PVN (in the left calcarine), SMN (in the left cerebellum) and in the VAN (in the left occipital middle, parietal inferior, in the bilateral frontal middle and angular and in the right frontal superior) (Table 1). When performing the task, participants with higher PSS scores deactivated more the left temporal superior (AN) and the left cerebellum (SMN) regions (Table 2). Interestingly, there was trend for a negative correlation between the volume of the DMN and PSS scores ($T = -1.5556$, $P = 0.12$).

Between GDS scores and functional patterns, only negative correlations were found. During resting state, the DMN (in the right frontal middle, in the left cuneus, calcarine and anterior cingulate and in the bilateral frontal medial orbitofrontal), PVN (in the right cuneus) and in the VAN (in the right angular, parietal inferior and in the bilateral frontal middle) presented less functional connectivity in subjects with higher GDS scores (Table 1). During deactivation the DMN (in the bilateral posterior cingulate, right precuneus and left frontal superior medial) was more active in subjects with higher GDS scores (Table 2). The total SMN volume was negatively correlated with the GDS scores ($T = -2.3629$, $P = 0.02$).

Interactions of age with stress and mood on the RSNs

In the DMN, specifically in the right frontal middle region, for low and medium PSS scores, the functional connectivity decreased with age; however, in subjects with high scores in the PSS an opposite pattern, with an increased connectivity was found. Similarly, for the functional connectivity of the right parietal inferior and angular and the left occipital middle of the VAN a decreased connectivity with age for low PSS scores, but an increase for higher scores was observed. On the SN, more precisely in the left frontal middle and bilateral anterior cingulate, at low PSS scores the functional connectivity

increased with age; individuals with higher scores, however, displayed a decreased connectivity with aging (Table 3 and Figure 1A).

On the interaction age*GDS, the functional connectivity of the AN (specifically in the left temporal superior) decreased with age for low GDS scores, but increased for higher scores. In the right temporal middle of the DMN and the right frontal middle of the VAN, the functional connectivity decreased with age at low and medium scores in the GDS, but increased for higher scores. In contrast, in the left cerebellum of the SMN, for low GDS scores the functional connectivity increased with age, whereas it decreased for higher scores (Table 3 and Figure 1A).

In task-induced deactivations only significant correlations with the DMN were found. Specifically, in the bilateral frontal superior medial and precuneus regions, for low PSS scores the deactivation decreased with aging, while for higher scorers an opposite pattern with increasing age was noted (Table 4 and Figure 1B). In what regards the interplay of age and GDS scores, the deactivation of the DMN, particularly in the left anterior cingulate increases with age for low and medium GDS scores, while for high scores, during task performance, deactivation is decreased (Table 4 and Figure 1B).

Combined interactions between Age*PSS*GDS on RSNs

During resting state, the functional connectivity of the DMN (in the left frontal medial orbitofrontal and in the right anterior cingulate), the HVN (in the right fusiform) and of the VAN (in the left parietal inferior) increased with age for both low and high PSS and GDS scores, and decreased for the combination low PSS and high GDS scores and vice-versa. On the contrary, the functional connectivity in the bilateral temporal superior of the AN, right frontal superior medial of the DMN and right frontal middle of the VAN decreased for both low and high PSS and GDS scores, increasing for combined low PSS and high GDS scores and vice-versa (Table 3 and Figures 1A and 2A).

During task performance, in the SMN, particularly in the right precentral region, the deactivation increased with age for both low and both high PSS and GDS scores, but it decreased for mixed low/high PSS and GDS scores and vice-versa. In the right frontal superior medial of the DMN, the deactivation decreased with aging for low PSS and GDS scores, increasing for combined low PSS and high GDS scores and the opposite, and for combined high scores (Table 4 and Figures 1B and 2B).

4. Discussion

Recent studies have consistently described the impact of the aging process, stress and mood on brain RSNs (Andreescu et al., 2011;Ferreira and Busatto, 2013;Kerestes et al., 2013;Soares et al., 2013a). However, most of the neuroimaging studies focused on the effect of individual factors, precluding the critical influence of the complex interplay among various concurrent processes across the lifespan, such as stress exposure (or perceived experienced stress) and/or variations in depressive mood. Herein, we dissect the influence of these life events and how they interplay and impact throughout life, using a multimodal neuroimaging approach. The present data: i) replicates the most consistent findings in the literature regarding the influence of the aging course, stress and major depression on RSNs, both during rest and task-induced deactivation, supporting our subsequent analyses; ii) reveals that different stress and mood levels have different impact across aging; iii) indicates that the constant interplay between stress and mood states has significant effects in the brain RSNs during lifespan.

Functional brain effects of aging, stress and mood

In line with the central tendencies of previous findings regarding age-induced differences in resting state functional connectivity, we found a global connectivity decrease across the RSNs (Damoiseaux et al., 2008;Allen et al., 2011;Ferreira and Busatto, 2013). Paralleled with this decrease, participants also showed a decreased deactivation of the DMN with increasing age, illustrating the reported difficulty in adaptive switching from a "default mode" to task-performance mode in aged individuals (Grady et al., 2010). Interestingly, however, the VAN displayed increased connectivity with aging, suggesting that the aging process is heterogeneous in its consequences at the brain network level (Filippini et al., 2012;Mowinckel et al., 2012).

The few previous studies, including ours, investigating the effect of stress on RSNs point to a generalized increase of functional connectivity under prolonged stress periods, followed by a selective recovery pattern (Soares et al., 2013a;Soares et al., 2013b;Vaisvaser et al., 2013). In this study, and in line with our previous findings in young participants, we found an increased connectivity in the more stressed participants (that is, higher PSS scores) in the AN, DMN, PVN, SMN and VAN, suggesting augmented self-reflective thoughts and emotional processing (Soares et al., 2013a). During task performance, these participants presented an increased deactivation of the AN and of the SMN

pointing to an easier disconnection of auditory and sensorial stimuli when shifting from task to resting periods. In terms of volumetric analysis, we found a tendency for global DMN atrophy for higher PSS scores, as we showed before in young participants (Soares et al., 2013a), suggesting the existence of both structural and functional reorganizations under stress periods along the lifespan.

Additionally, herein we also observed a decreased functional connectivity in the DMN, PVN and VAN in participants with higher GDS scores (that is, more depressive mood), along with an increased difficulty to deactivate the DMN during cognitive task performance. The results support other findings pointing to a decrease in RSNs connectivity in elderly depressed subjects (Veer et al., 2010;Andreescu et al., 2011;Wu et al., 2011) accompanied by a failure in deactivation during cognitive performance (Sheline et al., 2009), underlying the difficulty in switching between rest and cognitive demanding periods and in connecting internal and external attention in monitoring the world around (Greicius et al., 2003).

Functional brain effects of stress and mood and their interplay across the lifespan

In the DMN, particularly in the right frontal middle region, the functional connectivity, for low and medium stress levels decreased with age, in line with the typical aging findings (Damoiseaux et al., 2008;Hafkemeijer et al., 2012). Interestingly, however, for higher stress levels it increased with aging, as in young stressed subjects (Soares et al., 2013a;Vaisvaser et al., 2013), which shows that stress exposure has a similar effect in RSNs across the lifespan. The same behavior was observed in the right temporal middle of the DMN relative to mood*age interaction, as the subjects with higher GDS scores, also displayed an increased functional connectivity in the posterior regions of DMN, which fit other observations (Andreescu et al., 2011). Both results point to a more pronounced stress and depressive mood effect (at high levels) over the normal aging pattern on DMN connectivity decline. These apparently contrasting effects observed in the DMN across aging that result from the superimposition of concomitant factors are also observed during cognitive performance, in the bilateral frontal superior medial and the precuneus of the DMN, where the deactivation decreases for low stress levels but increases for medium and high levels with age. In fact, for subjects with low stress levels the typical decline in DMN during aging prevails, whereas for medium and high stress levels there is an increase in the deactivation, suggesting a stress predominant effect, and curiously contrary to what occurs in young stressed participants, whom show decreased deactivation when compared with controls (Soares et al., 2013a). In the DMN, specifically in the left anterior cingulate, the deactivation increases with age

for low and medium mood levels, but it decreases for high depressive levels, confirming the increased difficulty of older and depressed participants to deactivate the DMN during task performance (Damoiseaux et al., 2008;Sheline et al., 2009).

The differential pattern of age effects on the RSNs when combined with stress and GDS scores is, however, not confined to the DMN. For example in the VAN, in subjects with low stress levels, the connectivity in the right parietal inferior and angular and in the left occipital middle of the VAN decreases during aging, while for medium and, even more accentuated for high stress levels, the increase is in line with the aging and stress separated effects (Filippini et al., 2009;Mowinckel et al., 2012;Soares et al., 2013a). The mood*age interaction also shows that in the right frontal middle of the VAN, for low and medium mood levels the connectivity decreases with age, though it increases in subjects with high GDS score levels. These results suggest that aged subjects with the most stressed and lower depressive mood present higher bottom-up attention processes, mediation of exogenous stimulus-driven and salience processing during the aging course(Corbetta and Shulman, 2002;Seeley et al., 2007).

It is also noticeable that the functional connectivity in the SN, particularly in the left frontal middle and the bilateral anterior cingulate present an increased pattern for low stress levels with age, but reverse to a decreased connectivity for medium and more pronounced for high levels. This decrease for higher stress levels, may cause an abnormal integration of sensory data to segregate the most relevant among internal and external stimuli in order to guide behavior and also difficult the decision-making process (Seeley et al., 2007;Menon and Uddin, 2010;Ferreira and Busatto, 2013). Interestingly, we have previously shown that chronic stress impacts reversibly the decision-making process (Soares et al., 2012).

In the interaction age*mood, it was interesting the finding showing that in the left cerebellum of the SMN, there was an increase with aging for low depressive mood levels and a decrease for medium and high levels. These functional patterns were paralleled by a volumetric reduction of the total SMN during aging and also in participants with higher depressive mood levels. These results show that depressed participants may have even more difficulties in sensorial and motor tasks with age, compared to the ones associated with the normal aging process (Fox and Raichle, 2007;Habas et al., 2009).

The subsequent analysis in this study considered a more complex interplay. In fact, stress and mood are intrinsically connected states that interplay over the lifespan (Calabrese et al., 2009). This interplay is most significant at combined high stress and depressive mood levels (but also for combined low levels). The increase in functional connectivity in the DMN (left frontal medial orbitofrontal and right anterior cingulate) and in the HVN (right fusiform) suggests that these networks are particularly sensitive to this interplay, and only for mixed high and low stress and mood levels they follow the normal aging pattern of connectivity decline (Allen et al., 2011;Mowinckel et al., 2012). The critical effect of this interplay on the DMN and the stress contribution, leads to an increase in the functional connectivity, contrary to the decreases associated with the natural aging process and depression state (Damoiseaux et al., 2008;Andreescu et al., 2011;Wu et al., 2011). Curiously, the left parietal inferior region of the VAN presented the same pattern, but here with a strong stress influence.

Another finding of this study relates to the notion that when stress and mood are concordant (both low or high scores in PSS and GDS), the age pattern on the bilateral temporal superior of the AN, the right frontal superior medial of the DMN and the right frontal middle of the VAN operates as expected (Allen et al., 2011); however, in subjects with either only high PSS or GDS scores, these factors influence more than the typical aging effect.

During task performance, in the DMN, specifically in the right frontal superior medial region, there was a typical decrease in deactivation with age but only in subjects presenting both low stress and depressive mood levels. In fact, we found increased deactivation with age in individuals presenting high stress, independently of their mood levels, illustrating the predominance of the stress effect. Noticeably, for both low and high levels of stress and mood, there was an increase in the deactivation of the right precentral region of the SMN with aging, which shows the important impact of these states (especially the depressive mood level) on the normal aging course.

Limitations and conclusions

This study presents some important limitations that should be noticed. The functional analyses do not cover the whole cortex and thus do not provide a full description of brain functional architecture; for example the limbic system, crucial in the stress and mood states, was not analyzed. Another limitation relates to the fact that stress and mood evaluation was based only in psychological scales, without

complimentary assessment of biological markers. Finally, the study sample included only older adults and was a cross-sectional design, precluding a complete lifespan assessment, from childhood to elderly ages and longitudinal characterization.

To the best of our knowledge there are no prior reports that evaluate the interplay between stress and mood on RSNs in aging. In this study we have shown the critical influence of stress and mood, especially at higher levels, in different RSNs and in different regions of the same network. These combinatorial approach may contribute to clarify some literature inconsistencies regarding alterations in functional connectivity and deactivation patterns in aging and depression studies (Allen et al., 2011;Mowinckel et al., 2012;Wang et al., 2012;Chou et al., 2013;Ferreira and Busatto, 2013;Kerestes et al., 2013). Moreover, we expect also to enlighten the association between RSNs activation/deactivation abnormalities and several states that may lead to neuropsychiatric disorders (Liao et al., 2010;Meda et al., 2012;Whitfield-Gabrieli and Ford, 2012) that prevail in elder individuals.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tables

Table 1

Effect of age, stress and mood on resting state brain networks (multiple regressions, corrected for multiple comparisons, p <0.05).

Effect	Correlation	RSN	Regions	Peak MNI coordinates	Cluster size (voxels)	Maximum Z score
Age	Positive	VAN	Frontal superior (left)	-21, 21, 60	71	3.23
			Frontal superior (right)	24, 36, 42	53	2.99
	Negative	DAN	Parietal inferior (left)	-51, -27, 42	82	3.65
		DMN	Anterior cingulate (right)	3, 42, 3	601	3.98
			Frontal medial orbitofrontal (right)	3, 54, 0		3.41
			Precuneus (right)	12, -63, 24	145	3.61
		PVN	Calcarine (left)	0, -72, 15	180	2.97
			Calcarine (right)	12, -75, 6		2.79
		SMN	Cerebellum (left)	-3, -54, -12	104	3.11
			Cerebellum (right)	15, -60, -18		2.55
		SN	Frontal superior (left)	-18, 6, 63	142	3.76
			Frontal superior medial (left)	-3, 27, 42		2.66
			Superior motor area (left)	0, 18, 54		2.51
PSS	Positive	AN	Insula (left)	-45, -9, -3	40	2.80
			Temporal superior (left)	-57, 0, -6		2.74
		DMN	Frontal superior (left)	-21, 39, 42	63	3.34
			Frontal middle (right)	30, 36, 45	52	3.25
			Middle cingulate (left)	-12, -45, 33	87	2.94
			Posterior cingulate (right)	6, -39, 30		2.87
			Precuneus (right)	12, -45, 6	63	2.68
			Occipital middle (left)	-36, -87, 27	68	2.47
			Frontal medial orbitofrontal (left)	-3, -51, -3	52	2.35
		PVN	Calcarine (left)	-12, -54, 6	65	3.43
		SMN	Cerebellum (left)	-3, -60, -18	146	3.46
		VAN	Occipital middle (left)	-42, -72, 33	233	3.72
			Parietal inferior (left)	-30, -78, 42		3.61
			Angular (left)	-48, -63, 30		2.66
			Frontal middle (left)	-39, 15, 36	44	3.32
			Frontal middle (right)	36, 36, 39	162	2.96
			Frontal superior (right)	24, 24, 48		2.72
			Angular (right)	45, -69, 36	44	2.62
GDS	Negative	DMN	Frontal middle (right)	27, 39, 42	69	3.68
			Cuneus (left)	-6, -66, 24	59	3.29
			Calcarine (left)	-9, -63, 15		2.51
			Frontal medial orbitofrontal (left)	-3, 60, -9	154	2.66
			Anterior cingulate (left)	-3, 54, 0		2.64
			Frontal medial orbitofrontal (right)	9, 39, -6		2.60
		PVN	Cuneus (right)	6, -75, 18	51	2.83
		VAN	Angular (right)	48, -60, 36	118	3.41
			Parietal inferior (right)	57, -60, 42		2.59
			Frontal middle (right)	39, 15, 60	50	3.09
			Frontal middle (left)	-36, 15, 39	45	2.99

Table 2

Effect of age, stress and mood on task induced deactivations (multiple regressions, corrected for multiple comparisons, p <0.05).

Effect	Correlation	RSN	Regions	Peak MNI coordinates	Cluster size (voxels)	Maximum Z score
Age	Negative	DMN	Fusiform (left)	-30, -40, -14	53	2.89
PSS	Positive	AN	Temporal superior (left)	-51, -1, -8	104	3.52
		SMN	Cerebellum (left)	-18, -37, -26	57	2.55
GDS	Negative	DMN	Posterior cingulate (right)	9, -40, 25	226	2.92
			Posterior cingulate (left)	-9, -49, 25		2.48
			Precuneus (right)	18, -49, 13		2.45
			Frontal superior medial (left)	0, 62, 13	64	2.43

Table 3

Effect of the interplay between stress, mood and aging on resting state brain networks (multiple regressions, corrected for multiple comparisons, p <0.05). M- Mean; SD - Standard Deviation; LPSS - Low Perceived Stress Scale scores; HPSS - High Perceived Stress Scale scores; LGDS – Low Geriatric Depression Scale scores; HGDS – High Geriatric Depression Scale scores.

Interaction	Correlation	RSN	Regions	Peak MNI coordinates	Cluster size (voxels)	M-SD/M/M+SD	Maximum Z score
Age*PSS	Positive	DMN	Frontal middle (right)	3, 39, -15	78	-0.0683/-0.0120/0.0290	3.46
		VAN	Parietal inferior (right)	45, -45, 45	163	-0.0503/0.0114/0.0731	4.13
			Angular (right)	42, -51, 36			3.79
			Occipital middle (left)	-42, -72, 33	73	-0.0199/0.0247/0.0693	2.92
	Negative	SN	Frontal middle (left)	-36, 45, 21	101	0.0023/-0.0399/-0.0821	3.24
			Anterior cingulate (right)	6, 27, 24	93	0.0178/-0.0171/-0.052	3.07
			Anterior cingulate (left)	-6, 24, 24			2.84
Age*GDS	Positive	AN	Temporal superior (left)	-54, -6, -3	40	-0.0297/0.0033/0.0362	2.67
		DMN	Temporal middle (right)	42, -66, 21	76	-0.0414/-0.0078/0.0258	2.92
		VAN	Frontal middle (right)	33, 15, 48	90	-0.0368/-0.0092/0.0184	2.97
	Negative	SMN	Cerebellum (left)	-6, -57, -9	62	0.0254/-0.0230/-0.0714	4.02
						LPSS_LGDS/LPSS_HGDS/HPSS_LGDS/HPSS_HGDS	
Age*PSS*GDS	Positive	DMN	Frontal medial orbitofrontal (left)	-6, 45, -9	135	0.0102/-0.0513/-0.0734/0.0275	3.57
			Anterior cingulate (right)	9, 33, 3			2.73
		HVN	Fusiform (right)	27, -84, -6	90	0.0484/-0.0123/-0.1012/0.0356	3.27
		VAN	Parietal inferior (left)	-30, -81, 45	47	0.0201/-0.036/-0.0581/0.0783	2.53
	Negative	AN	Temporal superior (right)	57, -12, 9	41	-0.006/0.0402/0.0909/-0.0047	2.81
			Temporal superior (left)	-54, -27, 12	83	-0.0439/0.0927/0.0759/-0.0048	2.68
		DMN	Frontal superior medial (right)	6, 48, 33	96	-0.0627/0.0191/0.0217/-0.0544	3.88
		VAN	Frontal middle (right)	51, 24, 45	116	-0.0267/0.0841/0.0698/-0.0191	3.85

Table 4

Effect of the interplay between stress, mood and aging on task induced deactivation (multiple regressions, corrected for multiple comparisons, p <0.05). M- Mean; SD - Standard Deviation; LPSS - Low Perceived Stress Scale scores; HPSS - High Perceived Stress Scale scores; LGDS – Low Geriatric Depression Scale scores; HGDS – High Geriatric Depression Scale scores.

Interaction	Correlation	RSN	Regions	Peak MNI coordinates	Cluster size (voxels)	M-SD/M/M+SD	Maximum Z score
Age*PSS	Positive	DMN	Frontal superior medial (left)	-9, 44, 43	335	-0.0111/0.0061/0.0233	2.80
			Frontal superior medial (right)	12, 53, 16			2.72

			Precuneus (right)	9, -58, 34	69	-0.0094/0.0064/0.022	2.59
			Precuneus (left)	0, -61, 40			2.51
Age*GDS	Negative	DMN	Anterior cingulate (left)	-9, 44, 1	94	0.0161/0.0015/-0.0131	2.85
						LPSS_LGDS/LPSS_HGDS/HPSS_LGDS/HPSS_HGDS	
Age*PSS*GDS	Positive	SMN	Precentral (right)	36, -16, 58	313	0.0172/-0.017/-0.0142/0.0143	2.57
	Negative	DMN	Frontal superior medial (right)	12, 53, 22	65	-0.0208/0.0166/0.0445/0.0091	2.86

Table S1

Standardized regression coefficient of the significant Age*PSS*GDS interactions.

Region / Factor	**Resting state networks - cluster peak**							**Task-induced deactivation - cluster peak**	
	Frontal medial orbitofrontal (left)	Fusiform (right)	Parietal inferior (left)	Temporal superior (right)	Temporal superior (left)	Frontal superior medial (right)	Frontal middle (right)	Precentral (right)	Frontal superior medial (right)
Age	-0.2490	-0.0614	0.0072	0.1498	0.1902	-0.2281	0.2675	0.0037	0.4862
PSS	0.0374	0.0153	0.3181	0.0640	0.1298	-0.0336	-0.2382	0.0070	-0.2157
GDS	-0.0438	-0.0711	-0.1968	-0.1514	-0.0844	-0.2126	0.1476	-0.1841	0.1078
Age*PSS	-0.0138	-0.2122	0.0598	0.0187	0.0354	0.0326	-0.0164	-0.0010	0.5690
Age*GDS	0.1129	0.1587	0.1332	-0.1760	0.0888	0.0170	0.0544	-0.0583	0.0202
Age*PSS*GDS	0.4645	0.4119	0.3197	-0.3529	-0.3449	-0.4713	-0.4942	0.6357	-0.7168

Figures

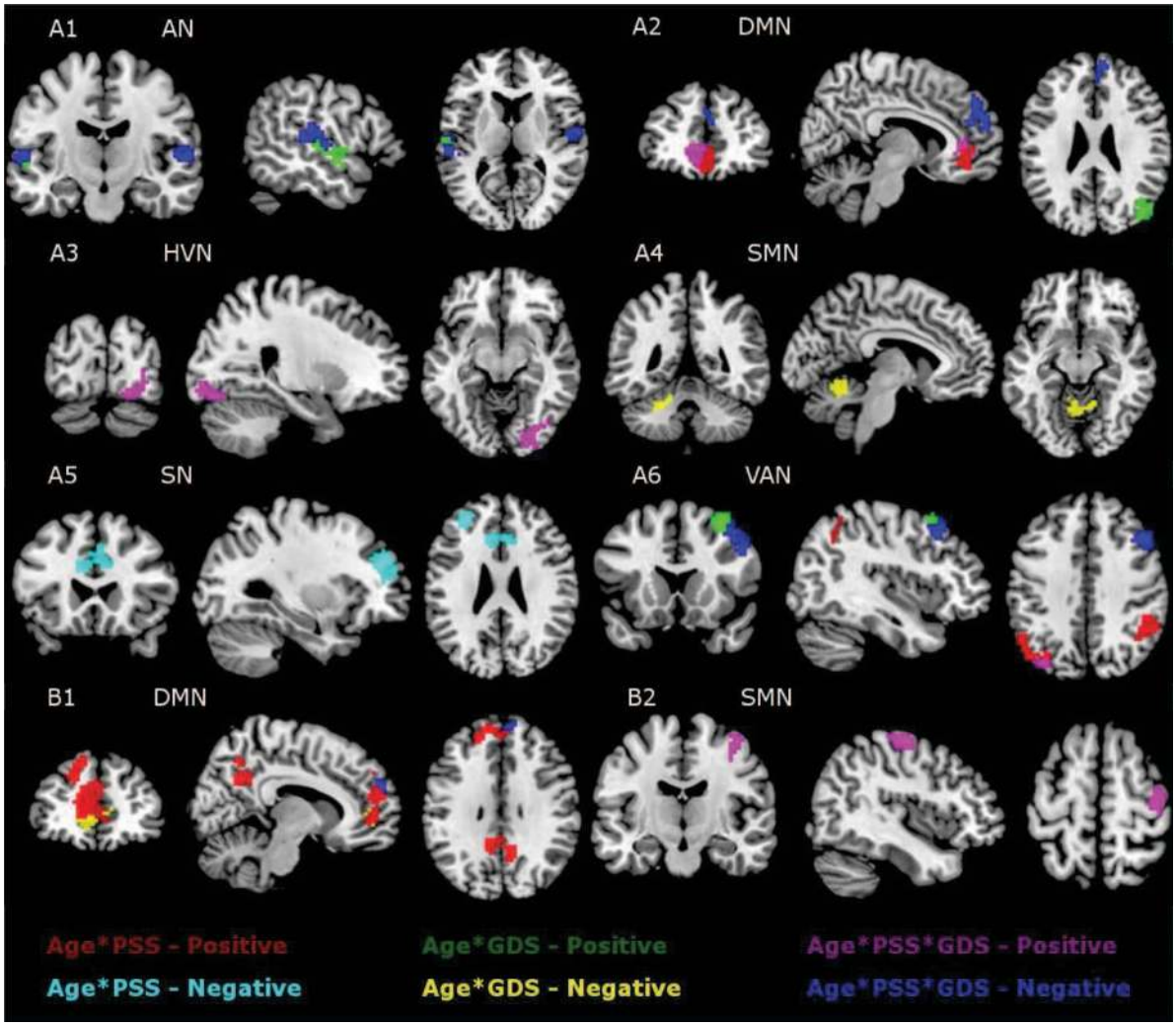

Figure 1. The interplay between stress and aging and stress and mood in Resting State Networks (RSNs) during resting periods (A's) and task-induced deactivations (B's). The images depict areas with significant interaction effects of age*PSS positive (in red) and negative (in cyan), age*GDS positive (in green) and negative (in yellow) and age*PSS*GDS positive (in violet) and negative (in blue) (results considered significant at a corrected for multiple comparisons $p<0.05$ threshold); A1 auditory network (AN); A2 and B1 default mode network (DMN); A3 high visual network (HVN); A4 and B2 sensorimotor network (SMN); A5 salience network (SN); A6 ventral attention network (VAN).

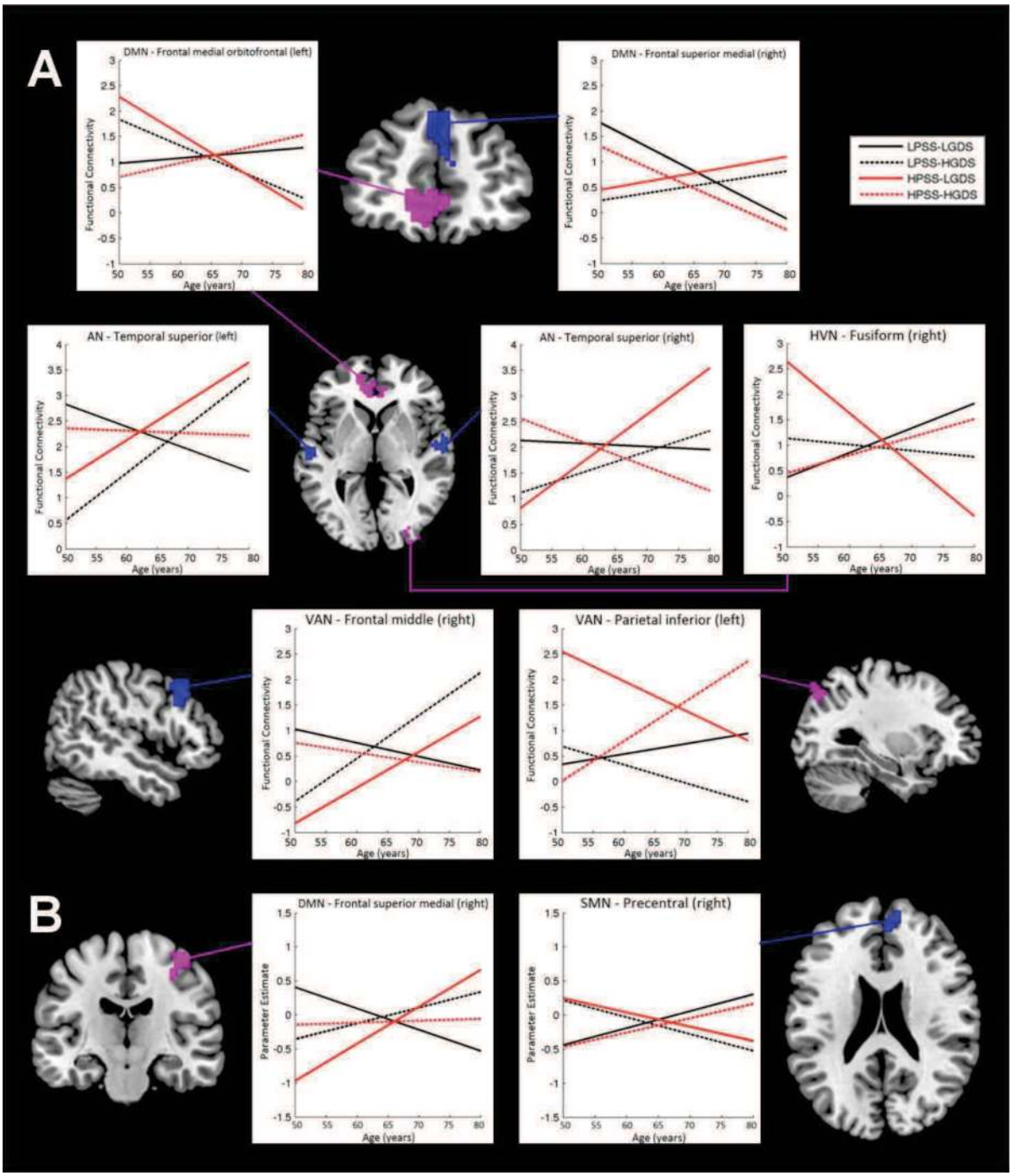

Figure 2. Regions showing significant age*PSS*GDS interactions and corresponding graphical representation in Resting State Networks (RSNs) during resting periods (A) and task-induced deactivations (B). Each graphic line is the regression line between age and functional measures for: Low PSS values (LPSS, black lines), combined with Low GDS (LPSS-LGDS, solid line) or with High GDS (LPSS-HGDS, dotted line); and High PSS value (HPSS, red lines), combined with Low GDS (HPSS-LGDS, solid line) or High GDS (HPSS-HGDS, dotted line).

Chapter 4

Discussion

4 - Discussion

Aging and stress are two intrinsically correlated concepts that have nowadays reached an inestimable importance as factor shaping brain structure and function. With the constant and fast medical and technological developments, life expectancy has reached unprecedented high rates worldwide. On the other hand, in modern societies, with increasing human competitive attitude, ever changing life styles and extreme workloads, stress has triggered unparalleled health damages, including anxiety, depression and disease states (Lucassen et al., 2014). Investigating the brain structural and functional alterations during the lifespan, in combination with understanding the effect of experienced or perceived stress, has become one of the most crucial challenges in the comprehension of aging and cognitive function in later ages.

The main research question addressed in this thesis is how maladaptive stress impacts on the sequential structural and functional modulation of stimulated and resting brain networks in healthy aging. Firstly, the stress influence on the brain dynamics underlying the decision making processes and its potential reversibility was explored (presented in Chapter 3.1). The work was complemented by the study of stress effects during the resting state, and exploring the deactivation behavior, to investigate the stress impact (Chapter 3.2) and its reversibility in this states (Chapter 3.3). Finally, the influence of aging, stress and mood, and their constant interplay on brain structure and function in a cohort of older adults were addressed (Chapters 3.4 and 3.5).

4.1 Stress: Structural and Functional Brain Changes in Adulthood

Stress response is vital for the survival of every living organism, however maladaptive responses to stress can produce changes in the brain and affect cognitive processes, attention and executive functions (Sapolsky, 1996;Lupien et al., 2009;McEwen and Gianaros, 2011), such as decision-making (Starcke and Brand, 2012). Decision making tasks are one of the most frequent cognitive processes on a daily basis and, therefore, during lifespan. Interestingly, along with language, decision making is known to be relatively preserved during healthy aging (Sanfey and Hastie, 2000;Kovalchik et al., 2005).

The selection of the appropriate actions and life-altering choices in particular situations is an extremely dynamic process. Actions can be selected based on their consequences (for example, when we first select the best route to drive from home to work, depending on the traffic information, weather conditions and time available). This goal-directed behavior is crucial to face the ever-changing environment, but it demands an effortful control and response monitoring. To increase efficiency, one can automatize recurring decision processes as habits (or rules). Habitual responses no longer need the evaluation of their consequences and can be elicited by particular situations or stimuli (for example, after driving to work for some time in the established route, we automatically, when entering the car, go in that direction without much conscious thought or decision). The ability to shift back and forth between these two types of strategies is necessary for appropriate decision-making. For example, in a novel situation, it may be crucial to be able to inhibit a habit and use a goal-directed strategy (for example, if we need to go to another place first, besides work, it may be necessary to use a route different than our habitual one to work).

In Chapter 3.1 it was shown that subjects exposed to chronic stress rapidly shift toward habitual strategies (in other words, following the above example, stressed individuals are more likely to choose the habitual route, even when the more appropriate choice would be to go a different way). More specifically, findings demonstrated that prolonged exposure to stress triggers a reorganization of corticostriatal circuits that determine decisions under instrumental tasks (instrumental behavior is determined by the association between an action and an outcome, as tested in this thesis; in this form of responses, actions can be either 'goal-directed' or 'habitual'). By testing the participants from the two distinct conditions - control versus stressed - in a paradigm in which they work for a reward, the pattern of their instrumental responses can be discriminated. An atrophy of the associative corticostriatal circuit that rules goal-directed actions, in parallel with a hypertrophy of the sensorimotor corticostriatal network, responsible for habitual behaviors, was found in the young participants displaying signs and symptoms of stress. Moreover, these structural changes were associated with a decreased activation of this circuit in instrumental tasks. Most importantly, stressed individuals had a bias to habits in their decision-making processes. Interestingly, we also demonstrated the remarkable plasticity of these neuronal circuits, by showing that after a stress-free period, both the structural and

the functional changes were reverted and the pattern of decision in previously stressed subjects was again goal-directed.

Importantly, other studies, in distinct experimental conditions, have shown volumetric variations in a similar (or even shorter) time frame (Lai and Hsu, 2011;Younger et al., 2011). The literature on the neurobiology of stress has also demonstrated rapid structural changes triggered by stress exposure (Sousa et al., 2000;Popoli et al., 2012). These changes typically occur at the dendritic level and are likely to represent alterations in synaptic connectivity between different brain regions. Alterations in several molecules, including trophic factors and adhesion molecules, are assumed to underlie such structural changes, which occur in opposite directions in distinct brain regions. Importantly, these changes are associated with functional impairments at specific neural circuit level. Our results confirmed a divergent structural reorganization of corticostriatal circuits in humans exposed to prolonged stress, with hypertrophy/overactivation of the sensorimotor and atrophy/underactivation of the associative corticostriatal circuits. This frontostriatal reorganization was accompanied by a shift toward habitual strategies, affecting the ability of stressed individuals to perform actions based on their consequences.

With this work, it was for the first time demonstrated that such behavioral changes are linked to alterations in the frontostriatal networks in humans, thus providing insights into the neural circuits underlying the shift between goal-directed actions and habitual behavior, which can lead to dysfunctional decision-making upon exposure to stress. Noticeable, this stress-decision bias was found to be reversible after the end of the stress exposure, with signs of plasticity both at the structural and at the activational levels. This is in accordance with previous data in rodents and primates showing that stress-induced changes in the structure of the prefrontal cortex are reversible, at least in young subjects (Cerqueira et al., 2007). Additionally, these results expanded on previous studies showing that acute stress can modulate decision-making processes. In fact, it has been shown in young animals that stress causes reversible morphological remodelling of prefrontal neurons; however, in contrast, middle-aged and aged animals failed to show the same morphological reversibility (Bloss et al., 2010). In the same line with these findings, McEwen and Morrison have described particular impact of stress on both brain structure and function on the prefrontal cortex during infancy and in adolescence, with a decreasing stress resilience with aging (McEwen and Morrison, 2013).

As a consequence of the structural/activational reorganization, we found a behavioral restoration of decision-making strategies in subjects that had been exposed to stress. This novel finding is of paramount importance inasmuch as optimization of decision-making processes confers an important advantage in response to a constantly changing environment. Indeed, under conditions of maladaptive stress, there is a reduced ability to shift from habitual strategies to goal-directed behaviors, even when conditions would recommend that shift. However, it is also true that the frontostriatal networks, even after prolonged stress, preserve the plastic properties that allow for a functional recovery once the stressful stimuli are gone. Moreover, the described stability of decision-making processes along with the decreased prefrontal stress resilience during aging, raise the dichotomy of whether this decreased resilience overlaps the decision-making stability especially in older adults, or not. These results are not only of relevance to understand the mechanisms through which stress is modulating decision-making in both physiological and pathological conditions, but they certainly also pave the way for interventional therapies that empower stresscoping mechanisms during lifespan.

Apart from one of the most frequently used cognitive processes during the lifespan, decision-making, it is also of upmost importance to characterize the stress influence over the most prevalent state during life, resting condition. To this end, the work presented on Chapters 3.2 and 3.3 addresses the stress impact and reversibility effects on brain resting state networks.

We showed, for the first time, that stress increases the activation of the DMN at rest in the ventral medial prefrontal cortex, posterior cingulate cortex, adjacent precuneus and inferior parietal cortex. Based on previous studies highlighting the role of the DMN at rest (Greicius et al., 2003;Mason et al., 2007;Raichle and Snyder, 2007;Andrews-Hanna et al., 2010), these results suggest an augment in self-reflective thoughts but also an increased dynamic interaction between emotional processing (i.e., ventral regions) and cognitive functions (i.e., dorsal regions) in stressed participants, as a result of increased activity in the anterior components of the DMN. The increases in the posterior regions of the DMN observed in stressed participants, particularly the posterior cingulate cortex and the inferolateral parietal lobes, are likely associated with longer processing of emotionally salient stimuli and episodic memory retrieval (Maddock et al., 2003;Wagner et al., 2005). Interestingly, after stress recovery, a decreased resting functional connectivity in the DMN (DMN plasticity) and an increase in a specific region, in the right anterior cingulate cortex in controls when compared with stress-recovered

participants was observed. These results (Chapter 3.3) confirm the increased functional connectivity of the DMN at rest after chronic stress exposure (Chapter 3.2), and are also in line with the findings of Vaisvaser and colleagues using an acute social stress model (Vaisvaser et al., 2013). Notably, this increase in functional connectivity in the DMN after chronic stress was paralleled by a volumetric contraction, with specific reductions in the left posterior cingulate cortex and left and right parietal inferior regions. These are likely to reflect the stress-induced atrophic effects in cortical regions, observed in several previous reports (Dias-Ferreira et al., 2009;Liston et al., 2009), including the findings described in Chapter 3.1. Nevertheless, the alternative explanation of a reduction of the number of neurons recruited cannot be completely excluded at this moment. Contrary to the DMN volume reductions described, the study presented on Chapter 3.3 shows that while the functional remodeling of DMN endures, the structural changes (volumetry) of the brain areas involving this network are still absent after the recovery period. This difference in our results from distinct studies may be related with the limited sample size.

The characterization of changes in functional connectivity between brain networks subserving distinct psychophysiological functions is of relevance to understand the symptoms triggered by stress, namely mood and anxiety changes (Bessa et al., 2009). Indeed, stress is well-known to be a precipitating factor for mood changes, a finding confirmed in the present work by the increased scoring in a validated scale of depression. Interestingly, in depressed participants, an increased fMRI connectivity pattern between the ''dorsal nexus'' (a bilateral dorsal medial prefrontal cortex region) and the DMN has been reported (Sheline et al., 2010); importantly, this hyperconnectivity has recently been shown to be reduced by antidepressants (McCabe and Mishor, 2011;McCabe et al., 2011;Scheidegger et al., 2012). Strikingly, we found an increased functional connectivity between medial prefrontal cortex (part of dorsal nexus) and posterior cingulate cortex in stressed participants. The finding of increased activation in resting states of the anterior cingulate cortex is also of relevance for the affective processing of negative information, known to be altered in depressed patients (Greicius et al., 2007;Lemogne et al., 2012;Zhu et al., 2012), but may additionally be related to a higher vigilance and alertness in stressed participants.

Another behavioral dimension targeted by stress is emotional hyperactivity; again, herein we confirmed that stressed individuals score higher in the Hamilton anxiety scale. The finding of increased

connectivity between the superior parietal, right middle occipital and left medial and superior frontal in the stress group suggests that brain regions belonging to the DAN could play a role in emotional regulation and in the higher state of vigilance and awareness, which is typical of stressed-induced hyperemotionality. Interestingly, DAN did not reveal a functional recovery after the cessation of the exposure to stress, maintaining a sustained pattern of increased functional connectivity, even though subjects displayed a recovery of anxiety-symptoms. The DAN has been also associated with top-down attention processes as inhibitory control, working memory, and response selection. These cognitive processes depend upon the prefrontal integrity (dorsal frontal regions), which are brain regions vulnerable to the effects of stress, as already described (Cerqueira et al., 2007;McEwen and Morrison, 2013). Indeed, animal studies evidenced stress-related prefrontal remodeling (e.g., selective atrophy of the prefrontal cortex, elimination of dendritic spines) after chronic stress exposure (Cerqueira et al., 2007;Gourley et al., 2013). This stress-related prefrontal structural reorganization has been associated with impaired perceptual attention, behavioral flexibility, and decision making in rodents (Cerqueira et al., 2005;Dias-Ferreira et al., 2009) and humans (as described in Chapter 3.1 and by (Yuen et al., 2012)). Moreover, studies analyzing the recovery of posttraumatic stress disorder reported that an increased thickness of the dorsolateral prefrontal cortex was associated with greater symptomatic alleviation (Lyoo et al., 2011).

In accordance with these data evidencing emotional hyperactivity (translated by increased anxiety states and related vigilance and alertness), we observed a greater activation of the sensorimotor and primary visual networks in stressed participants. This suggests a hyperactivation of cortical and subcortical attention areas oriented to perception–action, brain systems required to stress-related fight or flight responses. Also, this is in line with disrupted sensorimotor gating mechanisms (necessary to mediate threat selective attention to the most salient signals and ignore other non-relevant signals that emerge simultaneously) found in stress conditions (Grillon and Davis, 1997). Therefore, our results suggest that stress induces an increase in the general level of alertness and motor response in the stress participants, as suggested by others (Pijlman et al., 2003). Following a stress free period, and after recovery we found a decreased functional connectivity in the SMN, and when compared to controls a decreased connectivity was also observed in both SMN and PVN. The concomitant SMN and PVN sustained increased functional connectivity is possibly associated with a motor and visual

readiness state that is required for the stress response. These results show a sustained pattern of increased functional connectivity in SMN and PVN, pointing to a less degree of plasticity when recovering from the impact of stress exposure.

Of relevance, our studies reveal not only differences in the pattern of activation of RSNs, but also important differences in deactivation of these networks. The VAN was found to be associated with task control function (Dosenbach et al., 2006;Dosenbach et al., 2007;Mantini et al., 2009) and to be implicated in ''salience'' processing (Seeley et al., 2007). Importantly, the greater functional connectivity found in the VAN during resting state fMRI in stressed participants, followed by its functional recovery after the cessation of the exposure to stress, is likely to be of relevance to understand the decreased functional deactivation of RSNs during task-focused behavior, suggesting a difficulty in moving from more oriented, self-related processes towards a tuning down task-focused behavior that requires allocation of attention and other cognitive domains. In fact, it has been shown that the VAN has an important role in cognitive control related to switching between the DMN and task-related networks (Sridharan et al., 2008), even though, the increased rest activity of the DMN in stressed participants might simply require an increased effort for its deactivation during the transition from rest to task-focused activity, which might impact on functional performance. In fact, the DMN deactivation has been associated with reallocation of attentional resources to cognitively demanding tasks (Hu et al., 2013), and task-induced RSNs deactivation is correlated with behavioral performance. As an example, stronger DMN deactivation in a working memory task predicts better performance (Uddin et al., 2009;Mayer et al., 2010). Increased deactivation was also observed in the control group and in the stress-recovered participants (compared to stressed individuals). Moreover, the present findings of increased functional connectivity in the posterior cingulate cortex in stressed participants than in controls, along with a decreased deactivation in precuneus is of notice. The posterior cingulate cortex and the precuneus are sometimes referred to as a "pivotal hub of the DMN" in social cognition and in theory of mind (Gentili et al., 2009). Our results are also in line with previous fMRI studies revealing a lower deactivation in the precuneus on anxious patients (Carey et al., 2004;Gentili et al., 2009). As a matter of fact, here the findings sustain the hypothesis that the precuneus would be able to suspend functional connectivity within the DMN, being related to the perception of socially relevant emotional state and self-related mental representations (Buckner et al., 2008). This is consistent with

previous studies showing that failure of RSNs deactivation is present in several neuropsychiatric diseases such as schizophrenia (Pomarol-Clotet et al., 2008), first-episode psychosis (Guerrero-Pedraza et al., 2012), mild cognitive impairment and mild Alzheimer's disease (Rombouts et al., 2005).

In the work presented on Chapters 3.2 and 3.3, it was analyzed how the RSNs respond and change after prolonged stress periods and following recovery. The hypothesis was that there would be a significant stress impact on RSNs, both during resting state and task-induced deactivations, followed by a continuous recovery effect, in which the connectivity would be decreasing from stress toward the control group. We confirmed for the first time an increased resting stress-induced functional connectivity in the DMN (paralleled by a volumetric reduction), DAN, VAN, SMN and PVN, and also a disrupted deactivation pattern in these networks during a decision-making task. The plasticity of the RSNs after recovery from the impact of chronic stress-induced changes was further explored, being shown for the first time that all RSNs, with the exception of the DAN and AN, display a functional recovery after the cessation of the exposure to stress. Notably, the comparison with controls allowed to observe a return to the initial levels the functional connectivity of the DMN, VAN, and AN, but still a sustained pattern of increased functional connectivity of the DAN, SMN, and VN networks. These results suggest that DAN, SMN, and PVN are less plastic when recovering from the impact of stress exposure. Besides the functional plastic recovery in the connectivity of the RSNs at rest, it was also observed a continuum in the pattern of deactivation; that is, there was an increased deactivation from stress toward the control group in all the RSNs.

In conclusion, while there is substantial evidence for an association between RSNs activation/deactivation abnormalities and psychiatric disorders (Liao et al., 2010;Hahn et al., 2011;Woodward et al., 2011;Brier et al., 2012;Meda et al., 2012;Wee et al., 2012), this was to the best of our knowledge the first study exploring the functional significance of RSNs patterns after sustained stress exposure and the functional remodeling of the activation-deactivation pattern of the RSNs following chronic-stress recovery. The similarities of the present findings with those evidenced by depressed and anxious patients clearly suggest that these patterns of abnormal activity of RSNs in stressed participants may represent a neurobiological marker for the stress-induced increased emotionality. The present data, however, also reveals a deficit in the deactivation of the RSNs that

reflects an impaired turning off of the un-activated state. Importantly, the specific pattern of plasticity suggests that some RSNs may be a tool for monitoring effective anti-stress interventions, similar to that proposed to verify the effect of the treatments in several neuropsychiatric diseases (Achard and Bullmore, 2007).

Although the RSNs studied herein provide a valuable framework through which alterations of functional connectivity driven by chronic stress exposure and after recovery can be assessed, they do not cover the whole cortex and thus do not provide a complete description of brain functional architecture. Another limitation of this work is related with the impossibility to provide information on the functional connectivity of RSNs with several regions of the limbic system. In addition, one must still be cautious about the neurophysiological relevance of RSNs, namely on the functional significance of these task-networks when dynamically assembled and modulated during different behavioral states. Our results should also be interpreted with caution mainly due to the reduced size of our sample; therefore, future studies should try to replicate these observations in a larger sample, ideally using exactly the same participants in all conditions, as controls, stressed, and after recovery.

4.2 Stress: Structural and Functional Brain Changes in Aging

Following the investigation and confirmation of the stress-induced alterations on RSNs, the next logical step was to validate these findings in a different population and under different stress factors (older adults), to explore the aging effects and, finally, to study the interplay between stress, mood and (healthy) aging. Several studies have consistently described the critical impact of the aging process, stress and mood on brain structure and function (Fjell et al., 2008;Andreescu et al., 2011;Walhovd et al., 2011;Ferreira and Busatto, 2013;Fjell et al., 2013;Tamnes et al., 2013). Nevertheless, most of the neuroimaging studies focused on the effect of individual factors, precluding the critical influence of the complex interplay among various concurrent processes across the lifespan, such as stress exposure (or perceived experienced stress) and/or variations in depressive mood. In this work, we dissected the influence of these life events and how they interplay and impact throughout life, using a multimodal neuroimaging approach. The present data, i) replicates the most consistent findings in the literature regarding the influence of the aging course, stress and major depression on brain GM and WM volumetry and RSNs activation/deactivation, ii) reveals that different stress and mood levels have

different impact across aging, and iii) indicates that the constant interplay between stress and mood states has significant effects in the brain volumetry and RSNs during lifespan.

With aging we found the typical tendencies of structural and functional decline. A global, as well as a regional, pattern of volumetric GM and WM decrease was observed, accompanied by an expansion of the ventricles, choroid plexus and CSF spaces, reflecting an atrophy of brain parenchyma. Total and subcortical GM was decreased with age, including the left accumbens, both hippocampi and total cortical WM, frontal, temporal, occipital and parietal WM regions also presented significant volumetric decreases with age. Similar findings have been consistently reported in the literature, both in cross-sectional and longitudinal studies (Raz et al., 2005;Smith et al., 2007;Abe et al., 2008;Fjell et al., 2009;Walhovd et al., 2011;Tamnes et al., 2013). Specifically, however, herein we found a higher decrease in global WM than GM, confirming a striker WM deterioration after the fifth decade (Gunning-Dixon et al., 2009;Lemaitre et al., 2012). This global WM volume decline, more pronounced in frontal regions (orbitofrontal, superior frontal and rostral middle frontal) was paralleled by an increase with age of the WM hypointensities volume, a measure of lesion burden (Leritz et al., 2014).

In resting state functional connectivity, a global connectivity decrease across the RSNs was found, which is in line with the central tendencies of previous reports regarding age-induced differences (Damoiseaux et al., 2008;Allen et al., 2011;Ferreira and Busatto, 2013). Paralleled with this decrease, participants also showed a decreased deactivation of the DMN with increasing age, illustrating the often reported difficulty in adaptive switching from a “default mode” to task-performance mode in aged individuals (Grady et al., 2010). Interestingly, however, the VAN displayed increased connectivity with aging, suggesting that the aging process is heterogeneous in terms of its consequences at the brain network level (Filippini et al., 2012;Mowinckel et al., 2012). In our older sample, and in line with the findings in young participants, an increased connectivity in the more stressed participants (that is, higher PSS scores) in the AN, DMN, PVN, SMN and VAN, was found. This global increase in the functional connectivity of the RSNs suggests augmented self-reflective thoughts and emotional processing triggered by stress during the lifespan. During task performance, these participants presented an increased deactivation of the AN and of the SMN pointing to an easier “disconnection” of auditory and sensorial stimuli when shifting from task to resting periods. Interestingly, this sensorial disconnection is similar to what occurs in young subjects. In terms of RSNs volumetric analysis, a

tendency for global DMN atrophy for higher PSS scores was noted, similar to what takes place in young participants, and suggesting the existence of both structural and functional reorganizations under stress periods along the lifespan.

Additionally, we also observed a decreased functional connectivity in the DMN, PVN and VAN in participants with higher GDS scores (that is, more depressive mood), along with an increased difficulty to deactivate the DMN during cognitive task performance. These results support other findings pointing to a decrease in RSNs connectivity in elderly depressed subjects (Veer et al., 2010;Andreescu et al., 2011;Wu et al., 2011).

The impact of stress and mood, either separately or in combination, differs across different life stages (Calabrese et al., 2009;Sousa and Almeida, 2012). In the work herein presented, in the volume of the left frontal pole GM, there was an inversion from decreases at low stress levels to increases at high levels with age. Functionally, in the DMN, particularly in the right frontal middle region, the functional connectivity, for low and medium stress levels decreased with age, in line with the typical aging findings (Damoiseaux et al., 2008;Hafkemeijer et al., 2012). Interestingly, however, for higher stress levels it increased with aging, as we showed in young stressed subjects and in line with the report from Vaisvaser and colleagues, which shows that stress exposure has a similar effect in RSNs across the lifespan (Vaisvaser et al., 2013). These results show the critical stress impact in the frontal regions, especially at higher stress levels, leading to inversions from the normal aging patterns (Cerqueira et al., 2007;Lupien et al., 2009). Importantly, behavioral stress affects, with possible reversibility, both structure and function of the prefrontal cortex, a region where neurons become less efficient with aging (McEwen and Morrison, 2013). Additionally, on the left frontal pole WM, there is an increase in the volume reduction with age for higher depressive mood levels, in line with several previous findings (Konarski et al., 2008;Kong et al., 2014), showing the higher susceptibility and variability of this region.

In the right temporal middle of the DMN, relative to the mood*age interaction, the subjects with higher GDS scores, with a similar behavior to what occurs at higher stress levels, displayed an increased functional connectivity in the posterior regions of DMN, which fit other observations (Andreescu et al., 2011). These results point to a more pronounced stress and depressive mood effect (at high levels)

over the normal aging pattern on DMN connectivity decline. These apparently contrasting effects observed in the DMN across aging that result from the superimposition of concomitant factors were also observed during cognitive performance, in the bilateral frontal superior medial and the precuneus of the DMN, where the deactivation decreases for low stress levels but increases for medium and high levels with age. In fact, for subjects with low stress levels the typical decline in DMN during aging prevails, whereas for medium and high stress levels there is an increase in the deactivation, suggesting a dominancy of the stress effect, and curiously contrary to what occurs in young stressed participants (who displayed decreased deactivation when compared with controls). Also in the DMN, specifically in the left anterior cingulate, the deactivation increases with age for better mood levels, but it decreases for higher depressive levels, confirming the increased difficulty of older and depressed participants to deactivate the DMN during task performance (Damoiseaux et al., 2008;Sheline et al., 2009).

The differential pattern of age effects on the RSNs when combined with stress and GDS scores is, however, not confined to the DMN. For example in the VAN, in subjects with low stress levels, the connectivity in the right parietal inferior and angular and in the left occipital middle of the VAN decreases during aging, while for medium and, even more accentuated for high stress levels, the increase is in line with the aging and stress separated effects (Filippini et al., 2009;Mowinckel et al., 2012). The mood*age interaction also shows that in the right frontal middle of the VAN, for low and medium mood levels the connectivity decreases with age, though it increases in subjects with high GDS score levels. These results suggest that aged subjects with the more stress and lower depressive mood present higher bottom-up attention processes, mediation of exogenous stimulus-driven and salience processing during the aging course (Corbetta and Shulman, 2002;Seeley et al., 2007). It is also noticeable that the functional connectivity in the SN, particularly in the left frontal middle and the bilateral anterior cingulate present an increased pattern for low stress levels with age, but reverted to a decreased connectivity for medium and more pronounced for high stress levels. This decrease for higher stress levels, may cause an abnormal integration of sensory data to segregate the most relevant among internal and external stimuli in order to guide behavior and also difficult the decision-making process (Seeley et al., 2007;Menon and Uddin, 2010;Ferreira and Busatto, 2013). Interestingly, this finding is in line with our observation that chronic stress impacts reversibly the decision-making process.

Regarding the interaction age*mood, it was interesting the finding showing that in the left cerebellum of the SMN, there was a functional increase with aging for low depressive mood levels and a decrease for medium and high levels. These functional patterns were paralleled by a volumetric reduction of the total SMN during aging and also in participants with higher depressive mood levels. These results show that depressed participants may have even more difficulties in sensorial and motor tasks with age, compared to the ones associated with the normal aging process (Fox and Raichle, 2007;Habas et al., 2009). Structurally, several studies have reported volumetric reductions in temporal regions associated with mood disorders (Drevets et al., 2008;Son et al., 2013), and herein we observed the depressive mood impact, especially at higher levels, in the increased volumetric reduction of the WM and GM in the left temporal pole. In this study we also found an increased volumetric reduction with age at high depressive mood levels in the left superior parietal WM, in line with the literature pointing to WM decreases in older patients with major depressive disorder (Zeng et al., 2012).

The final analysis in this study considered a more complex interplay. In fact, stress and mood are intrinsically connected states that interplay over the lifespan (Calabrese et al., 2009). Functionally, this interplay was found to be most significant at combined high stress and depressive mood levels (but also for combined low levels). The increase in functional connectivity in the DMN (left frontal medial orbitofrontal and right anterior cingulate) and in the HVN (right fusiform) suggests that these networks are particularly sensitive to this interplay, and only for mixed high and low stress and mood levels they follow the normal aging pattern of connectivity decline (Allen et al., 2011;Mowinckel et al., 2012). The critical effect of this interplay on the DMN and the stress contribution, leads to an increase in the functional connectivity, contrary to the decreases associated with the natural aging process and depression state (Damoiseaux et al., 2008;Andreescu et al., 2011;Wu et al., 2011). Curiously, the left parietal inferior region of the VAN presented the same pattern, but here with a strong stress influence. Another important finding of this study relates to the notion that when stress and mood are concordant (both low or high scores in PSS and GDS), the age pattern on the bilateral temporal superior of the AN, the right frontal superior medial of the DMN and the right frontal middle of the VAN operates as expected (Allen et al., 2011); however, in subjects with either only high PSS or GDS scores, these factors influence more than the typical aging effect.

During task performance, in the DMN, specifically in the right frontal superior medial region, there was a typical decrease in deactivation with age but only in subjects presenting both low stress and depressive mood levels. In fact, we found increased deactivation with age in individuals presenting high stress, independently of their mood levels, illustrating the predominance of the stress effect. Noticeably, for both low and high levels of stress and mood, there was an increase in the deactivation of the right precentral region of the SMN with aging, which shows the important impact of these states (especially the depressive mood level) on the normal aging course.

Volumetrically, the most affected WM regions during aging by the interplay stress and mood are the left paracentral lobe and the left superior frontal. Indeed, the superior frontal WM volume is known to present an accelerated decline with increasing age (Salat et al., 2009). The volume of these regions decrease for all stress and mood level combinations, however the decrease is much more pronounced for high stress and low mood levels. This indicates that for better mood (i.e. less depressive), the effect of stress is negative since it increases the negative relation between age and volume; this negative impact of stress does not seem to operate so obviously for subjects with higher depressive mood. These higher volumetric reductions with age, especially under high levels of stress, in WM of these regions, may be associated with the lower predisposition to action and extraception observed with increasing age. Interestingly, the lateralization effect found in the volumetric interplays, with more pronounced atrophy in structures localized in the left hemisphere, is in good line with previous studies on this topic (see for review (Cerqueira et al., 2008)). Additionally, the impact of stress and mood during the lifespan seems to be higher in the WM compared to the GM volumetry, in line with the described faster WM deterioration after the fifth decade of life (Gunning-Dixon et al., 2009).

In the structural and functional investigation of the interplay between stress and aging, considering also the impact of mood, some important limitations should be noticed. Stress and mood evaluation was based only in psychological scales, without complimentary assessment of biological markers. In these analyses, the sample included only older adults and it was performed in a cross-sectional design, precluding a complete lifespan assessment, from childhood to elderly ages with a stronger longitudinal characterization. Functionally, the analyses do not cover the whole cortex and thus do not provide a full description of brain functional architecture; for example the limbic system, crucial in the stress and mood states, was not yet analyzed.

To the best of our knowledge there are no prior reports that evaluate the interplay between stress and mood on brain structure and function during aging. Herein we have shown the critical influence of stress and mood, especially at higher levels, in the brain. High levels of stress and/or depressive mood may accelerate the typical age-induced decline, or alternatively, reduce the age impact. We showed also that for the effects of stress and mood in brain structure and function, timing is crucial. Indeed, these combinatorial approaches may contribute to clarify some literature inconsistencies associated with the aging decline. Moreover, with this study we expect also to enlighten the association between brain structure and function abnormalities and several states that may lead to neuropsychiatric disorders (Liao et al., 2010;Kroes et al., 2011;Meda et al., 2012;Whitfield-Gabrieli and Ford, 2012;Durkee et al., 2013;Kuhn and Gallinat, 2013) that prevail in elder individuals.

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Chapter 5

Conclusions

5 - Conclusions

The present work explored the effects of stress on the active and "resting" human brain and its interplay with different mood levels during healthy aging. With this thesis we are able to conclude that:

1. Chronic stress biases decision-making strategies in humans toward habits, causing a reversible shift in the activation from the associative to the sensorimotor circuits, paralleled by atrophy of the medial prefrontal cortex and the caudate, and by an increase in the volume of the putamina;

2. Stress impacts on activation/deactivation pattern of RSNs, along with a simultaneous volumetric atrophy of the DMN;

3. There is a network-specific functional remodelling and plasticity of the activation-deactivation pattern following recovery from chronic-stress, which is not accompanied by significant structural plasticity;

4. Stress and mood have a critical influence, especially at higher levels, in brain structure and function.

Chapter 6

Future Perspectives

6 – Future Perspectives

Due to the extreme complexity and importance of understanding the interaction between aging, stress and mood, there is a world of opportunities for future work. However, following a logical way, based on the presented thesis, some future developments for the present work, include:

- Based on the promising results of stress and plasticity effects on decision-making and resting-state networks, it could be of great potential to replicate the work that we described in this thesis with a larger sample and ideally using exactly the same participants as controls, stressed and stressed recovery (longitudinal-design).
- As functional connectivity may reflect direct structural connectivity among regions, it would be of interest to assess the effects of the interplay between stress and aging using combined multimodal analysis of fMRI and DTI, incorporating other connectivity measures as graph theory.
- Specific areas such as hippocampus and amygdala, known to be implicated in the stress-related vulnerability to chronic health conditions, should be assessed using tailored functional imaging paradigms.
- To infer brain structure and function alterations on cross-sectional data alone is not the most accurate research strategy. Therefore, longitudinal studies could lead to new insights into brain aging, stress-effects, their interplay and plasticity. In fact, as exposure to chronic stress during lifetime is a risk factor for several psychiatric disorders, and little is known about the long-lasting effects, could be of interest to re-scan and analyse the same subjects 10-20 years from now.
- In line with the neuroimaging future tendencies, apart from brain structure and function characterizations, applying interventional approaches such as Transcranial Magnetic Stimulation, Deep Brain Stimulation or using the MRI scanner as a treatment apparatus, to faster reverse possible brain alterations would be a challenging but promising pathway.