Evidence of Neuronal Growth Spurts During Development in Healthy Children and Adolescents Using A Multi-voxel *In Vivo* ^{31}P Spectroscopy at 4 Tesla

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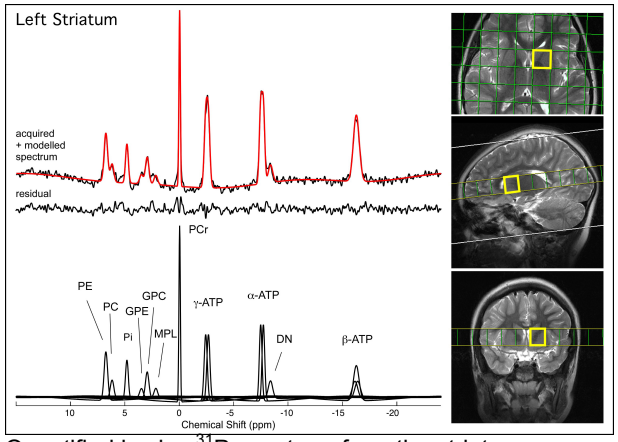
BACKGROUND: Because the prevalence of childhood onset psychiatric disorders is high, raises the importance of understanding healthy neurodevelopmental trajectories in the molecular biochemistry as the brain matures. Longitudinal MRI studies of healthy children have shown temporal trajectories of grey matter density/volume shaped similar to an inverted "U" (i.e., increasing values in children followed by a decrease in adolescents) reflecting the pruning of overproduced neuronal processes as the brain matures and becomes more efficient. In vivo ^{31}P spectroscopy also has been shown to be sensitive in detecting biochemical changes as the brain develops at 1.5 Tesla [1] by the measurement of precursor and breakdown product levels of membrane phospholipids (MPLs) [i.e., [phosphoethanolamine (PE) and phosphocholine (PC), and glycerophospho-ethanolamine (GPE) and glycerophosphocholine (GPC)]. The structural wall separating the various cellular entities such as the branching of dendrites and synaptic connections is composed of MPLs in a bilayer conformation. Early in postnatal brain development of animals, PE levels are high, and GPC and GPE levels are low reflecting the high demand of MPL synthesis for the development of cell membrane structures required in dendritic and synaptic connections. As the brain develops, PE levels decrease, and GPC and GPE levels increase with age. In the context of rapid proliferating tissue, elevated levels of MPL precursor levels, specifically PC, have been observed at the time and site of neuritic sprouting in the hippocampus following unilateral lesions of the entorhinal cortex in rats. Therefore, the purpose of this study is to investigate changes in MPL metabolites of healthy children and adolescents to discern developmental growth spurts in cortical and subcortical structures using *in vivo* ^{31}P spectroscopy.

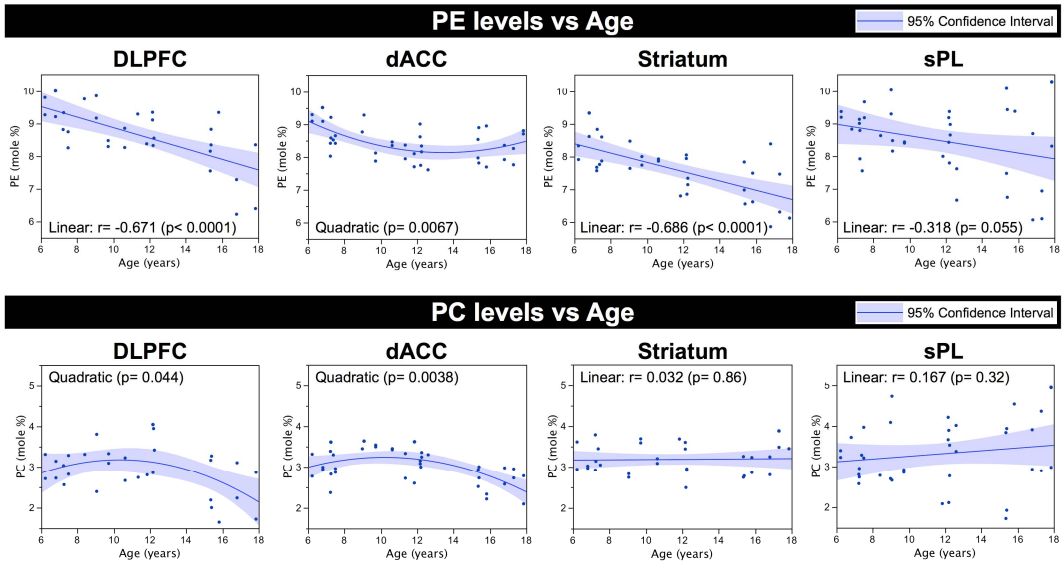


Quantified in vivo ^{31}P spectrum from the striatum.

SUBJECTS AND METHODS: A total of 23 healthy children and adolescents without any DSM-IV Axis I psychopathology (9M+14F; mean age 11.3±3.7 yrs; 6.2 to 17.9 years in age range) participated in this study. No sedation was used on any subjects during the MR examination.

A 3D whole-brain, multi-voxel ^{31}P spectroscopy measurement was collected in each subject on a 4-Tesla Bruker MedSpec scanner using a dual-tuned ^{31}P-^{1}H head coil (Bruker BioSpin MRI Inc.). An 80-cm thick axial slab was placed parallel to the AC-PC line to cover the whole-brain. The acquisition parameters of the ^{31}P spectroscopy included: FOV= 280x280x160mm, phase encoding steps= 14x14x8, zero-filled to 16x16x8 (nominal voxel dimension= 1.75x1.75x2.0cm^3 and estimated effective voxel size= 14cm^3), TR= 0.54sec, flip-angle= 33^0 reflecting the Ernst angle where the average T_1 value of phosphocreatine (PCr), PE, PC was 3sec, complex data points= 2,048, spectral bandwidth= 4.0kHz, 24 averages (weighted-average and elliptical k-space sampling, pre-acquisition delay time of 1.3ms.

For each bilateral region of interest (DLPFC [BA 9/46], dorsal anterior cingulate [dACC], striatum and superior parietal lobe [sPL]), the 16x16x8 grid was shifted in all three directions relative to the MRI images accordingly to ensure optimal voxel placements using 3DiCSI (Hatch MR Research Center, Columbia University). The MR signals of those voxels were then extracted, apodized (5Hz Gaussian), and modeled in the time domain with 15 Gaussian-damped sinusoids [i.e., PE, PC, Pi, GPE, GPC, *broad*-PDE, PCr, dinucleotides (DN) and ATP (two doublets and a triplet)], as shown on the right. Though cross-sectional, metabolite levels were modeled using either a linear or quadratic function to determine best fit based on the residual of the fitting.



RESULTS: In healthy development, PE levels significantly decreased with age linearly in prefrontal and striatal areas [DLPFC: r= -0.671 (p< 0.0001); dACC: r= -0.392 (p= 0.0091); striatum: r= -0.686 (p< 0.0001)]; the PE-age association in the sPL failed to reach significance [r=-0.318 (p=0.055)]. The quadratic term also was significant for PE in the dACC (p=0.0067). Regarding the trajectory of PC levels with age, the behavior was quadratic in prefrontal cortices [DLPFC: peaked at ~ 10.5 yrs (p= 0.044); dACC: peak at ~ 10 yrs (p= 0.0038)] but not in the striatum or sPL. GPE levels significantly increased with age linearly in the DLPFC (r= 0.393; p= 0.0044) and dACC (r= 0.365; p= 0.0049).

CONCLUSIONS: The decreasing PE levels with age appear to reflect a possible reduction in the demand MPL synthesis of neuronal and synaptic processes. In contrast, the inverted "U" behavior in PC levels with age in prefrontal cortices appears to reflect growth spurts in these later developing brain areas. During a growth spurt, which involves the branching out of dendrites and the formation of new synapses in the neuropil, one would expect increased MPL precursor levels of PC reflecting this increased proliferation of cortical tissue. The cross sectional nature of this study warrants appropriate caution in interpreting the results. Nevertheless, they provide an exciting possibility in investigating the molecular biochemistry of temporal trajectories in healthy development and psychiatric disorders in appropriately designed longitudinal studies.

[1]Stanley JA, et al. *Arch. Gen. Psychiatry* 2008; 65: 1419-28.