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SHORT COMMUNICATION

Perinatal effects on in vivo measures of human brain serotonin synthesis in adulthood: A 27-year longitudinal study

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Abstract

There is an increasing evidence that prenatal and early postnatal stressors have life long impacts on physical and mental health problems. Animal studies have shown that this could include enduring changes to brain serotonin neurotransmission. In the present study, we tested whether perinatal adversity in humans has a long-term impact on brain serotonin neurotransmission in adulthood. Twenty-six healthy males, recruited from a 27-year longitudinal study, underwent a positron emission tomography scan with the tracer alpha-[^{11}C]methyl-L-tryptophan (^{11}C-AMT), as an index of serotonin synthesis capacity. The trapping constant is taken as a proxy for the regional 5-HT synthesis. Birth complications, especially a delivery where the fetus showed signs of physiological distress, predicted lower ^{11}C-AMT trapping in the hippocampus and medial orbitofrontal cortex. Lower ^{11}C-AMT trapping in the medial orbitofrontal cortex was also predicted by maternal smoking and lower birth weight. There were no effects of

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childhood or recent adversity. This is the first human study reporting associations between perinatal adversity and adult ^{11}C-AMT trapping in the hippocampus and medial orbitofrontal cortex. The associations suggest that limbic serotonin pathways may be particularly vulnerable to environmental challenges during the period when they undergo the most prominent neurodevelopmental changes. In combination with other risk factors, perinatal stressors may contribute to increased vulnerability for psychiatric disorders in which serotonin plays a major role.

1. Introduction

There is increasing evidence that prenatal and early postnatal stressors have life long impacts on physical and mental health problems (Gluckman et al., 2008). Serotonin (5-hydroxytryptamine; 5-HT) is crucial for the development and maturation of the mammalian and human brain (Gaspar et al., 2003). Consistent with this, studies in animals have shown that early disruptions in the 5-HT system can lead to subtle changes in brain pathway development, differentiation and maturation, with adverse consequences for emotion regulation and stress-regulation in adulthood (Gaspar et al., 2003). Similarly, a number of studies have shown persisting alterations in 5-HT innervation in the offspring following asphyxia or exposure to neurotoxins during gestation e.g. (Slotkin et al., 2006; Strackx et al., 2008). Whether early perinatal disruptions also affect the 5-HT system in adulthood in humans is not known.

In humans, brain regional 5-HT synthesis can be estimated in vivo by positron emission tomography (PET) and alpha-[(^{11})C]methyl-L-tryptophan (^{11}C-AMT) blood-to-brain clearance/trapping (K*). The trapping constant is taken as a proxy for the regional 5-HT synthesis (Diksic and Young, 2001).

The aim of the present study was to test in vivo whether early perinatal adversity would explain part of the variance in brain 5-HT synthesis in brain regions involved in emotion regulation, using the ^{11}C-AMT method. As the 5-HT system undergoes the most prominent developmental changes in utero and very early life (Jacobs and Azmitia, 1992), we expected that adversity before and around birth would be stronger predictors of brain 5-HT synthesis than adversity occurring in childhood or adulthood. The study was conducted in a sample recruited from a 27-year population-based longitudinal cohort of healthy males.

2. Experimental procedures

2.1. Participants and procedures

Twenty-six adult males (mean age ± *SD* = 27.1 ± 0.7) free of psychiatric or neurological disorders were recruited from a longitudinal study in a French-speaking community sample (*N* = 1037). Participants were part of a PET-^{11}C-AMT study investigating brain 5-HT synthesis in relation to childhood developmental aggression (Booij et al., 2010). The main exclusion criteria were major medical/neurological illness likely to confound PET analysis or results; testing positive on a urine toxicology screen for illicit drugs of abuse on the day of the study; history of exposure to 3,4-Methylenedioxy-methamphetamine (MDMA) in the past month; and current medication likely to affect the function of the central nervous system.

Following a psychiatric interview (First et al., 2002) and medical evaluation, participants underwent a 60-minute dynamic PET scan, conducted with an ECAT HR+scanner following intravenous injection of 10 mCi (370 MBq) of alpha-[^{11}C]AMT injected over a 2-minute period. Each participant also underwent high-resolution magnetic resonance imaging using a 1.5-T superconducting magnet system (Philips Gyroscan; Philips Medical Systems, Eindhoven, the Netherlands) for the purpose of PET/MRI co-registration. All participants provided written informed consent. The study was approved by the research ethics committee at the Montreal Neurological Institute (MNI) and was conducted in accordance with the Declaration of Helsinki.

2.2. Adversity

2.2.1. Perinatal adversity

Obstetrical complications (OC), birth weight and maternal smoking during pregnancy were selected as the primary variables of interest for perinatal adversity. The variables were chosen for the following reasons. Each has been consistently associated with 1) affect and behavioral inhibition problems in which 5-HT plays a major role (Arseneault et al., 2002; Huijbregts et al., 2008; van Os et al., 2001), 2) altered 5-HT neurotransmission in animal experiments (Himpel et al., 2006; Slotkin et al., 2006; Strackx et al., 2008), and 3) structural or functional brain alterations (Lotfipour et al., 2009). Finally (4), their presence in our sample was variable, providing a representative range. Maternal smoking during pregnancy was defined as smoking at any time point during pregnancy and was based on self-report measures completed around the birth of the participant. Birth weight and obstetrical complications were extracted from hospital delivery records. *Obstetrical complications* were indexed by composite scores, as previously described in this cohort (Arseneault et al., 2002). The index consists of three scales: atypical presentation situation, cardiac or respiratory distress situation, and deadly risk situation (Arseneault et al., 2002). Variability in the latter deadly risk situation was minimal, and thus this subscale was left out in further analyses.

2.2.2. Childhood adversity

The index of *family adversity* is a composite score of the degree of social adversity in families ranging from 0 to 1 used in previous studies with this cohort (Arseneault et al., 2002). Family adversity was measured at age 6, and again at ages 10–16. Mean scores were calculated over all assessments (age 6–16) for those subjects with less than 50% missing data points (*N* = 22).

2.2.3. Recent psychosocial adjustment

Psychosocial stressors were indexed by items extracted from the 'Questionnaire about the development of the young adult', a self-report follow up survey completed around the same time as the current study. It included questions related to their current health, psychosocial status and relationships (Booij et al., 2010).

2.3. Image processing and statistical analyses

Functional K* images of α[^{11}C]MTrp trapping rate constants were generated, re-sampled into MNI305-mm isotropic stereotaxic space using a standard automatic algorithm (Collins et al., 1994), and smoothed to a 14-mm resolution FWHM using a Gaussian filter. In

order to cancel out global effects on regional K* values, regional K* values were normalized by the mean global K* of the gray matter to 100.This normalization procedure is the same as used in our other previous ^{11}C-AMT studies, and was achieved by dividing each participant's parametric K* image with their respective mean global K* of the gray matter, and then multiplied by 100. Though it could be argued that adversity may also lead to global, independent changes in 5-HT synthesis, only the normalized data were used in the analyses, given our specific questions on the effects of adversity on the key regions involved in emotion regulation and to reduce error variance and maximize power. Five voxels of interest (VOI) were identified (hippocampus, orbitofrontal cortex (OBFC), caudate, parietal and occipital lobe) on each participant's MRI. The first three were selected based on their prominent role in emotion regulation, the latter two (parietal and occipital lobe) were 'control regions' in order to investigate to what extent the effects of perinatal adversity are specific for brain regions that are primary involved in emotion regulation.

General Linear Models and linear regression analyses were used to assess the predictive power of adversity on normalized K* in each of the a-priori VOIs. To achieve an acceptable predictor/sample size ratio, only two predictors per a priori model were evaluated at the same time. First, the effects of OC and childhood adversity on normalized K* were evaluated. Next, the effects of BW and maternal smoking were simultaneously evaluated, given their strong interrelationship (Wang et al., 2002). Third, the effects of recent psychosocial adjustment were calculated. We evaluated stability and generalizability of the models with the best fit(s) by using multicollinearity diagnostic tests and a leave-one-out ('jackknife') procedure, and performed post hoc power calculations (Cohen et al., 2003).

Additional details regarding assessments and imaging procedures are in the supplementary section.

3. Results

3.1. Obstetrical complications (OC)

Regression analyses including OC and childhood adversity as predictors (N=21) showed that cardiac or respiratory stress predicted lower normalized K* in the right hippocampus (standardized beta=−0.61, t=−3.31, p=0.004) and right medial orbitofrontal cortex (OBFC) (standardized beta: −0.49, t=−2.37, p=0.03) (Figs. 1 and 2; Table S1). There were no effects of the OC subscale atypical presentation situation. There was also no effect of childhood adversity, neither alone nor in interaction, in any of the other investigated VOIs.

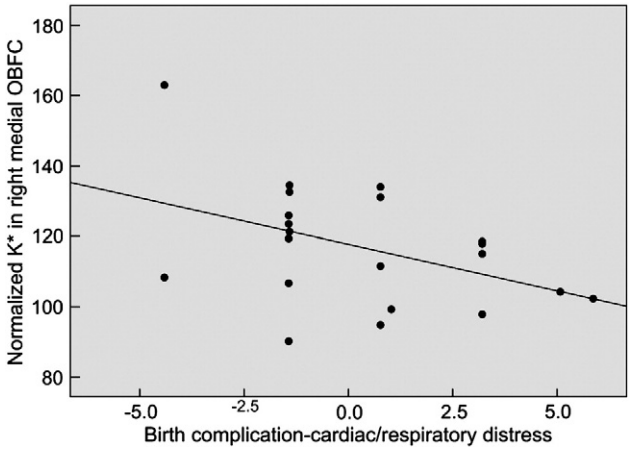

Figure 1 Correlation between fetal cardiac-respiratory distress and normalized K* in the right medial OBFC in adulthood (N=21) (r=−0.63, p=0.002). A high score on the fetal distress scale indicates a delivery situation in which the fetus showed signs of cardiac or respiratory distress along with several medical interventions. A higher score on the distress scale indicates more fetal stress during delivery.

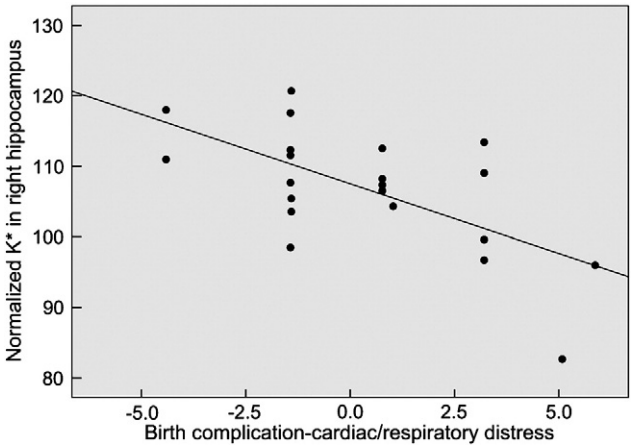

Figure 2 Correlation between fetal cardiac-respiratory distress and normalized K* in the right hippocampus in adulthood (N=21) (r=−0.43, p=0.049). A high score on the fetal distress scale indicates a delivery situation in which the fetus showed signs of cardiac or respiratory distress along with several medical interventions. A higher score on the distress scale indicates more fetal stress during delivery.

3.2. Birth weight and maternal smoking

GLM analyses including lower BW (mean±SD in grams: 3522±631) and maternal smoking (yes/no: N=4 vs. 14) showed that both were independent predictors of lower normalized K* in the right medial OBFC (standardized beta=0.43, t=2.3, p=0.04 and −0.47, t=−2.48, p=0.026, respectively) (Figs. S1 and S2, Table S1). Maternal smoking but not BW was also associated with *higher* normalized K* in the right caudate (standardized beta: 0.53, t=2.49, p=0.03). There were no effects in any of the other investigated VOIs.

The prediction models above all had adequate stability and large effect sizes (Table S1). All observed effects were specific for the right hemisphere (Figs. S3 to S6).

3.3. Recent psychosocial adjustment

There were no relationships between recent life stressors and normalized K* in any of the investigated VOIs (Table S2).

3.4. Relationship between obstetrical complications, birth weight and maternal smoking

A stepwise regression analysis indicated that, among the three perinatal variables of interest, maternal smoking (standardized

beta = −0.47, t = −2.48, p = 0.026) and lower BW (standardized beta = 0.43, t = 2.26, p = 0.04) were the strongest predictors for lower normalized K* in the right medial OBFC. Fetal cardiac/respiratory distress (standardized beta = −0.48, t = −2.18, p = 0.045) was the strongest predictor for normalized K* in the right hippocampus (Table S3).

4. Discussion

The present study with a prospectively followed sample of healthy adult males demonstrated for the first time that obstetrical complications, lower BW and maternal smoking were independent predictors of lower brain 5-HT synthesis in adulthood, in the medial OBFC and hippocampus.

Perinatal adversity specifically predicted lower 5-HT synthesis in those brain regions that are primarily involved in emotion regulation (frontal-limbic), and not in the control regions. The association between lower 5-HT neurotransmission in the hippocampal and frontal regions and perinatal adversity, is consistent with animal studies e.g. (Himpel et al., 2006; Strackx et al., 2008). Of particular interest is the consistency of the results with a very recent ^{11}C-AMT study in newborn rabbits, showing lower 5-HT metabolism in the frontal cortex in those exposed to endotoxin in utero, relative to control rabbits (Kannan et al., 2010). The present study showed that perinatal factors influence 5-HT synthesis in the frontal-limbic regions in humans.

Lower brain 5-HT neurotransmission in the OBFC has most consistently been associated with aggression problems (Booij et al., 2010). Interestingly, a previous study from our research group showed that birth complications (assessed in a similar way as in the present study) predicts violent behavior in late adolescence, when exposed to postnatal psychosocial adversity (Arseneault et al., 2002). Since none of the participants in the present study displayed violent behaviors and postnatal psychosocial adversity levels were relatively low, the results of the present study suggest that in utero adversity may lead to subtle and durable changes in 5-HT neurotransmission, which may set the stage for maladaptive behavior, when exposed to further life stressors.

Interestingly, the effects on adversity on brain 5-HT synthesis were specific for the right hemisphere. Though the underlying mechanism is not known, these laterality effects are remarkably consistent with some studies in monkeys and humans showing that early developmental stressors had a greater impact in the right hemisphere than in the left hemisphere on blood flow (Jones et al., 2011), SERT binding (Ichise et al., 2006) and OBFC brain volume (Hanson et al., 2010). Also of interest are the findings of a study investigating 5-HT neurotransmission in rats separated from their mother around birth vs. controls. Though maternally separated rats had lowered 5-HT neurotransmission in both the right and left frontal cortex and hippocampus relative to control rats (Matthews et al., 2001), calculation of effect sizes of the means provided in their Table 1 suggests that group differences in 5-HT in the right hemisphere in these regions were about 20% higher than for the left hemisphere. The present study showed laterality differences in human brain 5-HT neurotransmission, as a function of early adversity.

The relationships were independent of postnatal adversity. Though childhood adversity has been shown to be an important moderator between perinatal stress and other neurobiological features (e.g. brain structure or hypothalamic-pituitary-adrenal axis regulation (Lupien et al., 2009)), the results are consistent with animal models demonstrating direct effects of in utero adversity on adult 5-HT neurons (Strackx et al., 2008; Himpel et al., 2006). In rats, the first raphe neurons are already generated at 12 days of gestation, and the mature pattern of density and innervation of 5-HT fibers is reached by the end of postnatal week three, much earlier than other monoamines (Jacobs and Azmitia, 1992). Research in mice has shown that dorsal raphe neurons are the main source of 5-HT in the hindbrain and in later stages in the forebrain (after about E16.5), whereas at earlier embryonic stages, 5-HT levels in the fetal forebrain are modulated by an exogenous placental source of 5-HT. A comparable pattern of development has been observed in humans, with a capacity for placental 5-HT synthesis occurring at 11 weeks after gestation (Bonnin et al., 2011).

Postnatally, the most significant changes in ^{11}C-AMT uptake and trapping in humans occur over the first two years of life (Chugani et al., 1999). Hence, the association with perinatal adversity, together with the observation of a relatively weak relationship between brain 5-HT synthesis and adversity in childhood and adolescence or recent psychosocial adjustment, suggests that the 5-HT system may be most vulnerable to long-lasting environmentally induced alterations during the period when it undergoes the most prominent neurodevelopmental changes.

The validity and significance of the observations presented in this study, however, rest upon the following considerations: 1) although the sample size was appropriate for a PET study, the sample was too small to have sufficient power to correct for multiple comparisons. However, the variables and VOIs were all carefully chosen on the basis of prior research, applied in a prospectively followed and well documented sample, and the obtained regression coefficients had remarkable power and stability. 2) Adversity-induced alterations in brain 5-HT synthesis may develop in many different ways, including via changes in intracellular factors, interactions with other neurotransmitters, or stable changes in genetic expression caused by epigenetic mechanisms (Szyf et al., 2009). Furthermore adversity also affects structural brain development (Lotfipour et al., 2009). The findings of the present study are based on correlations and the study was not designed to take into account other putative neurobiological factors. Thus, whether the observed alterations in 5-HT synthesis, resulting from perinatal adversity, are primary or secondary is not known. 3) The present study was limited to a sample of healthy males in which the amount of perinatal stress was modest. It would be of much interest to investigate the extent to which the present results apply to psychiatric samples, females or to samples with clear birth defects. Nevertheless, the finding of a relationship between perinatal adversity and brain 5-HT synthesis in such a healthy sample suggests that the estimation of the impact of perinatal adversity on brain 5-HT synthesis may have been a conservative one.

Notwithstanding these limitations, the present study provides the first in vivo evidence for perinatal influences

on human brain 5-HT synthesis in adulthood. In combination with other biological and social risk factors, perinatal stressors may contribute to subtle, but durable changes in regional brain serotonergic neurotransmission, thereby putatively altering susceptibility for psychiatric disorders in which 5-HT plays a major role.

Supplementary materials related to this article can be found online at doi:10.1016/j.euroneuro.2011.11.002.

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Contributors

Linda Booij, Mélissa Lévesque and Chawki Benkelfat performed the experiment. Linda Booij, Paul Gravel, Louise Arseneault and Mirko Diksic analyzed the data. Linda Booij wrote the paper. Chawki Benkelfat, Richard Tremblay, Marco Leyton, Frank Vitaro, Louise Arseneault and Mirko Diksic critically reviewed the paper. Chawki Benkelfat and Richard Tremblay share senior authorship. All authors contributed to and have approved the final manuscript.

Conflict of interest

All other authors declare that they have no conflicts of interest.

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