Structural and neurochemical correlates of Tourette’s disorder and attention-deficit hyperactivity disorder
Forde, Natalie

IMPORTANT NOTE: You are advised to consult the publisher’s version (publisher’s PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 6

Multi-Modal Imaging Investigation of Cortical Cytoarchitecture in Neurodevelopment

Submitted as:


*Shared first authorship
†Shared last authorship
Abstract

Background: Multi-modal imaging may improve our understanding of the relationship between cortical morphology and cytoarchitecture. To this end we integrated the analyses of several magnetic resonance imaging (MRI) and spectroscopy (MRS) metrics within the anterior cingulate cortex (ACC). Considering the ACCs role in neurodevelopmental disorders, we also investigated the association between neuropsychiatric symptoms and the various metrics.

Methods: T1 and diffusion-weighted MRI and 1H-MRS (ACC voxel) data along with phenotypic information were acquired from children (8-12 years) with various neurodevelopmental disorders (n=95) and healthy controls (n=50). From within the MRS voxel mean diffusivity (MD) of the grey matter fraction, intrinsic curvature (IC) of the surface and concentrations of glutamate, N-acetylaspartate and myo-inositol were extracted. Linear models were used to investigate if the neurochemicals predicted MD and IC or if MD predicted IC. Finally, measures of various symptom severities were included to determine the influence of symptoms of neurodevelopmental disorders.

Results: All three neurochemicals inversely predicted MD (all $p_{\text{uncorrected}} < 0.04$, $\beta=0.23-0.36$). There was no association between IC and MD or IC and the neurochemicals (all $p>0.05$). Severity of autism symptoms related positively to MD ($p_{\text{uncorrected}} = 0.002$, $\beta=0.39$).

Conclusion: Our findings support the notion that the neurochemicals relate to cytoarchitecture within the cortex. Additionally, we showed that autism symptoms across participants relate to the ACC cytoarchitecture.
Introduction

Neuroimaging studies continuously falter when it comes to the interpretation of morphological measures. The ability to link morphology with the underlying cytoarchitecture and, even more importantly, with cellular functioning could prove paramount to understanding both healthy functioning and deviations from it that lead to behavioural changes and symptoms seen in neurodevelopmental disorders (Casanova and Trippe 2009; Kotagiri et al. 2014; Bakhshi and Chance 2015). Here we have applied a multi-modal approach to determine how several metrics, all purportedly related to the cytoarchitecture albeit measured at different scales, relate to each other. It is likely that one biological process (whether it is deviant or not) is reflected not in just one modality, but to varying extents in several modalities. By using a multi-modal approach the strengths of the different modalities complement each other, providing a more complete picture of the topic under investigation (Curiel et al. 2007).

Many morphological measures of the brain can be derived from T1-weighted magnetic resonance imaging (MRI) data. Intrinsic curvature (IC) is a relatively new morphological index of the cortical surface and it has been proposed to relate to the underlying cell density (Ronan et al. 2011). It is posited that expansion of the surface progresses at different rates across the surface dependent on the cytoarchitecture within the cortex. This process of differential expansion results in changes to the surface, measurable as IC. The degree of differential expansion is less in areas of greater cell density due to the accumulative tangential tension applied by the cells hindering expansion (Ronan et al. 2011; Ronan and Fletcher 2015). This association suggests IC can be used as a quantifiable measure of the cortical cell density.

Mean diffusivity (MD), an index derived from diffusion-weighted MRI data (dMRI), also relates to the cytoarchitecture. Diffusion-weighted MRI is a technique sensitive to the Brownian movement of water molecules within the tissue. MD is the average amount of diffusion by water in any direction within a voxel. Cell bodies and axons form obstacles to water diffusion, thereby reducing MD. MD therefore will vary dependent on cell density (Beaulieu 2002), where higher cell density will result in lower MD.

Proton MR Spectroscopy (1H-MRS) allows the non-invasive in vivo quantification of several neurochemicals simultaneously within a selected area of the brain. These include neurochemicals that due to their individual cellular localisation can also be used as measures of cell density. For instance, the intracellular concentration of glutamate is several thousand times higher than its extracellular concentration (Danbolt 2001). We therefore propose that glutamate may be used as a proxy for cell density. N-Acetylasparate (NAA) has been proposed as a marker of neuronal integrity although its concentration varies across neuronal populations limiting its interpretability (Moffett et al. 2007). Finally, myo-inositol (mI) has been proposed as a glial cell marker (Brand et al. 1993).

Several studies to date used multimodal neuroimaging to investigate associations between different imaging measures and symptoms in different neurodevelopmental
disorders. For instance, one study used anatomical MRI, dMRI and 1H-MRS to investigate differences between patients with autism spectrum disorder (ASD) and controls (Libero et al. 2015). Differences between groups were found within all measures in several different brain regions. Similarly, a study investigating bipolar disorder found differences in grey matter (GM) volume, white matter (WM) microstructure and functional connectivity in several regions throughout the brain (Johnston et al. 2017). However, neither study integrated the analyses of the different measures nor did they use the same region of interest across the different measures.

The anterior cingulate cortex (ACC), an important region for cognitive control, attention and emotion regulation (Allman et al. 2001), has been widely implicated in various neurodevelopmental disorders, including Tourette syndrome (TS), obsessive-compulsive disorder (OCD), attention-deficit/hyperactivity disorder (ADHD) and ASD. Studies have reported structural (Müller-Vahl et al. 2009; Nakao et al. 2011; Frodl and Skokauskas 2012; Kühn et al. 2013; Ecker et al. 2015), neurochemical (Brennan et al. 2013; Naaijen et al. 2015; Freed et al. 2016) and functional (Stern et al. 2000; Hart et al. 2013; Neuner et al. 2014; Brennan et al. 2015; Hull et al. 2017) changes, but findings have been inconsistent. TS, OCD, ADHD and ASD may all show increased impulsive and compulsive behaviour, although to different extents. The ACC has been hypothesised to be involved in regulating these behaviours through top-down cognitive control (Dalley et al. 2011), which makes it an interesting region to investigate across various neurodevelopmental disorders with partially overlapping symptoms.

Here, based on the abovementioned assumptions regarding the relations between the different imaging metrics and cell density, we integrated the analyses of different metrics within the ACC. To this end we used anatomical data, diffusion weighted imaging and 1H-MRS from a cohort of healthy children and children with various neurodevelopmental disorders, including TS, OCD, ADHD and ASD. We hypothesised that the MRS metrics (Glu, NAA and ml concentrations) would be predictive of MD and the degree of IC. Similarly, we expected that MD would predict IC. Which MRS neurochemical best predicts MD and IC may suggest which cellular population has a greater impact on the respective measures. Furthermore, we investigated whether symptoms related to neurodevelopmental disorders were associated with the metrics.

Methods
Participants

Participants in this study were all reported on previously (Forde et al. 2016; Naaijen et al. 2016; Naaijen et al. 2017). Briefly, healthy control children were recruited mostly through local schools. Children with neurodevelopmental disorders, including TS, OCD, ADHD and ASD, were recruited via child and adolescent psychiatry/neurology departments and patient associations throughout the Netherlands. Written informed consent was provided by the parents/guardians of all participants and written assent was also given by participants who were 12 years of age. The study
was approved by the regional ethics board (CMO Regio Arnhem Nijmegen, numbers: NL42004.091.12 & NL48377.091.14). After quality control of the MRI and MRS data, n=50 healthy controls and n=95 patients (with primary diagnosis of TS, n=40; OCD, n=8; ADHD, n=38; ASD, n=9) were available for statistical analysis. Diffusion MRI data were available for n=36 healthy controls and n=46 patients. See Quality assessment section for details.

Inclusion and exclusion criteria along with information on phenotypic data collection were extensively reported previously (Forde et al. 2016; Naaijen et al. 2016; Naaijen et al. 2017). Briefly, all participants were 8-12 years old with an IQ>70, were of Caucasian decent, had no contra indications for MR assessment, no previous head injuries or neurological disorders and no major medical illness. Participants of the healthy control group were free of psychiatric disorders as determined by domain scores in the normal range on screening questionnaires (Child behavior checklist [CBCL] and Teacher Report Form [TRF]; Bordin et al. 2013). A key inclusion criterion for the patient group was meeting DSM-IV criteria for the respective disorders.

Phenotypic information

TS diagnosis and tic severity were determined and rated using the Yale Global Tic Severity Scale (YGTSS; Leckman et al. 1989). The Children’s Yale-Brown Obsessive Compulsive Scale (CY-BOCS; Scahill et al. 1997) was used to confirm diagnosis of OCD and determine severity of obsessions and compulsions (in all participants if symptoms were present irrespective of diagnosis). To determine the presence of ADHD and other psychiatric disorders the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS; Kaufman et al. 1997) interview was administered to the parent(s) of all participants. The screening module was used, followed if needed by disorder-specific modules. ASD was evaluated with the autism diagnostic interview revised (ADI-R; Lord et al. 1994), a structured developmental interview to assess ASD symptom severity and classify diagnosis. All interviews on psychiatric symptoms were conducted about an unmedicated period. Information about tic severity was based on the previous week.

Phenotypic traits across disorders were further assessed with parental questionnaires including the Conners’ Parent Rating Scale – Revised Long version (CPRS-RL; Conners et al. 1997) to rate total ADHD severity, the Children’s Social Behavioral Questionnaire (CSBQ; Luteijn et al. 2000) to rate core autistic traits, a subscale of the Repetitive Behavior Scale (RBS-R; Lam and Aman 2007) to rate repetitive/compulsive behaviours and a medication questionnaire to determine medication history. Full-scale IQ was estimated from four subtests of the Wechsler Intelligence Scale for Children-III (WISC-III; Wechsler 2002).

T1-weighted MRI acquisition

All MRI datasets were acquired on a 3T Siemens Prisma (Siemens, Erlangen, Germany) scanner, located in the Donders Institute for Brain, Cognition and Behavior, Nijmegen,
the Netherlands. T1-weighted anatomical images were acquired with a sagittal, 3D magnetization prepared rapid gradient echo (MPRAGE) parallel imaging sequence with the following parameters: TR=2300 ms, TE=2.98 ms, TI=900 ms, FoV=256 mm, slice thickness=1.20 mm, flip angle=9 degrees, in plane resolution=1.0×1.0 mm, acceleration factor=2, acquisition time=5:30 minutes.

**Diffusion-weighted MRI acquisition**

Diffusion-weighted datasets were acquired, on a subsample of participants (n=102). After quality control, n=82 remained (n=46 patients and n=36 controls). A pulsed gradient spin-echo echo-planar imaging (EPI) sequence was used. Two non-diffusion weighted reference images (b=0 s/mm$^2$) and 64 images with a diffusion gradient (b=1500 s/mm$^2$) were acquired at each of 72 transversal slices with the following parameters: TE=103 ms, TR=12,000 ms, slice thickness=2 mm, in-plane resolution=2 x 2 mm and acquisition time=13:48 minutes.

**MRS acquisition**

Proton spectra were acquired using a point resolved spectroscopy (PRESS) protocol with chemically selective suppression (CHESS) water suppression (Haase et al. 1985). A 2×2×2 cm voxel was placed on the midline covering pregenual ACC (TR=3000 ms, TE=30 ms, number of averages = 96, bandwidth = 5kHz, number of points = 4096). Unsuppressed water reference spectra (16 averages) were also acquired. Total acquisition time was six minutes. Voxel location was adjusted for each participant to maximize GM content. See Figure 1A and 1D for the location of the voxel and an example spectrum, respectively. T1-weighted images were used for voxel placement and for tissue segmentation.

**Quality assessment**

Anatomical, dMRI and MRS data were all evaluated for data quality. Anatomical and dMRI datasets for each participant were visually inspected (for imaging- and motion artefacts) and evaluated by an experienced rater (NJF) who was blinded to group. Measures of quality such as signal-to-noise ratio (SNR) and motion parameters were investigated in addition to the visual evaluation. MR spectra were required to have a linewidth (full-width at half-maximum) ≤0.1 ppm and SNR ≥5 (all spectra were ≥19). To ensure similar data quality across the patient and control groups the Cramér-Rao lower bounds (CRLB) were compared for each metabolite of interest (Kreis 2015). These did not differ between patients and controls for any of the metabolites (all p-values > 0.1). CRLB estimated standard deviations for Glu, NAA and mI for the full sample were 4.3%, 2.1% and 3.3%, respectively. Mean voxel percentage GM, WM and cerebrospinal fluid (CSF) were 71.02 (6.14) %, 11.42 (2.26) % and 17.53 (6.28) % respectively. These also did not differ between the control and patient groups (all p-values > 0.05; see Supplementary table 2). Consistency of voxel placement across patients and controls can be seen in Figure 2. Participants with poor quality
anatomical/spectral data or with bad anatomical segmentation were excluded from analysis (n=29, these participants were not included in the demographic section/Table 1). An additional 20 participants were excluded from analyses of diffusion measures only based on their dMRI quality. Seven additional participants were excluded for having values of more than 3 standard deviations from the mean for our variables of interest. Our final samples for analyses consisted of n=145 (IC and the neurochemicals) and n=82 (analyses including MD).

Processing

T1-weighted images underwent processing with the unified segmentation procedure within the VBM8 toolbox of SPM8 (Statistical Parametric Mapping 8, UCL, London, UK) to produce GM, WM and CSF probability maps. Spectroscopy voxels were mapped onto these probability maps to generate fractions of GM, WM and CSF within each spectroscopy voxel ($f_{\text{GM}}$, $f_{\text{WM}}$, and $f_{\text{CSF}}$). MRS data were processed with Linear Combination Model (LCModel), version V6.03-01 (Provencher 1993; Provencher 2001), using a linear combination of in vitro metabolite solution spectra as a reference to identify and quantify the metabolite concentrations in in vivo spectra as described previously (Naaijen et al. 2016; Naaijen et al. 2017). Briefly, processing included eddy current correction and calculation of water-referenced concentrations in institutional units (i.u.). Each individual’s metabolite concentrations were corrected for partial volume confounds and differing amounts of water in each tissue type with the following formula:

$$\text{Metabolite}_{\text{corrected}} = \text{Metabolite}_{\text{Raw}} \times \left( \frac{43300 \times f_{\text{GM}} + 35880 \times f_{\text{WM}} + 55556 \times f_{\text{CSF}}}{35880} \right) \times \frac{1}{(1 - f_{\text{CSF}})}$$

where 43300, 35880, and 55556 are the water concentrations in mM for GM, WM, and CSF, respectively. $\text{Metabolite}_{\text{corrected}}$ represents the metabolite concentration from the grey and white matter proportion of the MRS voxel, following the LC Model assumption that CSF is free of metabolites (Provencher 2014).

White and pial cortical surfaces for each hemisphere were reconstructed from the T1-weighted data using the fully automated ‘recon-all’ procedure in FreeSurfer v5.3 (Fischl et al. 1999b; Dale et al. 1999; Fischl et al. 1999a; Fischl and Dale 2000). These cortical reconstructions were used in the Caret software (v5.65, http://brainvis.wustl.edu/wiki/index.php/Caret:About) to calculate the IC per vertex as described previously (Ronan et al. 2014; Forde et al. 2014).

Diffusion-weighted data were de-noised with a local principal component analysis (LPCA) noise filter as well as affine transformed to correct for motion and eddy current distortions (SPM8, London, UK). Susceptibility distortions were non-linearly corrected along the phase encoding direction to optimally match the T1-weighted image (Visser et al. 2010). Finally, diffusion tensors were robustly estimated using the PATCH (Zwiers 2010) algorithm to eliminate artifacts in the data from cardiac and head motion.
**Figure 1** Each processing step for one randomly chosen subject is shown. (A) Localisation of the MRS voxel (red) on the T1-weighted image. (B) Skull stripped T1-weighted image with tissue segmented voxel; red, orange and yellow correspond to grey matter, white matter and CSF, respectively. (C) Mean diffusivity (MD) image in T1-weighted space with grey matter fraction of the MRS voxel shown in red. (D) MRS spectrum with peaks corresponding to the major metabolites indicated. The thin black line represents the frequency-domain data, the red line is the LCModel fit. In the top panel the residuals are plotted (the data minus the fit). (E) MRS voxel is shown in orange having been transformed to FreeSurfer space. Green and red lines indicate the white/grey and grey/pial tissue borders, respectively, as defined by FreeSurfer. (F) Red indicates surface within the MRS voxel on the medial pial surface for the left and right hemispheres, respectively. Cho - Choline, Cre - creatine, Gln - glutamine, Glu - glutamate, Glx - Glu+Gln, MD - mean diffusivity, mI - myo-inositol, NAA - N-Acetylaspartate.
Figure 2 Average voxel placement per group; (A) controls, (B) patients and (C) combined for control and patients groups. Mid-line ACC voxels are superimposed on MNI template. Voxel placement was highly consistent within and across groups.

Extracting metrics from within the MRS voxel

A transformation matrix was generated by registering (fslregister) the T1-weighted image in native space to the FreeSurfer anatomical image. This transformation was then applied to the binarised MRS voxel file while converting it to a surface (mri_vol2surf), which resulted in a surface file that masked the region within the MRS voxel for each hemisphere in FreeSurfer space.

The IC files for each hemisphere were imported to Matlab (R2012b) where IC values from the region within the MRS voxel were isolated using the surface masks previously generated. These were then filtered to remove IC values inconsistent with the resolution of reconstruction (Ronan et al. 2012; Ronan et al. 2014) before calculating the absolute values of the remaining per-vertex IC measures. Values for left and right were combined before the skewness of the IC distribution for the region within the MRS voxel was computed (Ronan et al. 2012; Ronan et al. 2014). The distribution of cortical IC values is highly skewed towards zero (Pienaar et al. 2008; Ronan et al. 2011; Ronan et al. 2012). Therefore, it is more informative to use the dimensionless measure of skew, rather than mean or median, as a measure of the degree of differential expansion in the region. We infer that the less skewed the distribution the greater the degree of IC, which is proposed to reflect a lower cell
A transformation matrix from diffusion to T1-weighted space was generated by linearly registering the average $b=0$ s/mm$^2$ image to the T1-weighted image using FMRIB’s Linear Image Registration Tool (FLIRT; Jenkinson and Smith 2001; Jenkinson et al. 2002). This matrix was then applied to the MD image to transform it to T1 space. The GM fraction of the MRS voxel was isolated and binarised with fslmaths. Fslstats was then used to extract metrics from the MD image from within the GM masked region (Smith et al. 2004; Woolrich et al. 2009).

Statistics

Statistical analyses were conducted with the R statistical program (R Core Team 2013). Pearson’s chi-squared tests were used to examine the differences between the control and patient groups in categorical measures (sex and handedness). Group differences in continuous measures (age and IQ) were assessed with a Welch two sample $t$-test if assumptions of homogeneity of variance and normality of distributions were met ($p>0.05$ in Bartlett’s test of homogeneity of variance and Shapiro-Wilk normality test). If either of these assumptions were violated a non-parametric Mann-Whitney U test was used. Group differences in voxel tissue composition and spectral quality were assessed with Welch two sample $t$-test (if normal and homogenous, as above).

To address our primary question, i.e. are the MRS metrics, MD and IC associated, we used linear models including age and sex to determine if the respective metabolites (NAA, Glu and mI) predicted IC skew and MD (subset of data) and if MD predicted IC skew. When residuals of the model were not normally distributed we used a Box-Cox power transformation (Box and Cox 1964) to normalise the data. As our variables of interest were not totally independent, corrections for multiple comparisons (seven tests) were based on the effective number of tests (Nyholt 2004). This resulted in an adjusted alpha level of 0.009 (based on 5.6 effective tests rather than seven). Subsequently, we added phenotypic data to the models to address our second question; do cross-disorder symptoms of neurodevelopmental disorders significantly relate to the metrics? Here, ADHD severity T-score (CPRS; Conners et al. 1998), ASD core symptoms (CSBQ; Luteijn et al. 2000), repetitive and compulsive behaviours (RBS-R; Lam and Aman 2007), TS (yes/no; YGTSS; Leckman et al. 1989) and IQ were examined in addition to sex and age.

As a visual aid to understand the shared variance of the MR metrics we also conducted a principal component analysis (PCA) including participants for whom data for each metric were available (n=82). The principle components were not further analysed, as the above methods were deemed more suitable to address our specific research question and to make better use of the available data. The principle components are not specific to the metrics nor do they give information on how related each metric is to another. Results from the PCA analysis can be found in Supplementary table 3 and Supplementary figure 1.
Results

Demographics

Participant demographic details are reported in Table 1 (n=145). The patient group included participants with a primary diagnosis of TS, n=40; OCD, n=8; ADHD, n=38; ASD, n=9. For more detailed demographic information regarding the patient group and comorbidities, see Supplementary table 1. For the analyses including MD, the number of participants was fewer (n=46 and n=36 for patients and controls, respectively). This reduction did not affect the demographic distributions between groups (n=82; age, $t$=-1.36, $p$=0.18; sex, $\chi^2$=0.50, $p$=0.48; IQ, $t$=-3.11, $p$=0.003; handedness, $\chi^2$<0.01, $p$=0.99).

Table 1 Demographic details

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Control</th>
<th>Test statistic</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>95</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age years, mean(SD)</td>
<td>10.76 (1.31)</td>
<td>11.03 (0.99)</td>
<td>KW $\chi^2$= 0.99</td>
<td>0.32</td>
</tr>
<tr>
<td>Sex, m/f</td>
<td>59/36</td>
<td>36/14</td>
<td>$\chi^2$= 1.02</td>
<td>0.31</td>
</tr>
<tr>
<td>IQ, mean(SD)</td>
<td>102.8 (12.2)</td>
<td>109.6 (10.9)</td>
<td>$t$ = -3.44</td>
<td>0.0008**</td>
</tr>
<tr>
<td>Range</td>
<td>70.9-137.4</td>
<td>87.5-133.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handedness, r/l</td>
<td>82/13</td>
<td>46/4</td>
<td>$\chi^2$= 0.55</td>
<td>0.46</td>
</tr>
</tbody>
</table>

* IQ estimated from a subtest of the Wechsler Intelligence Scale for Children-III (WISC-III; Wechsler, 2002) rating. ** $p$ < 0.01

Neurochemicals predicting MD

In our basic models (including age and sex) we saw that all three neurochemicals predicted MD (NAA: $\beta$=0.32, $t$=2.94, $p$=0.004; Glu: $\beta$=0.36, $t$=3.36, $p$=0.001; mI: $\beta$=0.23, $t$=2.05, $p$=0.04), although mI failed to reach statistical significance following multiple comparison correction (adjusted alpha = 0.009). In each case there was a negative association between MD and the neurochemical (statistical analyses show a positive $t$-stat due to Box-Cox transformations), see Figure 3.

These findings were negligibly influenced by the inclusion of phenotypic data. None of the phenotypic information had a significant effect on the models (all $p$-values>0.02). However, autism symptoms (CSBQ core score) and compulsive behaviours (RBS compulsivity subscale) consistently appeared to affect the models although not significantly considering the adjusted alpha level (CSBQ: $p$=0.02-0.03; RBS $p$=0.04-0.06). We further investigated each of these phenotypic measures with respect to NAA, Glu, mI and MD individually using a linear model. This showed that autism traits significantly predicted MD ($\beta$=0.39, $t$=3.29, $p$=0.002, see Figure 4) but not any of the metabolite concentrations (all $p$-values>0.2). Compulsive behaviours did not significantly predict MD or any of the neurochemical concentrations ($p$>0.07).
**Figure 3** Figures show the raw data points and regression fit lines with their 95% confidence intervals (shaded areas) for association between the neurochemicals and mean diffusivity (MD). $R^2$ and $p$-values are derived from the linear models including age and sex. A similar pattern of negative association is seen between all neurochemicals and MD (Note: statistical analyses indicate positive $t$-stats due to the Box-Cox transformations). Glu – glutamate, mI – myo-Inositol, NAA – N-Acetylaspartate, i.u. - institutional units.

**Figure 4** Figure show the raw data points and regression fit line with its 95% confidence interval (shaded area) for the association between the Children's Social Behavior Checklist (CSBQ; Luteijn et al. 2000) core autism symptoms and mean diffusivity (MD). $R^2$ and $p$-values are derived from the linear model including age and sex.
Neurochemicals predicting IC

None of the neurochemicals significantly predicted IC skew (NAA: \(\beta=-0.15, t=-1.75, p=0.08\); Glu: \(\beta=0.05, t=0.62, p=0.54\); mI: \(\beta=0.10, t=1.17, p=0.25\)). The inclusion of phenotypic data did not significantly influence the model (all \(p\)-values>0.05).

MD predicting IC

MD did not predict IC skew \((\beta=-0.05, t=-0.43, p=0.67)\). However, sex had a significant main effect \((\beta=0.28, t=2.56, p=0.01)\).

To ensure our findings were not unduly affected by the inclusion of patients, we repeated our analyses regarding whether the respective measures were associated with each other in the healthy control group only (n=50 or n=36 when MD was investigated). As expected due to the decrease in power associated with reducing subject number none of our findings of neurochemicals predicting MD remained significant, however, the pattern and size of effects remained stable (NAA: \(\beta=0.32, t=1.88, p=0.07\); Glu: \(\beta=0.34, t=2.02, p=0.05\); mI: \(\beta=0.26, t=1.51, p=0.14\)). Results involving IC skew were also similar to those of the full group.

Discussion

In the current study we investigated whether there was an association between several different structural and neurochemical metrics, which are all theoretically related to cell density, within the ACC. As hypothesised, concentrations of all three neurochemicals investigated (NAA, Glu and mI) were related to MD, although mI concentration was not significantly related to MD after correction for multiple comparisons. In each case there was a negative association between MD and the neurochemical concentration (statistical analyses show a positive \(t\)-stat due to Box-Cox transformations). This supports the notion that water diffusion (quantified by MD) is hindered by cells (both neuronal and glial) and varies with cell density. Opposed to our hypothesis we saw no association between neurochemical concentrations or MD with the degree of IC of the surface. Finally, we investigated whether symptoms related to neurodevelopmental disorders, including autism symptom severity, attention, hyperactivity, compulsivity, obsessions and tics, were associated with the metrics. We showed a negative association between autism symptom severity and MD within the ACC region investigated. This suggests higher cell density in the ACC is associated with more severe autism symptoms across disorders.

We proposed that Glu concentration could be used as a proxy for cell density. The current finding of a negative correlation between Glu and MD supports this idea. As mentioned above, diffusion of water molecules (quantified by MD) is hindered by cells, thus with a higher cell density MD is lower. A previous study in a male group of healthy adults found a similar association between Glu concentration and MD, albeit in a different region of interest – posterior cingulate cortex (PCC; Arrubla et
Another previous study reported a negative association between NAA and MD in the centrum semiovale WM of controls and patients with cerebrovascular disease (Nitkunan et al. 2008) which they interpreted as relating to axonal loss or dysfunction. Our findings are in line with those of both studies and additionally show a similar association between MD and mI that has not previously been reported. However, the association here was not significant following multiple comparison correction and warrants replication. We chose to investigate the association with Glu, NAA and mI because of our understanding of their cellular localisation, but other metabolites may also relate to cell density.

There was no significant relationship between IC skew and neurochemical concentrations or between IC skew and MD indicating that IC does not directly relate to the underlying cortical cell density. This is not to say that during development of the cortex cell density does not influence differential surface expansion and thereby IC and cortical folding as hypothesised (Ronan et al. 2011; Ronan and Fletcher 2015). The only thing we can infer from our results is that at the age of our participants (8-12 years) the relationship is not present. This may be due to several other factors. For instance the cortex at this age is no longer expanding but is in fact reducing in thickness (Shaw et al. 2008; Ostby et al. 2009; Raznahan et al. 2011; Brown et al. 2012; Amlien et al. 2016), surface area (according to some sources [Ostby et al. 2009; Raznahan et al. 2011; Brown et al. 2012; Shaw et al. 2012]) and gyrification (Raznahan et al. 2011; Shaw et al. 2012). These processes may obscure any relationship that would once have been present. As well as this differential expansion leads to cortical folding which itself is thought to alter the cortical cell density in places which would in turn effect future surface expansion and result in a complex association of IC to cell density (Ronan and Fletcher 2015).

Severity of autism symptoms across disorders was inversely related to MD. From this we infer that those with more severe autistic traits have a higher cell density (which restricts water diffusion and results in a lower MD). This is in line with a former study of the same cohort where increased Glu concentrations were found in participants with ASD compared to controls in the same region of interest (Naaijen et al. 2016). Additionally, in this previous study Glu concentration in the ACC was positively correlated with a measure of repetitive behaviour (limited interest) across ASD and OCD patients, supporting the assumption of higher cell density across disorders with an increase in autism symptom severity. Previous research has suggested cytoarchitectural changes in ASD (Casanova and Trippe 2009). A study in adults with ASD showed atypical intrinsic cortico-cortical connectivity compared with controls at the level of grey matter, indicating cytoarchitectural abnormalities (Ecker et al. 2013). In another study of adults with ASD lower cell density was shown in the septal hypocellular gap of the subventricular zone, however, comparing this to the current study is difficult as a sub-cortical region was investigated and layer specific abnormalities were found (Kotagiri et al. 2014). Several of these studies refer to a strong genetic component involved in cytoarchitectural changes. It would therefore be worthwhile to investigate genetic factors underlying these cytoarchitectural changes along with the neurochemicals and MD in future studies.
Strengths of the current study were the investigation of a relatively large group of children with a restricted age range (8-12 years). Although investigating a heterogeneous group of participants with several different neurodevelopmental disorders can have limitations, by utilising dimensional measures across the groups we could address our research questions better than would have otherwise been possible. This dimensional approach has been advocated by Robbins and colleagues (Robbins et al. 2012) and the research domain criteria (RDoC) initiative (Cuthbert 2014) as opposed to categorising participants based on arbitrary thresholds. Furthermore, to account for the possible confound of heterogeneity caused by the different diagnostic groups, we performed an internal replication in the healthy controls, which showed similar results, although non-significant due to reduced power. A further strength of the current study was the use of several metrics, all with a theoretical relationship to cell density, in one region of interest. These metrics complement each other to give more information than investigating them individually.

In conclusion, the current study showed that the concentrations of various neurochemicals are inversely associated with MD supporting the notion that they all relate to cell density within the cortex. IC on the other hand was unrelated to the other metrics investigated suggesting that it is not directly related to the cell density within the cortex. Finally, our findings support evidence that autism symptoms across disorders relate to the cytoarchitecture in the ACC. Whether this extends to other areas needs further examination.

References


Fischl B, Sereno MI, Tootell RB, Dale AM (1999b) High-resolution intersubject averaging and a coordinate system for the cortical surface. Hum Brain Mapp 8:272–84. HBM10>3.0.CO;2-4


Multi-Modal Imaging Investigation of Cortical Cytoarchitecture in Neurodevelopment


R Core Team (2013) R: A language and environment for Statistical computing.


### Supplementary table 1 Demographic details on the controls and patient sub-groups

<table>
<thead>
<tr>
<th></th>
<th>TS</th>
<th>ADHD</th>
<th>ASD</th>
<th>OCD</th>
<th>Control</th>
<th>Test statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>38</td>
<td>9</td>
<td>8</td>
<td>50</td>
<td>K-W χ²=4.03</td>
<td>0.40</td>
</tr>
<tr>
<td>Age years, mean(SD)</td>
<td>10.51 (1.48)</td>
<td>10.88 (1.17)</td>
<td>11.19 (1.20)</td>
<td>10.95 (1.18)</td>
<td>11.02 (0.99)</td>
<td>χ²=23.99</td>
<td>0.00008**</td>
</tr>
<tr>
<td>Sex, m/f</td>
<td>34/6</td>
<td>21/17</td>
<td>1/8</td>
<td>3/5</td>
<td>36/14</td>
<td>χ²=23.99</td>
<td>0.00008**</td>
</tr>
<tr>
<td>'IQ, mean(SD)</td>
<td>105.4 (11.3)</td>
<td>101.6 (12.8)</td>
<td>99.0 (9.8)</td>
<td>99.1 (15.4)</td>
<td>109.6 (10.9)</td>
<td>F=3.81</td>
<td>0.006**</td>
</tr>
<tr>
<td>Range</td>
<td>80.6-126.3</td>
<td>70.9-137.4</td>
<td>82.0-113.9</td>
<td>80.6-122.2</td>
<td>87.5-133.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handed, r/l</td>
<td>35/5</td>
<td>33/5</td>
<td>8/1</td>
<td>6/2</td>
<td>46/4</td>
<td>χ²=2.13</td>
<td>0.71</td>
</tr>
<tr>
<td>'OC-symptoms, mean(SD)</td>
<td>6.83 (7.88)</td>
<td>4.67 (11.43)</td>
<td>NA</td>
<td>17.33 (6.35)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Tic severity, mean(SD)</td>
<td>20.1 (8.82)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'ADHD severity, mean(SD)</td>
<td>63.03 (12.08)</td>
<td>70.39 (11.22)</td>
<td>68.89 (10.90)</td>
<td>63.25 (13.09)</td>
<td>45.44 (5.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBS compulsivity, mean(SD)</td>
<td>2.13 (2.57)</td>
<td>1.12 (1.80)</td>
<td>1.56 (1.51)</td>
<td>5.63 (4.00)</td>
<td>0.10 (0.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSBQ core autism score, mean(SD)</td>
<td>14.31 (10.36)</td>
<td>12.43 (7.67)</td>
<td>24.67 (4.97)</td>
<td>13.00 (10.16)</td>
<td>1.46 (1.92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Medication</td>
<td>Stimulant</td>
<td>9</td>
<td>25</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atomoxetine</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antipsychotic</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clonidine</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSRI</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>'Comorbidities</td>
<td>ADHD (18)</td>
<td>OCD (1)</td>
<td>ADHD (3)</td>
<td>ADHD (2)</td>
<td>NA</td>
<td>GAD (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OCD (4)</td>
<td>ODD (2)</td>
<td></td>
<td></td>
<td></td>
<td>MD (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GAD (1)</td>
<td>ODD (2)</td>
<td></td>
<td></td>
<td></td>
<td>PDDNOS (1)</td>
<td></td>
</tr>
</tbody>
</table>

Supplementary table 2 ACC voxel composition (partial volumes) comparison across patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>Test statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% grey matter</td>
<td>70.8 (5.9)</td>
<td>71.3 (6.7)</td>
<td>K-W $\chi^2 = 0.70$</td>
<td>0.40</td>
</tr>
<tr>
<td>% white matter</td>
<td>11.5 (2.2)</td>
<td>11.3 (2.4)</td>
<td>K-W $\chi^2 = 0.89$</td>
<td>0.35</td>
</tr>
<tr>
<td>% CSF</td>
<td>17.6 (6.3)</td>
<td>17.4 (6.2)</td>
<td>K-W $\chi^2 = 0.04$</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Principle component analysis of MR metrics

Supplementary table 3 Variance and loadings from principle component analysis

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of variance</td>
<td>55%</td>
<td>21%</td>
<td>16%</td>
<td>92%</td>
</tr>
<tr>
<td>IC skew</td>
<td>0.12</td>
<td>0.84</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>0.25</td>
<td>-0.54</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>mI</td>
<td>-0.54</td>
<td>-0.03</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>-0.57</td>
<td>0.03</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>NAA</td>
<td>-0.55</td>
<td>-0.07</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

All data (n=82) were log transformed, centred and scaled for principle component analysis. Glu – glutamate, IC – intrinsic curvature, MD – mean diffusivity, mI – myo-inositol, NAA – N-acetylaspartate.

Supplementary figure 1 The first 3 principle components (PC) graphed against each other for n=82 participants. Together these PCs account for 92% of the variance of the magnetic resonance (MR) metrics. Patients with neurodevelopmental disorders are represented with black points and controls with grey. The correlation circles are plotted in red. All data were log transformed, centred and scaled for principle component analysis. Plots were produced with ggbiplot in R. PCA analysis allowed the visualisation of the shared variance of MR metrics. All three neurochemicals were closely grouped indicating a high degree of shared variance (PC1). Components 2 and 3 were highly influenced by both IC skew and MD. There appear to be more underlying relationships (e.g. negative correlations between IC skew and MD) but these are not significant possibly due to a lack of power. This PCA complements the results reported in the main manuscript. Glu – glutamate, IC – intrinsic curvature, MD – mean diffusivity, mI – myo-inositol, NAA – N-acetylaspartate, var – variance.