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Structural and Functional Brain Abnormalities in Children with Subclinical Depression

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AVANT-PROPOS

Ce mémoire est présenté sous forme d'articles et a été autorisée par la faculté des études supérieures. Deux articles scientifiques composent ce mémoire, qui sont soumis. L'auteur de cette thèse est également le premier auteur des 2 articles.

Le premier article intitulé «Dorsolateral prefrontal-anterior cingulate cortices activation in children with depressive symptoms during the external induction of sadness » soumis à *Biological Psychiatry* en Décembre 2005

Le deuxième article intitulé «Structural and functional corticolimbic abnormalities found in children with subclinical depressive symptoms» soumis à Nature Neuroscience en Mars 2007

Accord des coauteurs des articles

Résumé

Le traitement de l'information d'ordre émotionnel constitue un élément primordial de la dépression. Plusieurs hypothèses explicatives qui ont été soulevées dans la littérature suggèrent des anomalies anatomiques et fonctionnelles du circuit cortico-limbique. Cependant, la plupart de ces études ont été menées auprès de populations adultes et/ou âgées, recevant des traitements pharmacologiques et/ou psychologiques longtemps après le déclenchement des symptômes de la dépression.

Par conséquent, la présente étude vise d'abord à éclaircir les différents mécanismes cérébraux fonctionnels impliqués dans le traitement de l'information d'ordre émotionnel chez les enfants de 8 ans. En second lieu, elle vise à étudier la différence de l'anatomie de la matière blanche chez les enfants de 8 ans avec et sans symptômes dépressifs.

À cette fin, nous avons mené deux études à l'aide de l'imagerie par résonance magnétique nucléaire fonctionnelle (IRMf) et l'imagerie du tenseur de diffusion (ITD).

Première étude : Cette étude décrit principalement les résultats de l'IRMf portant sur les substrats neurobiologiques (du cortex préfrontal) de l'émotion triste chez des enfants normaux de 8 ans et des enfants avec des symptômes dépressifs. Vingt-cinq enfants (10 avec des symptômes dépressifs et 15 normaux) ont passé un examen d'IRMf durant le visionnement des extraits de films triste et neutre. Les résultats ont indiqué des différences

significatives entre les deux groupes. Les sujets normaux ont principalement activé le cortex préfrontal dorsolatéral (CPFdl) le cortex cingulaire antérieure (CCA). Par contre, les enfants avec des symptômes dépressifs ont activé seulement le cortex cingulaire antérieur. Nous concluons de ces résultats que les enfants dans le groupe des sujets normaux, en raison de l'activation du CPFdl, étaient « cognitivement » en mesure de réguler et moduler les stimuli émotifs de tristesse. Toutefois, les enfants avec des symptômes dépressifs ont principalement activé le CCA, traitant seulement ainsi les stimuli tristes d'une perspective émotionnelle sans implication cognitive permettant afin de réguler et moduler cette situation triste (aucune activation de CPFdl n'a été notée). Les résultats obtenus suggèrent en effet l'existence d'une différence significative dans le traitement de l'information d'ordre émotionnel entre les sujets avec et sans symptômes dépressifs.

Second étude: Cette étude décrit les résultats de l'IRMF et de l'ITD. Quarante-trois enfants de 8 ans ont participé à cette étude. Des 43 sujets, 20 enfants avec symptômes dépressifs (11 filles, 9 garçons) et 23 enfants sans symptômes dépressifs (10 filles, 13 garçon) ont passé un examen d'IDT, alors que 20 enfants avec symptômes dépressifs (12 filles, 8 garçons) et 22 enfants sans symptômes dépressifs (8 filles, 14 garçons) on passé un examen d'IRMf. Tous les sujets étaient droitiers, et appariés selon l'éducation parentale. L'étude a été approuvée par les comités d'éthique et scientifique du centre hospitalier de l'Université de Montréal (Hôpital Notre-Dame et hôpital Sainte Justine). Tous les parents ont donné leur consentement par écrit.

L'ITD a prouvé que les sujets avec des symptômes dépressifs avaient des réductions prononcées de l'intégrité de la matière blanche dans des régions du cerveau impliquées dans la dépression, telles que: l'hippocampe, le cortex fronto-médial et fronto-latéral, et le cortex cingulaire antérieur – soit toutes des régions liées à la dépression chez l'adulte. La pathologie de la matière blanche a permis de prédire 50% de la variance de la sévérité des symptômes dépressifs. L'analyse de l'activité fonctionnelle de ces régions, durant une émotion de tristesse induite expérimentalement, a également indiqué des changements significatifs de l'activité neurale et de la connectivité fonctionnelle parmi les sujets symptomatiques. Ces résultats suggèrent fortement que les anomalies fonctionnelles et anatomiques liées à la dépression de l'adulte se présentent tôt dans l'enfance, dès la première apparition des symptômes. Ceci implique que la neuropathophysiologie précède le début, et contribue à la pathogenèse, de la dépression.

L'ensemble des résultats de ce mémoire suggère fortement que les mécanismes neuronaux qui sous-tendent le traitement de l'information émotionnelle sont différents entre les deux groupes d'enfants de 8 ans. De plus, ces différences et anomalies fonctionnelles et anatomiques existent dès l'apparition des symptômes de la dépression.

Mots-clés : Imagerie par résonance magnétique fonctionnelle, imagerie du tenseur de diffusion, dépression, désordre dépressif principal, hippocampe, cortex préfrontal latéral, cortex préfrontal médial, cortex orbitofrontal, cortex cingulaire antérieur et subgenual rostral.

Abstract

Anatomical and functional anomalies are commonly observed in the corticolimbic circuit in adults with major depressive disorder (MDD). To explore causality and control for potential confounds of illness duration and treatment on this relationship, we used multimodal neuroimaging in unmedicated, subclinically depressed 8-year-old children.

First study: Reports mainly the results of fMRI investigating the neural correlates of sad emotion processing in 8 years old normal children and children with depression tendencies in the prefrontal cortex (PFC). Twenty-five (15 normal controls and 10 with depression tendencies) 8 years old children were scanned during the viewing of emotional and neutral film clips. Results revealed significant differences between the two groups. Normal subjects mainly activated the dorsolateral and anterior cingulated cortices. Conversely, children with depression tendencies activated the ACC. We conclude that children in the normal control group, by activation of the DLPFC, were able to "cognitively" process the emotional sad stimuli in addition to "emotionally" process the stimuli by activating the cingulate gyrus. Conversely, children with depression tendencies mainly activated the cingulate gyrus, thus only processed the sad stimuli from an emotional perspective without the implication of cognition and affect regulation (no DLPFC activation was noted).

Second study: Reports results of diffusion tensor imaging and functional magnetic resonance imaging (fMRI) investigating the fronto-cortico-limbic system. Forty-three 8-year-old children took part in this study. All subjects underwent fMRI then DTI scanning. Of the 43 subjects, 20 children with subclinical symptoms of depression (DS) (11 girls, 9 boys) and 23 asymptomatic children (AC) (10 girls, 13 boys) children completed DTI scanning, while 20 DS (12 girls, 8 boys) and 22 AC (8 girls, 14 boys) children completed fMRI scanning. All subjects were right-handed, matched for parental education and medication free except for two AC subjects receiving treatment for bronchial asthma. The study was approved by the ethics review boards of Ste. Justine Hospital and Centre hospitalier de l'Université de Montréal Hôpital Notre-Dame. All parents gave written informed consent. Diffusion tensor imaging showed that DS but not AC subjects exhibited pronounced reductions in white matter fiber tract integrity in the hippocampus, lateral prefrontal, medial prefrontal, orbitofrontal, rostral anterior and subgenual anterior cingulate cortices –all regions associated with adult depression. White matter pathology predicted 50% of severity of depressive symptoms. Functional analysis of those regions during induced sadness also revealed profoundly altered patterns of neural activity and functional connectivity among symptomatic subjects. Finding that the circuit associated with adult depression presents such pervasive structural and functional abnormalities so early in development implies that neuropathology precedes the onset, and contributes to the pathogenesis, of MDD.

Keywords: Functional magnetic resonance imaging, diffusion tensor imaging, depression, major depressive disorder, hippocampus, lateral prefrontal cortex, medial prefrontal cortex, orbitofrontal cortex, rostral anterior and subgenual anterior cingulate.

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Liste des abréviations

ACC:	Anterior cingulate cortex
AC:	Asymptomatic children
ATh:	Anterior thalamus
BA:	Brodmann's area
BOLD:	Blood oxygen level dependent
CBT:	Cognitive behavioural therapy
CHUM :	Centre Hospitalier de l'Université de Montréal
CIHR:	The Canadian Institute of Health Research
DICA-R:	Diagnostic Inventory for Children and Adolescents-Revised
DISC-2.3:	the Diagnostic Interview Schedule for Children Version 2.3
DLPFC:	Dorsolateral prefrontal cortex
DS:	Children with subclinical symptoms of depression
DSM IV:	Diagnostic and statistical manual of mental disorders, 4th edition
DSM IV-TR:	Diagnostic and statistical manual of mental disorders, 4th edition, revised text
DTI:	Diffusion Tensor Imaging
DWI:	Diffusion Weighted Imaging
DZ	Dizygotic
EPI:	Echo planar imaging
FA:	Fractional anisotropy
FDG-PET:	F-deoxyglucose Positron Emission Tomography

fMRI:	Functional magnetic resonance imaging
FRSQ :	Fonds de recherche en santé du Québec
Hipp:	Hippocampus
IAPS:	International Affective Picture System
IFG:	Inferior frontal gyrus
K^+:	Potassium ion
LFPs:	Local field potentials
LPFC:	Lateral prefrontal cortex
MDD:	Major Depressive Disorder
MDFC:	Medio-dorsal frontal cortex
MPFC:	Medial prefrontal cortex
MRI:	Magnetic Resonance Imaging
MZ	Monozygotic
Na^+:	Sodium
PC:	Posterior cingulate
PET:	Positron emission tomography
PFC:	Prefrontal cortex
PICA-III-R:	The Pictorial Instrument for Children and Adolescents
PPI:	Psychophysiological interactions
OFC:	Orbitofrontal cortex
rACC:	Rostral Anterior cigulate
rCBF:	Regional cerebral blood flow

SD:	Standard deviation
SPECT:	Single single photon emission computed tomography
SSRIs:	Selective serotonin reuptake inhibitors
sACC:	Ssubgenual cingulate
QNTS:	The Quebec Newborn Twin Study
VLPFC:	Ventrolateral prefrontal cortex
WM:	White matter

Dédicace

To my dear parents and family,

I have always admired you and appreciated what you have gone through in order to make me what I am, your affection, love, dedication, and encouragement...your compromise of what is dear to yourselves to provide what is dear and important to me. I bow to you with love, respect and gratitude for the many hours you spent helping me understand the difficult math and physics equations, for making history and geography fun and for teaching me that it is never too late to be what I want to be. Your earnest efforts to provide me with the best education possible, the sleepless nights you spent awake in the next room to make sure I don't fall asleep before I revise my last chapter before an exam...and even though it was hard for you to see me part you gave me the opportunity to travel and see the world. All you have done did not go in vain...this masters and the work invested in it are dedicated to you, to thank you for making me believe in what I can do, a "thank you" that is a very small drop of gratefulness in return for the oceans of generosity you have given me.

To my dear wife and kids,

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To my sons Zein and Gawad and my daughter Chiraz, I dedicate this work, that was not driven by the need for knowledge *alone*, but also driven by my wish to make you proud of your dad, and to inspire you to seek and follow your dreams.

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INTRODUCTION

.....I was becoming terribly disheartened owing to one circumstance which was beyond my control, namely, the conviction which was gaining upon me that nothing in the whole world made any difference. ...I suddenly felt that it made no difference to me whether the world existed or whether nothing existed anywhere at all. I began to be actually conscious that nothing existed in my own lifetime (Dostoevsky, 1817)

It got dark, and I felt more and more depressed. Painful thoughts of all kinds beset me. I kept fancying that I should die at last in Petersburg. (Dostoevsky, 1861)

1. A brief history on Depression

The Ebers papyrus dated back to 1550 BC is considered to be one of the most important ancient Egyptian medical papyri. The Ebers, the Edwin Smith (c.1600 BC) and the Brugsch (c.1300 BC) papyri are the oldest medical documents still preserved and accessible to man. The Ebers papyrus is written in hieratic script and provides us with the richest record of ancient Egyptian medicine known. The papyrus is made up of 110-page scroll containing about 700 medical notes, reports and treatments. Among the medical conditions described,

depression appears as a disease for the first time in the human history. Mental disorders are described in a special chapter of the papyrus named the Book of Hearts. The descriptions of these disorders suggest that Egyptians did not discriminate between mental and physical diseases and handled both in the same manner where the heart and mind were synonymous. Depression was described as: "fever in the heart", "dryness of the heart", "falling of the heart", "debility of the heart" and "kneeling of the mind" (Scholl, 2002). Treatments were also detailed, for example, psychotherapy was applied by "incubation" or "temple sleep", where the patient was instructed to spend a night in a court of a temple (Ebbel, 1937).

Hippocrates, (4^{th} century B.C), believed that depression was the results of excessive black bile in the spleen. He termed this condition "melancholia" (black bile). Black bile was considered by Hippocrates to be one of the four fluids (blood, phlegm, and yellow bile) that constitute the human physiology. In *Anatomy of Melancholy* (1621), the English writer and scholar Robert Burton stated that melancholic people "are born to melancholic parents." In his book he recognized specific environmental factors, such as lack or excessive sleep, diet, and alcoholic consumption in the pathogenesis of depression. Interestingly, the Arab physician Ishaq Ibn Imran attributed melancholia, at least partly, to prenatal injury, "as the result of the father's sperm having been damaged." (Badal, 1989; Contreras Mas, 2003). More recently, (19th century) depression was considered as a temperamental weakness that was inherited from the parents. In the first half of the 20th century, the emergence of psychoanalytic theory brought with it an emphasis on early life trauma in the development of adult psychopathology (Angst, 2000). Sigmund Freud also emphasised the effect of early

life events, as poverty and loss of dear ones in the development of depression without excluding the possible role of genetic predisposition. Through careful observation of his patients, Freud concluded that guilt, inferiority and self-criticism related to loss during childhood are important criteria for depression. Anger or hostility is usually directed at the parents for not providing the child with the love and affection needed for a normal and healthy development. These negative feeling are also directed to oneself in the form of self blame, guilt and self-criticism. Freud also suggested that grief for the loss of a loved one in adult life may bring back similar childhood experiences and may lead to blame directed at either oneself or at the loved one for dying or deserting them leading to internalized anger and depression (Abraham, 1924; Freud, 1917, 1937).

In the following decades, depression was described as an internalizing disorder that produces intense sadness and suffering to those affected, their families and close ones. Depression is clinically known as Major Depressive Disorder (MDD) or Unipolar Depressive Disorder. MDD is a medical condition that includes a wide range of abnormalities of affect and mood: MDD as a mental disease is replete with symptoms characterized by emotional, behavioural and physiological impairments.

2. Major depressive disorder

The lifetime prevalence of MDD is estimated between 12.2% and 17% in the general population. MDD was found to be more common in women than in men (Blazer et al., 1994; Kendler et al., 2001c; Maciejewski et al., 2001; Patten et al., 2006) and (DSM-IV-TR

(APA, 2000)) and in girls more than boys (Bailey et al., 2007; Costello et al., 2003) but the difference becomes smaller with advancing age (Patten et al., 2006) and (DSM-IV-TR (APA, 2000). A study by Patten et al (Patten et al., 2006) showed that the peak annual prevalence in Canada occurred in individuals aged 15 to 25 years. The same study also show that chronic medical conditions, unemployment, and income but not the level of education are associated with higher prevalence of major depression. While the effect of marital status on depression might vary with age, married people show the lowest prevalence of MDD. Men who never married have higher annual prevalence of depression as they get older (Patten et al., 2006; Whisman, 2007). The risk of recurrence of depression is about 70% at 5 year follow up and at least 80% at 8 year follow-up (Blazer et al., 1994; Patten et al., 2006).

After the first episode of MDD, there is a 50%-60% chance of having a second episode, and a 5-10% chance of having a Manic Episode (i.e., developing Bipolar I Disorder). After the second episode, there is a 70% chance of having a third. After the third episode, there is a 90% chance of having a fourth (DSM-IV-TR (APA, 2000).

The greater number of previous episodes is an important risk factor for recurrence. As has long been suspected, MDD is probably the most common of psychiatric disorders. According to Fava and Kendler (Fava and Kendler, 2000) four risk factors are consistently associated with MDD and some evidence suggests that some are contributory: 1- gender, 2- stressful life events (Kendler et al., 2001a; Kendler et al., 2001b; Kendler et al., 2001c; Maciejewski et al., 2001; Zimmermann et al., 2007), for example: job loss, marital

difficulties, major health problems, and loss of close personal relationships. 3-adverse childhood experiences (difficulties in childhood including physical and sexual abuse, poor parent-child relationships, and parental discord and divorce), and 4-certain personality traits (e.g., "Neuroticism") (Blazer et al., 1994). A recent WHO report (Murray and Lopez, 1996) ranked depression as the fourth medical condition with the greatest disease burden worldwide, measured in Disability-Adjusted Life Years, which express years of life lost to premature death and years lived with a disability of specified severity and duration. Furthermore, comorbid disorders are of major concern to the patients and their families. On one hand, a large population of patients with MDD also have anxiety symptoms (Levine et al., 2001) (e.g., anxiety, obsessive preoccupations, panic attacks, phobias, and excessive health concerns); separation anxiety may be prominent in children.(Axelson and Birmaher, 2001). Mood congruent delusions or hallucinations may accompany severe MDD (Black and Nasrallah, 1989), in addition to several comorbidities as substance abuse, panic disorder, obsessive compulsive disorder, anorexia nervosa, bulimia nervosa, and borderline personality (Beesdo et al., 2007; Pigott et al., 1994; Raffray and Pelissolo, 2007; Rowan, 2001) and (DSM-IV-TR (APA, 2000)).

DSM-IV-TR states that between 20-25% of patients with severe chronic medical illness (e.g., diabetes, myocardial infarction, carcinomas, stroke) develop depression. Similar findings were reported by Tsimmerman & Tsimmerman (Tsimmerman Ia and Tsimmerman, 2007) in patients with gastric conditions, carcinomas (Roscoe et al., 2007), diabetes, epilepsy, asthma, chronic obstructive pulmonary disease and hypothyroidism (Filipcic et al., 2007). About 5% of patients diagnosed with MDD are later found to have

another medical illness which was the cause of their depression, as is the case in endocrinal disturbances (hypothyroidism, hyperparathyroidism, Cushing's disease, and diabetes mellitus) or neurological diseases (multiple sclerosis, Parkinson's disease, epilepsy, encephalitis, and brain tumours.) ((DSM-IV-TR (APA, 2000)) (Filipcic et al., 2007)

According to Mueller and Leon (Mueller and Leon, 1996), a substantial proportion of patients experience their first episodes of MDD during childhood and adolescence. In this vein, family studies have reported an approximately 3-fold increased risk for MDD in the first-degree relatives (parents, siblings, offspring) of individuals with MDD versus the general population (Sullivan et al., 2000). Indeed, in 1999, Kendler and colleagues (Kendler et al., 1999) reported that MDD patients with family history of depression experience recurrent episodes, more sever impairments and possibly an earlier onset. At this point, it is worthy to mention a recent study done by our group, (Cote et al., 2007), conducted using the same cohort studied in this Master's project. The authors used fMRI in 104 pairs of 8-year-old twins (47 monozygotic [MZ] and 57 dizygotic [DZ] twins) to assess genetic-environmental contributions to individual differences in neural activation in two prefrontal cortex (PFC) areas repeatedly found in the literature to be involved in the emotional processing of sadness. This study showed that environmental factors entirely accounted for the individual variation in brain activation patterns related to sadness. These findings emphasise the relevance of early childhood events in the pathogenesis of MDD. At this point it is worthy to mention that twin studies in mental disorders are particularly

informative to examine the relative contribution of genetic and environmental risk factors in the brain morphological changes underlying a wide spectrum of mental diseases.

3. Why study major depressive disorder in children

Children are capable of experiencing episodes of depressive mood that meet standard DSM-IV criteria for MDD (Birmaher et al., 1996; Ryan et al., 1987; Valla et al., 1994). According to Kashani et al., (Kashani et al., 1983) the prevalence of depression is 2% in children and 15% according to Lewinsohn et al., (Lewinsohn et al., 1993). Another review by Birmaher et al (Birmaher et al., 1996) found the prevalence of depression to be between 0.4% and 2.5% in children and up to 8.3% in adolescents. Within 5 years of its onset, 70% of depressed children will experience a recurrence (Rao et al., 1995). A trajectory study conducted by Costello et al (Costello et al., 2003) showed that 7% to 12% of screened healthy children between the ages of 9-13 went on to develop depression by the age of 16 (Costello et al., 2003). Notably, MDD in children and adolescence is associated with significant morbidity and mortality (Birmaher et al., 1996; Pelkonen and Marttunen, 2003). 25% of depressed adolescents will develop a substance abuse disorder (Birmaher et al., 1996) and 10% will complete suicide within 15 years of their initial episode (Rao et al., 1993)

According to the DSM-IV (APA, 1994) MDD may result in several physical and cognitive symptoms ranging from abnormal appetite, disturbed sleep, fatigue or loss of energy accompanied with psychomotor retardation, in addition, psychological symptoms as

abnormal self-reproach or guilt, decreased self-esteem, pessimism, and hopelessness are present. Indeed negative thinking, as intense subjective misery, persistent negative mood state, anhedonia, hopelessness caused by depression is a key factor in producing the associated suicidal ideation and actual suicidal attempts often encountered in MDD patients. A study involving children 6-13 years found that the symptom most highly correlated with suicidal ideation was hopelessness. Suicidal attempts were found in 40% of children included in this study. Of those, children with MDD and experiencing feeling of hopelessness had the highest number of suicidal attempts (Rosembaum Asarnow, 1989). Socially, MDD patients are distant from others, have slow response to social interactions, and have distinctly sad appearance (APA, 1994; Calles, 2007; Fava and Kendler, 2000). Furthermore, a growing body of evidence suggests that MDD is associated with cognitive deficits (Den Hartog et al., 2003; Fossati et al., 2002; Paelecke-Habermann et al., 2005; Stip et al., 1994), particularly those requiring central executive functioning (Fossati et al., 2002; Ottowitz et al., 2002). Poor concentration or indecisiveness is often early symptoms of this disorder. In addition to the marked memory deficits often associated with MDD, people with depression become easily fatigued when asked to perform any mental tasks as reading, memorizing, studying, or when asked to solve complicated problems (Wong, 2002). As it worsens, abnormal morbid thoughts of death or suicide emerge.

The former paragraph clearly demonstrates the primordial importance of studying depression early enough in children even before the actual onset of MDD. In other words, the risk of high mortality, learning and memory deficits are of primordial concern during

childhood. Unfortunately, depression has a negative effect on memory and hence, learning. More importantly, cognitive impairments are an almost invariable component of MDD. The cognitive deficits seen in major depression patients affect mostly mnemonic processes (see review in (Fava and Kendler, 2000) which are responsible for building their personal life experience. For example, given MDD clinical heterogeneity, Bench, Dolan and their colleagues (Bench et al., 1990; Bench et al., 1995; Bench et al., 1993; Bench et al., 1992) attempted to correlate, in a series of positron emission tomography (PET) investigations of resting brain activity neuropsychological components with rCBF alterations in specific brain regions. Using correlational analysis, these authors found that global memory function and attentional processes were significantly correlated with rCBF decreases in the medial prefrontal cortex (MPFC). This finding correlates with the fact that the same area is activated during memory encoding in normal subjects (Nyberg et al., 1996).

Sadness is a principal characteristic of MDD. How the brain encodes sadness, which areas are involved, and their degree of involvement are important in our further understanding of the disorder. Encoding is defined as the processes by which new information is attended to and processed when first encountered. The extent and nature of this encoding are critically important for determining how the learned material will be perceived, analyzed and remembered at later times.

Recent meta-analytic reviews suggest a significant relationship between depression and memory deficits (Burt et al., 1995; Danion et al., 1991; Kindermann and Brown, 1997;

Teasdale, 1999; Watkins et al., 2000; Watkins et al., 1992). Of relevance to our study is mood-congruent memory (MCM), which is the tendency to recall information that is congruent with one's mood (Watkins et al., 1992). We believe according to our hypothesis that one could do the same also during encoding. Therefore, the content or affective valence of information is only relevant to the mood-state.

Memory deficits in depression have been demonstrated both on the explicit and implicit levels. In explicit memory the subject is asked to explicitly attempt to remember some earlier encoded or learned material. Implicit memory show evidence of learning, but no explicit reference is made to the earlier learning experience. Williams et al, (Williams and Scott, 1988) found that in depression MCM bias is found in explicit memory but not in implicit memory, which is relevant to the technique we used in this study.

Another important point in the question of why study negative emotion processing in children is that in the temperament literature, emotionality refers to the reactive component of emotion. Emotionality has been described as the individual differences in thresholds of reaction, latency, intensity, and recovery time (Hagekull and Bohlin, 2003; Hagekull., 2004; Rothbart, 1989) that is, how easily and how intensely emotions are aroused (Murphy, 1999). With regard to emotion regulation, most researchers agree that it involves successful management of emotional arousal to secure effective social functioning. In this vein, negative reactivity (sadness, anger, irritation, and anxiety) (Rothbart, 1989) and regulatory capacities have appeared most frequently in studies of the emotional basis of social

behaviour. High levels of negative emotionality and low regulation are presumably associated with high levels of behaviour problems (Eisenberg et al., 2000). For example, the NIMH describes a depressed child as "may pretend to be sick, refuse to go to school, cling to a parent, or worry that the parent may die. Older children may sulk, get into trouble at school, be negative, grouchy, and feel misunderstood" (NIMH:, 2007). These hypothesized relations have received support, to a large extent on the basis of studies by Eisenberg and colleagues (Eisenberg et al., 1995), on preschool and school-age children. The results show that high negative emotionality and low regulation have been associated with internalizing problems while low levels of negative emotionality and high levels of regulation have been associated with social competence in terms of good peer relation, healthy social behaviour, and better social skills.

4. Understanding the pathophysiology of major depressive disorder

A recent stereotactic meta-analysis by Steele and colleagues (Steele et al., 2007) concluded that despite the variability of reports in the literature, activity reported to be abnormal in MDD is particularly localized to brain areas representing the substrate for normal emotional experience in healthy subjects. These areas included the MPFC (reported abnormal when healthy subjects experience emotion, Broadmans Area (BA) 32 but extends into BA 25) and the lateral prefrontal cortex (LPFC) (orbitofrontal region (BA 47) for emotion processing and the other centred on a dorsolateral prefrontal cortex (DLPFC) region (BA 46 and 9) associated with cognitive tasks). Another important evidence of brain abnormalities in MDD comes from the path modeling metanalysis published by Mayberg's

group (Seminowicz et al., 2004). The authors conducted a metanalysis of effective connectivity in MDD using F-deoxyglucose positron emission tomography (FDG-PET) data and Structural Equation Modeling. The study tested several depression models based on current theories of limbic-cortical dysfunction in MDD and anatomical connectivity literature. A 7-region model consisting of lateral prefrontal cortex (LPFC BA9), anterior thalamus (ATh), anterior cingulate (ACC BA24), subgenual cingulate (sACC BA25), orbital frontal cortex (OFC BA11), hippocampus (Hipp), and medial frontal cortex (MPFC BA10) was identified. The above model was stable for MDD patients and healthy controls. Path differences in limbic-cortical connections (LPFC BA9- sACC BA25- OFC BA11-Hipp) differentiated drug treatment responders from nonresponders MDD patients. Nonresponders had also disturbed limbic-subcortical abnormalities in limbic-subcortical pathways (ATh-rACC BA24-sACC BA25-OFC BA11-Hipp), while differences in the Hipp-LPFC BA9 and the OFC BA11-MPFC BA10 pattern, distinguished between cognitive behavioural therapy (CBT) responders and pharmacotherapy responders.

Current studies view depression as a condition that results from developmental perturbations in information processing (Pine et al., 1998), and in emotion regulation (Davidson et al., 2002). These studies emphasize the role of perturbed attention allocation, particularly as a function of affective context, in mood and anxiety disorders (Beuke et al., 2003). Although such theories are generally well supported in studies of adults, virtually no research in children employs basic neuroscience measures to explore associations between mood or anxiety disorders and either perturbed attention control or contingency-related

processing (Austin et al., 2001). This limitation hinders efforts to move developmental models beyond simple descriptive formulations into neuroscience-based theories.

5. Neurobiological findings in depression

Despite continuing advances in the development of antidepressant drugs, the condition of about 30% of patients remains refractory to drug treatment (Hirschfeld, 1999). Importantly, children and adolescents have been found to differ from depressed adults in the neurobiological correlates and treatment response (Kaufman et al., 2001). Nevertheless, the most consistent finding across populations is alterations in structure, metabolism, chemistry and receptor binding in prefrontal cortical circuits. Such abnormalities have been demonstrated in postmortem and in vivo neuroimaging findings, both in adult and pediatric patients (Arango et al., 1997; Baxter et al., 1989; Drevets et al., 1997; Nolan et al., 2002). Most neuroimaging studies of depression have examined resting metabolic rate or blood flow using PET or single photon emission computed tomography (SPECT). These studies have focused on differences between *adult* patients and healthy comparison subjects when the patients were in the midst of a depressive episode as well as on changes in these parameters of brain function as a consequence of both antidepressant medication and behavioral or psychological treatment (Baxter et al., 1989; Bench et al., 1992; Brody et al., 2001). One of the most consistent findings has been that a variety of antidepressants increase the metabolic rate in the dorsolateral prefrontal cortex of depressed individuals, concomitant with symptom remission (Kennedy et al., 2001; Mayberg et al., 1999).

Neuroanatomical and neurochemical evidences through neuroimaging studies support a

Neuroanatomical and neurochemical evidences through neuroimaging studies support a role for dysfunction within the prefrontal cortical and striatal systems that normally modulate limbic and brain stem structures involved in mediating emotional behaviour and memory in the pathogenesis of depressive symptoms (Drevets, 2000, 2001; Drevets et al., 1997). Interestingly, the brain activity in the dorsolateral prefrontal cortex (DLPFC) (corresponding to Brodmann's areas [BA] 9/46) has been found to be decreased and conversely to be increased in the ventrolateral prefrontal cortex (VLPFC) (corresponding to BA 10/45/47) and ventral ACC during active sad thoughts (Brody et al., 2001). Other regions found abnormal in baseline studies include the temporal lobe, and basal ganglia. Impaired explicit memory is associated with left prefrontal and medial temporal dysfunction and imapired sustained attention with right prefrontal and parietal dysfunction (Goodwin, 1996; Kennedy et al., 1997; Ketter, 1996; Kimbrell et al., 2002; Mayberg, 1997; Soares and Mann, 1997).

In addition, advances in functional brain imaging have led to substantial progress in elucidating the physiology of induced sadness. Findings in these studies include decreased activity in the dorsal frontal cortex and dorsal ACC and posterior cingulate (PC) (Prado et al., 1993) and conversely, increased activity in the orbitofrontal cortex (OFC), the ventral ACC, the inferior frontal cortex and the MPFC (Beauregard et al., 1998; Mayberg et al., 1999).

The DLPFC circuit originates in BA 9 and 10 on the lateral surface of the anterior frontal lobe and projects to the dorsolateral head of the caudate nucleus. Neurons from this site project to the lateral part of the mediodorsal globus pallidus interna and rostrolateral substantia nigra pars reticulata as the direct pathway. The fibers from the basal ganglia project to parvocellular portions of the ventral anterior and mediodorsal thalamus. The mediodorsal thalamus sends fibers back to the circuit origin in the DLPFC (Burruss et al., 2000). A variety of evidence from experimental lesion studies in animals, and clinical neuropsychological and functional brain mapping studies in humans, indicating that the DLPFC is a key structure involved in willed actions (Frith and Dolan, 1996), with the holding in mind of information on which an action is to be based (Fuster, 1999; Goldman-Rakic, 1987; Roberts and Wallis, 2000), and with reappraisal, which is a cognitive form of emotion regulation (Ochsner et al., 2002). According to Baxter and colleagues (Baxter et al., 1989), the DLPFC may play an especially critical role in the pathogenesis of MDD. More recently, numerous other authors have also advocated the role the DLPFC plays in MDD. For example, Farchione et al., (Farchione et al., 2002) have reported, using proton magnetic resonance spectroscopic imaging in pediatric depression, a significant increase in choline compounds in left but not right DLPFC in MDD patients versus control subjects (32.5% higher). The authors concluded that this could lead to neuronal signal transduction abnormalities in pediatric MDD, resulting in functional anomaly of the DLPFC.

The ACC circuit originates in the anterior cingulate cortex (BA 24). The neurons project to ventral striatum, which includes the ventromedial caudate, ventral putamen, nucleus accumbens and olfactory tubercle (limbic striatum). Projections from the ventral striatum pass to the rostromedial globus pallidus interna, ventral pallidum and rostrodorsal substantia nigra. The ventral pallidum connects to the ventral anterior nucleus of the thalamus. The anterior cingulate circuit is closed with projections from ventral anterior thalamus back to the ACC. Limbic system connections involve both the ACC and medial frontal regions (Burruss et al., 2000). The ACC plays a pivotal role in the interoceptive and exteroceptive detection of emotional signals (Lane et al., 1997a). A great body of evidence has also implicated the ACC in the pathology of MDD. Anatomical, post-mortem and in vivo magnetic resonance imaging (MRI) studies have demonstrated reduced volume in the subgenual region of the ACC in patients with MDD compared with healthy controls (Botteron et al., 2002; Drevets et al., 1997; Drevets et al., 1992; Ongur et al., 1998). Furthermore, functional neuroimaging studies have also repeatedly found metabolic and perfusion abnormalities in the ACC (Bench et al., 1995; Ebert and Ebmeier, 1996; Mayberg et al., 1997). Two previous studies done by our group (Beauregard et al., 1998; Fahim et al., 2004) reported increased activation of the ACC in response to emotional film clips and aversive pictures. It is worth noting that these finding have been limited by using either adult depression patients or pediatric subjects already diagnosed with depression.

Based on the above, two studies were performed, 1- a pilot study involving 10 children with subclinical symptoms of depression (DS) and 15 asymptomatic children (AC) that

Based on the above, two studies were performed, 1- a pilot study involving 10 children with subclinical symptoms of depression (DS) and 15 asymptomatic children (AC) that aimed at investigating two brain areas: the dorsolateral prefrontal cortex (DLPFC) and ACC during the processing of sad stimuli. In the study, based on prior data and theory, we predicted that DS children would differ from AC subjects in their brain activation in response to the sad emotional stimuli. Specifically we predicted that DS children would only significantly activate regions implicated in emotional processing (ACC), on the other hand, normal children would activate regions implicated in emotional processing (ACC) and affect regulation (DLPFC) (Please see Article 1)

2- The second study aimed at increasing the statistical power by including more subjects, investigating a wider range of brain regions involved in the pathogenesis of depression and in emotional processing, and to test the model proposed by Mayberg and colleagues (Seminowicz et al., 2004) in children with only subclinical symptoms of depression. (Please see Article 2)

Article 1

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Dorsolateral prefrontal-anterior cingulate cortices activation in children with depressive symptoms during the external induction of sadness

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Abstract

Objective: We sought to compare brain activation patterns in healthy and depressed children during the external induction of sadness using functional magnetic resonance imaging (fMRI).

Method: Fifteen normal (N) controls and ten children with depressive symptoms (DS) were scanned with fMRI during the passive viewing of sad and emotionally neutral stimuli.

Results: Both groups activated the anterior cingulate cortex (ACC), but DS children exhibited less right dorsolateral prefrontal cortex (DLPFC) activation during sad stimuli relative to normal subjects.

Conclusion: Normal children activated the ACC and DLPFC and thus were able to process and regulate sadness. However, DS children activated the ACC only, thereby processing sad stimuli without the normal involvement of emotion regulation. These results suggest that depressive symptoms in childhood may be primarily related to a disturbance of the DLPFC-mediated down-regulation of sadness– which in turn may lead to the persistent and recurrent negative affect generally observed in depression.

Introduction

Children suffering from major depressive disorder (MDD) have been found to differ from depressed adults in terms of neurobiological correlates and treatment response (Kaufman et al 2001). Neuroimaging and postmortem studies have shown that alterations in structure, metabolism, chemistry and receptor binding in prefrontal cortical circuits are the most consistent abnormalities found among depressed children (Drevets et al 1997).

Several lines of evidence indicate that prefrontal cortical abnormalities play a key role in MDD (Steffens and Krishnan 1998). Functional neuroimaging studies conducted by our group and others have demonstrated that the dorsolateral prefrontal cortex (DLPFC) is crucially involved in emotion regulation (Beauregard 2004; Byrum et al 1999). Mounting evidence suggests that a dysregulation of emotion constitutes the core feature of MDD (Kring 1999). According to Baxter and colleagues (Baxter et al 1989), the DLPFC probably plays a critical role in the pathogenesis of MDD. Similarly, several authors have recently stressed the involvement of the DLPFC in depression. For example, Farchione et al (Farchione et al 2002) reported a significant increase in choline compounds in left but not right DLPFC in MDD patients versus controls – probably leading to a functional disturbance of the DLPFC.

Given that sadness is the prevailing mood in depression, we compared brain activation in healthy (N) and children with depressive symptoms (DS) during the external induction sadness. Analysis focused on the DLPFC and anterior cingulate cortex (ACC), two regions previously implicated in emotion processing and depression (Steffens and Krishnan 1998). The ACC is involved in the interoceptive and exteroceptive detection of

emotional signals (Lane et al 1997), and a large body of evidence has implicated this region in the pathophysiology of MDD (Davidson et al 2002). Anatomical, post-mortem and in vivo magnetic resonance imaging (MRI) studies have demonstrated reduced volume in the subgenual region of the ACC in patients with MDD compared with healthy controls (Botteron et al 2002; Drevets et al 1997; Drevets et al 1992; Ongur et al 1998). Similarly, functional neuroimaging studies have repeatedly found metabolic and perfusion abnormalities in the ACC of depressed patients (Bench et al 1995; Ebert and Ebmeier 1996; Mayberg et al 1997). Two previous studies performed by our group (Beauregard et al 1998; Fahim et al 2004) reported increased activation of the ACC in response to sad film excerpts and aversive pictures.

Based on these prior data and theory (Farchione et al 2002; Mayberg et al 1997), we predicted that the DLPFC and ACC would be differentially recruited in healthy and depressive children.

Methods

Fifteen normal (N) (age: 8 years; 8 girls/7 boys) and 10 DS children (age: 8 years; 3 girls/7 boys) matched for sex (Pearson χ^2= 1.326 p= .250) participated in this study. All subjects were right handed and medication free. Depression symptoms were assessed using the Dominic-R, a self-report DSM-IV-based computerized cartoon designed to assess psychiatric disorders in children 6-11 years of age (Valla et al 2000). This instrument compares favorably with child psychiatric interviews (PICA-III-R, DICA-R, DISC-2.3) in reliability and criterion validity, notably in the case of internalized disorders (Valla et al

2000). A recent study (Arseneault et al 2005) showed that the Dominic-R also successfully distinguished children meeting DSM-IV criteria for research diagnoses of conduct disorder. Mean Dominic-R depression scores were 1.53 (SD=1.3) in the N group and 11.91 (SD=0.83) in the DS group (p=0.0001).

Blood oxygen level dependent (BOLD) signal changes were measured while subjects passively viewed five blocks of emotionally neutral film excerpts (reference task) followed by five blocks of sad film excerpts (activation task). The latter depicted the death of a father extracted from the film *The Champ* (1979), validated (Gross 1995) and used in several studies of sadness induction (Christie and Friedman 2004; Eugene et al 2003; Levesque et al 2003; Sloan 2004). As subjective emotional responses persist on average 32sec after presentation of aversive pictures (Garrett and Maddock 2001), this design was used to avoid contamination of neutral by sad stimuli. Each block lasted 39sec and was separated by 15sec- resting periods during which subjects viewed a white cross on a black screen. A multiple-choice visual analog scale designed to identify and rate emotions felt while viewing the excerpts was presented to subjects immediately after scanning. Children first identified the primary emotions they felt (happiness, anger, sadness, fear, surprise, disgust). If a child identified sadness he/she was asked to rate its degree (sad, very sad, extremely sad, saddest ever). Subjects underwent a semi-structured interview where they described in detail what they viewed and how they felt during the sad and neutral stimuli.

Echoplanar images (EPI) were acquired on a 1.5-Tesla system (Magnetom Vision, Siemens Electric, Erlangen, Germany). Twenty-eight slices (5mm thick) were acquired every 2.65sec in an inclined axial plane, aligned with the AC-PC axis. These T2* weighted functional images were acquired using an EPI pulse sequence (TR=0.8msec, TE=54msec, Flip=90°, FOV=215mm, Matrix=64x64, Voxel size=3.36mm x 3.36mm x 5mm). Following functional scanning, high-resolution data were acquired via a Tl-weighted 3-D volume acquisition obtained using a gradient echo pulse sequence (TR=9.7msec, TE=4msec, Flip=12° FOV=250mm, Matrix=256x256, Voxel size=0.94mm^3). Data were analyzed using Statistical Parametric Mapping software (SPM2, Wellcome Department of Cognitive Neurology, London, UK). Statistical parametric maps for each contrast of the t statistic were calculated on a voxel-by-voxel basis. T-values were transformed to unit normal distribution yielding z-scores. Using a random-effect model, 1-sample t-test was conducted for each group to subtract brain activity associated with neutral from that associated with sad stimuli (sad minus neutral). Two-sample t-test was then performed to compare brain activity observed in both groups for the same contrast. An *a priori* search strategy was used, and a small volume correction was performed in the brain regions of interest (ROI) defined *a priori.* The search volume corresponding to these ROIs (ACC and DLPFC) was based on the neuroanatomic boundaries of these regions noted in the MR reference image (MNI template) and the Talairach & Tournoux Atlas (Talairach 1988). For this *a priori* search, a probability threshold for multiple comparisons of $P<0.05$ corrected was used. Only clusters showing spatial extents of at least five contiguous voxels were kept for analysis.

Results

Behavioral data: Comparison of self-reported sadness revealed no difference between the two groups (N: Mean=1.88, SD=1.36; D: Mean=2.73, D=1.49 P=0.91).

fMRI data:

For the sad minus neutral contrast, significant loci of activation were noted in the right DLPFC (Brodmann area–BA9/46) for the N group, and the right ACC (BA24/32) for the DS group (Table 1, Figure 1). For the same contrast, greater activations were found (1) in the left ACC (BA24/32) and right DLPFC (BA10/46) for the N relative to the DS group (N minus DS), and (2) in the right ACC (BA24) for the DS relative to the N group (D minus N) (Table 1, Figure 1).

Using the simple regression function in SPM2, a positive correlation was found between depression scores across all subjects and BOLD activation in the affective division of the right ACC (BA24) (Bush et al 2000) (Table 1, Figure 1).

Discussion

The correlation found between BOLD activation in the ACC and depression score across all subjects is in agreement with previous studies suggesting the key role of the ACC in the neurobiology of depression (Davidson et al 2002). The loci of activation found in the ACC

also accord with results of previous studies showing that this cortical region is implicated in sadness (Beauregard 2004).

The fact that DS children exhibited less right DLPFC activation during the sad excerpts may reflect a decreased capacity in these subjects for self-regulation of negative emotional states – otherwise experienced equally in all subjects following the sad stimuli. Studies conducted by our group and others have shown that robust activation in the DLPFC is indeed associated with increased capacity to voluntarily regulate negative emotion (Jackson et al 2000; Levesque et al 2003). A number of PET studies have also found reduced regional cerebral blood flow within the DLPFC in MDD subjects (Drevets 2000), and sad thoughts and feelings have been linked with decreased activity in the DLPFC (Brody et al 2001). Together, the present results suggest that depressive symptoms in children are not associated per se with differences in subjective experience of, and brain activation related to, sadness, but rather to a disturbance of the DLPFC-mediated down-regulation of this emotion. In turn, this disturbance may lead to the persistence and recurrence of negative affect that are characteristic of depression.

Compared with studies on depression in adults, involving children with depressive symptoms minimizes potential confounds such as illness duration (Ballmaier et al 2004), treatment (Anand et al 2005) and hormonal effects (Nottelmann et al 1987). Our method for inducing sadness was previously validated using psychophysiological measures (Gross 1995), and we did not collect such data in the present study. Although we focused on the DLPFC and ACC, other prefrontal regions such as medial prefrontal and orbitofrontal

cortices have also been implicated in emotion regulation (Beauregard 2004; Levesque et al 2003) and in the pathogenesis of depression (Cotter et al 2005; Neumeister et al 2004).

Figure 1. Legend:

N= normal controls; DS= children with depressive symptoms; R=right; L=left; BA=Brodmann area; MPFC=medial prefrontal cortex; ACC=Anterior cingulate; DLPFC=dorsolateral prefrontal cortex.

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Table 1. BOLD activation during the viewing of the film excerpts in normal children (N) (n=15) and children with depressive symptoms (DS) (n=10).

Brain region		Talairach Coordinates					
	Brodmann area	x	y	z	Number	Z	Corrected
I) 1 sample t-test in N, contrast = sad *minus* neutral							
a. R MPFC	9	6	51	25	141	3.56	0.04
b. R ACC	32	24	30	36	73	3.17	0.04
c. R DLPFC	9	27	37	37	45	3.39	0.02
d. R DLPFC	46	38	21	24	7	3.24	0.03
II) 1 sample t-test in DS, contrast = sad *minus* neutral							
e. R ACC	32/24	5	40	3	16	3.63	0.001
III) 2 sample t-test in N minus DS, contrast = sad *minus* neutral							
f. L ACC	32/24	-17	29	21	13	3.33	0.007
g. R DLPFC	10/46	20	35	20	15	3.29	0.04
IV) 2 sample t-test in DS minus N, contrast = sad *minus* neutral							
h. R ACC	24	3	38	4	6	3.43	0.04
V) Correlation between depression scores and BOLD activation across all subjects.							
i. R ACC	24	0	38	4	7	3.67	0.04

R=right; L=left; MPFC=medial prefrontal cortex; ACC=Anterior cingulate; DLPFC=dorsolateral prefrontal cortex

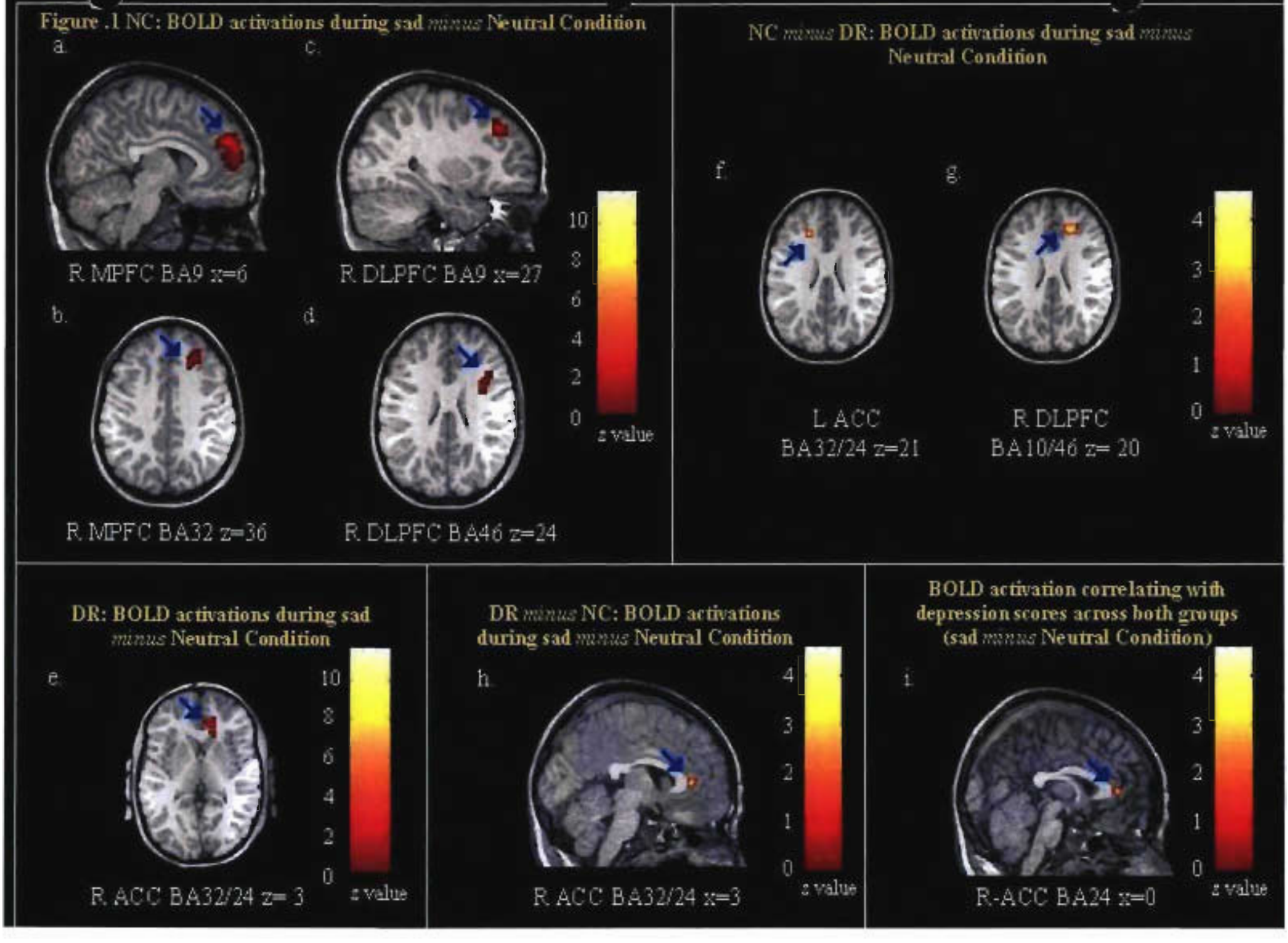
Figure .1 NC: BOLD activations during sad minus Neutral Condition
a.
c.
R MPFC BA9 x=6
R DLPFC BA9 x=27
b.
d.
R MPFC BA32 z=36
R DLPFC BA46 z=24
10
8
6
4
2
0
z value
NC minus DR: BOLD activations during sad minus Neutral Condition
f.
g.
L ACC
BA32/24 z=21
R DLPFC
BA10/46 z= 20
4
3
2
1
0
z value
DR: BOLD activations during sad minus Neutral Condition
e.
R ACC BA32/24 z= 3
10
8
6
4
2
0
z value
DR minus NC: BOLD activations during sad minus Neutral Condition
h.
R ACC BA32/24 x=3
4
3
2
1
0
z value
BOLD activation correlating with depression scores across both groups (sad minus Neutral Condition)
i.
R-ACC BA24 x=0
4
3
2
1
0
z value

Proof of submission for Article 1

-----Message d'origine-----

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List all author names (in order of appearance on the manuscript title page):

Adham Mancini-Marïe, Mario Beauregard, Boualem Mensour, Gilles Beaudoin, Michel Boivin and Daniel Pérusse

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Article 2

The following work was submitted on March 2007 to the Journal *Nature Neuroscience* (see attached proof of submission) under the title: Structural and functional corticolimbic abnormalities found in children with subclinical depressive symptoms

Structural and functional corticolimbic abnormalities found in children with subclinical depressive symptoms

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Abstract

Anatomical and functional anomalies are commonly observed in a corticolimbic circuit in adults with major depressive disorder (MDD). To explore causality and control for potential confounds of illness duration and treatment on this relationship, we used multimodal neuroimaging in unmedicated, subclinically depressed 8-year-old children. Diffusion tensor imaging showed that subjects with subclinical depressive symptoms versus asymptomatic controls exhibited pronounced reductions in white matter fiber tract integrity in hippocampus, lateral prefrontal, medial prefrontal, orbitofrontal, rostral anterior and subgenual anterior cingulate cortices –all regions associated with adult depression. White matter pathology predicted 50% of severity of depressive symptoms. Functional analysis of those regions during induced sadness also revealed profoundly altered patterns of neural activity and functional connectivity among symptomatic subjects. Finding that the circuit associated with adult depression presents such pervasive structural and functional abnormalities so early in development implies that neuropathology precedes the onset, and contributes to the pathogenesis, of MDD.

The World Health Organization Global Burden of Disease study[1] indicates that major depressive disorder (MDD) ranks second only to ischemic heart disease in magnitude of disease burden, and is the leading cause of disability worldwide among individuals age 15 and over. MDD is characterized by persistent sadness, dysphoria, anxiety, alterations of motivation and social behavior, psychomotor changes, and appetite and sleep disturbances (DSM-IV)[2]. Up to 25% of the population will experience a major depressive episode at some point in their lives[3]. The prevalence of childhood depression ranges from 2%[4] to 15%[5], depending on age and assessment method. Within five years of its onset, 70% of depressed children experience a recurrence[6]. Childhood depression is associated with important morbidity and mortality. Twenty-five percent of depressed adolescents develop a substance abuse disorder[7], and 10% complete suicide within 15 years of their initial episode[8].

Alterations in structure, metabolism, chemistry and receptor binding in prefrontal cortical circuits constitute the most consistent neurobiological findings in individuals with MDD. Such abnormalities have been demonstrated in postmortem and *in vivo* neuroimaging studies. Current theories view MDD as a condition resulting from developmental disruptions in brain systems supporting emotion regulation[9] and information processing[10]. These theories emphasize the role of perturbed attention allocation as a function of affective context[11]. Although this view receives some empirical support in adults, virtually no research employing basic neuroscience methods has been carried out in children to explore associations between mood disorders, emotion regulation and contingency-related

information processing[12]. This is a serious limitation in understanding the developmental pathology of MDD.

Most neuroimaging studies of MDD have examined resting metabolic rate or regional cerebral blood flow with positron emission tomography or single-photon emission-computed tomography. Some studies have focused on differences between affected adults and healthy subjects, others on changes in brain function as a consequence of antidepressant medication or psychological treatment[13]. Collectively, neuroimaging findings indicate that an abnormal activity in a limited number of brain areas underlies MDD. A recent meta-analysis[14] of effective connectivity in MDD has led to the proposal of a seven-region model involving the hippocampus, medial prefrontal cortex, orbitofrontal cortex, rostral anterior cingulate cortex, subgenual anterior cingulate cortex, and anterior thalamus in the right hemisphere, as well as the lateral prefrontal cortex in the left hemisphere. However, these brain abnormalities cannot be readily interpreted to cause MDD since they are observed in adults who have generally been ill for years, who are in the midst of a major depressive episode, or who have been exposed to the prolonged effects of antidepressant medication or psychological treatment.

In an effort to resolve potential confounding effects of illness duration[15] and therapy[16] on the relationship between brain pathology and MDD, we combined different imaging techniques to characterize the adult depression circuitry[14] in unmedicated 8-year-old children displaying significant but subclinical depressive symptoms. We used diffusion

tensor imaging (DTI) to measure the cellular integrity of white matter fiber tracts in the same child brain regions as those that are maximally affected in adult depression[14]. We then induced a state of sadness –the prevailing mood in MDD– to investigate neural activity and functional connectivity in those same regions using functional magnetic resonance imaging (fMRI). Assuming that brain abnormalities related to MDD are causal to behavioral symptoms rather than the converse, we tested the following hypotheses: In those regions that are maximally associated with adult depression, young children showing depressive symptoms at subclinical levels will nevertheless display 1) reduced cellular integrity of white matter fiber tracts, 2) altered neural activation, and 3) disturbed functional connectivity during transient sadness. To our knowledge this is the first neuroimaging investigation of subclinically depressed children. It is also the first study to investigate depressive symptomatology using DTI.

Results

Structural connectivity

We first conducted a structural study using DTI, a powerful technique that enables the non-invasive *in vivo* imaging of white matter fiber tract integrity, based on the fact that the random, three-dimensional, diffusion-driven molecular motion of water is sensitive to nerve tissue structure at a microscopic scale[17]. Fractional anisotropy (FA) derived from DTI provides the best quantitative information regarding the degree of directionality of water diffusion in relation to white matter fibers[17-19]. Lower FA indicates reduced directionality

of water molecules due to decreased myelination and/or a decreased number of myelinated white matter fibers, and therefore reduced structural connectivity[18].

Relative to asymptomatic controls (AC), subjects with subclinical depressive symptoms (DS) showed significantly lower FA values in all six regions examined. The difference in FA between the two groups was large in all regions (Fig. 1a,b, Table 1). Depending on area, FA was reduced by 28%-68% in symptomatic (DS) compared to asymptomatic (AC) subjects. Given our relatively limited sample size, we computed Cohen's *d*, a measure of the magnitude of group differences that is independent of sample size[20]. We found that FA was lower by 0.74 to 1.49 full standard deviation in DS relative to AC subjects. In four of the six regions examined, the effect of belonging to the symptomatic versus asymptomatic group was equal to or greater than one FA standard deviation – corresponding to a non-overlap of over 55% in the FA distributions between the two groups. The greatest FA difference was observed in the medial prefrontal cortex (MPFC, BA10), yielding a non-overlap of 70% between DS and AC subjects. Overall, the two groups were almost as distinguishable on white matter integrity as they were on depressive symptomatology.

DS subjects also showed FA values well below the range for normal white matter[21-23] in three of the six regions examined, and at the lower end of that range in two other regions. Water diffusion was practically isotropic in the lateral prefrontal cortex (LPFC, BA9), the MPFC (BA10), and the orbitofrontal cortex (OFC, BA11), implying a serious impairment in white matter fiber myelination and compromised anatomical connectivity.

To examine whether these six regions belonged to an integrated circuit as hypothesized[14], we correlated FA values across areas. Significant correlations were found in approximately half of couplings, with the hippocampus (Hipp) being most constantly correlated across regions (Table 1). Remarkably, the distributions of regional FA within the hypothesized circuit were almost identical irrespective of symptomatology status –the variance in one group accounting for nearly 90% of the variance in the other group (Fig. 1c).

Together, these findings describe drastic reductions in white matter fiber tract integrity and serious impairment in anatomical connectivity among DS subjects in the very same regions maximally associated with adult MDD. The following fMRI analyses provide a means for examining these structural abnormalities at a functional level.

Neural activity during transient sadness

We contrasted neural activations in the same regions between the two groups during the external induction of sadness to identify functional differences between DS and AC subjects. In the AC group, the Sad minus Neutral contrast revealed two significant loci of activation, bilaterally, in the MPFC (BA10). In the DS group, a weaker and lateralized single locus of activation was found in the same region, as well as two other significant loci in the right anterior thalamus (AT) (Fig. 2, Fig.3a, Table 2).

To further characterize the differences between symptomatic and asymptomatic subjects, we then subtracted the DS group from the AC group for the same contrast (Sad minus Neutral). Activations in AC subjects minus activations in DS subjects were again significant in the left MPFC (BA10) as well as in the right OFC (BA11) and the anterior cingulate (ACC, BA24a, 32) cortices. In contrast, subtracting the AC group from the DS group yielded significant loci of activation only in the right ventrolateral prefrontal cortex (VLPFC, BA47) and the OFC (BA11), bilaterally (Fig. 2, Fig. 3b, Table 2).

Interestingly, the largest relative reduction in activation observed in DS subjects was found in the MPFC (BA10), the same region that showed the greatest reduction in white matter integrity in these subjects. Furthermore, DS subjects were uniquely characterized by significant activation in the right VLPFC (BA47), an area found to be maximally abnormal in a recent meta-analysis of forty-two studies of adult major depression[24]. Finally, only AC subjects showed activation in the ACC (BA32), a region also found to be consistently different between normal and depressed adults[24].

Overall, these findings demonstrate extensive neural activation differences during transient sadness between DS and AC children in the same regions belonging to the adult depression circuit.

Functional connectivity during transient sadness

Given the above results, we sought to test whether these different patterns of neural activity gave rise to varying pathways of functional connectivity between the two groups. We performed a psychophysiological interaction (PPI) analysis[25] to obtain an approximation of functional connectivity between regions showing maximum sadness-related activation. Using the peak activities from the Sad minus Neutral contrast for each subject, we determined seed locations and then identified brain areas showing co-activation with these regions. PPI networks were established for each subject and then entered into a group-level analysis to determine functional connectivity differences between the two groups (Fig. 4, Table 2).

In AC subjects, significant co-activations were first observed between the right MPFC (BA10) and itself, the ACC (BA24), and the midline posterior cingulate cortex (PC, BA 23/31). We also found significant interactions involving the left ACC (BA24/32) on the one hand, and the right ACC (BA24), the right OFC (BA11), the left MPFC (BA10), the right putamen, and the right inferior parietal lobule (IPL, BA40) on the other. Last, significant couplings were observed between the right VLPFC (BA47) and the right MPFC (BA10) as well as with the right OFC (BA11).

We identified strikingly different networks of interaction in DS subjects. The right MPFC (BA10) was found to connect significantly with the midline PC (BA30), the superior parietal lobule (SPL, BA7), and the left IPL (BA40). The AT was significantly coupled with the PC (BA30, 31), the left OFC (BA11), and the left IPL (BA40). Significant

interactions also involved the left OFC (BA11) and itself, as well as the left PC (BA30). Finally, we found significant connections between the right VLPFC (BA47) and the OFC (BA11), the IPL (BA7, 40), the right PC (BA31), and the left SPL (BA7).

Thus, sadness-related neural activations were found to give rise to crucially different pathways of functional connectivity between the two groups: in AC subjects, most interactions (8 of 11) occurred within the described depression circuit, a network also involved in non-pathologic sadness[14]. In contrast, the majority of connections in DS subjects (12 of 17) took place outside of this circuit. These findings reveal that in subclinically depressed children, abnormal functional connectivity closely parallels the structural connectivity impairment observed in the regions making up the adult depression circuit.

Brain abnormalities and behavioral alterations

To further explore the relationship between white matter integrity and depressive symptomatology, we tested whether FA in each region of the hypothesized circuit correlated negatively with depression scores across subjects. We found highly significant negative correlations for all regions, except for the subgenual ACC (sACC, BA25) where we observed a moderately significant negative correlation, and the rostral ACC (rACC, BA24a) where a trend in the same direction was detected (Table 1). To further test whether these regions were indeed integrated within a neural circuit, we performed a multivariate regression analysis entering FA values within each region as independent variables and

depressive score as the dependent variable. Remarkably, "circuit" white matter pathology was found to account for half of the severity of depressive symptoms [$F(6,33) = 5.06$, $p \leq 0.000$, $r^2 = 0.505$].

We also tested whether the magnitude of subjective sadness induced by the experiment differed between the AC and DS groups. We found that the level of sad feelings was significantly greater in DS subjects compared to AC subjects (DS group: Mean = 3.47, SD = 0.71; AC: Mean = 2.72, SD = 0.76; t= 3.14; p = 0.002). This suggests that brain activations and functional connectivity differences between normal and depressed subjects underlie a reduced capacity to regulate sadness in the latter. Unsurprisingly, we found a highly significant positive correlation between magnitude of sadness and severity of depressive symptoms across all subjects ($r = 0.47$, $p = 0.002$).

Discussion

Our strategy of using multimodal neuroimaging in unmedicated, subclinically depressed children has led to the identification of major abnormalities in structure, function and interconnections within a corticolimbic circuit found to be maximally implicated in adult MDD. The analysis of white matter integrity revealed lower FA in DS children in all brain regions examined, abnormal values in three regions, and marginally normal values in two others. White matter integrity was also correlated between regions and, notably, showed nearly identical inter-regional distributions irrespective of depressive status. These latter findings strongly suggest that the specific regions examined are indeed integrated in a

structurally connected circuit, a conclusion also supported by the known anatomical connections between these corticolimbic areas[14]. Not only was FA in each region negatively correlated with depressive symptoms, but white matter pathology considered at the level of an integrated circuit was found to account for 50% of the severity of depressive symptomatology. We know of no other measure that qualifies more decisively as a biological marker for MDD.

The functional analysis of the same regions, while subjects experienced sadness –the emotion most often associated with MDD–, revealed profoundly distinct patterns of neural activation. DS children showed the greatest reduction in activation in the MPFC (BA10), where they also displayed the largest reduction in white matter integrity. In direct comparisons between the two groups, we found that only DS children showed significant activation in the right VLPFC (BA47), whereas only AC children showed activation in the ACC (BA32). Both areas have been identified as maximally different in adults with MDD relative to healthy subjects[24]. Interestingly, increased VLPFC activity has been reported in association with sadness in adults with MDD[13]. We also found unique activation among symptomatic children in the OFC (in the inferior frontal area, BA11). In fMRI studies previously conducted by our group, we showed that this portion of BA11 plays a pivotal role in the self-regulation of sadness[26]. We also demonstrated that a greater difficulty to down-regulate sadness is associated with an increased activation of the brain regions underlying self-regulation of this emotion[27]. Thus, it is possible that the greater orbitofrontal activation measured here in DS children reflects a dysfunction in the neural

circuitry of emotion regulation, which may account for the chronic sadness typifying depressive disorders.

The PPI analysis revealed that the regions examined were functionally connected in completely different ways between DS and AC subjects. In the latter, functional connectivity mostly implicated cortical areas located anteriorly in the brain (e.g., OFC, MPFC and ACC). In contrast, functional connections heavily engaged posterior cortical regions in DS subjects, such as the PC, IPL and SPL. There is evidence that the PC is crucially involved in the regulation of both normal and pathologic negative emotions[28]. A recent anatomical MRI study found that adults with MDD have significantly smaller PC volumes compared with normal controls[29]. In addition, a meta-analysis of several neuroimaging studies conducted in healthy subjects indicates that the PC plays a critical role in emotional episodic memory, during presentation of emotionally aversive stimuli[30] and in autobiographical memory[31]. In this context, it is plausible that DS children experienced more sadness than AC children by overly engaging the PC, which might be involved in rumination about past negative emotional experiences. Rumination is typical of individuals with depression. As for the IPL and SPL (BA7, 40) a PET study[32] during sadness in healthy subjects found a deactivation of these posterior parietal areas, which have previously been implicated in the regulation of negative emotional arousal[33]. The abnormal pattern of functional connectivity involving these regions in DS children might thus reflect a deficit in the regulation of arousal associated with sad feelings.

Overall, our results indicate that both structural and functional brain abnormalities are found in unmedicated children with subclinical depressive symptoms. Specifically, extensive white matter pathology and seriously altered functional connectivity are already present in 8-year-olds at risk for depression in the very same brain regions known to be involved in adult MDD. At a network level, our findings provide a compelling childhood homolog to the most robust brain-based model of adult depression.

There is growing evidence from animal models of depression and anxiety that experimental manipulation of parental rearing generates long-lasting neurobiological and behavioral alterations in offspring[34]. A large body of data in humans also shows that early life events such as parental death, divorce, neglect, and abuse, possibly in conjunction with genetic susceptibility[35], increase risk for a wide range of psychiatric conditions in offspring, including major depression[36]. Early stress in childhood has been shown to generate structural and functional alterations in brain regions similar to those seen in adults with MDD[37]. Given this, we propose that early familial stressors are especially likely to alter the integrity of the child's rapidly developing white matter, which in turn contributes to reduced corticolimbic connectivity, altered functioning of the neural circuitry underlying negative emotion regulation, depressive symptomatology and, ultimately, MDD.

Methods

Subjects

Forty-three 8-year-old children took part in this study. All subjects underwent fMRI then DTI scanning. Of the 43 subjects, 20 DS (11 girls, 9 boys) and 23 AC (10 girls, 13 boys) children completed DTI scanning, while 20 DS (12 girls, 8 boys) and 22 AC (8 girls, 14 boys) children completed fMRI scanning. All subjects were right-handed, matched for parental education and medication free except for two AC subjects receiving treatment for bronchial asthma. The study was approved by the ethics review boards of Ste. Justine Hospital and Centre hospitalier de l'Université de Montréal Hôpital Notre-Dame. All parents gave written informed consent.

Psychiatric assessment

Depressive symptoms were assessed using the *Dominic-R*. This self-answered computerized instrument consists of 90 pictures showing a character named Dominic in a variety of situations, and is akin to a video game; the use of pictures avoids relying on the vocabulary of the child. In some of the pictures, Dominic's behavior is positive, but in most situations his/her reactions are pathological (e.g., Do you feel sad most of the time, even when others are having fun?). The present study focused on the 20 depressive items of the instrument. The cut-off points for the likelihood of MDD are: (1) There is no problem [0-10], (2) There could be a problem [11-13], and (3) There is a problem [14-20]. The *Dominic-R* has been through an extensive development and validation process since it was designed in the early 1980s, and has been used with children from various ethnic groups in clinical and research settings in Quebec (Canada)[38,39] and elsewhere[40-44]. Its psychometric

properties were found to compare favorably with other psychiatric child interviews (e.g., PICA-III-R, DICA-R, DISC-2.3) in reliability and criterion validity, notably on internalized problems such as depression and anxiety[45]. A recent study[43] showed that the *Dominic-R* successfully distinguished children meeting DSM-IV criteria for research diagnoses of conduct disorder. In the present study, depressive scores were in the symptomatic but subclinical range for the DS group (mean=11.90, SD=1.0), and minimal in the AC group (mean=2.63, SD=1.6, P=0.0001). Both DS and AC subjects showed scores on all other disorders that were in the "There is no problem" category of the *Dominic-R*. Also, both groups scored in the normal range on the Strengths and Competencies scale of the instrument.

Diffusion tensor imaging

Acquisition

Images were acquired on a 1.5 Tesla system (Magnetom Vision, Siemens Electric, Erlangen, Germany). Sixteen slices (4-mm thick) were acquired in an inclined axial plane aligned with the AC-PC axis (repetition delay=5.197sec, pixel size=2.50 x 1.88mm, scan time=24sec). Images were acquired using an echoplanar (EPI) pulse sequence (TR=3100msec, TE=100/100.06msec, TD=0msec). DTI was acquired for each slice with four sets involving diffusion gradients placed along non-collinear directions (diffusion sensitivity, b =1000 s/mm^2). Each of the four sets consisted of seven images (six in which b=1000/or/500 and one where b=0 (gradient directions [0 0 0]). For each non-zero b value,

the sequence acquired images with *b* in six directions (gradient directions = [1 1 0], [1 0 1], [0 1 1], [-1 1 0], [-1 0 1], [0 -1 1]).

Processing and analysis

Raw diffusion-weighted data were corrected for geometric distortion secondary to eddy currents using a registration technique based on the geometric model of distortions[46]. Diffusion tensor (DT) FA was calculated using in-house software based on the method described by Basser and Pierpaoli[18]. A fully automated DTI algorithm was implemented in MATLAB7 (The MathWorks, Inc.) and run as a toolbox in SPM2 (Wellcome Department of Cognitive Neurology, London, UK). The algorithm was created based on the methodology described by Basser and Pierpaoli[18] for the qualitative examination of the white matter. The DT map is a three-dimensional rendering of DT data, and provides information on FA levels of the brain. In axons, water diffusion is impeded by cell walls and myelin sheaths. As a result, water movement is much larger along the axis of an axon than perpendicular to it. DTI allows visualization of this movement by fully characterizing water diffusion in three-dimensional space. Since the movement of water molecules is restricted by the boundaries of the axons, visualization of that movement allows visualization of the structure and direction of axons within a DTI brain image. FA is derived from eigenvectors, which define the orientation of the principal axes of a diffusion ellipsoid in space[19]. FA is expressed as a numerical value between 0 and 1 without a unit. A higher value implies a greater degree of directionality of water diffusion. Water diffusion parallel to white matter tracts is less restricted than water diffusion perpendicular to them.

Consequently, normal white matter shows strong directionality of water diffusion and high FA: 0.2-0.6 in normal white matter and 0.6-0.8 in the corpus callosum[22,23,47].

DTI data were processed using a multistep procedure to achieve the following: (1) motion and residual eddy current distortion correction; (2) calculation of FA from DT; (3) spatial registration of the images into standard space using SPM2; (4) normalization of the anatomical image of each subject to the SPM stereotactic space using the template brain of the Montreal Neurological Institute (MNI) – this process involves both linear transformations (translation, rotation, scaling and shearing) and nonlinear deformations; (5) application of the deformation parameters of this normalization to the FA images; (6) application of a random-effects model (SPM2 ANOVA for the DS minus AC contrast and the AC minus DS contrast using the normalized FA); (7) measurement of volume-of-interest (VOI) FA values on the spatially normalized data of each subject, using the VOI function in SPM2; (8) calculation in MATLAB of the mean and SD for each of the VOIs, and performance of ANOVA between the two groups in each of the VOIs. The normalized FA images formed the basis for the VOI analysis and group comparisons. We used an *a priori* search volume after converting the peak Talairach coordinates reported by Seminowicz et al[14] for their seven-region model to MNI coordinates using MATLAB7. The coordinates for each region (with the exclusion of AT since it consists mainly of grey matter) were then entered in the VOI search for the contrast DS minus AC. FA values were found in MATLAB7 by: (1) typing xY.y; (2) x=[(Y(1:10),Y(11:20)] to separate the two groups; (3) mean (Y(1:10)) then mean (Y(11:20)) to obtain the mean for each group; (4)

Std (x) to obtain the SD for each group; and (5) typing [P,ANOVATAB,STATS]=anova1(x) to obtain the p values for the difference between the two groups in this particular VOI. These steps were repeated six times for the six VOIs. These regions consisted of the right Hipp (Talairach coordinates x=22, y=-14, z=-16); the right OFC (BA11, Talairach coordinates x=2, y=28, z=-20); the right MPFC (BA10, Talairach coordinates x=16, y=64, z=-4); the right rACC (BA24a, Talairach coordinates x=2, y=32, z=4); the right sACC (BA25, Talairach coordinates x=8, y=16, z=-8); and the left LPFC (BA9, Talairach coordinates x=-24, y=34, z=32). These VOIs were defined by spheres of 16.0-mm radius.

Functional magnetic resonance imaging

fMRI acquisition

Twenty-eight slices (5-mm thick) were acquired every 3sec in an inclined axial plane aligned with the AC-PC axis. These T2*-weighted functional images were obtained using an EPI pulse sequence (TA=2.65sec, Siemens TR=0.8msec-time required to acquire one line in the slice, TR=3sec, TE=54msec, Flip=90°, FOV=215mm, Matrix=64x64, Voxel size=3.36mm x 3.36mm x 5mm). High-resolution data were then obtained via a Tl-weighted 3-D volume acquisition using a gradient echo pulse sequence (TR=9.7msec, TE=4msec, Flip=12° FOV=250mm, Matrix=256x256, Voxel size=0.94mm^3).

fMRI procedure

BOLD signal changes were measured while subjects passively viewed five blocks of emotionally neutral film excerpts (reference task) followed by five blocks of sad film excerpts (activation task). As subjective emotional responses persist on average 32sec after presentation of aversive pictures[48] , this design was used to avoid contamination of the neutral stimuli by the sad stimuli. The sad excerpts, depicting a young boy witnessing the tragic death of his father, were extracted from the film *The Champ* (1979), validated[49] and used in several studies of sadness induction[50-53]. The neutral excerpts consisted of a news interview matched to the sad film excerpts with respect to the number and gender of the individuals depicted. Each block lasted 39sec and was separated by 15-sec resting periods during which subjects viewed a white cross on a black screen. Stimuli were presented via magnetic resonance (MR)-compatible goggles. Scanner noise was reduced with MR-compatible headphones and head motion was minimized with pediatric MRI pillows. After scanning, subjects identified the primary emotions (happiness, anger, sadness, fear, surprise, disgust) they felt following the sad and neutral excerpts. If a subject identified sadness, he/she was asked to rate its degree (sad, very sad, extremely sad, saddest ever). All subjects identified sadness as the primary emotion felt.

fMRI analyses

Data were analyzed using SPM2. Prior to analyses, 15sec of pre-stimulus baseline volumes (total of five) were excluded from the beginning of the functional scan to omit transient signal changes. All volumes were realigned to the first volume of each session to correct for subject motion, and spatially normalized to the standard space defined by the Montreal

Neurological Institute (MNI) template. Functional images were spatially smoothed with a 3D isotropic Gaussian kernel (full width at half-maximum of 12mm). Significant hemodynamic changes for each condition were examined using the general linear model (GLM) with boxcar functions convoluted with a hemodynamic response function. Statistical parametric maps for each contrast of the *t*-statistic were calculated on a voxel-by-voxel basis, resulting in a *t*-statistic for every voxel. These *t*-values were then transformed to unit normal distribution, resulting in z-scores. A random-effect model, which estimates the error variance for each condition across subjects and allows generalization of results to the pediatric population, was implemented for the group analysis. A one-sample t-test was conducted for each group to subtract brain activity associated with the neutral film excerpts from that associated with the sad film excerpts (Sad minus Neutral contrast). A two-sample t-test was then performed to compare brain activity observed in both groups for the Sad minus Neutral contrast. The search volumes corresponding to the ROIs were defined *a priori*, using small volume correction (SVC) and box volume function in SPM2. For this *a priori* search, a probability threshold of $p<0.05$ corrected for multiple comparisons was used and only clusters showing spatial extents of at least five contiguous voxels and a z-score >1.67[54] were kept for analysis. SVC was performed in the following ROIs defined *a priori*: Hipp, OFC (BA11); MPFC (BA10); LPFC (BA9); rACC (BA24a); sACC (BA25), and AT. Two search approaches were used. First, Talairach coordinates provided by Seminowicz et al[14] were utilized as search centers (see DTI section). The AT (TAL x=2, y=-6, z=4) was defined by a sphere of 16.0-mm radius. Second, a search center based on the neuroanatomic boundaries of the ROIs noted in the MR reference image (MNI

template) and the Talairach and Tournoux atlas[55] was utilized. For exploratory purposes related to laterality of activation in pediatric populations, a search was performed for each ROI bilaterally.

Psychophysiological interaction analyses

The PPI approach[25] was used to obtain an appropriate approximation of the neural interactions between ROIs during the transient state of sadness. Utilizing the Sad minus Neutral contrast created for each subject, a VOI search was performed using the peak activations obtained in the one-sample and two-sample t-tests. In the AC group, the activation peaks used were: MPFC (BA10, Talairach coordinates x=3, y=62, z=11); ACC (BA32, Talairach coordinates x=-18, y=35, z=6); and VLPFC (BA47, Talairach coordinates x=27, y=38, z=6). In the DS group, the activation peaks used were: MPFC (BA10, Talairach coordinates x=3, y=56, z=11); AT (Talairach coordinates x=15, y=-6, z=11); OFC (BA11, Talairach coordinates x=-24, y=28, z=-16); and VLPFC (BA47, Talairach coordinates x=36, y=20, z=-6). Each VOI was determined by a sphere of 6-mm radius (for further details see http://www.fil.ion.ucl.ac.uk/~wpenny/datasets/attention/README_GLM_PPI.txt). Once all VOIs were created for each subject, the PPI for each VOI within each subject was determined using the PPI tool in SPM2. This produced a MATLAB-PPI parametric file which was then modeled to fit the design of the experimental run. The modeling included three regressors representing: 1) the deconvolution of the hemodynamic response to produce an appropriate approximation of the neural response; 2) the BOLD responses

produced by the Sad minus Neutral contrast; and 3) the parameters of the VOI created *a priori*. The smoothed, normalized functional run was then modeled to the PPI parametric file obtained. The previous steps generated a Sad minus Neutral contrast that represents the neural interaction between the VOIs and other brain regions. A one-sample t-test was performed with the final contrast obtained for each individual. The one-sample t-test was repeated for each VOI and group separately. An exploratory approach search at P=0.01 with clusters showing spatial extents of at least five contiguous voxels was performed to determine the brain regions that interacted with the VOIs. Significant PPIs and VOIs were then plotted onto a flow chart. The flow chart also involved regions that showed significant PPIs but were not included in the Seminowicz et al. model[14].

Statistical analyses

Cohen's *d* (Table 1, Fig. 1b) was calculated as M_1-M_2/σ pooled, where σ pooled=$\sqrt{[(\sigma_1^2+\sigma_2^2)/2]}$, and M_1 and σ_1, and M_2 and σ_2 are the mean and SD for each region for both groups, respectively[20]. Pearson's correlations (Table 1) were computed using the Statistical Package for the Social Sciences (SPSS) Version 14.0, between individual FA values and *Dominic-R* depression scores for each region, and between regional FA values. Significance tests were 2-tailed and the α level was set to p=0.05. The graph presented in Fig. 1c was done using the Graph and linear regression curve-fitting function of the Statistical Package for Social Sciences (SPSS) Version 14.0, plotting the mean regional FA values on the x axis for the AC group and on the y axis for the DS group, respectively. The multiple regression analysis reported in the Brain abnormalities and behavioral alterations

section of the Results was performed using the multivariate regression function of the Statistical Package for the Social Sciences (SPSS) Version 14.0, entering individual FA values for each region as independent variables and individual depressive score as the dependent variable.

Author contributions.

A.M.-M.: designing experimental protocols, analyzing the data and writing the manuscript. C.F.: analyzing the data and writing the manuscript. B.M.: writing the DTI analysis program and analyzing the data. G.B.: designing experimental MRI acquisition protocols. M.B.: designing experimental protocols, analyzing the data and writing the manuscript. D.P.: recruiting the subjects, designing experimental protocols, analyzing the data and writing the manuscript.

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Figures and tables legends

Table 1. Differences between DS subjects and AC subjects in regional fractional anisotropy (FA) values[1] ranging from 0 to 1; 0 means an isotropic diffusion and 1 means a highly anisotropic or directional diffusion. Here, the DS group shows significant decrease in FA values in all regions. Correlations[2] between FA values and depression scores across groups were very significant in all regions except for rostral anterior cingulate cortex (rACC). Correlations[2] between FA values across areas were used to examine whether the ROIs were integrated in a specific circuit as proposed by our *a priori* hypotheses. Highest correlations were found between the hippocampus (Hipp) and subgenual anterior cingulate cortex (sACC); the orbitofrontal cortex (OFC) and rACC, followed by the Hipp and medial prefrontal cortex (MPFC), and the MPFC and lateral prefrontal cortex (LPFC). BA= Brodmann area, Chn'd= Cohen's *d*, Depression=*Dominic-R* depression scores, r=correlation coefficient, p=p value, M=mean, R= right, L= left, SD=standard deviation.

Table 2. Provides statistical parametric maps of BOLD activation during the Sad minus Neutral contrast. (a-b). c) Presents psychophysiological neural activity maps of ROIs in both groups. Each ROI activated in either group was used as a starting point to track neural activity to and from neighbouring brain areas. To visualize these neural interactions please see Figure 4 as well. L/R/M=Left / Right/Midline, AT=anterior thalamus, ACC=anterior cingulate cortex, IFG=inferior frontal gyrus, IPL=inferior parietal lobule, MPFC=medial

prefrontal cortex, OFC= orbitofrontal cortex, PC=posterior cingulate cortex, SPL=superior parietal lobule, VLPFC= ventrolateral prefrontal cortex, ** common voxels.

Figure 1. a. Regions showing reduced white matter fractional anisotropy (FA) value in DS subjects compared to AC subjects. Hipp: hippocampus, OFC: orbitofrontal cortex (BA11), MPFC: medial prefrontal cortex (BA10), LPFC: lateral prefrontal cortex (BA9), rACC: rostral anterior cingulate cortex (BA24), sACC: subgenual anterior cingulate cortex (BA25), R= right, L= left. **b.** Differences in regional fractional anisotropy (FA) values between AC and DS subjects. A significant decrease in FA values in the DS group was observed in all six brain regions investigated, with maximal decreases in descending order in the medial prefrontal cortex (MPFC, BA10), hippocampus (Hipp), lateral prefrontal cortex (LPFC, BA9), orbitofrontal cortex (OFC, BA11), subgenual anterior cingulate cortex (sACC, BA25), and rostral anterior cingulate cortex (rACC, BA24). **c.** This graph shows that there is an almost identical distribution of mean regional FA values within the hypothesized brain circuit, irrespective of symptomatology status of each group.

Figure 2. BOLD signals showing significant loci of activation during the sad minus neutral contrast (a.) represents activation observed in the AC groups in the left (L), right (R), and midline (M) medial prefrontal cortex (MPFC) (b.) On the other hand, the DS group showed activations in the right MPFC and right anterior thalamus (AT) (c.) Between-group comparison (AC minus DS) demonstrates activations in regions involved in processing and regulation of emotions: the left MPFC, right orbitofrontal cortex (OFC), left

and right anterior cingulate gyrus (ACC) (d). The inverse comparison (DS minus AC) shows clearly an over-engagement of brain regions involved in enhancement of emotional experience: the left and right inferior frontal gyrus (IFC), and right ventrolateral prefrontal cortex (VLPFC) without relative activation in the ACC, OFC or MPFC. The color bars indicate the intensity of each activation in z score.

Figure 3. Histograms depicting the spatial extent of BOLD activations for the Sad minus Neutral contrast. (a.) One sample t-test: right (R), left (L), medial prefrontal cortex (MPFC), Brodmann's area (BA), anterior thalamus (AT), (b.) Two sample t-test: orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), inferior frontal gyrus (IFG), ventrolateral prefrontal cortex (VLPFC).

Figure 4. Psychophysiological interactions (PPIs) between regions provide information about neural activity during the Sad minus Neutral contrast. • indicate the regions of interest (ROIs) activated for this contrast. Arrows represent the neural interaction between each ROI and other brain regions during the same stimulus condition. ◊ indicate deeper brain structures. Very distinct neural patterns are observed in each group (a.) PPIs in the AC group: Right (R), Left (L), Middle (M), medial prefrontal cortex (MPFC), anterior cingulate cortex (ACC), posterior cingulate cortex (PC), orbitofrontal cortex (OFC), inferior parietal lobule (IPL) ventrolateral prefrontal cortex (VLPFC). (b.) PPIs in the DS group: superior parietal lobule (SPL), anterior thalamus (AT), and inferior frontal gyrus (IFG).

Table.1 Differences between DS subjects and AC subjects in regional fractional anisotropy and correlations with depressive symptoms

Brain region	Fractional anisotropy values[1]								Correlations[2]											
	AC		DS						Depression		R Hipp		R OFC BA11		R MPFC BA10		L LPFC BA9		R rACC BA24a	
	M	SD	M	SD	f	p	Chn'd	p	r	p	r	p	r	p	r	p	r	p	r	p
R Hipp	0.28	0.06	0.20	0.05	17.15	0.0001	1.45	0.0001	-0.535	0.0001										
R OFC BA11	0.17	0.12	0.07	0.08	10.02	0.003	0.98	0.003	-0.442	0.004	0.384	0.015								
R MPFC BA10	0.25	0.14	0.08	0.08	20.06	0.0001	1.49	0.0001	-0.540	0.0001	0.479	0.002	0.150	0.356						
L LPFC BA9	0.17	0.11	0.06	0.08	12.03	0.002	1.14	0.001	-0.459	0.003	0.276	0.084	0.209	0.196	0.470	0.002				
R rACC BA24a	0.46	0.19	0.33	0.16	5.39	0.026	0.74	0.024	-0.296	0.064	0.385	0.014	0.550	0.0001	0.326	0.04	0.215	0.182		
R sACC BA25	0.26	0.10	0.19	0.08	6.05	0.020	0.77	0.018	-0.347	0.028	0.548	0.0001	0.289	0.071	0.283	0.07	0.237	0.140	0.293	0.067

Table 2. Statistical parametric maps of BOLD activation

Type of analyses	Group	L/R/M	Brain region	BA	Talairach coordinates			Z score	voxels	p value
					x	y	z			
a. One-sample t-test (Sad	AC	R	MPFC	10	3	62	11	4.30	43	0.001
minus Neutral contrast)		R	MPFC	10	3	61	5	3.87	81	0.007
		L	MPFC	10	-3	62	11	4.30	44	0.0001
		L	MPFC	10	-6	62	8	3.72	45	0.001
	DS	R	MPFC	10	3	56	11	2.96	25	0.04
		R	AT		15	-6	11	3.13	231	0.041
		R	AT		6	-11	6	3.09	77	0.045
b. two-sample t-test (Sad	AC minus DS	L	MPFC	10	-3	61	8	2.71	106	0.006
minus Neutral contrast)		R	OFC	11	27	38	-6	2.26	97	0.012
		L	ACC	24a	-18	35	6	2.22	60	0.013
		R	ACC	32	18	33	20	2.15	72	0.016

	DS minus AC		L	IFG	11	-24	28	-16	2.83	88	0.002
			R	IFG	11	24	31	-14	2.52	89	0.006
			R	VLPFC	47	27	23	-11	2.50	48	0.006
			R	VLPFC	47	36	20	-6	2.55	268	0.005
c. Psychophysiological	AC	R MPFC BA10	R	MPFC	10	5	48	12	3.45	1341**	0.0001
interactions between regions			R	ACC	24	5	35	15	3.36		0.0001
(PPIs)			L	ACC	24	-3	32	9	3.36		0.001
			M	PC	23	0	-54	13	3.36	1492	0.0001
		L ACC	R	OFC	11	3	43	-12	3.41	95	0.0001
		BA 24/32	L	MPFC	10	-5	58	13	3.20	498	0.001
			R	Putamen		23	-2	0	3.29	291	0.0001
			R	IPL	40	47	-44	40	2.80	23	0.003
			R	ACC	24	3	-1	41	3.72	19	0.006
		R VLPFC	R	MPFC	10	8	58	8	3.63	6304	0.0001
		BA 47	M	OFC	11	0	45	-15	2.19	23	0.014
	DS	R MPFC	M	PC	30	0	-60	11	3.72	903	0.0001

BA 10	L	SPL	7	-32	-52	55	2.98	1399**	0.001
	R	SPL	7	38	-46	57	2.70		0.003
	L	IPL	40	-38	-52	49	2.66		0.004
R AT	L	IFG	11	-26	25	-6	4.07	3379	0.0001
	R	PC	30	14	-62	22	3.88	490	0.0001
	L	PC	31	-5	-15	31	2.79	41	0.003
	L	IPL	40	-38	-48	24	3.08	86	0.001
L IFG BA11	L	OFC	11	-8	54	-17	3.32	84	0.0001
	L	PC	30	-3	-66	11	2.75	46	0.003
R VLPFC	R	OFC	11	23	34	-11	2.69	125	0.004
BA 47	R	IPL	40	32	-38	43	2.65	115	0.004
	L	IPL	40	-38	-53	44	2.57	211	0.005
	L	OFC	11	-3	51	-17	2.35	38	0.009
	R	OFC	11	3	45	-14	2.32	28	0.010
	L	SPL	7	-26	-57	11	2.16	23	0.016
	R	PC	31	3	-47	38	2.03	44	0.021

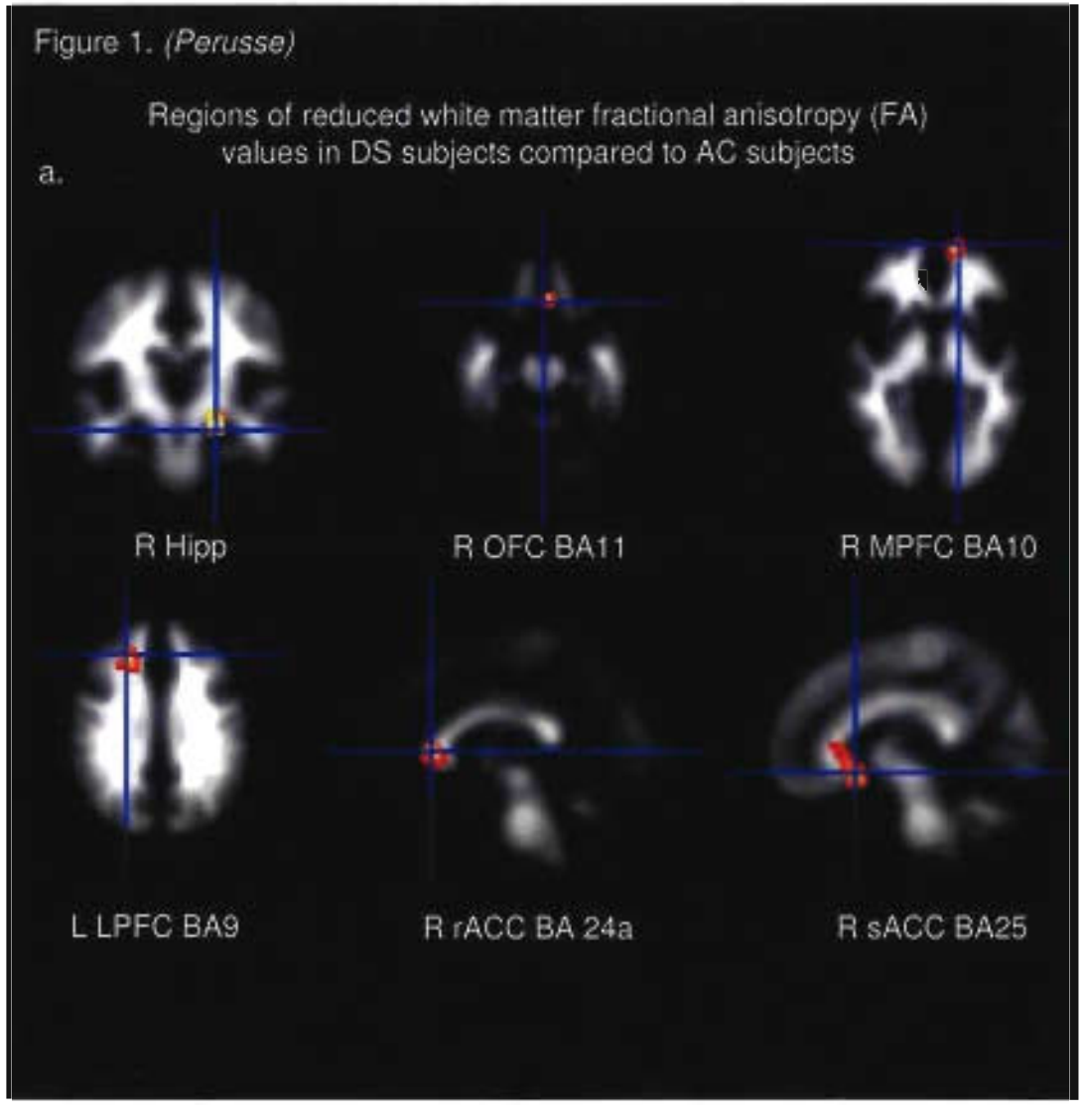
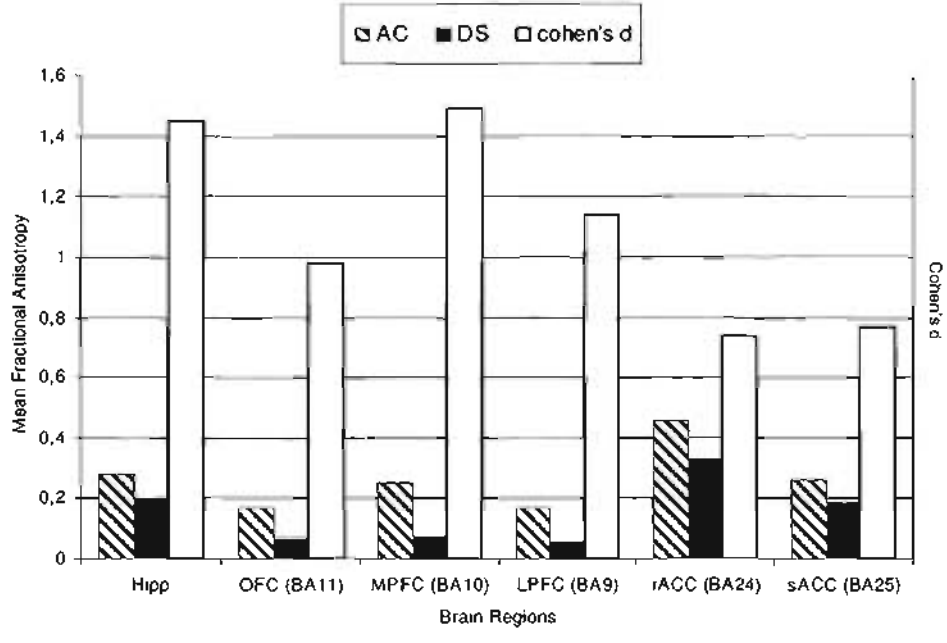


Figure 1. (Perusse)
Regions of reduced white matter fractional anisotropy (FA) values in DS subjects compared to AC subjects
a.
R Hipp
R OFC BA11
R MPFC BA10
L LPFC BA9
R rACC BA 24a
R sACC BA25

(Perusse)

Differences in regional fractional anisotropy values
between AC and DS groups

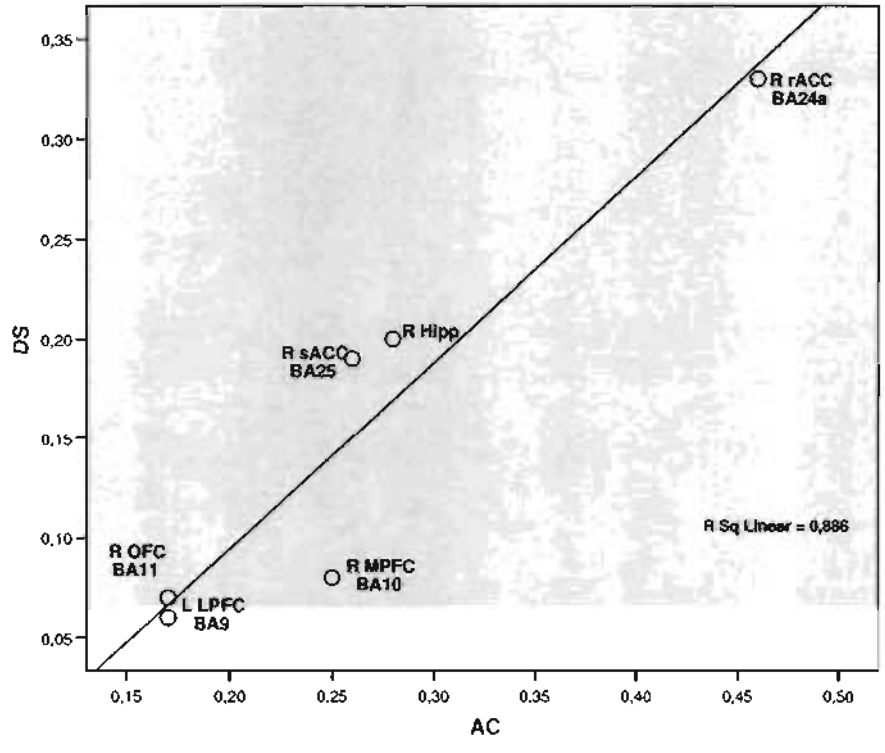
b.



(Perusse)

Fractional anisotropy relationships between regions

c.



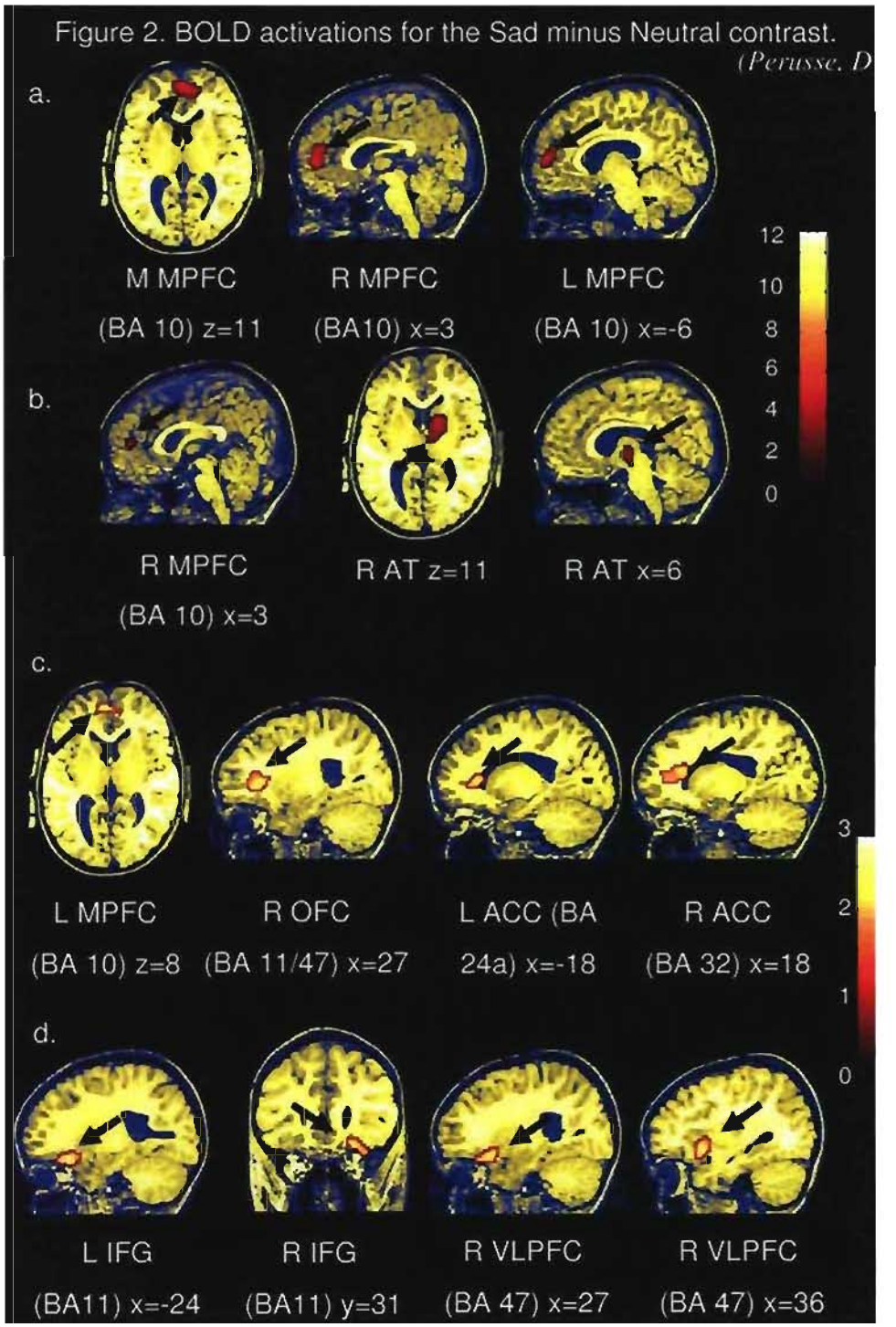
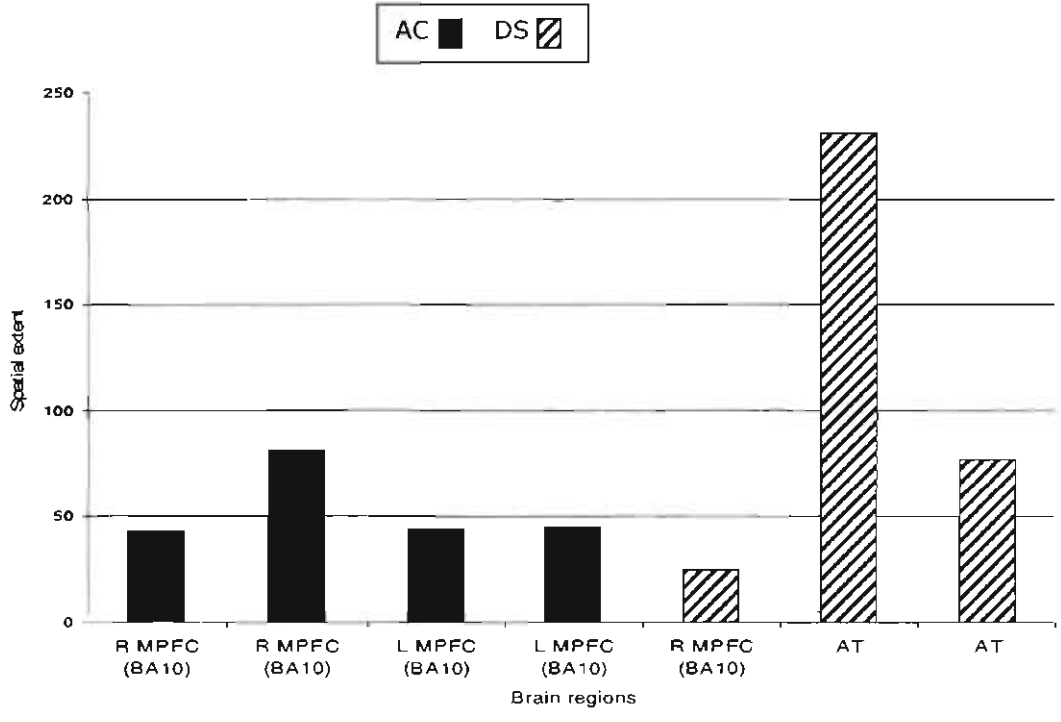


Figure 2. BOLD activations for the Sad minus Neutral contrast.
(Perusse, D
a.
M MPFC
(BA 10) z=11
R MPFC
(BA10) x=3
L MPFC
(BA 10) x=-6
12
10
8
6
4
2
0
b.
R MPFC
(BA 10) x=3
R AT z=11
R AT x=6
c.
L MPFC
(BA 10) z=8
R OFC
(BA 11/47) x=27
L ACC (BA
24a) x=-18
R ACC
(BA 32) x=18
3
2
1
0
d.
L IFG
(BA11) x=-24
R IFG
(BA11) y=31
R VLPFC
(BA 47) x=27
R VLPFC
(BA 47) x=36

Figure 3.

Significant BOLD activations for the Sad minus Neutral contrast

a.

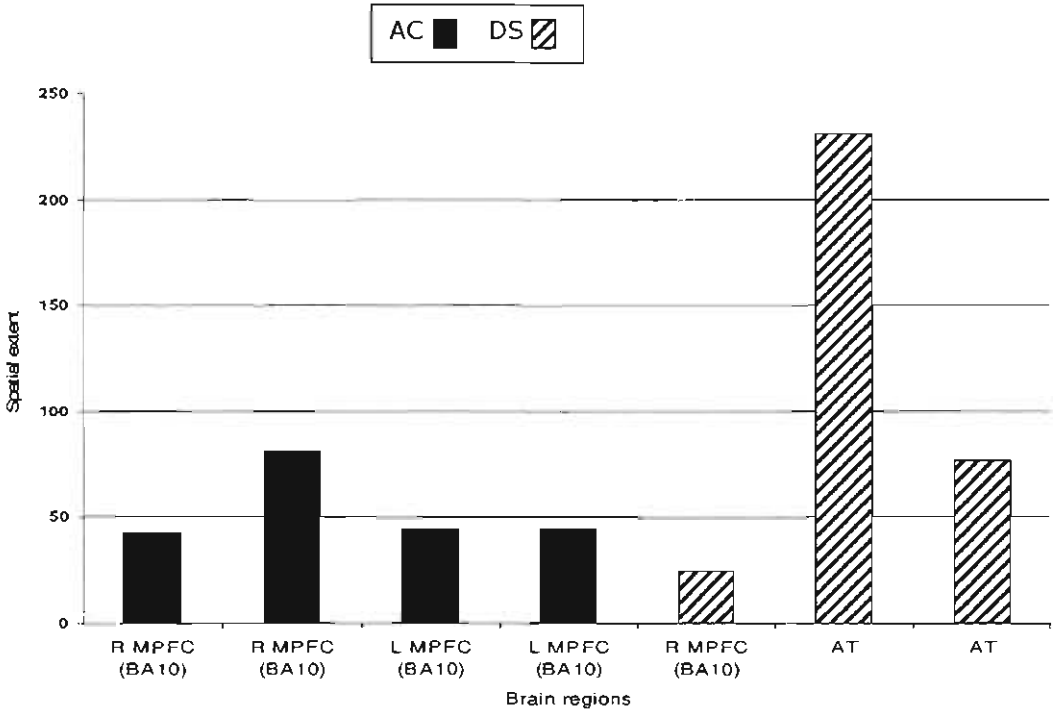


Perusse

Figure 3.

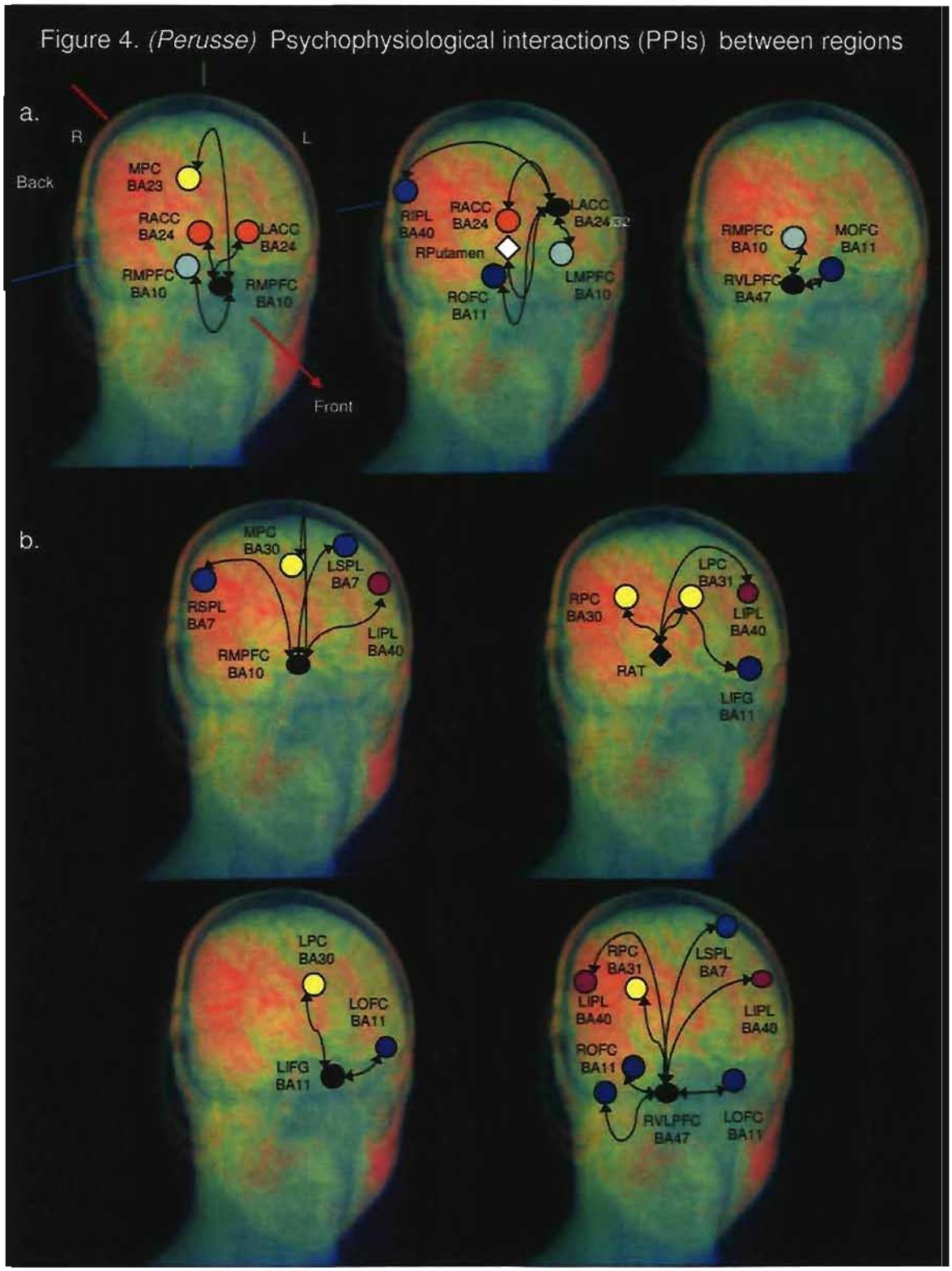
Significant BOLD activations for the Sad minus Neutral contrast

a.



Perusse

Figure 4. *(Perusse)* Psychophysiological interactions (PPIs) between regions



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Current Revision #	0
Submission Date	2nd Jun 07
Current Stage	Waiting for files to be sorted
Title	Structural and functional corticolimbic abnormalities found in children with subclinic depressive symptoms
Manuscript Type	Article
Corresponding Author	Daniel Pérusse (University of Montreal) .
Contributing Authors	Adham Mancini-Marie , Cherine Fahim , Boualem Mensour , Gilles Beaudoin , Dr. Mi Beauregard
Abstract	Anatomical and functional anomalies are commonly observed in a corticolimbic circ adults with major depressive disorder (MDD). To explore causality and control for p confounds of illness duration and treatment on this relationship, we used multimod neuroimaging in unmedicated, subclinically depressed 8-year-old children. Diffusior imaging showed that subjects with subclinical depressive symptoms versus asympt controls exhibited pronounced reductions in white matter fiber tract integrity in hipp lateral prefrontal, medial prefrontal, orbitofrontal, rostral anterior and subgenual ar cingulate cortices -all regions associated with adult depression. White matter patho predicted 50% of severity of depressive symptoms. Functional analysis of those rec during induced sadness also revealed profoundly altered patterns of neural activity functional connectivity among symptomatic subjects. Finding that the circuit associ adult depression presents such pervasive structural and functional abnormalities so development implies that neuropathology precedes the onset, and contributes to th pathogenesis, of MDD.
Key Words	functional imaging, motivation and emotion, psychiatric disorders
Dual Publication	No
Duality of Interest	No

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DISCUSSION

1. Neuroimaging emotional experience in 8-year-old children: What we found

We interpret our findings (based on the two methodologies fMRI and DTI) as suggesting, at the biological level, an underactivation of weakly connected networks in children with subclinical symptoms of depression from the limbic system to the prefrontal cortex. These findings also suggest that a key element of any network is its ability to maintain stability and control excitation. An emotional situation produces various levels of excitatory buildup. Hence, it could be argued that children with sub-clinical depression may produce a dysregulation of this network because of an "*over*" excitation from the limbic system to the prefrontal cortex. Indeed, behavioural data comparing self-reported sadness during fMRI revealed a significant difference between the two groups (DS group: Mean=3.47, SD=0.71; AC: Mean=2.72, SD=0.76; t=3.14; p=0.002) and a significant positive correlation with depression scores across groups (r=0.47, p=0.002). This could establish an important link between experimental findings and the clinical phenomenology of depression. Conversely, normal children displayed activation of the key brain regions involved in emotion processing, which coincides with their experiential response. I will now discuss the role of the limbic system and prefrontal cortex in emotion processing.

1.1. The limbic system: A circuit of midline structures circling the thalamus (which we have found to be very significantly different using both DTI and

fMRI methodology between children with sub-clinical depression and normal children) that plays a role in the control and production of emotional behaviour. By virtue of its connections with cortical and subcortical regions, it constitutes a sensory integration region and plays an important role in human emotion. For a review, see Augustine (1996). In this review, the author has reported that connections have been described between the limbic system (of particular relevance to our study, the hippocampus) and the orbital cortex and the frontal operculum. Specifically, it has a magnitude of local connections and projections to the cingulate gyrus and its different subdivisions. Furthermore, several studies provide evidence that the limbic system is a visceral sensory brain region engaged in interactions between the extrapersonal world (e.g., emotional stimuli) and the internal milieu (Mesulam and Mufson, 1982a, b; Mufson and Mesulam, 1982). Thereupon, we suggest that abnormalities (i.e., over activation) in the limbic system might be underlying the abnormal peripheral and behavioural responses to emotional stimuli that have been described in depression. We therefore propose that over activation of the hippocampus and its connections with pivotal other limbic regions may have played a role leading to the failure in the normal emotional process that permits children to adapt between internally (autonomic and visceral information) and externally generated sensory events (emotional stimuli depicted by the film excerpt). This inadaptation may lead, at least in part, to an "over-emotional negative response"

(i.e., resulting in the significant difference in the behavioural scores between the two groups).

1.2. The prefrontal cortex: Hypofrontality, that is hypoperfusion and hypometabolism in the frontal lobes, is a well-replicated finding in MDD (George et al., 1993; Soares and Mann, 1997). The role of the mesial prefrontal (MPFC) and medio-dorsal (MDFC) BA 10, inferior (IFG) and/or orbitofrontal (OFC) BA 11/47, and ACC BA 24/32 in voluntary emotional self-regulation (Beauregard et al., 2001; Levesque et al., 2003), social learning and in the internal representation of emotional experience (Arbib et al., 2000) was previously amply demonstrated (Phan et al., 2002). Of particular note, it is well established that damage within these regions dramatically alters the capacity of higher mammals to cope emotionally (Moll et al., 2002a; Moll et al., 2002b; Nauta, 1971) and socially (Anderson et al., 1999; Bechara et al., 1994; Moll et al., 2002a; Moll et al., 2002b) with different situations. According to Phillips and colleagues (Phillips, 2003; Phillips et al., 2003a, b), the information is conveyed to the prefrontal cortex from the limbic system for the regulation of emotional experiences and behaviour. On a related note, in a recent work by Beauregard et al., (Beauregard et al., 2001; Beauregard, 2004), the authors showed that the prefrontal cortex plays a role in conscious and voluntary emotional self-regulation, where the information is accessible to consciousness, thus providing an integrated perception of emotions. In sum, and of relevance to

our study, the limbic system and prefrontal cortex are considered to be key nodes of the neural circuit of emotional processing, the former a main signal generator and the latter a modulator (Davidson et al., 1999; Davidson and Irwin, 1999).

Overall, indeed the cortical and subcortical areas we have discussed have rich interconnections. None acts in a vacuum. Therefore, the functional anatomic modules that mediate emotional experience are abundantly interconnected and compose a modular network (Heilman and Gilmore, 1998). Emotional experience depends on the patterns of neural activation of this modular network, which we have seen is abnormal in children with sub-clinical depression.

2. Discussing methodological issues:

Investigating younger subjects who have only subclinical symptoms of depression but not clinically diagnosed with MDD minimizes potentially confounding factors such as illness duration (Ballmaier et al., 2004; Bremner et al., 2002; Frodl et al., 2003). Several studies have reported brain differences between first episode depression patients, chronic and elderly MDD patients (Ballmaier et al., 2004; Frodl et al., 2003). Further differences are also found between MDD patients falling in different age groups (Frodl et al., 2003). For example, Frodl and colleagues (Frodl et al., 2003) found larger amygdala volumes in first episode depression compared to recurrent major depression patients. Another important factor in creating heterogeneity in findings in MDD studies is treatment intervention

(Anand et al., 2005; Del-Ben et al., 2005). Evidenced based medicine show the effects of antidepressant medication (e.g. SSRIs) on clinical symptoms of depression (Ruhe et al., 2006a, b; Seminowicz et al., 2004; Taylor et al., 2006). These effects are obviously achieved through the chemical and neurophysiological changes at neurotransmitter and neuroreceptor levels in the brain (Meyer, 2007; Ruhe et al., 2006a, b; Taylor et al., 2006). These dramatic changes also involve cerebral activity pattern changes in MDD patients receiving treatment (Mayberg, 2003; Mayberg et al., 1997). Therefore, to avoid the above mentioned effects of medication, it was ideal to include medication naïve subjects in our study. With relevance to our study, hormonal effects on the onset of depression have been well reported in the literature (Angold and Costello, 2006; Nottelmann et al., 1987a; Nottelmann et al., 1987b; Susman et al., 1987b). For example, hormonal levels were related to emotional dispositions and aggressive behavior in boys (Susman et al., 1987a), while increased risk for depression in adolescent girls was found to be strongly associated with mid-puberty (Angold and Costello, 2006) and with early maturation (Ge et al., 2003). That being said, it is advantageous that this study was conducted in pre-pubertal children.

2.1. Why we chose the Dominic?

The *Dominic-R* is a child-answered interactive computerized cartoon-based psychiatric interview developed in Quebec that combines visual and auditory stimuli and was shown to yield valid DSM-IV symptoms in children as young as 6 years including: attention deficit-hyperactivity, oppositional, conduct, major depressive, separation anxiety, generalized anxiety, and specific phobias disorders. It is a children's self-report consisting

of 90 pictures showing a character named Dominic in a variety of situations. Dominic interactive is akin to a video game; the use of pictures avoids having to rely only on the vocabulary of the child. The cut-off points for the mental disorders investigated by the Dominic are grouped into three columns (i) there is no problem (normal controls), (ii) there could be a problem (children with tendencies, susceptibility to develop the disorders, but yet actually not diagnosed), (iii) there is a problem (children who already developed the disorder and are diagnosed according to the DSM-IV). In this vein, one of the advantages of the *Dominic-R*, is that it would enable us to examine the differences in brain structures between children who already developed the disorder, children who carry some symptoms and are at risk for developing the disorder, without yet being diagnosed, and normal children.

The *Dominic-R* has been through extensive development and validation processes since it was designed in the early 1980s and has been used with children from various ethnic groups in clinical and research settings in Québec (Rousseau et al., 2005; Valla et al., 1994) and elsewhere (Arseneault et al., 2005; Linares et al., 2006; Murphy et al., 2000; Scott et al., 2006; Valla et al., 2002). Agreements between Dominic-R scores and expert psychiatric assessments are high (kappas=0.6-0.85). To our knowledge, this was the first time that depression was self-assessed during childhood. This procedure contributes to unbiased phenotypic assessments and unbiased estimates of genetic and environmental effects. One of the strengths in the studies that have used DSM interview data is the fact that DSM contains the diagnostic criteria for the phenotype of interest. Weaknesses of using DSM

diagnoses as markers for genetic studies include the fact that DSM does not provide normative data that allow for gender- or age-specific discrimination. As an alternative to the DSM categorical approach is the use of empirically derived instruments, such as the *Dominic-R* interactive used in this Masters work. Depressive behaviour as defined by the *Dominic-R* has been shown to be highly predictive of DSM MDD (Valla et al., 1994; Valla et al., 1997; Valla et al., 2000; Valla et al., 2002). Its psychometric properties were found to compare favorably with other psychiatric child interviews (e.g., PICA-III-R, DICA-R, DISC-2.3) in reliability and criterion validity, notably on internalized problems such as depression and anxiety (Valla et al., 2000). Another recent study used the *Dominic-R* to successfully distinguish children meeting DSM-IV criteria for research diagnoses of conduct disorder (Arseneault et al., 2005)

2.2. **Why we chose a passive viewing method?**

We deliberately chose a passive viewing method in the study to replicate real life situations where the child implicitly processes daily emotional events, interactions with others, people interacting together at home and school. This method avoids the use of a cognitive task in conjunction with the presentation of the stimuli because of the known interactions between cognition and emotion (Simpson, 2000). Functional imaging studies have corroborated the notion that the amygdala activation appears to depend on relatively passive or implicit processing emotion, whereas requiring subjects to label the emotion can instead result in deactivation (Hariri et al., 2000) and concomitant suppression of emotional psychophysiological responses (Kapler, 2001). The reduction of amygdala responses to

emotional expressions, when the demand for explicit emotion recognition is increased, is a common observation across studies (Critchley et al., 2000) and may be mediated by the amygdala's inhibition by frontal cortex. One the other hand, explicit tasks may activate or over activate regions that otherwise would not be detected. A study by Cunningham and colleagues (Cunningham et al., 2004) showed that only explicit evaluation of social situation, as being good or bad activated the anterior cingulate, frontal pole, and lateral areas of the orbital frontal cortex. Another study on linguistic syntactic processing by Suzuki and colleague (Suzuki and Sakai, 2003) found more inferior frontal gyrus activation during explicit processing than in implicit processing. Of particular relevance to our study, passive emotional tasks with minimal cognitive demands activate the amygdala and other subcortical regions more often than emotional tasks with greater cognitive demands (Phan et al., 2002). We hypothesize that the lack of a specific cognitive instruction profoundly influenced the activity in the prefrontal cortex. In our study, we successfully observed robust activation in widespread cortical and subcortical regions as reported in previous studies (Lane et al., 1997a; Lane et al., 1997b; Lane et al., 1997c; Reiman et al., 1997). These activations were reasonably attributed to emotional not cognitive processes.

2.3.Why we chose dynamic emotional stimuli?

Studying the neuronal processing of emotions was predominantly performed via static emotional stimuli that usually involved only facial expressions. Few studies (Christie and Friedman, 2004; Kilts et al., 2003; Sato et al., 2004; Sloan, 2004) mostly performed by our research group (Beauregard et al., 1998; Beauregard et al., 2001; Beauregard et al., 2006; Cote et al., 2007; Eugene et al., 2003; Fahim et al., 2007; Levesque et al., 2003; Levesque et al., 2004; Mancini-Marie et al., 2006; Mancini-Marie et al., 2004; Stip et al., 2005a; Stip et al., 2005b) used dynamic emotional stimuli which are more natural, vivid, resemble real life situations and communicate more accurately emotional cues (e.g facial) in a contextual format (Lander et al., 2006). Dynamic emotional stimuli result in a stronger activation in distinct emotion-processing areas as the amygdala, fusiform gyrus, prefrontal cortex (LaBar et al., 2003; Sato et al., 2004) when compared to static images [for example the International Affective Picture System (IAPS) created by Lane and colleagues (Lang, 1997)]. At the level emotional experience, higher arousal responses where reported by subjects observing dynamic versus static stimuli (Sato et al., 2004). Therefore, it was important for us to use dynamic stimuli that depict complex sad situations closer to real-life. As previously mentioned, this paradigm has been validated (Gross, 1995) and used extensively by our group to investigate sadness in healthy control (adults and children) and patients.

2.4. fMRI as a method for emotion processing and DTI for functional/anatomical information: Are they of scientific relevance?

Affective neuroscience is a considerably new field of research that encompasses several disciplines as behavioural neuroscience, neuropsychology, and psychophysiology. Emotional neuroimaging provides a non-invasive method of measuring cerebral correlates (Kim et al., 2003; Kim et al., 2004) of emotion (Peper, 2006; Peper et al., 2006). This technique provides a mean for identifying the correlates of emotional experience with an increasing temporal and spatial precision of patterns of activation associated with specific emotional tasks or stimuli. Accordingly psychophysiological changes experienced by an individual during a specific condition could be identified in the form of measurable cerebral activation. Testing and developing models of emotion and depression have been revolutionized with the advancement of neuroimaging techniques. The functional neuroanatomy of neural patterns involved in emotional processing and in the pathogensis of MDD can now be described with increased accuracy (Beauregard et al., 1998; Drevets et al., 1992; Mayberg et al., 1999; Phan et al., 2002; Wager et al., 2003). Such advance still holds several limitations. One important problem is the presence of several mental processes that add up to produce the cerebral outcome measured during affective neuroimaging. For example, during the scanning time the child may perform several mental processes successively or simultaneously e.g. perception of the emotional stimulus, decoding of exteroceptive or interoceptive events, evaluation, memory retrieval, encoding, recognition etc. Since emotional processes are not only the result of localised but also of

widely distributed activity in the brain, representative assessment models are needed that allow to systematically relate the hierarchy of high- and low-level emotion constructs with the corresponding patterns of functional connectivity in the brain. To this end, we used another technique of data analyses [Psychophysiological interactions- PPI] that also uses the fMRI BOLD signals, to estimate underlying neural activity resulting from the psychophysiological interactions that occurred in association with the emotional task. Through the deconvolution of the hemodynamic response, this method provides an appropriate approximation of the neural response and interaction between brain regions. The findings were then be fitted to our a priori 7-region model proposed by Mayberg and her team (Seminowicz et al, 2004).

In the current work, we used Echo planar imaging (EPI) which is relatively robust with respect to movement artefacts and allows for a spatial resolution of 2 mm. EPI is the method of choice for exploratory investigation that are not necessarily hypothesis driven. The BOLD contrast originates from the intravoxel magnetic field inhomogeneity induced by paramagnetic deoxyhemoglobin (deoxyHb) found in red blood cells. The magnetic susceptibility differences between the deoxyHb-containing compartments and the surrounding space generate magnetic field gradients around the boundaries of these compartments. Therefore, changes in neuronal activity emotional tasks produce parallel changes in regional deoxyHb content which in turn alters the signal intensities in MR images (Kim et al., 2004; Kim et al., 1993). Interestingly, a study by Logothetis and Wandell (Logothetis and Wandell, 2004), shows that BOLD-responses mainly reflect the

input and local processing of neuronal information rather than the output signals. So in other words, synaptic potentials are the strongest cause of the BOLD responses and reflect the neuronal input to a specific brain region and how it is processed. That being said, it is reasonable to believe that many cerebral processes can not be detected due to sub-threshold changes in brain vascularization although increases in neural activity and energy metabolism are taking place. To demonstrate this point, white matter (WM) for example has an energy consumption that is ¼ of that consumed by grey matter tissue. Thus activation of the white matter has been rarely reported in the neuroimaging literature (Mosier and Bereznaya, 2001; Tettamanti et al., 2002) and most colleagues in the neuroimaging field would reject the idea of the presence of BOLD signal in white matter.

In conclusion, even though fMRI BOLD contrast provides information about which regions are involved in a specific mental process, it does not provide any information about how does such process take place (Kim et al., 2003; Kim et al., 2004). DTI is a new MRI technique that, similar to fMRI is non invasive. DTI allows us to examine neuronal connectivity in vivo and opens the door for more comprehensive studies of brain functions. For the above mentioned reasons, fMRI, PPI and DTI were used as complementary tools in the current work.

3. Taking into consideration risk for the children participating in the study

To protect the rights and confidentiality of the subjects involved in this Masters work, all records and data were collected for research purpose only. Confidentiality of records is and will be maintained. All results were and will be published in aggregate form and will not

reveal any individual identifying information. Furthermore, both the parents and children Parents were asked to participate voluntarily and to sign detailed informed consents prior to participation in the study following a home visit where they were shown a video tape of the MRI machine and the procedure that the child will undergo prior, during and after scanning

As an example of the tests administered to the children is the *Dominic-R*. The Dominic Interactive was developed in North America to assess a child's perception of her/his own symptoms, which is critical to balance parents' and school professionals' perception. Most children complete the Dominic Interactive 90 situations within 10-15 min. In some of the pictures, Dominic's behavior is positive, but in most situations his/her reactions are pathological (e.g., Do you feel sad most of the time, even when others are having fun?). These questions and the presented images may create a feeling of shyness, sadness or fear. If suicidal thoughts should appear during the Dominic test, the research assistant will ask a series of predetermined questions (do you ever think of death, do you even think of killing yourself) that will allow him or her to take any necessary action. If replies to these questions are positive, the parents are contacted and informed.

Children have passed the fMRI and DTI scans at the age of 8. This procedure took approximately 45 min (First scan started in May 23, 2004). Some children feel anxious in the enclosed space. If a child while being scanned felt, fear or anxiety, the child was immediately taken out of the machine. During the scan, the MRI machine produces powerful sounds similar to those heard on construction sites or strong humming- especially

during the functional part. To protect the children from these sounds, children wore headphones, to allow them to hear the film excerpts during the functional run, and to hear soft music, etc during the anatomical and DTI runs. The scan always started with the functional run then followed by the anatomical and DTI runs. To make the scan a more pleasant experience, following the functional run, the children watched a cartoon/children's movie of their choice during both the anatomical and DTI runs. This approach helped relax the child, decreased the noise from the machine, and made the scan a more pleasant experience.

However, the purpose of showing a sad film (during the functional run) was to evoke feelings of sadness and empathy. We anticipated that some children would have a stronger than expected reaction (e.g., children with sub-clinical depression, or children with separation anxiety, etc.). To this end, we created a de-dramatization protocol that would be followed with the child in case such a reaction occurs. A research assistant was always nearby to systematically evaluate the child's emotional reaction. If there is a stronger than normal reaction, the assistant had the necessary training to de-dramatize the scene and take the appropriate action. On a clinical level, when any abnormal findings (e.g mass, cyst, fracture, metal fragments, etc.) were seen in a child's brain by either the MRI technician, research assistant or the student, the responsible neurologist and radiologist on the ward were alerted, and a specific protocol was followed to advise the parents and the child's physician of the findings.

4. Potential benefits of the current masters project to the participants and others

The Quebec Newborn Twin Study (QNTS) was ascertained from all twin births occurring in the Province of Quebec between 1 April, 1995 and 31 December, 1998. This ongoing longitudinal study was followed at 5 (Boivin et al., 2005; Dubreuil et al., 2003), 18 (Tremblay et al., 2004), 30 (Dionne et al., 2003), 48, and 60 (Brendgen et al., 2005a; Brendgen et al., 2005b) months focusing on a variety of child- and family-related characteristics. A sixth set of data was completed at 6 years of age to assess children's social adaptation in kindergarten. During the beginning of the current work (8 years of age), all twins were undergoing neuroimaging/neuropsychological/neurological and psychiatric examinations. The QNTS is operated by a research group of over 30 researchers under the direction of Dr. Daniel Perusse (PI) (Sainte Justine Hospital), designated as an Emergent New Team investigating inattention, impulsiveness, aggression, emotion dysregulation, and hyperactivity in childhood: heritability, genetics, neuropsychology, neurophysioology, and anatomy.

The overall objective of the QNTS was the creation of a database of behavioural (neuropsychological and psychiatric and neurological assessments), genetic (molecular and quantitative) and neuroimaging (functional, structural/anatomical, and diffusion tensor imaging) from 672 twin pairs (229 MZ, 443 DZ). Such a database is to be shared with researchers and the clinical community. This project is extremely valuable because it would provide a basis for characterizing normal and abnormal brain morphology in relation to behaviour/mental disorders and gender differences, thus serving as "control data" for

neurological and psychiatric studies in children. The QNTS offers unique opportunity for studying the development of mental disorders in childhood: 1- It systematically used a "blind" procedure by which all measures including behavioural assessments are collected and rated by research personnel who were not exposed to both members of a twin pair, reducing potential biases related to the perceived zygosity of subjects. 2- It systematically used multi-informant, multi-method measurements of phenotypes. 3- Opposite-sex DZ pairs were included, enabling the exploration of potentially important sex differences in aetiology. 4- The first behavioural assessment was done when the twins were aged 5 months, providing the rare opportunity to chart risk factors and developmental changes from very early on in life. 5- A number of relevant measures seldom found in other studies have been collected such as chorionicity and amnionicity for monozygotic twins, obstetrical records for pre-and prenatal medical conditions, infant heart rate, infant feeding ability, infant salivary testosterone and cortisol samples, infant urine neurotransmitter metabolites, detailed 4-day diaries for the recording of infant states and parent-infant interactions, peer interactions in toddlerhood, longitudinal parental interactions throughout childhood, peer interactions at school age, etc. These assessments were followed within two weeks by a home visit to obtain social, demographic, health, and further behavioural data on the twins and their families. The home assessments were accomplished through three methods: interview of both parents, self-reported questionnaires filled out by both parents and direct observation of the infant, home, and neighbourhood by the interviewer. Interviews were done in French or English, according to the language of the respondent.

A total of 322 pairs of 5-month old twins were evaluated at home and in the laboratory. Home visits without laboratory assessments were also conducted on another 325 pairs also aged 5 months (Boivin et al., 2005; Dubreuil et al., 2003). The total sample (final N = 672 pairs) was then followed longitudinally at 18, 30 and 48 months using a similar protocol (Brendgen et al., 2005a; Brendgen et al., 2005b; Dionne et al., 2003; Tremblay et al., 2004) A fifth wave was started in June 2001 as the twins reached 60 months and continued at 72 and 84, focusing on mental disorders and school-readiness variables such as verbal and non-verbal IQ, reading ability, numeracy, executive functioning and peer-interactions during kindergarten in the complete sample. One other wave was acquired at 100 months collecting the same variables and was the source of data for this Masters work.

The Canadian Institute of Health Research (CIHR) funded the QNTS project for the collection of functional, anatomical magnetic resonance and diffusion tensor imaging data at 100 months (8.33 years). Further funding also was provided by the Fonds de recherche en santé du Québec (FRSQ) and Fondation Hôpital Ste-Justine. The Masters candidate of the present work (Adham Mancini-Marïe) is the recipient of *The Genes, Environment and Health Training Program CIHR award* for the amount of CA$10,000/year for 2 years (2006-2007).

All data collection (genetics, neuropsychology, electroencephalography, sociometry), except anatomical/functional anatomical magnetic resonance imaging and DTI were carried out at Sainte Justine Hospital. Anatomical/fMRI and DTI scan acquisition were acquired at

Understanding the biological basis of mental diseases, through a combination of functional brain activations, morphology and behaviour, is increasingly seen as a way of achieving insight into disease pathogenesis, with the ultimate goal of improving phenotyping, sub-typing, prevention, diagnosis, and treatment. Traditionally the biological investigation of human disease has focused on monogenic disorders, which are relatively uncommon. More recently, increasing efforts have been made to study complex multifactorial diseases such as hypertension, diabetes, asthma, cardiovascular disease, cancer and mental disorders, which occur at frequencies higher than 1-10% and constitute an immense social burden. However, in spite of consistent support for the presence of a genetic susceptibility in the aetiology of these conditions and significant technological advances, little progress has been achieved in identifying genes that play an important role. Here may come the role of discovering alternative controlled quantitative indices of disease liability or risk, termed endophenotypes, that predict the risk of such diseases. For example, blood pressure and cholesterol levels have proved fruitful as endophenotypes for heart disease. Part of the endophenotyping problem, based on brain morphology, is related to the fact that most studies have used relatively small samples that are generally poorly characterized with regard to the phenotype of interest. Moreover, the genetic architecture and mode of transmission of complex disorders are unknown and therefore rarely taken into account in genetic epidemiological designs.

In this vein, genetic influences on behaviour are complex and, as such, the effect of any single gene is likely to be modest. Neuroimaging measures may serve as a biological

intermediate phenotype to investigate the effect of genetics (using a twin cohort) on human behaviour. In particular, using fMRI and DTI makes it possible to achieve knowledge by including samples of subjects where the distribution of phenotypic variance is both wide and under heritable influences. Here, we use this approach to learn about the neurobiological basis of depression.

In our opinion, the QNTS offers an unparalleled scientific opportunity to study some of the most important childhood disorders, i.e., depression, brain morphology in order to achieve possible neurophenotyping. In addition, achieve new insights into the relationships between brain structures and functions (using fMRI and DTI), also to identify effects of environmental factors and interactions (through data supplied by the QNTS personnel to Dr. Mancini).

Potential benefits to the subjects involved in this research were the early detection of possible psychiatric and/or brain anomalies. In summary, parents would benefit from a systematic evaluation of their child's cognitive/emotional development, get results and updates from the Dominic evaluation, fMRI and DTI data if they requested. This unique amount of knowledge would contribute to a growing knowledge of children's development. And offer unique features for the study of the development of depression in childhood.

A QNTS passport was given to the children as a souvenir of his or her participation in each step of the study. As well, a booklet containing information and explanations of the activities that took place at previous visits was given to the parents.

The potential scientific impact of the current Masters project is substantial. Thus far, the scientific advances in depression have been limited with regard to a better understanding of the neuropathophysiology of depression in the elderly and/or adult patients long after the actual onset of the disease. By means of a comprehensive and multidisciplinary approach (behavioral, fMRI and DTI), the proposed project has the potential to efficiently overcome previous limitations. In addition, studies on depression in children focus on the most severely depressed subgroup of children. Waters et al conclude that "Major gaps exist in our knowledge of the range of depressive symptomatology in pre-pubertal children, the relationship of developmental factors to depressive symptoms, the reliability and validity of existing diagnostic tools, and the effectiveness of traditional child psychiatric treatment modalities for depression." (Waters and Storm, 1985)

In this vein, the information obtained during my Masters candidacy (using 43 subjects) will provide some of the essential knowledge for scientists for years to come and can help us understand childhood depression, and its development through adulthood into addiction, substance abuse and suicide.

5. Limitations

The results of this thesis need to be considered in the context of several potential limitations. First, while fMRI use is widespread, there is insufficient knowledge of the physiological basis of the fMRI signal to interpret the data confidently with respect to neural activity (Arbib et al., 2000). Logothetis and colleagues (Logothetis et al., 2001) conducted the first simultaneous intracortical recordings of neural signals and BOLD responses. The authors simultaneously scanned and recorded from cortex in anesthetised monkeys, using fMRI and electrophysiological recording. The monkeys viewed moving checkerboard patterns while electrodes placed in primary visual cortex measured single- and multi unit neural spiking activity, as well as local field potentials (LFPs), which reflect the dendro-somatic inputs of the neural population. Simultaneous fMRI scanning was used to define a region of interest near the electrode tip that showed a significant BOLD response to the stimulus. They found that the BOLD response was significantly correlated with the LFPs, which were stronger and more sustained than the single- and multi unit neural spiking activity. These results indicate that the BOLD signal does indeed reflect an increase in neural activity.

Second, it should be noted that our task could be regarded as an emotion-induction task. However, the finding needs to be interpreted cautiously because, strictly speaking, our task was testing the access to autonoetic perception of elicited emotions. It might be possible that the ability of children with sub-clinical depression to access their emotions (categorization of feeling) was different from that of normal controls. Our behavioural

results might not necessarily reflect gut-level elicited emotion that drives emotional behaviour. Autonomic data such as skin conductance responses would help to measure gut-level emotional response.

6. Conclusion

These findings provide preliminary evidence of abnormal fMRI and WM microstructure in children with subclinical depressive symptoms, before the actual onset of the disease, in comparison with same-age healthy children, as inferred from magnetic resonance imaging data. Theses methods used MRI to visualize the BOLD and diffusion of water molecules within axons, thereby allowing investigation of regional brain activity and WM tract structure, respectively, beyond simple volumetric measurements. WM microstructural alterations, as assessed via DTI, may reflect abnormalities in the myelin sheath and/or directional coherence of fiber tracts, which plays an important role in brain connectivity. In this vein, our study findings of significantly smaller fractional anisotropy (FA) values, specifically in the frontal gyri, in DS children suggest an abnormality in developmental myelination, that may occur early in development before the onset of the disease. Indeed, given the hypothesized involvement of the prefrontal cortex in the circuit paths responsible for affect modulation and the integration of thought and emotion, we propose that brain WM abnormalities, in above mentioned areas, have their effect by disrupting connections between cortical and subcortical regions involved in mood regulation, further disruption of these circuits may result in the actual diagnosis of depression. Potentially, if enough connecting WM tracts are impaired, depression may become treatment refractory. Therefore, it is possible that these findings may represent measurable functional changes that can predispose an individual to develop the cognitive and emotional changes that occur in depression. Worth mentioning, is the path modeling metanalysis by Mayberg and her team (Seminowicz et al., 2004) of functional abnormalities in depression. This model is

considered by the authors to be a first step toward full characterization of the depression phenotype at the neural systems level. It will be important for future studies to investigate whether identification of altered brain FA values have clinical utility in early diagnosis, treatment response monitoring, or the development of new treatments. This study is limited by the relatively small number of subjects. However, FA differences between the two groups are extremely large, with DS exhibiting FA values ranging between 0.74 to 1.49 full standard deviation in DS relative to AC. Indeed, the effect size is independent of sample size and effect sizes found here are so large as to make the two groups -who are completely distinguished on depressive symptoms- almost completely non-overlapping in WM structure. Clearly, further work is needed to investigate whether these changes resolve over time or lead to the development of depression and to understand and predict the respond to treatment interventions. Nevertheless, these findings should be considered preliminary until replicated in larger samples using additional diagnostic and neuropsychiatric screening tests. In summary, these abnormalities were identified in 8-year-old children, they may predate symptom development, and point to a neurodevelopmental abnormality that may contribute to risk of depressive illness in this population.

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