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# Master thesis

## Large-scale Network Configuration Before and After Stress Exposure in Anxious and in Resilient Individuals

Yana Schwarze
Supervised by Dr. Erno Hermans & Sophie Bögemann
Second reader: Dr. Floris Klumpers



Donders Institute
Centre for Cognitive Neuroimaging
Kapittelweg 29, Nijmegen
NL-6525 EN
The Netherlands
http://www.ru.nl/donders

## Contents

## Abstract

Stress plays an important role in the development of psychopathologies and stress-related disorders, such as anxiety, are becoming an increasingly larger problem for individuals and society worldwide. However, protective factors may keep individuals from developing mental health problems after stress exposure, which is known as resilience. Previous studies have found stress-induced connectivity changes within and between large-scale brain networks in the general population, but the exact mechanisms in individuals with trait anxiety or higher than average levels of resilience are less clear. To assess whether a person's resilience can be predicted from their stress-induced functional connectivity and to find out whether similar associations can be made from the level of trait anxiety, 47 individuals at risk for developing stress-related mental health problems were exposed to the ScanSTRESS task. In a preceding and following resting-state scan, functional connectivity changes of the executive control network (ECN), the salience network (SN), and the default mode network (DMN) were assessed. Stress induction resulted in decreased ECN connectivity, decreased SN connectivity, and decreased as well as increased DMN connectivity in the whole sample. Following that, participants were divided into a high or low anxiety and a high or low resilience group. There were differences in DMN - middle frontal gyrus and DMN - posterior cingulate gyrus connectivity between high vs. low anxious individuals, and a difference in ECN - superior frontal gyrus connectivity in high vs. low resilient individuals. In the SN, there was higher connectivity with the middle frontal gyrus in low resilient individuals, irrespective of stress. Based on these differences in resting-state functional connectivity in the resilience groups, it may be possible to predict a person's level of resilience at a later timepoint whereas a person's trait anxiety score does not seem to be a good predictor of resilience.

# 1 Introduction

Stress plays an important role in the development of psychiatric disorders (Butjosa et al., 2016). However, not everyone exposed to high levels of stress develops mental health problems (Kalisch et al., 2017). While the underlying mechanisms of individual differences are not quite clear yet, a difference in functional connectivity between large-scale brain networks has been previously implicated in several stress-related disorders (Hermans, Henckens, Joëls, & Fernández, 2014; Soares et al., 2013). Especially in at-risk individuals, as in the sample of this study, the effects of stress on mental well-being and protective mechanisms can be well studied. This study investigates the effects of stress on large-scale brain networks in a sample of at-risk individuals and whether resilience can be predicted from functional connectivity in the large-scale networks.

Stress-related disorders including major depressive disorder, generalized anxiety disorder (GAD), posttraumatic stress disorder (PTSD), and addiction have a large impact on both mental and physical health and are hence a burden to individuals and society worldwide (Håkansson & Jr, 2017). Specifically, anxiety and depression are rated as some of the main disorders causing global disability (depression 1st place, anxiety 6th place) (World-Health-Organization, 2017) and are the psychopathologies that are by far most commonly seen after chronic stress exposure (Ray, Gulati, & Rai, 2017). In line with that, both animal and human studies have shown correlations between stress and anxiety and stress and depression on the neural level (Batinić, Trajković, Duisin, & Nikolić-Balkoski, 2009; Campos et al., 2013; Hammack et al., 2009; Kinsey, Bailey, Sheridan, Padgett, & Avitsur, 2007; Risbrough & Stein, 2006; Tafet & Nemeroff, 2016). Thus, anxiety and depression are common neurobiological correlates of stress and can be caused by acute as well as chronic stress (Ray et al., 2017). But while stress can lead to anxiety or other stress-related disorders, not everyone exposed to high levels of stress develops those (Kalisch et al., 2017). Different factors may protect individuals from developing mental health problems after stress exposure which is usually referred to as resilience.

Even though there are many different definitions and understandings of the term resilience, it is usually understood as the quick recovery or maintenance of mental health after being exposed to a stressor that happens in an active, dynamic and adaptive manner (Kalisch et al., 2017). This means that people change in response to stressors and are able to develop new coping skills and competencies; it does not simply refer to the insensitivity or unresponsiveness to stressors (Kalisch et al., 2019). Previous studies have already demonstrated the effectiveness of several resilience-promoting strategies such as improving social interactions, mindfulness, and physical health (Lazar, 2014; Ozbay et al., 2007; Penedo & Dahn, 2005). But while there is a lot of evidence in favour of these alternative resilience-promoting strategies, the biological underpinnings of resilience are less well studied. Doing so, however, could deepen our understanding of resilience and stress-related disorders and lead to improved treatment in the future (Snijders et al., 2018). Considering the high occurrence of stress-related disorders worldwide, resilience research seems to be a promising strategy to help individuals to prevent future mental health problems. One study found that there is an overlap between brain regions involved in resilience and those regions involved in stress and emotion regulation (Bolsinger, Seifritz, Kleim, & Manoliu, 2018), but it remains unclear what the effects of resilience are on

brain processes following stress. The general stress response, however, has been investigated more thoroughly in the past years.

Generally, stressors are physical or psychological events that threaten homeostasis (de Kloet, Joëls, & Holsboer, 2005). Homeostasis is a state where an organism is in a condition that is optimal for its functioning and after being exposed to a stressor a series of behavioural and physiological events are triggered in order to restore homeostasis. At the molecular level, stress triggers neuroendocrine reactions (Hermans et al., 2014). The earliest part of the stress response is mediated by catecholamines (including epinephrine, norepinephrine and dopamine) and neuropeptides which exert immediate effects on the body. These effects include enhanced vigilance, perception, and attention, which are essential for survival. Acute stress also triggers the activation of the HPA-axis leading to cortisol production, with peak levels at around 20 minutes after stressor onset, and which exerts its effects through different receptors. These can be rapid non-genomic effects within minutes after stressor onset but also slow genomic effects several hours afterwards, causing long-lasting changes in gene expression. Rapid non-genomic effects can overlap and interact with catecholaminergic effects and allow the organism to quickly respond to threats (Joëls, Pasricha, & Karst, 2013). Slow genomic effects seem to reverse the effects of early stress hormones and are therefore thought to contribute to higher cognitive processes in the late part of the stress response (Hermans et al., 2014). Animal research has shown that all the neurotransmitters involved in the stress response have different effects on different brain regions and different timepoints. In the prefrontal cortex (PFC), for example, increased levels of norepinephrine (NE) and dopamine have an impairing effect (Birnbaum, Gobeske, Auerbach, Taylor, & Arnsten, 1999; Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007) while the stress-induced increase in NE enhances the activity of the basolateral amygdala (Giustino, Ramanathan, Totty, Miles, & Maren, 2020).

A group of brain regions that is specifically affected by catecholaminergic activity in the early parts of the stress response, lasting around 30 to 60 minutes, is the salience network (SN). The SN is comprised of the amygdala, dorsal anterior cingulate cortex, hypothalamus, anterior insula, thalamus, inferotemporal/ temporoparietal regions, striatum, and brainstem/midbrain nuclei (Hermans et al., 2014). This network plays an important role in mobilizing energy resources and reorienting attention to threats, so it only makes sense that during acute stress increased activity is observed in these brain regions. Enhancing vigilance and attention by promoting SN activity comes with a higher energy demand (Hermans et al., 2014). Hence, resources are reallocated from brain regions for higher-order functioning to regions to enhance vigilance and attention which are important for survival. Brain regions for higher-order function include the dorsolateral PFC, frontal eyefields, dorsomedial PFC, dorsal posterior partial areas and together form the executive control network (ECN)(Hermans et al., 2014). Thus, the ECN is downregulated in the early phases of the stress response. In the later phase of the stress response, which can last up to several hours, corticosteroids reverse the initial network configuration by down-regulating SN and upregulating ECN activity. Such brain networks are often examined with resting-state functional connectivity (RSFC), which measures the spontaneous BOLD signal in the absence of a task. If the spontaneous BOLD signals from spatially distributed regions are temporally correlated, these regions could form a functional network (Woodward & Cascio, 2015). In contrast to that, activity measures

differences in the amount of blood-oxygen-level-dependent signal (van Oort et al., 2017). RSFC has been used widely to explore the effects of stress on the brain (Maron-Katz, Vaisvaser, Lin, Hendler, & Shamir, 2016; Quaedflieg et al., 2015; Vaisvaser et al., 2013).

Besides the ECN and the SN, another brain network consisting of functionally correlated brain areas is the default mode network (DMN), which is characterized by larger activity during rest than during the task (Alves et al., 2019). The DMN consists of regions in the brain including the ventromedial and lateral prefrontal, posteromedial and inferior parietal, and lateral and medial temporal cortices (Alves et al., 2019). Although the DMN is not commonly associated with stress, several studies have demonstrated an involvement of the DMN in the stress response. Various studies have found increased DMN activity following stress (Soares et al., 2013; van Oort et al., 2017), increased connectivity within the DMN following stress induction (Vaisvaser et al., 2013), and increased functional connectivity with other networks (Clemens et al., 2017). Evidence is contradictory, however, as another study found decreased intra-network and global connectivity of the DMN following stress induction (W. Zhang et al., 2019).

Stress-related disorders are characterized by altered RSFC (Soares et al., 2013). In depression and anxiety, for example, abnormal intrinsic functional connectivity within the DMN has been shown (Menon, 2011). In panic disorder, increased RSFC between the bilateral amygdala and bilateral precuneus (part of DMN) has been observed (Pannekoek, Veer, Tol, et al., 2013). Another disorder that has received quite a lot of attention with regards to functional connectivity analyses is PTSD, in which abnormal connectivity of frontal, limbic, and paralimbic regions (Admon, Milad, & Hendler, 2013) and increased connectivity within the salience network (Abdallah et al., 2019) was found. These findings have also been confirmed in a meta-analysis and review by Koch et al. (2016), who reported enhanced SN connectivity but decreased DMN connectivity in PTSD. Many alterations in functional connectivity have also been observed in various types of anxiety. During a resting state scan alterations within the DMN in PTSD, socialized anxiety disorder (SAD) and obsessive-compulsive disorder, and for the SN in SAD and GAD were found (Peterson, Thome, Frewen, & Lanius, 2014). Also, Pannekoek, Veer, van Tol, et al. (2013) found differences in RSFC of limbic and salience networks in SAD patients. Further, increased activity of the anterior insula as part of the SN has been implicated in anxiety disorders consistently (Menon & Uddin, 2010). All of this points towards abnormal functional connectivity in individuals with stress-related disorders.

But while there is lots of evidence for abnormal functional connectivity in stress-related disorders, only few studies so far have demonstrated altered patterns of functional connectivity in individuals with stress-related disorders following stress. This is particularly interesting as the deviations from the commonly observed changes in network configurations following acute psychosocial stress could help deepen our understanding of the underlying mechanisms of the stress response in stress-related disorders. In a study with (initially) healthy soldiers who were assessed before and after stress exposure during their military service, a reduction in post-exposure hippocampal-vmPFC connectivity was observed in soldiers with more PTSD-related symptoms (post-exposure) (Admon et al., 2012). A study by X. Zhang, Li, Steffens, Guo, and Wang (2019) is one of the few studies looking at the effects of acute stress on the ECN, SN, and DMN in participants concerned about depression and who felt vulnerable to stress. In this study, participants were divided into a high depression and a low depression group and RSFC was assessed

in both groups following a stress task. They further developed a recovery index to assess participants' ability to recover from stress which was based on RSFC changes post-stress. In the high depression group, a lower recovery ability from stress was associated with higher depression severity one year later. Thus, vulnerability to stress-related disorders like depression seems to be correlated with altered resting-state brain activity. However, the pattern of functional connectivity following acute stress in individuals with (vulnerability to) other stress-related disorders like anxiety remains largely unknown. Given that previous studies have already demonstrated abnormal functional connectivity in the SN and DMN in several types of anxiety during rest and that there is a strong relationship between anxiety and stress, it seems likely that also large-scale networks configuration during stress is altered in individuals with high anxiety compared to individuals with low anxiety.

While it is clearly important to investigate these potentially altered large-scale network configurations in various stress-related disorders, most people don't develop stress-related disorders after stress exposure. Therefore, it might even be more important to investigate whether functional connectivity changes can also serve as protective factors for mental health. In a similar study to Zhang et al., 2019, participants were again divided into two groups, this time based on their level of resilience, and changes in RSFC following acute stress in both groups were assessed (Shao, Lau, Leung, & Lee, 2018). In the high resilience group, a decrease of left subgenual anterior cingulate – right anterior insula connectivity relative to baseline was found after stress induction, with changes being the opposite in the low resilience group (low connectivity before, high connectivity after stress induction). Further, it has been found that in the absence of a task, increased resilience is associated with a decrease in functional amygdala connectivity with the SN and decreased amygdala connectivity with the DMN (Bolsinger et al., 2018). But how exactly the large-scale networks are configured under stress in individuals with different levels of resilience remains largely unknown. A study by Leeuwen et al. (2020) compared RSFC of the ECN and SN in an at-risk sample of siblings of schizophrenia patients to healthy controls. They found that the at-risk group lacked the increase in SN connectivity that was found in healthy controls and is known from the literature. Schizophrenia patients were chosen for this study because they are at increased risk to develop stress-related psychopathologies (Leeuwen et al., 2020); their resilience can, however, only be assumed here.

The study of underlying mechanisms in a sample of at-risk individuals is, however, of great importance. In a longitudinal study, the selection of at-risk individuals would allow for the observation of fluctuations in resilience over time, which is more informative than studying individuals with a stable level of resilience. Even though resilience will be assessed at only one timepoint here, a sample with varying levels of resilience will enable us to look at the influence of resilience on neural networks in the brain. Notably, the sample does not comprise any individuals with an anxiety- or other stress-related disorder (except for an ongoing mild depressive episode). However, the sample is still quite enriched, consisting of at-risk individuals during stressful phases of life. To ensure this, participants were only included if they had experienced three or more burdening life events (adaptation of life events from Cochrane and Robertson (1973)) and scored in a medium to high range (>20) on the General Health Questionnaire (GHQ) (Goldberg & Hillier, 1979; Koeter & Ormel, 1991), indicating higher levels of distress.

To our knowledge, no study so far looked at RSFC in the three large scale networks in an actual at risk-population. Therefore, the main aim of this study is to see whether resilience can be predicted from the acute stress response. Thus, resilience will be the prospective outcome measure of the study. Additionally, anxiety is a known risk factor for stress-related psychopathologies. Therefore, a second aim of the study is to see whether similar associations between participants' rather stable trait anxiety level and their acute stress response can be found. In contrast to the relatively stable trait anxiety score assessed before the MRI session, the dynamic resilience score will be assessed only around 1 week after the MRI scan. Thus, we will be able to show if the functional connectivity changes after acute stress are potential protective factors for mental health or whether trait anxiety already predicts resilience equally well.

Therefore, we will perform a resting-state analysis to look at the effects of stress induction on RSFC within and between the ECN, SN, and DMN in an at-risk population. To further investigate the neurobiological mechanisms underlying stress-related disorders, we will explore the stress-induced connectivity changes in two independent subsets of the data, comparing a) high vs. low anxiety and b) high vs. low resilience. For a), participants will be divided into two groups based on their scores in the trait part of the Staite-Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) using a cutoff of 40 which is commonly used to define probable clinical levels of anxiety (Emons, Habibović, & Pedersen, 2019). For b), participants are divided into a high and low resilience group based on their mental health problems (assessed by the GHQ) in relation to the number of stressors experienced (assessed by score on the Daily Hassle questionnaire (Chmitorz et al., 2019)), in a residualized approach proposed by Kalisch et al. (2020).

Based on the currently existing literature, we hypothesise that there will be a main effect of stress: while we expect SN connectivity to increase after stress exposure, we expect ECN connectivity to decrease. In line with previous studies, we further expect an increase in DMN connectivity after stress.

Further, we also expect to find a different pattern of RSFC in individuals with high vs. low anxiety and with high vs. low resilience after stress exposure. Specifically, we do not expect large differences in ECN connectivity between high and low anxiety and high and low resilience as most studies didn't find ECN connectivity to be altered in stress-related disorders. Based on findings from Leeuwen et al. (2020), we expect an increase in functional connectivity of the SN in high compared to low resilience and similarly also for low compared to high anxiety. Finally, while the exact role of the DMN in the stress response is not clear, it has been found to be involved in negative self-referential processing (Muscatell et al., 2015) and another study has found associations between increasing DMN activity and depressive rumination (Hamilton et al., 2011). As it is possible that individuals with high anxiety or low resilience have more negative thoughts about the preceding stress task, we expect a larger increase in DMN connectivity in high anxiety and low resilience compared to low anxiety and high resilience.

# 2 Methods

## 2.1 Participants

All participants included in this thesis were recruited for the DynaMORE Observational Study (DynaM-OBS), a multicentre study on stress resilience. In this thesis, only a subset of the measurements and participants will be included.

47 subjects were included in this analysis (mean age = 21.1, females = 25, right-handed=41) and were recruited via Sona, the subject database of the Radboud University, advertisements on Facebook and Instagram, emails to students and flyers that were distributed on the campus of Radboud University. Before being invited to the institute, participants had to fill in an online pre-screening (`https://www.soscisurvey.de/DynaM-OBS_mc/`) to determine their eligibility. Invitations were sent to those who had experienced at least 3 burdening life events (e.g. loss of job, separation, death of friend/family member, etc.) (according to the life events from Cochrane and Robertson (1973)), scored above 20 on the GHQ (Goldberg & Hillier, 1979; Koeter & Ormel, 1991), and were MRI compatible. Further, they had to be students between 18 and 25 years of age and free of any current psychiatric disorders except for a mild depressive episode (assessed in more detail by Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998) on the first baseline day). Out of the 55 invited participants, six participants had to be excluded from the study based on the M.I.N.I, one person dropped out after the first baseline day, and one had to be excluded because of missing questionnaire data. For a full list of in- and exclusion criteria see appendix 1.

The study was approved by the local ethics committee and all participants gave written consent after being informed about the experimental procedures. All data were collected at the Donders Centre for Cognitive Neuroimaging in Nijmegen, The Netherlands.

## 2.2 Psychological assessments

Several questionnaires were used to assess participants' (mental) health and stressor exposure. To assess fluctuations, these were filled in on several occasions: during the pre-screening, within a week after the first baseline day (baseline questionnaires), in bi-weekly questionnaires during the first six months and later in monthly follow-ups. For this analysis, a subset of questionnaires is taken from the pre-screening, from the baseline questionnaires, and from the first biweekly questionnaire one week after the MRI assessment (Month 1 week 1 = M1W1). If participants did not fill in the M1W1 questionnaire, the missing data were substituted by the subsequent questionnaire (M1W3).

The State-Trait Anxiety Inventory (STAI) is typically used to measure state and trait anxiety. In this study, we only used the 20 trait anxiety items. STAI-T scores were assessed during the baseline questionnaires.

The Daily Hassles (DH) questionnaire (Chmitorz et al., 2019) was used to assess annoyances and hassles that occur in daily life. For each of the 58 items, participants were asked to consider the last seven days and indicate to what extent the situation caused mental strain on a scale from 0 (not at all) to 4 (very straining).

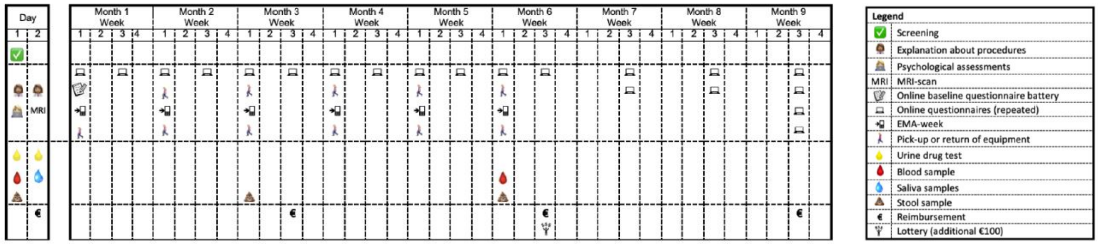
The 28-item General Health Questionnaire (GHQ) with its four subscales somatic symptoms, anxiety and insomnia, social dysfunction, and severe depression, was used

to assess participants' general mental health, with higher scores indicating higher levels of distress. To participate in the study, participants had to have a sum score of above 20 in the pre-screening. However, as for the current analyses the GHQ score from the M1W1/M1W3 was taken, scores may be lower than 20 if participants' health had improved in the time since the pre-screening. As the resilience score was calculated from the DH and GHQ score and the DH scores were only assessed during M1W1, we chose to use the M1W1 GHQ score over the pre-screening GHQ score to have both measures taken at the same timepoint.

During the MRI assessment, we further assessed participants' subjective stress levels at 11 points throughout the scan as well as shortly before and after. For this, participants were asked to indicate their current feeling of stress on a scale from 0 (not stressed at all) to 10 (very stressed). However, only stress ratings from before and after the stress task are considered here.

### 2.3 Experimental design and procedure

For the Dutch DynaM-OBS study, participants came to the Donders Institute on two separate days for baseline behavioural and MRI assessments, followed by nine months of questionnaires and ecological momentary assessments (questionnairs sent to participants phones). An overview of the complete study timeline can be found in figure 1. On the first baseline day, participants were screened for psychiatric disorders using the M.I.N.I. and only if they did not currently or in the past nine months meet the criteria for a psychiatric disorder except for a mild depressive episode, tobacco dependence, and substance abuse, the testing day continued. Further, a drug test was administered to confirm that all participants were free of drugs or any other psychoactive substances before drawing participants' blood and continuing with the neuropsychological assessments, which assessed processing speed, cognitive flexibility, and executive functioning. Further, participants received a stool kit to use at home and were instructed to fill in the baseline questionnaires within a week.




(a) Timeline (b) Legend

**Fig. 1. DynaM-OBS study timeline.** Overview of the longitudinal design of the DynaM-OBS study. In this thesis, only the MRI data from day 1 and questionnaire data from month 1 is being analysed.

On the second baseline day, participants came back for the MRI assessment. Before going into the scanner, another drug test was performed to ensure that participants had not consumed any drugs or other psychoactive substances that could influence the MRI measurements and all tasks were explained. To keep participants' naïve about the nature

of the stress task, it was described as the concentration task. The MRI session always took place between 13:00 and 18:00 because cortisol levels are relatively stable during this time (although this is not relevant for this thesis). To further ensure stable cortisol levels, participants were instructed to refrain from sports and alcohol for 24 hours, to not eat or smoke for two hours, and to wake up at least four hours before the start of the experiment. Stress was induced using a modified version of the ScanSTRESS task (Streit et al., 2014). During the task, participants had to perform two alternating cognitive tasks while being watched by an unknown stressor via a webcam. The cognitive tasks included mental arithmetic and spatial mental rotations. Participants had to respond to the tasks under time pressure, with a countdown bar at the bottom to visualize the remaining time. After every trial participants received feedback on their current performance on the screen ("work faster", "error"). To ensure that participants make mistakes throughout the task, speed and difficulty of the task were adapted according to their performance. After a practice session inside the scanner, participants were told by the stressor that with their performance they would risk the usability of the data and they were asked to try harder and concentrate more during the following scan. The whole task excluding the practice session took around six minutes with three blocks of spatial mental rotations and mental arithmetic each. In contrast to the original ScanSTRESS task, our version of the ScanSTRESS task did not include a control session, where no stress was induced, and there was only one stressor watching the participant instead of two as in the original task. An outline of the ScanSTRESS task including the preceding and following resting-state scans can be found in figure 2.

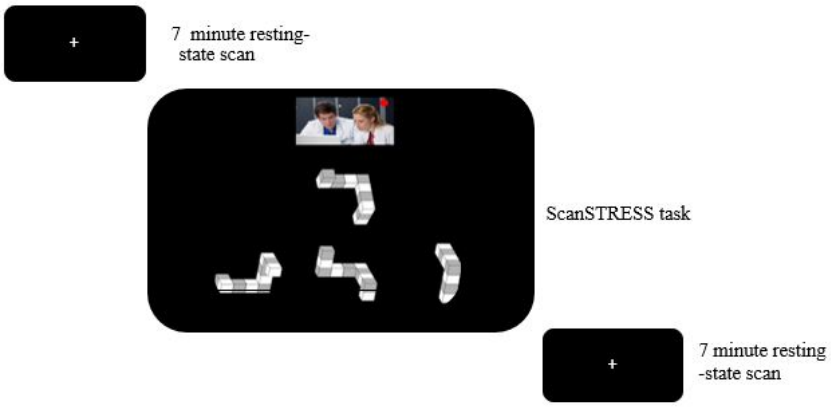



**Fig. 2. Stress induction and resting-state scans.** The ScanSTRESS task was used to induce acute stress in participants, which was preceded and followed by a 7 minute resting-state scan. The image shows an example of the spatial mental rotations task during which participants were observed by a stressor via a webcam. The second condition, in which participants had to perform mental arithmetic, is not shown here.

40 minutes after the end of the MRI session when the last salivette was taken participants were debriefed about the stress task. The complete MRI session lasted around two hours (scan time 90 minutes) during which two anatomical scans, five functional scans with tasks, two resting-state scans, and diffusion tensor imaging were done. The stress task was the last of the five tasks, so participants had already been in the scanner for

around one hour and were thus familiar with the scanning procedure. The stress task was preceded and followed by a resting state scan of seven minutes each during which participants were instructed to lie still and keep their eyes fixed to the cross in the middle of the screen. The resting-state scans are the only scans that will be analysed in this thesis.

## 2.4 MRI data acquisition

All images were acquired with a 3T Siemens Magnetom Prisma in Nijmegen, The Netherlands using a 32-channel head coil. Functional images were acquired using a T2*-weighted multiband echo planer sequence with an acceleration factor of 8 (interleaved) (TR = 800 ms, TE = 3700 ms, flip angle = 52°, voxel size = 2.0 x 2.0 x 2.0 mm, FOV = 208mm, slices = 72). High resolution anatomical images were acquired using a T1-weighted MPRAGE sequence (TR = 2500 ms, TE = 2.22 ms, flip angle = 8°, voxel size = 0.8 x 0.8 x 0.8 mm, FOV = 256mm, slices = 208).

## 2.5 Data analysis

### Behavioural and physiological measures

The STAI-T was scored by summing over all items on the trait-subscale with scores for anxiety absent items being reversed (Julian, 2011). A cut-off of 40 was chosen to split the sample into a low and a high anxiety group.

To split participants into a high and a low resilience group, first, their stressor reactivity was calculated. The stressor reactivity is based on the relationship between the GHQ score (mental problems: P) and the DH score (experienced stressors: E) (more details in Kalisch et al. (2020). Both scores were calculated as sum scores from the respective questionnaire. Following that, an E-P regression line was fitted, from which residuals were calculated for each participant separately. That way, each participant was given a positive or negative stressor reactivity score. Finally, this score was inverted to get a resilience score for each participant, with a (more) positive resilience score indicating higher levels of resilience and a (more) negative score indicating lower levels of resilience compared to the rest of the sample. Based on the sign of their resilience score (positive or negative), participants were divided into a high and a low resilience group. Afterwards, Mahalanobis distance was used to detect outliers (De Maesschalck, Jouan-Rimbaud, & Massart, 2000).

To ensure successful stress induction through the stress task, subjective stress ratings from before and after the stress task were compared. Within the whole sample, pre-stress ratings were subtracted from post-stress ratings and a one-sample t-test was used to check if the ratings are significantly different from zero. Stress ratings were also compared between the high and low anxiety and the high and low resilience groups. Again, pre-stress ratings were subtracted from post-stress ratings for the high and low groups respectively, and a two-sample t-test was used to compare stress ratings between the groups. To further investigate whether subjective stress already differed at baseline (before stress induction), pre-stress ratings were compared between the high and low anxiety and high and low resilience groups.

### MRI preprocessing

Preprocessing of fMRI data was done using fMRIPrep 20.2.1 (Esteban, Markiewicz, et al., 2018). For the preprocessing of the anatomical data, the T1-weighted reference image was skull-stripped using antsBrainExtraction.sh and normalized to standard space (MNI152NLin6Asym) through nonlinear registration with antsRegistration (ANTs 2.3.3). Brain tissue segmentation was done using FSL FAST v5.0.9. Functional data were slice time corrected using AFNI and motion-corrected using mcflirt (FSL 5.0.9). Following that, functional images were co-registered to the T1w reference using bbregister (FreeSurfer). Non-aggressive ICA-based Automatic Removal Of Motion Artifacts (ICA-AROMA, (Pruim et al., 2015b)) was used to remove motion-related independent components of fMRI data. For further details on fMRIPrep-preprocessing see appendix 2. The non-aggressively denoised 4D NIFITI files were smoothed at 6-mm FWHM Gaussian kernel. Finally, a high-pass filter of 125s was applied.

### MRI data analysis

To analyse the effects of stress on the functional connectivity of the ECN, SN, and DMN a seed analysis was performed for each of the networks individually. All analyses were performed in SPM12 (`http://www.fil.ion.ucl.ac.uk/spm`). To investigate functional connectivity with the networks of interest, the timeseries of all voxels within each of the three networks were extracted from predefined networks (templates were taken from Shirer, Ryali, Rykhlevskaia, Menon, and Greicius (2012)). Templates for Left Executive Control Network, Right Executive Control Network, Anterior Salience Network, Posterior Salience Network, Dorsal Default Mode Network, and Ventral Default Mode Network were taken and both templates of each network were combined into one larger network to yield one mask for each of the three networks. Timeseries extraction was performed using fslmeants (FSL: `http://www.fmrib.ox.ac.uk/fsl`) per subject, per session. Then, for each participant, one general linear model (GLM) was created per network, which included averaged timeseries per session from the respective network. No further motion regressors were included in the GLM because motion-related artefacts were already removed using non-aggressive ICA-AROMA.

To investigate changes in functional connectivity on the subject level, two contrast images were created per participant and per network, showing the difference in RSFC between pre- and post-exposure to stress (pre-stress > post-stress and pre-stress < post-stress), resulting in six contrast images per subject. These contrast images were tested for significance on the group level (i.e., six group-level tests in total).

To investigate functional connectivity changes between the respective anxiety and resilience groups, separate 2-sample t-tests were performed for each contrast image (pre-stress, post-stress, pre-stress > post-stress, and pre-stress < post-stress). This resulted in 4 (contrasts) x 3 (networks) x 2 (groups) GLMs, investigating either high vs. low anxious individuals or high vs. low resilient individuals.

To further explore the direction of our group-level findings, we created a 2 (groups: high and low) x 2 (timepoints: pre- and post-stress) full factorial design for each network and each group (3 x 2). For this, pre- and post-stress contrast images were added for each participant in the respective high or low group. Following that, contrast estimates

were plotted, showing the strength and direction of effect in both groups before and after stress.

An initial cluster-forming threshold of $p < 0.001$ (uncorrected) was used and statistical maps were then thresholded at a cluster-level whole-brain family-wise error-corrected threshold of $p < 0.05$.

# 3 Results

## 3.1 Demographics

| | Complete sample |
|---|---|
| N | 47 |
| Gender(f/m) | 25/22 |
| Age (years) | 21.11 |
| STAI-T (mean + std) | 41.15 + 7.58 |
| GHQ (mean + std) | 21.98 + 5.53 |
| Daily hassles (mean + std) | 50.23 + 19.58 |
| Number of life events | 6.77 |
| Pp with current episode | 5 |
| Pp with previous episode | 30 |

**Table 1. Demographics of the complete sample.** Current episodes include a mild depressive episode or tobacco abuse/dependence within the past nine months. Previous episodes refer to episodes of mental disorders more than nine months ago. Current and past episodes were assessed by the M.I.N.I.

All participants (N=47) completed the resting-state scans without excessive movement. A table of demographics can be found in table 1. To look at the effects of resilience and anxiety, participants were divided into a) a high or low anxiety group as well as b) a high or low resilience group. Based on Mahalanobis distance calculations, one participant was excluded from the resilience analyses. For three additional participants, not all behavioural data were collected, so that no resilience scores could be calculated. Thus, only 43 participants were included in one of the resilience groups. Of these 43, four participants did not complete M1W1 questionnaires, so that their resilience was calculated from M1W3 questionnaires. Demographics and plots of division into both groups can be found in table 2 and figure 4.

The Pearson's correlation between trait anxiety and resilience scores is negative and statistically not significant ($r = -0.03$, $t(41) = -0.21$, $p = 0.837$). Further correlation analyses revealed that there was no significant correlation between trait anxiety and mental health (GHQ scores) ($r = 0.23$, $t(41) = 1.53$, $p = 0.133$), but between trait anxiety and stressor exposure (DH scores) ($r = 0.32$, $t(41) = 2.17$, $p = 0.036$), indicating that more anxious people report higher stressor exposure.

## 3.2 Subjective stress levels

Subjective stress increased in response to the ScanSTRESS task (pre ± SD = 2.53 + 1.85; post ± SD = 6.38 + 1.94), indicating a successful stress-induction on the behavioural level ($t(46) = 14.247$; $p < 2.2e\text{-}16$). There was no difference in the change in subjective stress levels between the high vs. low anxiety ($t(44.935) = -1.3629$; $p > 0.05$) and the

| | High anxiety | Low anxiety | Group differences |
|---|---|---|---|
| N | 24 | 23 | |
| Gender(f/m) | 12/12 | 13/10 | |
| Age (years) | 21.25 | 20.96 | t=0.452, p=0.654 |
| STAI-T (mean + std) | 47.25 + 4.89 | 34.78 + 3.48 | t=10.103, p=0 *** |
| Resilience (mean + std) | 0 + 0.86 | 0 + 1.15 | t=0.026, p=0.98 |
| GHQ (mean + std) | 22.82 + 4.7 | 21.1 + 6.28 | t=1.015, p=0.317 |
| Daily hassles (mean + std) | 54.91 + 17.86 | 45.33 + 20.52 | t=1.629, p=0.111 |
| | High resilience | Low resilience | Group differences |
| N | 24 | 19 | |
| Gender(f/m) | 13/11 | 11/8 | |
| Age (years) | 21.21 | 21.32 | t=-0.154, p=0.878 |
| STAI-T (mean + std) | 40.12 + 7.97 | 41.95 + 6.96 | t=-0.799, p=0.429 |
| Resilience (mean + std) | 0.67 + 0.6 | -0.84 + 0.72 | t=7.31, p=0 *** |
| GHQ (mean + std) | 19.04 + 4.12 | 25.68 + 4.87 | t=-4.752, p=0 *** |
| Daily hassles (mean + std) | 49.54 + 18.58 | 51.11 + 21.27 | t=-0.253, p=0.802 |

**Table 2. Demographics of anxiety and resilience groups** The total number of participants in the whole sample and the number of participants in the anxiety groups is 47. Due to missing questionnaire data no resilience scores could be calculated for three individuals, resulting in 43 participants in the resilience groups.

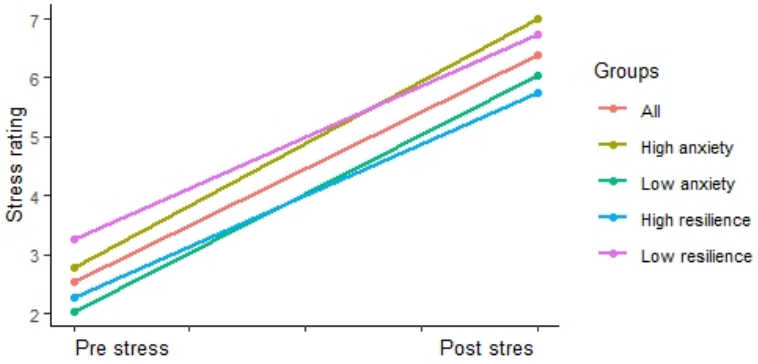


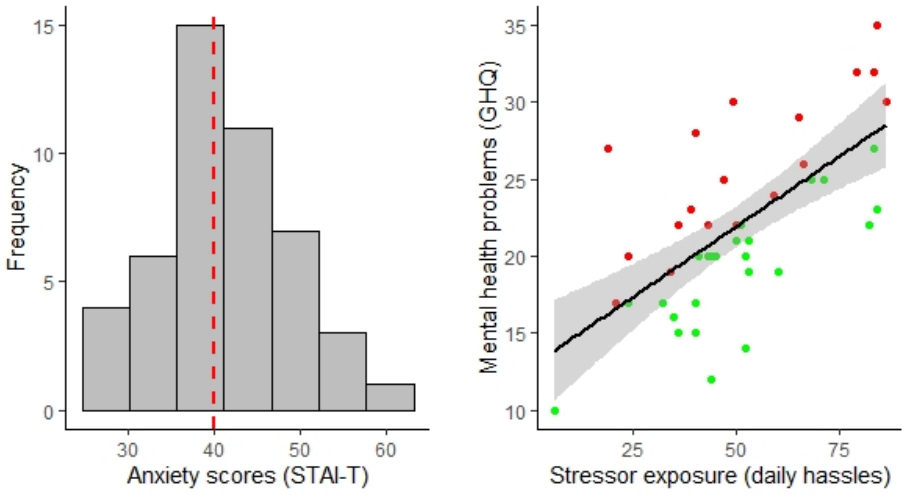

**Fig. 3. Trajectories of pre- and post-stress ratings.** Mean stress ratings before and after stress exposure in the complete sample and in all subgroups.

high vs. low resilience (t(40.968) = -0.92832; p > 0.05) group. While there was also no difference between the anxiety groups in stress levels before stress induction (t(40.358) = 0.99021, p > 0.05), pre-stress levels significantly differed between the high (pre ± SD = 2.04 + 1.6) and low (pre ± SD = 3.26 + 1.99) resilience group (t(34.091) = -2.1715, p = 0.03694). Mean trajectories of subjective stress ratings before and after stress induction can be found in figure 3.

### 3.3 Main effects of stress

We investigated changes in functional connectivity after acute stress in three different networks of interest. Results will be presented per network.

In the ECN, we expected to find decreased functional connectivity. Indeed, there was a significant decrease in the functional connectivity of the ECN with several occipital and parietal clusters. These included the left cuneus (peak MNI coordinates: -12 -66 14, pFWE-corr = 0.00, cluster size = 245), left and right precuneus (peak MNI coordinates: -6 -54 6, pFWE-corr = 0.00, cluster size = 191), posterior cingulate gyrus (peak MNI coordinates: 6 -44 22, pFWE-corr = 0.00, cluster size = 130), and a frontal cluster in the




(a) Anxiety scores (b) Resilience scores.

**Fig. 4. Division into anxiety and resilience groups.** a) Division into a low and high anxiety group, based on STAI-T scores using a clinical cutoff of 40. b) Division into a low (red) and high (green) resilience group by using a residualization approach based on GHQ and DH scores.

right superior frontal gyrus (peak MNI coordinates: 6 60 -2, pFWE-corr = 0.001, cluster size = 114). Further, ECN connectivity was decreased in two cerebellar clusters: right cerebellum exterior and right lingual gyrus (peak MNI coordinates: 18 -56 -12, pFWE-corr = 0.00, cluster size = 183) and left cerebellum exterior (peak MNI coordinates: -10 -70 -16, pFWE-corr = 0.001, cluster size = 114; peak MNI coordinates: -40 -68 -22, pFWE-corr = 0.025, cluster size = 62; peak MNI coordinates: -6 -74 -46, pFWE-corr = 0.049, cluster size = 54). All areas to which ECN connectivity was significantly decreased can be found in figure 5. Of these, the left cerebellum was the only area within the ECN that was decreased after stress. The results indicate that there was mainly a decrease in connectivity of the ECN with areas of the DMN, rather than decreased within ECN connectivity. As expected, there was no significant increase in ECN connectivity after stress.

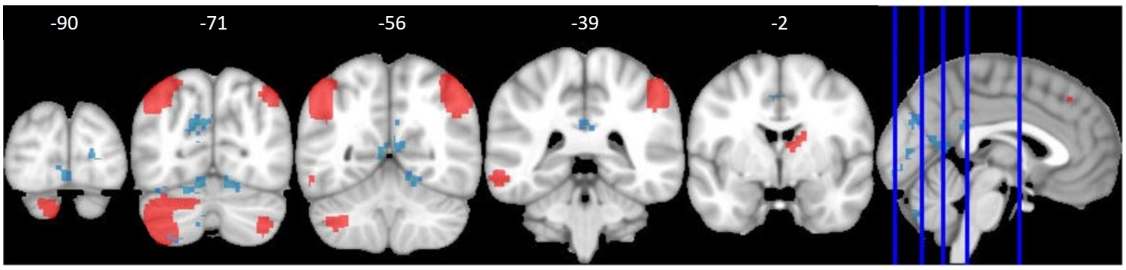
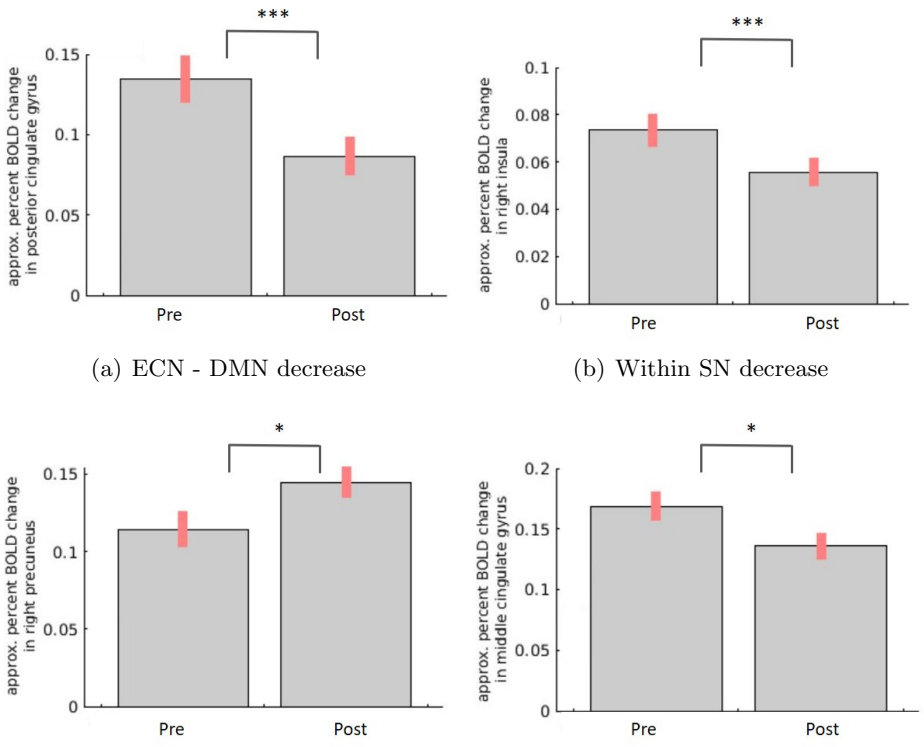



**Fig. 5. Decrease in ECN connectivity after stress (pre-stress > post-stress).** Decreased functional connectivity of the ECN (red) with several regions in the brain (blue). The only difference in connectivity within the ECN was in the left cerebellum.

To explore whether the effect was driven by less (positive) connectivity or whether stress induced anti-correlation, contrast estimates of significant regions were created for all three networks (figure 6). In all significant areas of the ECN, contrast estimates after acute stress were significantly decreased. An example of this can be found in figure 6(a). However, contrast estimates were in the positive range, suggesting decreased functional connectivity rather than anti-correlation.




(a) ECN - DMN decrease

(b) Within SN decrease

(c) Within DMN increase

(d) DMN decrease (outside)

**Fig. 6. Examples of contrast estimates in ECN, SN, and DMN.** a) Contrast estimates of the ECN with the posterior cingulate gyrus as an example of the decrease in ECN - DMN connectivity, b) contrast estimates showing the decrease of the SN with the right insula, c) contrast estimates of the DMN increase with the right precuneus within the DMN, and d) contrast estimates of the DMN decrease with the middle cingulate gyrus outside the DMN. The percent BOLD signal change is an approximation depending on the scaling of the data, the scaling of the predictor(s) that are involved in the selected contrast, and the scaling of the selected contrast. For more details see (*SPM plot units, 31/07/12, Neuroimaging Statistics Tips & Tools*, 2012). Red bars indicate 90% confidence intervals.

In contrast to our expectations, there was no significant increase in connectivity after stress in the SN. There was, however, a decrease in SN functional connectivity in the right insula (peak MNI coordinates: 36 24 4, pFWE-corr = 0.00, cluster size = 189) as part of the SN after stress induction, which can be seen in figure 7. Thus, the results indicate decreased instead of increased within SN connectivity. Contrast estimates showing the direction of this effect can be found in 6(b).

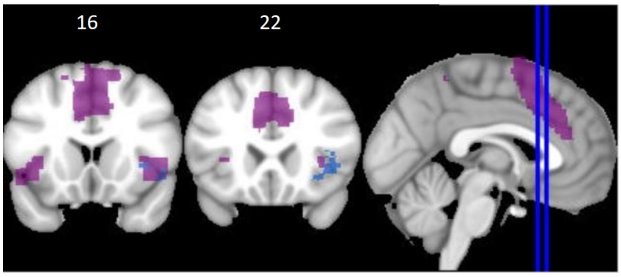



**Fig. 7. Decrease in SN connectivity (pre-stress > post-stress).** Decreased functional connectivity of the SN (purple) with the right insula (blue) after stress.

In a post-hoc analysis, changes in functional connectivity in the SN were assessed only in the low anxiety group. Here, we found an increase in connectivity of the SN with the middle frontal gyrus (peak MNI coordinates: -24 38 40, pFWE-corr = 0.038, cluster size = 45). Being partly within the SN, this was in line with our original hypothesis of increased SN connectivity after acute stress, however, this effect was only found in the low anxiety group and not in the complete sample.

In the DMN, we expected an increase in functional connectivity of the DMN. Indeed, there was increased functional connectivity of the DMN with the right precuneus (peak MNI coordinates: 2 -48 48, pFWE-corr = 0.016, cluster size = 74) within the DMN after stress. Additionally, there was also a decrease of DMN functional connectivity with the middle cingulate gyrus (peak MNI coordinates: 0 -26 32, pFWE-corr = 0.023, cluster size = 69) just outside the DMN. Increases and decreases in functional connectivity can be found in figure 8 and contrast estimates showing the direction of effects in both regions can be found in figure 6(c) and figure 6(d) respectively. The results suggest distinct roles of the DMN in the stress response, leading to increases as well as decreases in DMN connectivity.

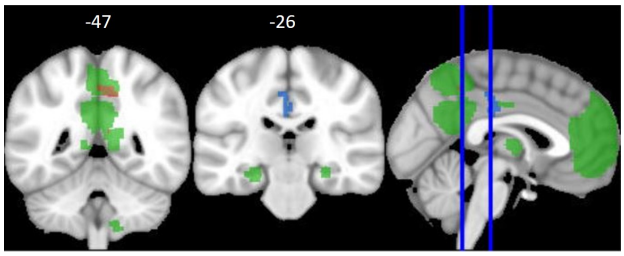



**Fig. 8. Increase and decrease in DMN connectivity (pre-stress < post-stress, pre-stress > post-stress).** Increased connectivity of the DMN (green) with the right precuneus (orange) and decreased functional connectivity of the DMN with the middle cingulate gyrus (blue) after stress.

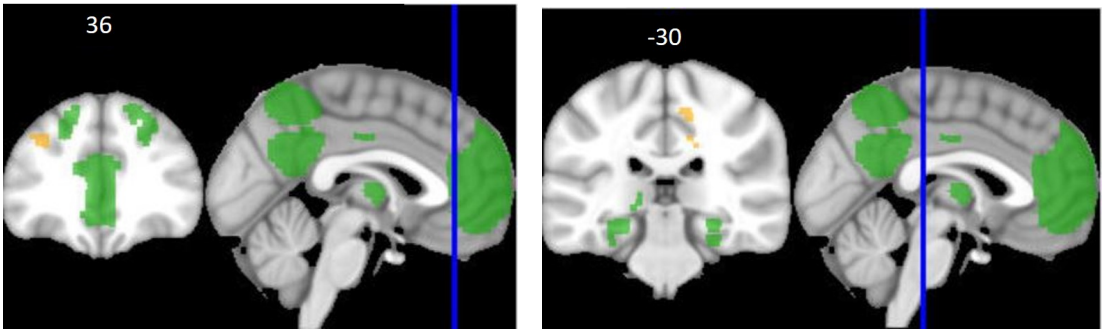
### 3.4 Connectivity changes in the anxiety groups

To further investigate the effects of anxiety on the functional connectivity of these three networks, participants were divided into a high and a low anxiety group. Between the high and the low anxiety groups, contrast images pre-stress, post-stress, pre-stress > post-stress, and pre-stress < post-stress were compared with a two-sample t-test.

We expected to find increased SN connectivity in low anxiety compared to individuals with high anxiety as well as increased DMN connectivity in high anxiety compared to the low anxiety group. In the ECN, we did not expect significant differences between the groups.

In line with our hypotheses, there was a significant difference in DMN functional connectivity post-stress between the high and low anxiety groups with the middle frontal gyrus (peak MNI coordinates: -34 38 34, pFWE-corr = 0.011, cluster size = 122) as part of the SN. In this area, individuals with low anxiety showed higher connectivity than individuals with high anxiety. The difference in post-stress DMN connectivity can be found in figure 9(a).

There was further a significant difference between the groups in the decrease in connectivity after stress. Compared to the low anxiety group, the high anxiety group showed significantly higher functional connectivity of the DMN with the right precentral gyrus and right posterior cingulate gyrus (peak MNI coordinates: 12 -36 42, pFWE-corr = 0.015, cluster size = 75), right on the edge of the DMN (figure 9(b)). This was confirmed by the opposite contrast, in which individuals with low anxiety showed higher connectivity with this area compared to those with high anxiety. Thus, there seems to be an increase of functional connectivity of the DMN with the right precentral gyrus and right posterior cingulate gyrus in individuals in the low anxiety group, while individuals with high anxiety show a decrease in connectivity with this area. Contrast estimates of the difference between groups in DMN - right precentral gyrus/right posterior cingulate gyrus connectivity can be found in figure 10(a).

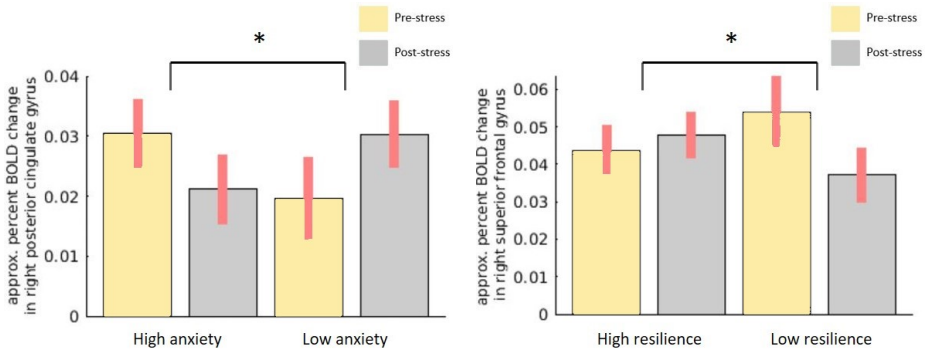



(a) Post-stress connectivity changes. (b) Stress-induced connectiviy changes in the DMN.

**Fig. 9. Differences in functional connectivity in high vs. low anxiety.**
a) Higher DMN - middle frontal gyrus connectivity after stress in low anxiety compared to high anxiety individuals. b) Increased DMN - right precentral gyrus/right posterior cingulate gyrus connectivity in individuals in the low anxiety group, decreased functional connectivity with this area in the high anxiety group after stress.

In the SN, the expected increase in low anxiety was not observed. However, there was a trend visible between the high and low anxiety groups in post-stress connectivity in the left superior parietal lobe (peak MNI coordinates: -16 -60 68, pFWE-corr = 0.066, cluster size = 75), with low anxiety individuals showing higher connectivity. Even though this is not significant here, this effect might become significant in a larger sample.

In line with our expectations, there was no difference in ECN connectivity between the groups. The results indicate that differences between individuals with high and low anxiety mainly concern the DMN.




(a) DMN change in anxiety (b) ECN change in resilience

**Fig. 10. Contrast estimates in the high and low anxiety/resilience groups.** a) Contrast estimates of the difference between high and low anxiety in DMN connectivity with the posterior cingulate gyrus, b) contrast estimates of the difference between high and low resilience in ECN connectivity with the right superior frontal gyrus. The percent BOLD signal change is an approximation depending on the scaling of the data, the scaling of the predictor(s) that are involved in the selected contrast, and the scaling of the selected contrast. For more details see (*SPM plot units, 31/07/12, Neuroimaging Statistics Tips & Tools*, 2012). Red bars indicated 90% confidence intervals.

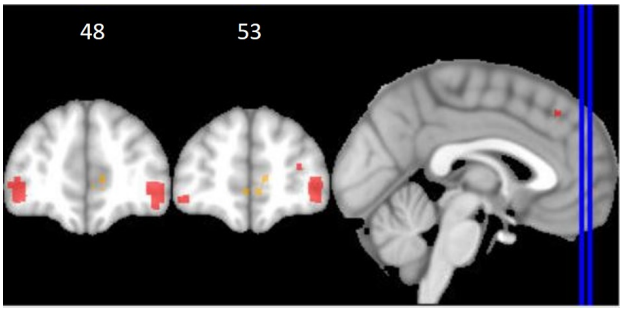
### 3.5 Stress-induced connectivity changes in the resilience groups

Further, differences in functional connectivity in individuals with high and low resilience scores in the three networks were investigated. As before, contrast images pre-stress, post-stress, pre-stress > post-stress, and pre-stress < post-stress were compared with a two-sample t-test.

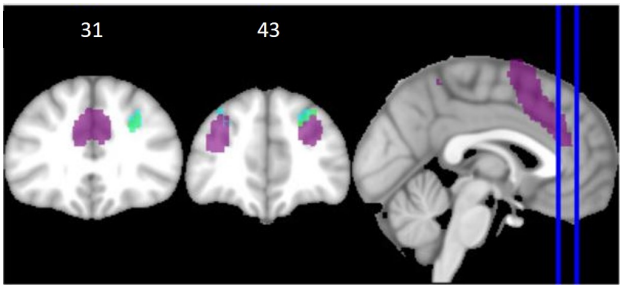
Similar as in the high and low anxiety groups, we expected to find increased SN connectivity in high resilience compared to individuals with low resilience and increased DMN connectivity in low resilience compared to the high resilience group. Again, we did not expect to find significant differences in ECN connectivity.

Contrary to our predictions, there was, however, a decrease in ECN connectivity after stress with the right superior frontal gyrus (DMN) in the low resilience group compared to the high resilience group (peak MNI coordinates: 10 54 4, pFWE-corr = 0.021, cluster size = 64), which can be seen in figure 11(a). This was again confirmed by the contrast in the opposite direction, in which the high resilient group showed an increase in functional connectivity with the right superior frontal gyrus. Contrast estimates of this difference in ECN connectivity can be found in figure 10(b).

In contrast to our expectations, there were also no differences in stress-induced connectivity changes in the DMN and SN. There was, however, more connectivity of the SN with the middle frontal gyrus in low resilient individuals which was observable pre-stress (right: peak MNI coordinates: 28 32 32, pFWE-corr = 0.018, cluster size = 203; left: peak MNI coordinates: -30 46 32, pFWE-corr = 0.00, cluster size = 68) as well as post-stress (right: peak MNI coordinates: 28 40 38, pFWE-corr = 0.003, cluster size = 140) (partly SN), indicating that there was a general effect in the right middle frontal gyrus, irrespective of stress induction. Figure 11(b) shows this difference in SN connectivity.




(a) Stress-induced connectivity change in the ECN




(b) Connectivity change in the SN irrespective of stress

**Fig. 11. Differences in functional connectivity in high vs. low resilience.** a) Increased ECN (red) - superior frontal gyrus connectivity in high resilient individuals (orange), decreased connectivity with this area in the low resilient group after stress, b) increased SN (purple) - middle frontal gyrus connectivity in low resilient individuals, pre-stress (light blue) and post-stress (light green).

## 4 Discussion

In this study, we looked at the effects of stress on resting-state functional connectivity (RSFC) in at-risk individuals. Firstly, the aim was to investigate the effects of acute stress in the executive control network (ECN) and salience network (SN) as proposed by Hermans et al. (2014), as well as in the default mode network (DMN). A second aim was to investigate whether functional connectivity changes after acute stress constitute protective factors and whether the same association can be found between functional connectivity changes and trait anxiety as well. To do so, we investigated functional connectivity changes due to stress in individuals with higher vs. lower anxiety and higher vs. lower resilience. In agreement with previous studies, we found large-scale connectivity changes following acute stress. The direction, however, sometimes differed. In the ECN and SN, we observed decreased functional connectivity. In the DMN there was increased connectivity within the DMN and decreased connectivity with an area outside the DMN. When comparing the anxiety groups there were significant differences in stress-induced functional connectivity in the DMN, while differences in the resilience groups were found in the ECN. There was also a difference in within SN functional connectivity between the resilience groups that was observable pre- and post-stress, and thus irrespective of stress induction. The findings demonstrate that different levels of anxiety and resilience are reflected in a distinctive pattern of stress-induced RSFC changes.

**Main effects of stress in the whole sample**

In the ECN, we expected to find a decrease in functional connectivity within the ECN as well as with other brain areas (Hermans et al., 2014; Qin, Hermans, van Marle, Luo, & Fernández, 2009). Within the ECN, we only found decreased functional connectivity with the left cerebellum. With the whole brain, the connectivity of the ECN was further reduced in the left cuneus, left and right precuneus, posterior cingulate gyrus, left and right cerebellum, and right superior frontal gyrus. Except for the cerebellum, all these regions are part of the DMN, indicating a reduction of ECN connectivity with several areas of the DMN after stress. Similar results were also found in a meta-analysis of RSFC in anxiety disorders, where anti-correlations between the ECN and the DMN were associated with anxiety (Xu et al., 2019). To look at the direction of the effect and to find out whether there might be an anti-correlation of the ECN with the DMN we plotted contrast estimates. The plot in figure 6(a) showed that there was a significant decrease in connectivity after stress. However, these were all in a positive range, suggesting a decrease in connectivity rather than anti-correlation of the ECN with the DMN. It is possible, however, that these results were influenced by the choice of preprocessing steps. Generally, previous studies have demonstrated the influence of preprocessing steps on functional connectivity (DeSalvo, 2020; Gavrilescu et al., 2007) and another study specifically demonstrated that global signal regression can induce artificial anti-correlations (Weissenbacher et al., 2009). Thus, it is possible that the observed anti-correlations between ECN and DMN from previous studies were influenced by the choice of preprocessing steps. There could have also been an influence of the chosen stress induction paradigm, however, it is not clear whether different stress induction procedures lead to differences in stress states (van Oort et al., 2017).

Additionally, it needs to be noted that none of the participants in our sample had a diagnosis of an anxiety disorder and that only half of the individuals in this sample show anxiety scores that indicate clinical levels of anxiety. Therefore, it makes sense that the results differ from the results by Xu et al. (2019). Besides that, the decrease in connectivity between the ECN and the DMN may be driven by the within DMN change. While there was barely any within ECN change, the within DMN connectivity was altered after stress, indicating that the DMN might play a role in the decrease in connectivity between the ECN and the DMN.

In the SN, we expected to find increased functional connectivity within the SN as well as with areas outside the SN. In contrast to our expectations, there was, however, no increase in SN functional connectivity. An effect in the opposite direction was found, with decreased connectivity of the SN with the right insula (contrast estimates can be found in figure 6(b)). Thus, except for the decrease in connectivity with the right insula, the connectivity of the SN after stress remained unchanged. Even though decreased ECN connectivity as well as increased SN connectivity as proposed in Hermans et al. (2014) has been replicated by many studies and seems to be a quite robust effect, some inconsistent findings concerning ECN, SN, and DMN functional connectivity after stress have been reported (Y. Zhang et al., 2020). One study, for example, reported deactivation of several regions of the SN after stress (Pruessner et al., 2008), which was, however, not a functional connectivity analysis. Similarly, Pruessner et al. (2008) also used a psychosocial stressor

with high cognitive load. Thus, again it is possible that the choice of the task had an effect on the resulting connectivity pattern.

Additionally, SN functional connectivity has been found to be weakened in anxiety disorders (Xu et al., 2019). As half of the sample shows anxiety scores above the normal range, it could be the case that the usual effects in the SN after stress are weakened. A posthoc exploratory analysis revealed that there was indeed an increase in SN functional connectivity with the middle frontal gyrus (partly in and partly outside of SN) (peak MNI coordinates: -24 38 40, pFWE-corr = 0.038, cluster size = 45) in the low anxiety group, but not in the high anxiety group. Further, there was no significant decrease in SN connectivity in low anxiety individuals. Thus, it could indeed be the case that functional connectivity of the SN is weakened or disrupted in people with higher levels of anxiety and that this leads to the unexpected decrease found here.

Two further possible reasons for the lack of increased SN connectivity were already proposed by Leeuwen et al. (2020), who also investigated functional connectivity changes after stress in an at-risk sample. Either, functional connectivity is unaffected before stress but fails to upregulate in response to stress, or SN connectivity is already elevated so that no further increase of SN connectivity is possible. Since the comparison between high and low anxiety groups did not reveal a difference in pre-stress connectivity, the first proposed option is more likely. As the same failure to upregulate the SN as in this at-risk sample was also found in siblings of schizophrenia patients, it could be a possible mechanism that is altered in (vulnerability to) psychopathology.

Alternatively, previous research has implicated the insula in switching between brain networks like the ECN and DMN as well as in the experience of emotion and subjective awareness of positive and negative feelings (Menon & Uddin, 2010). As all of this is expected to be altered after acute stress, the decrease in connectivity may be driven by a change in insula activity. Thus, the insula seems to play an important role under stress, and future research should examine its role in the stress response more closely.

It is further possible that there is an influence of the stress induction method or a transfer effect from a previous task that led to the unexpected decrease in SN connectivity. In comparison to other stress induction methods, the ScanSTRESS task is rather cognitively focused compared to methods like the cold pressor test, which includes a physical component. Additionally, while often male stressors or stressors of the opposite sex are used in stress studies, only female stressors were used in this study. One study, for example, showed that cortisol only increases when stress was induced by a stressor of the opposite sex (Duchesne, Tessera, Dedovic, Engert, & Pruessner, 2012). It is possible that these methodological differences led to a decrease instead of the expected increase in SN connectivity, although it would be surprising that the use of a different stress induction method or a difference in the gender of the stressor can induce such a strong effect in the opposite direction. Since the stress task is the last of five functional tasks participants perform in the scanner, there might also be a transfer effect from one of the previous tasks leading to elevated baseline SN connectivity already before stress induction. It was indeed the case that stress ratings before stress induction were significantly higher in the low resilience group. Similarly, the low resilience group also showed increased SN connectivity before stress induction so that it is plausible that no further increase in SN connectivity in response to stress was possible (second explanation for disrupted SN upregulation proposed in Leeuwen et al. (2020)). In future analyses, participants' subjective

stress levels before the first resting-state scan could be assessed as well, giving an idea of the trajectories of subjective stress in the different groups. This could give an indication of whether baseline stress levels were already increased in anticipation of the stress task.

An additional difficulty is to disentangle the acute stress phase and the recovery phase. As the stress response is a continuous process, no clear distinction between both phases can be made (van Oort et al., 2017). Thus, it is not possible to say whether participants are still in their acute stress phase or have already entered some kind of recovery phase. If this was the case, it could be a possible explanation for the lack of SN increase following acute stress. Generally, it is, however, assumed that participants are still in a continued stress phase right after stress induction and that the degree to which the participant has recovered from stress then depends on the length of the resting-state scan (van Oort et al., 2017). As the exact timings of the stress response are still unclear, it could potentially be the case that the connectivity of the SN is indeed still increased right after stress induction at the beginning of the second resting-state scan, but that it decreases again during the course of the scan.

In the DMN, there was an increase in functional connectivity with the right precuneus (DMN) and decreased functional connectivity of the DMN with the middle cingulate gyrus outside of the DMN. The increased functional connectivity within the DMN is in line with previous work (Vaisvaser et al., 2013). Also, similar interactions between DMN and precuneus have been found previously (Fransson & Marrelec, 2008). The DMN is further often divided into an anterior (centres on medial prefrontal cortex) and a posterior part (centres on posterior cingulate cortex and precuneus) (Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010; Buckner, Andrews-Hanna, & Schacter, 2008). While the anterior part is mostly implicated in self-referential processing and memory regulation, the posterior part has been found to be involved in consciousness and memory processing (Andrews-Hanna et al., 2010; Leech & Sharp, 2014). Increased within DMN connectivity with the precuneus in the posterior part of the DMN may thus reflect enhanced memory processing and reflections about preceding stressful experiences (Fransson & Marrelec, 2008; Vaisvaser et al., 2013).

The decrease in functional connectivity with the middle cingulate gyrus is similar to W. Zhang et al. (2019) who also found decreased functional connectivity to several regions in- and outside of the DMN after stress (W. Zhang et al., 2019). Another study found decreased RSFC of the DMN with the posterior cingulate gyrus (Quaedflieg et al., 2015). The middle cingulate gyrus is located right next to the posterior cingulate gyrus, which is part of the DMN according to the used mask and most of the literature (Leech & Sharp, 2014; Wang, Chang, Chuang, & Liu, 2019). Even though the middle cingulate gyrus was just outside the DMN mask, it could be considered as belonging to the DMN because of its proximity to the posterior cingulate gyrus and the DMN. However, it is difficult to make a clear decision on whether to consider it DMN or not. As the significant difference that was observed here was located right on the edge of the posterior cingulate gyrus, the decrease in connectivity with the middle cingulate gyrus might have resulted from similar reasons as the decreased connectivity of the DMN with the posterior cingulate gyrus. Previous studies regarded this decrease as a deactivation of the DMN (Quaedflieg et al., 2015) which is important for focused attention (Weissman, Roberts, Visscher, & Woldorff, 2006). Thus, it would mean that the DMN is partly upregulated because

participants were reflecting about the stress task, and partly down-regulated because participants' focused attention on the task led to a deactivation of the DMN.

**Influence of anxiety and resilience on functional connectivity changes**
Dividing participants into high and low anxiety groups revealed several further differences. Firstly, post-stress functional connectivity of the DMN with the middle frontal gyrus was significantly higher in the low anxiety group. Secondly, there was a difference in DMN - right precentral gyrus/right posterior cingulate gyrus connectivity, with low anxiety individuals showing increased and high anxiety individuals showing decreased RSFC with this area (contrast estimates can be found in figure 10(a)). In anxiety disorders, many differences in RSFC especially in the SN and DMN have been demonstrated in previous studies. It has been found, for example, that DMN connectivity is altered in individuals with anxiety disorders (Menon, 2011; Peterson et al., 2014) and that SN connectivity is weakened (Xu et al., 2019), which seems to be in line with the findings of the current study.

As discussed earlier, there seems to be a lack in SN increase, potentially driven by the high anxiety group. If this is indeed the case, the lack of DMN increase with the middle frontal gyrus in high anxiety may be driven by the failure to upregulate SN connectivity following acute stress. The decreased DMN – right precentral gyrus/right posterior cingulate gyrus connectivity in high anxiety individuals compared to the increased connectivity in low anxiety individuals may be driven by the commonly observed deactivation of the DMN with higher demand (Zhao et al., 2007). Even though the significant region in the posterior cingulate gyrus is again just outside the DMN mask, it is commonly considered as a hub region of the DMN. Further, the posterior cingulate gyrus has been found to be deactivated during cognitively demanding tasks and that its activity varies with arousal state (Leech & Sharp, 2014). It is not unlikely that task demand and arousal state vary between high and low anxiety individuals, leading to decreased functional connectivity within the DMN in the high anxiety group because they experience higher arousal and higher demand during the stress task.

Further differences were found after dividing participants into a high and a low resilience group. Firstly, higher SN connectivity with the middle frontal gyrus (mostly part of SN) was found in the low resilience group, pre- and post-stress. Secondly, there was a decrease in functional connectivity of the ECN with the superior frontal gyrus (part of DMN) in the low resilience group, while the opposite pattern was observed for the high resilience group. The contrast estimates in figure 10(b) further show that the main difference was in the low resilience group, where a decrease in connectivity from pre- to post-stress was observed, while connectivity in the high resilience group increased minimally from pre to post. The decrease in the low resilience group is similar to the main results of the study that also showed a decrease in ECN connectivity with the superior frontal gyrus of the DMN and is also in line with our expectations.

This difference in ECN-superior frontal gyrus connectivity might be similar to the decoupling between the ECN and the DMN found in anxiety disorders (Xu et al., 2019). It could be possible that individuals with low resilience show a similar pattern of ECN - DMN connectivity as individuals with high anxiety. In the current study, however, we did not find a significant difference in ECN - DMN connectivity when comparing the

high and low anxiety groups. The increase in connectivity of the ECN with the DMN in the high resilience group was rather unexpected since downregulation of the ECN after stress is commonly observed. However, this might be a result of differences in ECN or DMN connectivity. As discussed earlier, DMN connectivity depends, among others, on task demand and arousal level, which might be different between the two groups. Further, it is also not unlikely that participants in the high and low resilience groups recruit their ECN differently, leading to an increase in connectivity, rather than a decrease. Finally, it would be possible that in high resilient people, less resources need to be reallocated to other regions following stress, representing a potential protective factor. However, future studies need to investigate this possible protective mechanism further.

Similar to the higher within SN connectivity in the low resilience group found here, increased SN connectivity has also been demonstrated in other stress-related disorders like PSTD (Abdallah et al., 2019; Koch et al., 2016). Thus, individuals with low resilience might express similar patterns of SN connectivity as individuals with stress-related disorders like PTSD. Further, comparisons of the stress ratings between the resilience groups revealed that the low resilience group was already significantly more stressed before stress induction than the high resilience group, possibly explaining the higher SN levels at baseline. The connectivity pattern observed in low resilience seems to be different from that in high anxiety, however. While the results indicate that individuals with anxiety fail to upregulate their SN connectivity in response to stress, SN connectivity in low resilience is already increased before stress induction, indicating different patterns of connectivity in these vulnerable groups.

The absence of a correlation between trait anxiety and resilience further indicates that both are distinct factors and that it is not possible to predict resilience from trait anxiety. Interestingly, while there is no correlation between trait anxiety and mental health problems (GHQ scores), there was a positive correlation between trait anxiety and stressor exposure (DH scores based on stressor count method; more details on stressor count and stressor severity method in Veer et al. (2021), supplementary material). In future analyses, it would also be interesting to see whether more anxious people actually experience more stressors or whether they just experience the stressors as more burdening by comparing stressor count and stressor severity scores.

These correlational findings between trait anxiety and resilience may, however, not be transferable to the general population. Resilience is calculated from the EP regression line in the current sample and may be different from that in the general population. A comparison of this EP-line to that of a larger sample from the general population could give an indication whether this sample shows resilience levels comparable to that of the general population. With a mean score of 41.15, trait anxiety scores in this sample also seem to be a bit higher than those in the general population, where mean scores around between 35 and 36 have been found (Donzuso, Cerasa, Gioia, Caracciolo, & Quattrone, 2014; Fountoulakis et al., 2006). Therefore, high and low anxiety and high and low resilience should be considered only with respect to this sample and may not be transferable to the general population.

**Importance and limitations**

While some of the findings of this study are perfectly in line with previous studies, other findings like the decrease in SN connectivity after stress are unexpected. However, there have also been discrepancies in other studies regarding increases and decreases in RSFC in the ECN, SN, and DMN following stress induction (van Oort et al., 2017; Y. Zhang et al., 2020). Potential reasons for these differences are discussed in van Oort et al. (2017) and include the influence of different experimental settings such as stress induction methods, analysis methods (seed analysis vs. ICA), and inconsistencies in which brain regions belong to which network. Furthermore, different preprocessing steps and analyses make comparisons between different studies even more difficult. Especially the heterogeneity of the different stress studies make it hard to determine whether the different stress induction paradigms lead to differences in the processing of the stressful task (van Oort et al., 2017).

Nevertheless, the research of functional connectivity changes in response to stress is of great importance. This study showed that there are significant differences between individuals with different levels of anxiety and different levels of resilience after stress and unravelling the underlying mechanisms and differences further will help us deepen our understanding of resilience and stress-related disorders, which can improve treatment options in the future (Snijders et al., 2018). Between the anxiety groups, differences were mainly found in the DMN, which could be a potential risk factor for anxiety disorders. The increased connectivity of the ECN with the superior frontal gyrus in the high resilience group, as opposed to the decrease in the low resilience group, may point towards a possible protective factor.

However, the current study also has a few potential limitations. One limiting factor is the lack of a control session, where no stress is induced. Ideally, participants would come back for a control session on a separate day, which is again preceded and followed by a resting-state scan. This way, the change in connectivity from the stress and the control session could have been compared directly, ensuring that the observed changes are due to stress. Also, an additional resting-state scan later that day would have been useful to look at the late effects of recovery and to disentangle between stress and recovery phases. Both options were not feasible within the DynaM-OBS study but would have been an interesting addition.

Furthermore, it should also be mentioned that due to missing data, the resilience groups are smaller than the anxiety groups, and while there are about the same number of participants in the high and the low anxiety group (high anxiety = 24, low anxiety = 23), there is a larger difference in group sizes between the resilience groups (high resilience = 24, low resilience = 19). Also, again due to missing questionnaire data, not all resilience scores could be calculated from the same timepoint (in four participants, data from M1W3 instead of M1W1 was used). As stressed at-risk individuals were selected for this study to capture fluctuations in resilience, it is not unlikely that resilience scores have changed from one timepoint to the next (two weeks later) and therefore don't reflect the level of resilience in the week after the MRI session. Thus, results from the comparison of the resilience groups should be considered more carefully than those of the anxiety groups.

The most important limitation of the current study is that saliva samples have not yet been analysed and that therefore, no cortisol measurements are available. Thus, no

biological marker for stress could be included in the analysis and the only indication for participants' stress levels are their subjective stress ratings. While this already gives an indication of whether stress was successfully induced using the stress task, there is no information on whether participants' HPA-axis was activated and cortisol was released in response to stress. Previous studies have found that while cortisol increases in response to stress in some participants (cortisol responders), no such an effect can be found in other participants (cortisol non-responders) and differences in connectivity between responders and non-responders have been found in several studies (Dimitrov et al., 2018; Quaedflieg et al., 2015). Group differences could thus be strongly influenced by cortisol non-responders (Voges et al., 2021).

While the current study with a sample size of 47 is already quite large and it should already be possible to draw conclusions from this, the intended sample size of the DynaM-OBS study of 250 is a lot larger. Thus, current results need to be confirmed in the whole sample of 250 participants in future analyses. It would also be interesting to see if correlations between connectivity changes and anxiety or resilience scores can be found in the whole sample, which was not done here because of the anticipated rather small sample size. The addition of cortisol measurements can hopefully clarify inconsistencies there are at this time. Finally, DynaM-OBS is a longitudinal study in which changes in resilience over time are assessed. Here, only one timepoint is measured and therefore fluctuations in resilience and its dynamic nature cannot be analysed. It will hopefully be possible to confirm the identified risk and protective factors later in time in the DynaM-OBS study.

## 5 Conclusion

The study aimed to investigate whether individuals with different anxiety and resilience scores differ in their stress-induced functional connectivity and whether it is possible to predict resilience from the acute stress response. In the whole sample, ECN and SN connectivity was decreased and DMN connectivity was altered following acute stress. While these effects are partially consistent with previous findings, deviations from the literature might result from including an enriched sample comprising at-risk individuals. In the anxiety groups, differences in connectivity were mainly found in the DMN, which could point towards a potential risk factor in anxiety disorders, while differences in the resilience groups were found in ECN and SN. The difference in ECN - superior frontal gyrus connectivity in the high resilience group may point towards a possible protective factor and it may be possible to predict resilience based on this difference in functional connectivity after stress. Finally, RSFC patterns associated with the stable risk factor anxiety, seem to be distinct from the RSFC patterns associated with the dynamic resilience factor.

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# Appendix 1

## In- and exclusion criteria

### Inclusion criteria

In order to be eligible to participate in this study, a participant must meet all of the following:

- The participant agrees to participate by providing written informed consent. Participant is able to obtain full insight into the objectives of the study and is fully contractually capable. Participant is willing and able to comply with the protocol.
- The participant is 18-25 years of age on the day of signing informed consent.
- The participant has experienced at least 3 life events from the "Life Events Questionnaire, LEQ", which were each evaluated by the participant as burdening.
- The participant is currently studying or in vocational training
- The participant is proficient in Dutch (minimum level of C1 in the Common European Framework of Reference for Languages).

### Exclusion criteria

A potential participant who meets any of the following criteria will be excluded from participation in this study:

- The participant currently meets criteria of a relevant psychiatric disorder except for a mild depressive episode (ICD F32.1), tobacco dependence (ICD F12), or substance abuse as established using the Mini-International Neuropsychiatric interview.
- In the past 9 months, the participant has met criteria for a relevant psychiatric disorder except for a mild depressive episode (ICD F32.1), tobacco dependence (ICD F12), and substance abuse.
- The participant reports the use of any psychoactive substances 4 weeks prior to Baseline Day 1 and the MRI appointment. The participant receives hormonal treatment other than oral contraceptives and/or takes steroids.
- The participant has ever been diagnosed with a severe mental or organic disorder that affects neurodevelopment due to its pathological mechanism or treatment (e.g. schizophrenia, bipolar disorder, anorexia/bulimia nervosa, attention deficit hyperactivity disorder during adulthood, autism spectrum disorder, meningitis, epilepsy, multiple sclerosis, stroke, brain cancer, brain concussion, coma).
- The subject's body mass index is lower than 18 or higher than 27.
- The participant is currently in psychiatric treatment.
- The participant has participated in a previous fear conditioning and/or stress induction paradigm
- The participant is not eligible for functional magnetic resonance imaging:
  - Abnormal hearing or (uncorrected) vision
  - Hearing impairment
  - Claustrophobia
  - Non-removable ferromagnetic metal in/at the body
  - Pregnancy

- The participant is not eligible to wear the Chill+:
  - Skin disease around the wrist
  - Skin irritations after an initial try-on test
  - A known allergy to adhesive Ag/AgCl electrodes
  - The use of medication with phototoxic side effects (i.e. Tetracylines, Doxycycline, Phenothiazines, Dacarbazine, Ketoprofen, Lomefloxacin)
  - Unremovable measuring devices or implanted active devices

## Appendix 2

### fMRIPrep details

Results included in this manuscript come from preprocessing performed using *fMRIPrep* 20.2.1 (Esteban et al. (2018); Esteban, Blair, et al. (2018); RRID:SCR_016216), which is based on *Nipype* 1.5.1 (K. Gorgolewski et al. (2011); K. J. Gorgolewski et al. (2018); RRID:SCR_002502).

**Anatomical data preprocessing** A total of 1 T1-weighted (T1w) images were found within the input BIDS dataset.The T1-weighted (T1w) image was corrected for intensity non-uniformity (INU) with `N4BiasFieldCorrection` (Tustison et al., 2010), distributed with ANTs 2.3.3 (Avants, Epstein, Grossman, & Gee, 2008, RRID:SCR_004757), and used as T1w-reference throughout the workflow. The T1w-reference was then skull-stripped with a *Nipype* implementation of the `antsBrainExtraction.sh` workflow (from ANTs), using OASIS30ANTs as target template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using `fast` (FSL 5.0.9, RRID:SCR_002823, Y. Zhang, Brady, & Smith, 2001). Brain surfaces were reconstructed using `recon-all` (FreeSurfer 6.0.1, RRID:SCR_001847, Dale, Fischl, & Sereno, 1999), and the brain mask estimated previously was refined with a custom variation of the method to reconcile ANTs-derived and FreeSurfer-derived segmentations of the cortical gray-matter of Mindboggle (RRID:SCR_002438, Klein et al., 2017). Volume-based spatial normalization to two standard spaces (MNI152NLin6Asym, MNI152NLin2009cAsym) was performed through nonlinear registration with `antsRegistration` (ANTs 2.3.3), using brain-extracted versions of both T1w reference and the T1w template. The following templates were selected for spatial normalization: *FSL's MNI ICBM 152 non-linear 6th Generation Asymmetric Average Brain Stereotaxic Registration Model* [Evans, Janke, Collins, and Baillet (2012), RRID:SCR_002823; TemplateFlow ID: MNI152NLin6Asym], *ICBM 152 Nonlinear Asymmetrical template version 2009c* [Fonov, Evans, McKinstry, Almli, and Collins (2009), RRID:SCR_008796; TemplateFlow ID: MNI152NLin2009cAsym].

**Functional data preprocessing** For each of the 1 BOLD runs found per subject (across all tasks and sessions), the following preprocessing was performed. First, a reference volume and its skull-stripped version were generated by aligning and averaging 1 single-band references (SBRefs). Susceptibility distortion correction (SDC) was omitted. The BOLD reference was then co-registered to the T1w reference using `bbregister` (FreeSurfer) which implements boundary-based registration (Greve & Fischl, 2009). Co-registration was configured with six degrees of freedom. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated before any spatiotemporal filtering using `mcflirt` (FSL 5.0.9, Jenkinson, Bannister, Brady, & Smith, 2002). BOLD runs were slice-time corrected using `3dTshift` from AFNI 20160207 (Cox & Hyde, 1997, RRID:SCR_005927). First, a reference volume and its skull-stripped version were generated using a custom methodology of *fMRIPrep*. The BOLD time-series (including slice-timing correction when applied) were resampled onto their original, native space by applying the transforms to correct

for head-motion. These resampled BOLD time-series will be referred to as *preprocessed BOLD in original space*, or just *preprocessed BOLD*. The BOLD time-series were resampled into standard space, generating a *preprocessed BOLD run in MNI152NLin6Asym space*. First, a reference volume and its skull-stripped version were generated using a custom methodology of *fMRIPrep*. Automatic removal of motion artifacts using independent component analysis (ICA-AROMA, Pruim et al., 2015a) was performed on the *preprocessed BOLD on MNI space* time-series after removal of non-steady state volumes and spatial smoothing with an isotropic, Gaussian kernel of 6mm FWHM (full-width half-maximum). Corresponding "non-aggresively" denoised runs were produced after such smoothing. Additionally, the "aggressive" noise-regressors were collected and placed in the corresponding confounds file. Several confounding time-series were calculated based on the *preprocessed BOLD*: framewise displacement (FD), DVARS and three region-wise global signals. FD was computed using two formulations following Power (absolute sum of relative motions, Power et al. (2014)) and Jenkinson (relative root mean square displacement between affines, Jenkinson et al. (2002)). FD and DVARS are calculated for each functional run, both using their implementations in *Nipype* (following the definitions by Power et al., 2014). The three global signals are extracted within the CSF, the WM, and the whole-brain masks. Additionally, a set of physiological regressors were extracted to allow for component-based noise correction (*CompCor*, Behzadi, Restom, Liau, & Liu, 2007). Principal components are estimated after high-pass filtering the *preprocessed BOLD* time-series (using a discrete cosine filter with 128s cut-off) for the two *CompCor* variants: temporal (tCompCor) and anatomical (aCompCor). tCompCor components are then calculated from the top 2% variable voxels within the brain mask. For aCompCor, three probabilistic masks (CSF, WM and combined CSF+WM) are generated in anatomical space. The implementation differs from that of Behzadi et al. in that instead of eroding the masks by 2 pixels on BOLD space, the aCompCor masks are subtracted a mask of pixels that likely contain a volume fraction of GM. This mask is obtained by dilating a GM mask extracted from the FreeSurfer's *aseg* segmentation, and it ensures components are not extracted from voxels containing a minimal fraction of GM. Finally, these masks are resampled into BOLD space and binarized by thresholding at 0.99 (as in the original implementation). Components are also calculated separately within the WM and CSF masks. For each CompCor decomposition, the $k$ components with the largest singular values are retained, such that the retained components' time series are sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components are dropped from consideration. The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. The confound time series derived from head motion estimates and global signals were expanded with the inclusion of temporal derivatives and quadratic terms for each (Satterthwaite et al., 2013). Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardised DVARS were annotated as motion outliers. All resamplings can be performed with *a single interpolation step* by composing all the pertinent transformations (i.e. head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces). Gridded (volumetric) resamplings were performed using `antsApplyTransforms` (ANTs), configured with Lanczos interpolation to minimize the smoothing effects of other kernels (Lanczos, 1964). Non-gridded (surface) resamplings were performed using `mri_vol2surf` (FreeSurfer).

Many internal operations of *fMRIPrep* use *Nilearn* 0.6.2 (Abraham et al., 2014, RRID:SCR_001362), mostly within the functional processing workflow. For more details of the pipeline, see the section corresponding to workflows in *fMRIPrep*'s documentation.

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