Neural Responses to Reward and Aversion as a Possible Biomarker for Depression

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

Anhedonia has been suggested as a possible biomarker for depression. Moreover many studies have shown that symptoms of anhedonia are associated with a dysfunction in the neural circuits for reward processing. Given that depression typically begins during adolescence and has high rates of reoccurrence, examining reward function in adolescence could help us understand the aetiology, pathophysiology and the course of depression as well as perhaps even aid the development of new treatments.

This thesis is a composition of four studies. The first of these studies examined the neural response to the anticipation, effort and consummation of reward and aversion in adolescents at increased risk for depression. This is the first study that we know of that has examined the neural response to the sub components of reward and aversion; anticipation, effort and consumption in adolescents at risk of depression. We found overall blunted response to both reward and aversion in the at risk group which is what we would have hypothesized given the previous work using a similar task in people both recovered from depression and young people with a family history of depression.

The second study extended this work by including a group of adolescents with a clinical diagnosis of depression and collapsing across the groups using a dimensional approach. Dimensional analysis was also then used to examine the relationship between brain activity in regions of interest and depression and anhedonia symptoms. Results of this study revealed decreasing brain responses in key brain regions implicated in the aetiology of depression for the anticipation, effort and consummation of reward and aversion with increasing symptoms of depression and anhedonia. This study extends the previous literature in a number of ways. Firstly, it is the first study examining the neural response to the sub components of reward and aversion processing: anticipation, effort and consummation in depression. Further it is the first study examining how this is related to a range of depression severities in young people. The results of this study finds that there are indeed deficits in key neural regions involved in the processing of reward

and punishment and that these deficits are correlated with symptom severity, suggesting possible neural biomarkers for depression.

Given that the key resting-state functional connectivity (RSFC) networks have been reported dysfunctional in depression, the remaining two studies of this thesis aimed at investigating the RSFC. Study 3 examined adolescents at risk of depression while study 4 again used a more dimensional approach by combining data of those at risk with adolescents also with a clinical diagnoses of depression. Results of these studies extend current literature in adults by investigating adolescents. Secondly they extend the current knowledge of RSFC in depression by examining the relationship between depression symptoms and RSFC in a dimensional manner. This work shows that young people across a range of depression symptoms show similar patterns of RSFC deficits to previous studies in adults. However this work extends the data also by examining correlations between the RSFC in key networks such as the Cognitive Control Network, the Default Mode Network and the Salience Network and depression symptoms and also anhedonia symptoms.

Developing behavioural and pharmacological treatments that target these specific deficits in young individuals might improve the success rate for depression treatment that is currently at a low level of around 40%.

Peer review publications arising from this work

Rzepa, E., Fisk, J. and McCabe, C. (2016) Blunted neural response to anticipation, effort and consummation of reward and aversion in adolescents with depression symptomatology. Journal of Psychopharmacology, 31(3), pp. 303-311. ISSN 1461-7285 doi: 10.1177/0269881116681416

Rzepa, E. and McCabe, C. (2016) Decreased anticipated pleasure correlates with increased salience network resting state functional connectivity in adolescents with depressive symptomatology. Journal of Psychiatric Research, 82. pp. 40-47. ISSN 0022-3956 doi: 10.1016/j.jpsychires.2016.07.013.

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Chapter 1

1. General introduction

Major Depressive Disorder (MDD) is characterized by persistent sadness, loss of interest or pleasure, feeling of worthlessness or guilt, sleep disturbances, poor concentration, suicidal thoughts (Holtzheimer & Mayberg, 2011) and is one of the most occurring mental disorders causing many lifelong detrimental impacts (Kessler et al., 2003). With a lifetime prevalence of approximately 16 %, it is predicted that by 2020 MDD will be the second most disabling condition behind the ischemic heart disease (Ferrari et al., 2013; Murray & Lopez, 1996). MDD affects people of all ages, backgrounds, lifestyles, nationalities and genders, however twice as many women are affected as men. MDD is claimed to be heritable in around 30%-40% indicating involvement of both genetic and environmental risk factors (Sullivan, Neale, & Kendler, 2000). The economic burden of depression is significant. It has been estimated that in England, the cost of depression reaches around £9bn per year with the biggest effect on productivity, as self-reported depression has been the main cause of absenteeism in the UK (Cyhlarova, 2010).

It is crucial to emphasize that between 8-15% of adolescents are affected by depression by the age of 18 (Merikangas et al., 2010; Saluja et al., 2004). As recognition of MDD in adolescents has increased in recent years, more attention has been placed on the etiology and the consequences of early onset depression.

Adolescence is a transition period from childhood to adulthood. There are no clear age boundaries identifying the adolescence period, with some studies classifying 9 years old as adolescents and others 14 years old (Ernst et al., 2005; Van Leijenhorst et al., 2010). The upper bound of the adolescence period is also not established, with 18 years old classified as adolescents in Europe and 21 years old in the USA (Galvan, 2010).

Furthermore, adolescence is a critical period for the formation of identity, interpersonal relationships, and independence from caregivers. It is also accompanied by many, physical, psychological and social changes. However, vulnerability to depression increases markedly during adolescence as some of these changes expose young people to additional life stresses e.g. school change, failures of romantic relationships, disappointments, which might contribute to developments of mental health problems including MDD (Davey, Yucel, & Allen, 2008).

The neurobiological changes associated with the development of emotional and cognitive abilities also occur around the transitional period from childhood to adulthood (Weir, Zakama, & Rao, 2012). The most obvious neural changes are associated with greater myelination of cortical connections and synaptic pruning (Weir et al., 2012). These processes are very important as they result in an increase of speed of neural transmission as well as they create more dedicated neural networks for cognitive processing (Sowell, Trauner, Gamst, & Jernigan, 2002). Furthermore, studies on working memory or response inhibition in children and adolescents have directly linked the neural re-modeling of the brain with the brain functionality where both working memory and response inhibition improve significantly as a function of age (Nagy, Westerberg, & Klingberg, 2004; Tamm, Menon, & Reiss, 2002).

Moreover, the neural circuits of cognition and emotion undergo a maturation process. However, different brain areas mature independently from one another and at their own pace (Casey, Jones, & Hare, 2008). For example, the frontal cortical areas such as the prefrontal cortex (PFC) mature much later in adolescence or in early adulthood. At the same time, the subcortical limbic structures seem to be already developed (Casey et al., 2008). Studies supporting this observation have reported an enhanced nucleus accumbens activity to rewarding stimuli in healthy adolescents, which was similar to reports in adults and different from results found in children where the nucleus

accumbens activation was decreased (Galvan et al., 2006). Furthermore, this increased nucleus accumbens activation in adolescents was accompanied by the orbitofrontal cortex (OFC) activity that was more similar to children than to adults (Galvan et al., 2006). These results suggest that nucleus accumbens maturation precedes that of the OFC and possibly suggests that the bottom-up emotional processing in subcortical regions is enhanced in adolescents relative to less effective top-down regulation from the prefrontal regions (Galvan et al., 2006). This imbalance between emotional processing and the control systems influenced by a combination of environmental factors might contribute to an increased risk of affective disorders in adolescents such as MDD.

Many longitudinal studies that have focused on the consequences of early-onset depression suggest that adolescent depression has a detrimental impact on normal development and leads to poorer outcomes in adulthood. For example, (Harrington, Fudge, Rutter, Pickles, & Hill, 1990) reported that 60% of adults with adolescent onset depression experienced one or more episodes of MDD in the adulthood compared to 27% in the control group. More recent studies have additionally reported significantly increased risk of later anxiety disorder, nicotine dependence, alcohol abuse, suicide attempt, educational underachievement and unemployment in adults with the early onset depression when compared with healthy controls (HC) (Fergusson & Woodward, 2002; Weissman et al., 1999).

In summary, there are many changes associated with the transition from puberty to adolescents and then to adulthood. The interrelation of the socio-psychological and neurodevelopmental changes, additionally accompanied by the environmental stress factors put young people at increased risk for MDD. Moreover, statistics show that early-onset depression has long-term socio-economic and health consequences and has become a serious problem in the society. Thus studying the etiology of MDD in young people could improve current psychological and pharmacological treatments for depression.

Moreover, results of such studies could offer more practical advantages e.g. improve initial diagnosis, help to identify people at risk for depression and to plan prevention programs.

1.1. Anhedonia in MDD - diagnostic tools

One of the key diagnostic criterions for MDD, as recognized by two major psychiatric diagnostic systems: the Diagnostic Statistical Manual (DSM) (American Psychiatric Association), and the International Statistical Classification of Diseases (ICD) (World Health Organization), is anhedonia. The clinical definition of anhedonia describes it as a markedly diminish interest or pleasure in all, or almost all, activities previously enjoyed.

The severity of anhedonia is mostly assessed by self-report depression-targeted instruments with internal items emphasizing the experience of pleasure in response to positive stimuli (e.g. Snaith-Hamilton Pleasure Scale (SHAPS) (Snaith, 1995), Chapman Anhedonia Scale (Chapman, Chapman, & Raulin, 1976) or Fawcett Clark Pleasure Capacity Scale (FCPS) (Fawcett, Clark, Scheftner, & Gibbons, 1983) and that assesses pleasurable experiences in different situations. However, some of these scales are characterized by a small number of items assessing anhedonia severity including the Hamilton Depression Rating Scale (Hamilton, 1960), and the Beck Depression Inventory Scale (Beck et al., 1996) which assess anhedonia in 1 item out of 17, and 4 items out of 21 respectively. This is potentially problematic as psychiatric reports and studies have shown that anhedonia is a heterogeneous concept, which may prove anhedonia in MDD diagnosis difficult if not recognized adequately. Therefore attention must be placed on addressing this issue and also on the constructs of anhedonia as a concept itself (I will discuss these in the next section). Thus, studies usually only look at the presence or lack of anhedonia but in this thesis I will also examine the anticipatory and consummatory

aspects of anhedonia. To do this, I have used the Temporal Experience of Pleasure Scale (TEPS) (Gard D.E, 2006).

1.2. Anhedonia in MDD – behavioural studies

The nature of anhedonic symptoms has been broadly investigated in laboratory settings, focusing mostly on passive processing of positive stimuli. Most of these results suggested that subjects with MDD process positive information as less positive and less arousing than control subjects (Sloan, Strauss, & Wisner, 2001) (Rottenberg, Kasch, Gross, & Gotlib, 2002). However, these studies did not take into account negative information processing which presented to be problematic, as it was not clear whether MDD patients experience a general reduction in affective responsiveness to positive and negative stimuli or a reduction specific to processing of pleasure. This was further resolved by designing studies looking at responsiveness to negative stimuli as well as positive (Eshel & Roiser, 2010; Forbes & Dahl, 2012; Garavan, Pendergrass, Ross, Stein, & Risinger, 2001; McCabe, Woffindale, Harmer, & Cowen, 2012). However, findings from these studies have been mixed and three competitive views (the Positive Attenuation View, the Negative Potentiation View, the Emotion-Context Insensitivity Hypothesis) of how depression alters emotional reactivity with supporting experimental evidence have emerged.

In order to understand how humans respond to emotional and rewarding stimuli and how this is related to the interplay between mood and emotion, it is crucial to define these two terms first. *Mood* is defined as diffuse, slow moving feeling state that is weakly tided to specific objects or experiences, and might last for days altering feeling and cognition (Watson, 2000). On the contrary, *emotion* refers to a short in time reaction that happens when we process meaningful stimuli (Ekman, 1992). Emotions can affect many response systems modulating feelings, behavior and physiology for minutes or seconds.

However, prolonged moods can significantly alter an experience of the short-term emotion or reward. In relation to people with MDD it is thought that a lack of positive mood would attenuate experience of positive emotion, and excessive negative mood would potentiate experience of negative emotion (Bylsma, Morris, & Rottenberg, 2008).

The first view on how depression alters emotional reactivity, the Positive Attenuation holds that depressed individuals will have reduced response reactivity to positive emotional stimuli because they exhibit low positive mood (Bylsma et al., 2008). Indeed, one of the core symptoms of MDD is anhedonia- the inability to experience pleasure that has been shown to correlate with reduced responses to reward (American Psychiatric Association). Also, some individuals with MDD present other symptoms that indicate dysregulation of appetitive motivation, which additionally helps supporting this conception (Salamone, 1994). As mentioned previously, there are many studies showing that depressed patients have attenuated reactivity to various kinds of positive stimuli (Eshel & Roiser, 2010; Forbes & Dahl, 2012; Garavan et al., 2001; McCabe et al., 2012).

The Negative Potentiation Hypothesis states that depressed individuals will have potentiated response reactivity to negative emotional stimuli because they exhibit high negative mood (Bylsma et al., 2008). The idea that negative moods reinforce negative emotions is broadly applied in cognitive sciences where negative moods are thought to aid negative cognitive processing resulting in negative interpretations and dysphoric reactions (Rush & Beck, 1978). Indeed, the strongest support for this hypothesis comes from studies of dysphoric individuals but there is no convincing evidence that this generalizes to MDD subjects (Gotlib, 1984; Lewinsohn, Lobitz, & Wilson, 1973) Additional support for this hypothesis is very limited and challenged by studies showing reduced responses to negatively valence stimuli (Allen, Trinder, & Brennan, 1999; Thomas et al., 2001).

In light of these contradictory results related to processing of negative information, the third view was presented. The Emotion-Context Insensitivity Hypothesis (ECI) assumes that depressed individuals will have reduced reactivity to all emotional stimuli, regardless of valence (Rottenberg, Gross, & Gotlib, 2005). This view is derived from an evolutionary perspective where depression is seen as a defensive motivational state that promotes environmental disengagement in order to protect an organism from potentially dangerous situations. Thus emotional reactivity is overall reduced. ECI holds on similar predictions to the Positive Attenuation view of reduced response to positive stimuli, but presents opposite prediction to the Negative Potentiation view of potentiated response to negative stimuli. Thus, the ECI holds that there is reduced responsiveness to both positive and negative stimuli. Support for this hypothesis comes from many studies and the most robust evidence comes from studies using within-subject designs where depressed participants are presented with both positive and negative stimuli (Dichter, Tomarken, Shelton, & Sutton, 2004; Rottenberg et al., 2002). Furthermore, a recent meta-analysis of studies that looked at affective responsiveness of individuals with MDD to various types of stimuli, such as viewing of pleasant and unpleasant pictures, sad and happy films, or reward and punishment stimuli, revealed that depression was associated with blunted reactivity to both positive and negative stimuli (Bylsma et al., 2008).

Interestingly, most studies used passive viewing of stimuli (pictures), however other approaches to assess anhedonia have also been used. For example, (McFarland & Klein, 2009) looked at different aspects of reward and punishment processing in people who were currently depressed, recovered depressed and HC. They used a paradigm in which participants had to actively rate emotions across 10 dimensions in response to different condition: anticipating monetary reward, anticipating unpleasant sensory stimulus that was a cold press, no change, and avoiding unpleasant sensory stimulus. Results for reward anticipation showed that currently depressed participants had significantly reduced rating of positive emotions when compared with HC and marginally

lower than previously depressed participants. On the contrary, there were no differences in non-rewarding stimuli and anticipation or avoiding unpleasant stimuli when compared with controls and recovered depressed participants (McFarland & Klein, 2009). The authors concluded that depressed individuals had decreased emotional reactivity to reward anticipation only and that this might be a state effect as recovered depressed individuals did not show this effect. Also, Pizzagalli and colleagues (2008) used reinforcement paradigms to further investigate anhedonia in depression (Pizzagalli, Iosifescu, Hallett, Ratner, & Fava, 2008). In this study participants were asked to categorize presented stimulus as belonging to one of two categories. Subjects were more rewarded for guessing category A as opposed to category B. Control subjects tended to develop a response bias towards the more rewarding category whereas depressed subjects did not. Both of the mentioned studies point out that individuals with MDD have deficits in processing of reward-relevant information however it remains unclear whether this is related to specific deficits in hedonic capacity, emotion reactivity or motivation. However, studies that measured hedonic capacity in a direct way by using tastes of a different sucrose concentration showed that there was no difference in ratings of subjective hedonic experience between MDD and healthy individuals (Amsterdam, Settle, Doty, Abelman, & Winokur, 1987; Berlin, Givry-Steiner, Lecrubier, & Puech, 1998; Kazes et al., 1994). However, it is possible that the lack of differences on this task is caused by different sensitivity to tastes among individuals that is not picked up by the rating measures rather than a fully intact hedonic capacity in the MDD subjects.

In summary, studies directly looking at both reward and aversive processing have shown that majority of subjects with MDD have decreased hedonic capacity but also might experience a general flattening of affect which is in line with the ECI theory.

1.3. Anhedonia as a trait marker for depression

Anhedonia has been seen as a specific feature of depression (Fawcett et al., 1983) and has been directly linked to functional abnormalities of neural circuits involved in reward-related processes (Wacker, Dillon, & Pizzagalli, 2009). This association between anhedonia and dysfunction of the brain reward system suggests anhedonia as a possible endophenotypic marker of depression. However, the evaluation of endophenotype is based on specific criteria described as endophenotype being: 1) strongly associated with the disease of interest, 2) heritable, 3) state-independent, 4) co-segregate in families, 5) more prevalent among relatives of ill probands than in healthy population (Hasler, Drevets, Manji, & Charney, 2004). Support for this potential hypothesis comes from epidemiological studies (Dryman & Eaton, 1991) whereby the presence of anhedonic symptoms often precede the onset of MDD over a year following the initial assessment. Another study that investigated the relationship between anhedonia and depression severity showed that in a sample of acutely depressed patients followed for a year, anhedonic scores remained constant despite a general reduction in depression severity (Schrader, 1997). Moreover, the same study suggested also a genetic link between anhedonia and vulnerability to depression, as anhedonia scores were associated with depression in first-degree relatives of depressed patients, and many other studies further supported this hypothesis (Goodman & Gotlib, 1999; Hammen, Brennan, Keenan-Miller, & Herr, 2008). As mentioned in the previous section, deficits in the processing of positive information has also been noted in those suffering from depression (McFarland & Klein, 2009; Pizzagalli et al., 2008) as well as in non-clinical individuals with anhedonia symptoms (Harvey, Pruessner, Czechowska, & Lepage, 2007). These data suggest that anhedonia might have trait-like rather than state-like characteristics.

Identifying anhedonia as a trait marker for depression could aid initial diagnosis, help identify people at risk for depression and help plan treatment and prevention strategies.

1.4. Neural circuits of reward processing in relation to anhedonia

The majority of neuroimaging studies that have examined the neural bases of anhedonia have involved patients diagnosed with MDD or schizophrenia and have mostly focused on the processing of rewarding stimuli. These studies investigating neural responses in healthy populations used a variety of rewarding stimuli such as viewing attractive faces (Aharon et al., 2001; Senior, 2003), emotional pictures (Lane, Fink, Chau, & Dolan, 1997), pleasurable music (Blood & Zatorre, 2001) or responding to monetary rewards (Knutson, Adams, Fong, & Hommer, 2001; O'Doherty, Kringelbach, Rolls, Hornak, & Andrews, 2001). Results of these studies showed increased activation in the limbic regions such as the amygdala, the ventral striatum thus the nucleus accumbens (NaCC), the caudate, the putamen, and the cortical regions of the prefrontal cortex including medial prefrontal cortex (mPFC) and ventromedial prefrontal cortex (vmPFC). Also, studies that investigated more specific aspects of reward such as predictive appetitive reward value or reward anticipation found additionally a prominent involvement of the orbitofrontal cortex (OFC) and the insula (Ernst et al., 2004; Gottfried, O'Doherty, & Dolan, 2003). Moreover, the anterior cingulate cortex (ACC) seems to be involved in a variety of reward-related tasks such as reward based decision-making or reward encoding (Bush et al., 2002; Sallet et al., 2007). However, it remains unclear whether these brain regions respond directly to the rewarding value presented in these tasks or their responsiveness is a secondary effect of other reward-related cognitive processes such as decision-making or encoding.

Studies looking at reward processing in a depressed population have found diminished responses in the ventral striatum, the amygdala and the vmPFC to emotional faces, which additionally were correlated with symptoms of anhedonia, but not depression severity per se (Keedwell, Andrew, Williams, Brammer, & Phillips, 2005). Also, (Epstein et al., 2006) found that MDD patients had decreased activation in the ventral striatum and the dorsomedial frontal cortex to positive words, which also correlated with high scores on anhedonia. Alternations in a similar neural network were also observed during a gambling task, which investigated responses to feedback information (Steele, Kumar, & Ebmeier, 2007). In this study, depressed patients presented with decreased activations in the ventral striatum and the ACC for both 'winning' and 'loosing' feedback and did not adjust their reaction time appropriately, when compared with control subjects. This behavioural deficit additionally correlated with self-reported anhedonia for both patients and controls.

However, as the research into reward processing has progressed, anhedonia must be considered as the complex construct that it is with a variety of subtypes. For example, many reviews suggest now that anhedonia can be further divided into: anticipatory anhedonia, that is related to an inability to anticipate or predict expected reward; consummatory anhedonia, that is related to deficits in the hedonic response to reward; motivational anhedonia that is related to diminished motivation to pursue rewards; decisional anhedonia that is related to impaired decision making in the context of reward (Argyropoulos & Nutt, 2013; Der-Avakian & Markou, 2012; Treadway & Zald, 2011).

Indeed, studies have shown that seeking out pleasurable experiences and then acting on them is differently distributed in MDD subjects. For example, the only behavioural study that is available in an MDD sample was run by (Sherdell, Waugh, & Gotlib, 2012b). They asked MDD patients and control participants firstly to rate humorous and non-humorous cartoons on how much they liked the cartoons, and then

participants had to exert effort to view a particular cartoon. They found diminished anticipatory reward in the MDD group when compared with control group that additionally predicted reduced motivation to obtain reward in people with MDD. No differences were found for the consummatory rewards. Further, human neuroimaging studies in MDD have identified many brain regions activated in the response to anticipation and consummation of a variety of rewards. The monetary incentive delay task revealed decreased basal ganglia activity in the putamen to monetary reward anticipation and decreased activation in the caudate to monetary gains in the MDD subjects when compared with the HC (Pizzagalli et al., 2009). Another study that also used a monetary task found hyperactivation in the dorsal ACC in the MDD group when compared with HC for anticipating of monetary gains (Gorka et al., 2014) while Knutson and colleagues (2008) found the dACC had hypoactivation when anticipating monetary loses (Knutson, Bhanji, Cooney, Atlas, & Gotlib, 2008). Both of the studies did not find group differences in the limbic structures between depressed and HC. Furthermore, a card guessing task that investigated neural activation during anticipation and receipt of monetary reward found decreased reward receipt in the ACC for the MDD versus the HC group (Chase et al., 2013).

Similar alterations in the neural correlates were also reported in children and adolescents with depression in response to monetary rewards that aimed at disseminating anticipatory and consummatory aspects of reward. Studies run by (Forbes et al., 2006; Forbes et al., 2009) have consistently reported decreased caudate activation to both monetary reward anticipation and outcome and increased responses in the dorsolateral prefrontal cortex (dlPFC) and mPFC in depression when compared with HC. Additionally, decreased caudate activation was associated with lower subjective positive affect in adolescents with MDD (Forbes et al., 2009). There is also evidence for alterations in the OFC, ACC and the amygdala in children with MDD for the monetary task (Forbes et al., 2006). However, Stringaris and colleagues (2015) study showed that

adolescents with MDD diagnosis had decreased activation in the ventral striatum and the middle and medial superior frontal gyrus for the anticipation of monetary rewards but not consummation when compared with HC (Stringaris et al., 2015).

The monetary incentive reward task was also used in a sample of remitted depressed individuals and the results revealed the hyperactivation of brain regions such as the ACC, the frontal gyrus and the caudate to reward anticipation and hypoactivation of the OFC, the insula, the frontal pole to reward outcomes (Dichter, Kozink, McClernon, & Smoski, 2012) suggesting that neural abnormalities can be still presented in behaviourally non-depressed subjects. Neural hyperactivation in remitted depressed individuals when compared with HC was also found in the PFC, hippocampus and the amygdala for monetary reward anticipation with no group differences during reward delivery (Ubl et al., 2015). More interestingly, another study, which used a direct paradigm involving the sight and taste of rewarding and unpleasant stimuli, showed that both anticipatory and consummatory aspects were reduced in unmedicated recovered depressed patients when compared to controls. Specifically, consummation of rewarding taste revealed decreased responses in the ventral striatum and the ACC with decreased responses for anticipation of unpleasant stimuli in the ACC and consummation of the unpleasant taste in the putamen, amygdala and the insula for recovered depressed versus HC (McCabe, Cowen, & Harmer, 2009). There were no group differences for the anticipation of reward. Moreover, these alterations were observed despite the lack of differences between the two groups in ratings of pleasantness for the stimuli suggesting that subjective mood of recovered depressed individuals had improved significantly with recovery but with persistent neural dysfunction remained.

Importantly, dysfunction of the reward system was also observed in healthy subjects at risk for depression. Gotlib and colleagues (2010) assessed neural responses of reward and loss in young girls at familial risk for depression and reported attenuated

putamen and insula activation during the anticipation of gains and attenuated ventral striatum and putamen activation to receipt of reward, and decreased activation in the ventral striatum for anticipation of loss with increased dorsal ACC responses and decreased activation in the dorsal striatum during loss outcome, when compared with controls (Gotlib et al., 2010). Also, another study reported decreased reward anticipation but not consummation for monetary task in healthy adolescents with family history of depression when compared with HC that was further correlated with increasing scores on depression measures in the at risk group only (Olino et al., 2014). Furthermore, our group investigated brain responses to sight and taste of rewarding and aversive stimuli and found that relative to controls, young people at familial risk for depression presented diminished neural responses in the OFC to rewarding stimuli, and increased activations in the insula and the OFC to aversive stimuli (McCabe et al., 2012). Interestingly, 'at risk' individuals did not show impaired ventral striatal response, which distinguishes them from recovered depressed patients for which the same experimental paradigm was used (McCabe et al., 2009). Thus, it is possible that the impaired ventral striatal response in the recovered depressed patients is a scar of having had depression rather than a real biomarker.

These studies indicate that individuals at increased risk for developing depression have abnormalities in the anticipatory and consummatory processes of rewarding and unpleasant/punishment stimuli, and that those abnormalities in the neural representation of reward may be present in individuals 'at risk' prior to the onset of depression.

Furthermore, there have been attempts to investigate motivational anhedonia as studies have pointed out that individuals with anhedonia might have difficulties to engage in rewarding behavior due to decreased motivation. It is difficult to measure motivation to obtain rewards however, effort-based decision making tasks have been proposed by (Treadway, Buckholtz, Schwartzman, Lambert, & Zald, 2009). In such paradigm, on each

trial participants are presented with a choice of a 'hard task' on which they need to invest more button presses or an 'easy task' with less button presses in order to earn different amounts of money. Trials are also presented with different levels of probability for monetary receipt. This task aims at measuring how effort-based decision making is modulated by reward magnitude, probability of reward receipt and expected value. A behavioural study in the healthy subjects that used the described task and self-reported measures of anhedonia found that individuals with increased scores on anhedonia measures had reduced willingness to make 'hard task' choices in exchange for a higher reward (Treadway et al., 2009). A similar result was found in the MDD population where those individuals were generally less willing to expend efforts for rewards than HC (Sherdell, Waugh, & Gotlib, 2012a; Treadway, Bossaller, Shelton, & Zald, 2012; Yang et al., 2014). To date, there is only one fMRI study in MDD that used this paradigm (Yang et al., 2016). This study reported reduced neural responses in the left caudate for 'high reward-low reward' and reduced neural responses in the right caudate and the superior temporal gyrus for 'high probability- low probability' in the MDD versus the HC groups.

Taken together, findings of the presented studies suggest the presence of a well-established dysfunctional reward system with anticipatory, consummatory and effort deficits reported in adolescent and adult depressed patients and in individuals at increased risk for depression. Some of the presented brain activation results have also been related to the behavioural symptoms of anhedonia suggesting a direct brain-behaviour relationship. Moreover, abnormalities in the brain reward system have been observed in recovered patients and individuals with a family history of depression that were independent of subjective anhedonia symptoms. This suggests that abnormalities in the brain reward system might be a trait marker for depression.

However, besides significant attempts to tease apart brain circuits that initiate the hedonic experience from those that might be consequences of experienced pleasure, we

still find overlapping brain regions for what we think are distinct reward aspects. This leads us to question the types of experimental paradigms we use (what they measure exactly) or the clinical populations we recruit (medication status, psychiatric comorbidities, age and gender differences). Nevertheless, further exploration of reward processing that will address the above issues are needed as results of such studies would have practical implications for designing diagnostic tools that could offer greater specificity for anhedonia diagnosis as well as designing new treatments that could potentially target the neurochemical systems directly linked to the sub-types of anhedonia.

1.5. Computational modelling in the study of depression

Computational modelling in the field of affective neuroscience tries to develop formal mathematical algorithms to provide insight to the neural computations of variety of brain processes (Huys, Maia, & Frank, 2016). In depression, computational modelling has been mostly used in studying disrupted learning and decision-making, especially in experimental paradigms of reinforcement learning (RL) (Eshel & Roiser, 2010). RL is a process that aims at maximizing rewards and minimizing losses by modulating behaviour. More specifically, subjects choose actions according to mathematical value functions that determine the expected value of the action. Furthermore, value functions can be updated based on previous trials and error experience (the difference between the received and expected value is called prediction error) or by prospective planning based on the previously learned models (Eshel & Roiser, 2010).

As pointed out in the review by (Chen, Takahashi, Nakagawa, Inoue, & Kusumi, 2015) that focuses on the RL, the main advantages of the computational analysis approach are, that it allows testing predictions about the link between the brain and the

behaviour in a mathematical manner. Moreover, the computational approach allows better specification of particular processes of the experimental paradigms (e.g. learning rate, reward sensitivity) which may be particularly important in attempts of breaking down broad concepts such as e.g. anhedonia into more redefined concepts (Treadway & Zald, 2011). Finally, it could allow examination of the interactions between varieties of factors that contribute to the mental illness (e.g. genetics, cognition, neurobiology), because it uses multiple levels and types of data to address the heterogeneity of the psychiatric disorders (Huys et al., 2016).

In one of the first studies using RL modelling in depressed patients vs. HC, a monetary decision-making task during fMRI recording was used (Steele, Meyer, & Ebmeier, 2004). Participants were asked to make a choice between two cards that lead to either a monetary gain or loss after their choice. The feedback relating to the 'correct' card choice was fully predetermined, but the participants were instead instructed that the 'correct' card was determined by a changing rule during the task. Analysis of the reward prediction error (RPE) value and its further correlations with the BOLD responses revealed involvement of the PFC and the striatal regions in the RPE in both groups (Steele et al., 2004). Moreover, the further group contrasts showed greater RPE in the ACC and the parahippocampal gyrus in the MDD group and this was further correlated with increasing depressive symptoms and anhedonia. The authors concluded that patients with MDD had diminished ability to use reinforcement to modulate their behaviour when compared with HC. Moreover, this study showed that computations in the brain regions involved in the reward-related decision-making in MDD are disrupted which might contribute to the maintenance of depression (Steele et al., 2004).

Given the limitations of the monetary tasks (discussed in section 9.3.), experimental paradigms with the use of primary reward have been implemented in RL studies. For example, Kumar and colleagues (2008) run a study in the antidepressant

treatment resistant MDD patient group and the HC group that used water for a positive reward and lack of water as a negative reward (Kumar et al., 2008). Moreover, the HC group was scanned on two occasions: in an unmedicated state and after 3 days of SSRI treatment. The results showed that in comparison with the HC group, the MDD had reduced |RPE signals in the ventral striatum and dACC and increased RPE signals in the ventral tagmental area, rostral ACC, retrosplenial cortex and hippocampus. Finally, the severity of MDD was associated with the weaker RPE signals in the hippocampal and rostral ACC signals and greater RPE signal in the ventral tagmental area and amygdala. Interestingly, the medicated HC subjects showed blunting in a similar network to those with MDD and with strength of responses between the unmedicated HC and the MDD. This finding indicates that the reduced reposes in the medicated subjects (whether HC or MDD) might be possibly related to the SSRIs treatment administration (Kumar et al., 2008).

A similar study also found reduced RPE signals in the striatum, thalamus, midbrain and the right hippocampus of patients with MDD (Gradin et al., 2011). Further, the reduced signal in the striatum and midbrain correlated with increased anhedonia. Moreover, the mathematical estimation of the expected value signal revealed that the MDD patients had reduced expected value signal in the right hippocampus and parahippocampus gyrus, which was independent of the severity of depression or anhedonia. This finding is opposite the Kumar et al., (2008) findings. These differences might be related to the fact that Kumar et al., (2008) study looked at a sample of antidepressant resistant MDD patients while Grading et al., (2011) looked at a sample of medicated MDD with no distinction whether they were non-responsive or responsive to the given treatment.

Taken together, the above studies showed reduced RPE in the hippocampus, ACC, striatum, and thalamus in depressed patients. Some of these findings were also

associated with depression and anhedonia severity. Furthermore, (Kumar et al., 2008) showed that the blunted RPE responses might be related to the action of the medication. For example, studies showed that inhibition of noradrenaline reuptake significantly improves response inhibition and inhibition of serotonin reuptake significantly impairs probabilistic learning without the opposite effect on one another (Chamberlain et al., 2006). In other studies, treatment with SSRIs diminished responses to both reward and punishment in healthy participants (McCabe, Mishor, Cowen, & Harmer, 2010). These studies indicate that different neurotransmitters can modulate a variety of reward processes in the brain.

Furthermore, there have been studies that have specifically looked at the anticipatory aspect of the reward learning task such as the expected value and found reduced expected value in the MDD subjects in the brain regions of hippocampus and parahippocampus. (Gardin et al., 2011) and (Dombrovski et al., 2010) found similar results in the hippocampus. Moreover, their study revealed that reduced expected value signal in the vmPFC was specifically associated with the number of suicide attempts. In light of this finding the authors proposed that reduced motivation to obtain rewards might be further related to the deficits in motivation and thus contribute to the maintenance of depression (Dombrovski et al., 2010). These studies suggest that the reduced processing of the expected value and reward outcome are differentially distributed in the brain and seem to be served by different neurotransmitters' systems e.g. anticipation is mostly served by the dopamine.

However, recent work by (Rutledge et al., 2017) that looked at how the RPE signals are affected in depression with a focus on the dopamine related ventral striatal area, found opposite results to those mentioned above. Their study implemented the Probabilistic Reward Task and the Risky Decision Task that were devoid of any learning requirements and they also measured momentary mood. Contrary to previous results in reinforcement learning tasks, individuals with moderate depression showed intact RPE

signals in ventral striatum. Furthermore, depression symptom severity correlated with baseline mood parameters however, participants with depression showed an intact association between RPEs and happiness in a computational model of momentary mood dynamics that was not attenuated compared with controls. The authors concluded that in this specific non-learning context, the effect of RPE on ventral striatal activity is intact in depression questioning the previous statements that dopamine plays a central role in MDD. Furthermore, the authors suggest that diminished ventral striatal signalling found in previous studies of reinforcement learning (Gradin et al., 2011; Kumar et al., 2008; Robinson, Cools, Carlisi, Sahakian, & Drevets, 2012) may be related to a mistaken understanding of the environment (model-based valuation) rather than a fundamental failure of the dopaminergic computation of RPEs (Rutledge et al., 2017).

Overall, computational studies of reward learning and decision-making may contribute to a better understanding of abnormalities in the neural systems in psychiatric illness. However studies thus far showing RPE deficits in depression need to be replicated as recent findings indicate that there is no longer consistency on this in the literature.

1.6. Dimensional vs. a Categorical approach to study the neurobiology of depression.

Majority of studies that investigate mental illness are based on a categorical approach. Such an approach assumes that an individual either does have or does not have a mental illness (Potuzak, Ravichandran, Lewandowski, Ongur, & Cohen, 2012). This approach has many practical advantages. For example, it allows international linguistic consistency regarding the psychiatric diagnosis, and the presence or absence of a particular condition may allow clinicians a better determination about the treatment. Moreover, in the research arena of psychology and clinical neuroscience, the categorical classification has allowed the drawing of a map of behavioral and neurobiological

dysfunctions associated with depression. However, it has also revealed an important issue i.e. diagnosis based upon existing symptoms might not capture underlying mechanisms of dysfunction due to heterogeneity of mental illness. This is problematic as it limits our ability to investigate the etiology of mental health disorders and slows the development of new treatments and preventative strategies (Insel et al., 2010). Thus recently, more emphasis has been on investigating neurobiological signatures across nosological boundaries of mental illness. Such approach allows the dimensional conceptualization and assessment of psychiatric symptoms and can be used to identify core neurobiological dysfunctions across mental illness e.g. common neural circuits for processing of reward and punishment and how these could be associated with a specific behaviour (Potuzak et al., 2012).

Over the past 5 years, a number of studies have been published that incorporate the dimensional approach. These studies have focused on different symptoms and dysfunctions observed in psychiatry. For example, a study by (Hagele et al., 2016) investigated the neural responses to affective pictures in a group of patients with a variety of psychiatric disorders: alcohol dependence, schizophrenia, MDD, bipolar disorder, attention deficit/hyperactivity disorder as well as in HC. Results of this study revealed activation in the vmPFC, temporal gyri and the precuenus across all subjects in response to positive pictures, and in the amygdala and temporal gyri in response to negative pictures. However, there was no relationship between the brain activation and the behavioural symptoms of depression or anxiety for either of the contrasts. There were also no significant differences between the diagnostic groups. These results show brain dysfunction that is common across psychiatric conditions that would not have been revealed if only a categorical approach was used. Other studies that specifically looked at reward dysfunction using a dimensional approach have consistently reported decreased activation in the ventral striatum across patients of various psychiatric conditions. For example, Hagele and colleagues (2015) looked at the neural responses to reward

anticipation in patients with alcohol dependence, schizophrenia, MDD, bipolar disorder, attention deficit/hyperactivity disorder when compared to HC (Hagele et al., 2015). The results showed decreased ventral striatum activation in the alcohol dependence, schizophrenia and MDD groups. Interestingly, brain activation correlated with the depressive symptoms regardless of diagnosis. Another study that specifically looked at responses to reward anticipation across the spectrum of risk for depression also reported decreased ventral striatal activation in adolescents with subthreshold depression and clinical diagnosis (Stringaris et al., 2015). Interestingly, the ventral striatum activation across the spectrum of depression was associated with anhedonia but not with low mood. This is interesting given that low ventral striatum activation predicted transition to subthreshold or clinical depression in previously healthy or 'at risk' subjects (Stringaris et al., 2015). It also indicates that anhedonia could be a true biomarker of depression as it is persistent across the depressive spectrum. The findings of these studies emphasize the importance of studying mental illness both categorically and dimensionally to derive a holistic understanding of mechanisms implicated in mental illness.

1.7. Resting-state fMRI: explanation of the concept

It has been long claimed that cognitive and emotional functions result from operations in independent brain areas as investigated in a task-dependent environment. Accumulating evidence however, has shown that such an approach might be limiting as experimental paradigms differ in terms of the stimuli used, general design, and implementation protocol. This can be potentially problematic for results interpretation and if one is trying to compare brain functionality e.g. across different clinical samples. However, recent developments in neuroscience suggest that cognitive and emotional functions can also emerge from the interaction of distributed brain areas operating as large-scale brain networks (Smith et al., 2009). This approach provides new insight into the neural basis of cognition and emotion by characterizing them in terms of temporal

relationships between activations in different brain regions, known as functional connectivity. Thus, the functional connectivity framework allows looking into functional brain organization from a different and broader perspective.

Research into neural networks has mostly focused on resting-state functional connectivity (RSFC) that is investigated when people are not engaged in any external tasks. These networks arise from spontaneous and low frequency fluctuations of the blood oxygen level dependent signal (BOLD) at rest. These spontaneous BOLD signal fluctuations show temporal correlations between different brain areas or networks that occur in brain regions recognized to serve similar cognitive processes. Thus, those networks may reliably map functional connectivity patterns across the entire brain and are claimed to characterize the baseline activity of the human brain at rest (Beckmann, DeLuca, Devlin, & Smith, 2005; Buckner, Krienen, & Yeo, 2013; Greicius, 2008).

In this 'state of rest', people are thought to engage in a wide-range of internal and environmental monitoring behaviours, such as a simulation of episodic events and thoughts, interoception, and evaluation of external cues (Whitfield-Gabrieli & Ford, 2012). fMRI connectivity studies have identified several distinct neural networks during resting-state that show specific patterns of synchronous activity (Seeley et al., 2007). For example, the Default Mode Network (DMN) shows interconnectivity of brain areas in parts of the medial temporal lobe, the mPFC, the precuneus cortex and the posterior cingulate cortex (PCC). Another network, the Central Executive Network (CEN) has been identified in the dlPFC, and the posterior parietal cortex. The Salience Network (SN) consists of the ventrolateral PFC, the anterior insula and the ACC as well as subcortical structures such as the amygdala, the ventral tegmental and the thalamus (Smith et al., 2009). Other identified networks are sensorimotor, auditory and visuospatial attention networks anchored in the motor and somatosensory cortices, the temporal lobe and in the occipital and lateral visual areas, respectively (Smith *et al*., 2009).

The resting-state networks are also systematically engaged in task-related information processing, and as their key areas are anchored in different brain regions, they are claimed to play different roles. For example, the key nodes of the DMN are activated during tasks that involve autobiographical memory and self-referential processes and greater suppression of the DMN is related to a better performance on attention demanding tasks (Buckner & Carroll, 2007). The CEN nodes show increased activation for tasks that require engagement of cognitive control, specifically in the processes of decision-making and in the maintenance of information in working memory (Dosenbach et al., 2006). The brain regions of the SN respond to varied forms of salience including emotional, cognitive and homeostatic salience of stimuli (Seeley et al., 2007). Contrary to the DMN, the SN and the CEN show strong task-related activation and are less active at rest, which further suggests that they serve different cognitive operations (Sridharan, Levitin, & Menon, 2008).

1.8. Resting-state fMRI studies in depression and in population at increased risk for depression

It has now been well established that individuals with MDD present abnormalities in their RSFC networks. The majority of RSFC studies have investigated the DMN, CEN, and the SN networks and have mostly reported increased functional connectivity across these networks in MDD subjects when compared with HC. For example, (Li et al., 2013; Manoliu, Meng, et al., 2013) reported increased DMN connectivity between the PCC and the precuneus in patients with current MDD and in treatment resistant MDD patients when compared with HC. However, there are also studies reporting decreased RSFC in the DMN in MDD. For example, (van Tol et al., 2013) found decreased RSFC between the DMN and the dmPFC region in medicated MDD patients. Similar results of decreased RSFC between the PCC and the precuneus

were also found in medication naïve mildly depressed patients (Zhu et al., 2012). Moreover, these RSFC abnormalities are thought to be related to a symptoms of negative rumination in depression as the key nodes of the DMN are anchored in brain regions associated with self-referential thoughts (Whitfield-Gabrieli & Ford, 2012).

Decreased RSFC has been also reported for the CEN. Studies found decreased RSFC between the dlPFC and other parts on the CEN in treatment resistant patients (Liston et al., 2014) and also in medicated first episode patients (Ye et al., 2012) and medication free participants (Lui et al., 2011). However, others have found increased RSFC with the dorsal medial prefrontal cortex (dmPFC) in medication free MDD patients with a history of antidepressant medication when compared with HC (Sheline, Price, Yan, & Mintun, 2010). It has been suggested that abnormalities in the CEN in the MDD subjects may reflect deficits in attentional control over emotional stimuli, difficulties with suppression of unwanted thoughts, problems with information encoding and maintenance in working memory and thus they may contribute to the maintenance of depression.

Mixed results have been also found for the SN where increased RSFC was reported for the insula and the pgACC in acutely depressed medicated patients (Horn et al., 2010) and in the bilateral ACC for medicated MDD patients (Manoliu, Riedl, et al., 2013). However, the same study also found decreased connectivity within the anterior insula that additionally showed negative correlation with depression severity. Moreover, many other studies in MDD subjects also reported decreased RSFC between the amygdala and the insula (Ramasubbu et al., 2014; Tahmasian et al., 2013). However, a contrary result of increased RSFC was found for the amygdala and the temporal pole which was also correlated with depression severity in MDD subjects when compared with HC (Ramasubbu et al., 2014). Abnormalities in the SN in MDD patients might reflect difficulties with emotion recognition, regulation and processing that can lead to inadequate behavioural responses.

There are a small number of RSFC studies in adolescents with MDD. The available studies have mostly focused on investigating the SN and they report decreased RSFC between the amygdala and other brain regions (hippocampus, parahippocampus and brain stem), which additionally correlate negatively with depression severity and correlate positively with well-being in adolescents with MDD when compared with HC (Cullen et al., 2014). These results suggest a lack of appropriate regulation of networks that contribute to cognitive and emotional processing and have been related to the behavioural manifestation of depression. The same study also showed increased RSFC between the amygdala and the precuneus when compared with control subjects which could relate to difficulties with suppressing negative thoughts in MDD. Although, authors claimed to record the RSFC in this study, participants were allowed to listen to music of their choice, which can be a limitation when comparing this study with other resting-state studies that did not include auditory presentations. Another study, also found increased RSFC between the subgenual ACC (sgACC) and the SN and decreased RSFC between the sgACC and the DMN in MDD adolescents compared to HC (Connolly et al., 2013). Moreover, in this study depressive symptoms were correlated with decreased RSFC in the DMN (Connolly et al., 2013) suggesting a relationship between the brain connectivity and the behavior in MDD subjects. Mixed results were also reported in another study in MDD adolescents who showed increased RSFC between the amygdala and the parietal cortex and decreased RSFC between the amygdala and the frontal regions including the pgACC, the frontal pole and the paracingulate gyrus (Pannekoek et al., 2014). Taken together, studies suggest that the dysfunction in the RSFC networks is related to depressive behavior in MDD patients thus it can be considered as a possible biomarker for depression. However, there are also many inconsistencies in the direction of the connectivity which might be related to the differences in used protocols and selected participants.

To my knowledge there is only one study investigating RSFC in healthy adolescents females with a parental history of depression, thus at increased risk for developing depression themselves (Clasen, Beevers, Mumford, & Schnyer, 2014). In this study, the authors investigated the RSFC of brain regions associated with the cognitive control. They found that the adolescents had decreased RSFC between the inferior and the middle frontal region and both the middle frontal region and the supramarginal gyrus –parts of the CEN, when compared with HC. All these regions are part of the attention control network that is implicated in behaviour inhibition, thought suppression and control over emotional stimuli. Interestingly, the severity of the parent's worst depression episode was correlated with lower levels of RSFC between the nodes of the CEN in the children. The authors concluded that there is a possibility that the development of the CEN in daughters of parents with MDD may be altered by the severity of the parent's depression history which could possibly increase their vulnerability to depression.

Taken together, studying RSFC may allow a broader understanding of the neural underpinning of dysfunctional cognition and information processing in MDD by revealing how those dysfunctions may arise from interaction within and between distributed brain systems (Bressler & Menon, 2010). Many resting-state networks have been investigated in MDD and the majority of results show increased DMN, CEN and SN functional connectivity. However, many mixed results have also been reported. These inconsistencies in results might be related to differences in the MDD population studied for example; adults vs. elderly vs. adolescents. Further, the lack or presence of antidepressant medication as well as depression severity may also play a role in the inconsistencies across studies. Dysfunction of the resting-state networks were also found in adolescent depression which is important as it shows that aberrant brain networks linked to various emotional and control related processes, are apparent even in younger people, which in turn could be a treatment target. Abnormalities in the RSFC were also reported in adolescents at increased risk for developing depression which shows that

neural changes in the networks may be present before the onset of the illness and in absence of depression symptoms. Thus, there is a window here for therapeutic interventions that could aim to restore functional connectivity networks to that similar to healthy subjects.

1.9. Dimensional vs. categorical approach to study neurobiology of depression: resting –state studies.

Section 1.5. discusses the importance of implementing both the categorical and dimensional approach to study mental illness. Studies that investigate RSFC using fMRI in clinical populations have also incorporated the dimensional approach. For example, (Elton, Alcauter, & Gao, 2014; Elton, Di Martino, Hazlett, & Gao, 2016) studies aimed at characterizing both categorical and dimensional variations in RSFC networks, in order to identify connectivity mechanisms of Attention deficit-hyperactivity disorder (ADHD) and Autistic Spectrum Disorder (ASD), respectively. The findings of both of these studies revealed the existence of dimensional mechanisms that were specific for each of the disorders. Moreover, there were also direct brain connectivity- behaviour relationships, independent of diagnostic category. However, there were also categorical differences in the RSFC networks and the brain-behaviour relationships. These finding are important as they support the need for dual characterization (dimensional and categorical) of those disorders in RSFC.

To my knowledge, there is only one study that directly looked at the categorical and dimensional conceptualizations in relation to MDD and anxiety disorder. This study showed that when looking at the dimension of symptoms, some of them, such as general distress, were common for both depression and anxiety, whilst others such as anxious arousal or anhedonia were specific only to anxiety or depression, respectively (Oathes, Patenaude, Schatzberg, & Etkin, 2015). These differences were further related to

functional connectivity: anxious arousal was specifically associated with connectivity in the sgACC and ventral striatum. Moreover, when data was analyzed categorically, anxiety was associated with functional connectivity in the limbic and the paralimbic regions, while MDD was associated with greater functional connectivity of amygdala and subcortical regions. These findings show that brain mechanisms should be investigated using both approaches to better address the etiology of mental illness as it allows the illumination of mechanisms that are both common and distinct to psychiatric disorders. In turn this allows the utilization for e.g. of treatments that work well in one disorder to be examined in another and it also allows treatments to be developed specifically for particular deficits that are disorder specific.

1.10. Summary

In this chapter I provide a general background to the experiments described in this thesis. I have shown that depression is a significant social problem that affects people of different age and socio-economic status with an increasing number of adolescents affected by depression. I also discussed the consequences of early-onset depression on the social, economic and health outcomes in adulthood and emphasized the need for a better understanding of the etiology of MDD. Moreover, I presented evidence suggesting that the key diagnostic symptom of depression- anhedonia can be related to the neural abnormalities of the reward system and that anhedonia is considered a possible biomarker of depression. Furthermore, I discussed evidence suggesting that anhedonia is a complex heterogonous concept and that patients with MDD can have deficits in different aspects of anhedonia such as anticipatory, motivational or consummatory and these may be further represented in the neural circuits of reward. In light of this evidence I was interested to investigate further how adolescents at increased risk of developing depression and young people with a current depression diagnosis process different aspects of reward and aversion and whether abnormalities in these circuits can be related

to anhedonia. Furthermore, I have introduced a background to RSFC studies and showed that individuals with MDD and those at increased risk for developing depression have abnormalities in RSFC networks when compared with HC. Those abnormalities are presented in different networks involved in variety of emotional and cognitive processes which might be important for the development and maintenance of depression. In turn these networks could be targets for treatment development and even interventions in young people at risk.

1.11. Outlines of Papers

The four papers included in the thesis address some of the gaps in the existing literature associated with the abnormalities of the neural reward and punishment systems as well as the abnormalities in RSFC in individuals as risk for MDD and in those with a clinical diagnosis. The following sections provide an overview of the research questions addressed by each of the papers.

1.11.1. Paper 1: Blunted neural response to anticipation, effort and consummation of reward and aversion in adolescents with depression symptomatology.

As outlined above, symptoms of anhedonia are related to abnormalities in the brain's reward mechanisms and are suggested as a possible biomarker of depression. Previous studies have shown abnormalities in the reward system in individuals with a family history of depression, clinical diagnosis of depression and in those recovered from depression (McCabe et al., 2009; McCabe et al., 2012)(Admon & Pizzagalli, 2015). However it has been unclear from these studies whether these abnormalities are a state marker or a scar of having had depression. To tackle this issue it is important to look at another group of individuals- adolescents at increased risk of developing depression by virtue of elevated depressive symptoms. Moreover, given that anhedonia is a

heterogeneous concept associated with reward processing, it is important to investigate the specific subtypes of anhedonia to better understand the actual deficits in the brain. Thus in this paper, we have addressed these issues by looking at the neural subtypes of reward and punishment by introducing a novel experimental model that aims to separate the neural response to reward and punishment into the anticipation, effort and consummation of reward and punishment/aversion. Thus, the aims of the first study were as follow: 1) to investigate the neural responses to reward and aversion in adolescents with elevated depressive symptoms but without a clinical diagnosis of MDD; 2) to investigate whether there would be any neural differences between adolescents with elevated depressive symptoms and HC on different aspects of reward and aversion processing, namely: the anticipation of reward and aversion, the effort to obtain reward or to avoid aversion and the consummation of reward and aversion; 3) to investigate how the neural differences of reward and aversion processing might be related to symptoms of depression and anhedonia in adolescents with elevated depressive symptoms. The findings of the study, discussion and implications are considered in the paper.

Although data on aversive information processing was collected, this thesis focuses on reward processing as the neural correlate of the symptom of anhedonia

1.11.2. Paper 2: Decreased Neural Anticipation, Effort and Consummation of Reward and Aversion with Increased Anhedonia in Adolescents: An RDOC Dimensional approach.

As discussed above, individuals with MDD and those at risk of MDD have deficits in the neural processing of reward and punishment. Recent studies have also suggested that it is useful to investigate the neurobiology of mental illness from a categorical AND dimensional perspective as they offer a novel understanding of the neural underpinnings of mental illness. Thus, the aims of the second study were as

follow: 1) to investigate how the neural differences of reward and aversion processing might be related to symptoms of depression and anhedonia in young people with depression diagnosis; 2) to investigate the neural correlates of different aspects of reward and punishment processing, namely: the anticipation of reward and aversion, the effort to obtain reward or to avoid aversion and the consummation of reward and aversion across depression symptom severity using a dimensional approach and how these results can relate to severity of anhedonia and depression symptoms; 3) to investigate the neural responses to reward and aversion in young people with a clinical diagnosis of depression compared to matched age and gender heathy control subjects; 4) to investigate whether there would be any neural differences between young people with clinical depression and HC on different aspects of reward and aversion processing, namely: the anticipation of reward and aversion, the effort to obtain reward or to avoid aversion and the consummation of reward and aversion.

Results of these studies will show how anhedonia symptoms and depression severity are related to different aspects/subtypes of reward function. These results will extend our knowledge on the neural underpinnings of anhedonia as a possible biomarker for depression.

Although data on aversive information processing was collected, this thesis focuses on reward processing as the neural correlate of the symptom of anhedonia.

1.11.3 Paper 3: Decreased anticipated pleasure correlates with increased salience network resting state functional connectivity in adolescents with depressive symptomatology.

As mentioned above, previous studies have found dysfunctional RSFC in depressed patients, individuals at familial risk of depression, and in those recovered from depression (Clasen et al., 2014; Sheline et al., 2010). However RSFC studies in young

people at risk of depression due to increased depressive symptomatology have been limited.

Thus the third study aimed at: 1) investigating whether there would be any differences in the RSFC networks in adolescents with elevated depressive symptoms when compared with HC; 2) investigating whether abnormalities in the RSFC between the adolescents with elevated depressive symptoms when compared with HC will be associated with depression and anhedonia symptoms. Findings from this study, discussion and implications are considered in the paper.

1.11.4. Paper 4: Increasing depression and anhedonia severity correlates with decreasing resting-state functional connectivity in dorso-medial prefrontal cortex in adolescents: an RDOC dimensional approach

As mentioned above, previous studies have found dysfunctional RSFC in depressed patients, individuals at familial risk of depression, and in those recovered from depression (Clasen et al., 2014; Sheline et al., 2010). Moreover, recent studies have emphasized the importance of implementing both the categorical and dimensional approach to study mental illness. Thus, the final study aimed at: 1) investigating whether there would be any differences in the RSFC networks in young people with depressive symptoms when compared with heathy controls; 2) investigating how abnormalities in the RSFC networks studies in individuals with depressive symptoms would relate to severity of anhedonia and depression symptoms.

Studying RSFC framework may allow a broader understanding of the neural underpinnings of dysfunctional cognition and information processing in depression. RSFC can reveal how these dysfunctions may arise from interactions within and between distributed brain systems, and how they can relate to anhedonia and depression symptoms.

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Chapter 2:

Paper 1: Blunted neural response to anticipation, effort and consummation of reward and aversion in adolescents with depression symptomatology

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Title:

Blunted Neural Response to Anticipation, Effort and Consummation of Reward and Aversion in Adolescents with Depression Symptomatology.

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Key words: depression, fMRI, reward, aversion, adolescents, at risk, anhedonia, biomarker.

Abstract

Neural reward function has been proposed as a possible biomarker for depression. However, how the neural response to reward and aversion might differ in young adolescents with current symptoms of depression is as yet unclear. Thirty-three adolescents were recruited, 17 scoring low on the Mood and Feelings Questionnaire (low risk group) and 16 scoring high (high risk group). Our functional magnetic resonance imaging task measured; anticipation (pleasant/unpleasant cue), effort (achieve a pleasant taste or avoid an unpleasant taste) and consummation (pleasant/unpleasant tastes) in regions of interest; ventral medial prefrontal cortex, pregenual cingulate cortex, the insula and ventral striatum. We also examined whole brain group differences. In the regions of interest analysis we found reduced activity in the high risk group in the pregenual cingulate cortex during anticipation and reduced pregenual cingulate cortex and ventral medial prefrontal cortex during effort and consummation. In the whole brain analysis we also found reduced activity in the high risk group in the prefrontal cortex and the precuneus during anticipation. We found reduced activity in the hippocampus during the effort phase and in the anterior cingulate/frontal pole during consummation in the high risk group. Increased anhedonia measures correlated with decreased pregenual cingulate cortex activity during consummation in the high risk group only. Our results are the first to show that adolescents with depression symptoms have blunted neural responses during the anticipation, effort and consummation of rewarding and aversive stimuli. This study suggests that interventions in young people at risk of depression, that can reverse blunted responses, might be beneficial as preventative strategies.

Keywords: Depression, functional magnetic resonance imaging task, reward, aversion, adolescents, at risk, anhedonia, biomarker

Introduction

Anhedonia (loss of interest and pleasure) is one of the two main diagnostic criteria for depression (American Psychiatric Association, 2013) and is related to abnormalities in the brain's reward mechanisms and suggested as a possible biomarker of risk for depression (Argyropoulos and Nutt, 2013; Hasler et al., 2004; Nutt et al., 2007). Identifying biomarkers such as the neural response to reward could help develop preventative treatments for young people at increased risk of clinical depression.

Anhedonia is multi-dimensional, with the anticipatory (appetitive/wanting) and consummatory (hedonic/liking) dimensions being the most widely examined in depression (Frey et al., 2015, McCabe, 2014, Nutt et al., 2007). Studies in depression have found reduced anticipatory and consummatory responses to reward in the ventral and dorsal striatum (Epstein et al., 2006; Forbes et al., 2009; Pizzagalli et al., 2009; Smoski et al., 2009; Ubl et al., 2015; Zhang et al., 2013) with increased activity to the anticipation of gains in the anterior cingulate (Knutson et al., 2008). However, few studies investigate the separate dimensions of anhedonia within the same task (Treadway and Zald, 2011; Zhang et al., 2013). Yet a recent behavioural study suggests another possible conceptual dimension of anhedonia that of effort expenditure for reward. The authors found that effort expenditure was impaired in depressed patients (Sherdell et al., 2012; Treadway et al., 2012; Yang et al., 2014).

Interestingly the results of neural responses to aversive stimuli in depressed patients are less consistent, with some studies finding increased responses in the amygdala (Knutson and Greer, 2008; Sheline et al., 2001; Surguladze et al., 2004) whilst others find blunted responses in the amygdala and lateral orbitofrontal cortex (lOFC) (Bylsma et al., 2008; Luking et al., 2015; McCabe et al., 2009). Studies that find blunted responses to both reward and aversion in depression (see meta-analysis in Bylsma et al., 2008) however fit with the theory of emotion context insensitivity (Rottenberg et al.,

2005). This theory indicates a reduced reactivity to all emotion cues, regardless of valence (Rottenberg, 2007; Rottenberg et al., 2005).

To assess the neural response to reward and aversion, we have developed an experimental model that examines the anticipation, effort and consummation of pleasant and unpleasant sights and tastes (Dean et al., 2016). In an attempt to examine neural biomarkers we have shown previously that participants recovered from depression have decreased responses to anticipation and consummation (sight and taste of chocolate reward) in both ventral striatum and anterior cingulate cortex (ACC) (McCabe et al., 2009). In a follow-up study we examined young people (16–21 years) with a family history of depression (Beardslee et al., 1998) but no personal experience of depression and found diminished neural responses in the orbitofrontal cortex (OFC) and the dorsal anterior cingulate cortex (dACC) to rewarding stimuli (McCabe et al., 2012) in the at risk group. Consistent with this, a recent behavioural study found reduced risk-taking in young people at increased familial risk of depression (Mannie et al., 2015).

Despite adolescence being a critical period of neural development that increases vulnerability to depression (Davey et al., 2008), studies report conflicting results regarding the direction of developmental changes (Forbes et al., 2010). Further, few studies report how current depression symptoms map onto neural responses to reward. One recent study has found that decreased ventral striatal responses to monetary reward predict depressive symptoms in adolescents (Hanson et al., 2015). Thus, the aim of this study was to extend this work by investigating neural responses during reward and aversion processing (anticipation, effort and consummation) in younger adolescents (13–18 years) with current depressive symptoms but no clinical diagnosis.

Materials and methods

Participants

Thirty-three participants were recruited for the study. Seventeen volunteers were classified as low risk (LR) and 16 volunteers as high risk (HR) for depression based on scores on the Mood and Feelings Questionnaire (MFQ) (Angold et al., 1995). The MFQ scores were <15 for LR and >27 for HR. Participants who scored between 15–27 were excluded from the study. The University of Reading Ethics Committee approved the study, and written informed consent from all participants was obtained.

Potential participants were assessed with the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1997) schedule to exclude a personal current or previous history of major depression or any other Axis 1 disorder. Further, no subjects had ever been diagnosed with depression or had sought treatment for depression. We also excluded pregnancy and any contraindications to magnetic resonance imaging (MRI). With the exception of the contraceptive pill, volunteers took no medication.

All subjects completed the: MFQ; Beck Depression Inventory (BDI; Beck et al., 1961); Fawcett–Clarke Pleasure Scale (FCPS; Fawcett et al., 1983); Snaith–Hamilton Pleasure Scale (SHAPS; Snaith et al., 1995), the Temporal Experience of Pleasure Scale (TEPS; Gard et al., 2006), a 'chocolate questionnaire' to measure liking, craving, and frequency of eating chocolate (Rolls and McCabe, 2007). Body mass index (BMI) in the normal range was part of the inclusion criteria.

Overall design

Participants were asked to refrain from consuming chocolate 24 hours prior to scanning. Before and after each scan, volunteers completed the Befindlichskeits scale

(BFS) of mood and energy (Von Zerssen et al., 1974) and a mood visual analogue scale (VAS).

The task was adapted from (McCabe et al., 2010) to include an effort stage. The task (40 trials) had four conditions based on the trial type (reward/aversive) and its level of difficulty (easy/hard). Trial type was cued by a visual stimulus (chocolate picture or picture of mouldy drink (2 s)), which indicated either to work to win the chocolate taste or to avoid the aversive taste (effort phase). Difficulty was determined by the amount of effort required to complete the effort stage (easy=24, hard=45 button presses). This required volunteers to press a button as fast as possible (<6 s) to move a bar towards the pleasant chocolate picture (reward) or away from the unpleasant mouldy picture (aversive), allowing enough time to complete easy trials but not hard trials. If on reward trials volunteers were successful they received the taste (5 s delivery and 2 s swallow cue) of chocolate and if not they received the tasteless solution. If on aversive trials volunteers were successful they received the tasteless solution and if not they received the unpleasant taste. A grey image (2 s) was presented at the end of each trial. Each condition was repeated 10 times, chosen by random permutation. Jitters were used for both interstimulus intervals and inter-trial intervals. To sustain effort, four trials (two reward/two aversive) were longer at 9 s each. Volunteers also rated 'wanting', 'pleasantness' (+2 to −2) and 'intensity' (0 to +4) on a VAS on each trial.

Stimuli

We used a picture of liquid chocolate (reward), a mouldy drink (aversive) and a grey image (control). The rewarding taste was a Belgian chocolate drink and the aversive taste was a combination of the chocolate drink mixed with beetroot juice, providing a similar texture. The tasteless solution (25×10–3mol/L KCL and 2.5×10–3mol/L NaHCO3

in distilled H2O) was also used as a rinse between trials. Solutions were delivered through three Teflon tubes allowing 0.5 mL of solution to be manually delivered.

Functional magnetic resonance imaging task (fMRI) scan

The experimental protocol consisted of an event-related interleaved design. A Siemens Magnetom Trio 3T whole-body MRI scanner and a 32-channel head coil were used. Multi-band accelerated pulse sequencing (version no. RO12, Center for Magnetic Resonance Research, University of Minnesota, USA, EPI 2D BOLD/SE/DIFF sequence) was used with an acceleration factor of 6. T2*-weighted echo planner imaging slices were obtained every 0.7 s (repetition time (TR)). Fifty-four axial slices with in-plane resolution of 2.4×2.4 mm and between-plane spacing of 2.4 mm were attained. The matrix size was 96×96 and the field of view was 230×230 mm. Acquisition was performed during task performance, yielding ~3500 volumes. An anatomical T1 volume with sagittal plane slice thickness 1 mm and in-plane resolution of 1.0×1.0 mm was also acquired.

fMRI analysis

Statistical Parametric Mapping (SPM8) was used for realignment and normalisation to the Montreal Neurological Institute (MNI) coordinate system and spatial smoothing with a 6 mm full-width-at-half-maximum Gaussian kernel and global scaling (Collins et al., 1994). The time series at each voxel was low-pass filtered with a hemodynamic response kernel. Time series non-sphericity at each voxel was estimated and corrected for (Friston et al., 2002), and a high-pass filter with a cut-off period of 128 s was applied.

In the single-event design, a general linear model was then applied to the time course of activation in which stimulus onsets were modelled as single impulse response functions and then convolved with the canonical haemodynamic response function (Friston et al., 1994). Linear contrasts were defined to test specific effects. Time derivatives were included in the basis functions set. Following smoothness estimation (Worsley et al., 1996), linear contrasts of parameter estimates were defined to test the specific effects of each condition (pleasant/unpleasant cue – grey image and pleasant/unpleasant taste – rinse) with each individual dataset. Voxel values for each contrast resulted in a statistical parametric map of the corresponding t statistic (transformed into the unit normal distribution (SPM z)). Movement parameters for each person were added as additional regressors.

Second-level fMRI analyses examined simple main effects of task with one-sample t-tests for all scans (Supplementary Material, Table S1). Independent samples t-tests were used to examine between groups differences using SPM8. Results were thresholded at $p=0.001$ and whole-brain cluster corrected ($p<0.05$ family-wise error (FWE) for multiple comparisons) with age, gender and BMI added as covariates of no interest. Thresholding at $p=0.001$ with a cluster threshold of $k=30$ was our attempt at reducing both Type 1 and Type 11 errors in our results. Given that we have run this particular design in our previous studies, we believe we are less likely to attribute real activation to noise (Type I errors are not likely to replicate across multiple studies) and more likely instead to miss effects by increasing the p threshold. Therefore we increase the cluster threshold to 30 in an attempt to rebalance the Type 1 and Type 11 error rate. We also think this is appropriate given that these are healthy human volunteers and so differences in reward subtype correlations might have relatively subtle effects (Lieberman and Cunningham, 2009).

We also report results from atlas-based regions of interest (ROIs) analysis in SPM8 using Wake Forest University (WFU) PickAtlas toolbox to create 8 mm spheres

from coordinates selected from previous studies; pregenual anterior cingulate cortex (pgACC) (3 36 2) (McCabe et al., 2009), insula (−34 14 4) (McCabe et al., 2009), ventral medial prefrontal cortex (vmPFC) (8 56 −12) (McCabe et al., 2009) and ventral striatum (10 8 −4) (Rolls and McCabe, 2007) as these regions have been found to be activated by our task. Plots of contrast estimates were extracted with plots tool in SPM8, and Wake Forest University PickAtlas toolbox was used to display neural activation, with error bars representing the standard error of the mean (Maldjian et al., 2003).

Correlations with anhedonia measure

Using Pearson correlations we examined the relationship between mood (anhedonia) and the extracted beta values from our significant fMRI results.

Results

Demographic and clinical data

Analysis (Table 1) revealed no significant age and gender differences between LR and HR groups. There were significant differences; BMI $p<0.03$, MFQ, BDI, SHAPS, FCPS, TEPS all $p<0.02$ (Table 1).

Table 1: Demographics

Measure	HR (*n*=16) Mean (SD)	LR (*n*=17) Mean (SD)	*p*-value
Age (years)	16.63 (1.21)	16.24 (1.6)	.438
Gender (male)	4/12	6/11	.535
BMI	22.08 (2.6)	20.25 (2.1)	.033
MFQ	40.75 (6.14)	4.71 (5.13)	<.001

BDI	30.31 (12.95)	2.24 (4.25)	<.001
FCPS	120.13 (18.85)	137.76 (21.9)	.019
SHAPS	30.44 (5.57)	4.8 (5.57)	<.001
TEPS	65.25 (9.2)	83.65 (10.11)	<.001
Chocolate craving	6.44 (1.62)	5.97 (2.01)	.471
Chocolate liking	8.63 (1.02)	7.85 (1.5)	.096
Chocolate frequency	1.93 (1.52)	2.47 (2)	.396

BDI: Beck Depression Inventory; BMI: body mass index; FCPS: Fawcett–Clarke Pleasure Scale; HR: high risk; LR: low risk; MFQ: Mood and Feelings Questionnaire; SD: standard deviation; SHAPS: Snaith–Hamilton Pleasure Scale; TEPS: Temporal Experience of Pleasure Scale.

Mood, energy and affect scores

For the BFS we used a repeated measures ANOVA with within subject factor of time (before and after scan) and between subject factor of group (HR and LR). Results revealed that there was no significant main effect of time ($F(1.31)=.005$; $p=.943$) a significant main effect of group ($F(1.31)=216.73$; $p=.002$) and significant interaction between time (before and after scan) and group (LR and HR) ($F(1.31)=5.657$; $p=.024$). The results meant that the HR group had lower mood than LR both before and after the scan. Further paired sample t-test analysis revealed that there was a significant difference for time in the LR group ($t(16)=-3.24$; $p=.005$) meaning the LR group had a lower mood after the scan with no effect in the HR group ($t(15)=1.28$; $p=.221$) (Supplementary Material, Table S1).

For the VAS we used a repeated measures ANOVA with within subject factor of time, two levels (before and after scan) and within subject factor, emotion, on nine levels (alertness, disgust, drowsiness, sadness, happiness, anxiety, withdrawn, faint, nausea) and

between subject factor of group (LR and HR). Results revealed that there was no significant main effect of time ($F(1.31)=0.199$; $p=0.658$) and no significant main effect of group ($F(1.31)=2.5$; $p=0.124$). There was a significant main effect of emotion ($F(8.248)=54.75$; $p<0.001$) yet no significant interaction between the time, emotion and group ($F(8.248)=1.329$; $p=0.229$). Further paired sample t-test analysis revealed that there was a significant difference for emotion in LR group for disgust with increasing disgust after the scan ($t(16)=-2.615$, $p=0.019$) and in the LR group for drowsiness with increasing drowsiness after the scan ($t(15)=-3.23$; $p=0.006$) (Supplementary Material, Table S1) but not in the HR group.

Subjective ratings of stimuli

Ratings of wanting, pleasantness, and intensity for the stimuli were obtained during scanning on each trial for cues and the tastes. All subjects rated chocolate taste as pleasant and the aversive taste as unpleasant (Supplementary Material, Table S2). Using repeated measures ANOVA with ratings as the first factor, three levels (wanting, pleasantness, intensity) and condition as the second factor, two levels (chocolate, aversive) and between subject factor of group (LR and HR) we found no significant main effect of group ($F(1.31)=1.1$; $p=0.303$), a significant main effect of condition ($F(1.31)=683.34$; $p<0.001$), i.e. chocolate and aversive were rated differently and a significant effect of ratings $F(2.62)=484.64$; $p<0.001$) as expected (Supplementary Material, Table S2) but no significant group×condition×ratings interaction ($F(2.62)=3.68$; $p=0.055$) (Supplementary Material, Table S2).

Effort

The number of button presses as well as the time needed to complete the effort part of the task was also recorded. No significant group differences were found for the number of button presses or the time needed to complete the effort part of the study (p>0.05) (Supplementary Material, Table S3).

Main effects of stimuli on blood oxygen level-dependent responses

Supplementary Material, Table S4 provides a summary of the main effects of one-sample t-tests in all subjects for the anticipation, effort and consummation phases. As expected, the anticipation of the rewarding stimuli activated reward-relevant circuitry including the prefrontal cortex and striatum. The anticipation of the aversive cue activated similar areas and also the insula. Effort to achieve rewards activated the precentral gyrus and also the posterior cingulate and hippocampus. Effort to avoid aversion activated the precentral gyrus, posterior cingulate cortex, precuneus and caudate. Consummation of the pleasant chocolate taste activated the striatum, the anterior cingulate, amygdala and the hippocampus, whilst the aversive taste activated the same regions but also the insula (Supplementary Material, Table S4).

Effects of mood on blood oxygen level-dependent responses

Anticipatory phase:

Blood-Oxygen-Level-Dependent (BOLD) responses to aversive cue

Relative to LR, the HR group exhibited less BOLD responses in the pgACC ROI (Figure 1) and the medial frontal gyrus, posterior cingulate cortex/precuneus, inferior

frontal gyrus and frontal pole to the unpleasant cue during whole brain analysis (Tables 2 and 3). There were no group differences for the pleasant stimulus.

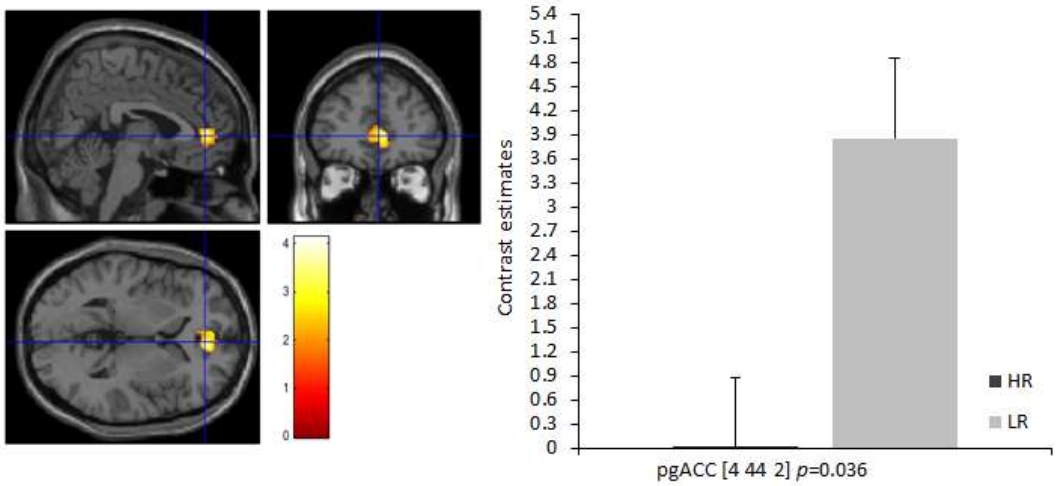

Figure 1. Anticipation: (left panel) Aversive cue, left panel, axial, sagittal and coronal image of pregenual anterior cingulate cortex (pgACC) activation in low risk (LR) vs high risk (HR) (z=2.98, p=0.036; region of interest (ROI) analysis with Wake Forest University (WFU) PickAtlas); (right panel) contrast estimates for pgACC centred at (4 44 2).

Effort phase

Blood-Oxygen-Level-Dependent (BOLD) responses to effort

Relative to LR, the HR group exhibited less BOLD responses in the pgACC ROI (Figure 2) and vmPFC ROI and the hippocampus for the chocolate hard trials vs chocolate easy trials during the whole brain analysis. Relative to LR, the HR group exhibited less BOLD responses in the medial frontal gyrus, the precentral gyrus and the superior temporal gyrus for the chocolate hard trials vs aversive hard trials during the whole brain analysis. Relative to LR, the HR group exhibited less BOLD responses in regions such as the central operculum, frontal pole and the superior frontal gyrus for the chocolate easy trials vs aversive easy trials during the whole brain analysis (Tables 2 and 3).

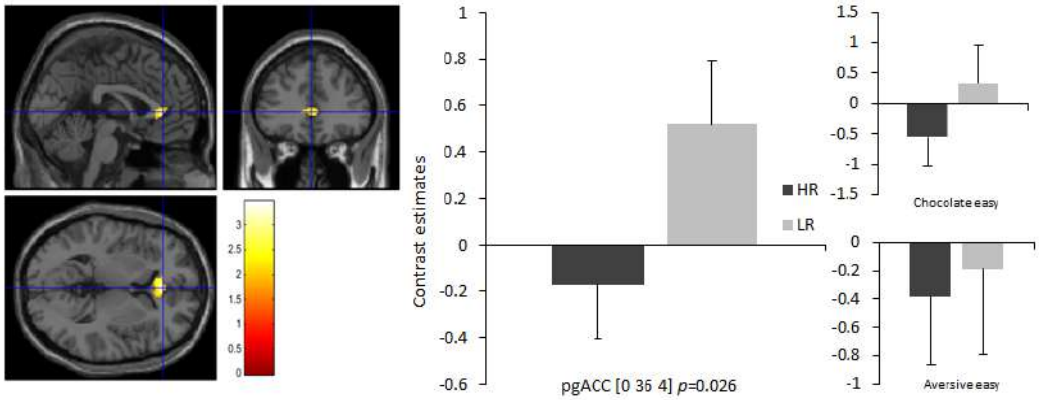

Figure 2. Effort: (left panel) chocolate easy–aversive easy, axial, sagittal and coronal image of pregenual anterior cingulate cortex (pgACC) activation in low risk (LR) vs high risk (HR) (z=3.13, p=0.026; region of interest (ROI) analysis with Wake Forest University (WFU) PickAtlas); (right panel) contrast estimates for pgACC centred at (0 36 4). Far right panel, contrast estimates for HR and LR separately for choc easy and aversive easy.

Consummatory phase

Blood-Oxygen-Level-Dependent (BOLD) responses to chocolate taste

Relative to LR, the HR group exhibited less BOLD responses in the pgACC ROI and the vmPFC ROI (Figure 3, Table 2.)

Blood-Oxygen-Level-Dependent (BOLD) responses to aversive taste

Relative to LR, the HR group exhibited less BOLD responses in the pgACC ROI and the vmPFC ROI and in the ACC/frontal pole for the unpleasant taste during whole brain analysis (Tables 2 and 3).

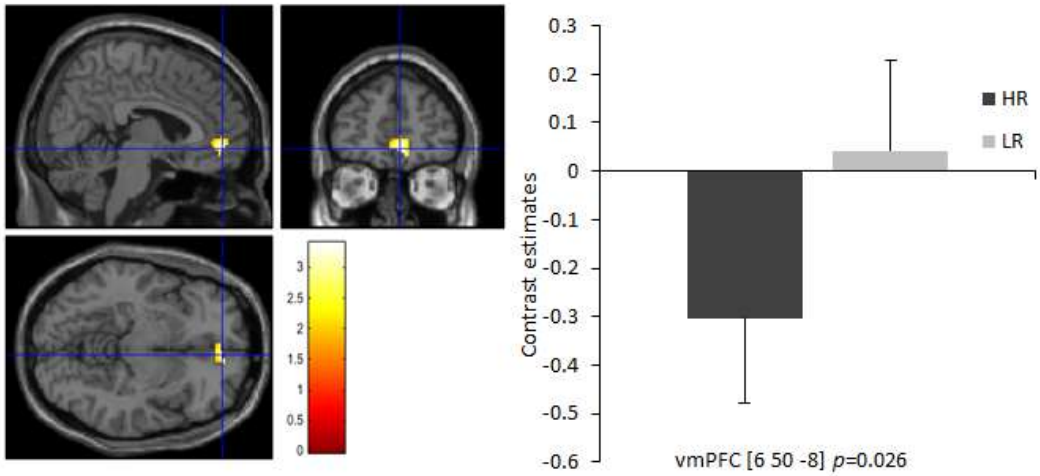

Figure 3. Consummation: (left panel) chocolate taste, axial, sagittal and coronal image of ventromedial prefrontal cortex (vmPFC) activation in low risk (LR) vs high risk (HR) (z=3.08, p=0.016; region of interest (ROI) analysis with Wake Forest University (WFU) PickAtlas); (right panel) contrast estimates for vmPFC centred at (6 50 −8).

Correlational analysis

Correlational analysis results revealed significant negative correlations between the FCPS scores and the pgACC ROI activation to the chocolate taste [8 36 2] (r=−0.606; p=0.013) in the HR group but no significant correlation in the LR group (r=0.144, p=0.581). This shows that as the anhedonia scores increased in the HR group the brain activity in the pgACC decreased (Figure 4).

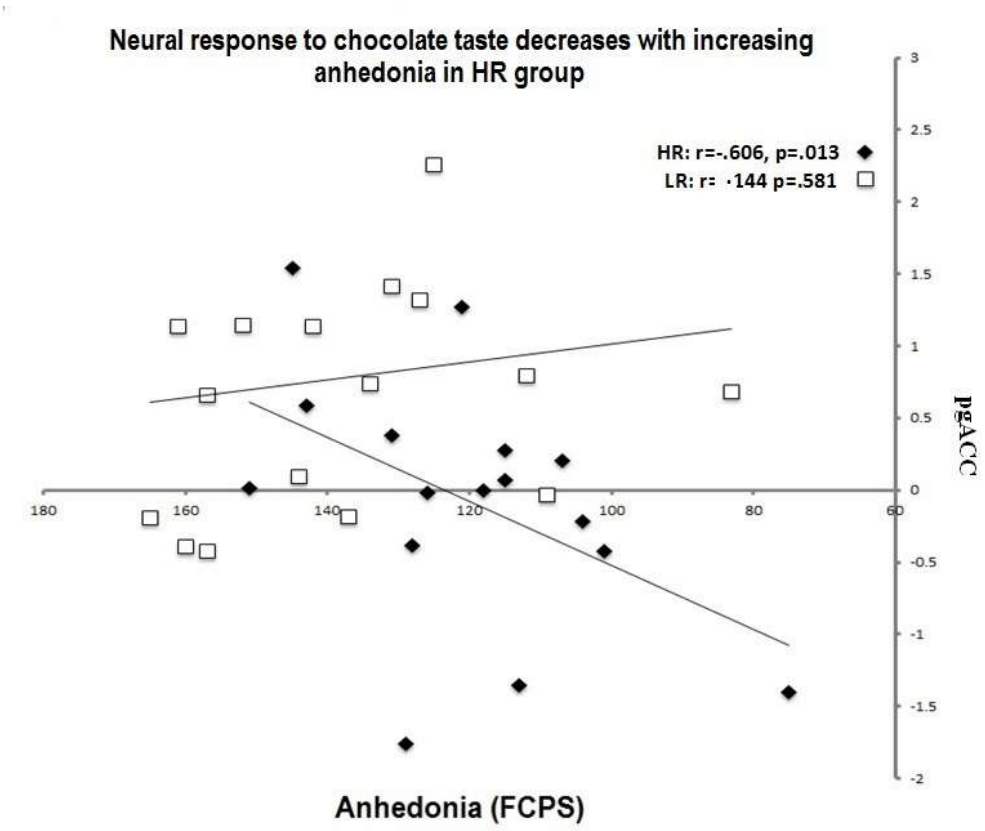

Figure 4. Correlations between pregenual anterior cingulate cortex (pgACC) activation to chocolate taste and anhedonia measures (Fawcett–Clarke Pleasure Scale (FCPS)) in the high risk (HR) group ($r=-0.606$, $p=0.013$) and low risk (LR) group ($r=0.144$, $p=0.581$).

Table 2. Significant group differences from region of interest (ROI) analysis using Wake Forest University (WFU) PickAtlas.

ROI seed	X	Y	Z	z-score	P value
Anticipation					
Aversive cue					
pgACC	4	44	2	2.98	0.036
Effort					
Chocolate easy-					
Aversive easy					
pgACC	0	36	4	3.13	0.026
vmPFC	10	6	-4	3.03	0.034
Consummation					
Chocolate taste					
pgACC	8	36	2	3.06	0.03
vmPFC	6	50	-8	3.08	0.016
Mould taste					
pgACC	8	34	0	4.03	0.001
vmPFC	8	56	-12	3.06	0.016

vmPFC- ventromedial prefrontal; pgACC- pregenual anterior cingulate
Family wise error corrected $p<0.05$ for multiple comparisons.

Table 3. Regions showing significant effect of mood of each of the groups on each of the conditions covaried for age, gender and body mass index (BMI).

	MNI coordinates				
Brain Region	X	Y	Z	*Z*-value	*P*-value
Anticipatory					
Aversive cue: LR>HR					
MFG	48	32	36	4.56	<.001
IFG	54	22	26	4.34	<.001°
Frontal Pole	14	44	48	4.16	<.001
PCC/Precuneus	6	-34	40	3.47	<.001
Effort					
Chocolate hard-chocolate easy: LR>HR					
Hippocampus	-26	-38	6	3.98	=0.002°
Chocolate hard-aversive hard: LR>HR					
MFG	-52	18	38	3.55	<.001°
Precentral gyrus	60	-2	32	3.43	<.001°
STG	56	-4	-8	3.39	<.001°
Chocolate easy-aversive easy: LR>HR					
Central operculum	-54	-8	20	4.05	<.001
Frontal Pole	-12	62	26	3.30	<.001
SFG	20	30	50	3.15	<.001

Consummatory					
Aversive taste: LR>HR					
ACC/Frontal Pole	14	44	32	3.33	=0.04

ACC: anterior cingulate cortex; HR: high risk; IFG: inferior frontal gyrus; lOFC: lateral orbitofrontal; MFG: middle frontal gyrus; PCC: posterior cingulate; pgACC: pregenual anterior cingulate cortex; SFG: superior frontal gyrus; sgACC: subgenual anterior cingulate cortex; STG: superior temporal gyrus; vmPFC: ventromedial prefrontal cortex. Thresholded p=0.001. Family-wise error corrected p<0.05 for multiple comparisons. °Results that do not survive MFQ as a covariate.

Discussion

Our findings show blunted neural responses during anticipation, effort and consummation of rewarding and aversive stimuli, despite no significant differences in behavioural responses, in adolescents with depressive symptomatology. Our results are consistent with the theory of emotion context insensitivity in depression whereby reduced reactivity to positive and negative stimuli is predominant (Rottenberg, 2007, Rottenberg et al., 2005).

Specifically we found reduced response in the HR group during the anticipation of the unpleasant cue in the pgACC ROI. This region is involved in reward anticipation (Kim et al., 2010; Sescousse et al., 2013) and has been found blunted to the anticipation of reward and aversion in adults with a history of depression (McCabe et al., 2009) and in currently depressed adults (Knutson and Heinz, 2015; Price and Drevets, 2010; Smoski et al., 2009; Ubl et al., 2015, Zhang et al., 2013).

We also found decreased medial and inferior frontal gyrus activation in the HR group compared to the LR group during anticipation (aversive cue). These are regions involved in cognitive control over emotional stimuli (Ochsner and Gross, 2005; Wager et al., 2008) and found dysfunctional in volunteers with depression symptoms (Beevers et al., 2010).

Despite relatively few studies examining neural responses in adolescents at risk of depression our results, of decreased pgACC ROI activity is similar to that of our previous study examining young people with a parent with depression where we also found evidence of diminished ACC activity to the anticipation of reward and aversion (McCabe et al., 2012). The pgACC is claimed to be a node of communication between the dACC, important for error detection or attention, and the more ventral ACC implicated in emotion processing and regulation as well as salience detection (Ball et al., 2014). Aberrant neuronal activation patterns of the pgACC have been found in depressed patients (Walter et al., 2009) and in remitted depressed patients in a task combining pleasant and unpleasant experiences of music and emotional faces (Aust et al., 2013). Therefore the reduced pgACC/ACC activations in our study in the HR group during the anticipation of aversive stimuli could be a mechanism by which those at risk of depression have problems using negative information to guide appropriate actions. This in turn could lead to an increased risk of depression.

During the effort phase we found more neural activity under hard trials than easy in all subjects. Specifically we found increased hippocampus and insula activity during chocolate hard trials and increased caudate activity for aversive hard (Supplementary Material, Table S4). The hippocampus is implicated in task performance and effort (Gur et al., 1997; Hosking et al., 2016; Pribram and McGuinness, 1975) and when comparing whole brain analysis between groups we found decreased activation in this region in the HR group compared to the LR group which is interesting given that we found no behavioural differences between the groups in their effort expended (Table 2). We also found decreased middle frontal gyrus (MFG) activations for hard chocolate trials versus hard aversive trials in the HR group compared to the LR group and decreased pgACC ROI for easy chocolate trials versus easy aversive trials in the HR group compared to the LR group. These are regions involved in reward processing, motor responses (Liljeholm and O'Doherty, 2012; Scholl et al., 2015) and in the avoidance of aversion (Kerr et al.,

2012) and in the willingness to expend effort in cost-benefit scenarios (Green et al., 2015; Schmidt et al., 2012). As such, these regions are important in underlying motivation both cognitive and physical (Schmidt et al., 2012). In summary we find that brain regions involved in effortful motivation to win reward and avoid aversion are reduced in adolescents at increased risk of clinical depression.

During the consummation phase we found decreased vmPFC ROI activation and pgACC ROI activation for the chocolate taste in the HR group. The pgACC decrease was significantly correlated with increasing depression symptomatology in the HR group (Figure 4). As described above, aberrant neuronal activation patterns of the pgACC have been found in depressed patients (Walter et al., 2009) and in remitted depressed patients in a task combining pleasant and unpleasant experiences of music and emotional faces (Aust et al., 2013). Thus our decreased pgACC activations to chocolate taste may indicate a biological marker of difficulty engaging with the experiences of reward. The vmPFC is reported as important for hedonic processes in many studies in animals and humans and is thought to mediate internally driven motivational processes such as satiety (Bouret and Richmond, 2010; Robbins and Everitt, 1996). In our previous study of those with a history of depression we found decreased responses in vmPFC to the consummation of chocolate (McCabe et al., 2009), similar to our current results and supporting the notion that neural deficits to reward also predate clinical depression onset.

We also found decreased pgACC and vmPFC ROI activations for the aversive taste in the HR group, which is interesting given that reports in the depression literature assume elevated responses to aversive stimuli (Rottenberg et al., 2005). Further increased activity in regions like the vmPFC (part of the Default Mode Network) have been reported in the processing of fear in depression (Grimm et al., 2009). However, studies also report blunted responses to a variety of negative and positive stimuli in depressed patients with a recent meta-analysis being the first quantitative review of emotional reactivity finding that depression involves consistent reductions in both positive AND

negative reactivity (Bylsma et al., 2008). Our result of blunted responses to positive and negative stimuli is also similar to our previous study with a recovered depressed sample (McCabe et al., 2009), suggesting that blunted aversion might also be a biomarker detectable both before depression onset and a residual trait marker of depression.

Interestingly, we did not find either ROI or whole brain differences between the groups in the ventral striatum which is consistent with our previous study examining young people at familial risk of depression but no personal depression experiences (McCabe et al., 2012). Also our results are unlike the large differences in striatal response to reward found in our previous study examining those recovered from depression (McCabe et al., 2009). This suggests that perhaps striatal differences (in this task) are only detectable after having experienced clinical depression and is thus a state rather than a trait marker of depression. Further longitudinal studies are necessary to clarify this.

In conclusion, our results show that adolescents with depression symptomatology have reduced neural responses to both reward and aversion. This is in line with the emotion context insensitivity theory of depression whereby depression is characterised by an emotional flattening to all stimuli, both positive and negative. This study suggests that there are biological markers of depression symptoms before clinical onset that may improve diagnosis and be important targets for early treatment interventions. Further, longitudinal studies with larger sample sizes are needed to clarify and replicate these results. Studies examining other groups at risk of depression such as those with a family history are needed to identify how reward function interacts with heritability, prognosis and treatment outcome in those who develop depression.

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Chapter 3: Addendum for Paper 1: Further Analyses and Discussion:

3.1. Rationale and Aims

Paper 1 presented brain activations for the main contrasts of interest across high risk and low risk groups. However, in order to fully explore the brain processing of the experimental stimuli, other contrasts should be also looked at. Thus, this chapter will present additional analyses and discussion for Paper 1.

The analysis was extended to show whether there were any group differences on how the control conditions were processed in the brain giving more insight into the baseline processing of the experimental stimuli. It is also important to present how the brain activation differed for the same condition in both of the groups, e.g. what the brain responses to the chocolate cue, aversive cue, chocolate taste, aversive taste were. This way, it is possible to show and directly compare the brain responses to each of the conditions. Furthermore, Figures 1 and 3 from Paper 1 were additionally updated with a bar graph presenting contrasts estimates for high and low risk groups separately for each of the conditions.

3.2. Results

3.2.1. Blood-Oxygen-Level-Dependent (BOLD) responses: ROI analysis

Control conditions:

Relative to the low risk group, the high risk group presented increased brain responses in the insula ROI only in response to the grey control cue (32 18 10; z=3.55, p=0.007; family-wise error corrected at p=0.05).

There were no statistically significant group differences in how the brain processes the tasteless control conditions.

Anticipation of rewarding and aversive stimuli:

There were no statistically significant group differences between the high and the low risk participants in how the brain processes the chocolate cue in any of the ROI regions (pgACC, insula, vmPFC, ventral striatum).

Relative to low risk group, the high risk group presented reduced brain activation in the insula ROI in response to the aversive cue (-36 18 0; z=3.01, p=0.035; family-wise error corrected at p=0.05).

Consummation of rewarding and aversive stimuli:

Relative to the low risk group, the high risk group presented increased brain responses in the insula ROI only in response to the consummation of rewarding stimuli (-34 8 8; z=3.17, p=0.025; family-wise error corrected at p=0.05).

Relative to the low risk group, the high risk group presented reduced brain responses in the pgACC ROI only in response to the consummation of rewarding (2 32 2; z=3.67, p=0.005; family-wise error corrected at p=0.05).

Relative to the low risk group, the high risk group presented reduced brain responses in the pgACC ROI only in response to consummation of aversive taste (0 34 2; z=3.70, p=0.005; family-wise error corrected at p=0.05).

3.2.2. Blood-Oxygen-Level-Dependent (BOLD) responses*:* Whole brain analysis

Control conditions:

There were no statistically significant group differences in how the brain processes the control conditions for both the grey box (control condition for the anticipatory phase) and tasteless solution (control condition for the consummatory phase).

Anticipation of rewarding and aversive stimuli:

There were no statistically significant group differences between the high and the low risk participants in how the brain processes the chocolate cue.

Relative to low risk group, the high risk group presented reduced brain activation in the precentral gyrus/middle frontal gyrus (36 8 28; z=4.61, p=0.007; covaried for age, gender and BMI; thresholder at p=0.001; family-wise error corrected at p=0.05) in response to the anticipation of aversive cue.

Consummation of rewarding and aversive stimuli:

There were no statistically significant group differences between the high and the low risk participants in how the brain processes the chocolate.

There were no statistically significant group differences between the high and the low risk participants in how the brain processes the aversive taste.

3.3. Further analysis of the contrasts estimates for significant group differences:

3.3.1. Addendum to Figure 1 from Paper 1

Additional contrasts estimates for HR and LR separately for the aversive cue and the grey cue for pgACC centred at (4 44 2) have been added.

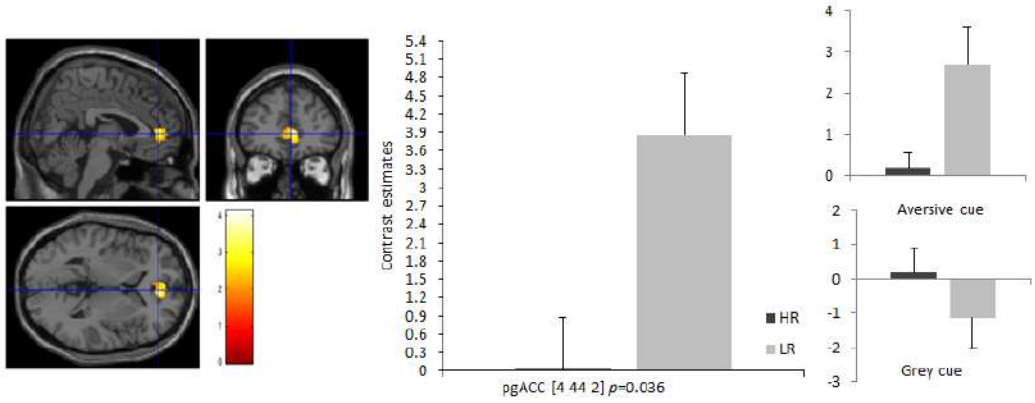

Figure 1: Anticipation: (left panel) Aversive cue, left panel, axial, sagittal and coronal image of pregenual anterior cingulate cortex (pgACC) activation in low risk (LR) vs high risk (HR) (z=2.98, p=0.036; region of interest (ROI) analysis with Wake Forest University (WFU) PickAtlas); right panel, contrast estimates for pgACC centred at (4 44 2); far right panel, contrasts estimates for HR and LR separately for aversive cue and grey cue for pgACC centred at (4 44 2).

3.3.2. Addendum to Figure 3 from Paper 1

Additional contrasts estimates for HR and LR separately for the chocolate taste and the rinse for vmPFC centred at (6 50 -8) have been added.

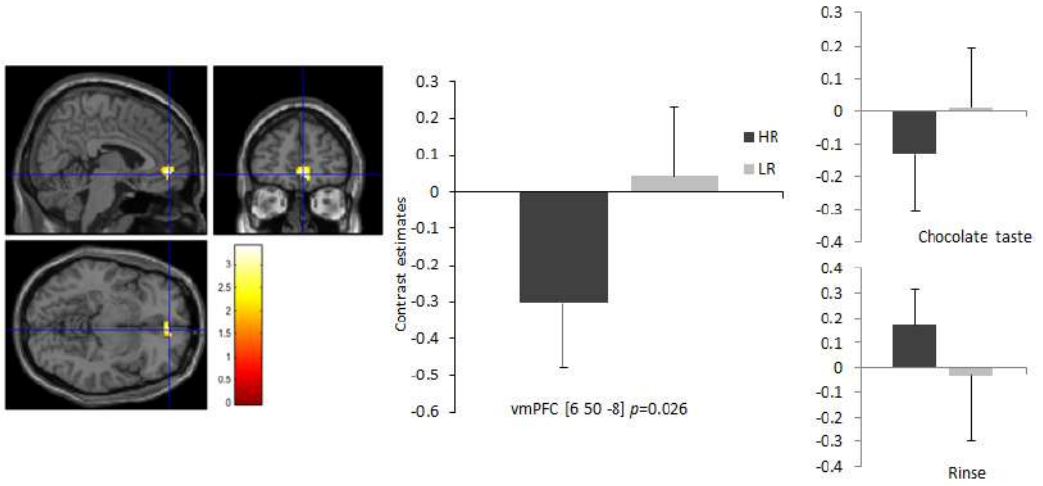

Figure 3. Consummation: (left panel) chocolate taste, axial, sagittal and coronal image of ventromedial prefrontal cortex (vmPFC) activation in low risk (LR) vs high risk (HR) (z=3.08, p=0.016; region of interest (ROI) analysis with Wake Forest University (WFU) PickAtlas); right panel, contrast estimates for vmPFC centred at (6 50 −8); far right panel, contrasts estimates for HR and LR separately for chocolate taste and rinse for vmPFC centred at (6 50 -8).

3.4. Discussion

The findings of the further analysis suggest that high and low risk groups did not differ in the way they processed the control conditions neither for the whole brain or the ROI analysis. This indicated that the brain differences in activation for other conditions (anticipation of reward and punishment, effort, consummation of reward and punishment) are attributed to a distinct processing of those stimuli in the brain of low risk and high risk individuals.

Moreover, the results also revealed decreased responses to the anticipation of aversive cue (aversive cue-aversive cue) in the insula ROI in the high risk versus low risk group. Alongside this result, there were also increased brain responses to the consummation of reward (chocolate taste-chocolate taste) in the insula ROI in the high

risk versus low risk group. Insula is a brain regions that have been implicated in the processing of salience and specifically in the processing of aversive stimuli (monetary loss and/or aversive sight and tastes (McCabe, Cowen, & Harmer, 2009; O'Doherty, Kringelbach, Rolls, Hornak, & Andrews, 2001; O'Doherty, Rolls, Francis, Bowtell, & McGlone, 2001). It is thus of interest that high risk individuals showed increased activation to chocolate taste by its own given that overall they have blunted responses to all conditions of the experimental task. To my knowledge, there is not a study available that looked at rewarding taste processing in subjects at risk for depression and reported increased insula involvement. However, this difference might be attributed to increased sensitivity in high risk subjects to positive stimuli which might serve as a possible compensatory or even protective mechanism by which these high risk subjects are still able to process salience information in relatively normal manner and have not yet progressed into clinical depression. However, this is only a possibility that should be further tested.

The results also revealed reduced brain responses in the pgACC ROI in the high risk versus low risk group in response to the consummation of both rewarding and aversive tastes suggesting that the responses to the rewarding and aversive tastes only were lower in the high risk group. This result is in line with previous findings that are presented in Paper 1 of blunted responses in the pgACC across all conditions. As mentioned in the discussion for Paper 1, the pgACC is claimed to be a node of communication between the dorsal ACC important for error detection or attention and the more ventral ACC implicated in emotion processing, regulation and salience detection (Ball, Stein, & Paulus, 2014). Thus it is possible that reduced responses in the pgACC in the at risk group could possibly reflect problems in integrating inputs of positive information and influencing affect regulation which in result lead to decreases in a hedonic response and a feeling of negativity.

The results also revealed reduced brain responses for the anticipation of aversive cue in the precentral gyrus/MFG in the high risk versus low risk group for the whole brain analysis. This result suggests that processing of aversive cue only was reduced in this brain region in the high risk group. This is in line with the main findings presented in the Paper 1and discussion over this finding is also available in the discussion section of Paper 1.

Overall, results of this extended analysis indicate that high risk subjects have reduced responses to anticipation and consummation of reward and aversion (except increased processing of chocolate taste).

3.5. References

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Chapter 4:

Paper 2: Decreased Neural Anticipation, Effort and Consummation of Reward and Aversion with Increased Anhedonia in Adolescents: An RDOC Dimensional approach.

Manuscript under review in the Biological Psychiatry journal.

Title:

Decreased Neural Anticipation, Effort and Consummation of Reward and Aversion with Increased Anhedonia in Adolescents: An RDOC Dimensional approach.

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Key words: depression, fMRI, reward, aversion, adolescents, at risk, anhedonia, biomarker.

Abstract

Recent studies have emphasized that a dimensional approach to studying the symptoms of depression may be beneficial given depressions heterogeneous nature. As reward function has been proposed as a possible biomarker for depression we are interested in how neural response to the anticipation, effort and consummation of reward is related to depression symptom severity in young people.

84 participants were recruited for the study. 43 participants presented with a range of depression symptoms (DS), including 27 participants with a current diagnosis of major depressive disorder (MDD) and 41 individuals were age and gender matched healthy controls (HC). We examined with functional magnetic resonance imaging anticipation (pleasant/unpleasant cue), effort (to achieve a pleasant taste or avoid an unpleasant taste) and consummation (experience of a pleasant/unpleasant taste) in regions of interest (ROI); amygdala, ventral striatum, insula and hippocampus. We measured how mood and anhedonia relate to neural responses to reward and aversion in ROIs.

Dimensional analysis revealed increased anhedonia correlated with decreased ventral striatal activity to reward anticipation and consummation in all subjects. Further analysis revealed that this effect was driven by those with depression symptoms and to a greater degree by those with MDD. We also found a correlation between increased anhedonia and decreased amygdala responses during reward consummation in the MDD group. Contrast ROI analyses revealed reduced ventral striatal and amygdala reward and aversion responses in the symptomatic groups. Despite no significant differences in subjective rating of the stimuli in the task, the DS and MDD groups also exerted less effort for reward compared to controls. Further the MDD group had reduced hippocampal response during the effort for reward and reduced insula response during effort to avoid aversion compared to controls.

These results show that ventral striatal and amygdala responses are related to trait anhedonia measures and that the greater the experience of anhedonia the greater the neural blunting to reward and aversion. Given that young people with depression symptoms have reduced neural response during effort and also exert less physical effort for reward, we suggest that therapies such as Behavioural Activation (increased exposure to reward) may be beneficial for young people with depression symptoms especially those who are anhedonic. Increased exposure in turn might prevent the onset of clinical depression in those at increased risk.

Keywords: Depression, functional magnetic resonance imaging task, reward, aversion, anhedonia, biomarker, dimensional approach, categorical approach

Introduction

It has been suggested that traditional diagnostic boundaries are not entirely useful for capturing the fundamental underlying mechanisms of psychiatric dysfunction (Insel et al., 2010). Rather, examining clinical symptoms as a continuum across symptom severity ranges may be more useful for identifying neurobiological signatures and risk markers. Reports suggest that a dimensional approach to studying the symptoms of depression may be more beneficial than a categorical approach especially given the heterogeneous nature of depression (Insel et al., 2010).

Anhedonia is defined as a loss of interest and pleasure, and is a key symptom of depression (AmericanPsychiatricAssociation, 2013). Moreover, anhedonia is related to abnormalities in the brain's reward mechanisms and is suggested as a possible biomarker for depression (Argyropoulos & Nutt, 2013; Hasler, Drevets, Manji, & Charney, 2004; Nutt et al., 2007). However anhedonia as a depression symptom is challenging to study because of its multi-faceted nature. Anhedonia has been described in terms of both anticipatory and consummatory processes with studies in depression finding both facets blunted in the ventral and dorsal striatum (Epstein et al., 2006; Forbes et al., 2009; Pizzagalli et al., 2009; Smoski et al., 2009; Ubl et al., 2015; Zhang, Chang, Guo, Zhang, & Wang, 2013). However few studies investigate the separate facets of anhedonia within the same task (Treadway & Zald, 2011; Zhang et al., 2013). We have developed an experimental model that utilizes pleasant sights and tastes of food to examine anticipation and consummation of reward. We have shown previously that adults at risk of depression have blunted neural responses to reward (McCabe, Cowen, & Harmer, 2009; McCabe, Woffindale, Harmer, & Cowen, 2012). Our recent follow up to this work finds that adolescents with depression symptomatology, but no current clinical diagnosis, also have blunted responses to reward consumption in the ventromedial prefrontal cortex and the

pregenual anterior cingulate cortex which negatively correlates with anhedonia measures (E Rzepa, Fisk, & McCabe, 2016).

Few studies have examined effort expenditure as another facet of reward processing in depression. The data available suggests that, at the behavioural level, depressed patients show reduced effort expenditure compared to healthy controls (Sherdell, Waugh, & Gotlib, 2012; Treadway, Bossaller, Shelton, & Zald, 2012; Yang et al., 2014). To examine this further we have also developed our task to examine the neural response during effort expenditure to win reward. Using this we found blunted responses during effort to win rewarding taste in young people with depression symptoms in the prefrontal cortex and the hippocampus (E Rzepa et al., 2016).

However we are also interested in how depression symptom severity is related to negative processing in the brain. Previous studies have found increased responses in the amygdala (Knutson & Greer, 2008; Sheline et al., 2001; Surguladze et al., 2004) whilst others have found *blunted* responses in the amygdala and the lateral orbitofrontal cortex (lOFC) during negative processing in depression (Bylsma, Morris, & Rottenberg, 2008) (Luking, Neiman, Luby, & Barch, 2015; McCabe et al., 2009). We have developed our experimental model to also examine the anticipation of aversive food (sight), effort to avoid aversive tastes and consumption of aversive tastes. Using this model we have found mixed results with both increases and decreases to the anticipation and consummation of aversive food in those at (McCabe et al., 2009; McCabe et al., 2012) which may be related to risk markers vs. scars of illness. However, in our recent study examining adolescents with depression symptoms we found blunted responses to both the anticipation and consumption of aversion in regions like the anterior cingulate cortex (E Rzepa et al., 2016). Interestingly these results support others that find blunted responses to both reward and aversion in depression (Luking et al., 2015) and fit with the theory of Emotion Context Insensitivity (Rottenberg, Gross, & Gotlib, 2005), whereby responses to

both positive and negative stimuli are blunted in depression. However, how the brain responds to reward (anticipation, effort and consummation) in adolescents across a range of depression symptoms from mild and moderate to clinical depression, is unknown. Therefore in this study we will examine the neural response to reward and aversion in young people with varying degrees of depression symptoms. We hypothesize that neural responses will correlate with depression and anhedonia symptoms across the spectrum. Further, in a categorical analysis, we will examine for the first time how adolescents with a clinical diagnosis of depression respond to the neural anticipation, effort and consummation of reward and aversion compared to healthy age and gender matched controls.

Methods

Participants

84 (13-21) participants completed the study. All participants were assessed with the Structured Clinical Interview for DSM-IV Axis I Disorders Schedule (SCID) (First, Spitzer, Gibbon, & Williams, 1997). 43 adolescents were deemed as having DS as they scored >27 on the Mood and Feelings Questionnaire (MFQ) (Angold, Costello, Messer, & Pickles, 1995). 41 individuals, age and gender matched, were considered healthy controls (HC) through the SCID and scoring < 15 on the MFQ. 27 major depression disorder MDD participants with a current diagnosis of major depression disorder (MDD) from either their GP, clinical psychologist or psychiatrist were also in the DS group. 14 MDD participants were on antidepressants. 6 MDD participants had an antidepressant history (see Table S1). We excluded pregnancy any other medications except the contraceptive pill and any contraindications to MRI. The National and University Research Ethics Committees approved the study and written informed consent was obtained from all participants.

Overall design

Participants were asked to refrain from consuming chocolate 24 hours prior to scanning. Before and after each scan, volunteers completed the Befindlichskeits scale (BFS) of mood and energy (von Zerssen, Strian, & Schwarz, 1974) and a mood visual analogue scale (VAS).

The task is previously published in Dean et al., 2016. The task (40 trials) had 4 conditions based on the trial type (reward/aversive) and its level of difficulty (easy/hard). Trial type was cued by a visual stimulus (chocolate picture or picture of moldy drink (2 sec)), which indicated either to work to win the chocolate taste or to avoid the aversive taste (effort phase). Difficulty was determined by the amount of effort required to complete the effort stage (easy = 24, hard = 45 button presses). This required volunteers to press a button as fast as possible (< 6 sec) to move a bar towards the pleasant chocolate picture (reward) or away from the unpleasant moldy picture (aversive), allowing enough time to complete easy trials but not hard. If on reward trials volunteers were successful they received the taste (5 sec delivery and 2 sec swallow cue) of chocolate and if not they received the tasteless solution. If on aversive trials volunteers were successful they received the tasteless solution and if not they received the unpleasant taste. A grey image (2 sec) was presented at the end of each trial. Each condition was repeated 10 times, chosen by random permutation. Jitters were used for both interstimulus intervals and inter-trial intervals. To sustain effort, 4 trials (2 reward/2 aversive) were longer at 9 sec each. Volunteers also rated 'wanting', 'pleasantness' (+2 to –2) and 'intensity' (0 to +4) on a VAS on each trial.

Stimuli

We used a picture of liquid chocolate (reward), a moldy drink (aversive) and a grey image (control). The rewarding taste was a Belgian chocolate drink and the aversive

taste was a combination of the chocolate drink mixed with beetroot juice, providing a similar texture.

The tasteless solution (25 x 10^{-3}mol/L KCL and 2.5x10^{-3}mol/L $NaHCO_3$ in distilled H_2O) was also used as a rinse between trials. Solutions were delivered through three teflon tubes allowing 0.5 mL of solution to be manually delivered.

fMRI Scan

The experimental protocol consisted of an event-related interleaved design. A Siemens Magnetom Trio 3T whole-body MRI scanner and a 32-channel head coil were used. Multi-band accelerated pulse sequencing (version no. RO12, Center for Magnetic Resonance Research, University of Minnesota, USA, EPI 2D BOLD/SE/DIFF Sequence) was used with an acceleration factor of 6. T2*-weighted echo planner imaging slices were obtained every 0.7 s (TR). Fifty-four axial slices with in-plane resolution of 2.4 × 2.4 mm and between-plane spacing of 2.4 mm were attained. The matrix size was 96 × 96 and the field of view was 230 × 230 mm. Acquisition was performed during task performance, yielding ~3500 volumes. An anatomical T1 volume with sagittal plane slice thickness 1 mm and in-plane resolution of 1.0 × 1.0 mm was also acquired.

fMRI analysis

Statistical Parametric Mapping (SPM8) was used for realignment and normalization to the Montreal Neurological Institute (MNI) coordinate system and spatial smoothing with a 6-mm full-width-at-half-maximum Gaussian kernel (Collins, Neelin, Peters, & Evans, 1994). The time series at each voxel was low-pass filtered with a hemodynamic response kernel. Time series non-sphericity at each voxel was estimated and corrected for (K. J. Friston et al., 2002), and a high-pass filter with a cut-off period of 128 sec was applied.

In the single-event design, a general linear model was then applied to the time course of activation in which stimulus onsets were modeled as single impulse response

functions and then convolved with the canonical hemodynamic response function (K.J. Friston, Worsley, Frackowiak, Mazziotta, & Evans, 1994). Linear contrasts were defined to test specific effects. Time derivatives were included in the basis functions set. Following smoothness estimation (Worsley, Marrett, Neelin, Friston, & Evans, 1996), linear contrasts of parameter estimates were defined to test the specific effects of each condition (pleasant/unpleasant cue – grey image or pleasant/unpleasant taste – rinse) with each individual dataset. Voxel values for each contrast resulted in a statistical parametric map of the corresponding *t* statistic (transformed into the unit normal distribution (SPM *z*)). Movement parameters for each person were added as additional regressors.

Simple main effects of task were examined with one-sample *t*-tests thresholded at $p<0.001$ and report p values <0.05 FWE corrected (Table S4). Regions of interest (ROI) were identified for each condition based on activity in healthy controls using this task in a previous study (Dean, Horndasch, Giannopoulos, & McCabe, 2016). For anticipation we selected the ventral striatum [6 4 -6]) for consummation we selected the (insula [36 10 -14] amygdala [20 -4 -20] and also ventral striatum [6 4 -6]). These ROIs were created with Wake Forest University Pickatlas toolbox in SPM8, with 8 mm spheres for the insula and amygdala seeds and 6mm sphere for the ventral striatum seeds. We also created a hippocampus structural ROI with Wake Forest University Pickatlas, as the hippocampus was previously found involved in physical effort (Gur et al., 1997) (Pribram & McGuinness, 1975). ROI analysis across all conditions was carried out with Wake Forest University Pickatlas in SPM8. We extracted peak voxel brain activity in the ROIs during the anticipation, effort and consummation of reward and aversion and correlated with depression and anhedonia measures in all subjects and in the DS and MDD groups separately, using Pearson correlations. We also ran ROI analyses for the DS group vs. HC and the subgroup of MDD vs. HC, thresholded at $p<0.001$ and report p values <0.05 FDR corrected. We also further Bonferroni corrected for the 4 ROIs examined (meaning a $p<0.01$ had to be achieved) **(**Table 2). We also ran whole brain exploratory analyses for the DS group vs. HC and the subgroup of MDD vs. HC, thresholded at $p<0.001$ and with

p values <0.001 FWE corrected for multiple comparisons (Table 3, 4). All analyses had age, medication status and BDI added as covariates of no interest. Plots of contrast estimates were extracted with plots tool in SPM8, and Wake Forest University Pick Atlas toolbox was used to display neural activation.

Results

Demographic and Clinical Data

Table 1 shows there were no significant differences between the DS and HC group or the MDD subgroup and HC group for age, gender, BMI, chocolate craving, liking and the frequency of chocolate eating. However the groups differed on mood and anhedonia measures as expected (Table 1a and 1b).

Table 1a: Demographics for all participants

Measure	All Participants (n=84)
Age (years)	18.09 (1.89)
Age range	13-21
Gender	F=64, M=20
BMI	21.47 (2.31)
Chocolate: craving	6.66 (2.01)
liking	8.56 (1.34)
frequency	2.55 (2.07)

Table 1b: Demographics for each group separate

Measure	**DS (n=43) Mean (SD)**	**HC (n=41) Mean (SD)**	**p-value**	**MDD (*n*=27) Mean (SD)**	**HC (*n*=31) Mean (SD)**	***p*-value**
Age (year)	18.16 (1.84)	18.02 (1.97)	.74	19.07 (1.51)	18.94 (1.09)	.689
Age range	15-21	13-21	-	15-21	17-21	-
Gender	F33, M10	F31, M10	.904	F21, M6	F26, M5	.563
BMI	21.83 (2.18)	21.09 (2.41)	.144	21.68 (1.94)	21.63 (2.3)	.930
BDI	29.88 (12.77)	3.32 (4.1)	<.001	29.62 (12.93)	3.65 (3.64)	<.001
FCPS	113.7 (25)	137.0 (19.4)	<.001	109.89 (27.63)	138.8 (17.16)	<.001
SHAPS	30.9 (7.37)	20.58 (7)	<.001	31.19 (8.34)	20.83 (7.52)	<.001
TEPS-A	36.28 (8.78)	48 (5.85)	<.001	35.93 (9.52)	47.65 (6.02)	<.001
TEPS-C	30.35 (6.24)	36.78 (7.05)	<.001	29.7 (6.39)	37.13 (6.43)	<.001
Chocolate:						
craving	6.77 (2)	6.55 (2)	.622	6.96 (2.23)	6.8 (2)	.779
liking	8.6 (1.33)	8.52 (1.37)	.786	8.59 (1.5)	8.77 (1.28)	.621
frequency	2.67 (2.31)	2.43 (1.82)	.588	3.1 (2.6)	2.2 (1.6)	.120

DS: depression symptoms, MDD: Major Depressive Disorder, HC: Healthy Controls, BMI:Body Mass Index. BDI: Beck Depression Inventory, FCPS: Fawcet Clarke Pleasure Scale, SHAPS: Snaith-Hamilton Pleasure Scale, TEPS: Temporal Experience of Pleasure Scale A: Anticipation, C: Consummation.

Mood, Energy and Affect Scores

To investigate whether the scanning session had any effect on the mood of participants, the BFS and VAS scores were collected before and after each of the scanning

sessions. Results of this investigation are presented in the supplementary materials SR1.

Subjective Ratings of Stimuli

Ratings of wanting, pleasantness, and intensity for the stimuli were obtained during scanning on each trial for cues and the tastes. All subjects rated chocolate taste as pleasant and the aversive taste as unpleasant (Table S3). We used repeated measures ANOVA with ratings as the first factor, three levels (wanting, pleasantness, intensity) and condition as the second factor, two levels (chocolate, aversive) and between subject factor of group (DS vs. HC). We found no significant main effect of group ($F(1.82)=729.07$; $p=.094$), a significant main effect of condition ($F(1.82)=530.93$; $p<.001$), i.e. chocolate and aversive tastes were rated differently and a significant main effect of ratings ($F(1.116,91.53)=932.816$; *pGreenhouseGeisser-corrected*<.001) as expected. We found no significant group x condition x ratings interaction (DS and HC: ($F(2.164)=1.670$; *pGreenhouseGeisser-corrected*=.199) (Table S2). For the MDD vs. HC we found no significant main effect of group ($F(1.56)=13.56$; $p=.064$), a significant main effect of condition ($F(1.56)=1261$; $p<.001$), and a significant main effect of ratings as expected ($F(2.88)=808.8$; *pGreenhouseGeisser-corrected*<.001) but no significant group x condition x ratings interaction (MDD and HC*:* $F(2.64)=0.29$; *pGreenhouseGeisser-corrected*=.895)

Effort behaviour.

The number of button presses and time taken to complete the effort part of the task was also recorded. There were significant group differences between the DS and HC ($t(82)=-2.225$, $p=0.029$) and between the MDD and HC ($t(56)=-2.25$, $p=0.028$) for the number of button presses in the chocolate hard condition, meaning the DS and MDD groups exerted less effort for reward compared to controls. There were no significant group differences for the aversive conditions or in the time taken to complete the effort part of the study ($p>.05$) (Table S3).

Main Effects of Reward and aversion on Brain Blood Oxygen Level-Dependent Responses

Table S4 provides a summary of the main effects of one-sample t-tests in all healthy controls for the anticipation, effort and consummation phases. As expected, the anticipation of the rewarding stimuli activated reward-relevant circuitry including the prefrontal cortex and striatum. The anticipation of the aversive cue activated similar areas but also the insula. Effort to achieve rewards activated the precentral gyrus and also the posterior cingulate and hippocampus. Effort to avoid aversion activated the precentral gyrus, posterior cingulate cortex, precuneus and caudate. Consummation of the pleasant chocolate taste activated the striatum, the anterior cingulate, amygdala and the hippocampus, whilst the aversive taste activated the same regions but also the insula (Table S4).

Relationship between Brain and Depression Symptoms/Anhedonia:

Ventral Striatum ROI: Neural Anticipation correlates with Depression Symptoms

We run correlational analyses for all participants together and each of the groups separately but only significant correlations are reported here. We found a significant negative correlation between the ventral striatum activation to the rewarding cue and the BDI scores (r=-.242; p=.028) in all participants. This shows that as the depression scores increased, the ventral striatum response to the reward cue decreased (Fig 1a). We also found a significant positive correlation between the ventral striatum activation to the rewarding cue and the scores on the TEPS anticipatory (r=.254, p=.02) (Fig 1b) and consummatory (r=.263, p=.016) (Fig 1c) scales in all subjects. This means that as trait anticipatory and consummatory pleasure increases there is increased ventral striatum. Further, the ventral striatal activation to the reward cue correlated with the TEPS consummatory scale in the DS (r=.357, p=.019) (Fig 2a) and MDD (r=.583, p=.001) (Fig

2b) sub groups, showing that the higher the trait anhedonia the lower the ventral striatal anticipatory activity.

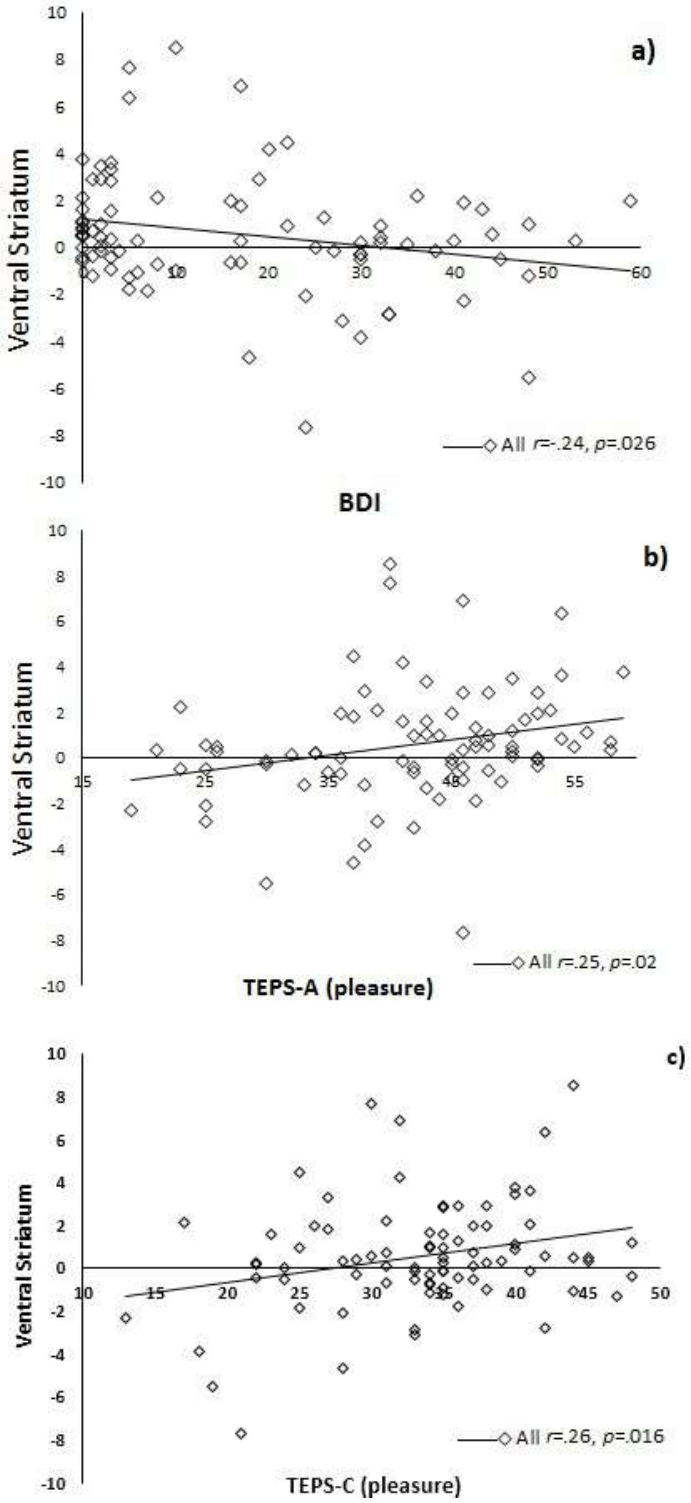

Figure 1. a) Correlations between the ventral striatum ROI activation to reward cue and depression measure (BDI) for all participants (r =-.242, p=.026); **b)** and TEPS A for all participants (r =.254, p=.02). **c)** and TEPS C for all participants (r=.263, p=.016).

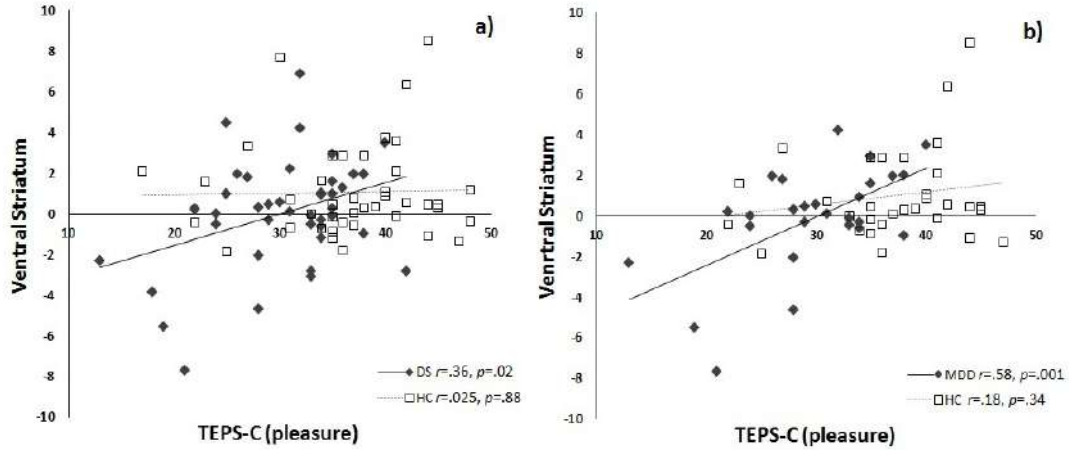

Figure 2. a) Correlations between the ventral striatum ROI activation to reward cue and TEPS C for DS (r=.357, p=.019) and HC (r=.025, p=.88); **b)** and MDD (r=.583, p=.001) and HC (r=.18, p=.34).

Ventral Striatum ROI: Neural Consummation correlates with Depression Symptoms

There was a significant negative correlation between the ventral striatum ROI during reward taste and the SHAPS scores (r=-.39; p=.01) in the DS group, meaning that the higher the anhedonia the lower the ventral striatal consummatory response but only in those with depression symptoms (Fig 3).

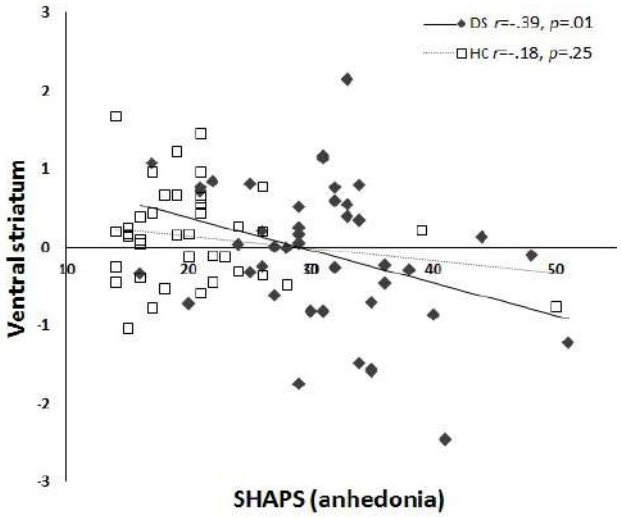

Figure 3. Correlations between the ventral striatum ROI activation to rewarding taste and SHAPS scale for DS (r=-.39; p=.01) and HC (r=.18, p=.25).

Amygdala ROI: Neural Consummation correlates with Depression Symptoms

There was a significant negative correlation between the amygdala during the reward taste and the SHAPS scores (r=-.670, p=.001), the FCPS scores (r=.402, p=0.038) and the TEPS anticipatory scale (r=.44 p=.022) in the MDD group, meaning that the higher the trait anhedonia the lower the amygdala consummatory reward response. There was also a significant positive correlation between the amygdala during the aversive taste and the TEPS consummatory scale (r=.290, p=.008) in all subjects, meaning that as trait consummatory anhedonia increased, the amygdala consummatory response to aversion also increased. This correlation was also significant in the HC group (r=.462, p=.002).

Hippocampus ROI: Neural Effort correlates with Physical Effort

There was a significant correlation between the hippocampus during effort for reward (chocolate hard-chocolate easy) and the number of button presses to complete the chocolate hard trials (*r*=.586, *p*=.001) and the time taken to complete the chocolate hard trials (*r*=-.381 *p*= 0.034) in the HC group (Fig 4). Meaning that for HC the higher the hippocampus activity the more effort was expended and the quicker they made their responses. This was not evident in the MDD or the DS groups.

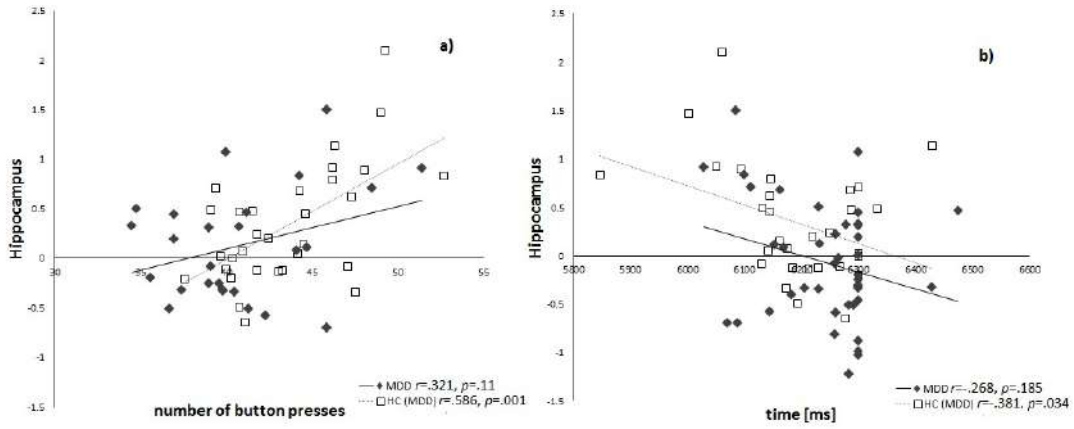

Figure 4. **a)** Correlations between the hippocampus ROI activation to effort (chocolate hard-chocolate easy contrast) and number of button presses for chocolate hard trials for HC (MDD) group (r=.586, p=.001) and the MDD group (r=.321, p=.1); **b)** Correlations between the hippocampus ROI activation to effort (chocolate hard-chocolate easy contrast) and time taken to complete the chocolate hard trials for the HC group (r=381, p=.034) and MDD group (r=-.268, p=.185).

Between Group Differences (ROI Analyses) (Table 2):

Anticipatory phase (Reward)

Relative to HC, the DS exhibited less BOLD responses in the ventral striatum, and the amygdala to the rewarding cue (Fig 5). Relative to HC, the MDD group also exhibited less BOLD responses in the bilateral ventral striatum to the rewarding cue (Fig 5).

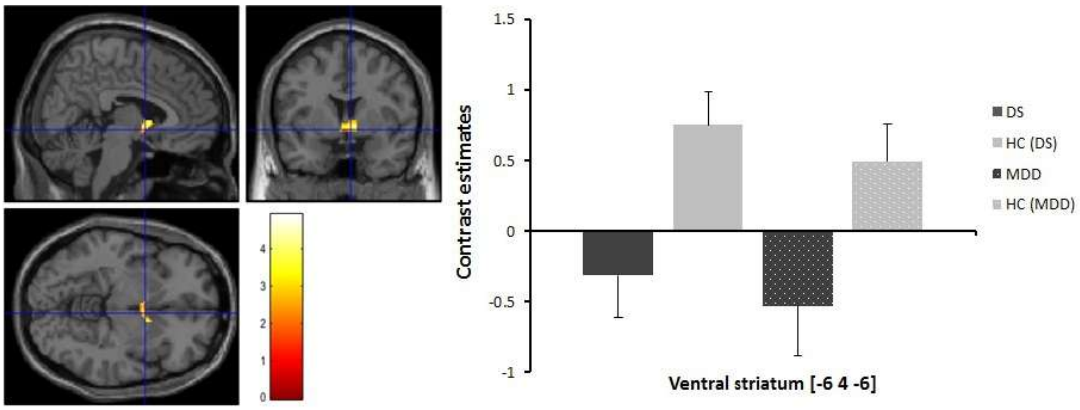

Figure 5. Anticipation: Rewarding cue, *left panel,* axial, sagittal and coronal image of ventral striatum [6 4 -6] ROI activation: HC vs. DS (z=3.19, p=0.035); HC vs. MDD (z=4.45, p=0.001); *right panel,* contrast estimates for ventral striatum for HC and DS and HC and MDD groups.

Anticipatory phase (Aversive)

Relative to HC, the MDD group exhibited less BOLD responses in the ventral striatum ROI to the aversive cue.

Effort Phase (Reward)

Relative to HC, the MDD group exhibited less BOLD response in the hippocampus ROI for the chocolate hard trials vs. chocolate easy trials.

Effort Phase (Aversive)

Relative to HC, the MDD group exhibited less BOLD response in the insula ROI to the aversive hard trials vs. aversive easy trials.

Consummatory Phase (Reward)

Relative to HC, the MDD group exhibited less BOLD responses in the right ventral striatum, bilateral amygdala and right insula (Fig 6) during the reward consummatory phase.

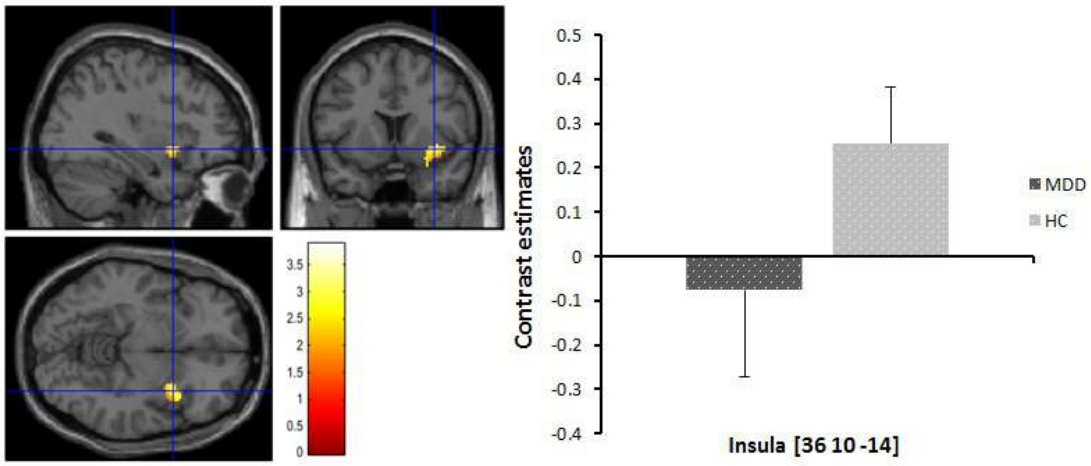

Figure 6. Consummation: Rewarding taste, *left panel,* axial, sagittal and coronal image of insula ROI [36 10 -14] activation: HC vs. MDD (z=3.53, p=0.015); *right panel,* contrast estimates for insula for HC and MDD groups.

Consummatory Phase (Aversive)

Relative to HC, the MDD group exhibited less BOLD responses in the amygdala ROIs during the unpleasant taste condition.

Table 2:
ROI analyses: Effect of Depression Symptoms (DS) and Clinical Depression (MDD).

				HC vs DS		HC vs MDD	
ROI seed	X	Y	Z	z-score	P value	z-score	P value
Anticipation							
Reward cue							
Ventral striatum	6	4	-6	-	-	3.91	0.001
Ventral striatum	-6	4	-6	3.86	<.001	5.49	<.001
Amygdala	20	-4	-20	3.62	0.005	-	-
Aversive cue							
Ventral Striatum	-6	4	-6	-	-	4.07	0.001
Effort							
Chocolate hard-Chocolate easy							
Hippocampus	26	-6	-28	-	-	3.83	0.010
Aversive hard-aversive easy							
Insula	36	10	-14	-	-	3.77	0.003
Consummation							
Reward taste							
Ventral striatum	6	4	-6	-	-	3.13	0.015
Amygdala	20	-4	-20	-	-	3.47	0.01
Amygdala	-20	-4	-20	-	-	4.03	0.001
Insula	36	10	-14	-	-	3.53	0.015
Aversive taste							
Amygdala	-20	-4	-20	-	-	3.83	0.003

Thresholded p<0.001. p values <0.05 FDR corrected. Results covaried for age, gender and medication.

Discussion

Using a dimensional approach this study aimed to investigate the relationship between neural responses to reward and symptoms of depression, specifically anhedonia, in a group of adolescents, in line with the NIMH Research Domain Criteria (RDoC) initiative.

Specifically we found that in the ventral striatum ROI there were correlations between increased depression severity and decreasing brain responses to the rewarding cue in all subjects. Furthermore increasing TEPS anticipatory and consummatory scores also correlated with increasing brain activity in the ventral striatum to the rewarding cue in all subjects. Interestingly, we find that these correlations were driven by the adolescents with depression symptoms and to a larger degree by those with a clinical depression diagnosis. We also found increasing anhedonia correlated with decreasing amygdala response to reward anticipation in the DS group. Supporting this, contrast analysis revealed blunted ventral striatal and amygdala responses to reward anticipation compared to controls and blunted ventral striatal responses to anticipation of aversion in the MDD group. These results are in line with our hypothesis in that we did find blunted neural responses to reward and aversion in our ROIs in those with depression symptoms and those with clinical depression. Our results are consistent with the previous studies that report the ventral striatum's role in the processing of rewarding and aversive information (Carter, Macinnes, Huettel, & Adcock, 2009; Kohls et al., 2013), and its involvement in the anticipation and consummation of reward and aversion in healthy people (Dean et al., 2016) and depressed patients (Pizzagalli et al., 2009). Furthermore, some studies in clinically depressed patients showed correlations between symptomatic anhedonia measures and decreased ventral striatal activity (Keedwell, Andrew, Williams, Brammer, & Phillips, 2005). Further, the amygdala has been implicated in emotion regulation and in the processing of emotional stimuli especially those ones associated with threat (Drevets, 2003; LeDoux, 2000; Stein et al., 2007) (Bressler & Menon, 2010). These emotional processes have been shown to be dysregulated in MDD (Drevets, 2003),

and in individuals with subclinical depression (Chan, Norbury, Goodwin, & Harmer, 2009). Moreover, (Smoski et al., 2009) reported decreased amygdala activation for monetary reward outcome in depressed patients suggesting its involvement in reward processing. Our results are consistent with the theory of Emotion Context Insensitivity which states a reduced reactivity to positive and negative stimuli in depression (Rottenberg et al., 2005).

Together this data show that not only is the striatal and amygdala responses to reward related to subjective experiences of pleasure and thus anhedonia but that this is more pronounced in those with clinical depression. Given the dimensional nature of these results this work further supports the notion that anhedonia and the neural response to reward anticipation may be a biomarker for depression. We also found a correlation between increasing anhedonia and decreasing ventral striatal response to reward consummation in adolescents but only in those who report depression symptoms. Supporting this, contrast analyses revealed blunted ventral striatum, amygdala and insula to reward consummation and blunted amygdala to aversive consummation in the MDD group compared to controls. The insula is considered a part of the primary taste cortex and we find this part of the brain more active during the consummatory phase in previous taste studies (McCabe et al., 2009; McCabe et al., 2012) yet blunted here in clinical depression. These results are interesting given that most studies report only reward anticipatory deficits in those with depression and find it difficult to distinguish between anticipatory and consummatory deficits (Treadway & Zald, 2011). However the few studies that have tried use monetary reward, and thus it could of course be argued that they are, are not in fact measuring real consummatory responses (Argyropoulos & Nutt, 2013; Treadway & Zald, 2011).

This is the first study that we know of examining the neural responses to effort to attain reward or avoid aversive taste stimuli in depression. Interestingly we did find reduced effort expended in those with depression symptoms despite no significant differences in subjective ratings (pleasantness, wanting and intensity) of the rewarding

and aversive stimuli. We also found the hippocampal ROI response during effort blunted in those with clinical depression. Hippocampus is implicated in task performance and effort (Gur et al., 1997; Hosking, Cocker, & Winstanley, 2016; Pribram & McGuinness, 1975) and a previous study that used the same experimental paradigm also found decreased hippocampal activity in adolescents with increased depression symptomatology when compared with matched HC (E. Rzepa, Fisk, & McCabe, 2017). Consistent with this the hippocampal response during effort for reward also correlated with increased effort and faster reaction times during hard reward trials in the HC group but not in those with clinical depression. We also found reduced activity in the insula in those with clinical depression during the effort to avoid the unpleasant taste, even though there were no behavior differences in effort for the unpleasant conditions between the groups. The insula is a brain regions implicated in the processing of salient and sometimes aversive stimuli (monetary loss and/or aversive sight and tastes (McCabe et al., 2009; O'Doherty, Kringelbach, Rolls, Hornak, & Andrews, 2001; O'Doherty, Rolls, Francis, Bowtell, & McGlone, 2001). Altogether our results suggest that blunted effort to avoid unpleasant stimuli is less pronounced than the blunted effort to gain reward in depression. This certainly is in keeping with the notion that in depression there is actually a focus on negative information with studies finding that compared to non-depressed controls, depressed individuals respond faster to stimuli cued by negative words (Gotlib, Krasnoperova, Yue, & Joormann, 2004; Mathews, Ridgeway, & Williamson, 1996; Mogg, Bradley, & Williams, 1995).

Effects of depression symptoms on the whole brain analysis also revealed similar results to that of our ROI analyses. However, prefrontal cortex deficits were also apparent specifically in the precuneus, PCC, ACC for those with depression symptoms for reward and aversion anticipation and in the limbic structures of the putamen and caudate for reward anticipation only. These brain regions have been implicated previously in neurobiology of depression and have been found dysfunctional in tasks examining

emotion processing, motivation and reward (Kerestes, Davey, Stephanou, Whittle, & Harrison, 2014; Smoski, Rittenberg, & Dichter, 2011).

In summary our results support the idea that interventions such as Behavioural Activation that work by increasing engagement with pleasurable activities might be beneficial specifically for those adolescents with pronounced anhedonia during their depression. Clinical trials are needed to establish this, by examining the relationship between anhedonia, depression symptoms and brain activity before and after Behavioural Activation.

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Chapter 5: Addendum for Paper 2: Further Analyses and Discussions

Note as requested after PhD defence: The DS group consisted of 16 high risk (HR) individuals from Chapter 2 (Study 1) and 27 MDD individuals as indicated in Chapter 4 (Study 3).

5.1. Rationale and Aims

Paper 2 presented brain activations for the ROI's of interest (ventral striatum, amygdala, insula, and hippocampus) across dimension of depressive symptoms and in those with a clinical diagnosis of depression. However, in order to fully explore the brain processing of the experimental stimuli for both dimensional and categorical approaches, whole brain analysis should be addressed. I will also present results for experimental stimuli that were not considered in chapter 4. Thus, this chapter will present additional analyses and discussion for Paper 2.

The analysis was extended to address the effects of depressive symptoms and clinical diagnosis on the processing of experimental stimuli in the whole brain between the DS vs. HC and the MDD vs. HC. Moreover, this chapter also addresses whether there were any group differences on how the control conditions were processed in the brain giving more insight into the baseline processing of the experimental stimuli. It is also important to present how the brain activation differed for the same condition across the group, e.g. what the brain responses to the chocolate cue, aversive cue, chocolate taste, aversive taste were. This way, it is possible to show and directly compare the brain responses to each of the conditions. Furthermore, Figures 5 and 6 from Paper 2 were additionally updated of bar graphs presenting contrasts estimates for high and low risk groups separately for each of the conditions.

5.2. Addendum to the results section of Paper 2:

5.2.1. Results for whole brain analysis DS vs. HC and MDD vs. HC groups

Note as requested after viva: The DS group consisted of 16 high risk (HR) individuals from Chapter 2 (Study 1) page 48 and 27 MDD individuals as indicated in Chapter 4 on page 83.

Anticipatory phase (Reward)

Relative to HC, the DS group exhibited less BOLD responses in the precuneus, PCC, ACC/PCC, frontal pole, superior frontal and the middle temporal gyrus, insula, lateral occipital cortex, and the putamen/caudate (Table 1).

Relative to HC, the MDD group exhibited less BOLD responses in all the same regions and including the ventral striatum (Table 2).

Anticipatory phase (Aversive)

Relative to HC, the DS group exhibited less BOLD responses in the frontal pole, precuneus, PCC/ACC, the superior frontal and middle temporal gyri and in the lateral occipital cortex (Table 1).

Relative to HC, the MDD group exhibited less BOLD responses in the precuneus, PCC/ACC, the superior frontal gyrus, the lateral occipital cortex, the caudate and the putamen (Table 2).

Effort Phase (Reward)

Relative to HC, the DS group exhibited less BOLD responses in the insula, ACC, putamen, and precuneus for the chocolate hard trials versus chocolate easy trials (Table 1).

Relative to HC, the MDD group exhibited less BOLD responses in the lingual gyrus, occipital fusiform gyrus, paracingulate gyrus/ACC and the PCC/Precuneus for the chocolate hard trials versus chocolate easy trials (Table 2).

Relative to HC, the DS group exhibited less BOLD responses in the superior temporal gyrus/frontal pole, the middle and inferior frontal gyrus for the chocolate hard trials versus aversive hard trials (Table 1).

Effort Phase (Aversive)

There were no significant group differences for the unpleasant effort conditions.

Consummatory Phase

There were no significant group differences for the consummatory phase.

Table 1.

Whole brain: Effect of Depression Symptoms (DS).

Brain region p<0.001	X	Y	Z	Z score	P value	Cluster size
Chocolate cue HC-DS						
Precuenus	10	-70	48	5.08	<0.001	5873
SFG/MTG	26	6	54	4.28	<0.001	5873
PCC	4	-26	44	4.20	<0.001	5873
ACC/PCC	-6	-12	42	4.13	<0.001	5873
Frontal Pole	-38	48	4	5.03	<0.001	1138
MFG	-26	36	30	4.22	<0.001	1138
Insula	42	8	0	4.39	=0.009	327
Putamen/Caudate	20	18	2	4.23	=0.009	327
LOC	36	-68	8	3.89	=0.027	251
Mould cue HC-DS						
Frontal Pole	-38	48	-2	4.77	<0.001	1112
MFG/IFG	-50	30	32	3.63	<0.001	1112
Precuneus	-4	-74	46	4.29	<0.001	1465
LOC	16	-70	50	4.21	<0.001	1465
PCC/Precuneus	8	-30	42	3.95	<0.001	1465
ACC/PCC	8	-10	40	3.78	<0.001	1465
Paracingulate gyrus/SFG	8	36	36	3.61	<0.001	643
SFG/MFG	26	6	54	4.12	=0.012	318
MFG/Frontal Pole	38	34	40	3.65	=0.01	338
Chochard-choceasy HC-DS						
Insula	32	16	-2	4.22	<0.001	1159
ACC	14	34	20	4.15	<0.001	1159
Putamen	26	8	-4	3.83	<0.001	1159
Precuenus	-10	-52	54	3.80	=0.046	227
Chochard-averhard HC-DS						
STG/Frontal Pole	-14	34	54	4.80	<.001	945
MFG/IFG	-52	20	34	4.71	0.038	249
IFG	60	14	22	3.85	=0.001	536

Thresholded p<0.001. p values <0.05 FWE cluster corrected. Results covaried for age, gender and medication; ACC-anterior cingulate cortex, PCC-Posterior Cingulate Cortex, LOC- lateral Occipital Cortex, STG- Superior Temporal gyrus, MFG-Middle Frontal Gyrus, IFG- Inferior temporal gurus, SFG- Superior Frontal Gyrus, MFG- Medial frontal gyrus,

Table 2.

Whole brain: Effect of Clinical Depression (MDD).

Brain region p<0.001	X	Y	Z	Z score	P value	Cluster size
Chocolate cue HC-MDD						
Putamen	-10	6	0	6.05	<0.001	528
Caudate	12	10	0	4.69	<0.001	528
Ventral striatum	4	4	-2	4.01	<0.001	528
LOC	14	-72	52	5.43	<0.001	5521
PCC/ACC	-6	-14	42	5.27	<0.001	5521
Precunues	-4	-74	44	5.18	<0.001	5521
MTG	-52	-58	-6	3.54		
SFG	4	20	54	4.32	<0.001	484
Frontal Pole	-36	46	18	4.13	<0.001	208
Mould cue HC-DA						
Caudate	-10	6	2	5.08	=.046	182
Putamen	-18	14	2	3.94	=.046	182
Precuneus	4	-62	32	4.64	<0.001	588
PCC/ACC	-6	-16	42	4.41	<0.001	552
SFG	4	20	54	4.23	=.02	226
LOC	-40	-62	26	4.17	.036	194
Chochard-choceasy HC-DA						
Occipital fusiform gyrus/Lingual gurys	-14	-68	-10	4.44	=.037	226
Paracingulate gurus/ACC	12	34	24	4.25	<0.001	604
PCC/Precuenus	-8	-30	42	4.06	=.01	319

Thresholded p<0.001. p values <0.05 FWE cluster corrected. Results covaried for age, gender and medication. ACC-anterior cingulate cortex, PCC-Posterior Cingulate Cortex, LOC- lateral Occipital Cortex, STG- Superior Temporal gyrus, MFG-Middle Frontal Gyrus, IFG- Inferior temporal gurus, SFG- Superior Frontal Gyrus, MFG- Medial frontal gyrus,

5.2.2. Region of interest and whole brain analysis for individuals classified as having depressive symptoms, splitting to two separate groups (increased depressive symptoms and clinical MDD diagnosis).

To compare whether there were any group differences between those who had a clinical MDD diagnosis (MDD group) and those who presented with increased depressive symptoms (high risk: HR), ROI and whole-brain analysis were run.

No significant group differences were found for the ROI analysis (amygdala, ventral striatum, hippocampus, insula) between the MDD and the HR groups for either of the conditions. The whole brain analysis revealed that relative to the HR group, the MDD group presented decreased brain activation in the precuneus (14 -58 52, z=3.94, p=.037) and in the lateral occipital cortex (16 -70 52, z=3.82, p=.038) for the anticipation of rewarding cue. There were no group differences for any other conditions.

5.2.3. Blood-Oxygen-Level-Dependent (BOLD) responses*:* ROI analysis for DS vs. HC

Control conditions:

There were no group differences between the MDD and HC in the amygdala, hippocampus, insula or ventral striatum ROIs in the response to the control cue (grey picture) and taste (rinse) conditions alone (without any control being subtracted).

Anticipation of rewarding and aversive stimuli:

There were no group differences between the MDD and HC in the amygdala, hippocampus, insula or ventral striatum ROIs in the response to the rewarding and aversive cue conditions alone (without any control being subtracted).

Consummation of rewarding and aversive stimuli:

There were no group differences between the MDD and HC in the amygdala, hippocampus, insula or ventral striatum ROIs in the response to the rewarding and aversive taste conditions alone (without any control being subtracted).

5.2.4. Blood-Oxygen-Level-Dependent (BOLD) responses*:* ROI analysis for MDD vs. HC

Control conditions:

There were no group differences between the MDD and HC in the amygdala, hippocampus, insula or ventral striatum ROIs in the response to the control cue and taste (rinse) conditions alone.

Anticipation of rewarding and aversive stimuli:

There were no group differences between the MDD and HC in the amygdala, hippocampus, insula or ventral striatum ROIs in the response to the rewarding and aversive cue condition alone (without any control being subtracted).

Consummation of rewarding and aversive stimuli:

There were no group differences between the MDD and HC in the amygdala, hippocampus, insula or ventral striatum ROIs in the response to the rewarding and aversive taste conditions alone (without any control being subtracted).

5.2.5 Blood-Oxygen-Level-Dependent (BOLD) responses*:* Whole brain analysis DS vs. HC

Control conditions:

Relative to HC group, the DS group presented increased brain activation in the in response to the control cue condition in the precuneus (-2 -76 46; z=4.08, p<.001), in the caudate (16 16 6, z=4.28, p<.001), in the frontal pole/MFG (-42 42 28, z=4.65, p<.001), superior parietal lobule (32 -46 44, z=3.74, p<.001) and in the lateral occipital cortex (42 -76 24, z=4.48, p=.009) (thresholded: 0.001; family-wise error corrected at p=0.05).

There were no statistically significant group differences between DS and HC for the control taste (rinse alone) condition.

Anticipation of rewarding and aversive stimuli:

Relative to HC group, the DS group presented decreased brain activation for the rewarding cue in the frontal pole (30 36 30; z=4.2, p=.021) (thresholded: 0.001; family-wise error corrected at p=0.05).

There were no statistically significant group differences between DS and HC for the aversive cue alone condition.

Consummation of rewarding and aversive stimuli:

There were no statistically significant group differences between DS and HC for the rewarding taste condition (without any control being subtracted).

There were no statistically significant group differences between DS and HC for the aversive taste condition (without any control being subtracted).

5.2.6 Blood-Oxygen-Level-Dependent (BOLD) responses*:* Whole brain analysis MDD vs. HC

Control conditions:

Relative to HC group, the MDD group presented increased brain activation in the in response to the control cue condition in the lateral occipital cortex (14 -72 54; z=5.3, p<.001), in the putamen (-22 12 -8, z=3.65, p=.011), in the superior frontal gyrus (12 32 52, z=4.79, p<.001), PCC/ACC (-8 -16 42, z=4.68, p<.001) and in the frontal pole (46 50 16, z=4.57, p=.016) (thresholded: 0.001; family-wise error corrected at p=0.05).

There were no statistically significant group differences between MDD and HC for the control taste condition.

Anticipation of rewarding and aversive stimuli:

There were no statistically significant group differences between MDD and HC for the rewarding and aversive cue condition.

Consummation of rewarding and aversive stimuli:

Relative to HC, the MDD group presented decreased responses in the insula (40 16 -2, z=4.76, p=.001) for the rewarding taste (thresholded: 0.001; family-wise error corrected at p=0.05).

There were no statistically significant group differences between MDD and HC for the aversive taste condition.

5.3. Addendum to Figures from Paper 2.

5.3.1. Addendum to Figure 5 from Paper 2:

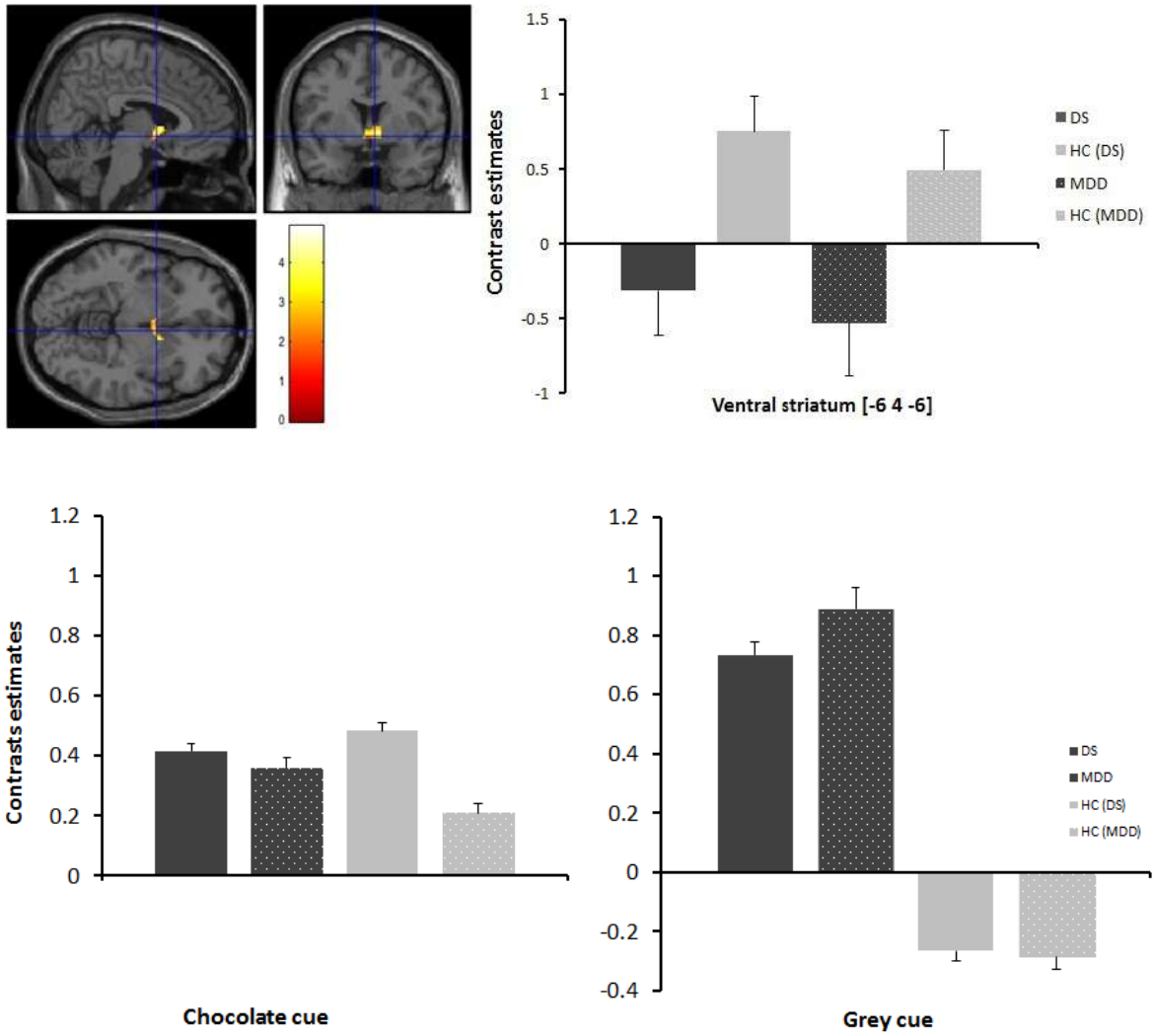

Figure 5. Anticipation: Rewarding cue, *left top panel,* axial, sagittal and coronal image of ventral

striatum [6 4 -6] ROI activation: HC vs. DS (z=3.19, p=0.035); HC vs. MDD (z=4.45, p=0.001); *right top panel,* contrast estimates for ventral striatum for HC and DS and HC and MDD groups;); *left bottom panel*, contrast estimates for all groups for ventral striatum ROI centered at (-6 4 -6) for chocolate cue only; *right bottom panel*, contrasts estimates for all groups for ventral striatum ROI centered (-6 4 -6) for grey cue only.

5.3.2. Addendum to Figure 6 from Paper 2:

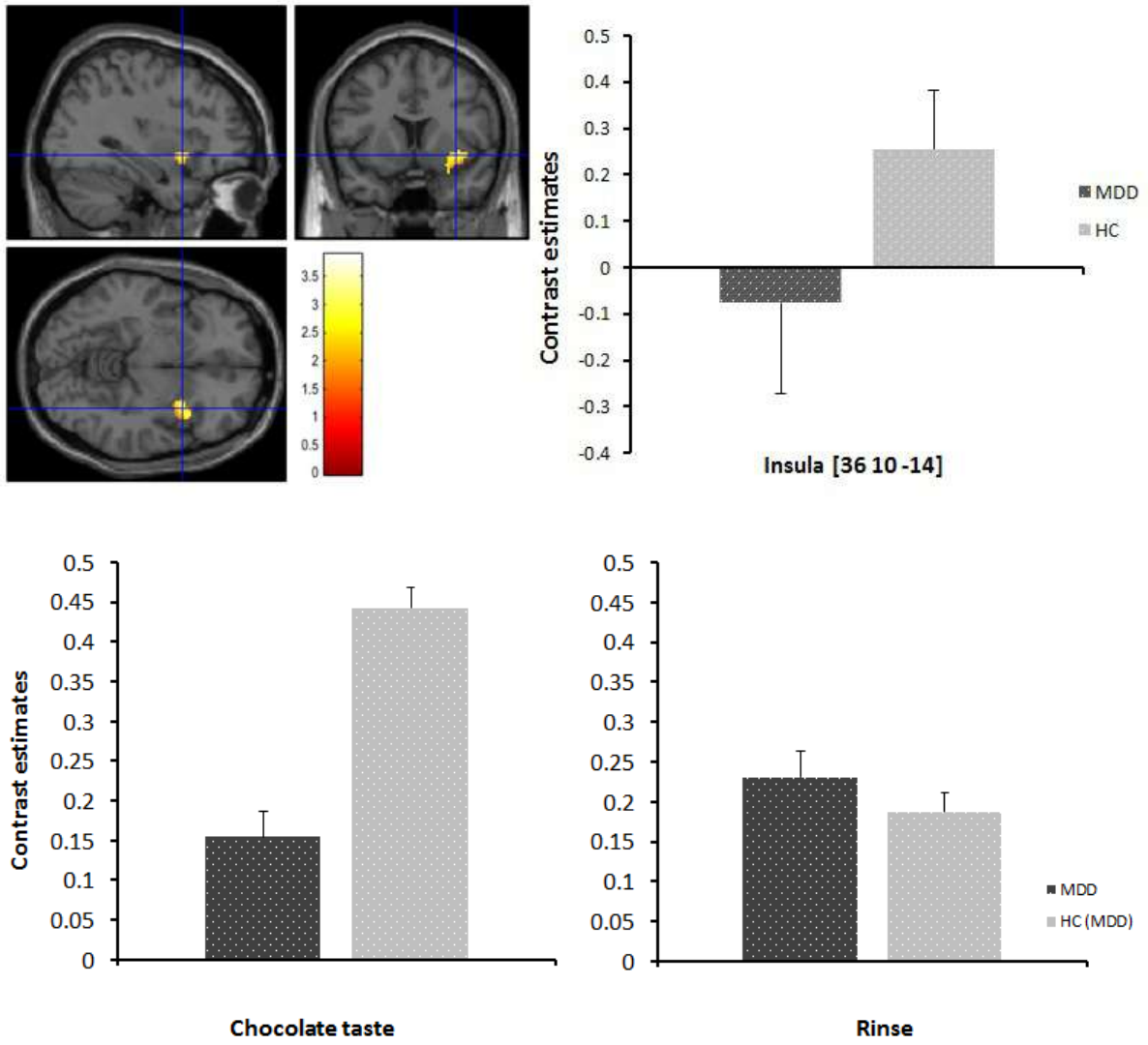

Figure 6. Consummation: Rewarding taste, *left top panel,* axial, sagittal and coronal image of insula ROI [36 10 -14] activation: HC vs. MDD (z=3.53, p=0.015); *right top panel,* contrast estimates for insula for HC and MDD groups; *left bottom panel*, contrast estimates for MDD and HC(MDD) groups for insula ROI centered at (36 10 -14) for chocolate taste only; *right bottom panel*, contrasts estimates for MDD and MC (MDD) groups for insula ROI centered (36 10 -14) for rinse only.

5.4. Comparison of the number of positive outcomes for the taste stimuli:

We found decreased brain responses for each of the task conditions in our samples with depressive symptoms. As receiving rewarding or aversive taste was dependent on the performance on the effort trials, there was a possibility that the more anhedonic subjects would have a smaller number of positive outcomes thus smaller number of responses to the taste conditions of interests. To check for this possibility, a comparison between the number of positive outcomes between the DS and the HC, the MDD and the HC, and the HR and the LR groups was run. There were non-significant results between the DS vs HC for obtaining the chocolate taste: DS (M=9.67, SD=1.97), HC (M=10.37, SD=1.43), t(82)=-1.83, p=.07 and for obtaining the aversive taste: DS (M=9.07, SD=2.04), HC (M=9.027, SD=1.4), t(82)=.-418, p=.606. There was a significant difference between the MDD and the HC groups for obtaining the chocolate taste: MDD (M=9.4, SD= 2.24), HC (M=10.55, SD=1.48) t(56)=-2.24, p=.029 and a non-significant difference for obtaining the aversive taste: MDD (M=8.67, SD=2.42), HC (M=9.13, SD=.99) t(56)=-.98, p=.33. There was non-significant difference between HR and LR groups for obtaining the chocolate taste: HR (M=10.06, SD=1.39), LR (M=10.06, SD=1.0) t(32)=.017, p=.99 and non-significant difference for obtaining the aversive taste: HR (M=9.75, SD=.86), LR (M=9.61, SD=1.72) t(32)=.29, p=.77. The results suggest that the blunted brain responses for the clinical and subclinical subjects in response to taste conditions of interest were not influenced by the number of positive responses on the effort trials. Even though, the MDD subjects had significantly lower number of positive chocolate taste outcomes when compared with HC, it is very unlikely that the decreased brain responses to the rewarding taste were caused by it given that the MDD subjects also had decreased responses to passive viewing of the rewarding cue as presented in the figure 5 for the 5.3. Addendum as well as during the effort outcomes.

5.5. Discussion:

Effects of depression symptoms on the whole brain analysis also revealed decreased brain activity for the anticipation of and the effort for rewarding and aversive stimuli. Decreased activation to the anticipation of reward and aversion in the DS vs. HC groups was additionally found in the precuneus, PCC, ACC and in the limbic structures of the putamen and the caudate for reward anticipation and effort to obtain reward only. Relative to the HC group, the MDD group showed additional activation in in the ventral striatum for the anticipation of reward and in the dorsal striatum for the anticipation of reward and aversion. Those brain regions have been implicated previously in the neurobiology of depression and have been found dysfunctional in tasks looking at emotion processing, motivation and reward (Kerestes et al., 2014; Smoski et al., 2011). Specifically, dorsal striatum (caudate, putamen) has been implicated in decision-making and in the processing of anticipatory and consummatory aspects of reward (Gabbay et al., 2013; Yang et al., 2016). Moreover, caudate has been involved in the processing of emotion of disgust (Fitzgerald et al., 2004; Shapira et al., 2003), and this is of interest given the results of decreased responses in the caudate for the anticipation of aversive cue in the MDD group when compared with HC, which was not found for the DS vs. HC groups. Furthermore, decreased activation in the putamen is also reported for the anticipation of aversive cue in the MDD vs. HC groups. This might suggest that individuals with MDD have deficits in the dorsal striatum for anticipation of both reward and aversion which might be related to the severity of depression. Interestingly, there was a significant negative correlation between the decreased responses in the putamen and increasing anhedonia as measured by SHAPS (r=-.434, p=.024) in the MDD group, which was non-significant in any other groups.

We did not find any whole brain group differences for the consummation of reward or aversion in either of the groups, which is interesting as many studies showed involvement of the cortical and subcortical structures in the consummatory aspects of reward. However, a conservative threshold (.001) for data reporting was adopted which

could have influenced the final results and possibly lead to type II error. If this is the case, then possibly a larger sample size could increase the sensitivity of the statistical tests.

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Chapter 6:

Paper 3: Decreased anticipated pleasure correlates with increased salience network resting state functional connectivity in adolescents with depressive symptomatology

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Title:

Decreased anticipated pleasure correlates with increased salience network resting state functional connectivity in adolescents with depressive symptomatology.

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Key words: fMRI, depression, biomarker, resting-state, connectivity, salience network, adolescent, DMN.

Abstract

Previous studies have found dysfunctional resting state functional connectivity (RSFC) in depressed patients. Examining RSFC might aid biomarker discovery for depression. However RSFC in young people at risk of depression has yet to be examined. 35 healthy adolescents (13–18 yrs old.) were recruited. 17 scoring high on the Mood and Feelings Questionnaire (MFQ > 27 (High Risk: HR), and 18 scoring low on the MFQ < 15 (Low Risk: LR) matched on age and gender. We selected seed regions in the salience network (SN: amygdala and pregenual anterior cingulate cortex (pgACC)) and the central executive network (CEN: dorsal medial prefrontal cortex (dmPFC)). Mood and anhedonia measures were correlated with brain connectivity. We found decreased RSFC in the HR group between the amygdala and the pgACC and hippocampus and precuneus. We also found decreased RSFC in the HR group between the pgACC and the putamen and between the dmPFC and the precuneus. The pgACC RSFC with the insula/orbitofrontal cortex correlated inversely with the anticipation of pleasure in all subjects. Increased RSFC was observed between the pgACC and the prefrontal cortex and the amygdala and the temporal pole in the HR group compared to the LR group. Our findings are the first to show that adolescents with depression symptoms have dysfunctional RSFC between seeds in the SN and CEN with nodes in the Default Mode Network. As increased connectivity between the pgACC and the insula correlated with decreased ability to anticipate pleasure, we suggest this might be mechanism underlying the risk of experiencing anhedonia, a suggested biomarker for depression.

Keywords: fMRI, Depression, Biomarker, Resting-state, Connectivity, Salience network, Adolescent, DMN

Introduction

Adolescence is a crucial developmental period where the incidence of depression increases significantly, and reports have emphasised that around 8% of adolescents are affected by depression by the age of 16 (Saluja et al., 2004). Identifying biomarkers such as dysfunctional neural networks could help develop preventative treatments for young people at increased risk of clinical depression.

It is been shown that patients with major depressive disorder (MDD) have abnormalities in their resting state functional connectivity (RSFC) in networks such as the Salience Network (SN) the Central Executive Network (CEN) and the Default Mode Network (DMN) (Sheline et al., 2010).

The Salience Network (SN) and the central executive network (CEN) show strong task-related activation and are less active at rest in healthy controls. The SN which consists of regions such as the anterior insula, pregenual anterior cingulate and amygdala are implicated in the processing of various aspects of salient stimuli whereas the CEN, which consists of regions such as the dorsolateral, dorsal medial prefrontal cortex and the posterior parietal cortex, is involved in cognitive functioning including attention and working memory (Bressler and Menon, 2010). In MDD, abnormalities in these networks are thought to reflect deficits in attentional control over emotional stimuli, difficulties with suppression of unwanted thoughts and difficulties with emotion recognition. However there have been inconsistencies in the direction of effects with some studies finding increased SN and CEN (Horn et al., 2010, Manoliu et al., 2014, Ramasubbu et al., 2014, Sheline et al., 2010), whilst others find reduced connectivity (Liston et al., 2014, Tahmasian et al., 2013, Ye et al., 2012) in these regions in MDD. These inconsistencies might be related to differences in the MDD population studied (adults, elderly, adolescents), medication history and depression severity.

There is small number of RSFC studies in adolescents with MDD. These studies have mostly focused on investigating the SN and they report decreased RSFC between the amygdala and the hippocampus, parahippocampus and brain stem which has also been shown to correlate with depression severity (Cullen et al., 2014). The same study however, also showed increased RSFC between the amygdala and the precuneus in depressed adolescents. Mixed results were also reported by Pannekoek et al. who found both increased RSFC between the amygdala and the parietal cortex in MDD adolescents and decreased RSFC between the amygdala and regions such as the pgACC, frontal pole and the paracingulate gyrus (Pannekoek et al., 2014).

The authors suggested that risk for depression may be related to dysfunction in brain areas involved in supporting self-relational processes and reward prediction. Another study examining familial risk for depression in adolescents found decreased RSFC between the prefrontal cortex and parts of the CEN, in those with a parent with depression which also correlated with the parents depression severity (Clasen et al., 2014). The authors suggested that an increase in vulnerability to depression may thus be underpinned by altered development of the CEN in young people at risk.

Our current study aims to examine RSFC in another at risk group, adolescents with increased depression symptomatology but with no clinical diagnoses. Based on the previous literature, we selected seed regions that have been shown dysfunctional in depressed patients and adolescents at familial risk of depression, amygdala and pgACC seeds from the SN and the dmPFC highlighted as a key node of within the CEN and dysfunctional RSFC in depressed patients (Sheline et al., 2010). We hypothesised that adolescents at risk of depression would also have decreased RSFC between key brain regions implicated in the aetiology of depression, supporting the notion that dysfunctional resting state neural networks may be biomarkers for depression.

Materials and methods

Participants

35 healthy adolescents (13–18 yrs old.) were recruited. 17 healthy adolescents scoring high on the Mood and Feelings Questionnaire (Costello et al., 1998) (MFQ > 27 (HR), and 18 adolescents scoring low on the MFQ < 15 (LR) matched on age and gender. Participants completed the MFQ; a self-report questionnaire designed to assess recent (last 2 weeks) presence and severity of depressive symptoms as specified in DSM-IV. The measure is composed of 33 statements corresponding to how often the individual has experienced particular behaviours and feelings during this time. There is considerable psychometric data for this child version, including good test–retest reliability for a score of 27 and above indicating increased depression symptom severity (Pearson's r ¼ 0.78) (Wood et al., 1995) and below 15 indicating healthy controls (Kyte et al., 2005).

Participants who scored between 15 and 27 were excluded from the study. The University of Reading Ethics Committee provided ethical approval and the investigation was carried out in accordance with the latest version of the Declaration of Helsinki. We obtained written informed consent from all participants before screening and after giving the complete description of the study. Exclusion criteria for all subjects consisted of current or past history of alcohol or drug dependency, pregnancy and any contradictions to MRI, e.g. pacemaker, mechanical heart valve, hip replacement, metal implants. Further, both of the groups were determined to be free from current or past axis 1 disorder (including anxiety disorders, depression, eating disorders psychosis and substance abuse) on the structured clinical interview for DMS-IV (Spitzer et al., 2004). None of the participant took current medication apart from the contraceptive pill. All subjects were rated on the following questionnaires: Mood and Feeling Questionnaire (MFQ; (Angold et al., 1995)), Beck Depression Inventory (BDI; (Beck et al., 1961)), the Fawcett–Clarke Pleasure Scale (FCPS; (Fawcett et al., 1983)), and the Snaith–Hamilton Pleasure Scale

(SHAPS; (Snaith et al., 1995)), the Temporal Experience of Pleasure Scale, consummatory subscale TEPS-C and anticipatory subscale TEPS-A (Gard et al., 2006), before scanning. Body mass index (BMI) for each individual was also calculated.

Overall design

MRI derived measures of brain function, based on blood-oxygenation-level-dependent (BOLD) contrast were used to compare brain responses at rest across the LR group and the HR group. The resting-state data were acquired before any other scans including the structural scan. Subjects were instructed to lie in dimmed light with their eyes open, think of nothing in particular, and not to fall asleep, similar to our previous studies (Cowdrey et al., 2012, McCabe and Mishor, 2011, McCabe et al., 2010, Rzepa et al., 2015) and a method found to have higher reliability than eyes closed (Patriat et al., 2013). To measure whether undergoing a scan had an effect on mood change, volunteers completed the Befindlischkeit scale on mood (BFS; (Hobi, 1985)) and an energy and affect Visual Analogue Scale (VAS) pre and post scan.

Image acquisition

A Siemens Magnetom Trio 3T whole body MRI scanner and a thirty-two-channel head coil were used. Multi-band accelerated echo planar imaging sequencing (Center for Magnetic Resonance Research, Minnesota) was used with an acceleration factor of 6 and iPAT acceleration factor of 2. T2*-weighted EPI slices were obtained every 0.7 s (TR = 0.7, TE = 0.03), these parameters were optimised given our scanner capability and used to increase sampling rates and increase our power to detect resting state networks as has been shown previously with multiband (Xu et al., 2013, Filippini et al., 2014). 54 transverse slices with in-plane resolution of 2.4 × 2.4 mm were attained and slice thickness was 2.4 mm. The matrix size was 96 × 96 and the field of view (FOV) were 230 × 230 mm. Acquisition was performed during resting-state scan, yielding 400 vol in total.

Sagittal 3D MPRAGE images were also acquired with an isotropic in-plane resolution of 1 × 1 × 1 (TI = 0.9 s, TR = 2.02, flip angle 9°, FOV = 250 × 250 mm) yielding 192 slices.

fMRI data analysis

Pre-processing

Imaging data were pre-processed and analyzed using FSL tools (www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). fMRI data pre-processing was carried out using FEAT (FMRI Expert Analysis Tool, Version 6.0, a part of FSL software), and included the following steps: non-brain removal (Smith, 2002), motion correction using MCFLIRT(Jenkinson and Smith, 2001), spatial smoothing using a Gaussian kernel of full-width at half maximum (FWHM) of 5 mm, grand mean intensity normalization of the entire 4D dataset by a single multiplicative factor and high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 64.0s). fMRI volumes were registered to the individual's structural scan and the MNI-152 standard space image (Montreal Neurological Institute, Montreal, QC, Canada) using FMRIB's Linear Image Registration Tool (FLIRT).

Time series extraction and higher level analysis

To study resting-state functional connectivity, a seed-based correlation approach was used. Using the Harvard-Oxford subcortical structural atlas (Kennedy et al., 1998) we created a structural bilateral amygdala seed as the amygdala is a small structure and not suitable for a ROI sphere. To maximize the exact coverage, the masks of these seed regions were threshold by 20% to include voxels having at least 80% of probability of being in these particular regions. We also created seeds for the dmPFC (18 34 29; −24 35 28) (6 mm sphere so as to not cross into other brain regions) coordinates from (Sheline et al., 2010) and pgACC (8 mm sphere with a center at 0 38 0 so as to not cross into other brain regions). The dmPFC and pgACC seeds were created with Wake Forest University

Pickatlas tool in SPM8 as in our previous study and can be seen in the figures (McCabe et al., 2011).

The mean time course within the left and right seeds of each ROI (except for the pgACC, only comprising one medial seed) was calculated and used as a regressor in a general linear model. In addition, white matter signal, cerebrospinal fluid signal, 6 motion parameters (3 translations and 3 rotations), and the global signal were used as nuisance regressors. We have obtained white matter and cerebrospinal fluid masks using FSL's FAST segmentation program. The resulting segmented images were then thresholded to ensure 80% tissue type probability. For each individual, the general linear model was analyzed by using the FMRI Expert Analysis Tool [version 5.4, part of FMRIB's Software Library (Smith et al., 2004)]. The resulting parameter estimate maps were then analyzed using higher level 1 sample t-tests for group averages and between samples t-tests for group differences. Clusters were determined by $Z > 2.3$ voxel-wise thresholding and a family-wise error-corrected cluster significance threshold of $P < 0.05$ (Worsley, 2001). From the results we then only report those that met the further correction for number of ROIs examined which gave $P < 0.016$ (i.e, $P < 0.05$ Bonferroni corrected for the 3 networks of interest: amygdala, dmPFC, pgACC (Davidson et al., 2003). Next, the % BOLD signal change data was extracted from the regions of significant effect (Table 2) using the FSL tool Featquery (www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004).

Correlational analyses

To examine the relationship between the scores on behavioural questionnaires and RSFC we extracted the % BOLD signal change using FSL Featquery and correlated with scores on our questionnaires.

Results

Demographic and clinical data

Demographic data analysis (Table 1) revealed no significant age and gender differences between HR and the LR groups. Results for BMI scores were calculated and there was a significant BMI difference between the HR and the LR group. There were also significant differences between the two groups on measures of mood, depression and anhedonia (Table 1) (MFQ, BDI, SHAPS, FCPS, TEPS-A, TEPS-C) (Table 1).

Table 1: Demographic and clinical characteristics

Measure	HR (*n*=17) Mean (SD)	LR (*n*=18) Mean (SD)	*p*-value
Age (years)	16.59 (1.18)	16.33 (1.6)	.598
Gender (male)	4/13	6/12	.535
BMI	21.82 (2.72)	20.1 (1.94)	.041
MFQ	40.41 (6.1)	4.4 (5.1)	<.001
BDI	29.82 (12.7)	2.28 (4.13)	<.001
FCPS	121.12 (18.7)	137.89 (21.3)	.019
SHAPS	30.11 (5.56)	20.77 (8)	<.001
TEPS -A	37.29 (7.58)	50.66 (5.14)	<.001
TEPS-C	31.76 (5.98)	36.66 (7.28)	<.001

BMI:Body Mass Index; MFQ- Mood and Feeling Questionnaire; BDI: Beck Depression Inventory, FCPS: Fawcet Clarke Pleasure Scale, SHAPS: Snaith-Hamilton Pleasure Scale, TEPS: Temporal Experience of Pleasure Scale A: Anticipation, C: Consummation.

Mood, energy and affect scores

Repeated measures ANOVA with within subject factor of time (before and after scan) and between subject factor of group (HR and LR) was employed to examine whether there would be any differences on scores of mood as measured by the BFS. Results for BFS revealed that there was no significant main effect of time ($F(1.33) = 0.011$; $p = 0.919$) but there was a significant main effect of group ($F(1.33) = 11.33$; $p = 0.002$) and significant interaction between time and group ($F(1.33) = 5.56$; $p = 0.025$). Further paired sample t-test analysis revealed that there was a significant difference for time in the LR group ($t(17) = -3.08$; $p = 0.007$) meaning they felt worse after the scan

and non-significant difference for time in the at HR group ($t(16) = 1.29$; $p = 0.216$) (Table S1).

Repeated measures ANOVA with within subject factor of time on two levels (before and after scan) and within subject factor of Emotion on nine levels (alertness, disgust, drowsiness, sadness, happiness, anxiety, withdrawn, faint, nausea) and between subject factor of group (healthy controls and at risk) was employed to examine whether there will be any differences between the LR and HR groups scores of energy and affect, as measured by VAS. Results for VAS revealed that there was no significant main effect of time ($F(1.33) = 0.385$; $p = 0.539$) and no significant main effect of group ($F(1.33) = 3.37$; $p = 0.075$) but there was a significant main effect of Emotion ($F(8.264) = 57.78$; $p < 0.001$) yet no significant interaction between the time, emotion and group ($F(8.264) = 1.28$; $p = 0.252$). Further paired sample t-test analysis revealed that there was a significant difference for time in the LR group for disgust ($t(17) = -2.949$, $p = 0.009$) feeling faint ($t(17) = -2.164$, $p = 0.045$) and in the HR group for drowsiness ($t(16) = 2.57$; $p = 0.02$) and anxiety ($t(16) = 2.14$; $p = 0.049$) all increasing after the scan except anxiety (Table S1).

Main effects of stimuli on blood oxygen level-dependent responses

Table S2 provides a summary of the main effects, i.e. the brain regions that had RSFC with the seed regions (baseline) for the LR group only. Overall, the patterns of connectivity associated with each of the seed regions are consistent with resting-state and functional connectivity experiments in healthy controls, subjects at risk for depression and depressed patients (Bebko et al., 2015, Cullen et al., 2014, Guo et al., 2015, Sheline et al., 2009, Sheline et al., 2010, Shen et al., 2015, Clasen et al., 2014)

Effects of mood on RSFC

There was no main effect of age, gender, BMI or MFQ on RSFC.

Decreased functional connectivity in the HR group

Left amygdala seed

There was decreased RSFC in the HR group compared to the LR group between the left amygdala seed and the pgACC and the precuneus (Fig. 1) and posterior cingulate cortex (PCC) (Table 2).

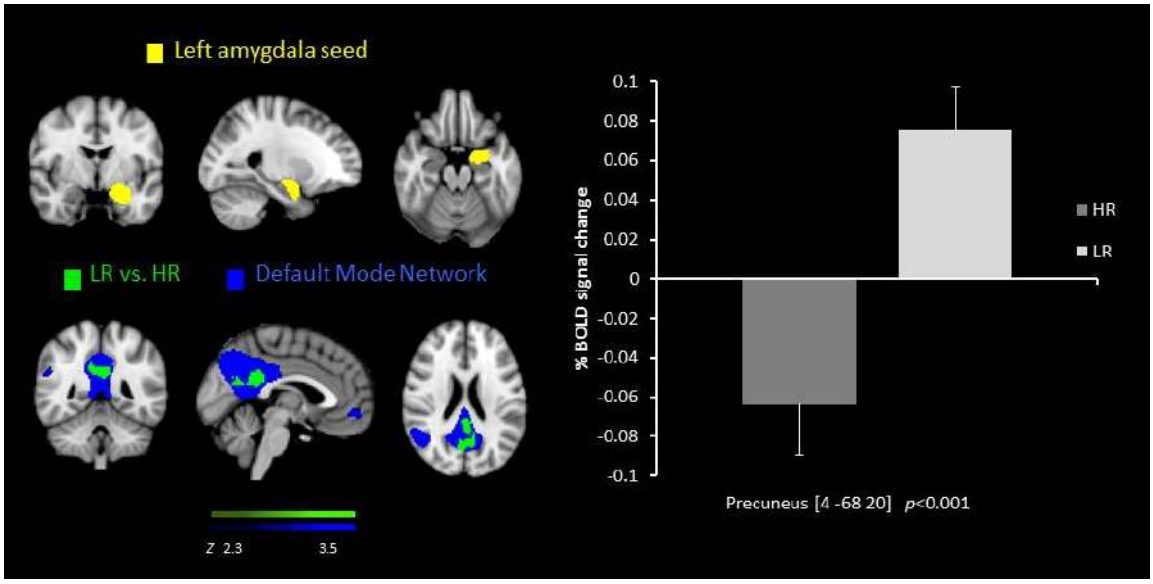

Fig 1: Resting state functional connectivity between the left amygdala seed region and the precuneus, lower in the HR group than the LR overlaid on the DMN . % BOLD signal change extracted for connectivity for both of the groups.

pgACC seed

There was decreased RSFC in the HR group compared to the LR group between the pgACC seed and the thalamus, palladium and the putamen (Table 2).

Right amygdala seed

There was decreased RSFC in the HR group compared to the LR group between the right amygdala seed and the hippocampus (Table 2).

Right dmPFC seed

There was decreased RSFC in the HR group compared to the LR group between the right dmPFC seed and the precuneus/cuneal cortex and the lateral occipital cortex (Table 2, Fig. 2).

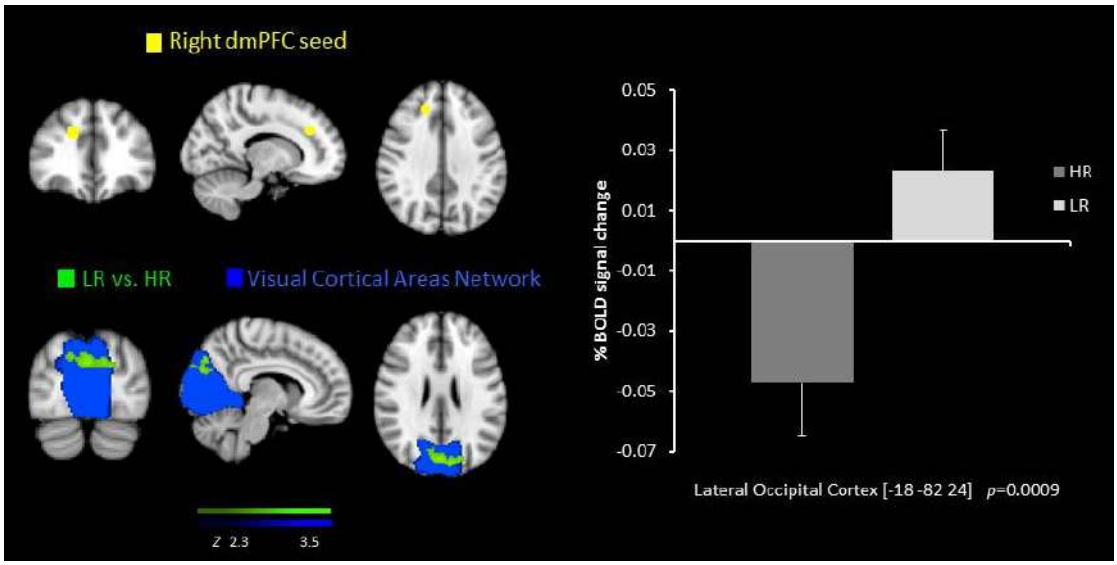

Fig 2: Resting state functional connectivity between the right dmPFC seed region and the lateral occipital cortex, lower in the HR group than the LR overlaid on the Visual Cortical Network . % BOLD signal change extracted for both of the groups.

Correlational analysis with behaviour

There was a negative correlation between increased RSFC of the pgACC seed and the insula/OFC and decreased anticipation of pleasure (TEPS-A) in both the HR ($r = -0.65$, $p = 0.004$) and LR groups ($r = -0.48$, $p = 0.04$) (Fig. 3).

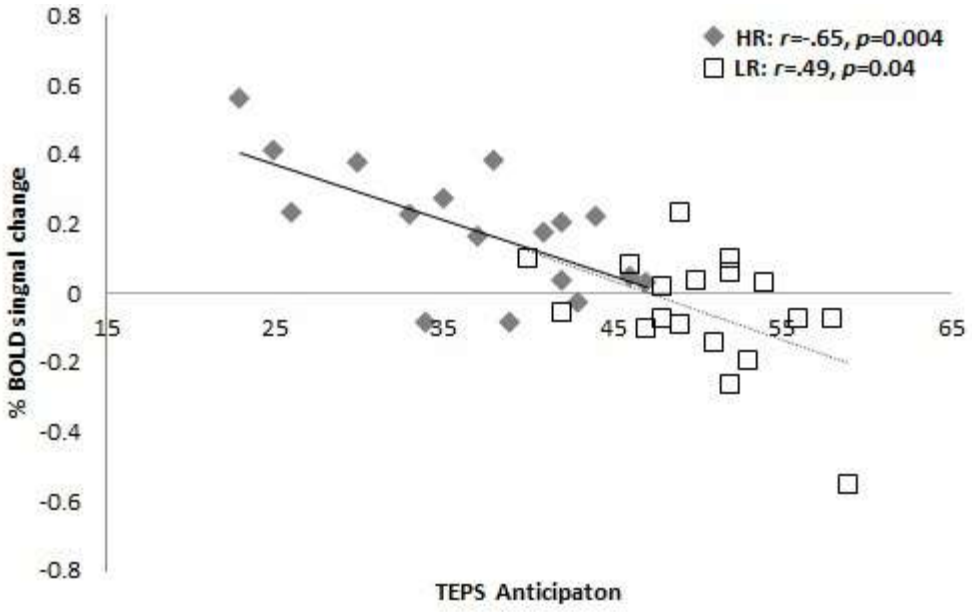

Fig 3: Increased resting state functional connectivity between the pgACC seed region and the insula/LOFC correlates with reduced anticipation of pleasure.

Increased functional connectivity in the HR group

Left amygdala seed

There was increased RSFC in the HR group compared to the LR group between the left amygdala seed and the temporal pole (Table 2).

pgACC seed

There was increased RSFC in the HR group compared to the LR group between the pgACC seed and the brain stem, the anterior cingulate cortex, the ventral medial prefrontal cortex and the OFC/insula (Table 2, Fig. 4).

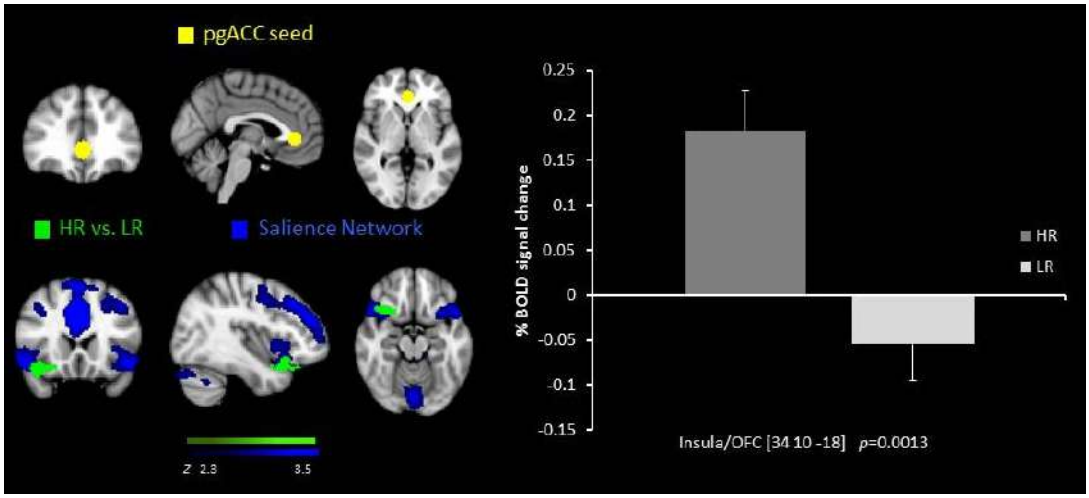

Fig 4: Resting state functional connectivity between the pgACC seed region and the insula/LOFC, higher in the HR group than the LR overlaid on the Salience Network . % BOLD signal change extracted for connectivity for both of the groups.

Table 2: RSFC between seed regions and whole brain compared between groups.

Brain region	MNI coordinates			z-score	Cluster size	p-value
	X	Y	Z			
LR> HR						
L Amygdala seed						
pgACC	2	36	-2	6.13	4447	<.0.001
Precuneus	4	-68	20	3.5	476	<0.001
PCC	0	-40	24	3.21	476	<0.001
pgACC seed						
Thalamus	0	-4	2	6.28	1150	<0.001
Pallidum	16	-4	-6	4.17	1150	<0.001*
Pallidum/Putamen	-16	6	-2	3.11	1150	<0.001
R amygdala seed						
Hippocampus	-14	-10	-18	4.05	357	0.00069
R dmPFC seed						
Cuneal Cortex/Precuneus	8	-76	36	3.15	401	0.0009
Lateral Occipital Cortex	-18	-82	24	3.1	401	0.0009
HR>LR						
L Amygdala seed						
Temporal pole	-46	4	-18	3.2	1473	<0.001
pgACC seed						
Brain stem	8	-30	-30	4.69	1156	<0.001
ACC	6	32	26	3.85	2552	<0.001
vmPFC	-6	50	-4	3.79	2552	<0.001
Lateral OFC	40	22	-16	3.57	369	0.0013
Insula/OFC	34	10	-18	3.27	369	0.0013

All *p*-values for clusters were firstly determined by Z > 2.3 voxel-wise thresholding and a family-wise error-corrected cluster significance threshold of $P < 0.05$, then further Bonferroni corrected for number of ROIs examined which gave $P < 0.016$ (i.e, $P < 0.05$ (Davidson et al., 2003)). OFC-orbitofrontal cortex, pgACC- pregenual anterior cingulate cortex, ACC-anterior cingulate cortex. All data except * remained significant even when the global signal was not used as a nuisance regressor.

Discussion

The main aim of our study was to investigate RSFC in young people at risk of developing major depressive disorder by virtue of having increased depression symptomatology. We hypothesised deceased RSFC in key regions that have been found decreased in adults with depression such as regions in the SN and CEN.

Specifically we found decreased RSFC in the HR group compared to the LR group between the amygdala seed and the pgACC. The pgACC is claimed to be a node of communicating between the dorsal ACC important for error detection and attention and the more ventral ACC implicated in emotion processing, regulation and salience detection (Ball et al., 2014). Further, studies in depressed patients, remitted depressed patients and young people with depression symptoms find dysfunctional ACC activity during tasks involved in processing emotions and rewards (Ho et al., 2014, Fitzgerald et al., 2008, McCabe et al., 2009; Rzepa et al., in press), and recently in a RSFC study in depressed adolescents (Pannekoek et al., 2014). Thus it has been suggested that reduced RSFC between the amygdala and the pgACC may reflect problems in integrating inputs of positive information and influencing affect regulation, resulting in decreased hedonic responses and increased feelings of negativity. We found decreased RSFC between the pgACC seed and the thalamus, palladium and the putamen. These are areas of the brain involved in reward and emotion processing and also found dysfunctional in depression (Alexander et al., 1990, Phillips et al., 2003). As our results are in line with the decreased pgACC to amygdala, pallidum and thalamus RSFC found in depressed patients (Anand et al., 2005) it suggests that dysfunctional cortico-limbic connectivity in young people at risk of depression might be a biomarker underpinning problems with the control and regulation of emotional processes.

We also found decreased RSFC in the HR group compared to the LR group between the amygdala seed region and the PCC and the precuneus which have been

implicated in self-referential processing in fMRI tasks and thought to underlie maladaptive rumination in depression (Nejad et al., 2013). Further these regions are key nodes of the default mode Network (DMN). The DMN is a network of distributed brain regions that show prominent activation during rest, and deactivation during the performance of cognitive tasks (Whitfield-Gabrieli and Ford, 2012). Studies have revealed that in healthy subjects, the DMN is associated with rumination and self-reflection and that greater suppression of DMN is related to a better performance on attention demanding tasks. Whilst some studies find the DMN overactive in MDD patients when compared with healthy controls, which has been suggested to underlie the symptom of negative rumination in depression (Whitfield-Gabrieli and Ford, 2012) others find decreased connectivity between the DMN and the ventral striatum and the sensorimotor cortex which also correlate negatively with behavioural inhibition in young people at increased familial risk of depression (Frost Bellgowan et al., 2015). However, reports of decreased amygdala RSFC with the precuneus in children at familial risk for depression and in depressed adolescents have been shown (Luking et al., 2011). Our results are in line with Luking at al. and suggest, that if adolescents with increased risk of depression have difficulty in emotion regulation this may be due to decreased amygdala-precuneus connectivity. To further explore this, future studies should examine the relationship between emotion processing and RSFC in young people at risk of depression.

We also found decreased RSFC in the HR group compared to the LR group between the amygdala seed and the hippocampus, similar to that found previously in MDD adolescents and in children with a family history of depression (Cullen et al., 2014, Luking et al., 2011). The amygdala possesses strong connections with the hippocampus which plays a crucial role in encoding and retrieval of emotional stimuli (Smith et al., 2006). Thus it could be suggested that decreased connectivity between the amygdala and the hippocampus might lead to less ability to suppress negative memories which in turn could be a risk factor for developing depression.

Our results also revealed decreased RSFC between the dmPFC seed and the precuneus part of the DMN and the lateral occipital cortex part of the visual network. The dmPFC is a key node in the CEN and is a structure implicated in many cognitive and emotional processes (Janssen et al., 2015, Jaworska et al., 2014). Similarly, recent studies have shown that RSFC between the CEN and the DMN is decreased in MDD (Abbott et al., 2013, Manoliu et al., 2013, Sheline et al., 2010) which has also been associated with patients' difficulties to disengage from self-referential processes that may lead to negative thoughts (Manoliu et al., 2013). Thus our results are an extension of this, as we also see a similar pattern in young people at high risk but not currently diagnosed. Further, the decreased connectivity between the dmPFC and the occipital cortex in the HR group, found in our study, may indicate a mechanism by which compromised control over emotional images in adolescents could increase the risk of depression.

Interestingly, we also found increased RSFC between the pgACC seed and the insula, brain stem and frontal regions such as the ACC, vmPFC, lateral OFC and the amygdala seed and the temporal pole. As described above the pgACC is a key node for emotion processing, regulation and salience detection (Ball et al., 2014) and a previous study by Horn et al. also found altered pgACC RSFC with the anterior insula which was also related to depression severity and glutamate levels (Horn et al., 2010). The insula is thought to be an integration center for emotion, visceromotor, autonomic and interoceptive information and is also key node in switching between the CEN and the DMN during task performance (Guo et al., 2015, Sridharan et al., 2008). Given that we found that increased connectivity between the pgACC and the insula correlated with decreased ability to anticipate pleasure, we suggest this might be mechanism underlying the experience of anhedonia and therefore a possible further biomarker for depression in adolescents.

Increased RSFC between the amygdala and the temporal pole in the HR group may also be important given that the temporal pole has been implicated in studies

examining Theory of Mind (the ability to predict other people's behaviour by attributing mental states such as believes and desires). For example a previous study in MDD patients reported increased amygdala to Temporal pole RSFC which also correlated inversely with depression severity (Ramasubbu et al., 2014). Interestingly, the authors argued that because the connectivity between these regions increased as depression severity decreased this may represent a compensatory mechanism by which subjects are more likely to maintain a balance in processing socially and emotionally relevant information. Intriguingly finding a similar pattern in our sample of young people at risk might also be related to resilience and protection against future depression development. Thus future studies would benefit from larger sample sizes and longitudinal designs to address this directly. Furthermore, our analysis examined the entire amygdala, but prior work has shown that amygdala subregions have known dissociable functional networks (Roy et al., 2009). Therefore future research should investigate how RSFC patterns in adolescents at risk of depression vary across amygdala subregions; interestingly such research would also benefit from the implementation of multiband sequencing that we have used in this study (Ugurbil et al., 2013).

Taken together we have shown that even in young people who are not currently depressed but who are at risk, due to depression symptomatology, there are decreased RSFC between key regions involved in the processing of salient stimuli and decision making. Further increased connectivity between amygdala and the temporal pole may also be an indicator of resilience to clinical depression in the future, we believe this is certainly worth investigating further.

Conflict of interest

The authors have no conflict of interest.

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Chapter 7:

Paper 4: Increasing Depression and Anhedonia Severity Correlates with Decreasing Resting-State Functional Connectivity in Dorso-Medial Prefrontal Cortex in Adolescents: An RDOC Dimensional Approach

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Title:

Increasing Depression and Anhedonia Severity Correlates with Decreasing Resting-State Functional Connectivity in Dorso-Medial Prefrontal Cortex in Adolescents: An RDOC Dimensional Approach

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Key words: fMRI, depression, biomarker, resting-state, connectivity, salience network, adolescent, DMN.

Abstract

Recent studies have emphasized that a dimensional approach to studying the symptoms of depression may be beneficial given the heterogeneous nature of depression. Moreover, previous examinations have indicated that resting-state functional connectivity (RSFC) might aid biomarker discovery for depression. Thus the aim of this study was to investigate RSFC in young people across a range of depression symptom severities.

86 participants were recruited for the study. 44 participants presented with a range of depression symptoms (DS), including 27 participants with a current diagnosis of major depressive disorder (MDD) and 42 individuals were age and gender matched healthy controls (HC). We measured mood and anhedonia in all subjects and correlated this with results from the RSFC investigation in key regions of interest (ROI) implicated in the aetiology of depression; amygdala, nucleus accumbens, pregenual anterior cingulate cortex (pgACC)- parts of the Salience Network and the dorsal medial prefrontal cortex (dmPFC)- part of the Central Executive Network.

Analysis revealed that RSFC between the dmPFC seed and the precuneus negatively correlated with scores of depression severity and positively correlated with the anticipation of pleasure in all participants. Further analysis revealed that these effects were driven by those with depression symptoms. We also found a positive correlation between dmPFC seed and the frontal pole and depression severity in all participants. We also found a negative correlation between the dmPFC seed and the ACC/paragingulate RSFC and the anticipation of pleasure in all participants. These correlations were non-significant when the participants were separated to the DS and the HC.

We found decreased RSFC between the dmPFC seeds and the lateral occipital cortex and the precuneus in the DS vs HC group and between the pgACC seed and the superior/middle frontal gyrus and the postcentral gyrus. We also found increased RSFC

between the dmPFC seed and the ACC/Paracingulate gurus and the frontal pole and between the nucleus accumbens seed and the precuneus and between the pgACC seed and the limbic structures in the DS vs HC groups.

Our findings show that individuals with a range of depressive symptoms have dysfunctional RSFC in key depression related networks. Further as we find that this dysfunction correlates with depression severity and pleasure scores this might be the mechanism underlying the risk of depression and perhaps even anhedonia, a suggested biomarker for depression.

Introduction

Major depressive disorder (MDD) is estimated to have a lifetime prevalence of approximately 16 %, with around 8% of adolescents being affected by depression by the age of 16 (Saluja et al., 2004). As recognition of MDD in adolescents has increased in recent years, more attention needs to be placed on the aetiology of the disorder. However, traditional diagnostic systems, that focus on categorising psychiatric disorders based on solid criteria, has been suggested as not entirely useful for capturing the fundamental underlying mechanisms of psychiatric dysfunction (Insel et al., 2010). Rather, examining clinical symptoms as a continuum across symptom severity ranges may be more useful for identifying neurobiological signatures and risk markers. Reports suggest that a dimensional approach to studying the symptoms of Major Depressive Disorders (MDD) may be more beneficial than the categorical approach especially given the heterogeneous nature of MDD (Insel et al., 2010).

It has been increasingly recognised that MDD is a disorder of abnormal neural networks. Resting-state functional connectivity studies (RSFC) have revealed that patients with MDD and individuals with depressive symptoms have abnormalities in the key RSFC networks such as the Central Executive Network (CEN), the Default Mode Network (DMN) and the Salience Network (SN) in adults and in adolescents (Rzepa & McCabe, 2016; Sheline, Price, Yan, & Mintun, 2010)

The Salience Network (SN) and the Central Executive Network (CEN) show strong task-related activation and are less active at rest, while the Default Mode Network (DMN) shows prominent activation during rest, and deactivation during a performance of cognitive tasks (Whitfield-Gabrieli & Ford, 2012). The SN which consists of regions such as the anterior insula, pregenual anterior cingulate and amygdala are implicated in the processing of various aspects of salient stimuli whereas the CEN, which consists of regions such as the dorsolateral, dorsal medial prefrontal cortex and the posterior parietal cortex, is involved in cognitive functioning including attention and working memory

(Bressler & Menon, 2010). Furthermore, the DMN is a network of distributed brain regions of the medial prefrontal cortex, the posterior cingulate cortex, and the precuneus (Bressler & Menon, 2010). In MDD, abnormalities in these networks are thought to reflect deficits in attentional control over emotional stimuli, emotion recognition, difficulties with suppression of unwanted thoughts and increased rumination. However there have been inconsistencies in the direction of effects with some studies finding increased SN, CEN and DMN (Horn et al., 2010; Li et al., 2013; Manoliu et al., 2014; Ramasubbu et al., 2014; Sheline et al., 2010), whilst others find reduced connectivity (Liston et al., 2014; Tahmasian et al., 2013; Ye et al., 2012; X. L. Zhu et al., 2012). Mixed results have also been presented for adolescents with a MDD diagnosis. Studies report decreased RSFC between the SN and the amygdala, hippocampus and the brain stem, which has also been shown to correlate with depression severity, and increased RSFC between the amygdala and the precuneus (Cullen et al., 2014). These inconsistencies might be related to differences in the MDD population studied (adults, elderly, adolescents), medication history and depression severity.

Moreover, studies that looked at individuals at familial risk of depression found decreased RSFC between the prefrontal cortex and parts of the CEN, which also correlated with the parent's depression severity (Clasen, Beevers, Mumford, & Schnyer, 2014). The authors suggested that an increase in vulnerability to depression may thus be underpinned by altered development of the CEN in young people at risk. Recently, we investigated another group at risk for depression- adolescents with depressive symptoms but lack of clinical diagnosis (Rzepa & McCabe, 2016). We found decreased RSFC in the at risk group when compared to HC between parts of the SN and the pgACC and the hippocampus and the SN and DMN. We also found decreased RSFC in the at risk group between the pgACC and the putamen and between the parts of the CEN and the DMN. Also, the increased pgACC RSFC with the insula/orbitofrontal cortex correlated negatively with the anticipation of pleasure in all subjects. Despite the small sample size

in that study we suggested that this brain-behaviour relationship might be a contributing factor of experiencing anhedonia- a key MDD symptom and a suggested biomarker for depression.

To our knowledge, there are no studies available that have directly examined the dimensional nature of depression severity in adolescents. There is only one RSFC study that examined anxiety symptoms with a dimensional approach in patients with clinical diagnoses of anxiety disorder, MDD and comorbid MDD/anxiety (Oathes, Patenaude, Schatzberg, & Etkin, 2015).

Our current study aims to combine the RSFC across a range of adolescents some with a clinical diagnosis of MDD and some with depressive symptoms (Rzepa & McCabe, 2016) but no clinical diagnosis. Therefore we have combined our previous data in those at risk of depression with adolescents with clinical depression (N=27) into a new depression symptom (DS) group (N=44). Based on the previous literature, we selected seed regions that have been shown dysfunctional in depressed patients and adolescents at increased risk of depression: amygdala, nucleus accumbens and pgACC seeds from the SN and the dmPFC seed highlighted as a key node within the CEN (Rzepa & McCabe, 2016; Sheline et al., 2010). The relationship between the RSFC and behaviour collected from clinical measures and questionnaires on anhedonia and depression severity were also examined. Based on the previous literature, we hypothesised decreased RSFC between key brain regions implicated in the aetiology of depression such as the dmPFC, amygdala, nucleus accumbens and the pgACC seeds. As our previous study with adolescents with elevated depressive symptoms found increased RSFC between the pgACC and the amygdala seeds, we would also expect to see similar findings.

Methods:

Participants:

86 participants (age range 13-21, M=18.06, SD= 1.88) were recruited for the study. All participants were assessed with the Structured Clinical Interview for DSM-IV Axis I Disorders Schedule (SCID) (First, Spitzer, Gibbon, & Williams, 1997). 44 participants presented with depressive symptoms (DS) and 42 individuals were age and gender matched healthy controls (HC). Adolescents were deemed as having DS if they scored >27 on the Mood and Feelings Questionnaire (MFQ) (A. Angold, Costello, Messer, & Pickles, 1995). 27 of the DS participants had a current diagnosis of major depressive disorder (MDD) either from their GP, clinical psychologist or psychiatrist. 13 MDD participants were medication free and 14 MDD participants were on antidepressants. 6 MDD participants had an antidepressant history (see Table S1). We also excluded pregnancy any other medications except the contraceptive pill and any contraindications to MRI. The National and University Research Ethics Committees approved the study and written informed consent was obtained from all participants.

All subjects were additionally rated on the following questionnaires: Mood and Feeling Questionnaire (MFQ; (A. Angold, Costello, E.j., Messer, S.C., Pickles, A., Winder, F., & Silver, D. , 1995)), Beck Depression Inventory (BDI; (Beck, 1961)), the Fawcett–Clarke Pleasure Scale (FCPS; (Fawcett, 1983)), and the Snaith–Hamilton Pleasure Scale (SHAPS; (Snaith, 1995)), the Temporal Experience of Pleasure Scale, consummatory subscale TEPS-C and anticipatory subscale TEPS-A (Gard D.E, 2006), before scanning. Body mass index (BMI) for each individual was also calculated.

Overall design:

MRI derived measures of brain function, based on blood-oxygenation-level-dependent (BOLD) contrast were used to compare brain responses at rest across the LR

group and the HR group. The resting-state data were acquired before any other scans including the structural scan. Subjects were instructed to lie in dimmed light with their eyes open, think of nothing in particular, and not to fall asleep, similar to our previous studies (Cowdrey, Filippini, Park, Smith, & McCabe, 2012; C. McCabe & Mishor, 2011; C McCabe et al., 2010) (Rzepa, Tudge, & McCabe, 2015) and a method found to have higher reliability than eyes closed (Patriat R et al., 2013). To measure whether undergoing a scan had an effect on mood change, volunteers completed the Befindlichkeit scale on mood (BFS; (Hobi, 1985)) and an energy and affect Visual Analogue Scale (VAS) pre and post scan.

Image acquisition:

A Siemens Magnetom Trio 3T whole body MRI scanner and a thirty-two-channel head coil were used. Multi-band accelerated echo planar imaging sequencing (Center for Magnetic Resonance Research, Minnesota) was used with an acceleration factor of 6 and iPAT acceleration factor of 2. T2*-weighted EPI slices were obtained every 0.7 seconds (TR=0.7, TE=0.03), these parameters were optimised given our scanner capability and used to increase sampling rates and increase our power to detect resting state networks as has been shown previously with multiband (Xu et al., 2013) (Filippini et al., 2014). 54 transverse slices with in-plane resolution of 2.4 x 2.4mm were attained and slice thickness was 2.4mm. The matrix size was 96x96 and the field of view (FOV) were 230x230mm. Acquisition was performed during 5 minutes resting-state scan, yielding 420 volumes in total. Sagittal 3D MPRAGE images were also acquired with an isotropic in-plane resolution of 1x1x1 (TI=0.9 seconds, TR=2.02, flip angle 9°, FOV=250x250mm) yielding 192 slices.

fMRI data analysis:

Pre-processing:

Imaging data were pre-processed and analysed using FSL tools (www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). fMRI data pre-processing was carried out

using FEAT (FMRI Expert Analysis Tool, Version 6.0, a part of FSL software), and included the following steps: non-brain removal (Smith, 2002), motion correction using MCFLIRT(Jenkinson & Smith, 2001), spatial smoothing using a Gaussian kernel of full-width at half maximum (FWHM) of 5mm, grand mean intensity normalization of the entire 4D dataset by a single multiplicative factor and high pass temporal filtering **(**Gaussian-weighted least-squares straight line fitting, with sigma=64.0s**).** fMRI volumes were registered to the individual's structural scan and the MNI-152 standard space image (Montreal Neurological Institute, Montreal, QC, Canada) using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson et al., 2002).

Time series extraction and higher level analysis:

To study resting-state functional connectivity, a seed-based correlation approach was used. Using the Harvard-Oxford subcortical structural atlas (Kennedy et al., 1998) we created a structural bilateral amygdala and bilateral nucleus accumbens seeds as the amygdala and the nucleus accumbens are small structures and are not suitable for a ROI sphere. To maximize the exact coverage, the masks of these seed regions were threshold by 20% to include voxels having at least 80% of probability of being in these particular regions. We also created seeds for the dmPFC (18 34 29; -24 35 28) (6 mm sphere so as to not cross into other brain regions) coordinates from (Sheline et al., 2010) and pgACC (8 mm sphere with a centre at 0 38 0 so as to not cross into other brain regions). The dmPFC and pgACC seeds were created with Wake Forest University Pickatlas tool in SPM8 as in our previous study and can be seen in the figures (C McCabe et al., 2010).

The mean time course within the left and right seeds of each ROI (except for the pgACC, only comprising one medial seed) was calculated and used as a regressor in a general linear model. In addition, white matter signal, cerebrospinal fluid signal, 6 motion parameters (3 translations and 3 rotations), and the global signal were used as nuisance regressors. We have obtained white matter and cerebrospinal fluid masks using FSL's

FAST segmentation program. The resulting segmented images were then thresholded to ensure 80% tissue type probability. For each individual, the general linear model was analyzed by using the FMRI Expert Analysis Tool [version 5.4, part of FMRIB's Software Library (Smith et al., 2004)]. The resulting parameter estimate maps were then analyzed using higher level 1 sample t-tests for group averages and between samples t-tests for group differences. Clusters were determined by $Z > 2.3$ voxel-wise thresholding and a family-wise error-corrected cluster significance threshold of $P < 0.05$ (Worsley, 2001). From the results we then only report those that met the further correction for number of ROIs examined which gave $P < 0.012$ (i.e, $P < 0.05$ Bonferroni corrected for the 4 networks of interest: amygdala, nucleus accumbens, dmPFC, pgACC (Davidson, Irwin, Anderle, & Kalin, 2003)). Next, the % BOLD signal change data was extracted from the regions of significant effect (Table 2) using the FSL tool Featquery (www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004).

Correlational analyses:

To examine the relationship between the scores on behavioural questionnaires and RSFC we extracted the % BOLD signal change using FSL Featquery and applied Person's correlations.

Results:

Table 1 shows the demographics for all the participants and for each group separately. There were no significant differences between the DS group and controls for age, gender and BMI. Differences were present between the subgroups for the: BDI, SHAPS, FCPS, TEPS (Table 1).

Table 1: Demographics

Measure	**DS (n=44)** **Mean (SD)**	**HC (n=42)** **Mean (SD)**	**p-value**	***All (n=86)***
Age(years)	18.11 (1.84)	18.02 (1.94)	.827	18.06 (1.88)
Age range	15-21	13-21	-	13-21
Gender	F33, M10	F31, M10	.907	F66, M20
BMI	21.73 (2.24)	21.09 (2.41)	.205	21.43 (2.34)
BDI	29.70 (12.69)	3.30 (4.1)	<.001	16.81 (16.3)
FCPS	117.23 (25)	137.01 (19.18)	<.001	125.4 (25)
SHAPS	30.8 (7.34)	21.21 (8)	<.001	26.1 (9.02)
TEPS-A	36.25 (8.67)	48 (5.78)	<.001	41.98 (9.43)
TEPS-C	30.61 (6.41)	36.76 (7)	<.001	33.62 (7.33)

F- females, M-males, BMI- Body Mass Index, BDI- Beck Depression Inventory, FCPS- the Fawcett-Clarke Pleasure Scale, SHAPS- the Snaith–Hamilton Pleasure Scale, TEPS-A- the Temporal Experience of Pleasure Scale, anticipatory subscale, TEPS-C- the Temporal Experience of Pleasure Scale, consummatory subscale

Mood, Energy and Affect Scores:

To investigate whether the scanning session had an effect on mood we collected the BFS and VAS scores before and after each of the scanning sessions. Results of this investigation are presented in the supplementary materials SR1, Table S2.

Correlational analysis of RSFC with depression severity and anhedonia measures: Dimensional approach.

Correlational analysis was performed for all participants together and each group separately, and only significant results are reported. There was a negative correlation between decreased RSFC of the right dmPFC seed and the left precuneus and increasing scores on BDI in all participants (r=-.321, p=.003). Also, this connectivity remained significant in the DS group (r=-.350, p=.023) and non-significant in the HC group (r=-.248, p=.104) (Figure 1).

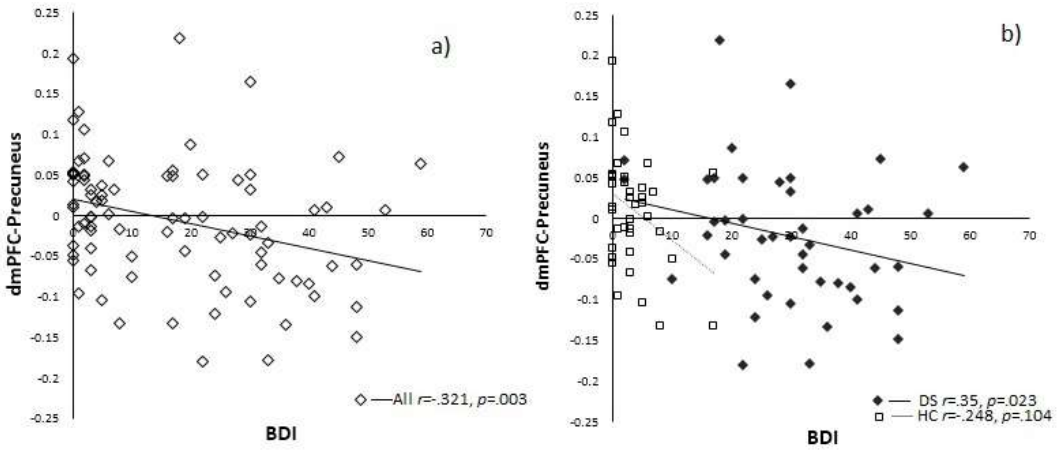

Figure 1: a) Negative correlation between decreased RSFC of the right dmPFC seed and the left precuneus and increasing scores on BDI in all participants (r=-.321, p=.003); b) and in the DS group (r=-.350, p=.023) and the HC group (r=-.248, p=.104).

There was a positive correlation between decreased RSFC of the right dmPFC seed and the left precuneus with increasing scores on TEPS anticipatory scale in all participants (r=.365, p=.001). Also, this connectivity reminded significant in the DS group (r=-.446, p=.003) and non-significant in the HC group (r=-.226, p=.104) (Figure 2).

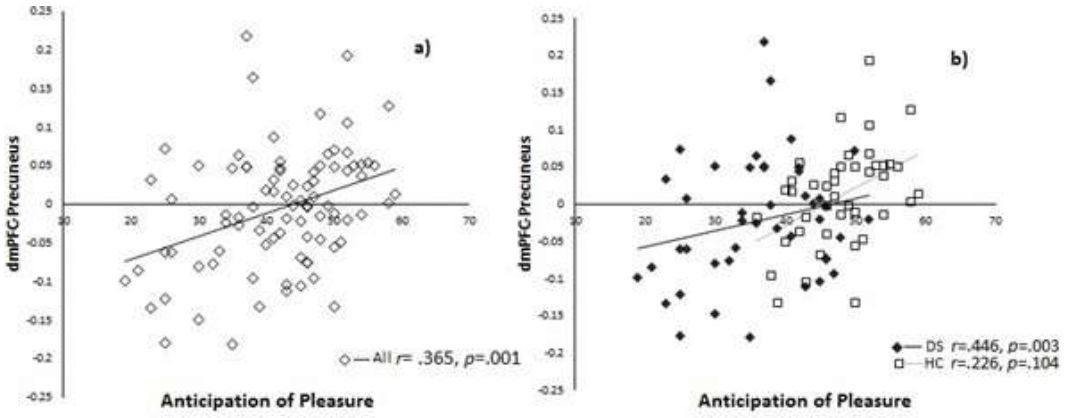

Figure 2: a) Positive correlation between decreased RSFC of the right dmPFC seed and the left precuneus and increasing scores on TEPS anticipatory scale in all participants (r=.365, p=.001); b) and in the DS group (r=-.446, p=.003) and the HC group (r=-.226, p=.104).

There was a positive correlation between increased RSFC of the right dmPFC seed and the frontal pole and increasing scores on the BDI in all participants (r=.308, p=.004). However, this connectivity did not correlate with the BDI when separated into the DS or HC groups (p>0.05).

There was a negative correlation between increased RSFC of the right dmPFC seed and the ACC/paracingulate gyrus with increasing scores on TEPS anticipatory scale in all participants (r=-.281, p=.009). However, this connectivity did not correlate with the TEPS anticipatory when separated into the DS or HC groups (p>0.05) (Figure 3).

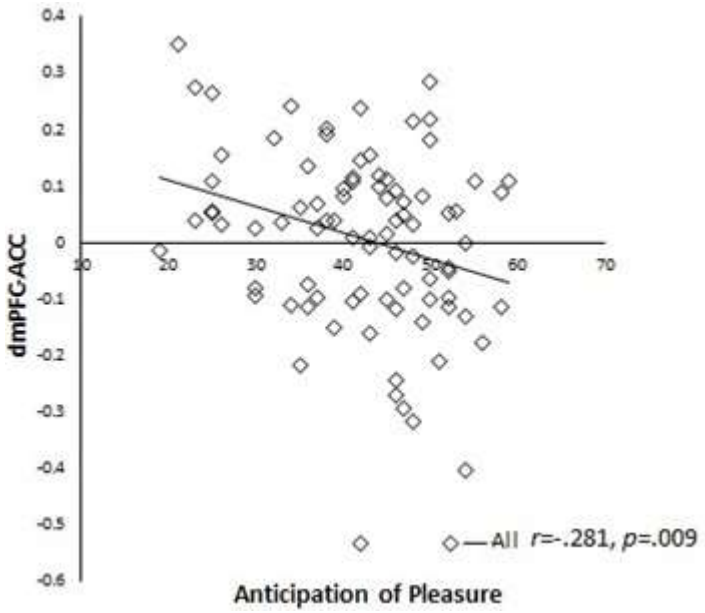

Figure 3: Negative correlation between increased RSFC of the right dmPFC seed and the ACC/paracingulate gyrus with increasing scores on TEPS anticipatory scale in all participants (r=-.281, p=.009).

RSFC over entire brain with seed regions in healthy controls:

Table S3 provides a summary of the main effects, i.e. the brain regions that had RSFC with the seed regions (baseline) for the HC group only. Overall, the patterns of connectivity associated with each of the seed regions are consistent with resting-state and functional connectivity experiments in healthy controls (Bebko et al., 2015; Clasen et al., 2014; Cullen et al., 2014; Guo et al., 2015; Sheline et al., 2009; Sheline et al., 2010).

Effects of depression symptoms (DS) on RSFC:

(HC vs. DS)

Left dmPFC seed

There was decreased RSFC in the DS group compared to the HC group between the left dmPFC seed and the lateral occipital cortex and the inferior/ middle temporal gyrus (Table 2) (Figure 4).

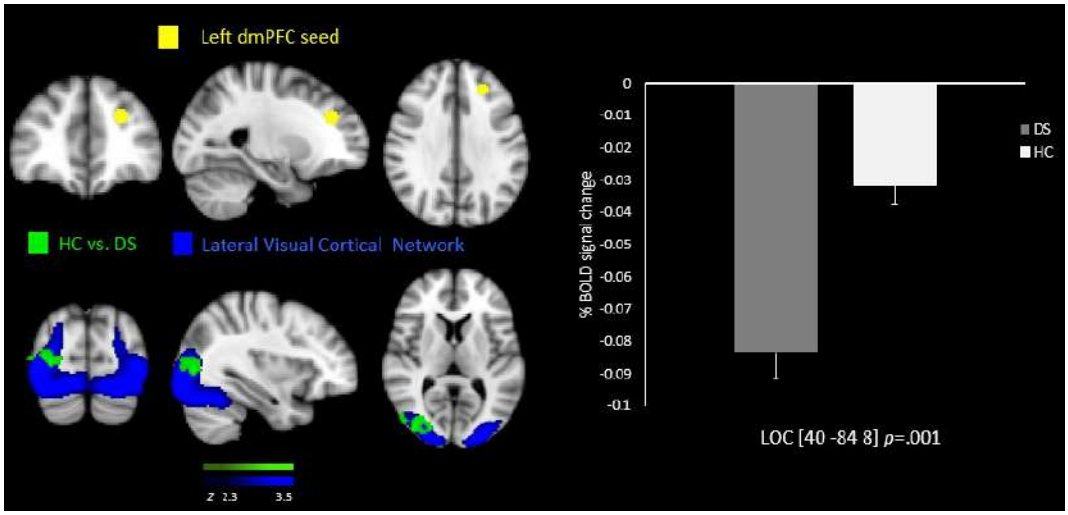

Figure 4: Resting state functional connectivity between the left dmPFC seed region and the LOC, lower in the DS group than the HC overlaid on the Lateral Visual Cortical Network. % BOLD signal change extracted for connectivity for both of the groups.

Right dmPFC seed

There was decreased RSFC in the DS group compared to the HC group between the right dmPFC seed and the precuneus and the cuneal cortex (Table 2).

pgACC seed

There was increased RSFC in the DS group compared to the HC group between the superior frontal gyrus/middle frontal gyrus and postcentral gyrus (Table 2).

DS vs HC

Right dmPFC seed

There was increased RSFC in the DS group compared to the HC group between the right dmPFC seed and the frontal pole and the ACC/paracingulate gyrus (Table 2).

Left NAcc seed

There was increased RSFC in the DS group compared to the HC group between the left NAcc seed and the precuenus (Table 2) (Figure 5).

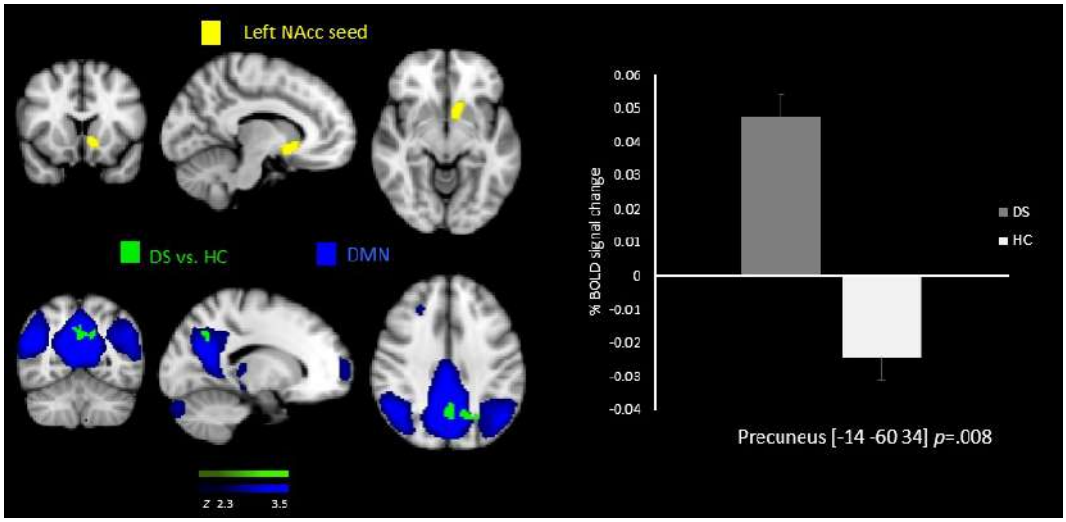

Figure 5: Resting state functional connectivity between the left NAcc seed region and the Precuneus, higher in the DS group than the HC overlaid on the DMN . % BOLD signal change extracted for connectivity for both of the groups.

pgACC seed

There was increased RSFC in the DS group compared to the HC group between the pgACC seed and the thalamus, putamen, caudate, NAcc and planum temporale (Table2).

Table 2: RSFC between seed regions and whole brain compared between DS and HC groups controlled for age, gender and medication status.

Brain Region	MNI Coordinates X	Y	Z	z-score	Cluster size	P value
HC>DS						
L dmPFC seed						
ITG/MTG	58	-22	-26	4.14	407	<0.001
LOC	40	-84	8	4.03	388	0.001
R dmPFC seed						
Cuneal cortex	-2	-82	26	4.09	328	0.002
Precuneus	8	-76	36	3.1	282	0.002
pgACC seed						
SFG/MFG	-22	16	44	3.88	385	0.001
Postcentral gyrus	-38	-22	60	3.98	269	0.013
DS>HC						
R dmPFC seed						
Frontal Pole	-32	32	12	4.11	485	<.001
ACC/Paracingulate	-8	25	22	3.2	485	<.001
L NAcc seed						
Precuneus	-14	-60	34	3.86	238	0.008
pgACC seed						
Thalamus	-2	-4	-4	4.27	655	<.001
Putamen	-26	4	0	4.12	655	<.001
Caudate	-10	8	16	3.8	655	<.001
NAcc	6	6	-2	3.51	655	<.001
Planum Temporale	-60	-38	14	4.64	286	0.008

All *p*-values for clusters were firstly determined by $Z > 2.3$ voxel-wise thresholding and a family-wise error-corrected cluster significance threshold of $P < 0.05$, then further Bonferroni corrected for number of ROIs networks examined which gave $P < 0.012$ (i.e, $P < 0.05$ (Davidson et al., 2003). pgACC- pregenual anterior cingulate cortex, ACC-anterior cingulate cortex, dmPFC-dorsal medial prefrontal cortex, LOC- lateral Occipital Cortex, NAcc- nucleus accumbens, STG-Superior Temporal gyrus, IFG- Inferior temporal gurus, SFG- Superior Frontal Gyrus, MFG-Medial frontal gyrus, IFG- inferior frontal gyrus

Discussion:

The aim of our study was to investigate RSFC across a range of depression severities in adolescents and to correlate RSFC with clinical measures of depression and anhedonia.

We found the decreased RSFC of the right dmPFC seed with the precuneus correlated with increasing scores on depression and decreasing anticipation of pleasure in all subjects. Interestingly, we found that these correlations were driven by the individuals with depression symptoms as when we examined the HC and the DS groups separately only the DS group correlation remained significant. This result was also supported by the decreased RSFC between these regions in the DS group compared to the HC group. The dmPFC is a key node in the CEN network (Seeley et al., 2007) and has been implicated in response activation and inhibition as measured by GO/NOGO tasks (Garavan, Ross, Murphy, Roche, & Stein, 2002). Interestingly this region had been reported dysfunctional in individuals with depressive symptoms and those with a clinical diagnosis of depression (Fonseka, Jaworska, Courtright, MacMaster, & MacQueen, 2016; Nixon, Liddle, Worwood, Liotti, & Nixon, 2013). (Sheline et al., 2010) argue that dysfunctional connectivity with the dmPFC might explain the symptoms of decreased ability to focus on cognitive tasks in depression.

The precuneus is a part of the DMN and has been implicated in self-referential thoughts and rumination. Similarly, previous studies have reported decreased RSFC between the CEN and the DMN in MDD and in individuals at risk for depression (Rzepa & McCabe, 2016; Sheline et al., 2010). Furthermore, studies find that decreased connectivity between these regions is associated with patients' difficulties to disengage from self-referential processes and that this in turn may lead to negative schemas, a hallmark of depression (Burkhouse et al., 2017; X. Zhu et al., 2012). In our study the connectivity between these regions was also correlated with decreased anticipation of pleasure in all subjects and in the DS group only. This shows for the first time, that as the ability to anticipate pleasure decreases, so does the RSFC between the CEN and the

DMN. Taken together these results may be a mechanism by which the symptom of anhedonia is experienced in those with and at risk of clinical depression, and therefore a neural biomarker for anhedonia.

The decreased RSFC with the dmPFC is of interest given our previous work examining the effects of antidepressant medications on RSFC. We and others have found that 7 days treatment with an SSRI in healthy volunteers also decreased functional connectivity with the dmPFC and parts of the DMN (C McCabe et al., 2010; van de Ven, Wingen, Kuypers, Ramaekers, & Formisano, 2013). Therefore it is possible that decreasing dmPFC RSFC leads to emotional blunting often cited with SSRI use (Opbroek et al., 2002, Price, Cole, Goodwin, 2009) especially since our more recent study examining the effects of 7 days treatment with bupropion found the opposite response. Bupropion is a dopaminergic and noradrenergic re-uptake inhibitor (DNRI) and reported to cause less emotional blunting than SSRIs. We found *increased* RSFC in healthy volunteers between the dmPFC seed region and the posterior cingulate cortex and the precuneus cortex, key parts of the default mode network, under bupropion treatment. As in this study we found the decreased dmPFC with precuneus connectivity correlating with increased depression and decreased pleasure this fits with the notion that bupropion may be a treatment that is better suited at treating anhedonia in depression

We also found increased RSFC of the right dmPFC seed with the frontal pole correlating with increasing depression symptoms and with the ACC/Paracingulate cortex correlating with decreasing anticipation of pleasure in all subjects. Although, these correlations were non-significant for either of the groups alone when the groups were directly compared for RSFC, there was increased connectivity in the DS group compared to the HC group. These regions are involved in attention, working memory and the anticipation of pleasant stimuli (Gorka et al., 2014; Rzepa, Fisk, & McCabe, 2017; Vasic, Walter, Sambataro, & Wolf, 2009) and been found dysfunctional during RSFC in MDD patients (Pannekoek et al., 2014). Thus, it is possible that increased connectivity between

the cognitive control nodes and the brain regions involved in attention and processing of salient information might underlie disrupted functioning and also anhedonia in MDD. In fact in our study only the dmPFC RSFC correlated with both depression severity and anhedonia scores. Importantly the study by (Sheline et al., 2010) from where we sourced our seed regions, reported that the dmPFC (coined dorsal nexus) is a brain region that serves as an intersection point for multiple brain networks and has greater connectivity and "hot-wiring" with other brain regions the more severe the depression. Thus our results are consistent with this but also go one step further showing that when using a dimensional approach dmPFC RSFC is also related to depression severity across a sample of adolescents both with and without depression. We also show for the first time that those with the greatest connectivity between the dmPFC and the frontal pole and ACC have the greatest problems experiencing pleasure, which in turn may be a mechanism for anhedonia.

We also found decreased connectivity between the dmPFC seed and the LOC in the DS group when compared with the HC group. The LOC is a part of the lateral visual cortical areas and has been involved with the processing of visual information (Bermpohl et al., 2006; van Wingen et al., 2011). Given the function of the dmPFC and the LOC, these results might indicate possible difficulties with a control over negative visual cues thus increasing the experience of depressive symptoms.

We also found decreased RSFC between the pgACC seed and the superior/medial frontal gyrus and the postcentral gyrus in the DS when compared with the HC groups. The pgACC is claimed to be a node of communicating between the dorsal ACC important for error detection and attention and the more ventral ACC implicated in emotion processing, regulation and salience detection (Ball, Stein, & Paulus, 2014). Furthermore, superior and middle frontal gyri are involved in self-referential tasks and attentional control (Goldberg, Harel, & Malach, 2006; Ochsner et al., 2004). Thus our result of decreased functional connectivity between these regions may possibly suggest problems

with integrating inputs of emotional information, resulting in abnormalities in affect regulation and thus a mechanism for depressive symptoms.

Interestingly, we also found increased RSFC between the pgACC seed and the limbic structures of the thalamus, putamen, caudate and the nucleus accumbens in the DS when compared with the HC group. As mentioned above, the pgACC is involved in integrating the salient and cognitive information. The limbic brain structures are involved in reward and emotion processing and also have been found dysfunctional in depression (Alexander, Crutcher, & DeLong, 1990; Phillips, Drevets, Rauch, & Lane, 2003). Interestingly, (Gabbay et al., 2013) also found increased pgACC- caudate RSFC in adolescents with MDD which additionally was related to anhedonia severity. However, it is important to mention that there are other resting-state studies that have reported decreased connectivity of the pgACC to striatum in adults (Philippi, Motzkin, Pujara, & Koenigs, 2015). These mixed findings might be related to different characteristics of depression such as anhedonic or anxious types, medication status, or even age which might possibly influence the pattern of connectivity.

We also found increased RSFC between the left NAcc seed and the precuenus/ posterior cingulate cortex in the DS when compared with the HC group. NAcc is a key reward region that has been reported dysfunctional in MDD across variety of reward-related tasks, and has also been associated with anhedonia (Rademacher, Salama, Grunder, & Spreckelmeyer, 2014). As mentioned above, precuneus is a part of the DMN and many studies have reported increased RSFC of these brain regions in MDD subjects. For example, a recent study reported increased RSFC between the NAcc and the precuenus in young individuals at risk for depression (Hwang et al., 2016), which is also in line with a recent meta-analysis that found increased RSFC within the DMN as well as between the DMN and networks of fronto-pariatal regions in adolescents and adults (Kaiser, Andrews-Hanna, Wager, & Pizzagalli, 2015). These results might therefore suggest that individuals with increased anhedonia levels, thus lowered reward

responsiveness, self-compensate by presenting increased RSFC between the NAcc and the precuneus.

In summary we examined the relationship between RSFC and depression and anhedonia scores across a sample of adolescents with a range of depression severities. Our findings show that individuals with depressive symptoms have dysfunctional RSFC in key cognitive, affective and visual networks. We found that decreased RSFC also correlated with depression severity and anhedonia (decreased anticipation of pleasure) but only connectivity with the dmPFC seed region, the same seed region decrease under SSRI treatment but increased under DNRI treatment. We suggest these results might point to neural networks as biomarkers of depression like the symptom of anhedonia and targets for specific symptom treatments.

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Chapter 8: Addendum for Paper 4: Further Analyses and Discussion:

8.1. Rationale and Aims

Paper 4 investigated how abnormalities in RSFC networks related to the severity of anhedonia and depression symptoms using a dimensional approach. However, it is also of interest to run further analyses that focus on investigating whether there are any differences in the RSFC networks in those with a depression diagnosis when compared with heathy controls, and between those who have elevated depressive symptoms thus are at increased risk of depression compared to those with a clinical diagnosis. It is also of interest to see how these differences might be related to differences in symptoms. Such investigation requires a categorical characterisation of participants. Thus, this chapter will present additional analyses and discussions that expand the previous Paper 4.

8.2. Methods

Note as requested after PhD defence: The DS group consisted of 17 high risk (HR) individuals from Chapter 6 (Study 3) and 27 MDD individuals as indicated in Chapter 7 (Study 4).

This methods section is exactly the same as the method section of Paper 4 (page 156-159) with a difference in the characterisation of the participant groups.

8.2.1. Characterisation of the participant groups

For the purpose of the categorical analysis the DS group from the previous chapter was split into three separate groups: 27 participants with a depression diagnosis (MDD), 17 individuals with elevated depressive symptoms thus at high risk for depression (HR) and 31 healthy controls (HC) (table 1 and table 2). For the high level RSFC analysis we compared the MDD and the HC groups and the MDD and the HR groups. The HR group vs controls is already published in Chapter 6.

8.3. Results

Table 1 shows the demographics for the MDD and the HC and Table 2 shows the demographics for MDD and HR. There were no significant differences between the MDD group and the HC group for age, gender and BMI. Differences were present between the MDD and HC for the: BDI, SHAPS, FCPS, TEPS_A and TEPS_C (Table 1). There were no significant differences between the MDD group and the HR groups for gender, BMI, BDI, SHAPS, FCPS, TEPS_A and TEPS_C ($p>.05$). There were significant group differences for age (Table 2)

Table 1: Demographics for the MDD and the HC groups

Measure	**MDD (*n*=27)** **Mean (SD)**	**HC (*n*=31)** **Mean (SD)**	***p*-value**
Age(years)	19.07 (1.51)	18.94 (1.09)	.689
Age range	15-21	17-21	-
Gender	F21,M6	F26, M5	.563
BMI	21.68 (1.94)	21.63 (2.3)	.930
BDI	29.62 (12.93)	3.65 (3.64)	<.001
FCPS	109.89 (27.63)	138.8 (17.16)	<.001
SHAPS	31.19 (8.34)	20.83 (7.52)	<.001
TEPS-A	35.93 (9.52)	47.65 (6.02)	<.001
TEPS-C	29.7 (6.39)	37.13 (6.43)	<.001

F- females, M-males, BMI- Body Mass Index, BDI- Beck Depression Inventory, FCPS- the Fawcett-Clarke Pleasure Scale, SHAPS- the Snaith–Hamilton Pleasure Scale, TEPS-A- the Temporal Experience of Pleasure Scale, anticipatory subscale, TEPS-C- the Temporal Experience of Pleasure Scale, consummatory subscale

Table 2: Demographics for the MDD and the HR groups

Measure	**MDD (*n*=27)** **Mean (SD)**	**HR (*n*=17)** **Mean (SD)**	***p*-value**
Age(years)	19.07 (1.51)	16.59 (1.18)	<.001
Age range	15-21	15-18	-
Gender	F21,M6	F13, M4	.922
BMI	21.68 (1.94)	21.82 (2.72)	.855
BDI	29.62 (12.93)	29.82 (12.7)	.961
FCPS	109.89 (27.63)	121.12 (18.7)	.148
SHAPS	31.19 (8.34)	30.11 (5.56)	.644
TEPS-A	35.93 (9.52)	37.29 (7.58)	.759
TEPS-C	29.7 (6.39)	31.76 (5.98)	.240

F- females, M-males, BMI- Body Mass Index, BDI- Beck Depression Inventory, FCPS- the Fawcett-Clarke Pleasure Scale, SHAPS- the Snaith–Hamilton Pleasure Scale, TEPS-A- the Temporal Experience of Pleasure Scale, anticipatory subscale, TEPS-C- the Temporal Experience of Pleasure Scale, consummatory subscale

Decreased functional connectivity: HC vs. MDD

Right Amygdala seed

There was decreased RSFC in the MDD group compared to the HC group between the right amygdala seed and the precuneus and the posterior cingulate cortex (Table 3).

Left dmPFC seed

There was decreased RSFC in the MDD group compared to the HC group between the left dmPFC seed and the lateral occipital cortex and the precentral/postcentral gyrus (Table 3) (Figure 1).

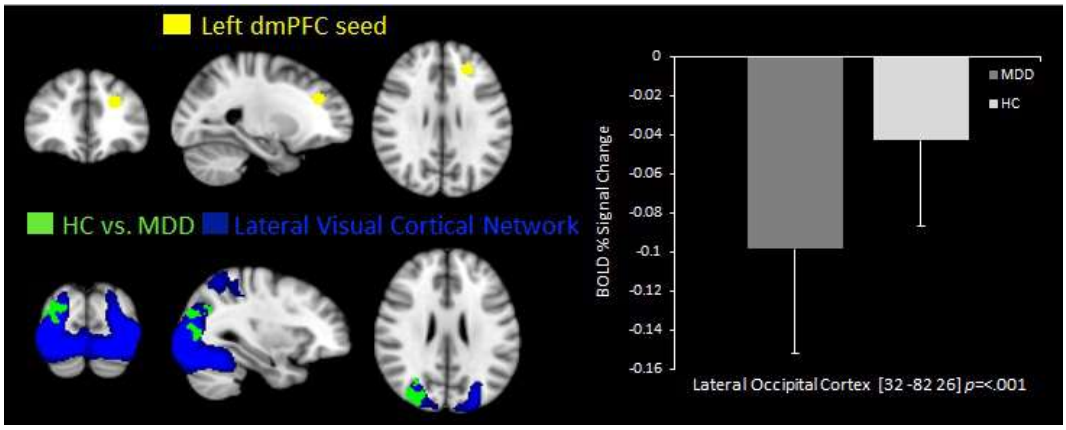

Figure 1: Decreased RSFC between the left dmPFC seed (CEN) and the lateral occipital cortex (visual network) in the MDD group compared to the HC group.

Left NAcc seed

There was decreased RSFC in the MDD group compared to the HC group between the left NAcc seed and the anterior/posterior cingulate cortex and the thalamus (Table 3).

Right NAcc seed

There was decreased RSFC in the MDD group compared to the HC group between the right NAcc seed and the posterior cingulate cortex/ precuneus (Table 3).

pgACC seed

There was decreased RSFC in the MDD group compared to the HC group between the pgACC seed and the lateral occipital cortex, the middle/inferior frontal gyrus, and the postcentral/precentral gyrus (Table 3).

Increased functional connectivity: MDD vs. HC

Left amygdala seed

There was increased RSFC in the MDD group compared to the HC group between the left amygdala seed and thalamus and the putamen/ pallidum (Table3).

Right amygdala seed

There was increased RSFC in the MDD group compared to the HC group between the right amygdala seed and the pallidum/caudate (Table3).

Right NAcc seed

There was increased RSFC in the MDD group compared to the HC group between the right NAcc seed and the middle/inferior frontal gyrus (Table3).

pgACC seed

There was increased RSFC in the MDD group compared to the HC group between the pgACC seed and the precuneus/posterior cingulate cortex (Table3).

Table 3: RSFC between seed regions and whole brain compared between MDD and HC groups controlled for medication status, age and gender.

Brain Region	MNI Coordinates			z-score	Cluster size	P value
	X	Y	Z			
HC>MDD						
R Amygdala seed						
PCC	4	-42	6	3.42	332	<.001
Precuneus	-4	-54	14	3.26	332	<.001
Precuneus	2	-54	26	3.02	332	<.001
L dmPFC seed						
LOC	32	-82	26	4.28	422	<0.001
Precentral gyrus	-30	-26	46	4.79	290	0.006
L NAcc seed						
ACC/PCC	6	-8	30	3.88	353	<.001
Thalamus	-8	-22	18	4.28	339	<.001
R NAcc seed						
PCC/Precuneus	12	-38	40	3.48	292	0.001
PCC/Precuneus	-2	-40	44	3.37	292	0.001
pgACC seed						
MFG	54	30	28	4.34	549	<.001
Postcentral gyrus	56	-26	56	3.97	540	<.001
LOC	-24	-78	24	4	321	0.002
LOC	28	-72	32	3.44	317	0.002
MDD>HC						
L Amygdala seed						
Thalamus	22	-26	14	4.28	895	<.001
Putamen	30	-12	0	3.59	285	0.001
Pallidum	24	-12	2	3.19	285	0.001
R Amygdala seed						
Pallidum	-10	-4	-10	3.97	396	<.001
Caudate	8	2	12	3.35	396	<.001
R NAcc seed						
MFG	52	18	32	3.4	236	0.005
IFG	42	22	18	3.37	236	0.005
pgACC seed						
Precuneus	-10	-58	16	4.39	1989	<.001

Precuneus	12	-50	16	3.98	1989	<.001

All *p*-values for clusters were firstly determined by Z > 2.3 voxel-wise thresholding and a family-wise error-corrected cluster significance threshold of $P < 0.05$, then further Bonferroni corrected for number of ROIs networks examined which gave $P < 0.012$ (i.e, $P < 0.05$ (Davidson, Irwin, Anderle, & Kalin, 2003). pgACC- pregenual anterior cingulate cortex, PCC- posterior cingulate cortex, ACC-anterior cingulate cortex, dmPFC- dorsal medial prefrontal cortex, LOC- lateral Occipital Cortex, NAcc- nucleus accumbens, MFG- Medial frontal gyrus, IFG- inferior frontal gyrus

Correlational analysis with behaviour: MDD and HC groups

There was a negative correlation between increased RSFC of the right amygdala seed and the pallidum and decreased consummation of pleasure (TEPS-C) in the MDD group only (*r*= -.533, *p*=0.004) (HC: *r*=-.033, *p*=.860) (Figure 2).

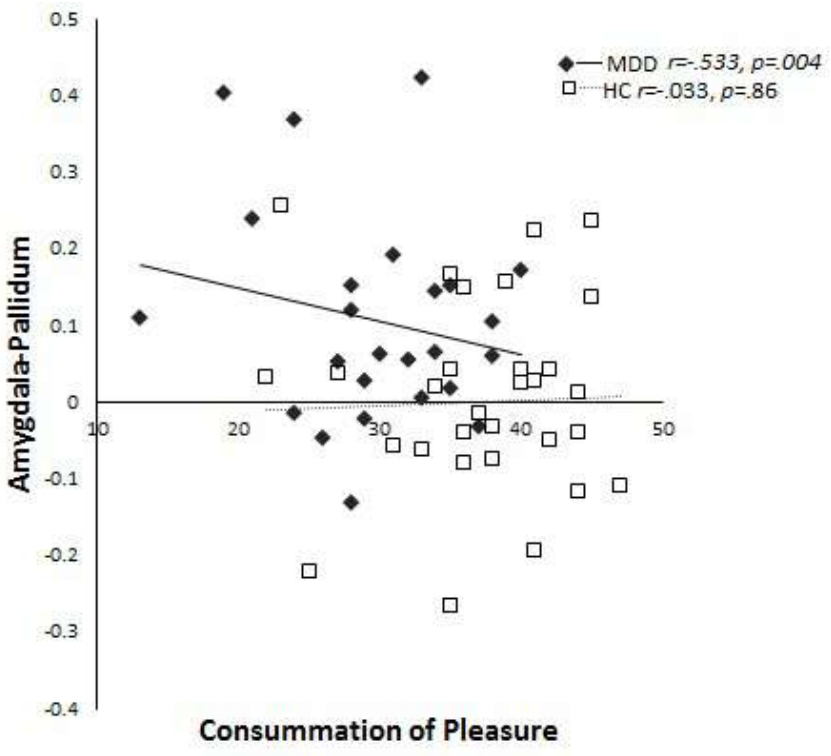

Figure 2: Correlation between increased RSFC of the right amygdala seed and the pallidum and decreased consummation of pleasure (TEPS-C) in the MDD (*r*= -.533, *p*=0.004) and the HC groups (*r*=-.033, *p*=.860).

There was a positive correlation between increased RSFC of the right amygdala seed and the posterior cingulate cortex and decreased consummation of pleasure (TEPS-C) in the MDD group only (r= -.497, p=0.008) (HC: r=-.129, p=.523).

There was a negative correlation between increased RSFC of the pgACC seed and the right precuneus and decreased consummation of pleasure (TEPS-C) in the MDD group only (r= -.485, p=0.01) (HC: r=-.093, p=.62) (Figure 3).

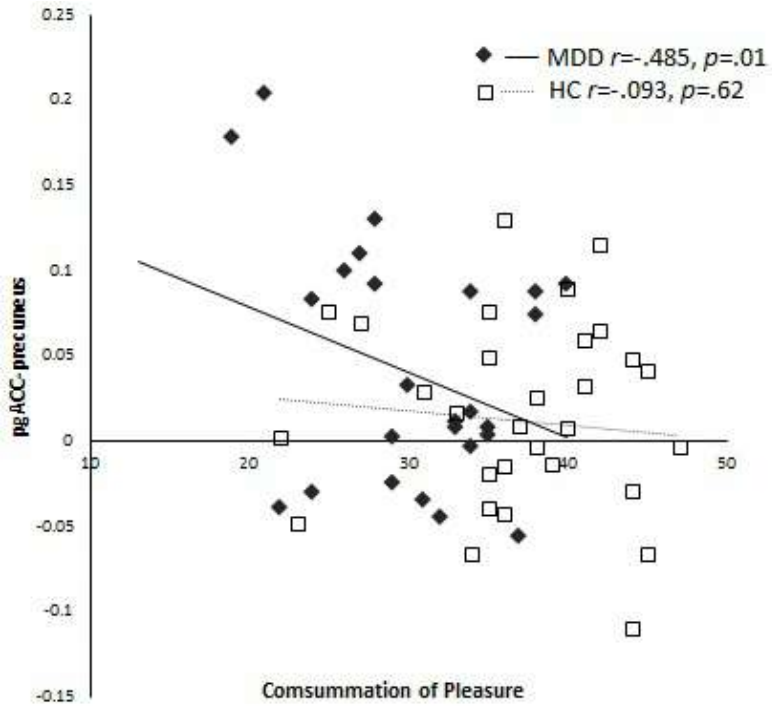

Figure 3: Correlation between increased RSFC of the pgACC seed and the right precuneus and decreased consummation of pleasure (TEPS-C) in the MDD (r= -.485, p=0.01) and the HC groups (r=-.093, p=.62).

Decreased functional connectivity in HR vs. MDD

Left dmPFC seed

There was decreased RSFC in the MDD group compared to the HR group between the left dmPFC seed and the Precentral gyrus, PCC, the Occipital Pole and the lateral occipital cortex (Figure 4) (Table 4).

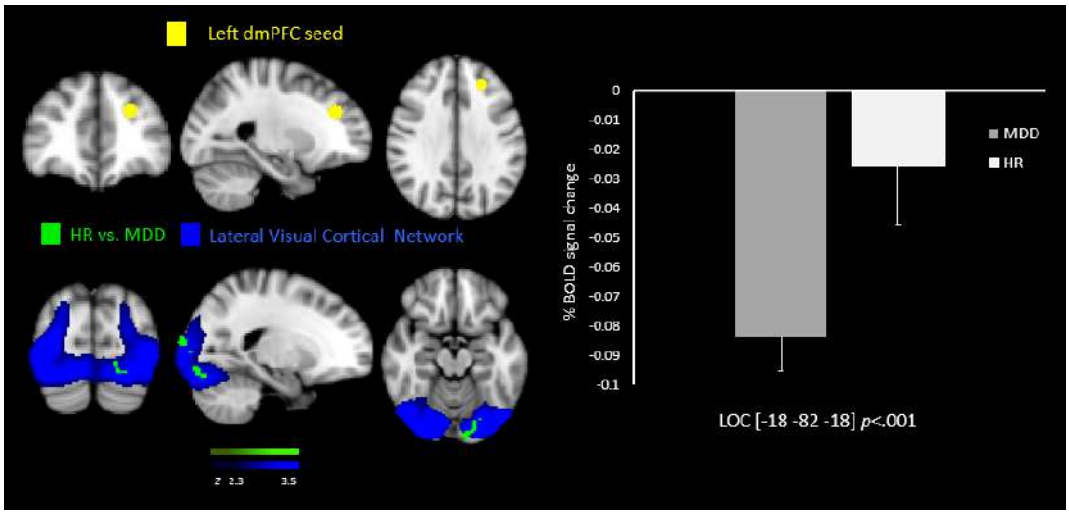

Figure 4: Decreased RSFC between the left dmPFC seed and the LOC in the MDD group compared to the HR group.

Left NAcc seed

There was decreased RSFC in the MDD group compared to the HR group between the left NAcc seed and the PCC and the thalamus (Table 4).

pgACC seed

There was decreased RSFC in the MDD group compared to the HR group between the pgACC seed and the lateral occipital cortex, the middle temporal gyrus, the middle/inferior frontal gyrus, the Thalamus, Caudate, NAcc, the postcentral and the precentral gyrus (Table 4).

Increased functional connectivity in MDD vs. HR

Left amygdala seed

There was increased RSFC in the MDD group compared to the HR group between the left amygdala seed and thalamus and the ACC/PCC (Table 4).

Right dmPFC seed

There was increased RSFC in the MDD group compared to the HR group between the right dmPFC seed and thalamus (Table 4).

Left NAcc seed

There was increased RSFC in the MDD group compared to the HR group between the left NAcc seed and the supplementary motor cortex, caudate and the ACC (Table 4).

pgACC seed

There was increased RSFC in the MDD group compared to the HR group between the pgACC seed and the paracingulate gyrus/ACC, paracingulate gyrus/frontal pole, precuneus, PCC and the Temporal pole (Table 4).

Table 4: RSFC between seed regions and whole brain compared between MDD and HR groups controlled for medication status, age and gender.

Brain Region	MNI Coordinates X	Y	Z	z-score	Cluster size	P value
HR>MDD						
L dmPFC seed						
Precentral gyrus	-34	-20	46	3.69	1008	<.001
PCC	0	-18	46	3.54	1008	<.001
Occipital Pole	-2	-100	0	3.74	453	<.001
LOC	-18	-82	-18	3.14	453	<.001
L NAcc seed						
PCC	4	-40	6	4.29	2401	<.001
Thalamus	-8	-22	18	4.04	2401	<.001
pgACC seed						
MTG	50	6	50	4.55	2370	<.001
LOC	26	-70	44	4.32	2370	<.001
IFG/MFG	52	16	16	3.82	1092	<.001
Thalamus/Caudate	12	0	10	4.74	814	<.001
Caudate/NAcc	12	14	-2	2.94	814	<.001
Postcentral Gyrus	-8	-44	76	3.61	451	<.001
Precentral Gyrus	-48	-6	44	3.47	385	<.001
MDD>HR						
L amygdala seed						
Thalamus	-14	-22	12	3.26	656	<.001
ACC/PCC	-4	-14	44	3.85	268	0.003
R dmPFC seed						
Thalamus	-18	-14	12	4.42	1808	<.001
L NAcc seed						
Motor Cortex	-8	4	44	4.32	862	<.001
Caudate	-10	10	18	3.72	862	<.001
ACC	2	8	36	3.39	862	<.001
pgACC seed						
Paracingulate/ACC	-2	48	4	5.81	4720	<.001
Paracingulate/Frontal Pole	0	54	2	5.2	4720	<.001
Precuneus	6	-54	30	4.94	2383	<.001

PCC	-4	-50	16	4.21	2383	<.001
Temporal Pole	58	10	-24	4.23	480	<.001

All *p*-values for clusters were firstly determined by Z > 2.3 voxel-wise thresholding and a family-wise error-corrected cluster significance threshold of $P < 0.05$, then further Bonferroni corrected for number of ROIs networks examined which gave $P < 0.012$ (i.e, $P < 0.05$ (Davidson et al., 2003). pgACC- pregenual anterior cingulate cortex, ACC-anterior cingulate cortex, PCC-Posterior Cingulate Gyrus, dmPFC- dorsal medial prefrontal cortex, LOC- lateral Occipital Cortex, NAcc- nucleus accumbens, MTG- middle Temporal Gyrus, SFG- Superior Frontal Gyrus, MFG-Medial frontal gyrus, IFG- inferior frontal gyrus

Correlational analysis with behaviour:

Significant correlations in the MDD group

There was a negative correlation between decreased RSFC of the left dmPFC seed and the LOC and increased depression severity (BDI) in the MDD group only (*r*= -.494, *p*=0.009) (HR: *r*=-.198, *p*=.446) (Figure 6).

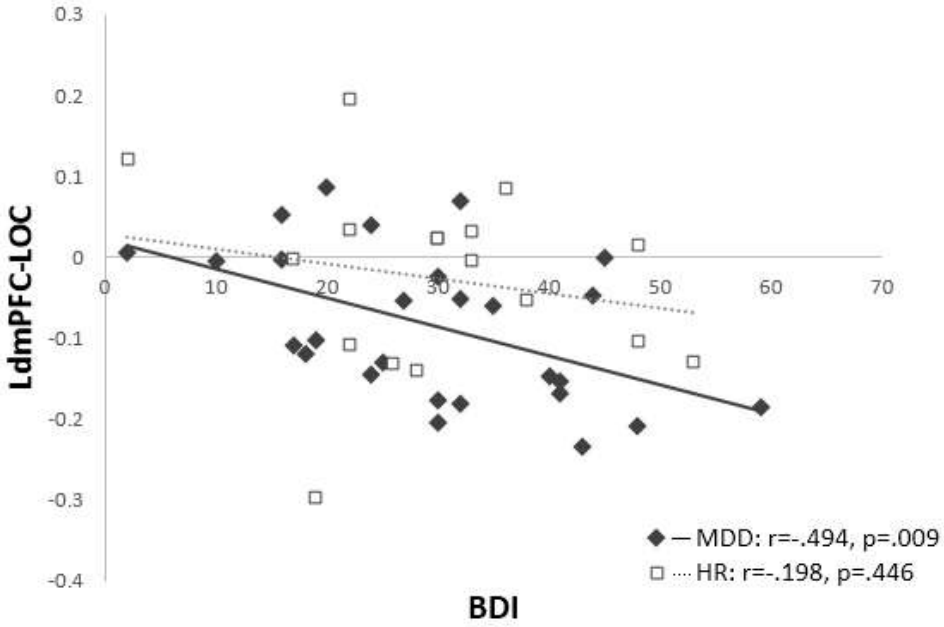

Figure 6: Correlation between decreased RSFC of the dmPFC seed and the LOC and increased depression severity (BDI) in the MDD group only (*r*= -.494, *p*=0.009) (HR: *r*=-.198, *p*=.446).

There was a positive correlation between decreased RSFC of the left NAcc seed and the PCC and decreased consummation of pleasure (TEPS_C) in the MDD group only (r= .485, p=0.01) (HR: r=.165, p=.527) (Figure 7).

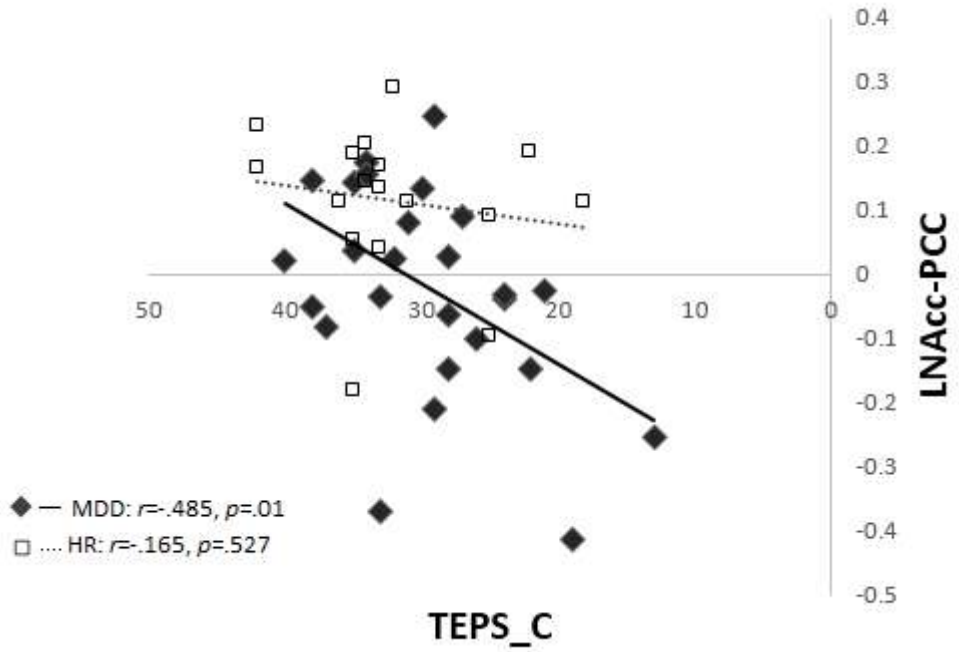

Figure 7: Correlation between decreased RSFC of the left NAcc seed and the PCC and decreased consummation of pleasure (TEPS_C) in the MDD group only (r=- .485, p=0.01) (HR: r=-.165, p=.527).

There was a positive correlation between increased RSFC of the right dmPFC seed and the insula and increased depression severity (BDI) in the MDD group only (r= .507, p=.007) (HR: r=.139, p=.594) (Figure 8).

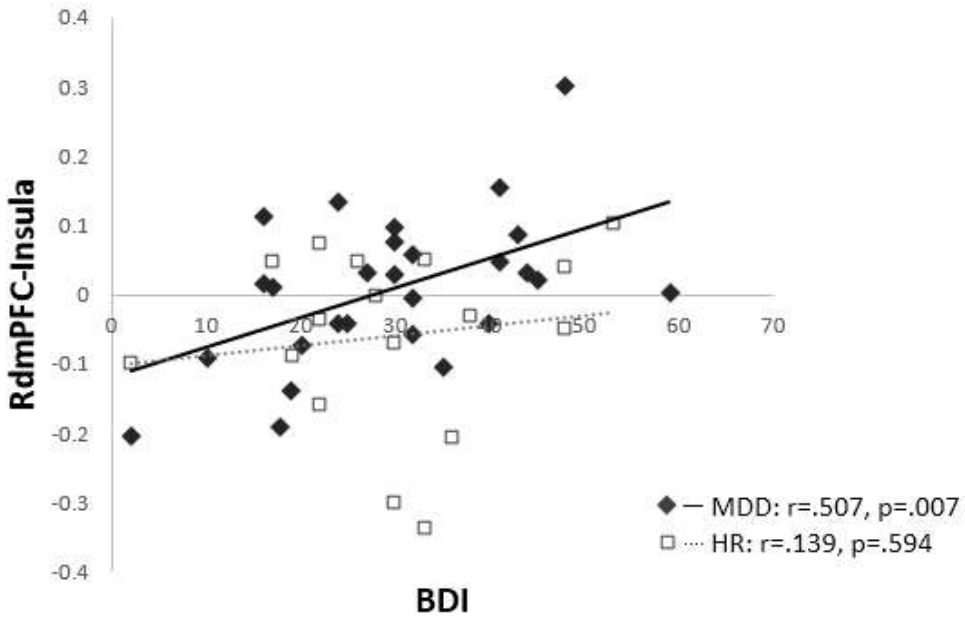

Figure 8: Correlation between increased RSFC of the right dmPFC seed and the insula and increased depression severity (BDI) in the MDD group only (*r*= .507, p=.007) (HR: *r*=.139, *p*=.594).

8.4. Discussion:

Our categorical analysis of the MDD vs. HC and MDD vs. HR groups revealed many similarities to the previous dimensional analysis as expected. However differences in the patterns of functional connectivity were also present as we now examine the HR group compared to the MDD group. I will now discuss the similarities and differences across these analyses.

Comparison of the RSFC results across all groups

We observed decreased RSFC between the left dmPFC seed and the LOC in the DS vs. HC groups, the MDD vs. HC groups and the MDD vs. HR groups. As mentioned in Chapter 7, the LOC is a part of the lateral visual cortical areas and has been involved in the processing of visual information (Bermpohl et al., 2006; van Wingen et al., 2011), whilst the dmPFC is involved in cognitive control and is a part of the CEN (Bressler & Menon, 2010). Moreover, there was a significant correlation between the dmPFC-LOC

RSFC and increasing depression severity in the MDD group which was non-significant in the HR group. This suggests that at the brain level, the dmPFC-LOC RSFC is already dysfunctional before the onset of clinical depression but as depression increases so to do the behavioural symptoms. Furthermore, given the functions of the dmPFC and the LOC these results indicate that individuals with depressive symptoms and depression might have difficulties with control over emotional visual cues thus further increasing their experience of depressive symptoms. Also, the observed correlation in the MDD group between the brain and behaviour suggests the dmPFC-LOC RSFC as a possible biomarker for depression.

Comparison of the RSFC results between DS vs. HC, MDD vs. HC and MDD vs. HR

We also found decreased RSFC between the right NAcc seed and the PCC/precuneus in the MDD vs. HC group which was increased under the dimensional analysis (Chapter 7). More interestingly, this decreased RSFC between the right NAcc and the PCC/precuneus correlated with the decreasing anticipation of pleasure in the MDD group only in this analysis. Moreover, we also found decreased RSFC between the left NAcc seed and the PCC in the MDD when compared with the HR group. These results were further correlated with decreasing consummatory pleasure in the MDD group only. These results therefore are in line with our previous findings that showed a positive correlation of the nucleus accumbens responses to rewarding cue and taste with anticipatory and consummatory pleasure scales in all and the MDD subjects (Chapter 4). This means that the dysfunctional RSFC between those brain regions is further related to the anticipatory and consummatory anhedonia in MDD individuals only suggesting that this connectivity might be a possible state marker of depression.

Comparison of the RSFC results between MDD vs. HC and the MDD vs. HR

We also found decreased RSFC of the left NAcc seed with the ACC/PCC and the thalamus in the MDD vs. HC group and the MDD vs. HR groups. The thalamus plays a role in reward processing due to its anatomical connections with the reward network including the nucleus accumbens and the orbitofrontal cortex (OFC) (Cauda et al., 2011; Kringelbach, 2005). Furthermore, the dorsal ACC is a node in the SN and, as described above, has been found dysfunctional in MDD. Also, previous RSFC studies in MDD reported decreased connectivity of the thalamus with the reward related brain region of the OFC (Tadayonnejad, Yang, Kumar, & Ajilore, 2015). Thus it is possible to suggest a further dysregulation in brain regions for both the reward and the salient stimuli processing. Also, as this RSFC dysfunction is present in both of the groups, it is possible that it might be a trait marker of depression.

We also observed decreased RSFC between the pgACC seed and the LOC in the MDD vs. HC and MDD vs. HR groups, and increased RSFC between the pgACC seed and the precuneus in the MDD vs. HC and MDD vs. HR groups. As mentioned in the previous chapters, the pgACC serves a node of communicating between the dorsal ACC important for error detection and attention and the more ventral ACC implicated in emotion processing, regulation and salience detection (Ball et al., 2014). Our previous study in adolescents at increased risk for depression also showed pgACC involvement in reward and aversion processing (Rzepa, Fisk, & McCabe, 2017). Previous studies in MDD also found that the precuneus, a key node of the DMN, shows increased RSFC, which is thought to reflect increased rumination (Whitfield-Gabrieli & Ford, 2012). Thus it is possible that individuals with depressive symptoms have difficulties in integrating visual and self-referential information the way healthy individuals are able to, which might further contribute to the experience of depression. As these deficits are found in both the HR and the MDD sample this suggests that these RSFC maps are possible

biomarkers for depression that if targeted and normalised might help in the treatment of depression.

The RSFC results for HR vs. LR (Chapter 6) and MDD vs. HC

The RSFC analysis also revealed decreased RSFC in the HR vs. LR group between the amygdala seed and the pgACC and the precuneus/PCC (Rzepa & McCabe, 2016). Similar results were found in the MDD vs. HC group. The amygdala is a part of the SN and has been implicated in the processing and regulation of emotional information (Smith, Stephan, Rugg, & Dolan, 2006). The precuneus is a part of the DMN and has been implicated in self-referential thoughts and rumination (Nejad, Fossati, & Lemogne, 2013). Those brain regions have been also found dysfunctional in the MDD and in the at risk for MDD populations. Thus it is possible that those subjects have difficulties in emotion regulation and it might be due to decreased amygdala-precuneus connectivity. Moreover, results of our current studies show that this dysfunction is present before the onset of clinical depression and in clinically depressed indicating that decreased amygdala-precuneus connectivity might a biomarker of depression. To further investigate this, longitudinal studies should be employed that will monitor whether those at risk of MDD and also with amygdala-precuneus dysfunction will progress to clinical depression.

The RSFC results for MDD vs. HC

Furthermore, we observed abnormalities in the RSFC between the amygdala seeds, which are part of the SN, and regions of the reward network and the DMN in the clinical depressed group. Specifically, we found decreased RSFC between the right amygdala and the precuenus and the PCC in the MDD versus the HC group that was also correlated with decreased scores on the consummatory pleasure scale in the MDD group. It is well established that patients with MDD present dysfunctional processing and regulation of emotional information. There are many studies that found abnormalities of

the amygdala activation in variety of emotional tasks and amygdala connectivity in resting-state studies in MDD (Kaiser, Andrews-Hanna, Wager, & Pizzagalli, 2015; Kerestes, Davey, Stephanou, Whittle, & Harrison, 2014). Also, recent studies have reported decreased amygdala RSFC with the precuneus in individuals at risk for depression and in depressed adolescents (Luking et al., 2011; Rzepa & McCabe, 2016). Moreover, decreased amygdala-precuenus connectivity might be a possible biomarker of depression as it is present in the at risk population and in those with a clinical diagnosis of depression.

Further, we also found increased RSFC between the right and left amygdala seeds and the reward related brain regions of the pallidum, putamen, caudate and thalamus in the MDD vs HC groups. Increased RSFC of the left amygdala to thalamus was also observed in the MDD vs. the HR groups. As mentioned above these brain regions are involved in rewarding and emotional information processing that have been reported dysfunctional in depression. Interestingly, the amygdala to pallidum connectivity correlated with decreased consummatory pleasure in the MDD group. Thus, our results suggest that if individuals with depression have difficulty in emotion regulation this may be due to dysregulation of the affective, reward and self-referential networks in the human brain.

To sum up, this chapter presented additional results and further discussions for Chapter 7 and categorical analyses for the current Chapter 8. The results revealed dysfunctional RSFC in key resting-state networks that were also correlated with increasing depression severity and anhedonia. Moreover, we found decreased dmPFC-LOC RSFC across all groups which further correlated with depression severity in the MDD group only. This results suggest that the dmPFC-LOC connectivity might be a possible biomarker of depression as it is present in individuals with MDD diagnosis and in those before the onset of depression and in MDD, and in those in MDD it is further associated with increased behavioural symptoms of depression.

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Chapter 9

9. General discussion

The overall aim of this thesis was to describe and better understand both the neural responses to reward and punishment and the RSFC in young people at increased risk for developing depression and in those clinically depressed. Further both a dimensional and categorical approach to data analysis was used. More specifically the reported studies focused on investigating how subtypes of reward and aversion processing (anticipation, effort, consummation) are represented in the brain in those at risk and with depression and how this is related to depression symptoms and the symptom of anhedonia specifically. Moreover, the papers also investigated how those with depressive symptoms or clinical depression differ in their brain RSFC networks and how this is related to the characteristics of depression. This collection of studies is an extension to the previous work by McCabe and colleagues who examined other adult groups at risk of depression (McCabe et al., 2009, McCabe et al., 2012). However it was not known before this thesis how the neural responses to the subtypes of reward and punishment or the RSFC might be related to actual symptoms of depression in adolescents. Thus the aim of the thesis was to identify neural biomarkers and targets for psychological and pharmacological treatments and interventions (Argyropoulos & Nutt, 2013; Hasler, Drevets, Manji, & Charney, 2004; Nutt et al., 2007).

This general discussion presents an overview of the findings for each of the papers, and then considers the collective findings in relation to implications for future research.

9.1. Overview of findings

9.1.1. Paper 1: Blunted neural response to anticipation, effort and consummation of reward and aversion in adolescents with depression symptomatology

Neural reward function has been proposed as a possible biomarker for depression (Hasler et al., 2004). However how the neural response to reward and aversion and their subtypes might differ in young adolescents with current symptoms of depression is as yet unclear. Therefore, Paper 1 (Chapter 2) reported on neural responses during the anticipation, effort and consummation of rewarding and aversive stimuli in adolescents at risk of depression by the virtue of having increased depressive symptomology. Thirty three adolescents were recruited for this study. Seventeen of them scored low on the MFQ thus they were classified as low risk (LR). Sixteen participants scored high on the MFQ and were classified as high risk (HR). Participants were also assessed with questionnaires relating to depression and anhedonia. The experimental fMRI paradigm measured: anticipation, effort and consummation of reward and aversion alongside measures of the exerted effort to obtain or avoid the taste stimuli, and subjective ratings of wanting, liking and intensity of the taste stimuli. The brain data was analysed utilizing a region of interest (ROI) analysis and whole-brain analysis using SPM8.

The findings showed that when compared to the LR participants, the HR group presented with depressive symptoms on the MFQ and BDI and anhedonia on the SHAPS, FCPS, TEPS. However these young people did not present with a clinical diagnosis as investigated by the SCID. The main brain results revealed blunted responses across anticipatory, effort and consummatory phases in the HR group when compared with the LR. Specifically, the ROI analysis showed that when compared with the LR group, the HR group showed decreased responses to the anticipation of aversive cue in the pgACC and decreased neural responses in the pgACC and vmPFC for the effort reward phase

(chocolate easy-aversive easy) and the consummation of pleasant and unpleasant tastes. The whole-brain analysis further revealed decreased activity in the prefrontal cortex and the precuneus during anticipation of aversive cue in the HR vs. LR group. In the effort phase, decreased activity was found in the hippocampus and the frontal gyrus in the HR vs. LR group for chocolate easy-aversive easy contrast. Also, decreased activity was found in the anterior cingulate/frontal pole during consummation of aversive taste in the HR vs. LR group. These brain differences were observed despite a lack of differences between the HR and LR groups on their subjective ratings of wanting, pleasantness and intensity of the rewarding and aversive stimuli, and the time taken to complete the effort parts or the number of button presses made in the effort phases. Moreover, there was an association between the activation in the pgACC to the rewarding taste and anhedonia scores that was only significant in the HR group.

Overall, the results suggest that adolescents with depression symptomatology have reduced neural responses to both reward and aversion with reduced anticipation specific to aversive stimuli and reduced consummation for both rewarding and aversive stimuli. For the effort phase, the HR group had decreased activation across all the effort contrasts when compared with the LR group. This is in line with the Emotion Context Insensitivity theory of depression whereby depression is characterised by an emotional flattening to all stimuli, both positive and negative. There have been many suggestions as to why individuals at risk or with MDD present blunted responses to reward and punishment. For blunted reward processing, it is suggested that patients with MDD have deficits in the approach related systems resulting in hyposensitivity to reward (Bylsma, Morris, & Rottenberg, 2008). However, two explanations of blunted responses to negative stimuli have been proposed. Firstly, hypersensitivity to punishments where experience with previous failure activates further failure-related responses which results in increased error rates in behavioural studies (Eshel & Roiser, 2010). Secondly, hyposensitivity to punishments where MDD individuals struggle to use feedback

appropriately to improve their performance (Eshel & Roiser, 2010). Thus, in our studies the at risk individuals might be unable to adapt their responses to reward and punishment appropriately due to the hyposensitivity of the systems, which results in what looks like blunted affect across all stimuli in our task. This study suggests that interventions in young people at risk of depression, that can reverse blunted responses, might be beneficial as preventative strategies.

Discussion over individual findings of this study is presented in Chapter 2.

9.1.2. Paper 2: Decreased Neural Anticipation, Effort and Consummation of Reward and Aversion with Increased Anhedonia in Adolescents: An RDOC Dimensional approach

Recent studies have emphasized the need to investigate the neurobiological signatures across nosological boundaries of mental illness. As mentioned in the previous chapters, neural reward dysfunction has been proposed as a possible biomarker for depression however how reward dysfunction is represented in the brain across a range of depression severities is as yet unknown. Thus this study aimed at investigating the neural responses to reward and aversion across a range of young people with depression symptoms including a clinical diagnosis of depression. Comparisons between those with and without a clinical diagnosis were also performed using the traditional categorical analysis approach.

Eighty four participants were recruited for the study. All participants were assessed with the SCID. Forty three participants presented with depressive symptoms (DS) and forty one individuals were age and gender matched healthy controls (HC). Adolescents were deemed as having depressive symptoms if they scored >27 on the MFQ. Twenty seven of the participants with depressive symptoms had a current diagnosis of MDD either from their GP, a clinical psychologist or a psychiatrist. Our participants were

also assessed with a battery of measures relating to depression. The experimental fMRI paradigm measured: anticipation, effort and consummation of reward and aversion alongside with behavioural measures regarding the exerted effort to obtain or avoid the taste stimuli, and subjective ratings of wanting, liking and intensity of the taste stimuli. The brain data for the dimensional and categorical approaches was analysed utilizing the region of interest (ROI) analysis and the whole-brain analysis.

The findings showed that when compared to HC, DS individuals and those with MDD significantly differed on depression measures such as the MFQ, BDI, SHAPS, FCPS, TEPS. We also found that when compared to HC, the DS and the MDD individuals exerted significantly fewer button presses and took significantly longer to complete the effort part.

Using a dimensional approach, an association between the brain and behaviour across all subjects revealed a significant negative correlation between the BDI scores and the ventral striatum activation to the chocolate cue, but not in the subgroups. This shows that as the depression scores increased, the brain activity in the ventral striatum decreased. We also found a significant positive correlation between the scores on the TEPS anticipatory and consummatory scales and the brain activation in the ventral striatum for the chocolate cue in all subjects. This means that as the experience of pleasure decreased so did the brain activation in the ventral striatum across all subjects. The results of this illuminate the fact that neural responses to reward are on a continuum and not simply binary, rather the blunting increases with increasing depression severity. This is important as it is consistent with the notion that symptoms of psychiatric disorders are on a spectrum and not ‘’all or nothing’’ which is what is currently expected for the DSM criterion. Our results further provide support for the neural response to reward as a possible biomarker of illness. Knowing that increasing symptoms are related to the biological decreased neural response suggests that finding ways to prevent further neural deficits could be a preventative measure, whilst increasing neural responses to reward

could be a possible marker of treatment response, either pharmacological or psychological.

When using a ROI analysis the DS individuals presented blunted responses for the anticipation of reward in the ventral striatum and the amygdala ROIs and to the anticipation of aversion in the ventral striatum ROI compared to HC. Moreover, there was a significant correlation between the ventral striatal activation to the chocolate cue and the TEPS consummatory scale in the DS individuals only and a significant negative correlation between the SHAPS scores and the brain activation in the amygdala ROI during the chocolate taste condition in the DS group only. This shows that the neural responses to anticipation and consummation of reward are directly related to increasing anhedonia.

The brain ROI results revealed that when compared to HC, the MDD individuals presented blunted responses for the anticipation, effort and consummation of reward and aversion. Specifically, the MDD individuals showed decreased responses to the anticipation of rewarding and aversive cue in the ventral striatum ROI when compared with HC. Relative to HC, the MDD individuals showed decreased brain responses in the hippocampus ROI for the effort to obtain rewarding taste (chocolate hard-chocolate easy) and in the insula ROI for the effort to avoid aversive taste (aversive hard-aversive easy). Relative to HC, the MDD individuals also showed decreased responses to the consummation of rewarding taste in the ventral striatum, amygdala and insula ROIs and decreased brain responses to the consummation of aversive taste in the amygdala ROI. Also, the ventral striatal activation during the chocolate cue correlated with the TEPS consummatory in the MDD individuals. Moreover, there was a significant positive correlation between the ventral striatal activation to the chocolate cue and the TEPS consummatory scale in the MDD individuals. Furthermore, the amygdala activation during the chocolate taste condition correlated with the SHAPS and the FCPS scores and

the TEPS anticipatory scale in the MDD group suggesting that as anhedonia increases the brain activation in amygdala to the consummation of chocolate decreases.

Overall, the correlational analysis showed that depressive symptoms are directly associated with decreased processing in the key reward related brain regions such as the ventral striatum. When looking at the brain responses in depression symptoms, those with DS have deficits mostly in the anticipatory and the effort aspects of reward and aversion processing. However, the categorical approach further revealed that the MDD subjects have deficits in all aspects of reward and aversion processing. Findings of these studies emphasize the importance of studying mental illness both categorically and dimensionally to derive a holistic understanding of the core mechanisms implicated in a scope of clinical entities. This is because a dimensional approach is more likely to reveal possible biomarkers of an illness as it looks at the spectrum of symptoms ignoring the current problematic classification system. On the other hand, a categorical approach is more likely to identify state markers of an illness as it looks at those who are currently ill compared to those not ill.

Discussion over individual findings of the study is presented in Chapter 4.

9.1.3. Paper 3: Decreased anticipated pleasure correlates with increased salience network resting state functional connectivity in adolescents with depressive symptomatology.

Recent developments in neuroscience suggest that cognitive and emotional functions can emerge from an interaction of distributed brain areas operating as large-scale brain networks (Smith et al., 2009). This novel approach provides a new insight into the neural basis of cognition and emotion by characterizing them in terms of temporal relationships between activations in different brain regions, known as functional

connectivity. This allows looking into functional brain organization from a different and a broader perspective. Interestingly, it has been shown that patients with MDD and those at familial risk for MDD have abnormalities in their RSFC networks such as the SN the CEN and the DMN (Clasen, Beevers, Mumford, & Schnyer, 2014; Sheline, Price, Yan, & Mintun, 2010). However, no studies are available that have looked at the RSFC in young people at risk of depression due to increased depressive symptoms. Therefore, Paper 3 reported abnormalities in the RSFC between the key brain regions implicated in the aetiology of depression.

Thirty five adolescents were recruited for this study. Eighteen of them scored low on the MFQ thus they were classified as LR. Seventeen participants scored high on the MFQ and were classified as HR. Participants were also assessed with a battery of measures relating to depression. The experimental fMRI paradigm consisted of five minutes resting-state data acquisition.

The findings showed that when compared to the LR participants, the HR group presented with depressive symptoms on such measures as the MFQ and BDI and anhedonia on the SHAPS, FCPS, TEPS, despite no clinical diagnoses. The main brain results revealed that, when compared to the LR, the HR group showed decreased RSFC between the amygdala seeds and the pgACC, hippocampus and the precuneus. There was also decreased RSFC in the HR vs. LR group between the pgACC seed and the pallidum/putamen, thalamus and between the dmPFC seed and the precuneus and the lateral occipital cortex. Increased RSFC in the HR vs. LR group was observed between the pgACC seed and the prefrontal cortex and between the amygdala seed and the temporal pole. Moreover, there was a negative association for the increased functional connectivity between the pgACC seed and the insula/orbitofrontal cortex and the anticipation of pleasure in all subjects.

Overall, the findings are the first to show that adolescents with depression symptoms have dysfunctional RSFC between the seed-ROI in the key RSFC networks such as the SN, CEN and the DMN (precuenus) and brain regions related to reward processing. Also, as increased connectivity between the pgACC seed and the insula/orbitofrontal cortex correlated with decreased ability to anticipate pleasure, it is suggested this might be a mechanism underlying the risk of experiencing anhedonia, a suggested biomarker for depression.

Discussion over individual findings of the study is presented in Chapter 6.

9.1.4. Paper 4: Increasing Depression and Anhedonia Severity Correlates with Decreasing Resting-State Functional Connectivity in Dorso-Medial Prefrontal Cortex in Adolescents: An RDOC Dimensional Approach

Recent studies have emphasized that dimensional approach to studying symptoms of depression may be beneficial given the heterogeneous nature of depression. As discussed in the previous chapters, studies have indicated that the RSFC investigations might aid biomarker discovery for depression. Thus the aim of this study was to investigate the RSFC in young people across the spectrum of depression symptom severity.

Eighty-six participants were recruited for the study. Forty-four participants presented with a range of depression symptoms (DS), including 27 participants with a current diagnosis of major depressive disorder (MDD) and 42 individuals were age and gender matched healthy controls (HC). We measured mood and anhedonia in all subjects and correlated this with results from the RSFC investigation in the ROI of the key resting-state networks implicated in the aetiology of depression: amygdala, nucleus accumbens, pgACC-SN; and dmPFC- CEN.

Analysis revealed that RSFC between the dmPFC seed and the precuneus negatively correlated with scores of depression severity and positively correlated with the anticipation of pleasure in all participants. Further analysis revealed that these effects were driven by those with depression symptoms. There also was a positive correlation between dmPFC seed and the frontal pole and depression severity in all participants. We also found a negative correlation between the dmPFC seed and the ACC/paragingulate RSFC and the anticipation of pleasure in all participants. These correlations were non-significant when the participants were separated to the DS and the HC.

There also was decreased RSFC between the dmPFC seeds and the LOC and the precuneus and between the pgACC seed and the superior/middle frontal gyrus and the postcentral gyrus in the DS vs. HC group. We also found increased RSFC between the dmPFC seed and the ACC/Paracingulate gurus and the frontal pole and between the nucleus accumbens seed and the precuneus and between the pgACC seed and the limbic structures in the DS vs. HC groups.

Overall, the findings show that individuals with a range of depressive symptoms have dysfunctional RSFC in key depression related networks. Further as we find that this dysfunction correlates with depression severity and pleasure scores, this might be the mechanism underlying the risk of depression and perhaps even anhedonia, a suggested biomarker for depression.

9.2. Comparison of results across Papers

In this section I will discuss how findings for each of the papers relate to one another. I will firstly compare task dependent fMRI findings between Papers 1 and 2. The aims of these studies were similar and they used the same experimental paradigm and a similar data analysis protocol, thus allowing a direct comparison. I will then compare

RSFC findings between Papers 3 and 4. The aims of these studies were also similar and they used a similar protocol for data analysis thus allowing a direct comparison.

9.2.1. Blunted neural responses to anticipation, effort and consummation of reward and aversion in individuals with depression symptomatology and clinical diagnosis of depression: comparison of Papers 1 and 2

As mentioned in section 9.1.1., the main aim of study 1 was to investigate how different aspects of reward and aversion processing (anticipation, effort, consummation) in the brain relate to depressive mood in adolescents without a clinical diagnosis of depression. The second study, mentioned in section 9.1.2., also aimed at investigating how different aspects of reward and aversion processing in the brain relate to depressive mood but in this study examined individuals with a range of depressive symptoms (with and without a current diagnosis of depression).

There were significant differences on the behavioural measures of depression and anhedonia between the HR vs. LR participants from study 1 and the MDD vs. HC groups and the DS vs. HC groups from study 2 as expected.

The ROI analysis revealed that the HR group had decreased brain responses when compared with LR for the anticipation of aversive cue (pgACC), the effort for reward (pgACC, vmPFC) and the consummation of rewarding and aversive taste (pgACC, vmPFC). In comparison, the MDD showed decreased brain responses when compared with the HC for the anticipation of reward and aversion (NAcc), the effort for reward and aversion (hippocampus, insula) and the consummation of reward (NAcc, amygdala, insula) and aversion (amygdala). As there are different regions blunted such as the pgACC in the HR group compared to the MDD with the NAcc and amygdala being blunted once depressed this might point to different neural regions involved in different

stages of illness. The pgACC could again be more of a biomarker whilst the NAcc and amgydala could be state effects that happen once depression is established.

As mentioned above, both the HR and the MDD groups showed reduced brain activation for the effort trials in the regions of pgACC and vmPFC (HR vs. HC) and the hippocampus and the insula (MDD vs. HC). These results are interesting given the lack of behavioural differences for the effort trials in the HR vs. LR groups and significant group differences between the MDD and the HC groups for the effort trials. This means that at the brain level the differences for the physical effort exerted to obtain reward or to avoid aversion are already present in HR, whilst those who are currently depressed also have brain differences (albeit in different brain regions) but these are additionally accompanied by behavioural differences too i.e. slower response times and lower number of button presses. This suggests that neural differences are apparent before behavioural symptoms. We also found a significant correlation between the number of button presses and the time to obtain reward and the activation in the hippocampus in the HC. This suggests that the higher the activation in the hippocampus the more button presses and the less time it takes for the HC group perform the effort trials which again links the behaviour of effort to particular brain regions. This effect was non-significant in the MDD group as they showed overall decreased brain responses in the effort condition when compared with HC. The results show that the anticipatory, effort and consummatory aspects of reward processing are blunted in MDD and in the at risk individuals in both cortical and subcortical brain regions.

Furthermore, correlational analysis between the ROI and the behavioural measures of depression and anhedonia revealed associations. Specifically, decreased neural responses to the consummation of reward in the pgACC ROI were associated with increasing anhedonia in the HR group. This shows the direct brain-behaviour association and possibly suggests that adolescents at increased risk for depression might have difficulty engaging with the experience of reward. Moreover, significant correlations

between decreased NAcc ROI responses and depression and anhedonia measures were found in both the DS and the MDD groups for both reward anticipation and consummation. These correlations were driven by adolescents with depressive symptoms and to a larger degree by those with a clinical diagnosis. The results are in line with previous studies that indicate ventral striatal involvement in the anticipatory and consummatory aspects of reward and aversion processing in both healthy subjects and those with MDD (Aharon et al., 2001; Keedwell, Andrew, Williams, Brammer, & Phillips, 2005; Knutson, Adams, Fong, & Hommer, 2001) (detailed discussion over these results is presented in Chapter 4).

Studies 1 and 2 could be directly compared for aspects of reward and aversion processing (anticipation, effort, consummation), as we used different ROIs in each study as we thought to use the same would be double dipping as some of the participants in study 1 were also included in study 2. Yet we can compare the whole brain analysis. Whole brain analysis revealed that across the studies those at risk and with the MDD diagnosis had decreased brain responses to all conditions of the experimental task. The HR group did not differ from the HC on the anticipation and consummation of rewarding stimuli but the DS and MDD groups did, which could be due to a small sample size (N=16) in study 1. However, regions such as the PCC and precuneus were reduced across all groups the HR, MDD and DS groups vs. HC for the anticipation of aversion, which suggests power was not an issue for detecting differences. The PCC and the precuenus are involved in the processing of episodic memories and reflection over the self (Nejad, Fossati, & Lemogne, 2013). The fact that these brain regions are decreased across all groups for the anticipation of aversive cue, might suggest these brain regions as a possible trait marker. Perhaps therefore when anticipating something negative the adolescents with depressive symptoms internalise the event more and relate it to themselves more than those with no symptoms of depression. Moreover, there were also decreased brain responses in the effort trials to obtain reward or aversion in the HR,

MDD, DS vs. HC groups. The differences in the HR group were revealed in similar brain regions to those found for the anticipation or consummation of reward or aversion (MFG, STG, SFG, frontal pole) in the HR vs. HC comparison. These brain regions were also found decreased in the DS group with additionally decreased activation in the insula, ACC, putamen and the precuneus in the effort to obtain reward, and in the MDD group in the ACC/paracingulate gyrus, PCC and the precuneus also in the effort to obtain reward. As mentioned above and in the discussions for the individual chapters, these brain regions have all been implicated in variety of salient, reward, effort and cognitive information processing. Moreover, measures of physical effort are thought be related to measures of motivation (Berridge, 2004), and decreased motivation is suggested to be one of the dysfunctional aspects of reward processing in depression. Thus, decreased responses on the effort trials might possibly relate to problems with motivation and further related to motivational anhedonia. This could be investigated if we had more sophisticated measurements of motivation and anhedonia questionnaires to correlate with brain processes. Moreover, it is of interest that the whole brain analysis revealed a lack of group differences in the reward or aversion processing in the ventral striatum in the HR vs. LR and the DS vs. HC groups but reduced ventral striatal activation in the MDD group only. The lack of any deficit in the HR group is consistent with another study that used a similar paradigm and examined young people with a familial risk of depression but no personal experience of depression (McCabe, Woffindale, Harmer, & Cowen, 2012), and is opposite to the current study results in the MDD group and to a previous study results examining those recovered from depression (McCabe, Cowen, & Harmer, 2009). This possibly suggests that the ventral striatal differences in this study are only detectable after having experienced MDD thus are rather a state than a trait marker of depression. Further, longitudinal studies are necessary to clarify this.

In conclusion, studies 1 and 2 showed that dysfunction in the neural processing of the anticipatory, effort and consummatory aspects of reward and aversion processing is

present before the onset of depression and in individuals with depression diagnosis. Furthermore, dimensional analysis showed significant correlations between decreasing brain activations in the key regions implicated in reward and aversion processing and increasing depression and anhedonia symptoms. These findings emphasize the importance of studying mental illness both categorically and dimensionally to help us understand which features may be state or trait markers.

9.2.2. Resting-state functional connectivity in individuals with depression symptomatology and a clinical diagnosis of depression: comparison of Papers 3 and 4

As mentioned in section 9.1.3., the main aim of study 3 was to investigate the RSFC in adolescents with increased depression symptomatology but without a clinical diagnosis of depression (HR). The aim of study 4, mentioned in section 9.1.4., was to investigate RSFC in a sample of adolescents with a range of depression severities (DS). Further, the addendum to study 4, separated the DS individuals to those with a clinical diagnosis (MDD) and those with increased depression symptomatology (HR).

This section will discuss similarities and differences between the RSFC results for studies 3 and 4 and only results that were similar across all resting-state studies presented in this thesis. Any other results have already been discussed in the individual chapters.

9.2.2.1. Comparison of the RSFC results between Papers 3 and 4

Studies 3 and 4 revealed decreased RSFC between the dmPFC seed and the cuneal cortex/precuneus in the HR vs. LR groups and in the DS vs. HC groups. The dmPFC is a part of the CEN and it has been implemented in cognitively demanding tasks including attention and response inhibition (Janssen, Heslenfeld, van Mourik, Logan, &

Oosterlaan, 2015). The role of the precuneus has been already discussed above. Many studies have reported decreased RSFC between the CEN and the DMN in MDD and in at risk individuals (Mulders et al., 2015). It has been suggested that decreased connectivity between these networks might contribute to patients' difficulties to disengage from self-referential processes that might lead to negative thoughts (Sheline et al., 2010). Interestingly, we reported a correlation between the dmPFC seed with increasing depressive symptoms and anhedonia in all participants, the DS and the MDD groups. No brain-behaviour correlations for the dmPFC seeds were found in the HC group. This suggests that those with depression symptoms were driving the correlation and that as connectivity increases symptoms increase. Interestingly, there was also decreased RSFC between the dmPFC seed and the LOC in all those with depressive symptoms (HR vs. LR, MDD vs. HC and the DS vs. HC groups). The LOC is a part of the Lateral Visual Network and the decreased connectivity between the dmPFC seed and the LOC might indicate impaired control over processing of visual stimuli including those of a negative valance thus contributing to increased processing of negative information

Both of the studies also revealed decreased and increased RSFC of the pgACC seed with many brain regions constituting different networks across all groups. For example, decreased pgACC-thalamus, pallidum, putamen connectivity was reported for the HR vs. LR groups and decreased pgACC-SFG connectivity reported for the DS vs. HC groups. On the other hand, increased pgACC -ACC, vmPFC, lOFC, insula connectivity was reported for the HR vs. LR groups and increased pgACC- thalamus, putamen, caudate, NAcc connectivity in the DS vs. HC groups. The pgACC is thought to be a node of communication between the dorsal ACC important for error detection and the more ventral ACC important for detection, processing and regulation of salient stimuli, which might be disrupted in our symptomatic participants (Ball, Stein, & Paulus, 2014). Moreover, we also found the brain-behaviour association reflected as correlations between the increased pgACC-insula RSFC and decreased ability to anticipate pleasure in

the HR group, and the increased pgACC-precuneus RSFC and decreased consummation of pleasure. These results might possibly reflect a mechanism underlying the experience of anticipatory and consummatory anhedonia.

9.2.2.2. Comparison of the RSFC results across all resting-state studies presented in this thesis

As already mentioned in the discussion of Chapter 8, we consistently observed decreased RSFC between the left dmPFC seed and the LOC in the DS vs. HC groups, the MDD vs. HC groups and the MDD vs. HR groups. Decreased dmPFC-LOC RSFC was also observed in a separate categorical analysis in chapter 6. As already mentioned in the previous chapters, the LOC is a part of the lateral visual cortical areas and has been involved in the processing of visual information (Bermpohl et al., 2006; van Wingen et al., 2011), whilst the dmPFC is involved in cognitive control and is a part of the CEN (Bressler & Menon, 2010). Moreover, there was a significant correlation between the dmPFC-LOC RSFC and increasing depression in the MDD group and non-significant in the HR group. This suggests that at the brain level, the dmPFC-LOC RSFC is already dysfunctional before the onset of clinical depression and as depression increases, it is further associated with the behavioural symptoms of depression suggesting this connectivity as a biomarker for depression. Furthermore, given the functions of the dmPFC and the LOC these results indicate that individuals with depressive symptoms and clinical depression might have difficulties with control over emotional visual cues which further increases the experience of depressive symptoms. However, longitudinal studies are needed to further investigate how the RSFC networks can better identify those at risk for depression and those who will progress to a clinical diagnosis over time.

9.3. Strengths and limitations of the current studies

The current studies specifically recruited adolescents. This is an important improvement over previous research, as the majority of studies investigate adults with MDD. Adolescence is a period of many changes including those related to the brain development, cognitive and emotional changes as well as psychological and social ones. The vulnerability to depression increases markedly during adolescence as some of these changes expose young people to additional life stresses e.g. school change, failures of romantic relationships, disappointments, which might contribute to developments of mental health problems including MDD (Davey, Yucel, & Allen, 2008). Thus it is crucial to investigate this group of people to better understand the neural circuits underlying the risk of depression in adolescents to possibly develop preventative strategies. Moreover, findings of studies focusing on adolescents might allow more accurate diagnosis and possibly more personalised psychological and pharmacological treatments. However, the current studies investigated individuals at particular age-related points in their life but in reality changes might be developing at different times in life. Therefore, there is still a need for longitudinal research with a large sample size that will follow individuals from early stage of development to adulthood and will monitor behavioural and neural changes over time.

Another strength is that our participants were recruited from different sources such as schools, universities, adolescents' mental health clinics, GP clinics, and via adverts placed in the surrounding area or on social media. Thus, the sample is likely to reflect a typical population of young people living in the local community in the South of England. Moreover, all participants were screened with standardised depression and anhedonia questionnaires and were also assessed with the structured diagnostic assessment (SCID) to confirm MDD diagnosis and to exclude any other mental health problems. Thus the recruitment of our participants was of high standard.

According to our knowledge, the experimental model used in the current studies (1, 2) is the first to investigate the separate subtypes of reward processing at the neural level in relation to depression and anhedonia. Anhedonia is a multi-dimensional concept with anticipatory and consummatory aspects being the most widely examined in depression (Der-Avakian & Markou, 2012). Recent results of behavioural studies in depressed patients have suggested another possible conceptual dimension of anhedonia that of effort expenditure for reward (Treadway, Buckholtz, Schwartzman, Lambert, & Zald, 2009). Moreover, many suggest that depression is characterised by a bias toward the processing of negative information (Roiser, Elliott, & Sahakian, 2012). Thus the current model aimed at investigating separate subtypes of reward in one task by looking at the anticipatory, effort and consummatory aspects of both reward and aversion processing.

Furthermore, the current experimental model uses primary rewards (food) to measure different aspects of reward processing and how this might be related to the symptoms of anhedonia measured behaviourally on questionnaires. This is important for several reasons. Firstly, the use of primary rewards compared to secondary monetary rewards in our work allows a more translational approach given that most preclinical studies use foods as incentives (Rizvi, Pizzagalli, Sproule, & Kennedy, 2016). Secondly, primary rewards tend to tap into more objective rather than subjective responses. This is because the experience of food, as a main source of nutritional support to an organism, is thought to be primary and objective unlike money which might have a different meaning to people depending on their life experience, values, beliefs and age (Rizvi et al., 2016). Following on from this, therefore the use of primary rewards is also more suitable for developmental and longitudinal studies. Many researchers have argued that the use of secondary rewards in children and adolescent is too abstract and symbolic and it is not built to engage the interest of the younger population (Helfinstein et al., 2013), while

humans are familiar with the concept and pleasurable experience of food from the day they are born.

Moreover, we also incorporated a dimensional approach to data analysis in study 2 and 4. Such an approach was aimed at allowing the investigation of neural responses that are related to reward, punishment and the symptoms of anhedonia and depression severity on a continuum instead of a categorical approach. This analysis was intended to capture neurobiological signatures of reward function and dysfunction across nosological boundaries of depression (Insel et al., 2010). Thus the current studies present findings for both categorical and dimensional analyses.

The main limitation of studies 1 and 3 was the sample size of individuals with elevated depressive symptoms (N=16 study 1; N=17 study 2). Murphy and Garavan (2004) showed that to achieve the required power of 80% for fMRI studies, a sample size of 24-25 participants is required (Murphy & Garavan, 2004). According to this criterion, study 1 is underpowered. However, being aware of this problem, we utilised conservative thresholding for fMRI findings (i.e. all fMRI results were family-wise error corrected for multiple comparisons ($p<.05$) and thresholded at $p=.001$). Moreover, we based our ROI hypotheses for brain activations for the current studies on results obtained from previous studies that either investigated a sample of people similar to ours or used a similar experimental model. This way we were able to build predictions for the current study and assure reliability of the findings.

The main limitation of study 2 and 4 was that the majority of individuals in our MDD sample (N=27) were either medicated (N=14) or had a history of antidepressant treatment (N=6) during the study. This is a potential problem as previous research has suggested a modulation of the brain activations in response to various psychological tasks as a function of pharmacological treatments (Dean et al., 2016)(Pringle, McCabe, Cowen, & Harmer, 2013). A recent review that looked at the effects of psychopharmacological

treatments (SSRI, NRI) in MDD individuals and healthy subjects revealed parallel findings of decreased activation in the limbic regions in response to negative stimuli in both healthy and depressed individuals (Wessa & Lois, 2015). A comprehensive review that looked at the effects of pharmacological treatment specifically on reward processing is as yet not available, however individual pharmacological studies that utilize the current model are reported. For example, from our own lab, studies have shown anti-depressants can reduce the brains response to reward and aversion (McCabe, Huber, Harmer, & Cowen, 2011; McCabe & Mishor, 2011) whilst other drugs that modulate dopamine and noradrenaline can increase activity (Dean, Horndasch, Giannopoulos, & McCabe, 2016). Further, we and others have also shown that RSFC can be modulated by antidepressant treatments (McCabe & Mishor, 2011; Rzepa, Dean, & McCabe, 2017) although perhaps not ideal we did however control for this in our studies by adding medication as a confound in the analyses.

9.4. Thesis specific future directions

9.4.1. Independent Component Analysis for resting state studies

One of the most common methods to investigate RSFC is the seed-region based correlation approach that we used in our studies. It incorporates timeseries of voxels that significantly correlate with the mean timeseries of voxels within a priori defined seed region of interest (ROI) (Zuo et al., 2010). However, the biggest limitation of this method is that the obtained components are highly dependent on the choice of the seed-ROI: the shape, the size and the location. Moreover, as this method only allows analyse of the relationship of the pre-defined seeds, obtaining a picture of the entire pattern of changes in the brain connectivity is limited.

To overcome this, Beckmann and colleagues (2005) were among the first to develop a method to identify resting-state networks in a purely data-driven manner by

using the Independent Component Analysis (ICA) (Beckmann, DeLuca, Devlin, & Smith, 2005). The ICA separates spatial and temporal signals from the mixed BOLD signals and looks at the most pronounced independence among these signals. As this multivariate approach is hypothesis-free, it allows characterizing various types of fluctuations and further delineating it to what is known as noise (e.g. physiological effects such as respiratory or cardiac fluctuation, motion or scanner artefacts) and components of interests, which could have been disregarded by the seed-based approach (Beckmann et al., 2005). Also, the ICA is capable of detecting interacting networks of remote regions by taking into account multiple voxels relationship, which the seed-based approach is unable to achieve.

Interestingly, results from the seed-based and the ICA methodologies are generally similar (Joel, Caffo, Zijl, & Pekar, 2011). Yet, a recent review on the RSFC in MDD has reported that at least regarding the DMN network, the ICA methods seems to reveal mostly increased functional connectivity, while the seed-based analysis presents increased and decreased functional connectivity results (Mulders, van Eijndhoven, Schene, Beckmann, & Tendolkar, 2015). This, however, was not the case for other networks such as the SN or the CEN where mixed results are presented for both methods. It is crucial to mention that the studies selected for the Mulders et al., (2015) review differed in their recruitment on depression symptom severity, age range and antidepressant medications being taken. This is thus a limitation of the review and makes it difficult to compare across their studies.

Nevertheless, in future it would be worth investigating whether applying the ICA in the current resting-state studies would deliver similar results to the ones obtained with the seed-based method.

9.4.2. fMRI statistical correction and the limitations of the statistical correction applied in many fMRI studies discussed in the light of the Eklund, Nichols and Knutsson (2016).

In recent years a lot of attention has been given to the paper "Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates", by Eklund, Nichols and Knutsson (2016) (Eklund, Nichols, & Knutsson, 2016). This work focuses on looking at how reliably different correction procedures, that are widely used in fMRI research, control for multiple testing.

To investigate this, the researchers tested multiple testing procedures that are implemented in some of the main software packages for different testing parameters (i.e. cluster defining threshold of p=0.01 and p=0.001). The investigated software were the SPM, FSL, AFNI and a permutation-based approach implemented in the BROCCOLI software. The researchers used a large set of resting-state data that they fitted a model of a task to. It is important to highlight here that applying an artificial task model to resting-state data should not reveal any valid activations. The researchers assumed that finding clusters in more than 5% of all would indicate that the used multiple testing correction method led to higher false positives than generally accepted (Eklund et al., 2016).

The main results revealed that a cluster defining threshold of p=.01 in a parametric analysis led to poor control of FWE (between 4-50%). However, a cluster defining threshold of p=.001 improved the FWE control (between 0-25%). This was regardless of software packages. Moreover, a cluster defining threshold of p=.001 was not sufficient if the cluster extent was not estimated but an arbitrary ad-hoc cluster extent was chosen instead e.g. when using a cluster size of k=10 was chosen then a cluster size of k=10 voxels or greater were identified as significant. In that case the FWE increased to 70% and this method should not be used as a method of controlling for multiple testing. Non-parametric permutation tests seemed most reliable and tended to control for the type

I errors at the expected 5% rate. However, even with the permutation test, the skewedness of data contributed to increased FWE in a case of 1-sample t-test (Eklund et al., 2016). Moreover, the permutation tests can be problematic where small effect size is expected, as the nonparametric tests have lower sensitivity to detect small effects than parametric tests (Chen, Taylor & Cox, 2015). Furthermore, the study also revealed a reason for the limitation of the use of parametric methods implemented in the mentioned software i.e. the assumption of the null distribution for fMRI data does not take the assumed Gaussian shape into consideration. This is problematic because the activity of voxels that are closer together is even more similar than assumed before. However, voxels that are further apart differ in the signal similarity depending on their location in the brain, the tissue type and the head size. This finding is important as it possibly contributed to the high rate of false positives in their study and also explains why more conservative thresholds for cluster forming, that tend to produce smaller clusters, bring more reliable results.

The current thesis implemented the SPM8 and FSL software to analyse task-depended and resting state data, respectively. The analysis of the thesis data was done in the year of 2015 and 2016, thus the versions of the software are not updated to account for one of the Eklund et al., (2016) findings related to the random field theory and the Gaussian shape (the newest versions of the software are now updated and recommended for use). However, the task dependent results were thresholded at p=.001 with a cluster threshold of k=30 and whole-brain cluster corrected at $p<0.05$ FWE for multiple comparisons. Moreover, thresholding at p=.001 with a cluster threshold of k=30 was an attempt at reducing both Type I and Type II errors in the results (Lieberman & Cunningham, 2009). Moreover, this particular design was run in the previous studies in our laboratory (McCabe & Mishor, 2011), thus it is believed that it is less likely to attribute real activation to noise (Type I errors are not likely to replicate across multiple studies) and more likely instead to miss effects by increasing the p threshold. Therefore the increase in the cluster threshold to k=30 was done in an attempt to rebalance the Type

I and Type II error rate (Lieberman & Cunningham, 2009). In terms of the region of interest whole brain resting state data analysis, a more lenient cluster defining threshold of $z > 2.3$ voxel-wise thresholding and a family-wise error-corrected cluster at significance threshold of $p < 0.05$ was applied. This might have allowed for a higher number of false positive thus the final results were subsequently Bonferroni corrected for the number of ROIs examined which gave $p < 0.016$ (i.e, $p < 0.05$ Bonferroni corrected for the 3 networks of interest: amygdala, dmPFC, pgACC) (Davidson, Irwin, Anderle, & Kalin, 2003). Both of the methods of controlling for multiple comparison issue that were used in the thesis aimed at minimizing the false positive rates. However, it is important to emphasize that even though the attempts have been made, there is still a possibility of false positives in the reported results.

Overall, the Eklund et al., (2016) study revealed that certain statistical correction methods in fMRI research are flawed. Many improvements and practical recommendation have been suggested to date to minimize the observed issues. Nevertheless, the researchers should be more aware of the issues and report and interpret their data with more caution. Furthermore future replication of data should allow confidence in the results.

9.5. Future directions and clinical implications

9.5.1. Reconsidering the definition of anhedonia

Anhedonia is defined as the inability to experience pleasure from activities usually found enjoyable, e.g. exercise, hobbies, singing, sexual activities or social interactions (AmericanPsychiatricAssociation, 2013). This definition was introduced by Theodule Armand Ribot in 1896 and has not changed much since (Treadway & Zald, 2011). However, clinical observations indicate that e.g. patients with anhedonia might present with low motivation to obtain reward rather than low experience of pleasure once reward is obtained (Forbes & Dahl, 2012) indicating that the concept of anhedonia might

be more complex than the definition assumes. The distinction between different aspects of anhedonia is crucial especially in studies that investigate the relationship between the neural pathways and specific symptoms of depression.

Over recent years, empirical studies have supported a heterogeneous conceptualisation of anhedonia pointing toward more defined deficits of reward processing such as the anticipatory, motivational, consummatory and learning aspects. Two excellent reviews on the neurobiology of anhedonia have indicated that the distinct concepts are found to rely on separate neural systems however, a window for an overlap has also been suggested (Der-Avakian & Markou, 2012; Treadway & Zald, 2011). This is mostly due to the fact that depression is multi-facet and very often comorbid with other disorders such as e.g. anxiety or obsessive- compulsive disorder (Hirschfeld, 2001; Overbeek, Schruers, Vermetten, & Griez, 2002). Of course it could also be because measuring the separate aspects of anhedonia is not that straight forward. Moreover, researchers suggest that a lack of an accurate definition is possibly one of the main reasons for many inconsistencies in the literature as the majority of experimental models are based on conceptual assumptions which, if not clearly defined, might lead to mixed findings (Forbes & Dahl, 2012; Treadway & Zald, 2011).

Reconsidering the definition of anhedonia in the light of empirical findings and clinical observations could improve diagnostic tools, and strengthen the foundation of research on reward dysfunction in MDD, this work is ongoing in our lab.

9.5.2. The role of age and gender

Age and gender have been suggested as developmental factors in the prevalence of MDD. It has been identified that the occurrence of MDD increases almost 5 times between the ages of 12 and 18 with around 20% of adolescents experiencing their first depressive episode around this age (Hankin & Abramson, 1999). Furthermore, teenage

females tend to report twice as many depressive episodes as teenage males (Hankin, Mermelstein, & Roesch, 2007). Reports suggest that this 2:1 ratio is constant through adulthood but other report indicate that the ratio even increases for females (3:1) in the adulthood (Lewinsohn, Pettit, Joiner, & Seeley, 2003). These data suggest that both age and gender are risk factors but still little is known about how those factors contribute to increased vulnerability in youth.

There is however, evidence from structural MRI and fMRI studies suggesting age and gender related differences in the brain volume and the processing of rewarding information. For example, on average the male brain is 9-12% bigger in the volume than the female brain (when accounted for the body size). Also, the total brain volume for females peaks around the age of 10 and the total brain volume for males peaks around 4 years later (Lenroot & Giedd, 2010; Sowell et al., 2007). Moreover, there are many regional differences such as a bigger caudate or hippocampal volume in females and a bigger amygdala volume in males. These differences are further associated with higher levels of estrogen (female sex hormone) receptors in the hippocampus and higher levels of androgen (male steroid hormone) receptors in the amygdala (Forbes et al., 2010). Moreover, Galvan and colleagues (2006) found that both healthy adolescents and adults showed enhanced NAcc activity to rewarding stimuli, whereas children showed decreased activation in the NAcc in response to the same rewards (Galvan et al., 2006). Furthermore, the same adolescents showed that the OFC activity was similar to the activity found in children and different from the activity found in adults. Findings of this study suggest that maturation of the NAcc precedes that of the OFC and possibly suggests that the bottom-up emotional processing in the subcortical regions is enhanced in adolescents relative to the less effective top-down regulation from the prefrontal regions. However, it is crucial to emphasize that the changes in the brain size and the processing of various types of information might still be happening at individual rates, which needs to be taken into account while addressing developmental studies. Nevertheless, future studies suggest

need to be aware that there are age and gender differences associated with both the structure and the function of the brain that might be contributing to increased risk of developing depression (Lenroot & Giedd, 2010; Sowell et al., 2007).

9.5.3. The comparison of current task-dependent and resting-state studies

This thesis has presented results of studies that looked at the brain activations for different task conditions and, for what is considered task-independent, resting-state functional connectivity studies. While task-dependent studies investigate activations in separate brain regions in response to a specific condition, the resting-state studies look at the temporal correlations of remote brain regions constituting networks. However, it would be of interest to investigate the relationship patterns of connectivity in distributed networks during performance on tasks. More specifically in terms of our studies, it would be worth testing whether individuals at risk of depression or with MDD have different RSFC patterns during anticipation, effort and consummation of rewarding and aversive stimuli compared with HC and how this is related to anhedonia symptoms. This can be achieved with a dual regression method. Thus future research should take advantage of this method which can deliver maps of correspondence for brain regions responding during task conditions and brain regions connecting in the state of rest thus providing a new way of looking at the dynamics of the brain.

9.5.4. Longitudinal research and the Research Domain Criteria initiative

Depression is a heterogeneous disorder with an extensive array of symptoms and subtypes. The direct causes of MDD are as yet unknown but there are many risk factors that have been identified in cross-sectional studies (genetics, biological, stress, environment, depressive mood, personality) (Galambos, Leadbeater, & Barker, 2004). For example, it is well known that children of parents with depression are 30-40% more likely to develop depression themselves (Beardslee, Versage, & Gladstone, 1998).

However, it is still unclear why some of those children will never develop depression and others will. Moreover, as mentioned in the general introduction, studies looking at neural function in healthy and depressed adolescents often report mixed results, which might be due to different ages of recruited participants. For example, one study found reduced ventral striatal activation in young females at familial risk for depression aged 10-14 years for monetary reward anticipation, (Gotlib et al., 2010) while another study that recruited females at familial risk aged 16-21 did not (McCabe et al., 2012). Another explanation of the differences might be different experimental paradigms (secondary vs. primary reward) but it is also possible that those in the McCabe et al., (2012) study who have not experienced depression by the age of 21 are a highly resilient sample. This is just one example of many debates that result from cross-sectional studies that longitudinal studies can help to resolve. Thus it is essential that future research implement longitudinal studies that will follow the same individuals from as early as puberty to adulthood. Such temporal observations will be able to address developmental changes in different domains of interest (e.g. behavioural changes, neural development, reward functioning) and significantly add to the research of biomarker discovery.

One of the aims of studies 2 and 4 was to respond to a recent RDoC call and to incorporate the dimensional approach to data analysis. This approach allowed investigating the relationship between the different aspects of reward processing and the symptoms of depression across depression severities (refer to chapter 1 and 4 for further discussion). Future studies should aim at adopting the RDoC initiative to a broader scale by examining how the symptom of anhedonia is related to the neural response to reward (anticipation, effort and consummation) across psychiatric disorders. Such an approach could allow findings from neuroscience and genetic research that reveal the link between the behaviour and the underlying biological system (Insel et al., 2010).

9.6. Clinical implications

Psychological treatments have been developed to treat depression such as Cognitive-Behavioural Therapy and Interpersonal Therapy (Shapiro et al., 1994). Outcomes of these therapies seem to be beneficial for some, with others report no improvement in symptoms (Shapiro et al., 1994)). It has been suggested that the success rate might be highly dependent on how detailed the phenotypic description of depressed individuals is and what the main symptoms are (Butler, Chapman, Forman, & Beck, 2006). Thus, developments of psychotherapeutic techniques that target specific symptoms are of particular interest. Behavioural Activation (BA) has been suggested as a suitable therapy for those with general anhedonia and in particular pronounced motivational anhedonia. The ultimate goal of this therapy is to provide patients with tools that allow them to distinguish between rewarding and non-rewarding behaviours and make choices that are likely to increase the engagement with pleasurable experiences (Dimidjian et al., 2017). One fMRI study that looked at the effects of the BA treatment on reward processing reported increased responses in the dorsal striatum to the anticipation of reward in the MDD group when compared to HC after the BA treatment, which was also associated with a decrease in depressive symptoms (Dichter et al., 2009). However how BA works is not clear, future work could combine the neural response to reward and BA treatment to see if we can predict those who would respond best to this treatment based on their initial neural response.

Many patients also fail to respond to pharmacological treatments. The most commonly prescribed antidepressants, such as the selective serotonin reuptake inhibitors (SSRI), often do no address anhedonia related symptoms (Calabrese et al., 2014), and even might reduce responsiveness to rewards (McCabe, Mishor, Cowen, & Harmer, 2010). Instead dopaminergic or norepinephrine-dopamine reuptake inhibitors (NDRI) have been suggested as more appropriate for targeting symptoms of anhedonia. (Treadway & Zald, 2011) in their review pointed out that the NDRI antidepressant

bupropion is superior over SSRI in treating anhedonia, especially motivational anhedonia. In fact, a recent study by our lab (Dean et al., 2016) that looked at the effects of 7 days bupropion/placebo treatment on reward and aversion processing in healthy individuals showed that when compared to placebo treatment, bupropion increased neural activation to reward and aversion during anticipation, effort and consummation. The authors suggested that such responses might suggest a mechanism of action that could promote reward-seeking and aversive-avoidant behaviours in patients with depression. This is one of many examples (Cassano et al., 2005; Corrigan, Denahan, Wright, Ragual, & Evans, 2000) that suggest that knowing the mechanism of action of particular drugs could help us tailor drugs to specific symptoms of depression for more successful treatment outcomes.

In summary, the clinical implications of our studies are that the neural responses to reward could be used to identifying targets for prevention and personalised treatments.

9.7. Conclusions

The studies reported in this thesis show that regardless of data analysis approach, the young individuals with depressive symptoms had decreased brain responses to the anticipation, effort and consummation of reward and aversion in key brain regions involved in reward and emotional processing such as the ventral striatum and amygdala. Furthermore, the greater the experience of anhedonia the greater the neural blunting to reward. Young individuals with depression symptoms also showed reduced neural response during effort and also exerted less physical effort for reward. These results are important as they suggest that young people with depression symptoms, especially those who are anhedonic, might benefit from therapies that aim at increasing exposure to reward e.g. BA.

Results of the RSFC studies showed that regardless of data analysis approach, the young individuals with depressive symptoms had abnormalities in the RSFC networks. Specifically, decreased RSFC between the CEN and the visual network emerged as an abnormality across all subjects, which additionally correlated with increasing depression severity in the depressed individuals only. This suggests that at the brain level, this decreased RSFC is observed before the onset of clinical depression and as depression increases, it is further associated with the behavioural symptoms of depression. This further suggests that this specific connectivity can be considered as a possible biomarker for depression.

9.8. References

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Chapter 10:

10. Supplementary data and Appendices

10.1. Supplementary data

10.1.1. Supplementary data: Paper 1

Supplementary Data

Blunted Motivational and Consummatory Neural Responses to Reward and Aversion in Adolescents with Depressive Symptomatology.

Running title:

Neural Reward and aversion response in adolescents with depressive symptoms.

Category: Regular research article

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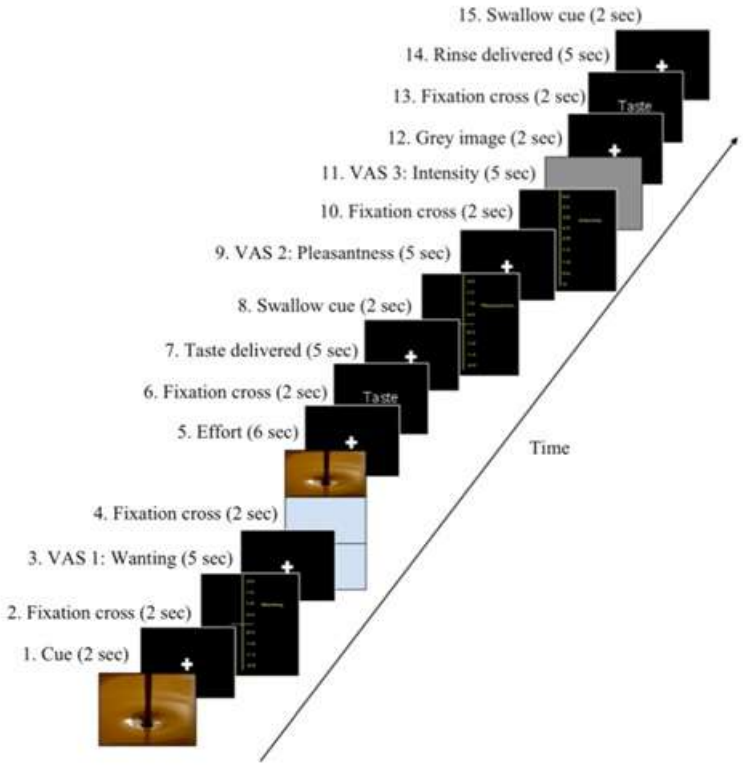
Figure S1. Visual depiction of a reward-hard trial.



Timings are approximate as the fixations were jittered as were the inter trial intervals. Timings also varied depending on the effort expended on each trial.

Table S1: Mood, energy and affect means and SD for BFS and VAS.

Measure	HR; mean (SD)	LR; mean (SD)
Before		
BFS	48.3 (17.22)	25 (17.57)
VAS alert	5.56 (1.59)	6.17 (2.04)
VAS disgust	1.84 (2.63)	.78 (1.42)
VAS drowsiness	3.43 (2.71)	2.19 (2.26)
VAS anxiety	3.37 (2.4)	1.42 (1.59)
VAS happiness	5.68 (2.18)	6.94 (2.36)
VAS nausea	2.18 (2.91)	1.02 (1.86)
VAS sadness	2.37 (2.53)	.79 (1.3)
VAS withdrawn	3.35 (3.17)	.86 (1.14)
VAS faint	1.25 (2.42)	.4 (.75)
After		
BFS	42.06 (14.24)	30.88 (14.8)
VAS alert	5.18 (2.43)	5.38 (2.78)
VAS disgust	1 (1.06)	2.21 (3.19)
VAS drowsiness	5 (2.76)	3.82 (3.38)
VAS anxiety	2.2 (2.32)	.69 (1.16)
VAS happiness	6.43 (1.73)	7.72 (1.59)
VAS nausea	1.2 (1.84)	2.33 (3.01)
VAS sadness	1.4 (1.47)	.69 (1.64)
VAS withdrawn	2.43 (2.1)	1.01 (2.28)
VAS faint	1.52 (2.12)	.86 (.91)

VAS: Visual Analog Scale

Table S2: Subjective ratings

	Group					
Ratings	LR	HR	LR	HR	LR	HR
	Chocolate		Aversive		Tasteless solution	
Picture wanting; mean (SD)	1.62 (.15)	1.52 (.46)	-1.78 (.13)	-1.71 (.33)		
Taste liking; mean (SD)	1.62 (.22)	1.56 (.45)	-1.65 (.16)	-1.6 (.35)	.16 (.17)	.16 (.41)
Taste intensity; mean (SD)	2.52 (.51)	2.48 (.96)	2.44 (.35)	2.16 (.91)	.87 (.43)	.74 (.51)

Table S3: Number of button presses and button presses time for the effort part of the task.

	LR; mean (SD)	HR; mean (SD)
Button presses		
Chocolate Easy	32.28 (1.5)	32.26 (0.5)
Chocolate Hard	42.48 (5.25)	41.39 (3.39)
Aversive Easy	31.97 (.99)	32.09 (.7)
Aversive Hard	43.94 (5.8)	41.79 (3.3)
Time (ms)		
Chocolate Easy	4956 (534.5)	5068 (427.5)
Chocolate Hard	6207 (100)	6251 (60.9)
Aversive Easy	4668 (632)	4937 (440.2)
Aversive Hard	6185 (106.1)	6218 (82)

Table S4: Regions showing main effects of task conditions in the LR group when covariate for age, gender and BMI.

	MNI coordinates				
Brain region	X	Y	Z	p-value	z-value
Anticipatory					
Chocolate cue					
Frontal pole	36	54	-6	4.38	<.001
Frontal pole	-28	58	8	3.43	<.001
MFG	32	28	32	3.96	<.001
Paracingulate gyrus	-12	52	0	3.85	<.001
ACC	2	18	34	3.81	<.001
Postcentral gyrus	50	-36	56	3.58	<.001
PCC	2	-18	30	3.55	<.001
Occipital pole	-12	-96	-2	4.47	0.037
Occipital fusiform gyrus	-26	-86	-10	3.29	0.037
Mouldcue					
Paracingulate gyrus	10	48	2	4.34	<.001
Frontal Pole	16	42	50	4.32	<.00*
MFG	50	28	38	4.20	<.001
pgACC	6	44	4	4.15	0.002ROI
PCC	6	-36	40	3.88	<.001
Occipital Pole	-12	-98	2	5.24	0.03
Occipital fusiform gyrus	-26	-86	-10	3.31	0.03
Effort					
Chocolate hard-chocolate easy					
Precentral gyrus	-30	*18	46	4.6	<.001
Insula	-32	-28	14	4.42	<.001
Chocolate hard-aversive hard					
Putamen	28	-6	16	4	<.001
Precentral gyrus	18	-34	40	3.78	<.001
Amygdala	22	-12	-14	3.54	<.001
Frontal Pole	4	60	28	3.26	<.001
Insula	38	-2	16	3.51	<.001

sgACC/vmPFC	6	44	-14	3.17	<.001
Chocolate easy-aversive easy					
Frontal Pole	24	54	34	3.71	<.001
Aversive hard-aversive easy	-28	-24	74	4.08	0.009
Precentral gyrus	0	-12	54	3.58	0.009
Supplementary Motor	16	6	74	3.23	0.009
Cortex	-4	-58	60	2.78	0.009
STG					
Precuneus					
Consummatory					
Chocolate taste					
Caudate	-14	4	20	3.77	0.013
Caudate	16	14	14	3.16	0.013
ACC	4	8	24	3.72	0.013
insula	-34	14	4	3.31	0.032ROI
Mould cue					
Paracingulate gyrus	10	48	2	4.34	<.001
Frontal Pole	16	42	50	4.32	<.001
MFG	50	28	38	4.2	<.001
PCC	6	-36	40	3.88	<.001
Occipital Pole	-12	-98	2	5.24	0.03
Occipital fusiform gyrus	-26	-86	-10	3.31	0.03
Mould taste					
Paracingulate gyrus/ACC	-10	16	38	3.32	0.009*
Amygdala	22	2	-20	2.96	0.023*

$p<0.01$ FWE, indicating p values voxel level small volume corrected
*-$p<.0.01$ FEW, small volume corrected; ROI- region of interest analysis
OFC- orbitofrontal cortex; PCC- posterior cingulate cortex; ACC- anterior cingulate cortex; pgACC- pregenual anterior cingulate cortex, sgACC- subgenual anterior cingulate cortex; vmPFC- ventral medial prefrontal cortex; MFG- middle frontal Gyrus; SFG- superior frontal gurus; STG- superior temporal gyrus

10.1.2. Supplementary data: Paper 2

Supplementary Data

Depression

Running title:

Decreased Neural Anticipation, Effort and Consummation of Reward and Aversion with Increased Anhedonia in Adolescents: An RDOC Dimensional approach.

Category:

Regular research article

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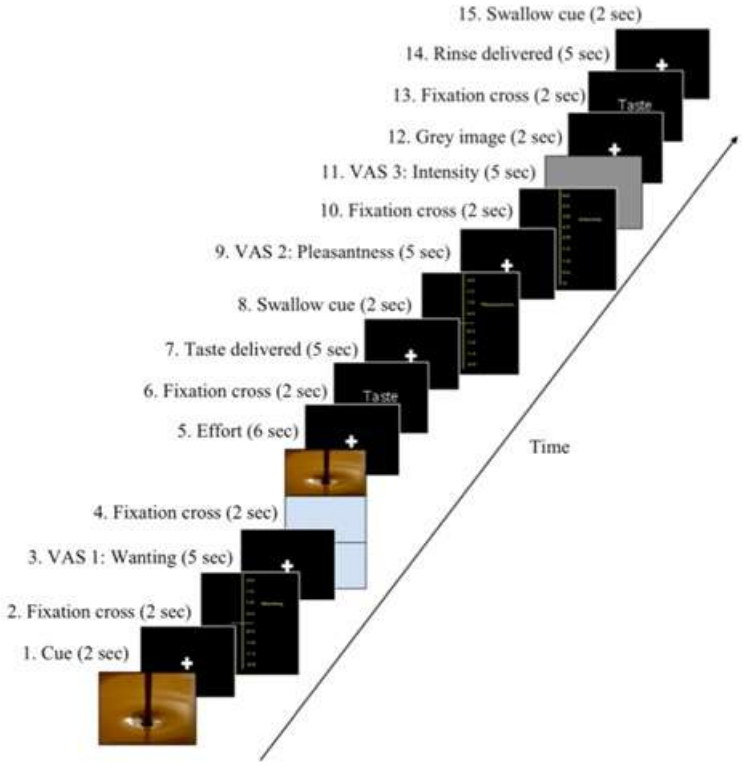
Figure S1**.** Visual depiction of a reward-hard trial.



Timings are approximate as the fixations were jittered as were the inter trial intervals. Timings also varied depending on the effort expended on each trial.

Table S1: Medication status of participants with MDD diagnosis.

Participant ID	Medicated during study	History of medications
1	Citalopram 10mg	Sertraline
2	-	Bupropion, Fluoxetine, Sertraline, Mirtazapine
3	Fluoxetine 50mg	-
4	Sertraline 100mg	
5	-	Fluoxetine 20-60mg, Sertraline 50mg
6	Sertraline 100mg	Citalopram
7	Sertraline	-
8	-	Fluoxetine 20mg
9	Citalopram 20mg	-
10	Citalopram 40mg	-
11	-	Citalopram 20mg
12	Citalopram 30mg	-
13	Fluoxetine 40mg	-
14	Sertraline 10mg	-
15	Fluoxetine 60mg	-
16	-	Fluoxetine 20mg
17	-	Citalopram 20mg
18	Paroxetine 20mg	-
19	-	Citalopram 20mg
20	-	Fluoxetine 20mg
21	Citalopram 40mg	-
22	Sertraline 50mg	-

Table S2: Mood energy and affect scores for DS and HC (DS), and MDD and HC (MDD).

Measure	DS; mean (SD)	HC (DS); mean (SD)	MDD; mean (SD)	HC (MDD); mean (SD)
Before				
BFS	52.23 (5.9)	48.73 (5.98)	53.29 (5.7)	48.11 (8.9)
VAS alert	5.35 (1.77)	6.18 (1.89)	5.21 (1.88)	3.91 (2.26)
VAS disgust	1.65 (2.29)	.98 (1.99)	1.53 (2.11)	2.23 (2)
VAS drowsiness	4.03 (2.59)	2.99 (2.52)	4.38 (2.49)	4.6 (2.53)
VAS anxiety	3.88 (2.46)	1.47 (1.88)	4.18 (2.49)	2.8 (2.23)
VAS happiness	4.6 (2.14)	6.83 (1.95)	3.96 (1.88)	4 (1.97)
VAS nausea	1.78 (2.44)	.83 (1.48)	1.55 (2.14)	2.53 (2.27)
VAS sadness	3.14 (3.67)	.91 (1.31)	3.6 (2.69)	3 (2.33)
VAS withdrawn	4.14 (2.87)	1.36 (1.980	4.6 (2.62)	4.35 (2.17)
VAS faint	1.34 (2.13)	.55 (.8)	1.39 (1.98)	2.13 (2.28)
After				
BFS	51.12 (6.35)	49.1 (6.05)	52.93 (6.06)	49.26 (6.11)
VAS alert	4.4 (2.38)	5.38 (2.51)	6.13 (2.1)	5.64 (2.6)
VAS disgust	1.77 (1.8)	1.9 (2.45)	1.06 (2.2)	1.8 (1.87)
VAS drowsiness	4.75 (2.59)	3.66 (2.7)	3.67 (2.65)	3.35 (2.62)
VAS anxiety	2.6 (2.26)	.67 (.94)	1.57 (2.02)	.63 (.75)
VAS happiness	4.91 (2.21)	6.89 (1.9)	7.13 (1.74)	6.8 (1.87)
VAS nausea	2.03 (2.2)	1.64 (2.47)	.81 (1.42)	1.5 (2.39)
VAS sadness	2.53 (2.18)	.65 (1.17)	.91 (1.3)	.49 (.67)
VAS withdrawn	3.63 (2.32)	.84 (1.57)	1.43 (2.22)	.57 (.6)
VAS faint	1.9 (2.22)	.91 (.97)	.59 (.82)	.87 (1)

VAS: Visual Analog Scale

Table S3: Subjective ratings for DS and HC (DS) groups and MDD and HC (MDD) groups.

	Group					
Ratings	DS	HC(DS)	DS	HC(DS)	DS	HC(DS)
	Chocolate		Aversive		Tasteless solution	
Picture wanting; mean (SD)	1.43 (.45)	1.62 (.25)	-1.75 (.33)	-1.75 (.25)		
Taste liking; mean (SD)	1.38 (.44)	1.56 (.28)	-1.52 (.47)	-1.55 (.48)	.67 (.9)	.57 (.85)
Taste intensity; mean (SD)	2.28 (.77)	2.37 (.63)	2.38 (.85)	2.51 (.74)	1.15(.87)	1.53 (1)
	Group					
Ratings	MDD	HC(MDD)	MDD	HC(MDD)	MDD	HC(MDD)
	Chocolate		Aversive		Tasteless solution	
Picture wanting; mean (SD)	1.37 (.44)	1.62 (.26)	-1.76 (.33)	-1.77 (.23)		
Taste liking; mean (SD)	1.29 (.39)	1.55 (.29)	-1.49 (.52)	-1.55 (.54)	.44 (.39)	.33 (.37)
Taste intensity; mean (SD)	2.15 (.65)	2.39 (.63)	2.52 (.76)	2.56 (.75)	.54 (.37)	.6 (.33)

Table S4: Number of button presses and button presses time for the effort part of the task.

	DS; mean (SD)	HC (HC); mean (SD)	MDD; mean (SD)	HC (MDD); mean (SD)
Button presses				
Chocolate Easy	31.77 (1.27)	32 (1.1)	31.48 (1.5)	31.86 (.62)
Chocolate Hard	41.25 (4.13)	43.33 (4.44)	41.17 (4.57)	43.6 (3.62)
Aversive Easy	31.42 (1.51)	31.81 (.91)	31.03 (.1.73)	31.74 (.8)
Aversive Hard	42.93 (4.67)	44.48 (4.69)	43.6 (5.26)	44.53 (3.69)
Time (ms)				
Chocolate Easy	5025.3 (567.5)	4793.6 (501.6)	5000 (642.7)	4714.4 (441.6)
Chocolate Hard	6236.7 (110.1)	6200.3 (112.3)	6227.8 (131)	6195.4 (114.3)
Aversive Easy	4718.9 (614.3)	4646.4 (546.4)	4587.6 (670)	4634 (468.8)
Aversive Hard	6194.6 (158.7)	6185.2 (104.3)	6180.6 (190)	6187.3 (100.5)

Table S5: Regions showing main effects of task conditions in all HC controlling for age.

	MNI coordinates				
Brain region	X	Y	Z	p-value	z-value
Anticipatory					
Chocolate cue					
Occipital pole	-20	-92	6	6.12	<.001
Cuneal cortex/Precuneus	-12	-80	34	4.33	<.001
LOC	32	-84	10	5.49	<.001
Frontal Pole	40	36	42	5.43	<.001
Planum Polare	-40	-24	0	4.17	<.001
Insula	-34	-26	6	4.16	<.001
ACC	-6	-4	32	3.9	0.002
SFG	-20	16	42	3.82	0.002
Putamen	28	-8	-8	4.55	<.001
Parahippocampal gyrus	28	-24	-22	4.05	<.001
Ventral striatum	6	4	-6	3.2	0.02 ROI
Ventral striatum	-6	4	-6	4.36	<.001ROI
Insula	36	10	-14	4.36	<.001ROI
Amygdala	20	-4	-20	3.26	0.017 ROI
Hippocampus	-30	-28	-14	3.53	0.027 ROI
Hippocampus	24	-14	-12	3.7	0.016 ROI
Aversive cue					
Occipital Pole	-20	-92	6	3.31	<.001
Cuneal cortex/ Precuneus	18	-76	32	5.57	<.001
Paracingulate gyrus	10	48	2	5.72	<.001
Frontal Pole	42	36	42	5.29	<.001
Supramarginal gyrus	60	-46	36	5.31	<.001
Precuenus/PCC	12	-36	46	5.07	<.001
LOC	-24	-76	48	3.71	0.001
Putamen	30	-6	-8	4.84	0.008
Amygdala	34	-2	-18	3.84	0.008
IFG/OFC	-32	34	6	4.72	<.001
STG	58	-28	8	4.3	0.008
Amygdala	-20	-4	-20	3.58	0.006 ROI
Amygdala	20	4	-20	3.2	0.02 ROI
Ventral Striatum	-6	4	-6	3.68	0.005 ROI
Hippocampus	-20	-6	-26	3.58	0.023ROI
Hippocampus	26	-40	-2	3.96	0.006 ROI
Effort					
Chocolate hard-chocolate easy					
Precentral gyrus	-32	-24	50	6.91	<.001
Putamen	28	4	-6	4.18	0.001

Insula	40	2	6	4.16	0.001
OFC	34	20	-18	3.57	0.001
Amygdala	30	2	-14	3.42	0.001
Paracingulate gyrus	14	36	24	4.17	0.024
ACC	6	32	24	3.85	0.024
Insula	36	10	-14	3.63	0.005ROI
Hippocampus	36	-24	-8	4.18	0.014ROI
Hippocampus	-36	-34	-6	3.92	0.006ROI
Aversive hard-aversive easy					
Precentral gyrus	-30	-24	72	5.83	<.001
Postecentral gyrus	-34	-36	-58	5.07	<.001
Supplementary motor cortex	-8	-6	54	4.25	<.001
Hippocampus	-30	-32	-2	3.92	0.006ROI
Ventral Striatum	6	4	-6	3.08	0.024ROI
Chocolate hard-aversive hard	-20	4	-20	3.14	0.019ROI
Amygdala	36	10	-14	2.79	0.047ROI
Insula					
Consummatory					
Chocolate taste					
Frontal Pole	40	38	20	4.55	<.001
Thalamus	-2	-2	8	4.31	0.031
Caudate	16	14	14	3.87	0.031
Paracingulate gyrus/ACC	-4	8	50	4.16	0.007
SFG	4	22	60	4.05	0.007
Precentral gyrus	-40	-18	62	3.98	0.009
Amygdala	-20	-4	-20	3.71	0.004ROI
Amygdala	20	-4	-20	4.51	<.001ROI
Hippocampus	-20	-6	-22	3.58	0.024ROI
Aversive taste					
Paracingulate gyrus/ACC	-6	12	46	4.7	0.008
Insula	36	10	-14	2.88	0.043ROI

$p<0.001$ FWE, whole brain fully corrected; ROI- region of interest analysis
OFC- orbitofrontal cortex; ACC- anterior cingulate cortex; MFG- middle frontal Gyrus; IFG- inferior frontal gurus; SFG- superior frontal gyrus; STG- superior temporal gyrus; LOC- lateral occipital cortex; PCC- posterior cingulate gyrus

10.1.3. Supplementary data: Paper 3

Supplementary Data

Decreased anticipated pleasure correlates with increased salience network resting state functional connectivity in adolescents with depressive symptomatology.

Running title:

Resting-state functional connectivity in adolescents at high risk for depression.

Category:

Regular research article

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Table S1: Mood, energy and affect means and SD for BFS and VAS.

Measure	HR; mean (SD)	LR; mean (SD)
Before		
BFS	48.47 (16.69)	26.4 (18.1)
VAS alert	5.75 (1.72)	6.22 (1.99)
VAS disgust	1.81 (2.55)	.74 (1.39)
VAS drowsiness	3.36 (2.64)	2.16 (2.19)
VAS anxiety	3.5 (2.38)	1.34 (1.58)
VAS happiness	5.82 (2.2)	7.0 (2.31)
VAS nausea	2.25 (2.83)	1.02 (1.81)
VAS sadness	2.32 (2.5)	.75 (1.28)
VAS withdrawn	3.27 (3.09)	.81 (1.13)
VAS faint	1.3 (2.36)	.37 (.9)
After		
BFS	42.53 (13.92)	31.8 (14.9)
VAS alert	5.29 (2.39)	5.44 (2.7)
VAS disgust	1.3 (1.65)	2.36 (3.16)
VAS drowsiness	4.93 (2.69)	3.8 (3.28)
VAS anxiety	2.18 (2.25)	.66 (1.13)
VAS happiness	6.16 (2.01)	7.46 (1.91)
VAS nausea	1.4 (1.96)	2.52 (3.03)
VAS sadness	1.49 (1.48)	.66 (1.6)
VAS withdrawn	2.37 (2.04)	.95 (2.23)
VAS faint	1.78 (2.33)	.89 (.9)

VAS: Visual Analog Scale

Table S2. Functional connectivity between seeds and whole brain in healthy control group only.

	MNI coordinates			
	X	Y	Z	z-score
Left Amygdala				
pgACC	-2	34	-2	8.47
Paracingulate gyrus	6	52	2	6.42
ACC	2	34	12	6.1
Lateral Occipital Cortex	-46	-76	40	4.02
Lateral Occipital Cortex	48	-60	30	3.82
Right Amygdala				
Amygdala	22	-4	-24	7.69
Insula	42	-6	4	4.59
Central Opecular Cortex	44	-2	6	4.51
Planum Temporale	60	-10	6	4.29
Hippocampus	-16	-10	-18	5.54
Amygdala	-18	-6	-16	5.13
Temporal Pole	-24	6	-24	4.84
Brain stem	8	-36	-42	3.38
Inferior Temporal Gyrus	50	-54	-14	3.84
Left dmPFC				
Middle Frontal Gyrus	-22	34	26	7.46
Frontal Pole	-28	46	16	5.35
Paracingulate Gyrus/ACC	-6	32	28	4.59
Middle Frontal Gyrus	28	30	36	4.68
Frontal Pole	32	40	34	4.56
Superior Parietal Lobule	-30	-42	56	3.9
Precuneus Cortex	-12	-46	50	3.71
Postcentral gyrus	-30	-34	54	3.28
Right dmPFC				
Frontal Pole	18	34	30	7.32
Paracingulate Gyrus	10	36	30	5.98
Superior Frontal Gyrus	16	32	42	4.75
Lateral Occipital Cortex	58	-60	34	4.01
Cuneal Cortex	6	-70	24	3.75

Precuneus Cortex	8	-58	8	3.54
Paracingulate Gyrus	-18	48	4	3.27
Middle Temporal Gyrus	62	-8	-16	3.78
pgACC				
Thalamus	-4	0	-2	8.38
Thalamus	2	-2	0	8.3
Putamen	-16	8	-2	4.52
ACC	6	42	6	4.28
Frontal Medial Cortex	-4	42	-12	4.09
Superior frontal Gyrus	-18	32	38	3.73
Frontal pole	-16	40	38	2.85

OFC- orbitofrontal cortex; PCC- posterior cingulate cortex; ACC- anterior cingulate cortex; pgACC-pregenual anterior cingulate cortex

Thesis specific additional information as requested after the PhD defence:
'Acquisition was performed during 5 minutes, resting-state scan, yielding 420 volumes in total'.

10.1.4. Supplementary data: Paper 4

Supplementary Data

Depression

Running title:

Increasing Depression and Anhedonia Severity Correlates with Decreasing Resting-State Functional Connectivity in Dorso-Medial Prefrontal Cortex in Adolescents: An RDOC Dimensional Approach

Category:

Regular research article

Ewelina Rzepa MSc, Ciara McCabe* PhD.

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Table S1: Medication status of participants with MDD diagnosis.

Participant ID	Medicated during study	History of medications
1	Citalopram 10mg	Sertraline
2	-	Bupropion, Fluoxetine, Sertraline, Mirtazapine
3	Fluoxetine 50mg	-
4	Sertraline 100mg	
5	-	Fluoxetine 20-60mg, Sertraline 50mg
6	Sertraline 100mg	Citalopram
7	Sertraline	-
8	-	Fluoxetine 20mg
9	Citalopram 20mg	-
10	Citalopram 40mg	-
11	-	Citalopram 20mg
12	Citalopram 30mg	-
13	Fluoxetine 40mg	-
14	Sertraline 10mg	-
15	Fluoxetine 60mg	-
16	-	Fluoxetine 20mg
17	-	Citalopram 20mg
18	Paroxetine 20mg	-
19	-	Citalopram 20mg
20	-	Fluoxetine 20mg
21	Citalopram 40mg	-
22	Sertraline 50mg	-

SR1. Mood, Energy and Affect Scores

Repeated measures ANOVAs were conducted to examine mood (BFS), with within subject factor of time (before and after scan) and between subjects factor of group, DS and HC. Results revealed that there was no significant main effect of time F(1.82)=.323; p=.571) and no significant interaction between time and group (F(1.82)=4.65; p=.034). There was a significant main effect of group F(1.82)=4.65; p=.034) which was due to a significantly lower mood in the DS group before the scan compared to controls $t(41)=2.08$, $p=.044$. Repeated measures ANOVAs were conducted to examine mood (BFS), with within subject factor of time (before and after scan) and between subjects factor of group, MDD and HC. Results revealed that there was no significant main effect of time F(1.56)=.243; p=.624;) and no significant interaction between time and group F(1.56)=.854; p=.359). There was a significant main effect of

group (MDD and HC: F(1.56)=9.42; p=.003) which was due to a significantly lower mood in the MDD group before the scan and after the scan compared to controls (before: *t*(26)=2.38, *p*=.025;) (after: (*t*(26)=2.24; *p*=.034).

Repeated measures ANOVA were used to examine emotion (VAS: alertness, disgust, drowsiness, sadness, happiness, anxiety, withdrawn, faint, nausea) with within subject factor of time, two levels (before and after scan) and between subjects factor of group, DS and HC. Results revealed that there was no significant main effect of time (*F*(1.82)=.228; *p*=.634,). There was a significant main effect of group (*F*(1.82)=684; *p*=.001,) and a significant main effect of emotion (*F*(8.656)=102.17; *pGreenhouseGeisser-corrected*<.001,) meaning that there were differences in how the emotions were experienced between the DS and the HC groups. There was no significant interaction between time and group (*F*(1.82=.919 *p*=.341, but a significant interaction between emotion and group (*F*(8.654)=24.68 *p*<.001) meaning that there were differences in emotions experienced depending on the group. There was also a significant interaction between time and emotion (*F*(8.654)=6.68 *pGreenhouseGeisser-corrected*<.001) but no significant interaction between time, emotion and group (*F*(8.654)=.575; *pGreenhouseGeisser-corrected*=.735) (Table S2). Meaning that emotions were experienced differently before and after the scan but this did not differ between the groups.

Repeated measures ANOVA were used to examine emotion (VAS: alertness, disgust, drowsiness, sadness, happiness, anxiety, withdrawn, faint, nausea) with within subject factor of time, two levels (before and after scan) and between subjects factor of group, MDD and HC. Results revealed that there was no significant main effect of time (*F*(1.56)=.801; *p*=.375). There was a significant main effect of group *F*(1.56)=12.62; *p*=.001) and a significant main effect of emotion *F*(8.243)=66.17; *pGreenhouseGeisser-corrected*<.001) meaning that there were differences in how the emotions were experienced between the MDD and the HC groups. There was no significant interaction

between time and group F(1.56)=.032 p=.858) but a significant interaction between the emotion and group F(8.243)=24.68 p<.001) meaning that there were differences in emotions experienced depending on the group. There was also a significant interaction between time and emotion F(8.282)=6.68 *pGreenhouseGeisser-corrected*<.001) but no significant interaction between time, emotion and group F(8.282)=.535; *pGreenhouseGeisser-corrected*=.751) (Table S2). Meaning that emotions were experienced differently before and after the scan but this did not differ between the groups.

Further analysis using paired sample t-tests to examine emotion revealed decreased alertness (t(46)=2.306, p=.026), anxiety (t(46)=3.45, p=.001) and sadness (t(46)=2.24, p=.03) in the DS group after the scan. The HC groups also had decreasing anxiety (t(46)=3.21, p=.003) after the scan but also increased disgust (t(46)=-2.11, p=.041) and faintness (t(46)=-2.19, p=.035) (Table S3). Further analysis using paired sample t-tests to examine emotion revealed increased happiness (t(26)=-6.65, p<.001) and decreased anxiety (t(26)=3.56, p=.001), sadness (t(26)=4.35, p=.001), and feeling withdrawn (t(26)=4.6, p=.001) in the MDD group after the scan. This was similar in the HC group who had increasing happiness after the scan (t(26)=-5.42, p<.001) and decreasing anxiety (t(26)=4.51, p<.001), sadness (t(26)=5.22 , p<.001) and feeling withdrawn (t(26)=8.61, p<.001) (Table S2).

Table S2: Mood energy and affect scores

Measure	MDD; mean (SD)	HC; mean (SD)	DS; mean (SD)	HC; mean (SD)
Before				
BFS	53.29 (5.7)	48.11 (8.9)	52.2 (5.81)	48.79 (7.85)
VAS alert	5.21 (1.88)	3.91 (2.26)	5.42 (1.82)	6.20 (1.87)
VAS disgust	1.53 (2.11)	2.23 (2)	1.64 (2.27)	.96 (1.96)
VAS drowsiness	4.38 (2.49)	4.6 (2.53)	3.99 (2.57)	2.96 (2.49)
VAS anxiety	4.18 (2.49)	2.8 (2.23)	3.92 (2.45)	1.43 (1.86)
VAS happiness	3.96 (1.88)	4 (1.97)	4.68 (2.18)	6.86 (1.94)
VAS nausea	1.55 (2.14)	2.53 (2.27)	1.82 (2.42)	.81 (1.46)
VAS sadness	3.6 (2.69)	3 (2.33)	3.1 (2.65)	.89 (1.3)
VAS withdrawn	4.6 (2.62)	4.35 (2.17)	4.1 (2.85)	1.32 (1.96)
VAS faint	1.39 (1.98)	2.13 (2.28)	1.35 (2.1)	.53 (.79)
After				
BFS	52.93 (6.06)	49.26 (6.11)	51.09 (6.27)	49.1 (5.97)
VAS alert	6.13 (2.1)	5.64 (2.6)	4.5 (2.38)	5.4 (2.48)
VAS disgust	1.06 (2.2)	1.8 (1.87)	1.89 (1.9)	1.98 (2.46)
VAS drowsiness	3.67 (2.65)	3.35 (2.62)	4.73 (2.57)	3.66 (2.67)
VAS anxiety	1.57 (2.02)	.63 (.75)	2.56 (2.23)	.66 (.93)
VAS happiness	7.13 (1.74)	6.8 (1.87)	4.83 (2.23)	6.8 (2)
VAS nausea	.81 (1.42)	1.5 (2.39)	2.09 (2.2)	1.74 (2.52)
VAS sadness	.91 (1.3)	.49 (.67)	2.54 (2.16)	.64 (1.16)
VAS withdrawn	1.43 (2.22)	.57 (.6)	3.59 (2.31)	.89 (1.58)
VAS faint	.59 (.82)	.87 (1)	2 (2.28)	.93 (.96)

VAS: Visual Analog Scale

Table S3. Functional connectivity between seeds and whole brain in healthy control group only.

Brain regions	MNI coordinates			
	X	Y	Z	z-score
Left Amygdala				
Amygdala	-18	-6	-16	12.4
Hippocampus	22	-20	-18	5.81
Insula	-42	-8	2	5.8
Planum temporale	-56	-16	6	5.7
Postcentral gyrus	-58	-8	26	4.06
Precentral gyrus	-58	-2	26	3.72
ACC	0	-12	40	4.1
PCC	-6	-22	44	3.16
Right Amygdala				
Amygdala	22	-2	-18	12.2
Hippocampus	-22	-18	-18	6.44
Central Opecular Cortex	54	-4	6	5.75
Temporal Pole	-40	6	-18	5.67
Insula	42	0	-8	5.44
Precentral gyrus	28	-20	72	4.02
Motor Cortex	6	-10	64	3.54
Postcentral Gyrus	32	-28	72	3.54
Middle Frontal Gyrus	-46	-34	20	4.63
OFC	-42	32	-22	4.29
Left dmPFC				
Middle Frontal Gyrus	-24	36	28	10.6
Frontal Pole	-24	46	24	7.79
Paracingulate Gyrus/ACC	-10	36	26	6.39
Middle Frontal Gyrus	28	30	36	4.68
Frontal Pole	32	42	34	6.28
Frontal Operculum Cortex	-40	20	2	4.13
Insula	-34	-14	-2	3.28
Right dmPFC				
Frontal Pole	18	34	30	10.7
Paracingulate Gyrus	2	44	28	6.5
Superior Frontal Gyrus	12	36	40	6.61
Middle Temporal Gyrus	64	0	-24	5.13
Temporal Pole	54	4	-38	4.26

LOC	8	-58	8	4.24
Cuneal cortex/Precuneus	4	-70	24	4.43
Precuenus	6	-54	18	4.03
L NAcc				
NAcc	-8	10	-6	13.6
OFC	20	8	-18	5.58
Paracingulate Gyrus	-2	54	4	5.58
OFC	-18	6	-24	5.36
Precuenus/PCC	-4	-52	10	4.21
Precuenus/PCC	12	-52	4	4.09
R NAcc				
NAcc	10	10	-8	13.7
Paracingulate Gyrus	16	2	-28	5.04
Frontal Medial cortex	-6	32	-18	4.76
Frontal Pole	22	38	-22	4.72
Precuenus/PCC	4	-54	6	4.03
PCC	8	-46	4	3.76
PCC	-4	-48	32	2.85
Frontal Pole	-44	52	0	3.61
Parahippocampal gyrus	-28	-38	-4	2.76
Hippocampus	-22	-32	-10	2.33
pgACC				
pgACC	0	32	0	8.8
ACC/Paracingulate	-6	46	4	8.37
Frontal Pole	-10	56	14	6.79
Middle Temporal Gyrus	64	-4	-16	6.5
OFC/Insula	30	14	-18	5.65
LOC	-52	-64	36	5.06
LOC	50	-66	40	3.24
Parahippocampal gyrus	26	-22	-18	6.13

OFC- orbitofrontal cortex; PCC- posterior cingulate cortex; ACC- anterior cingulate cortex; pgACC-pregenual anterior cingulate cortex, LOC-Lateral Occipital Cortex

10.2. Appendices

10.2.1. Ethics Approval: Studies 1 and 3

University of Reading

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Dr Ciara McCabe
School of Psychology and Clinical Language Sciences
University of Reading
RG6 6AL

26 March 2014

Dear Ciara

UREC 14/13: Effect of Mood on Neural Reward Processing in Adolescents. *Favourable opinion*

Thank you for the email (from Ewelina Rzepa, dated 20 March 2014 and including attachments) responding to my email of 14 March 2014 and addressing the minor issues raised by the UREC Sub-committee at their meeting on 11 March. I can confirm that the Chair is pleased to confirm a favourable ethical opinion on the basis of these documents.

Please note that the Committee will monitor the progress of projects to which it has given favourable ethical opinion approximately one year after such agreement, and then on a regular basis until its completion.

Please also find attached Safety Note 59: Incident Reporting in Human Interventional Studies at the University of Reading, to be followed should there be an incident arising from the conduct of this research.

The University Board for Research and Innovation has also asked that recipients of favourable ethical opinions from UREC be reminded of the provisions of the University Code of Good Practice in Research. A copy is attached and further information may be obtained here: http://www.reading.ac.uk/internal/res/QualityAssuranceInResearch/reas-RSqar.aspx.

Yours sincerely

Mike Proven

Dr M J Proven
Coordinator for Quality Assurance in Research (UREC Secretary)
cc: Dr John Wright (Chair); Dr Laurie Butler; Ewelina Rzepa

10.2.2. Ethics Approval: Studies 2 and 4

NHS
Health Research Authority

NRES Committee South Central - Hampshire B
Level 3 Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT
Tel: 01173421334

19 January 2015

Dr Ciara McCabe
Lecturer in Neuroscience
University of Reading
Department of Psychology, Earley Gate
Whiteknights Road
Reading
RG6 6AL

Dear Dr McCabe

Study title:	**Effects of Mood on Neural Reward Processing in Adolescents with Depression**
REC reference:	**14/SC/0102**
Amendment number:	**Amendment 3**
Amendment date:	**19 December 2014**
IRAS project ID:	**145827**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The amendment relates to the addition of a poster and an increase in the age range for participants.

- The Committee request that a small change is made to the poster to change "h" to "hr" so as to give "(around 2.5 hr in total)".

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Poster]	1	04 December 2014
Copies of advertisement materials for research participants [Leaflet]	1	04 December 2014
Covering letter on headed paper		23 December 2014
Notice of Substantial Amendment (non-CTIMP)	Sub Amend 3	19 December 2014
Participant information sheet (PIS) [Non Depressed Volunteers]	4	04 December 2014
Participant information sheet (PIS) [Depressed Volunteers 16-21]	4	04 December 2014
Participant information sheet (PIS) [Parent DA]	6	16 January 2015

NHS
Health Research Authority

Participant information sheet (PIS) [Parent Non-DA]	6	16 January 2015
Research protocol or project proposal	5	04 December 2014

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

14/SC/0102:	**Please quote this number on all correspondence**

Yours sincerely

PP

Professor Ron King (Chair)
Chair

E-mail: nrescommittee.southcentral-hampshireb@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Dr Mike Proven*

NRES Committee South Central - Hampshire B

Attendance at Sub-Committee of the REC meeting on 14 January 2015

Committee Members:

Name	*Profession*	*Present*
Professor Ron King (Chair)	Mathematician (Retired)	Yes
Dr Andrew Scott	Course Leader, M.Sc. Clinical Exercise Science	Yes

10.2.3. Mood and Feeling Questionnaire (MFQ)

The MFQ contains of 33 descriptive phrases that aim at measuring depression in adolescents. Each descriptive phrase is rated on 3-point scale ('not true', 'sometimes', 'true'). The range of scores differ from 0 to 66 with a higher score on the MFQ reflecting more depressive symptoms (Kent, Vostanis et al. 1997). There is considerable psychometric data for this questionnaire, including good test–retest reliability for a score of 27 and above indicating increased depression symptom severity (Wood, Kroll et al. 1995) and below 15 indicating healthy controls (Kyte, Goodyer et al. 2005).

10.2.4. Beck Depression Inventory (BDI)

The BDI contains of 21 descriptive phrases that aim at measuring the severity of depression from lack of depression to extreme clinical depression. Each item can be rated between 0 and 3 with a higher score indicating more severe depression. The range of scores differ from 0 to 63 (Beck 1961).

10.2.5. Fawcett-Clark Pleasure Scale (FCPS)

The FCPS contains of 36 items that describe pleasurable situations. Each item is rated on a 5-point scale and aims at assessing hedonic capacity. The range of scores differ from 36 (low hedonic capacity) to 180 (high hedonic capacity) (Fawcett, Clark et al. 1983).

10.2.6. Snaith-Hamilton Pleasure Scale (SHAPS)

The SHAPS consists of 14 items that measure hedonic capacity. Each item is rated on a 4-point scale between 1 and 4 with a lower score indicating less hedonic capacity. The range of scores is between 14 and 56 (Snaith 1995).

10.2.7. Temporal Experience of Pleasure Scale (TEPS)

The TEPS is a questionnaire with 18 items that specifically measures anticipatory and consummatory aspects of anhedonia. The questions are rated on a 6-point scale between 1 and 6. Higher score on each of the TEPS subscales indicates better experience of anticipatory and consummatory pleasure (Gard D.E 2006).

10.2.8. Befindischkeit Scale (BFS)

This questionnaire contains 57 pairs of words that aim at measuring the current emotional state. Participants are asked to put a cross next to a word that correspond more closely to their current feelings or in a 'neither' box that indicate none of the words described the current state (von Zerssen, Strian et al. 1974).

10.2.9. Visual Analogue Scale (VAS)

This scale is used to assess current mood on 9 different emotions (alertness, disgust, drowsiness, anxiety, happiness, nausea, sadness, withdrawn, faint). The visual scale is 10cm long and the beginning of the scale starts at 0 corresponding to 'not at all' experiencing the emotion at the moment and ends at 10 corresponding to 'extremely' experiencing this emotion at the moment. Participants are asked to mark a cross on the line at the point that best represents their current mood (Bond 1974).

10.2.10. Chocolate scale

Chocolate scale

1. On a scale from 1-10 how much would you say that you sometimes crave chocolate?

2. On a scale from 1-10 how much would you say that you like chocolate?

3. On a scale from 1-10 how much would you say that you like milk?

4. How frequently do you eat chocolate?

5. How much chocolate do you eat at a time? (To estimate this, please use as units a regular bar of chocolate)

6. Do you crave any other food?

If so, what is that food, and on a scale from 1-10 how much would you say that you crave that food?

(WITH 10 BEING THE HIGHEST)

If you participate in an fMRI experiment, please eat only a small lunch on the day of scanning and no chocolate 24hrs before the scan!