Pharmacological MRI and resting-state fMRI of functional brain organization in the serotonin transporter knock-out rat

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Introduction

The brain circuitry involving amygdala and prefrontal cortex (PFC) is known to mediate emotional processing and cognitive flexibility. Functional and structural changes in this circuit have been associated with a common human polymorphism in the serotonin transporter gene (5-HTTLPR) [1,2]. The serotonin transporter (SERT) modulates the serotonergic (5-HT) system throughout the brain and disrupted 5-HT homeostasis has been linked with a variety of neuropsychiatric disorders, including affective disorders, depression, anxiety and drug addiction. Neuroimaging studies have demonstrated that carriers of the dominant short (s) allelic variant show increased amygdala activity [3] and a strengthened coupling between amygdala and PFC [1] and cingulate cortex [2], when presented with aversive emotional stimuli. This has been ascribed to increased 5-HT levels and subsequent desensitization of inhibitory 5-HT_{1A} receptors [1]. The 5-HTTLPR genotype has also been correlated with alterations in baseline cerebral blood flow (CBF), which was increased in amygdala and decreased in ventromedial PFC in short (s/s) versus long allele (l/l) homozygotes [4, 5]. However, it still remains unclear whether the effects of compromised SERT-expression persist in the baseline functional organization of the brain. Resting-state fMRI (rs-fMRI) is a novel fMRI approach that allows assessment of spatial organization of brain function without stimulating a particular functional system, and may therefore provide additional insights in the differences in default functional organization of 5-HT-modulated pathways. The serotonin transporter knock-out rat ($SERT^{-/-}$) is a recently developed animal model to study the effects of compromised serotonergic signaling [6,7]. Since the $SERT^{-/-}$ rat does not express a functional SERT, extracellular 5-HT levels are 9-fold increased and reuptake is reduced, although not completely absent [7]. From behavioral studies, increased sensitivity for cocaine in $SERT^{-/-}$ rats has been observed [8]. Recent findings from manganese-enhanced MRI of the $SERT^{-/-}$ mouse suggest alterations in the neuronal pathways connecting PFC, nucleus accumbens, caudate-putamen, and thalamic nuclei [9]. Our aim was to assess differences in functional organization of the 5-HT-modulated neuronal pathways in the $SERT^{-/-}$ knockout rat. We therefore applied rs-fMRI-based functional connectivity (FC) analysis and pharmacological MRI (phMRI) with a cocaine challenge to study the effects of absent SERT on both baseline and stimulated states of neuronal functioning. We hypothesized that in the $SERT^{-/-}$ rat, 5-HT-modulated networks show an overall increased FC and specifically exhibit a stronger response to cocaine.

Methods

A total of 23 adult male Wistar rats were included in the study, of which 11 controls ($SERT^{+/+}$) and 12 $SERT^{-/-}$ ($Slc6a4^{1Hubr}$). Animals were anesthetized with 5% isoflurane (2-2.5% maintenance). Two tail veins were cannulated for administration of pancuronium bromide (2 mg/ml) and cocaine HCl dissolved in saline (1 mg/ml). The left femoral artery was cannulated for blood pressure measurements and blood gas analysis. MRI measurements were conducted on a 4.7T horizontal bore Varian MR system, with use of a Helmholtz volume coil (90 mm diameter) and an inductively coupled surface coil (25 mm diameter) for signal excitation and detection, respectively. Rats were mechanically ventilated with 1% end-tidal isoflurane in air/O_2 (2:1) and received a continuous infusion of 1 mg/kg/h pancuronium bromide to prevent movement. Blood oxygen saturation and heart rate, and expired P_aCO_2, were monitored during MRI, and body temperature was maintained at 37.0 ± 0.5 °C. Before and after rs-fMRI and phMRI, a sample of 0.15 ml arterial blood was analyzed to ensure P_aCO_2 levels were in normal physiological range. Based on physiological stability of the animal during MRI, as assessed by capnography or P_aCO_2 measurements, animals were included in the rs-fMRI or phMRI group, or both.

First, 10 minutes of rs-fMRI was performed using a T2*-weighted single-shot gradient echo EPI sequence (TR/TE=500/19 ms; 35° flip angle; 64×64 matrix; 0.5×0.5 mm^2 voxels; 7×1.5 mm coronal slices; 1200 BOLD images). Subsequently, gradient-echo multi-slice T2*-weighted phMRI was performed (TR/TE=468.75/35 ms; 60° flip angle; 64×64 matrix; 0.5×0.5×1.0 mm^2 voxels; 12×1.0 mm coronal slices) with 10 minutes (20 images) of baseline measurements followed by 40 minutes (80 images) acquisition after injection of 1 mg/kg cocaine. Anatomical images for registration purposes were obtained with a 3D gradient-echo sequence (TR/TE=6/2.576 ms; 40° flip angle; 256×128×128 matrix; FOV=60×40×40 mm^3).

Preprocessing for rs-fMRI entailed motion correction (FSL FLIRT, www.fmrib.ox.ac.uk/fsl), spatial smoothing, data correction with linear regression [10], and band-pass filtering ($0.01 < f < 0.1$ Hz) using AFNI (R.W. Cox, http://afni.nimh.nih.gov/afni). Anatomical images were registered non-rigidly (Elastix, http://elastix.isi.uu.nl) to a reference image, which was matched to a 3D model of a rat brain atlas [11]. FC was measured as the Fisher z'-transformed correlation coefficient. Preprocessing for phMRI entailed linear drift correction using AFNI, spatial smoothing and calculation of BOLD signal changes relative to the mean baseline value. BOLD signals were grouped in 10 bins covering 50 minutes (10 samples per bin). Bilateral regions-of-interest (ROIs) within the limbic system that are innervated by 5-HT projections, i.e. medial PFC (mPFC), amygdala, cingulate cortex, thalamus and nucleus accumbens (NA) were projected from the atlas onto the functional time-series for seed-based FC and phMRI analysis. Groups for rs-fMRI analysis consisted of eight $SERT^{+/+}$ and eight $SERT^{-/-}$ rats; phMRI groups contained six $SERT^{+/+}$ and six $SERT^{-/-}$ rats.

Results

During rs-fMRI and phMRI studies, arterial P_aCO_2 levels were within normal limits ($33.9 < P_aCO_2 < 44.6$ mmHg) for all included animals, except three for which no blood gas data could be obtained. FC analysis showed positive correlation between mPFC, cingulate cortex, NA and thalamus. Positive FC with amygdala was mostly confined to amygdaloid nuclei and caudate-putamen (Figure 1). However, more distal FC with amygdala could not be reliably assessed, since voxel-wise FC-values were close to zero. FC was not significantly different between $SERT^{+/+}$ and $SERT^{-/-}$. phMRI showed a robust brain-wide response to cocaine in both $SERT^{+/+}$ and $SERT^{-/-}$ animals. The cocaine-induced activation was significantly higher in $SERT^{-/-}$ rats in cingulate cortex (Figure 2). Amygdala activation was reduced in $SERT^{-/-}$ animals, though not significant. Other ROIs showed increased but not significantly higher activation in $SERT^{-/-}$.

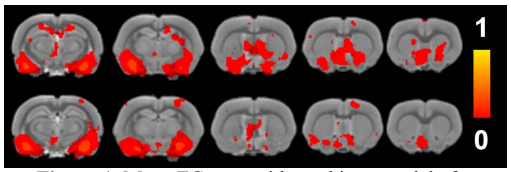

Figure 1. Mean FC-map with seed in amygdala for $SERT^{+/+}$ controls (top) and $SERT^{-/-}$ knock-outs (bottom).

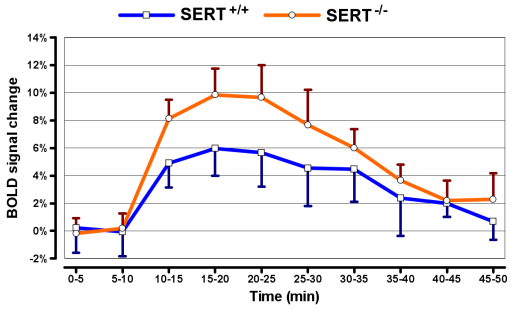

Figure 2. Mean BOLD phMRI signal in cingulate cortex.

Discussion

In this study we combined rs-fMRI with phMRI in the $SERT^{-/-}$ rat, to study the effects of persistently disrupted 5-HT homeostasis on functional organization during baseline and psychoactive stimulation. With rs-fMRI we observed positive FC among ROIs within the limbic system, but no difference with controls. We showed with BOLD phMRI that cocaine may elicit stronger activation responses in $SERT^{-/-}$ rats in specific limbic areas, which is in agreement with previously reported cocaine supersensitivity [8]. Whereas human studies have identified strong interactions between 5-HTTLPR genotype and functional coupling, e.g. amygdala-PFC [1,4] and amygdala-cingulate cortex [2], we were unable to detect similar differences in FC with rs-fMRI in rats. This may be due to biological differences between the species models, but could also reflect limited sensitivity of the rs-fMRI method (e.g. caused by partial volume effects and lower SNR in ventral and frontal areas, or masking due to global signal regression [10]).

References

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