Repeated fluoxetine treatment during adolescence causes regional brain atrophy in adulthood: A voxel-based morphormetry study in rat

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Introduction Fluoxetine (FLX) is the most popular prescription drugs for treatment of major depressive disorder in young populations [1]. Recent studies have shown that chronic exposure to selective serotonin reuptake inhibitors (SSRI), such as FLX, during adolescence or juvenile period may cause neuroplastic changes on some brain regions, such as amygdala [2] and prefrontal cortex (PFC) [3]. In this work, we constructed tissue probability maps (TPMs) of adult rat brain using high resolution volumetric T_2-weighted image datasets, and used them to perform VBM analysis on the structural changes caused by repeated FLX treatment in adolescent rats.

Materials and Methods *Subjects & MRI data acquisition:* Adolescent rats (postnatal 35d) received intraperitoneal injection of FLX (5 mg/kg) (n=8) or normal saline (n=8) twice daily (4 hrs apart) for 15 consecutive days, and scanned 45 days after the last rejection. T_2-weighted brain images were acquired with a RARE pulse sequence on a Bruker Biospec 7.0 T/20 cm scanner with the following parameters: RARE factor 4, matrix size 512×384, FOV 35 mm×35 mm, slice thickness 0.58 mm, 52 contiguous coronal slices, TR 5800 ms and TE_{eff} 40 ms. A volume coil were used for RF transmitter, and a 4-channel phase-array surface coil for signal reception. *Creation of template and TPMs:* High-resolution RARE images of 19 adult normal male SD rats were used to create TPMs. All images were first cropped to a size of 312×184 pixels, manually stripped out of the extra-cranium pixels and corrected for image intensity ununiformity artifacts. Then the fuzzy c-means clustering method (FCM) was used to evaluate the probabilities of each voxel belonging to gray matter (GM), white matter (WM) or cerebrospinal fluid (CSF), yielding three TPMs for each rat. An initial RARE template was created by resample a preprocessed rat image to voxel size of 0.1 mm×0.1 mm×0.1 mm, and smoothed with a 0.2-mm FWHM Gaussian kernel. All other preprocessed RARE images were spatially normalized to the initial template, and the affine transformation parameters obtained were applied to the corresponding TPMs of each rat. Finally, all the co-registered TPMs were averaged to obtain three TPM images with a matrix size 213×167×301 and a voxel size 0.1 mm×0.1 mm×0.1 mm. *VBM analysis on FLX-treated rats*: VBM analysis was performed twice using 'SEGMENT' in SPM8 with different TPMs: first with the TPMs created above and produced 'unmodulated normalized' tissue images for each rat brain, then with mixed group images averaged from all the tissue images in the first step, producing modulated normalized images containing volumetric information. These images were smoothed with a 0.2-mm FWHM Gaussian kernel and compared with two-sample *t*-tests.

Results Selected slices of the TPM templates obtained are shown in Fig. 1, illustrating the probability distribution of GM, WM and CSF. The results of VBM analysis are shown in Fig. 2. Compared to control, the FLX-treated animals showed atrophy in the medial PFC (mPFC), motor and somatosensory cortices, fimbria of the hippocampus and parts of the thalamic nuclei.

Discussion The TPM templates generated in this study could be used as target images in normalization and co-registration of T_2-weighted or similar contrast rat brain images. They could also give probability priori estimates of tissue segmentation when segmentation or VBM analysis on rat brain is performed with SPM. Varea et al reported that chronic FLX treatment changs the neuroplasticity of the medial prefrontal cortex [4]. Homberg el al suggested that 5-HTT blockade (genetic and pharmacological) during devolopment in rodents causes functional and neuroanatomical deficits in the layer IV of somatosensory cortex [5]. Our observation that mPFC and somatosensory/motor cortex are atrophic in the FLX-treated rats, related to control, is consistent with these previous findings.

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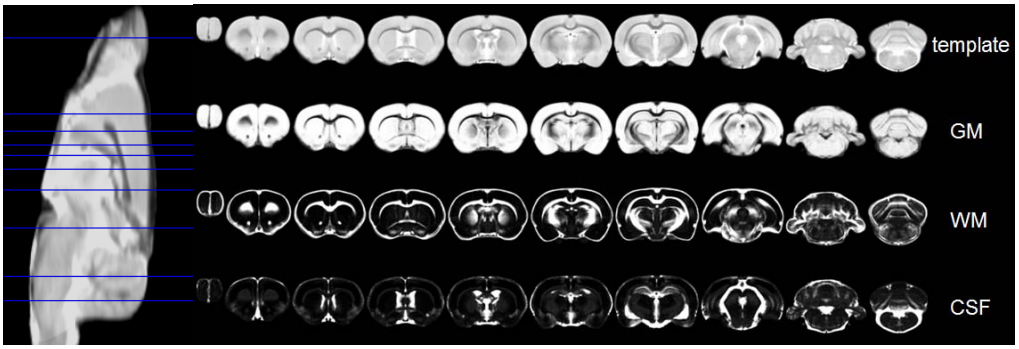
References [1] Safer DJ et al, Pediatric, 2006, 118:1248–1251. [2] Varea E et al, Neuropsychopharmacol, 2007, 32:803–812. [3] Ago Y, Psychopharmacology, 2011, 217:377 – 386. [4] Norrholm SD, et al, Brain Res, 2000, 883:205–215. [5] Homberg SR et al, Trends Pharmacol Sci, 2010, 31:60–65.



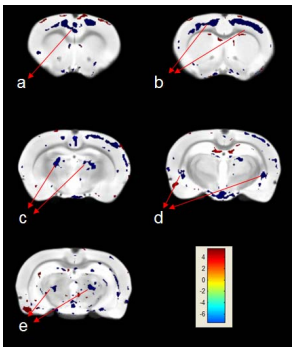


Figure. 1 Template RARE images and correponding TPMs of GM, WM and CSF of rat brain.

Figure. 2 The results of VBM analysis show regions with reduced volume in the FLX-treated group ($p<0.05$). a: mPFC; b: motor and somatosensory cortices; c: lateraldorsal thalamic nucleus; d: fimbria of the hippocampus; e: anterior pretectal nucleus.