Brain development during adolescence: metabolic, anatomical and functional characterisation in rats

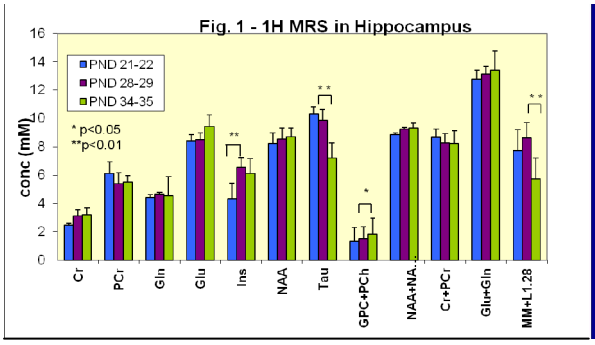
Luisa Altabella[1], Francesca Zoratto[1], Francesco De Pasquale[2], Giovanni Laviola[1], Walter Adriani[1], and Rossella Canese[1]

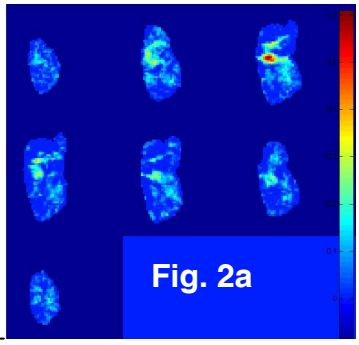
[1]*Cell Biology and Neuroscience, Istituto Superiore di Sanita', Rome, Rome, Italy,* [2]*ITAB Neuroscience and Imaging, University G. d'Annunzio, Chieti, Chieti, Italy*

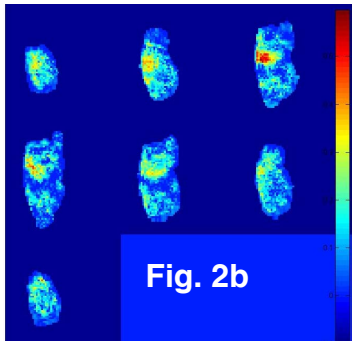
Introduction – It is well know that brain undergoes several morphological and functional changes during adolescence, in humans as well as in rodents. Magnetic resonance is the most suitable technique to study these changes in vivo because it can investigate brain metabolism (by ^{1}H MRS), anatomy (by DTI) and functionality (by functional connectivity) in a non-invasive way.

Methods - Wistar male rats at early (PND 21-24, n=12), middle (PND 28-31, n=12) and late adolescence (PND 35-38, n=12) were considered. 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 as receiver (RAPID Biomedical). Multislice spin echo axial images (TR/TE =2500/50 ms) were acquired to localise the regions of interest. ^{1}H localised MR spectra (PRESS sequence, TR/TE = 4000/23) were collected from the hippocampus (ROI = 18 μl) of 6 animals per group according to a quantitative protocol (1). Spectra were analysed by using LCModel fitting program (2).
Rat brain resting state fMRI exploiting the blood oxygen level dependent (BOLD) effect was studied (on 6 animals per group) by using a multi-slice sagittal gradient echo 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 three groups of animals were generated as described in (3). First, voxel-wise correlation maps were obtained corresponding to a given seed (nucleus accumbens (NAcc), the orbital frontal cortex (OFC) and hippocampus (HIP) in both hemispheres) for every session of every rat. In order to assess the statistical significant of the observed connections a bootstrapping technique has been used on the youngest group (control) to obtain 95% confidence interval. Connections folding outside such intervals have been labeled significant in the mean maps form the second and third week.
On the same animals, a DTI study was applied at middle and late adolescence using a spin-echo sequence with addition of the Stejskal-Tanner diffusion gradients. 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. Multi-slice DT images were acquired (15 slices of 1 mm thickness) in the coronal plan with 2 averages and TE/TR = 50/2000 ms. Using FSL software package (4) and ImageJ (5), diffusivity values (fractional anisotropy, FA and mean diffusivity, MD) were derived from the tensor. Three different regions were analyzed: HIP, OFC and NAcc. Significant differences of FA and MD values between the groups were assessed by a unpaired 2-tail t-test.

Results - Between early and middle adolescence, by quantitative MRS analysis we detected a significant increase of Ins (p=0.0004). Between middle and late adolescence, we detected an increase in total choline (p=0.03) while Tau and the lipid signal at 1.28 ppm decreased (both p=0.01). These results are summarized in a histogram (see Fig 1).
Our seed-based analysis in the connectivity study found differences in the functional maps of networks among the three different ages. Specifically, as consequence of the age-related development, an increase in the strength of connectivity was detected between early and middle or late adolescent for all the analysed seeds. As an example, for a seed positioned in the right Hip, we found an increase in the number of areas which are functionally connected and in the strength of such connection in late (Fig 2b) and in middle (Fig.2a) with respect to early adolescence.







Preliminary analysis of DTI data shows a significant decrease of FA in the brains of animals at late compared to middle adolescence in both NAcc (p=0.042) and OFC (p=0.016). Neither FA nor MD differences were detected for Hip.

Discussion and Conclusion – Present data provide a deeper view on the metabolic, morphological and functional modifications which occur during rodents' adolescence. Our MRS findings for the first two ages are in line with (6). Reduction of the 1.28 ppm signal, which can be considered as a neurogenesis marker (7), could suggest a progressive reduction of neurogenesis in Hip from early to late adolescence. The FA reduction suggest that important structural modification happen in the Nacc and OFC during adolescence. Finally, it is during adolescence that functional changes occur within forebrain networks like maturation of cortico-limbic loops.

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