Long term effects of developmental exposure to LP211, a new serotonin 7 receptor (5-HT7) agonist

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Introduction - LP211 is an agonist of serotonin (5-hydroxytryptamine, 5-HT) receptor 7 (5-HT7). It is proposed for a role in neurogenesis and in patho-physiological processes like anxiety / depression, cognitive / sleep disturbances, and impaired coping with stress (1). Evidences demonstrate that it has consistent psychoactive effects onto exploratory motivation, anxiety-related profiles, and spontaneous circadian rhythm (2).
Here, we investigated the long-term consequences of developmental exposure to LP211 on brain metabolism (by in vivo ^{1}H MRS), anatomy (by DTI) and functionality (by functional connectivity, an emerging tool for studying brain functional organization widely applied to the study of the human brain, more recently to preclinical species).

Methods - Wistar adolescent rats (38- to 42-day-old) were administered with LP211 (0.250 mg/kg/day, n=9) or saline (n=9) for 5 days, and tested at adulthood for potential carry-over effects. Experiments were performed on a VARIAN Inova MRI/MRS system operating at 4.7 T, by using a volume coil as transmitter and a surface coil constructed for rat head as receiver (RAPID Biomedical). Multislice fast spin echo sagittal images (TR/TEeff = 3000/70 ms, ns = 2, slice thickness 1 mm, matrix 128 x 256) were acquired to localised the regions of interest. ^{1}H localised MR spectra (PRESS sequence, TR/TE = 4000/23ms, ns=256) were collected from the hippocampus (ROI = 33.7 μl). Spectra were analysed by using LCModel fitting program (3). The unsuppressed water signal was used for metabolite quantification.
In the DTI study a spin-echo sequence with addition of the Stejskal-Tanner diffusion gradients was used. Diffusion gradients were applied along six spatial directions. Intensity, duration and diffusion time were set to 8.27 G/cm, 8 ms and 25 ms respectively, given a *b*-value of 700 s/mm^2. A field of view of 25 × 25 mm^2 was sampled on a 64 × 64 cartesian grid. Multi-slice DT images were acquired (15 slices of 1 mm thickness) in the coronal plane with 2 averages and TE/TR = 50/2000 ms. Using in house Matlab script (Mathworks, Natick, MA), diffusivity values (fractional anisotropy, FA and mean diffusivity, MD) were derived from the tensor. The program allows manual delineation of region of interest (ROI) on the FA and MD maps. Four different regions of the brain were analyzed: the hippocampus (HIP), the prefrontal cortex (PFC), the striatum (STR) and the nucleus accumbens (NAcc) at six different levels of the brain (i.e. six image planes). Significant differences of FA and MD values between the groups were assessed by a unpaired 2 tail t-test.
Rat brain resting state fMRI exploiting the BOLD effect was studied by using a multi slice sagittal gems sequence (TR/TE= 200/5 ms, 7 slices of 2 mm thickness, FOV 25x25 mm^2, 64x64 matrix,140 temporal points. Seed-based resting state fMRI connectivity maps for the two group of animals were generated as follows (4). First, voxel-wise correlation maps were obtained corresponding to a given seed (HIP, NAcc, PFC and orbito-frontal cortex (OFC) in both hemispheres) for every session of every rat. Then z-scores of connectivity are obtained from correlation values across all the sessions to compute significant connections at every voxel. This step is performed through a t-test in which the null hypothesis is that the connectivity is the same as a baseline value corresponding to the mean connectivity in the whole brain.

Results - The MRS data indicate that adolescent LP211 exposure produces long-term metabolic changes in hippocampus. Glutamate and total creatine concentration were found to be increased in the hippocampus of LP211-exposed rats (being Glu 10.9 and 9.8 mM and tCr 8.7 and 8.1 for treated and control animals, respectively) suggesting altered hippocampal function.
DTI analyses reveal a reduced FA in all the analysed ROIs. MD significantly decreased in PFC and HIP (Table 1).

Table 1- FA and MD mean values

	FA			MD		
	Control	**LP211**	**p***	**Control**	**LP211**	**p***
PFC	0.41±0.08	0.32±0.08	0.042	0.74±0.11	0.70±0.03	0.0481
HIP	0.38±0.09	0.27±0.11	0.033	0.88±0.05	0.81±0.05	0.0346
STR	0.34±0.06	0.29±0.05	0.029	0.74±0.04	0.71±0.05	0.055
NAcc	0.34±0.06	0.28±0.04	0.048	0.77±0.06	0.74±0.05	0.138

* Statistics: unpaired two-tailed t-test. Statistical significance for p<0.05.

The basic finding in a typical connectivity study is that in resting subjects, spontaneous, slow (<0.1 Hz) fluctuations of the blood oxygen level dependent (BOLD) signal are temporally coherent within widely distributed functional networks. Our seed-based analysis found differences in the functional maps of networks between the two groups of animals. Specifically, for all the seeds we analysed, an increase in the strength of connectivity has been detected as a long-term consequence of treatment. As an example, the differences (between treated and control rats), detected in the connectivity maps obtained positioning the seed in the right OFC, are shown in Fig.1. The right OFC is more functionally connected to ipsi / contra lateral thalamus and striatum. Therefore, connexions within fronto-striatal-thalamic circuits seem to have been potentiated by developmental exposure to LP211.

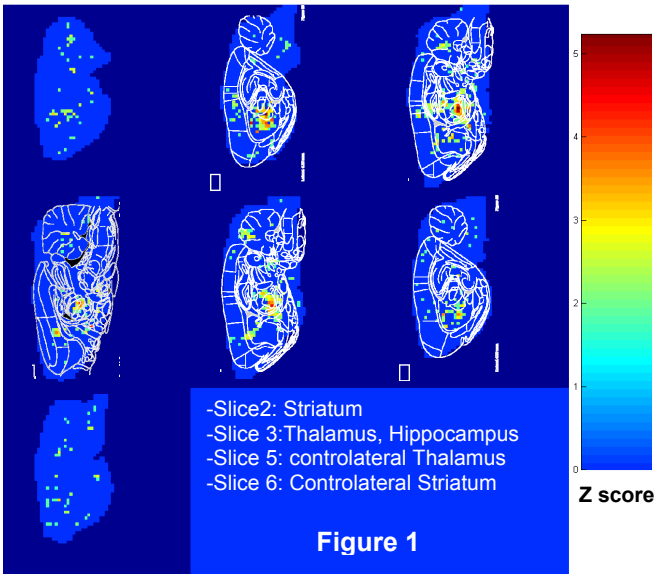

Figure 1

Discussion and Conclusion – Present data give a deeper view about the possible mechanisms of long-term actions, found for LP211 developmental exposure (5). Specifically, enduring behavioural alterations, such as decreased anxiety and potentiated spatial memory, was found in rats following adolescent LP211 exposure. These enduring MRS/MRI-detectable modifications may perhaps indicate rearrangement in forebrain networks which could account for profiles of observed behaviour.

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