

Evidence of Age Effects in Cortical Areas But Not in the Subcortex of ADHD Children: A Multi-voxel *In Vivo* ³¹P Spectroscopy Study at 4 Tesla

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BACKGROUND: Attention-deficit/hyperactivity disorder (ADHD) is one of the most prevalent neurodevelopmental disorders in children, which is not well understood. *In vivo* ³¹P spectroscopy is a neuroimaging method that is sensitive in detecting biochemical changes as the brain develops. Using ³¹P spectroscopy, we have shown subcortical and cortical deficits in the metabolism of membrane phospholipids (MPL) that are suggestive of an alteration of an earlier developing region influencing the maturational integration of later or slower developing regions^{1,2}. The purpose of this study is to further our investigation, using a high-field system, which dramatically improves regional specificity and biochemical resolution [e.g., individual phosphomonoesters, phosphoethanolamine (PE) and phosphocholine (PC)].

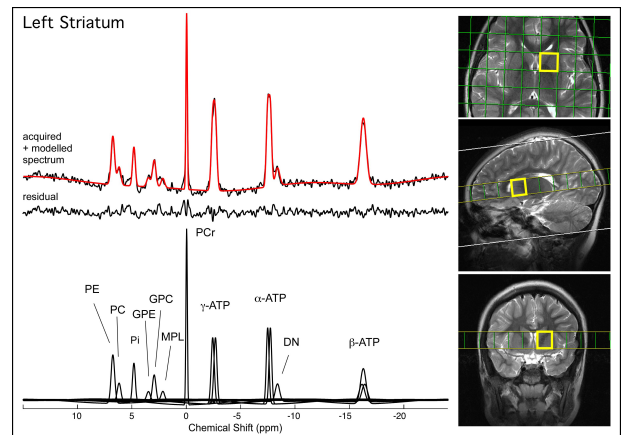
SUBJECTS AND METHODS: A total of 20 children with DSM-IV ADHD (13M+7F; mean age 9.1±1.4 yrs; 14 with the combined subtype and 6 with the predominantly inattentive type) and 16 HC (4M+12F; mean age 9.3±2.2 yrs) participated in this study. All ADHD children were not on any psychostimulant medication for at least a 24-hour period prior to the MR examination and no sedation was used on any subjects during the MR examination.

A 3D whole-brain, multi-voxel ³¹P spectroscopy measurement was collected in each subject on a 4-Tesla Bruker MedSpec scanner using a dual-tuned ³¹P-¹H 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 ³¹P spectroscopy included: FOV= 280x280x160mm, phase encoding steps= 14x14x8, zero-filled to 16x16x8 (nominal voxel dimension= 1.75x1.75x2.0cm³ and estimated effective voxel size= 14cm³), TR= 0.54sec, flip-angle= 33° reflecting the Ernst angle where the average T₁ 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 and acquisition time 23 minutes).

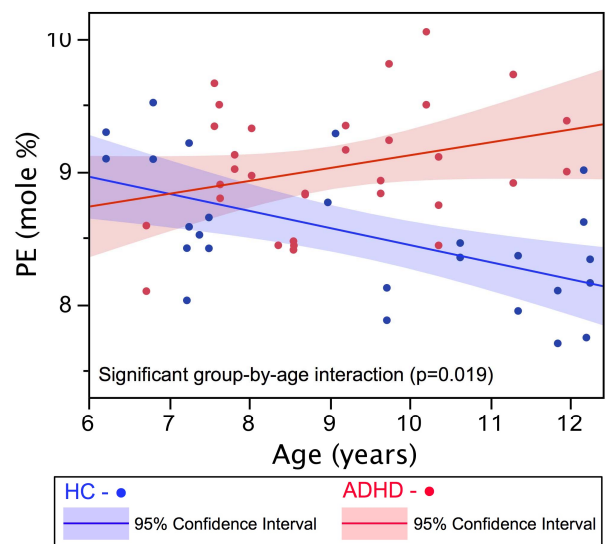
For each bilateral region of interest (DLPFC [BA 9/46], inferior frontal gyrus [iFG; BA 44/45], dorsal anterior cingulate [dACC], striatum, and superior parietal lobe [sPL]), the 16x16x8 grid was shifted in all three directions relative to the MRI images accordingly to ensure optimal voxel placements 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 in figure on the right. A generalized linear regression model (PROC GENMOD; SAS Institute Inc.) with subject group, gender and side (right vs. left side) as the main effects, was used as well as with additional terms for group-by-age interactions.

RESULTS: In the striatum of ADHD children compared with HC, PE levels were significantly lower and GPC levels tended to be higher (p=0.040 and p=0.069, respectively). Regarding prefrontal cortices, PE levels were significantly higher and GPC tended to be higher in the dACC of ADHD children compared with HC (p=0.0056 and p=0.068, respectively). In terms of age effects, there was a significant group-by-age interaction for PE in the dACC (p=0.019; figure bottom right), PC in the iFG (p=0.026) and PE in the sPL (p=0.0056), all with converging values in the younger subjects and diverging values with increasing age.

CONCLUSIONS: In healthy development, PE levels decrease with age reflecting a possible reduction in the demand MPL synthesis of neuronal and synaptic processes. In contrast, the other MPL precursor PC behaves differently by showing increasing levels followed by decreasing levels with age (i.e., similar to the inverted "U") at least in prefrontal cortices, which appear to reflect growth spurts in these later developing brain areas. Therefore, the lower PE in subcortical areas may reflect reduced density of dendrites and synaptic connections, which is consistent with an underdeveloped subcortical area in young ADHD children. In the dACC, the non-significant difference of PE levels in the younger subjects followed by a lack of progressive decreasing PE levels in ADHD with age compared with HC suggests a deviation in the developmental trajectory. Lastly, the increasing PC levels in the iFG of HC children are consistent with that of prefrontal areas experiencing a developmental growth spurt, which is underachieved in the ADHD children. Ultimately, longitudinal measurements will be required to definitively address whether developmental trajectories in the brain biochemistry are altered in pediatric ADHD.



Quantified *in vivo* ³¹P spectrum from the striatum.



Scatter plot of dACC PE levels vs Age (years).

¹Stanley JA, et al. *Psychiatry Res.* 2006; 148:217-221.

²Stanley JA, et al. *Arch. Gen. Psychiatry* 2008; 65 (12): 1419-1428.