Effect of Fluoxetine on the developing brain

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Background:

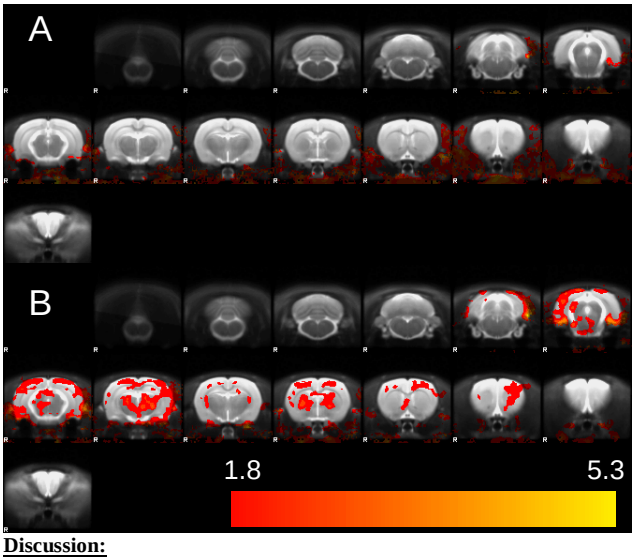
Approximately 500.000 children and adolescents are treated with serotonin (5-HT) reuptake inhibitors (SSRIs) for major depressive disorder (MDD) in the UK and Netherlands. Numerous trials have shown robust safety of SSRIs in adults. However, very limited data are available on their influence on brain development. Indeed, when fluoxetine (potent SSRI) is administered peri-natally to mice, abnormal emotional behaviors occur later in adult mice mimicking those of mice genetically deficient in 5-HT transporters (SERT) (Ansorge 2004). Increase in frontal cortex SERT has also been observed in peri-adolescent rats chronically treated with fluoxetine, which persists into adulthood (Wegerer 1999), whereas in adult animals and humans a reduction in SERT density and function is observed (Benmansour 1999, Kugaya 2003). Finally, there have been concerns about increased self-harm among children treated with SSRI's (Jureidini 2004), although the European Medicines Agency (EMEA) recently approved fluoxetine for use in children aged 8 years and older (Gibbons 2006). The aim of this study is thus to investigate whether the effects of the fluoxetine on the outgrowth of the 5-HT system are dependent on age.

Methods:

In all experiments the same treatment protocol was used. Fluoxetine 5mg/kg and saline was injected daily during 3 weeks in pre-adolescent rats (P28-50, n=3-4) and adult rats (P63-75, n=3-4) followed by one week off before the assessment of the brain functions through the ex-vivo SERT binding studies using [^{123}I]βCIT, and pharmacological MRI (phMRI). NMRI male mice (10/group at P25 and P75) were used for behavioural assessment (tail suspension test, Y maze, spontaneous activity and + maze). Binding ratios of hypothalamus and prefrontal cortex versus cerebellum were calculated 3 hrs after injection of the radiotracer. The amount of radioactivity was expressed as a percentage of the injected dose, multiplied by the body weight per gram tissue weight (% ID x kg/g tissue), as earlier described (Rijks 1996). For the phMRI, rats (n=4 for adult and pre-adolescent treated group, and n=2 and 1 for adult and pre-adolescent control groups respectively) were anaesthetized with isoflurane (5% induction and then reduced to 1.5-2% for maintenance of anaesthesia during surgery and scanning) given in a mixture of nitrous oxide (1.2-1.5 l/min) and oxygen (0.5 l/min). To monitor physiological conditions, the right femoral artery was cannulated for blood gas (AVL, Roswell, GA, USA) and blood pressure (Biopac Systems Corp., Goweta, USA) monitoring. The right femoral vein was also cannulated for injection of the fluoxetine (5 mg/kg) for the acute challenge during the phMRI experiments. PhMRI experiments were acquired on a Varian 4.7T. A cylindrical quadrature coil placed around the head of the animal was used to transmit and receive the signal. Temperature was monitored through a rectal probe and maintained at 37.5 ± 0.5 °C by a warm air heating system (SA Instruments, New York, USA). For each subject, we acquired a T2-weighted anatomical image volume using the rapid acquisition with relaxation enhancement (RARE) sequence with a RARE factor of 16, matrix = 256x 256, FOV = 50 mm, 30 contiguous 1 mm coronal slices, centered 8 mm caudal to the posterior edge of the olfactory bulb, 4 averages, TReff = 5112ms, and TEeff = 60 ms. The time series acquisition used the same sequence with a RARE factor of 8, 16 slices of 1 mm thickness centered to the same position as before with TReff = 4500 ms, TEeff = 60 ms and a matrix size of 128 x 128. Thirty two time points per subject (total scan time of 80 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 template (Schwarz et al., 2006) and analysed using FSL/FEAT v 4.0. The experimental design consisted of an initial period of baseline recordings followed by recording of functional activity induced by Fluoxetine.

Results:

Ex-vivo binding revealed significantly higher hypothalamic SERT binding ratios after fluoxetine treatment in the pre-adolescent group (P28 7.25 +/- 0.11) vs. controls (P28 5.48 +/- 0.74; P<0.01) and adult treated rats (P63 6.87 +/- 0.02 p< 0.01). Similar observations were made in the prefrontal cortex (4.55 +/- 0.42, 3.67 +/- 0.27 and 4.82 +/- 0.13, respectively). These findings were correlated with significant alterations in behavior. Indeed, the chronic treatment with fluoxetine elicits an age dependency effect in which the young population showed increased anxiety and motor activity (tail suspension and + maze tests). PhMRI also elicited a different pattern of brain activation between the two populations with a decrease of the response to acute challenge with fluoxetine in young group compared to adults (see figure).



A: Statistical parametric map of Fluoxetine (5 mg/kg i.v.) in young group (n = 4, P50) treated daily during 3 weeks with fluoxetine (5 mg/kg i.p.)

B: Statistical parametric map of Fluoxetine (5 mg/kg i.v.) in adult group (n = 4, P75) treated daily during 3 weeks with fluoxetine (5 mg/kg i.p.)

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.90, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/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

Discussion:

We demonstrate here using different read-outs that SSRIs could have a differential effect on the 5-HT system depending on the age of the subject. The decrease of the brain activity following acute challenge is directly correlated to an increase of anxious behaviour and an increase in SERT. Our hypothesis is that release of a growth factor from astroglial cells in response to stimulation by 5-HT of postsynaptic 5-HT receptors in the developing brain cause these 5-HT associated alterations (Whitaker-Azmitia 1989, Wegerer 1999). This pre-clinical assessment indicates that 5-HT plays an important role in the regulation of 5-HT outgrowth which is dependent on the age of exposure. We thus hereby raise serious concern on the safety of prescribing SSRIs to the pediatric population without relevant clinical trials.

References:

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