

Subcortical Glutamate Mediates the Reduction of Short-Range Functional Connectivity with Age in a Developmental Cohort

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Marked changes in brain physiology and structure take place between childhood and adulthood, including changes in functional connectivity and changes in the balance between main excitatory and inhibitory neurotransmitters glutamate (Glu) and GABA. The balance of these neurotransmitters is thought to underlie neural activity in general and functional connectivity networks in particular, but so far no studies have investigated the relationship between human development related differences in these neurotransmitters and concomitant changes in functional connectivity. GABA +/H₂O and Glu/H₂O levels were acquired in a group of healthy children, adolescents, and adults in a subcortical (basal ganglia) region, as well as in a frontal region in adolescents and adults. Our results showed higher GABA +/Glu with age in both the subcortical and the frontal voxel, which were differentially associated with significantly lower Glu/H₂O with age in the subcortical voxel and by significantly higher GABA +/H₂O with age in the frontal voxel. Using a seed-to-voxel analysis, we were further able to show that functional connectivity between the putamen (seed) and other subcortical structures was lower with age. Lower subcortical Glu/H₂O with age mediated the lower connectivity in the dorsal putamen. Based on these results, and the potential role of Glu in synaptic pruning, we suggest that lower Glu mediates a reduction of local connectivity during human development.

Key words: development; functional connectivity; GABA; glutamate; magnetic resonance spectroscopy; working memory

Introduction

The transition from childhood to adulthood is accompanied by changes in regional gray matter volume, increases in white matter volume (Giedd and Rapoport, 2010), as well as increases in long-range corticocortical networks, and decreases in short-range cortical and subcortical networks (Fair et al., 2007a, 2009; Supekar et al., 2009). Functional network changes in development are likely to be attributed to putative changes in neurotransmitter levels, such as the major inhibitory and excitatory neurotransmitters GABA and glutamate (Glu), respectively. It has been suggested that adjustments of the excitation–inhibition balance on both individual neurons and within networks are accompanied by

synaptic elimination during adolescence (Selemon, 2013). The main argument in support of this hypothesis originates from the specificity of loss, i.e., the excitatory synapses are selectively degenerated, whereas inhibitory synapses are spared in primates (Bourgeois and Rakic, 1993). However, the interaction between developmental changes in GABA and Glu, and functional network changes in humans is currently unresolved. GABAergic interneurons not only regulate nearly all key developmental steps in the cortex but also the experience-dependent refinement of local circuits (Di Cristo, 2007), and they play a central role in shaping developing hippocampal networks (Bonifazi et al., 2009). Glu has been linked to synchronizing neuronal networks (Rodriguez et al., 2013). Glu has been reported to be mostly positively associated with functional connectivity (Schmaal et al., 2012; Duncan et al., 2013; Kapogiannis et al., 2013), whereas GABA has been negatively associated with functional connectivity (Kapogiannis et al., 2013; Stagg et al., 2014).

To our knowledge, only one study investigated GABA and Glu in healthy adolescents (12–14 years) in addition to young adults, and reported an increase of GABA and a nonsignificant decrease of Glu with age (Silveri et al., 2013). There are no studies investigating GABA changes in the first years after birth, although a few reports of Glu changes including children and adolescents

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showed steep increases, reaching a plateau in childhood (Blüml et al., 2013; Degnan et al., 2014).

The aim of this study, therefore, was to answer two questions. First, how do GABA and Glu differ between late childhood to middle adulthood in subcortical and cortical regions? Second, do developmental differences in GABA/Glu contribute to age related differences in neural networks? For the first aim, we acquired GABA+/ H_2O and Glu/ H_2O levels in healthy children, adolescents, and adults in a subcortical (basal ganglia) region, as well as in a cortical (frontal) region in adolescents and adults. We focused on these two regions because they follow different developmental trajectories, e.g., in gray matter (Østby et al., 2009) and in microstructural maturation (Lebel et al., 2008). For the second aim, we examined the contributions of the subcortical neurotransmitter levels to the developmental differences of neural networks, assessed by functional connectivity. We expected to find lower Glu/ H_2O and higher GABA+/ H_2O associated with age. We hypothesized that this relative higher GABA+/ H_2O with age would be related to differences in local (i.e., short-range) and distal (i.e., long-range) connectivity.

Materials and Methods

Subjects. To investigate developmental differences in the major inhibitory and excitatory neurotransmitter levels, we acquired magnetic resonance spectroscopy (MRS) measures of GABA+/ H_2O and Glu/ H_2O in healthy children, adolescents, and adults during rest in a subcortical (basal ganglia) and in a frontal voxel. MRS values from the subcortical voxel were obtained from a total of 80 subjects. Of these, 40 were adults (21 females) with a mean age of 32.0 years (SD: 9.6; range: 21.0–53.3 years) and 40 were children and adolescents (19 females) with a mean age of 13.0 years (SD: 3.0, range 8.1–18.0 years). The MRS measurement in the frontal voxel was not conducted in one adult subject and only in subjects older than 13 years, resulting in a total of 56 subjects. Of these, 39 were adults (21 females) with a mean age of 32.0 years (SD: 9.7; range: 21–53.3 years) and 17 adolescents (9 females) with a mean age of 16 years (SD: 1.4; range: 13.6–18.0 years).

After MRS measurement participants performed a spatial working memory task. Task functional magnetic resonance imaging (fMRI) data were obtained from all children and adolescents and from a subset of adults, resulting in a total of 65 subjects. Of these, 25 were adults (15 females) with a mean age of 32.8 years (SD: 9.7; range: 21.0–50.6 years). The numbers for children and adolescents remained as stated above for the MRS values. Due to the smaller numbers of subjects for the task fMRI data and MRS measurements in the frontal voxel, we restricted our analysis on the relationship between age related differences in functional connectivity and MRS measurements to the subcortical voxel. No current neurological or psychiatric illness of the subjects was reported by them or their parents, respectively. All subjects gave written informed consent before the experiment. The study was approved by the Ethics Committee of the Canton of Zurich, Switzerland.

MRI data acquisition. MRI data were acquired with a 3.0 T GE HD.xt whole-body MRI scanner (GE Healthcare), using an 8-channel receive-only head coil and a body transmit coil. The MRI protocol included a 3D T1-weighted gradient echo pulse sequence [number of slices = 172, slice thickness (ST) = 1.0 mm, repetition time (TR) = 9.94 ms, echo time (TE) = 2.948 ms, inversion time = 600 ms, field-of-view (FOV) = 256 mm × 192 mm, flip angle = 8°, matrix = 256 × 192, reconstructed voxel resolution: 1 × 1 × 1 mm] used for positioning of the MRS voxels. GABA-edited MR spectra were acquired using the MEGA-PRESS method (Edden and Barker, 2007) with TE = 68 ms, TR = 2000 ms, 320 averages (160 pairs), and an eight-step phase cycle. We acquired spectra from a 28 × 40 × 25 mm³ voxel in a left subcortical region centered on the axial slice where the putamen was the widest, with the anterior border of the voxel aligned with the anterior margin of the putamen (Fig. 1, top row, insets). As estimated in native space, this approach shows the putamen comprising between 15 and 29% of the corresponding MRS voxel (mean 21.7%, SD = 2.9). The frontal voxel was 25 × 40 × 30 mm³ in size

and was positioned as described by Michels et al. (2012). The voxel was centered on the axial slice 1 mm above the superior margin of the left lateral ventricle. The length of the midline was measured on this slice and the center of the voxel along the anterior–posterior direction was placed at a point 1/3 of the distance down the midline from the anterior margin of the brain and along the left–right direction at a point 1/2 of the distance between the midline and the left lateral border of the brain, perpendicular to the midline (Fig. 1, bottom row, insets). During MRS measurements, subjects were instructed to relax but stay awake. Subsequently, fMRI images were acquired interleaved in 35 axial slices covering the whole brain with a multislice echoplanar imaging sequence (ST = 3 mm; 0.3 mm skip; 35 slices; TR = 1.925 s; TE = 32 ms; flip angle = 74°; matrix = 64 × 64; FOV = 240 mm).

fMRI task. We investigated functional connectivity related to spontaneous background activity (Fox et al., 2006; Fox and Raichle, 2007; Norman-Haignere et al., 2012) during a spatial working memory task. The task consisted of a display of 11 circles positioned on a circular grid. Either two or four of the stimulus circles were filled to indicate the positions to be memorized during three different retention intervals (2, 4, and 6 s, varying from trial to trial). In the subsequent probe phase of each trial, a question mark was shown in one of the 11 circles and subjects indicated by a button press (LuminaBox) whether or not that circle had been filled. The task lasted 14 min and contained 72 trials in total, with one-half of the trials showing two positions to memorize, and one-half with four (at most 2 trials with the same number in succession). Null trials were included (fixation cross for 2, 4, and 6 s) after four trials and motivation trials after 12 trials, displaying the number of correctly answered trials and a smile. The task was implemented in Presentation v16 (Neurobehavioral Systems) and synchronized to the fMRI acquisition. During the MRI acquisition, the task was projected onto a screen at the foot of the MR table, which subjects viewed via a mirror.

MRS analysis. T1-weighted images were segmented in native space using SPM8 with the “New Segment” module (Wellcome Department of Cognitive Neurology, London, UK) running in MATLAB 2012b (MathWorks) to correct the MRS results for partial volume CSF contamination and different water compartment relaxation times (Gasparovic et al., 2006). Metabolite relaxation corrections were performed using published values for the T1 and T2 relaxation times (Träber et al., 2004; Zaaraoui et al., 2007), according to the method detailed in the LCMModel manual (LCModel v6.3-1K). The spectra were coil combined with weighting factors derived from the first point of the free induction decay signal from the unsuppressed water lines acquired with each coil. Water-scaled metabolite concentrations were derived with LCMModel v6.3-1B (Provencher, 1993). Spectra were frequency aligned to account for frequency drift due to motion or other factors and separated into the edited (subtraction) spectra and the edit OFF subspectra. The edited spectra were analyzed with a simulated basis set including basis spectra for GABA, glutathione (GSH), *N*-acetylaspartate (NAA), glutamine (Gln), Glu, and *N*-acetylaspartylglutamate (NAAG; Fig. 1). The edit OFF subspectra were analyzed with a simulated basis set including basis spectra for NAA, NAAG, creatine (Cr), choline (Cho), Glu, Gln, GSH, GABA, lactate, and myo-inositol. GABA findings described subsequently are reported as GABA+ due to the contribution of a macromolecular component to the edited peak at 3 ppm in the assessment of GABA with MEGA-PRESS. GABA+/ H_2O and Glu/ H_2O were quantified from the edited spectra, using the control parameter *sptype* = ‘mega-press-2’. The Cramer Rao lower bound (CRLB) of the spectral fit for all GABA+/ H_2O and Glu/ H_2O values were <20% for all participants (Table 1). However, due to the spectral dispersion at 3T the derived Glu/ H_2O concentrations may also include small contributions from Gln/ H_2O . The linewidth reported by LCMModel ranged from 0.038 to 0.129 ppm (4.85–16.5 Hz) in the subcortical voxel, and from 0.029 to 0.067 ppm (3.7–8.6 Hz) in the frontal voxel for all participants (Table 1). Because particularly younger participants might move during MRS measurements, we estimated an individual motion parameter from the fMRI data. This “fingerprint” was estimated by calculating the mean and SD of distances from scan to scan across all scans in each individual and separately for translation and rotation realignment parameters resulting in a mean and SD distance for translation parameters, and mean and SD distance for rotation parameters.

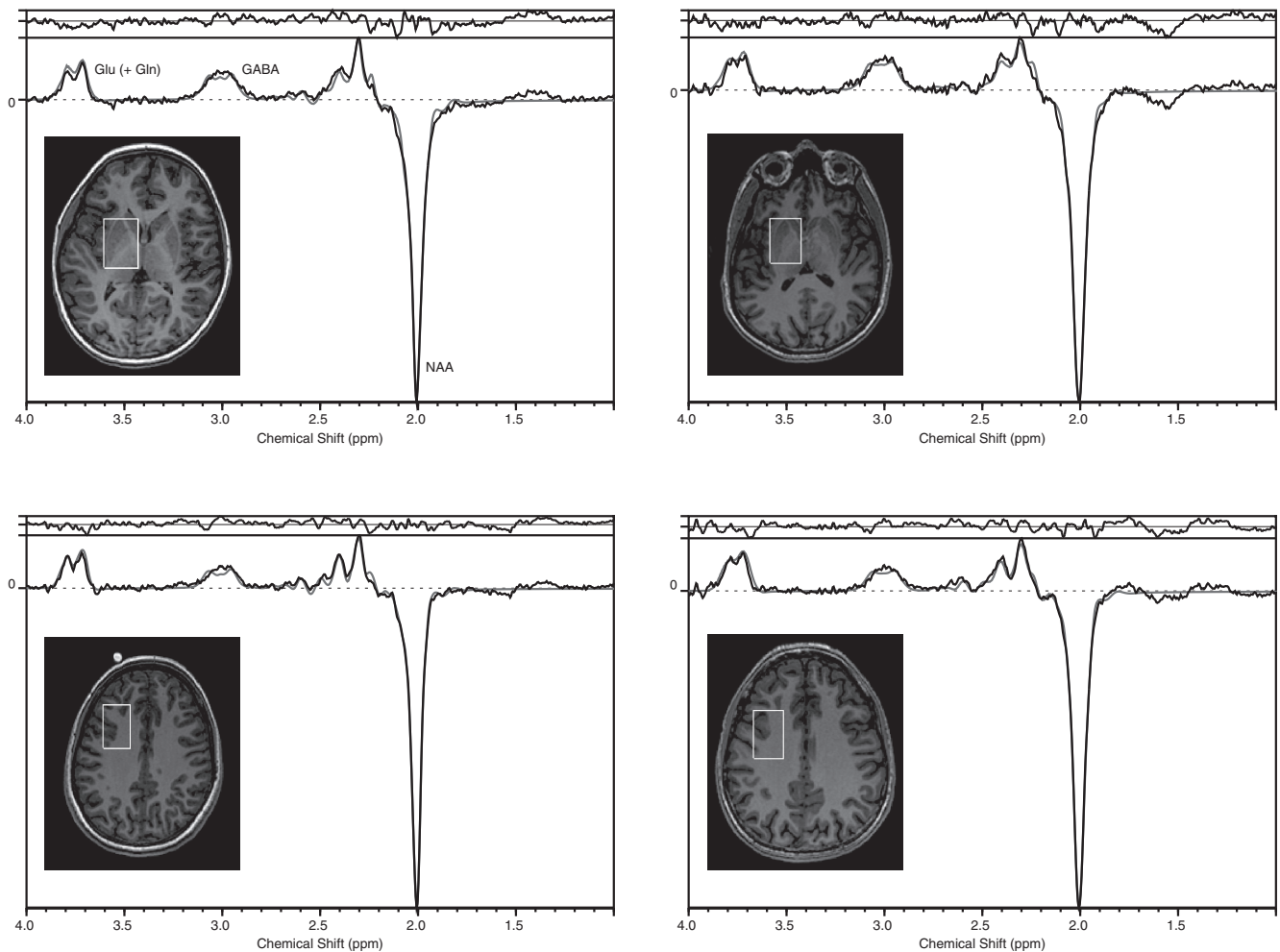


Figure 1. Examples of voxel positions and edited spectral fits in the left subcortical region (top row) of a child (9 years, left) and an adult subject (41 years, right), and in the left frontal region (bottom row) of an adolescent (15 years, left) and an adult subject (41 years, right). Spectra are shown in black, with the spectral fits overlaid in gray. The residuals are shown above each spectrum. Glu (+ Gln), glutamate with contributions from glutamine.

ters. Next, the SD/mean distance was averaged between translation and rotation parameters. These fingerprints were used to statistically control the associations between metabolites and age for movement.

Statistical analyses. Correlations (Spearman's r_s) between age and MRS values (Glu/ H_2O , GABA+/ H_2O , GABA+/ Glu) are reported. All statistical analyses (not including the functional connectivity analyses) were performed in IBM SPSS Statistics v20, and the plots were created using R (v3.1.0; ggplot from the ggplot2 package). We report data before and after Bonferroni correction ($p < 0.0125$).

Functional connectivity analyses. Functional connectivity was assessed with the conn toolbox v14.c (Whitfield-Gabrieli and Nieto-Castanon, 2012) using standard fMRI preprocessing. Spatial preprocessing of the task fMRI data included slice-timing correction, motion correction, coregistration of functional volumes to the anatomical volumes, and smoothing with a Gaussian filter of 8 mm. The voxel size for analyses was 2 mm. T1-weighted anatomical volumes were segmented into gray matter, white matter, and CSF and the resulting transformation parameters were used to normalize the anatomical and functional images. These preprocessed images were further bandpass filtered (0.01–0.1 Hz), and non-neuronal contributions from white matter and CSF were regressed out (5 principle components) at the subject level using the aCompCor strategy (Behzadi et al., 2007) implemented in the conn toolbox. Additionally, each subject's realignment parameters (3 translation, 3 rotation parameters, and their first temporal derivatives) were included as confounding factors. Finally, event-related task effects were also regressed out with five regressors (and their first temporal derivatives) consisting of two regressors modeling the retention phase with different durations

of correctly answered trials (starting from stimulus onset and lasting until probe onset) for the two- and the four-circle trials, respectively, two regressors modeling the retrieval phase of correctly answered trials (starting from probe onset and lasting until button press) for the two- and the four-circle trials, respectively, and one regressor modeling all button presses (duration of 0 s). This approach of analyzing functional connectivity from residuals of the entire time courses of event-related task fMRI data was described by Fair et al. (2007b) and applied by several studies (Harris et al., 2011; Zhang et al., 2014). These spontaneous correlations are meaningful and may help characterize the systems level interactions subserving cognitive processes (Norman-Haignere et al., 2012). Because the MRS subcortical voxel was centrally placed on the left putamen, this region was chosen as the seed region for the connectivity analyses. The region of interest was taken from the Harvard–Oxford Subcortical Structural Atlas available in FSL (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). The average blood oxygen level-dependent (BOLD) time series was computed across all the voxels within the left putamen and used for the seed-to-voxel (whole brain) bivariate correlation analyses. For second level analyses, i.e., regression models with age and the MRS values as between-subject effects, a Fisher transformation (inverse hyperbolic tangent function) was applied to the correlation measures to improve the normality assumptions of standard second-level general linear models (Whitfield-Gabrieli and Nieto-Castanon, 2012). Separate regression models were run in the conn toolbox across all available subjects to evaluate the association between seed-to-voxel connectivity (left putamen as seed) and the variables age and the different MRS values (Glu/ H_2O , GABA+/ H_2O , GABA+/ Glu from the subcortical voxel) as

Table 1. Metabolite concentrations, CRLB, linewidths, and voxel tissue composition

	Subcortical voxel		Frontal voxel	
	<25 years	>25 years	<25 years	>25 years
<i>n</i> (<i>n</i> females)	51 (25)	29 (15)	28 (15)	28 (15)
Age	15.1 (4.8)	35.6 (8.9)	18.5 (3.5)	35.6 (9.1)
MEGA-PRESS				
GABA + /H ₂ O (mm)	1.9 (0.2)	1.9 (0.2)	1.4 (0.1)	1.5 (0.2)
GABA + /H ₂ O CRLB (%)	3.5 (0.6)	3.9 (1.0)	4.6 (0.9)	4.4 (0.9)
Glu/H ₂ O (mm)	4.2 (0.5)	3.7 (0.5)	3.7 (0.4)	3.7 (0.3)
Glu/H ₂ O CRLB (%)	4.1 (1.0)	5.3 (1.9)	3.8 (1.2)	4.0 (0.9)
Gln/H ₂ O (mm)*	1.3 (0.5)	1.7 (0.7)	1.0 (0.3)	1.1 (0.3)
Gln/H ₂ O CRLB (%)*	15.3 (2.8)	16.2 (2.1)	13.9 (3.1)	14.4 (2.7)
NAA/H ₂ O (mm)	8.9 (0.5)	9.1 (0.5)	8.2 (0.4)	8.4 (0.4)
NAA/H ₂ O CRLB (%)	1.0 (0.3)	1.0 (0.2)	1.1 (0.3)	1.1 (0.3)
PRESS				
Cr/H ₂ O (mm)	5.3 (0.9)	5.2 (0.6)	5.0 (0.5)	5.4 (0.3)
Cr/H ₂ O CRLB (%)	1.9 (0.2)	2.0 (0.3)	2.0 (0.2)	1.9 (0.3)
Cho/H ₂ O (mm)	0.6 (0.1)	0.6 (0.1)	0.7 (0.1)	0.7 (0.1)
Cho/H ₂ O CRLB (%)	2.2 (0.4)	2.3 (0.5)	2.1 (0.3)	2.1 (0.3)
Linewidth (Hz)	8.0 (2.2)	9.4 (1.9)	5.9 (1.0)	6.7 (1.1)
Grey matter (%)	54.0 (4.9)	50.8 (3.0)	35.8 (3.4)	33.2 (3.1)
White matter (%)	42.6 (3.8)	44.8 (2.6)	61.2 (4.3)	62.6 (4.0)
Cerebrospinal fluid (%)	3.3 (3.0)	4.3 (1.4)	2.7 (1.5)	4.0 (2.3)
Grey/white matter ratio	1.3 (0.2)	1.1 (0.1)	0.6 (0.1)	0.5 (0.1)

Values are mean (SD).

*Only values considered with CRLB of Gln <20% (subcortical voxel: *n* = 30 for <25 years and *n* = 14 for >25 years; frontal voxel: *n* = 25 for <25 years and *n* = 21 for >25 years).

between-subject effects. Using the same event-related task effects in a separate SPM8 analysis, we verified whether the putamen was active during the task and found strong activity during probe recognition and button press, but not during the retention phase (data not shown). Reported functional connectivity results survived a height threshold of $p < 0.001$ (uncorrected) and an extent threshold of familywise error-corrected $p < 0.05$ at the cluster level.

As mentioned in the subject section, group sizes for different analyses varied due to differences in available data per subject. A summary of the numbers for the respective analyses is provided here: correlation between age and MRS values from the subcortical voxel: age range = 8.1–53.3 years; *n* = 80, 40 females; correlation between age and MRS values from the frontal voxel: age range = 13.6–53.3 years; *n* = 56, 30 females; regressions of functional connectivity by age and functional connectivity by MRS values from the subcortical voxel: age range = 8.1–50.6 years; *n* = 65, 34 females.

Results

Correlations MRS values with age

In the subcortical voxel (Fig. 2, top row), a negative correlation between Glu/H₂O and age was observed ($r_s = -0.61$, $p < 0.001$, left), whereas the correlation between GABA + /H₂O and age was not significant ($r_s = -0.07$, $p = 0.55$, middle). GABA + /Glu showed a positive correlation with age ($r_s = 0.58$, $p < 0.001$, right). The negative correlation between Glu/H₂O and age remained significant after correction for sex ($r = -0.54$, $p < 0.001$). The correlation also remained significant if we excluded subjects that had 2 SD more or less than average Glu/H₂O (3 outliers: $r_s = -0.58$, $p < 0.001$). Because the Glu and Gln multiplets partially overlap at 3T, we reran the Glu/H₂O correlations with age only in cases where Gln/H₂O was fit with a CRLB < 20%, and the correlation remained significant in the subcortical voxel ($r_s = -0.64$, $p < 0.001$). We additionally repeated and confirmed the correlation between age and Glu/H₂O from this region for participants with linewidth values <12 Hz (*n* = 73, $r_s = -0.61$, $p < 0.001$). Because our sample included subjects younger and older than 25 years of age, one might consider that age related

differences might not be related to development. We therefore reran the analyses in the group of younger subjects (<25 years) and older subjects (>25 years; Table 1 shows the number of subjects). These analyses replicated that Glu/H₂O significantly correlated with age in the younger group ($r_s = -0.58$, $p < 0.001$) and showed a trend to significance in the age group >25 years ($r_s = -0.36$, $p = 0.054$). For GABA + /H₂O we found a weak negative correlation with age < 25 years that was not significant after Bonferroni correction ($r_s = -0.28$, $p = 0.045$) and no significant correlation > 25 years ($r_s = 0.09$, $p = 0.629$). These results thus support the notion that the association between Glu/H₂O and age was prominent during development. Indeed when comparing regression models we found that logarithmic model slightly better explained the association between age and Glu/H₂O than the linear model ($R^2 = 0.34$ vs 0.29, both $p < 0.001$). Finally, because particularly younger subjects might move during MRS measurements, we reanalyzed the MRS data after controlling for motion (as described in the Materials and Methods). These analyses led to similar results, such that the correlation between Glu/H₂O and age was significant ($r = -0.47$, $p < 0.001$).

In the frontal voxel (Fig. 2, bottom row), the correlation between Glu/H₂O and age was not significant ($r_s = -0.18$, $p = 0.18$, left), whereas a positive correlation between GABA + /H₂O and age was observed that was not significant after Bonferroni correction ($r_s = 0.30$, $p = 0.02$, middle). GABA + /Glu also showed a positive correlation with age ($r_s = 0.42$, $p = 0.001$, right). The correlation between GABA + /H₂O and age remained significant after controlling for sex ($r = 0.32$, $p = 0.019$) and the correlation showed a trend to significance after excluding subjects who had 2 SD more or less than average GABA + /H₂O (3 outlier: $r_s = 0.25$, $p = 0.068$). When analyzing younger and older groups separately, we found no significant correlation between GABA + /H₂O and age and between Glu/H₂O and age. Finally, we reanalyzed the MRS data after controlling for motion, which led to similar results, such that the correlation between GABA + /H₂O and age was significant ($r = 0.37$, $p = 0.018$). Together these results indicate that the correlation between GABA + /H₂O and age in the frontal voxel was relatively weak and would not survive correction for multiple comparisons. By contrast, the correlation between Glu/H₂O and age in the subcortical voxel was highly robust and would survive multiple comparisons.

Correlations functional connectivity with age and MRS values

Figure 3 and Table 2 illustrate the results of the correlation analyses of functional connectivity with age and MRS values.

Age

The functional connectivity showed a negative correlation with age in a cluster encompassing the left putamen and neighboring regions, i.e., left pallidum, thalamus, amygdala, hippocampus, and parahippocampal gyrus (Fig. 3, first row).

MRS values from the subcortical voxel

A negative correlation between Glu/H₂O and functional connectivity was observed in a cluster encompassing the left ventral putamen and orbitofrontal cortex (Fig. 3, second row, blue). This cluster did not overlap with the above-mentioned cluster correlating negatively with age. A positive correlation between Glu/H₂O and functional connectivity was observed in a cluster encompassing the left dorsal putamen and adjacent external capsule (Fig. 3, second row, red). This cluster largely overlapped with the above-mentioned cluster correlating negatively with age (Fig. 3, third row, yellow). Several other regions that correlated with

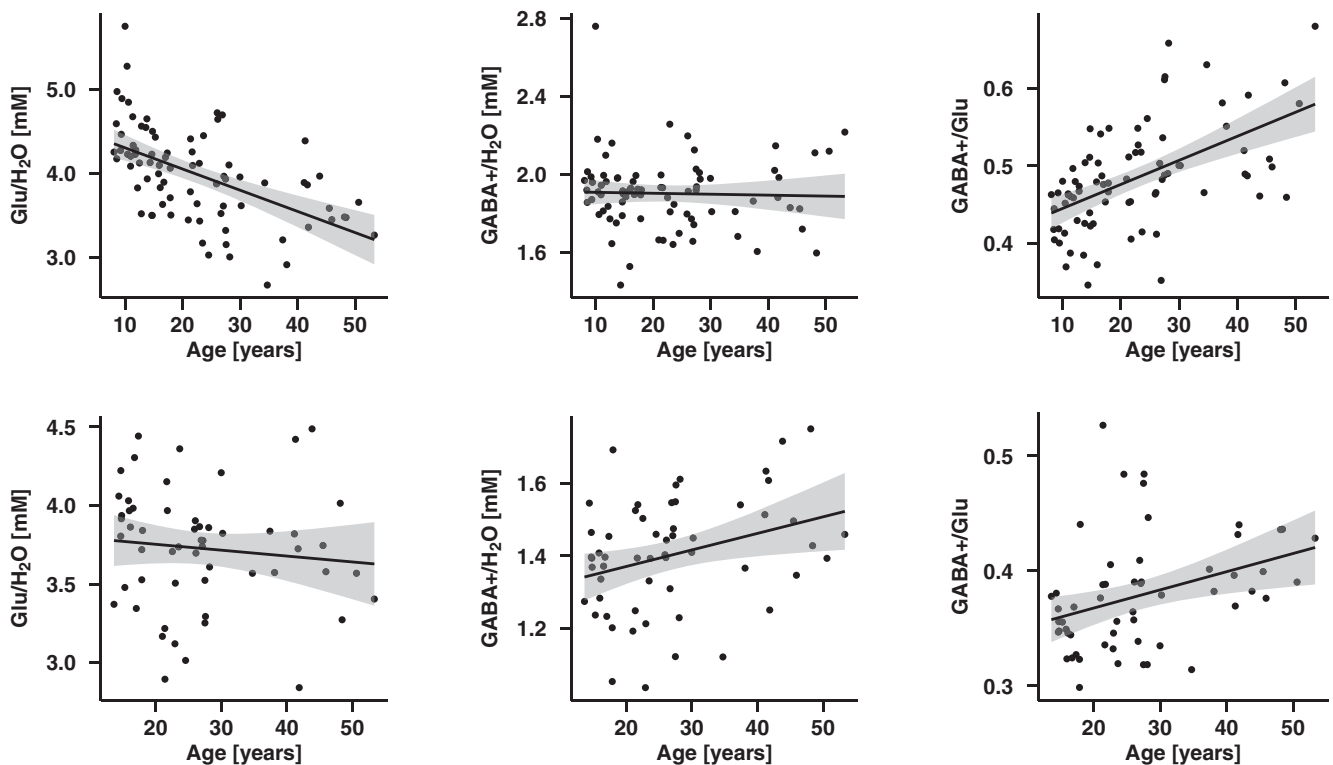


Figure 2. Spectroscopic measures in the subcortical voxel (top row) and the frontal voxel (bottom row) are differentially correlated with age. Glu/H₂O (left column) showed a significant decrease with age in the subcortical region ($r_s = -0.61, p < 0.001$; not significant in the frontal region, $r_s = -0.18, p = 0.18$). GABA + /H₂O (middle column) showed a significant increase with age in the frontal region ($r_s = 0.30, p = 0.02$; not significant in the subcortical region, $r_s = -0.07, p = 0.55$). GABA + /Glu (right column) showed a significant increase with age in both regions ($r_s = 0.58, p < 0.001$ for the subcortical region and $r_s = 0.42, p = 0.001$ for the frontal region).

age, e.g., thalamus and pallidum, showed no significant correlation with Glu/H₂O. No significant correlation between GABA + /H₂O and functional connectivity was observed. GABA + /Glu values correlated negatively with functional connectivity in a cluster encompassing the left dorsal putamen and adjacent external capsule (Fig. 3, fourth row). Because the colocalization between age related changes in connectivity and Glu/H₂O related changes in connectivity suggested a Glu/H₂O mediated change in connectivity with age, we tested the mediation by a correlation between age and putamen connectivity after controlling for Glu/H₂O. The results showed that the correlation between age and connectivity remained significant in all regions except for the dorsal putamen, indicating that the connectivity with the dorsal putamen was mediated by age related changes in Glu/H₂O. Similar results were found when we controlled for the GABA + /Glu ratio. Because male and female subjects might show different patterns of connectivity depending on metabolites and age, we repeated the analyses after statistically controlling for sex. These analyses provided similar results (data not shown). We also tested whether younger (<25 years) or older participants (>25 years) might drive age and Glu/H₂O associations with putamen connectivity. Here we found both age and Glu/H₂O correlations with putamen connectivity in younger participants only (data not shown), which highlighted connectivity with the dorsal putamen to be sensitive to lower Glu/H₂O during development.

Discussion

As hypothesized, age was negatively correlated with Glu/H₂O in the subcortical voxel, and positively correlated with GABA + /H₂O in the frontal voxel, resulting in a positive association between the GABA + /Glu ratio and age in both regions. The

observed age-related changes in Glu/H₂O fosters reports that Glu correlates negatively with age in the basal ganglia (Chang et al., 2009), but not in the frontal cortex (Sailasuta et al., 2008; Chang et al., 2009). The positive correlation between GABA + /H₂O and age in the frontal cortex, but not in other regions, concurs with studies in adolescents and adults (Silveri et al., 2013; Gaetz et al., 2014). These results might thus support region specific and functionally distinct developmental trajectories of Glu and GABA. Glu might be associated with pruning of subcortical gray matter and concurrent rewiring of gray matter structures (Lebel et al., 2008), whereas GABA links to myelination of frontal white matter trajectories by controlling oligodendrocyte precursor cell activity (Vélez-Fort et al., 2012). Pruning takes place at the synaptic level, and would not be measurable using macroscopic morphometry measurements (Bourgeois and Rakic, 1993; Paus et al., 2008). Yet, further studies are necessary to associate gray and white matter changes with Glu and GABA levels across the full developmental trajectory. Together, this study first reports that developmental changes of Glu and GABA levels depend on both the region and neurotransmitter investigated.

Functional connectivity in the dorsal putamen correlated positively with subcortical Glu/H₂O and negatively with age. We propose that Glu/H₂O mediates the negative association between local functional connectivity in the dorsal putamen and development. In support of this notion, the correlation between functional connectivity and age disappeared after controlling for Glu/H₂O levels. Glu has previously been positively (Schmaal et al., 2012; Duncan et al., 2013; Kapogiannis et al., 2013) and negatively associated with functional connectivity (Duncan et al., 2013), depending on the target region of interest. Our finding of

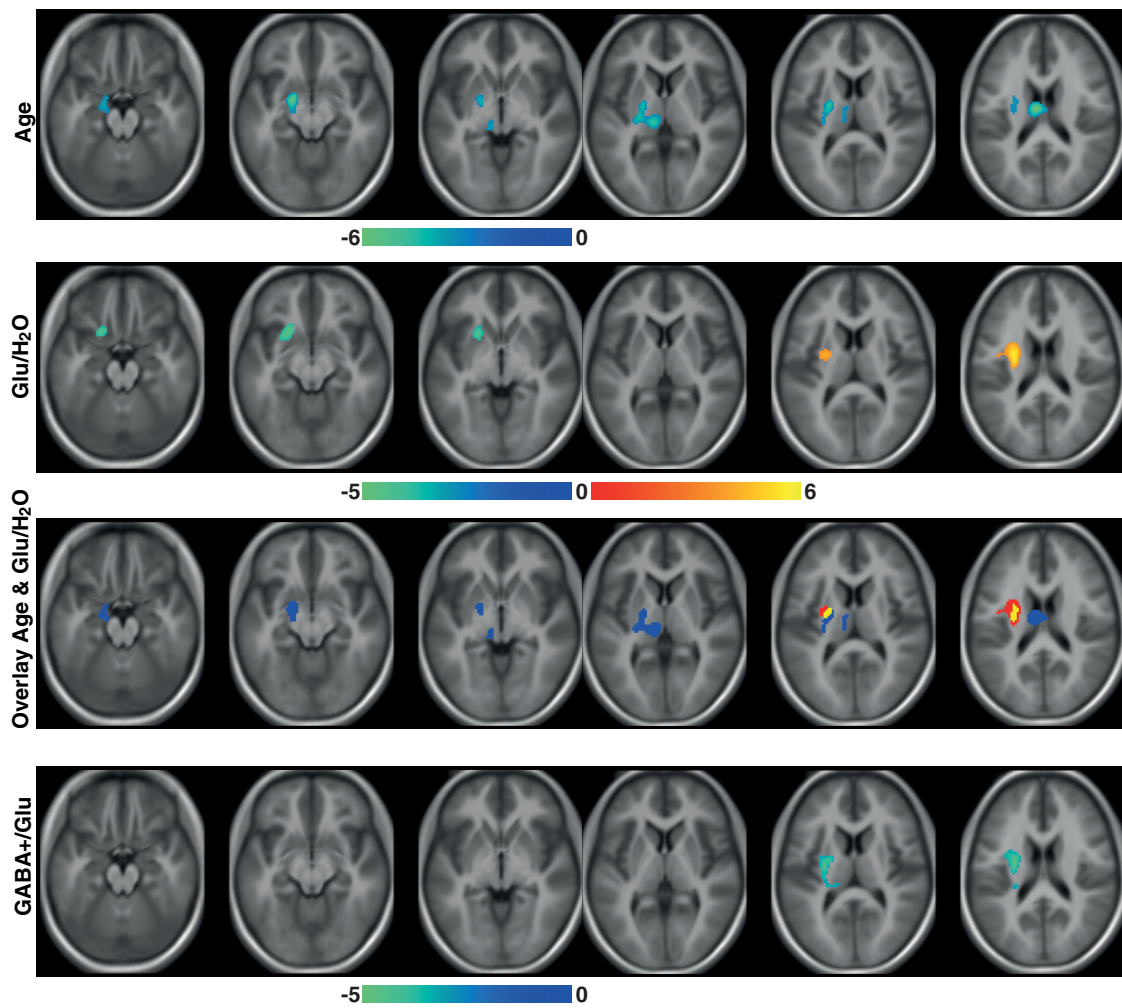


Figure 3. Correlations of functional connectivity (left putamen as seed region) with age, and the spectroscopic measures from the subcortical region. First row, functional connectivity correlated negatively with age in a cluster encompassing the left putamen and neighboring regions, i.e., left pallidum, thalamus, amygdala, hippocampus, and parahippocampal gyrus. Second row, functional connectivity correlated negatively with Glu/H₂O in a cluster encompassing the left ventral putamen and orbitofrontal cortex and positively in a cluster encompassing the left dorsal putamen and adjacent external capsule. Third row, overlay of the correlations between functional connectivity and age (blue), Glu/H₂O (red), and their overlap (yellow). The cluster showing a correlation with age overlapped in the dorsal putamen with the Glu/H₂O correlation cluster but not in other regions such as thalamus or pallidum. Fourth row, functional connectivity correlated negatively with GABA + /Glu values in a cluster encompassing the left dorsal putamen and adjacent external capsule. Results reported survived a height threshold of $p < 0.001$ (uncorrected) and an extent threshold of familywise error-corrected $p < 0.05$ at the cluster level. Color bar shows T values. Slices are at MNI z -coordinates [−20, −12, −4, 4, 12, 20].

Table 2. Correlations of functional connectivity (left putamen as seed region) with age, and the spectroscopic measures from the subcortical region

Correlation	Region	k	x, y, z (mm)	T	p
Age	Left thalamus	1403	−6, −12, 20	−6.23	< 0.001
	Left pallidum/amygdala /hippocampus		−18, −4, −10	−5.9	
	Left putamen/pallidum		−22, −8, 8	−5.34	
Glu/H ₂ O	Left dorsal putamen and external capsule	386	−28, −6, 18	5.85	0.024
Glu/H ₂ O	Left ventral putamen/frontal orbital cortex	330	−20, 18, −16	−4.71	0.042
GABA + /Glu	Left dorsal putamen and external capsule	487	−28, −14, 16	−5.26	0.009

Results reported survived a height threshold of $p < 0.001$ (uncorrected) and an extent threshold of familywise error-corrected $p < 0.05$ at the cluster level. k , Cluster size; x, y, z (mm), peak MNI coordinates.

positive and negative correlations between Glu/H₂O and functional connectivity in the dorsal and ventral putamen, respectively, suggests a dissociation of the effect of Glu/H₂O, because only the positive association was related to developmental changes in functional connectivity. We found no significant correlation between connectivity and GABA + /H₂O, whereas the association between GABA + /Glu ratio and connectivity across development mirrored the findings from Glu/H₂O. Other studies reported a negative association between GABA + /H₂O and functional connectivity (Kapogiannis et al., 2013; Stagg et al., 2014).

Those studies included adult subjects only and used other metabolites (i.e., creatine and NAA) for quantification of the GABA values. Note that both NAA and creatine change with age in adults (Haga et al., 2009; Duncan et al., 2014) and in younger (<18 years) subjects (Blüml et al., 2013). We used the unsuppressed water signal, which may therefore represent a more stable calibration standard for metabolite quantification in developmental MRS studies.

Glu receptor-mediated synaptic plasticity is a factor in synaptic pruning during brain development and probably involves ad-

justment of the excitation-inhibition balance on individual neurons and within networks (Selemon, 2013). Variations in this balance might underlie the BOLD signal as an indirect measure of neuronal activity (Logothetis, 2008; Isaacson and Scanziani, 2011; Lauritzen et al., 2012). Our findings concord with studies showing a developmental decrease of local functional connectivity (Fair et al., 2007a, 2009; Supekar et al., 2009), which might be supported by synaptic pruning and myelination (Power et al., 2010). We thus propose that an increase in synaptic pruning during development explains both the lower Glu/H₂O and the decrease of local connectivity from childhood to adulthood. The imbalance in the excitation-inhibition ratio has been linked to neurodevelopmental disorders (Gatto and Brodie, 2010) and our work thus is significant in further elucidating the role of Glu in fine-tuning functional connectivity networks.

Several methodological considerations need to be addressed. First, GABA and Glu are present in the brain in different pools (e.g., intracellular vs extracellular levels; Rae, 2014), which cannot be disentangled using current MRS measures. Nevertheless, the concentrations in these pools appear to be balanced, their total measured concentrations are neurophysiologically relevant (Kapogiannis et al., 2013) and any pool may be drawn on as a source for neurotransmitter GABA and Glu (Rae, 2014). Second, it is currently not satisfactorily possible to account for the macromolecular contributions to the GABA signal without compromising data quality or acquisition time (Mullins et al., 2014). Because the macromolecular contribution might be associated with age (Aufhaus et al., 2013), improvements of techniques accounting for this putatively confounding factor are relevant for future developmental studies investigating GABA. Third, age-related changes in tissue water concentration may also impact the derived water-scaled GABA+ and Glu concentrations, but recent developments in relaxometry methods could enable correction for developmental changes in water content in different tissue types in future studies (Kumar et al., 2011). Fourth, this is a cross-sectional study so that inferences on causality of age, metabolite, and connectivity related changes across development need to be investigated in future longitudinal studies. Fifth, we investigated the association between development and metabolites only with relation to the putamen connectivity. Because the acquisition of MRS values take a long time, the frontal voxel was not measured in younger children. This prevented us to directly compare age related changes in putamen connectivity and frontal connectivity. Sixth, both MRS voxels were large so that MRS values might partly be derived from other (gray matter) structures. Although the putamen was the largest structure within the subcortical MRS voxel, we cannot exclude that neighboring or connecting regions contribute to connectivity and to which extent gray or white matter changes with age affect this connectivity. Future studies require examination of the developmental trajectory of metabolites and connectivity in other regions and with comparable voxel sizes for acquiring connectivity and MRS values. Seventh, motion artifacts can bias estimates of functional connectivity, which is particularly important in developmental studies because motion can be related to subject age (Satterthwaite et al., 2013b). Yet, in-scanner motion putatively exerts minimal effects on the present finding of an age-related decrease in local connectivity, because motion appears to affect age-negative connections less strongly than age-positive connections (Satterthwaite et al., 2013b; Chai et al., 2014), and because subcortical areas are among the least affected by head motion because the pivot of head rotation lies near to these regions (Satterthwaite et al., 2013a). Eighth, we used task related background activity to

investigate developmental changes in functional connectivity. It has been shown that “hard-wired”, i.e., anatomical, connections underlie functional connectivity networks (Damoiseaux and Greicius, 2009), and that task instruction (Norman-Haignere et al., 2012), task performance (Sepulcre et al., 2010), and hormonal concentrations (e.g., oxytocin; Kirsch et al., 2005) can modulate connectivity. The function of ongoing activity is intimately related to cognition, and in both “resting” or active state this relation is inherent to the brain (Sadaghiani et al., 2010). Although the strength of functional connectivity is constrained by structural connectivity, it is modulated by mental states and current context, such that intrinsic activity “constitutes the brain’s internal context for processing external information and generating behavior (Kenet et al., 2003; Fontanini and Katz, 2008)” (Sadaghiani et al., 2010, p 12). We thus interpret our functional connectivity findings as representing intrinsic, yet context-dependent background activity and the context was given by our cognitive task. Several studies have reported the positive correlation of Glu also with resting state functional connectivity (Schmaal et al., 2012; Duncan et al., 2013; Kapogiannis et al., 2013). Our results thus extend knowledge by demonstrating this positive correlation also in task-related background activity. We argue that the positive relationship between Glu and functional connectivity is not limited to resting state data, but represents a more general role of Glu in the modulation of functional connectivity. Finally, the putamen has mainly been attributed to filtering efficiency in the context of working memory, i.e., hindering irrelevant information from entering working memory (Frank et al., 2001; McNab and Klingberg, 2008; Baier et al., 2010). The ability to inhibit irrelevant information increases with age (Robert et al., 2009), suggesting a potential role of striatal regions in the development of working memory networks possibly in cooperation with frontoparietal and hippocampal networks (Finn et al., 2010; von Allmen et al., 2014). Our results of lower functional connectivity in striatal regions might reflect the fine-tuning of these filtering processes, possibly mediated by a concomitant lower Glu/H₂O across development mirroring increased synaptic pruning. Future studies also investigating other tasks, especially with the addition of irrelevant stimuli, are needed to further elucidate this proposed role of Glu.

In conclusion, we found lower Glu/H₂O with age in a subcortical (basal ganglia) voxel and higher GABA+/H₂O with age in a frontal voxel, mirroring higher age-related GABA+/Glu in both regions. We further found lower connectivity between the putamen and other subcortical structures across development. Subcortical Glu/H₂O mediated the lower connectivity in the dorsal putamen with age. Given these results and the potential association between Glu and synaptic pruning, we suggest that age related changes in Glu mediate the decrease of local connectivity during development.

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