Differential effects of chronic fluoxetine use in young vs. adult rats: a phMRI study

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Background:
Although numerous trials have shown robust safety of fluoxetine (the only SSRI registered for use in children over 8 years) in adults, limited data is available on its effects on the developing brain during (pre)adolescence. Also, its clinical efficacy in this population remains debated (Hetrick et al., 2007). SSRIs like fluoxetine act upon the serotonergic (5-HT) system by blocking the 5-HT transporters and in this way increasing the amount of available 5-HT. Recent literature and ongoing pilot studies of our group suggest that fluoxetine treatment has a stimulatory effect on the outgrowth of the 5-HT system which can only be seen in the developing brain which is still ongoing in peri-adolescent rats (Wegerer et al., 1999; Gsell et al., 2008). Pharmacological MRI (phMRI) is a relatively new non-invasive method to assess brain function in both human and animals (Anderson et al., 2008; Martin & Sibson, 2008) by mapping the hemodynamic response induced by a drug challenge. The aim of this study is to assess the effects of chronic fluoxetine treatment on 5-HT function in the developing versus adult rat brain using a phMRI approach.

Methods:
Male Wistar peri-adolescent (PND25) and adult rats (PND65-70) (N=25, Harlan UK) were treated daily for 3 weeks with fluoxetine (p.o., 5mg/kg,) or sterile water followed by one week off before phMRI scanning. Animals were anaesthetized with isoflurane (5% induction, 1.5-2% maintenance during surgery and scanning) given in a 70/30 mixture of nitrous oxide. The right femoral artery was cannulated for blood gas (RapidLab 348, Siemens diagnostic) and blood pressure (Biopac Systems Corp., USA) monitoring. The right femoral vein was cannulated for acute injection of fluoxetine (5 mg/kg) during the phMRI experiments. Images were acquired on a Varian 4.7T MRI scanner using a cylindrical 72 mm diameter quadrature transceiver coil. Temperature was monitored through a rectal probe and maintained at 37.5 ± 1 °C by a warm air heating system (SA Instruments, USA). For each subject, a T2-weighted anatomical volume was acquired using a fast Spin Echo Multi-Slice (fSEMS) sequence with an echo train of 16, matrix = 256x 256, FOV = 50 mm, 30 contiguous 1 mm coronal slices, 4 averages, TReff = 5112ms, and TEeff = 60 ms. The time series acquisition used the same sequence with a echo train of 8, 20 slices of 1 mm thickness, TR=158 ms, TReff = 4914.8 ms, TEeff = 60 ms and a matrix size of 128 x 128. Thirty two time points per subject (total scan time of 84 min.) were acquired with injection of 5 mg/kg fluoxetine during the acquisition of 9th time point. The data were spatially normalized to a stereotaxic rat brain MRI atlas template (Schwarz et al., 2006). A fast analysis using Stimulate software (Strupp, 1996) was performed in order to determine the shape of the brain signal changes. FSL-FEAT 4.0 (Woolrich et al., 2001) was then used for the group analysis using a GLM model matching the brain changes determined previously in Stimulate.

Results:
Analysis of individual data sets in Stimulate showed a significant long-lasting effect of fluoxetine administration on T2-weighted signal intensity in localized brain regions in all animals (See Figure 1). Group analysis showed a reduction of the overall brain activation in the adult treated group (n=8) compared to the adult control group (n=7). Within the young animals, there was no significant difference between the mean activation patterns of the treated and untreated animals (n=5 for both groups). When comparing the control animals, there was no difference between the young and adult mean activation pattern. In the fluoxetine-treated animals however, the young animals showed more activation than the adult animals, mostly in subcortical regions (See Figure 2).

Discussion:
The long-lasting brain activation induced by the fluoxetine challenge corroborates the pharmacodynamic properties of the drug (Harvey & Preskorn, 2001). The main finding of our study is the decrease of brain activation in adult treated animals suggesting a central effect of the chronic treatment in this population. However, this decrease of activation has not been found in the young group and this could explain the differential effect of fluoxetine treatment between young and adult animals reported in the literature (Ansorge et al., 2008). Our (preliminary) interpretation would be that the developing brain is compensating the effect of chronic fluoxetine exposure by increasing the number of serotonin transporters as previously shown by our group using ex-vivo binding studies (Gsell et al., 2008). These results will add to the discussion on the clinical efficacy of this drug as a therapeutic agent in young patients. Further localized analysis using 5-HT-related regions of interest is ongoing in order to fully understand which specific brain regions are involved in this compensatory mechanism.

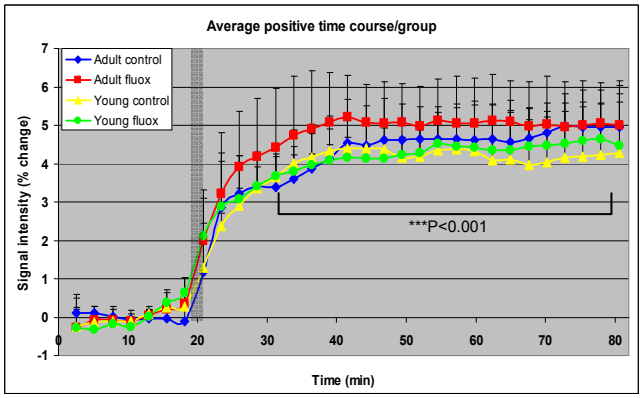

Figure 1: Average time courses of signal intensity change induced by injection of fluoxetine (5mg/kg, grey bar). Data including all activated voxels in the brain determined by t-test using Stimulate. *** P<0.001 paired t-test

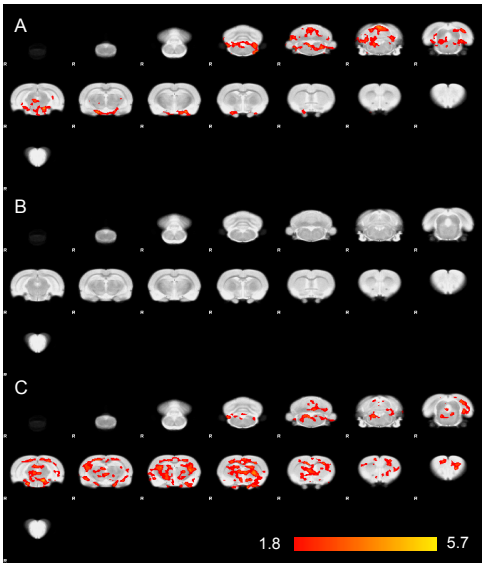

Figure 2: Statistical parametric maps of adult control versus adult fluoxetine (A), young control versus young fluoxetine (B) and young fluoxetine versus adult fluoxetine (C) (FEAT Version 5.90, part of FSL). Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>1.8 and a (corrected) cluster significance threshold of P=0.05.

References:
Anderson et al., Neuropharmacology 2008; 55(6):1029-1037; Ansorge et al., J Neurosci. 2008; 2;28(1):199-207; Gsell et al., Proc. Intl. Soc. Mag. Reson. Med. 16 (2008);2083; Harvey & Preskorn, J Clin Psychopharmacol. 2001; 21(2):161-6; Hetrick et al., Cochrane Database Syst Rev 2007; 18(3):CD004851; Martin & Sibson, Neuropharmacology 2008; 55(6):1038-1047; Schwarz et al., Neuroimage 2006; 32(2):538-550; Strupp, NeuroImage, 1996;3(3):S607; Wegerer et al., J Child Adolesc Psychopharmacol 1999; 9:13-24; Woolrich et al., NeuroImage 2001; 14(6):1370-1386.