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# Early impact of 5-HTTLPR polymorphism on the neural correlates of sadness

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ABSTRACT

Healthy adults carrying the short (S) allele of the human serotonin transporter gene linked polymorphism (5-HTTLPR) show increased amygdala activation during visual processing of emotionally negative stimuli compared to healthy adults homozygous for the long (L) allele. To determine whether abnormal brain responses during negative emotion appear early in life in S allele carriers, functional magnetic resonance imaging (fMRI) was used to measure brain activity during a transient state of sadness in children carrying the S allele (S group) or homozygous for the L allele (L group). Blood-oxygen-level dependent (BOLD) signal changes were measured while subjects viewed blocks of neutral film excerpts and sad film excerpts. During the sad condition (relative to the neutral condition), there was significantly greater activation in the S group compared to the L group in brain regions known to be involved in normal sadness and major depression. Given that the 5-HTTLPR polymorphism has been associated with mood disorders, it is plausible that the abnormal pattern of regional brain activity detected here, in children carrying the S allele, increases susceptibility to emotional dysregulation and depressive symptoms.



The serotonin (5-HT) transporter (5-HTT) plays a central role in 5-HT neurotransmission by determining the duration and intensity of the 5-HT communication with its pre- and postsynaptic receptors, and anomalies in emotional processing may be linked to 5-HTT dysfunction [11]. The fact that drug-free patients with mood and anxiety disorders exhibit significant reductions in brain 5-HTT binding relative to healthy controls [26] is consistent with this view.

A relatively common genetic polymorphism in the human 5-HTT gene (SLC6A4) located in the transcriptional control region on chromosome 17q11.1-q12 was identified more than a decade ago [13]. The 5-HTT gene linked polymorphic region (or 5-HTTLPR) is frequently defined by two variable nucleotide tandem repeat elements, a short (S) allele and a long (L) allele. In humans, having one or two copies of the short, 'S' allelic variant of this polymorphism is associated with significantly lesser 5-HTT binding in brain [15]. Moreover, individuals with either one or two copies of the S allele have a higher probability of developing depressive symptoms as compared to those who are homozygous for the L allele [23,33].

In a seminal study [12], functional magnetic resonance imaging (fMRI) was used to evaluate, in a small sample of healthy adult volunteers carrying the S or L allele, neural activation during perceptual processing of fearful and angry human facial expressions. This task consistently engages the amygdala, a brain region crucially involved in emotional arousal and reactivity. During the task, S allele carriers showed nearly five-fold greater amygdala activity than L homozygotes. This finding was replicated in a larger cohort of healthy adults [10]. Along the same lines, healthy adult carriers of the S allele were found to exhibit more robust activation of the amygdala during the presentation of emotionally negative pictures [14]. Additionally, another fMRI study carried out in healthy adults revealed a weaker functional connectivity between the anterior cingulate cortex (ACC) – a prefrontal area playing a pivotal role in various aspects of emotion – and the amygdala in S-allele carriers relative to L-allele homozygotes during presentation of angry and fearful faces [29].

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Collectively, the findings of these neuroimaging studies indicate that relative loss of 5-HTT gene function negatively affects the brain systems mediating emotional processing. A dysfunction of these systems could provide a putative neural substrate for the emotional vulnerability associated with genetic variation in the 5-HTT [11].

In keeping with what has been found in adults, it has been recently demonstrated that infants insecurely attached to their caregiver and homozygous for the S-variant of the 5-HTTLPR genotype develop a high level of negative emotionality and fear [28]. Furthermore, healthy adolescents with at least one copy of the S allele show stronger amygdala responses to fearful faces than healthy adolescents without this allele [22]. These findings suggests that the abnormal brain responses to emotionally negative stimuli may appear relatively early in life in S allele carriers.

To tackle this important issue, we used fMRI to measure brain activity, during a transient state of sadness, in children carrying the S allele and children homozygous for the L allele. This primary emotion was selected given that it is the prevailing mood in major depressive disorder (MDD), and that the 5-HTTLPR polymorphism appears to exert a certain effect on susceptibility to depression [17]. We predicted that the sad state would be correlated with greater activation of brain regions mediating sadness in the S allele carriers relative to the L allele carriers.

Within a prospective twin cohort study [27], 438 children of Caucasian ancestry were scanned when they were 8 years and 4 months of age. The study protocol was approved by the ethics review boards of Sainte-Justine Hospital and CHUM-Notre-Dame Hospital. Written informed consent was obtained from parents of all subjects as well as written assent from all subjects. In this cohort, 90 children were genotyped for the 5-HTTLPR polymorphism. They were divided into two equal groups on the basis of their 5-HTTLPR genotype: children homozygous for the L allele (L group, $n = 24$, 14 girls, 10 boys) and children with either one or two copies of the S allele (S group, $n = 24$, S/S = 17, S/L = 7, 11 girls, 13 boys). These children were unrelated (within and between groups). Subjects with head movements greater than 3 mm in the MRI scanner were excluded from this study.

Participants were assessed using the Dominic-R Interactive [36]. This self-answered, computerized, DSM-IV-based instrument designed to measure mental health in children 6–11 years of age has been used in epidemiologic samples in clinical and research settings [32] (see supplementary material). Both groups scored below the clinical cut-off scores on all mental disorders assessed with the Dominic-R (depression, separation anxiety, generalized anxiety, specific phobias, attention-deficit hyperactivity disorder, conduct problems, oppositional-defiant disorder). The depression scores were minimal and not statistically different between groups (S group: mean = 3.74, S.D. = 2.30; L group: mean = 5.35, S.D. = 3.27) ($P = 0.081$).

Blood-oxygen-level dependent (BOLD) signal changes were measured while subjects viewed five blocks of neutral film excerpts (control condition) followed by five blocks of sad-film excerpts (experimental condition) (see supplementary material). As subjective emotional responses persist on average 32 s after presentation of aversive pictures [9], 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), used in several studies of sadness induction [7,24]. The neutral excerpts consisted of a news interview. Each block lasted 39 s and was separated by 15-s resting periods during which subjects viewed a white cross on a black screen. After scanning, subjects identified the primary emotions (happiness, anger, sadness, fear, surprise, disgust) they felt during the sad and neutral excerpts using a visual analog scale. 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.

Echoplanar images (EPI) were acquired on a 1.5-Tesla system (Magnetom Vision, Siemens Electric, Erlangen, Germany). Twenty-eight slices (5 mm thick) were acquired every 2.65 s in an inclined axial plane, aligned with the anterior commissure–posterior axis. These T2*-weighted functional images were acquired using EPI pulse sequence (time repetition [TR] = 0.8 ms, time-echo [TE] = 54 ms, flip = 90°, field of view [FOV] = 215 mm, matrix = 64 × 64, voxel size = 3.36 mm × 3.36 mm × 5 mm). Following functional scanning, high resolution data were acquired via T1-weighted three dimensional volume acquisition obtained using a gradient echo pulse sequence (TR = 9.7 ms, TE = 4 ms, flip = 12°, FOV = 250 mm, matrix = 256 × 256, voxel size = 0.94 $mm^3$).

Data were analyzed using Statistical Parametric Mapping software (SPM5; Wellcome Department of Cognitive Neurology, London, UK). Images for all subjects were realigned to correct for artifacts due to small head movements. The images for all subjects were then spatially normalized into an MRI stereotactic space (Montreal Neurological Institute [MNI] template) using this masked mean image. The MNI template was used since Burgund et al. [2] have shown that even if there are some small anatomical differences between the brain's structures and sulci of adults (age range: 18–30) compared with those of children (age range: 7–8), such minimal differences do not compromise the usefulness of an adult stereotactic space for children. Images were also convolved in space with a three-dimensional isotropic gaussian kernel (12 mm full width half maximum [FWHM]) to improve the signal-to-noise ratio and to accommodate for residual variations in functional neuroanatomy.

The time series of the images were convolved with the delayed box-car function which approximates the activation patterns. Effects at each and every voxel were estimated using the general linear model. Voxel values for the contrasts of interest yielded a statistical parametric map of the $t$ statistic (SPM $t$), subsequently transformed to the unit normal distribution, (SPM $Z$). A "fixed-effects model" was implemented to contrast the brain activity associated with the viewing of the sad film excerpts and that associated with the viewing of the emotionally neutral film excerpts (Sad minus Neutral). This "fixed-effects model" produced individual contrast images, which were used as raw data for the implementation of a "random-effects model" used for group analysis. One-sample $t$-test was performed for both groups to determine brain activity for the Sad minus Neutral contrast. A two-sample $t$-test was performed to compare brain activity observed in the S and L groups (Sad minus Neutral contrast). A priori search strategy was used and a small volume correction was performed in the following brain regions of interest (ROIs): ACC (Brodmann areas [BA] 24 and 32), ventrolateral prefrontal cortex (VLPFC) (BA 47), anterior temporal pole (BA 21 and 38), insula, caudate nucleus, putamen, and amygdala. These regions have been consistently activated in previous neuroimaging studies of sadness [1]. The search volume corresponding to the ROIs was defined by creating an inclusive mask in the MARINA program (Bender Institute of Neuroimaging) and using the small volume correction and image volume functions in SPM5. For this a priori search, a corrected probability threshold for multiple comparisons of $P < 0.05$ corrected was used. Only clusters showing a spatial extent of at least 10 contiguous voxels were kept for image analysis.

The viewing of the sad film excerpts induced a transient state of sadness in all subjects. The mean level of reported sadness was not statistically different ($P = 0.24$) in the L group (mean: 2.75, S.D.: 1.16; range 1–4) compared to the S group (mean: 3.18, S.D.: 0.95; range 1–4). In addition, the viewing of the sad film excerpts did not produce other significant change of the emotional state than sad-