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MAPPING BRAIN NETWORKS AND THEIR DYSFUNCTION IN AUTISM: A
FUNCTIONAL CONNECTIVITY ANALYSIS

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Foreword

This master thesis was written as a completion to the Master of Science in Psychology: theoretical and experimental psychology. Working on this master thesis for two years has been both exciting and interesting. I learned a lot about Autism and resting-state fMRI analysis, and about my own future goals and research interests. Overall I am very grateful to all the people that have supported me throughout this project, without whom this would not have been possible.

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Abstract

Neural connectivity could be a possible explanatory framework for autism spectrum disorder (ASD). ASD is a common developmental disorder that is characterized by impaired social interaction, impaired communication, restricted interests, and repetitive behaviors. Two seemingly contradictory connectivity theories about etiology of ASD, namely the underconnectivity theory and the hyperconnectivity theory, could possibly be integrated in a more mixed and nuanced perspective. In the current study the publicly available dataset ABIDE is used to investigate the functional and structural connectivity patterns of young children (< 12 years old) with severe and mild ASD compared to matched controls. Independent Component Analysis (ICA) of functional data revealed within-network hyperconnectivity between the dorsal DMN and the left anterior insula as a possible biomarker for milder forms of autism, and within-network hypoconnectivity of the auditory network with the right superior parietal lobule as possible indication for more severe forms of autism. Additionally, Source-Based Morphometry (SBM) revealed structural abnormalities in a gray-matter network that consists mainly of left frontal areas with connections to the temporal and parietal lobe in both severity groups compared to healthy controls. Whilst our findings in functional connectivity could be used to differentiate between mild and severe forms of autism, our findings in structural connectivity point towards a more unified abnormality that is consistent across all degrees of ASD severity.

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Autism spectrum disorder (ASD) is clinically described as a ‘triad’ of several deficits: impaired social interaction, impaired communication, restricted interests, and repetitive behaviors (Belmonte et al., 2004). In eight-year-old children it has a prevalence of 1 in 88 (Baio, 2012). Geschwind and Levitt (2007) described ASD as a very broad syndrome instead of just one single disorder that is why they referred to it as ‘the autisms’. The autism spectrum includes Asperger’s syndrome, Pervasive Developmental Disorder-Not Otherwise Specified, Rett’s disorder and Childhood Disintegrative Disorder. The diagnosis of autism is made when six symptoms are present over the domain of the triad: impairment of social interaction, communication and repetitive behavior, interests and activities (Gotham, Bishop & Lord, 2011). Impairment of social interaction includes a lack of spontaneous seeking to share with others and a lack of social or emotional reciprocity. Impairment of communication (verbal and nonverbal) includes using stereotyped, repetitive language and difficulty in initiating or sustaining a conversation with others. The restricted, repetitive behavior involves inflexibility and sticking to (non-functional) routines or rituals (MacKenzie, 2008).

Abnormal neural connectivity can be used as an explanatory framework for the many faces of ASD (Belmonte et al., 2004). ASD was considered to be a ‘developmental disconnection syndrome’ (Geschwind and Levitt, 2007). Therefore recent research on etiology of ASD focusses on discovering neurological connectivity biomarkers that can serve as a diagnostic tool that indicates ASD. Consequently investigating brain connectivity in autism can help in understanding and treating children and adults with ASD.

Brain connectivity consists of anatomical connectivity or structural connectivity, functional connectivity and effective connectivity. In structural connectivity, anatomical patterns of units in a nervous system are investigated where in functional connectivity statistical dependencies between signals in different regions are calculated (like cross-correlations or coherence). Causal interactions are investigated within effective connectivity research (Sporns, 2007). In order to understand human functioning you need to know the elements and interconnections of the networks in the brain. A comprehensive understanding of the structural connections in combination with

functional connectivity lies at the base of increased insight in how functional brain states emerge (Sporns et al., 2005). Various types of neuroimaging techniques have indicated associations between major psychiatric disorders and abnormalities of brain function and brain structure (Savitz et al., 2013). A lot of psychiatric disorders (like depression, schizophrenia and autism) are known to have abnormal functioning of the default mode network (DMN), which is one of the networks that are found to be correlated and more active during rest. During rest the mind is free to wander. The DMN is associated to self-referential mental activity, stimulus-independent thought, monitoring somesthetic and vegetative information, inner speech, planning of future activities, creativity, self-awareness etc. (Callard and Margulies, 2014). Neuropsychiatric disorders influence these important aspects of human experience; they alter (or are caused by alteration of) DMN functioning (Whitfield-Gabrieli & Ford, 2012). This reveals the importance of resting-state fMRI in comprehension of psychopathology.

Publicly available datasets of functional MRI scan sessions on patients with ASD boost connectivity research in ASD. Earlier studies had often consisted of small sample sizes, but datasets such as the ‘1000 functional connectome project’ (Biswal et al., 2010) improved this shortcoming. The 1000 functional connectome project is freely available at www.nitrc.org/projects/fcon_1000/ and consists mainly of data from healthy subjects. The Human Connectome Project (HCP) is another publicly available dataset that focusses on characterization of brain connectivity and function and their variability in healthy adults (Van Essen et al., 2012). Other publically available datasets are more focused on patients with certain types of pathology, examples are the ‘Autism Brain Imaging Data Exchange (ABIDE)’ (Di Martino et al., 2013), the ‘Alzheimer’s Disease Neuroimaging Initiative (ADNI)’ (Jack et al., 2008) and the ‘Collaborative Informatics and Neuroimaging Suite (COINS)’. COINS (<http://coins.mrn.org>) consists of data from over 300 studies which is over 19,000 MRI, MEG, and EEG scan sessions together with more than 180,000 clinical assessments (Scott et al., 2011). The advantage of this very large source of information is that the data can be reorganized and used for many different problems and research questions. Second you can also take a lot of covariates such as handedness, ASD severity, intelligence etc. into account while analyzing your research questions.

We often use phenotypic measures to determine the severity of autism features in neuroscience. Two of the most commonly used measures are the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview-Revised (ADI-R) scales (Gotham et al., 2009). Their scores indicate that the patient had a higher number of items that represent core deficits and/or greater severity. Both scales are often used as an indication of severity, but the raw ADOS scores for example where not developed for comparison across individuals or time, and the raw scores are not normalized (de Bildt et al., 2011). So Gotham et al. developed a calibrated severity score for ADOS based on percentiles of raw total scores of each ADOS diagnostic classification (Gotham et al., 2009). Characteristic variables like chronological age and verbal IQ have less influence on the calibrated severity scores than on the raw ADOS scores. That is why we use these calibrated ADOS Gotham scores in our current research. The severity scores have a 10-point metric, a score of 1-3 indicates a nonspectrum ADOS classification, a score of 4-5 indicates an ASD classification and a score of 6-10 indicates an autism classification (Gotham et al., 2009; de Bildt et al., 2010).

One of the earliest indications of autism is macrocephaly (a head circumference above the 97th percentile), a large head size that was still within the normal range was even more common. This macrocephaly is not present at birth and there is no relationship with autism severity (Lainhart et al., 1997). More specifically, overgrowth in occipitofrontal circumference in children with ASD occurs during the second half of the first year of life (Webb et al., 2007). Macrocephaly in primary school children with autism (7-11 years old) is found to be a possible biomarker of abnormal neural connectivity (White, O'Reilly and Frith, 2009). So macrocephaly in ASD is not restricted to young infants. Other evidence that points out the role of network dysfunctions in autism is the increased cerebral white matter volume and increased cortical gray matter volume seen on structural MRI scans in very young children with autism. These increases cannot be seen in older children with autism (Minschew and Keller, 2010). These results support the developmental perspective of autism, which will be discussed further on.

Support for the importance of looking at cortical neurons as the unit of dysfunction in autism can also be found in the postmortem study of Courchesne et al. (2011) where

a higher neuron count was found in the prefrontal cortex of children with autism compared to controls. The excess in neurons was higher in the dorsolateral prefrontal cortex than in the medial prefrontal cortex. A connection with clinical symptoms can be found in the bilateral enlargement of the amygdala and the hippocampus in children with ASD (Sparks et al., 2002). Since the amygdala is important for responses to emotional stimuli, which for example is important for our interactions with others, people with autism usually have more problems in understanding emotional expressions. This could be a potential region of interest in our current study.

The findings of macrocephaly are inconsistent with the under-connectivity theories of autism. A lot of studies reported a weaker functional connectivity between brain areas during the execution of cognitive tasks in patients with ASD (Supekar et al., 2013). This phenomenon of underconnectivity is found during language comprehension tasks (Just et al., 2004), social processing (Koshino et al., 2008), visuospatial processing (Damarla et al., 2010), viewing faces (Khan et al., 2013), high-level inhibition (Solomon et al., 2009) and during many more task performances (for a review: Kana et al., 2011). Since a balance between specialization and integration in the brain is critical for functioning, this weaker functional connectivity could be a crucial cause for ASD. This convergence of evidence lead to the “underconnectivity theory” proposed by Just and colleagues (2012). The underconnectivity theory states that the disruption of inter-regional connective circuitry could lie at the origin of dysfunctions in autism. Especially tasks that are particularly dependent on integration of frontal and more posterior cortical regions are impaired (mostly more complex cognitive tasks). The focus of the theory lies on reduced frontal-posterior synchronization of activation during cognitive tasks, this causes an increase in reliance on posterior regions when frontal cortex involvement is required (Just et al., 2012). But empirical evidence that was inconsistent with this theory leads to a comparison study of Müller and colleagues (2011). Underconnectivity is mainly a conclusion of task-driven research in specific regions of interest without the use of low-pass filtering. Underconnectivity could thus be a result of specific methodological choices.

Contrary to the underconnectivity theory, the hyperconnectivity theory relates dysfunctions in ASD to patterns of hyperconnectivity in the brain. Findings of

macrocephaly in humans in combination with hyperconnectivity in animal autism models (Testa-Silva et al., 2012) were at the base of the hyperconnectivity theory. Di Martino and colleagues (2011) tried to reconcile these findings with human neuroimaging studies on ASD. They found excessive functional connectivity in the striatal-cortical circuits. Supekar and colleagues (2013) examined functional connectivity with a whole-brain analysis in a cohort study on school-age children (7-13 years old). Widespread functional brain hyperconnectivity was found at the whole-brain level but also at the level of major functional networks (between both proximal and distant anatomical regions).

These two seemingly contradictory theories might be reconcilable with a more developmental perspective. Since a normal brain development shows a transition from over-connectivity to pruning, which rewrites the connectivity at neural and at system level (evidence from resting state fMRI, Supekar et al., 2009) a similar or contradictory development could also be possible in ASD. The contradictory results in whole-brain functional connectivity in autism neuroimaging research could result from differences between children and adults. Studies of children under the age of 12 find evidence for the hyperconnectivity theory, studies based on data from adolescents and adults give an indication of functional underconnectivity (Uddin, Supekar & Menon, 2013). From this we can expect hyperconnectivity in our sample of ASD children under the age of 12.

Additionally, several studies have discovered mixed results when examining functional connectivity in children with ASD. Doyle-Thomas and colleagues (2015) found that, depending on the region, connections between the posterior cingulate cortex (PCC) and the default mode network regions showed either stronger or weaker connectivity in children with ASD. However, they also showed a decreased functional connectivity pattern between the PCC and medial prefrontal cortex with age, which is in line with our developmental perspective. These patterns of mixed increased and decreased functional connectivity in ASD can also be found in an adult sample (Hahamy, Behrman & Malach, 2015). Their findings could point towards a new characteristic of ASD, namely idiosyncratic distortions of the functional connectivity pattern compared to the typical template (Hahamy, Behrman & Malach, 2015). Interestingly their findings also point out the relation between the magnitude of these

idiosyncratic distortions and behavioral symptoms of ASD. ASD severity could thus be a determining factor in the distortions of the functional connectivity pattern. According to a review of Anderson (2014) three additional studies found signs of mixed functional connectivity during rest in autism, all of which focused on adults or adolescents with ASD. It would therefore be interesting to replicate findings of mixed functional connectivity found in more recent studies in our current study.

Within resting-state there are different methods to examine functional connectivity. A couple of studies on children under the age of 12 used seed-based methods to look at resting-state data. Seed-based analysis uses a bivariate measure (like correlation or coherence) between the time-series of different seeds (brain regions of interest) in order to look for areas that exhibit a high level of connectivity to your target seed (Neuroimaging in Python team, 2009). Hyper-connectivity was found in different studies: (a) hyper-connectivity of the striatum with the insula and the superior temporal gyrus (Di Martino et al., 2011) and (b) hyper-connectivity within the default mode network (Lynch et al., 2013). Washington and colleagues (2013) on the other hand found hypo-connectivity in the intermodal default mode network, also with seed-based methods. In contrast to seed-based functional connectivity you can also use independent component analysis (ICA). With ICA the fMRI signal is decomposed into independent component maps, where active voxels in every map are mostly non-overlapping. With every component map comes an associated component activation waveform. So in short ICA separates the fMRI signal in spatially independent maps of activity (McKeown et al., 1997). You have functional connectivity within one map. In our age group (12 and under) the ICA method also leads to findings of hyper-connectivity: (a) hyper-connectivity within the salience, default mode, fronto-temporal, motor and visual networks (Uddin, Supekar, Lynch et al., 2013) and (b) hyper-connectivity within default mode, visual and motor networks (Washington et al., 2013). We can again conclude that we will most likely find hyper-connectivity within our sample. But based on these studies a large hiatus appears, namely the absence of whole-brain analyses on children under the age of 12 with ASD. This will be the focus of the current study. We use ICA to look at the entire brain, separated into different neural networks. One of these networks is the default mode network, which seems to be an important network in ASD.

Aside from functional connectivity, structural connectivity can give further insight into how the brain is structured anatomically. Are the brains of patients with ASD simply wired in a different, and possibly malignant fashion? Previous research on these structural anomalies in ASD up until now has been inconsistent (Valk et al., 2015). However, literature based on univariate analysis techniques such as voxel-based morphometry (VBM) have shown repeated findings of structural differences in frontal lobe, precuneus, hippocampus, parahippocampal, amygdala, cerebellum and brain stem (Grecucci et al., 2015, under review). Additionally, Ecker and colleagues (2010) provided a predictive classification model of adult ASD by using structural scans in a whole-brain classification approach with a support vector machine (SVM). Applying SVM to gray matter structural MRI scans provided a correct classification of adults with ASD with a specificity of 86.0% and a sensitivity of 88.0%. The most important anatomical differences were found in middle frontal gyrus, inferior frontal gyrus, precentral gyrus, the temporal lobe, posterior cingulate gyrus, precuneus, medial occipital gyrus, hippocampal gyrus, fusiform gyrus, insular cortex, amygdala, and cerebellar cortex. Based on these previous findings we can pose that structural anomalies do play a role in the symptomatology of ASD. However, aside from findings of macrocephaly, overgrowth in occipitofrontal circumference and increased cortical gray matter volume in young children; not much is known about the structural anomalies of ASD in younger children. Which is why we will focus on structural connectivity of ASD in children under the age of 12 in order to fill this gap. Additionally we will zoom in on the relationship between these structural connectivity differences and autism severity.

We will use Source-based Morphometry (SBM) in order to examine these structural connectivity patterns. SBM applies the multivariate technique of Independent Component Analysis (ICA) to anatomical data (Kašpárek et al., 2010). This multivariate technique is proven to be more powerful than the univariate VBM (Xu et al., 2009). Additionally, univariate approaches are known to have issues with correctly characterizing the inherently multivariate brain morphology (Friston & Ashburner, 2004).

Current Study

The present study focusses on children under the age of 12. This is a very interesting age because previous findings show that for example the difference in brain size between children with ASD and normal controls disappears by the time they reach adolescence (Schumann et al., 2004). So it is important to study the effects of ASD closer to their onset because in adulthood compensatory mechanisms might have already taken place (Amaral, 2011).

From previous evidence we can conclude that a developmental framework is probably the best starting point. In this developmental framework hyper-connectivity is assumed to arise in children before the age of 12, after a puberty period this connectivity-pattern shifts to hypo-connectivity or under-connectivity (Uddin, Supekar & Menon, 2013). So we can expect patterns of hyper-connectivity within our resting-state fMRI sample. We used ICA to separate the signals from different networks within the brain and we will look at the entire brain as a whole. Based on previous research, we expect differences in the default mode, salience, fronto-temporal, motor and visual networks but we can't forget the striatum, the insula, the amygdala and the superior temporal gyrus. We will try to replicate previous findings and look for the other, yet unknown differences in resting-state functional connectivity and structural connectivity in children with ASD.

Method

Participants

Datasets from 28 children with ASD and 28 healthy controls from the COINS database (<http://coins.mrn.org>) were included in this study. Each dataset consists of a resting fMRI acquisition; all images were obtained with additional informed consent (Nielsen et al., 2013). For more information about the data acquisition, see <http://coins.mrn.org>. Inclusion criteria were age (12 or under), absence of comorbidity and availability of handedness, sex and IQ-scores. Control subjects were matched to participants with ASD based on age, handedness and IQ-score. All participants and controls were males. All patients had the DSM IV diagnose of autism without other comorbid disorders. Participants in the control group were between eight and twelve years old (mean = 10.6, SD = 1.24), participants in the ASD group were also between

Table 1 – Subject characteristics

	Control - Mild	Control - Severe	ASD – Mild	ASD - Severe
Number of participants	14	14	14	14
Handedness (R:L:Ambidextrous)	12:1:1	14:0:0	12:1:1	14:0:0
Age Mean (SD)	10.6 (1.27)	10.4 (1.27)	10.8 (1.13)	10.5 (1.29)
ADOS Gotham Severity Mean (SD)			5.29 (1.14)	9.07 (0.73)
ADOS Gotham Social Affect Mean (SD)			6.57 (1.91)	12.21 (2.78)
ADOS Gotham RRB Mean (SD)			1.79 (1.05)	4.86 (1.99)
ADOS Gotham Total Mean (SD)			8.36 (1.45)	17.07 (3.67)

eight and twelve years old (mean = 10.7, SD = 1.22). The ASD group was divided into the severe ASD group and the mild ASD group based on their ADOS Gotham severity scores (mild: score between 3 and 6, severe: score between 8 and 10). Both groups differed significantly on ADOS Gotham Social Affect sub scores ($t = -7.3491$, $p < 0.001$), ADOS Gotham Restricted and Repetitive Behaviors sub scores ($t = -5.636$, $p < 0.001$) and ADOS Gotham Total score ($t = -9.5019$, $p < 0.001$). For more detailed subject characteristics, see Table 1.

Preprocessing

Preprocessing of anatomical data.

Preprocessing of our anatomical images is done using FSL and the VBM8 toolbox in SPM (an fMRI data analysis software package called ‘Statistical Parametric Mapping’ that is used in combination with Matlab). During preprocessing of the anatomical data, the following sequence of analysis steps was executed:

- (1) Brain extraction and robust center estimation in FSL: Segmenting the anatomical head images into brain and non-brain. This was automatically carried out using FSL Brain extraction tool (BET, Smith; 2002).
- (2) Normalization and segmentation in VBM8: anatomical images of every subject are spatially normalized by warping them onto the MNI template. Afterwards all normalized images are segmented into gray matter, white matter and CSF.
- (3) Spatial smoothing in SPM: anatomical images are smoothed across their neighboring voxels in order to reduce noise in the data. The normalized gray matter images were smoothed with 8-mm full width at half-maximum (FWHM) Gaussian kernel.

Preprocessing of functional data.

Preprocessing the resting state fMRI data was done using AFNI (an fMRI analysis software package called ‘Analysis of Functional NeuroImages’) in combination with FSL (another fMRI data analysis package called ‘FMRIB Software Library’). Before preprocessing all anatomical brain images were skull-stripped (as explained in preprocessing of anatomical images) in AFNI for more precise coregistration of images. During preprocessing of the functional data the following sequence of analysis was executed:

- (1) Reorient the functional images into an FSL friendly space was done in AFNI. Files were reoriented to fit an RPI orientation, which means voxels are organized from Right to left to store a row, rows are ordered from Posterior to anterior to store a slice and slices are ordered from Inferior to superior to store a volume. Keep in mind that all preprocessing steps were done for each subject individually.
- (2) Realignment is necessary because our subjects do not lie perfectly still during the fMRI acquisition session. The realignment lines up each functional image with the previous one so that each voxel always samples the same region without blurring. Spatial alignment is executed, which is essentially a rigid body registration and correction per functional image. Acceptable movement is up to 6mm (Ashburner et al., 2013). Please note that we have to take the age of our subjects into consideration, since this study only focuses on children, which tend to move more than adults during scanning sessions.
- (3) Coregistration, done in FSL: This procedure attempts to align both the functional and structural data per individual. Coregistration is a critical step because it combines the high spatial resolution of our structural image in order to improve the spatial localization in the low spatial resolution functional images.
- (4) Removing head movement, white matter and cerebrospinal fluid (CSF) signals from functional images was performed in AFNI. This procedure removes noise and unwanted signals from our functional data in order to focus on grey matter signals.
- (5) Normalization in FSL: The focus of this study is comparing groups, not individuals. So we need to be able to compare the signal changes across groups

of individuals. Since there is a large variation between individual brains (even for major landmarks) we need to warp the images of every individual onto the same reference space (a template), this is called spatial normalization. The template that is used in this study is the MNI template (Montreal Neurological Institute template). Each individual subject's images are warped into the standard space based on the MNI template.

Post-processing analysis

Independent Component Analysis of functional data.

The preprocessed functional data were analyzed using the Group ICA of the GIFT toolbox to determine spatially independent and temporally coherent networks (Ashby, 2011). The minimum description length (MDL) principle was used to estimate a number of independent components. Independent component (IC) estimation was executed by using the Infomax algorithm, which is a neural network algorithm that tries to minimize the mutual information in our networks in order to identify natural grouping and maximally independent sources (Bell and Sejnowski, 1995). This was repeated 20 times in ICASSO in order to ensure maximum stability of our components (Himberg et al., 2004). GIFT identified 20 reliable independent components, an overview of these components can be found in Appendix 1. Only components of interest were selected for further group analysis.

Source-based Morphometry analysis of anatomical data.

SBM was also executed using the GIFT toolbox. MDL was used to estimate the number of independent components in our preprocessed anatomical MRI data. Nine different independent components were identified. The Infomax algorithm was used to perform a group ICA; ICA was repeated 20 times in ICASSO. The components were then clustered to ensure the reliability and consistence of our results. All of our resulting 9 components were deemed reliable ($I_q > 0.96$), which indicates a stable ICA decomposition.

During SBM each gray matter volume is converted into a vector, which leaves us with a matrix where the 56 rows represent our 56 subjects (28 controls, 14 ASD mild

and 14 ASD severe) and each column indicates a voxel. ICA decomposes this matrix in a “mixing matrix” and a “source matrix”. The mixing matrix consists of 56 rows (each row representing a subject) and 9 columns that represent our independent components. This mixing matrix thus represents how much one given subject expresses each of the components. The source matrix represents the relationship between our independent components and the voxels. For the visualization of our grey matter volume components, the source matrix is reshaped to a 3D image which is scaled to unit standard deviations (Z maps) and thresholded at $Z>2.5$. An overview of our components can be found in Appendix 2. The mixing matrix will be used to compute our group differences.

Results

Group analysis of functional data.

ICA extracted 20 independent components (for an overview see Appendix 1) based on our functional data. These 20 components represent group components across all of the subjects in our four groups. Independent components of each subject were then entered into a one-way ANOVA where we contrasted the ASD-mild and ASD-severe groups with their respective control groups; additionally we contrasted the ASD-mild group with the ASD-severe group. Age and motion during the scan were used as covariates. It is important to note that both of our ASD groups reported a lot more movement than the control groups. Mean motion estimates for the mild and severe ASD group were mean = 5.03 (SD = 10.10) and mean = 9.73 (SD = 24.08) respectively, means for the mild and severe control group were mean = 0.39 (SD = 0.58) and mean = 0.12 (SD = 0.17) respectively. Group results were corrected for multiple comparisons by using the family-wise error-rate (FWER). Group differences were found in component 1 and 17. Anatomical labels of regions in these components were obtained through the WFU PickAtlas (Tzourio-Mazoyer et al., 2002) and can be found in Table 2. Component 1 represents the dorsal DMN and component 17 represents the auditory network. Contrast maps of these two components revealed differences in activation of the left anterior insula (part of component 1, peak t-value = 6.04) between the Control-mild and the DSM-mild group, and the right superior parietal lobule (part of component

Table 2 – Anatomical regions of functional components 1 and 17

Component	Area	Brodmann Area	Volume (cc) L/R	Random effects: Max Value (x,y,z) L/R
1	Superior Frontal Gyrus	10, 11	9.3/9.0	6.0 (-27, 61, -13)/7.1 (24, 60, -16)
	Medial Frontal Gyrus	10, 11, 25	6.3/5.6	6.2 (-3, 46, -17)/5.9 (9, 67, -6)
	Orbital Gyrus	11, 47	1.4/1.7	5.9 (-3, 51, -20)/5.8 (3, 51, -20)
	Middle Frontal Gyrus	10, 11, 47	7.0/4.9	5.0 (-36, 52, -13)/5.8 (36, 58, -13)
	Rectal Gyrus	11	2.0/2.4	4.7 (-6, 28, -22)/5.1 (3, 48, -23)
	Sub-Gyral		1.4/0.8	4.4 (-9, 45, -22)/4.5 (9, 46, -20)
	Inferior Frontal Gyrus	10, 11, 47	5.2/2.8	4.3 (-24, 28, -22)/4.3 (21, 34, -22)
	Subcallosal Gyrus	25	0.4/0.6	3.5 (-3, 14, -13)/3.5 (3, 14, -13)
	Superior Temporal Gyrus	38	0.3/0.3	2.9 (-53, 17, -11)/2.8 (24, 10, -26)
	Uncus		0.1/0.0	2.7 (-21, 8, -23)/-
	Anterior Cingulate	25	0.0/0.1	-2.6 (3, 14, -8)
17	Transverse Temporal Gyrus	41, 42	0.8/1.1	6.7 (-62, -14, 12)/7.9 (62, -14, 12)
	Superior Temporal Gyrus	13, 22, 38, 41, 42	7.8/8.7	6.5 (-59, -11, 9)/7.5 (62, -8, 9)
	Postcentral Gyrus	1, 2, 3, 40, 43	7.2/8.3	6.7 (-62, -17, 15)/7.4 (59, -14, 15)
	Precentral Gyrus	3, 6, 13, 43, 44	12.9/16.8	6.6 (-59, -5, 25)/6.8 (53, -11, 12)
	Sub-Gyral		0.3/0.8	3.5 (-48, -8, 14)/5.5 (50, -11, 14)
	Insula	13, 40	4.9/7.9	4.3 (-48, -20, 15)/5.2 (45, -11, 9)
	Inferior Frontal Gyrus	9, 44, 45	1.0/1.6	4.2 (-59, 4, 22)/5.1 (59, 4, 22)
	Inferior Parietal Lobule	40	1.3/2.0	4.3 (-62, -22, 26)/4.9 (62, -22, 26)
	Claustrum		0.0/0.4	-3.6 (36, -8, 9)
	Middle Frontal Gyrus		0.0/0.1	-3.6 (56, 2, 39)
	Extra-Nuclear		0.1/0.4	3.0 (-36, -5, 9)/3.5 (48, 0, 3)
	Medial Frontal Gyrus	6	0.1/0.2	3.0 (0, 0, 50)/2.7 (3, 0, 53)
	Middle Temporal Gyrus		0.1/0.1	3.0 (-59, 0, -3)/2.8 (65, 0, -3)
	Cingulate Gyrus		0.1/0.0	2.8 (0, 2, 47)/-

17, peak t-value = 6.20) between the control-severe and the DSM-severe group. Contrast maps of these two components can be found in Appendix 3.

Group analysis of anatomical data.

We extracted 9 independent components with the SBM procedure (see Appendix 2). The mixing matrix obtained after the SBM procedure was used to verify differences in the expression of these components across our four groups. A MANOVA was performed with our four groups (Control-mild, Control-severe, ASD-mild and ASD-severe) as independent variable and the weights on each of our 9 independent components (found in the mixing matrix) as dependent variables. Differences between groups were found in component 5 ($F(3,52) = 3.99; p = 0.012$) and 8 ($F(3,52) = 5.034; p = 0.004$). However, component 5 did not survive our threshold ($Z > 2.5$), which leaves us with differences in component 8. Anatomical labels of regions in component 8 were obtained through the WFU PickAtlas (Tzourio-Mazoyer et al., 2002). Component 8

Table 3 – Changes of Gray matter volume of component 8

Area	Brodmann Area	Volume (cc) L/R	Random effects: Max Value Talairach (x, y, z) L/R
Inferior Frontal Gyrus	9, 44	3.1/0.7	6.2 (-43, 6, 30)/4.2 (42, 9, 30)
Middle Frontal Gyrus	6, 9, 10, 46	8.2/1.0	5.3 (-42, 10, 31)/3.9 (42, 12, 27)
Precentral Gyrus	4, 6, 9	7.6/0.1	5.2 (-43, 3, 37)/3.7 (45, 5, 36)
Sub-Gyral		2.8/0.3	5.1 (-42, 7, 23)/3.3 (42, 9, 24)
Anterior Cingulate	10, 24, 32	2.6/2.2	4.3 (-4, 43, 10)/4.2 (9, 45, 1)
Medial Frontal Gyrus	9, 10, 11, 32	2.4/1.9	4.2 (-6, 47, 2)/4.0 (6, 48, 0)
Superior Frontal Gyrus	9, 10	1.6/0.0	4.1 (-27, 43, 17)/ -
Postcentral Gyrus	3, 43	0.8/0.1	3.7 (-56, -12, 25)/3.2 (50, -20, 38)
Cingulate Gyrus	32	0.6/0.1	3.4 (-3, 32, 27)/3.1 (3, 32, 29)

consists mainly of frontal areas such as the Inferior and Middle Frontal Gyrus, the Precentral Gyrus, the Sub-Gyral, Anterior Cingulate, the Medial Frontal Gyrus, the Superior Frontal Gyrus, the Postcentral Gyrus and the Cingulate Gyrus (See Appendix 4). All gray-matter regions represented in component 8 are presented in Table 3. After the MANOVA, a Least Significant Differences (LSD) post-hoc test was performed. This group-wise comparison will uncover which group differences drove these significant results. Significant differences in the structural pattern of component 8 were found between both our control groups and both ASD groups. The Control-mild group showed significant differences ($p = 0.009$) from the ASD-mild group. Similarly, our Control-severe group was significantly different ($p = 0.008$) from the ASD-severe group (mean = 0.42, SD = 0.89). No significant differences were found between both ASD severity groups. Correlations between the loading coefficients of component 8 and our measures of ASD severity were not significant.

In summary we found small clusters that differ between our four groups. One as a marker for DSM-mild which indicates hyperconnectivity of the left anterior insula with the dorsal DMN (component 1), and one as a marker for DSM-severe which indicates hypoconnectivity of the right superior parietal lobule with the auditory network (component 17). From anatomical group-analysis we found an anatomical component mainly consisting of left frontal areas that differs between ASD-mild and matched controls, and ASD-severe and matched controls. But this significant difference didn't correlate with severity scores, which indicates that the brain structure in component 8 is different but does not predict severity.

Discussion

This study aimed to assess functional and structural connectivity in children with mild and severe Autism Spectrum Disorder compared to matched healthy controls.

Resting state scans and clinical assessments from children with ASD under the age of 12 and matched healthy controls were obtained from the ABIDE database. Functional and anatomical images were pre- and post-processed before group analysis. Functional connectivity was examined by means of ICA and structural connectivity was assessed by means of SBM. Results showed two possible biomarkers for functional connectivity and one for structural connectivity. Implications of these results will be discussed in further detail.

Functional connectivity

ICA of functional data revealed hyperconnectivity of the left anterior insula with the dorsal DMN in children with mild ASD compared to matched healthy controls. Previous studies have shown that atypical within-network functional connectivity of the DMN and the insula could be a possible brain marker for children with ASD (Bos et al., 2014; Nomi and Uddin, 2015). Within-network hyper-connectivity between the DMN and the insula has previously been found in an ASD sample of both children and adolescents by using a linear correlation approach (Bos et al. 2014). These within-network functional connectivity differences seem to disappear later in life (Nomi and Uddin, 2015). The atypical within-network functional connections of the DMN and the insula could, based on previous research, be a potential biomarker for ASD. However, our findings suggest that this might not be the end of the story since we found this specific hyperconnectivity of the left anterior insula with the dorsal DMN only in children under the age of 12 with a mild form of ASD. This could indicate a specific biomarker for milder forms of autism. Consequentially, this could lead to earlier detection of ASD severity, which could in turn be beneficial towards earlier and more specific treatments for each individual child with ASD. This hypothesis needs further research that focuses on connectivity measures applied specifically to the insula within the dorsal DMN. Additionally, the role of the left anterior insula within this DMN should be studies more extensively. Even though we know the specific functions of the DMN as a whole (self-referential mental activity, stimulus-independent thought, monitoring somesthesia and vegetative information, inner speech, planning of future activities, creativity, self-awareness etc.; Callard and Margulies, 2014), our current

findings point towards a possible role of the left anterior insula within this network as an underlying cause of disability within mild forms of autism.

Additionally, ICA of functional data showed hypoconnectivity of the right superior parietal lobule within the auditory network in children with severe ASD compared to matched healthy controls. The auditory network is a network that can easily be defined during resting state fMRI because it represents spontaneous BOLD fluctuations that are common to the whole brain particularly well (Fox et al., 2008). However, no studies have found abnormal functional connectivity within the auditory network during resting state in ASD. There does seem to be evidence supporting the hypothesis that the social and language impairment, which are key symptoms of ASD, could be related to abnormalities in the auditory network (Russo, 2008). Furthermore, abnormal cortical auditory processing has also been connected to language impairments in children with autism (Boddaert et al., 2004). The superior parietal lobule on the other hand is found to be activated in healthy controls, but not in adults with ASD, during execution of a theory of mind task (Baron-Cohen et al., 1999). As a conclusion we can hypothesize that the auditory network and more specifically the right superior parietal lobule plays an important role in social interaction. The within-network hypoconnectivity of the auditory network with the right superior parietal lobule in severe autism compared to healthy controls could possibly underlie the deficits in social interaction. Additional research is needed to further support this hypothesis, specifically within resting state fMRI.

As a conclusion, we can state that ICA of functional connectivity in mild and severe ASD compared to healthy controls revealed two possible biomarkers for different levels of severity in ASD. First of all, within-network hyperconnectivity between the dorsal DMN and the left anterior insula could point towards milder forms of ASD. Behavioral implications of this abnormality however is not yet known and needs further research. Within-network hypoconnectivity of the auditory network with the right superior parietal lobule on the other hand could point towards more severe cases of ASD. This abnormal functional connectivity pattern could point towards difficulties in social interactions occurring in people with ASD. Taken together, both findings seem to indicate the possibility of hemispheric differences in mild versus severe autism. This

could be an interesting hypothesis towards further research onto functional connectivity patterns in autism.

Structural connectivity

Lastly, multivariate morphometric network analysis by means of SBM of anatomical data revealed differences in the anatomical structure of component 8, which mainly consisted of left frontal areas, between both ASD-mild and matched controls, and ASD-severe and matched controls. For future convenience we will call this gray matter network of component 8 the Frontal – Cingulate network, named after its main components. This Frontal – Cingulate network, which is centered in the frontal lobe with some connections to the temporal lobe (Sub Gyral) and the parietal lobe (Postcentral Gyrus), showed an abnormal expression in both severity groups compared to matched controls. Regions include the Inferior and Middle Frontal Gyrus, the Precentral Gyrus, the Sub-Gyral, Anterior Cingulate, the Medial Frontal Gyrus, the Superior Frontal Gyrus, the Postcentral Gyrus and the Cingulate Gyrus. This replicates some of these regions found in the whole-brain classification study of Ecker and colleagues (2010) namely the middle frontal gyrus, the inferior frontal gyrus and the precentral gyrus.

There is evidence from previous research that suggests that the frontal lobe plays a very important role in a social cognition network that relates to the social deficits known in autism (Baron-Cohen et al., 1999). In this previous study, deficits in the left dorsolateral prefrontal cortex (Brodmann area 44, 45 and 46) and the left medial frontal cortex (Brodmann area 9) were found while executing a theory of mind task in ASD. These Brodmann areas are also represented in the Frontal – Cingulate network in the following regions: the Inferior and Middle Frontal Gyrus, the Precentral Gyrus, the Medial Frontal Gyrus and the Superior Frontal Gyrus (for a detailed overview of the Brodmann areas in the Frontal – Cingulate network see Table 3). A number of structural neuroimaging studies have found frontal lobe abnormalities in patients with autism (Carper and Courchesne, 2000, 2005; Herbert et al., 2003; Salmond et al., 2003; Sundaram et al., 2008). These abnormalities in prefrontal cortical regions are known to be correlated with deficits in social cognition, executive functioning and language in patients with ASD (Sundaram et al., 2008). This accumulating evidence that supports

our findings leads us to believe that abnormalities in frontal lobe gray matter might be a core mechanism that underlies deficits in social functioning in ASD.

Additionally, abnormalities in the structural Frontal – Cingulate network include the Pre and Postcentral Gyrus. These regions correspond to the motor cortex and the somatosensory cortex, which is important for proprioceptive feedback during movement. Increased gray matter in both the Pre and Postcentral Gyrus has previously been shown in children with ASD (Bonilha et al., 2008). Motor impairments such as hypotonia and motor apraxia are categorized as associated symptoms to ASD, especially in young children (Ming, Brimacombe and Wagner, 2007). Even though motor impairment is considered an associated symptom, recent studies show that it could still prove to be useful in the diagnostic process of ASD (Torres et al., 2013). Our findings could thus reflect the underlying mechanisms of the occurrence of these associated motor symptoms in ASD.

The Frontal – Cingulate network also contains the Anterior Cingulate and Cingulate Gyrus, which is part of the limbic system. Structural brain differences in both structures, which replicate previous findings (Hyde et al. 2010), represent key symptoms of ASD. The cingulate cortices play a role in linking the perception of socially relevant stimuli to motivation, emotion and cognition (Adolphs, 2001). The cingulate gyrus is proven to be a socially relevant brain area that is known to be atypical in ASD (Hyde et al., 2010). The anterior cingulate gyrus on the other hand is known for its involvement in repetitive behavior (Whiteside, Port and Abramowitz, 2004; Hyde et al., 2010). Both social deficits and repetitive behavior are key factors in the symptomatology of ASD, it is therefore unsurprising that their underlying neural mechanisms show structural abnormalities compared to healthy controls.

Lastly, the Frontal – Cingulate network includes the Sub Gyral, which is part of the temporal lobe. Previous studies have indicated reduced white matter volume in the left Sub Gyral in young children with ASD (Bonilha et al., 2008) and greater bilateral activity of the Sub Gyral during imitation in older children with ASD (Williams et al., 2006). Furthermore, our findings of abnormalities in the Sub-Gyral in ASD replicate findings in a recent study by Grecucci and colleagues (2015, under review). Difficulties with imitation might be one of the primary deficits in ASD (Rogers, 1999), which could

be due to underlying structural abnormalities in the Sub Gyrus. Future research into this connection between imitation difficulties and structural Sub Gyrus abnormalities in ASD is needed to further substantiate this hypothesis.

As a conclusion, we can pose that our structural connectivity analysis revealed structural abnormalities in regions that are linked to social deficits, theory of mind, executive functioning, language, motor impairment, repetitive behavior and imitation. All of these have been linked to impairments in ASD which leads us to believe that an atypical structural network as found in the Frontal – Cingulate network might prove to be a core source of impairment. The Frontal – Cingulate network tends to have a negative expression in children with both mild and severe ASD compared to matched controls. This results in less gray matter volume in the regions observed in the Frontal – Cingulate network. However, changes in intra-connectivity are also a viable explanation for this different structural expression pattern in ASD. Further research focusing on connectivity methods applied to these areas is needed to further support this hypothesis.

Conclusion

In this study we examined possible biomarkers of ASD by means of functional and structural connectivity analysis of resting state fMRI. We can conclude that functional and structural connectivity analysis of resting state fMRI data in children with mild and severe autism reveals abnormalities compared to matched healthy controls. We found two possible biomarkers by means of ICA and one by means of SBM. Within-network hyperconnectivity between the dorsal DMN and the left anterior insula might be a possible biomarker for milder forms of autism whilst within-network hypoconnectivity of the auditory network with the right superior parietal lobule might indicate more severe forms of autism. Additionally, we found structural abnormalities in a gray-matter network (the Frontal – Cingulate network) that mainly consists of left frontal areas with connections to the temporal and parietal lobe in both severity groups compared to healthy controls. Whilst our findings in functional connectivity could be used to differentiate between mild and severe forms of autism, our findings in structural connectivity point towards a more unified abnormality that is consistent across all degrees of ASD severity.

Limitations of the study

First of all we only used male participants in our study, which limits our generalizability. This is somewhat countered by the fact that the male-to-female ratio of prevalence in autism is 3.3:1, which indicates higher prevalence in males (Baird et al., 2006).

Another issue is the high amount of movement within both ASD groups compared to the healthy controls. Even though we included the motion estimation as a covariate in our analysis, the movement estimations are much higher than the normally accepted threshold.

Lastly, we have a small sample size of 14 subjects per group which means that we should consider our results as preliminary. Future research should focus on replicating our findings before actual conclusions can be drawn.

Nederlandstalige samenvatting

Neuronale connectiviteit zou mogelijks een verklarend framework kunnen bieden voor autisme spectrum stoornissen. Autisme spectrum stoornis is een veelvoorkomende ontwikkelingsstoornis die gekarakteriseerd wordt door verstoerde sociale interactie, verstoerde communicatie, beperkte interesses en repetitief gedrag. Twee ogenschijnlijk contradictorische theorieën over de etiologie van autisme spectrum stoornissen, de onder-connectiviteitstheorie en de hyper-connectiviteitstheorie, kunnen mogelijks geïntegreerd worden in een meer gemengd en genuanceerd perspectief. In de huidige studie wordt gebruik gemaakt van een publiek beschikbare dataset genaamd ABIDE. Er wordt onderzoek gedaan naar de functionele en structurele connectiviteitspatronen van jonge kinderen (< 12 jaar oud) met ernstig en milde vormen van autisme. Beide groepen worden vergeleken met gezonde gematchte controle groepen. Independent Component Analysis (ICA) van functionele data vertoonde binnen-netwerk hyperconnectiviteit tussen het dorsale Default Mode Netwerk (DMN) en de linker anteriere insula als een mogelijke biomarker voor mildere vormen van autisme, en binnen-netwerk onder-connectiviteit van het auditieve netwerk met de rechter superieure pariëtale lobule als mogelijke indicatie voor ernstigere vormen van autisme. Verder vertoonde de Source-Based Morphometry (SBM) analyse structurele abnormaliteiten in een grijze-stof netwerk dat voornamelijk bestaat uit linker frontale gebieden met connecties naar de temporale en pariëtale lob in beide autisme groepen vergeleken met de controle groepen. Terwijl onze bevindingen met betrekking tot functionele connectiviteit mogelijks zouden kunnen differentiëren tussen milde en ernstigere vormen van autisme, tonen onze bevindingen in structurele connectiviteit net een algemene abnormaliteit over alle gradaties van autisme binnen onze studie.

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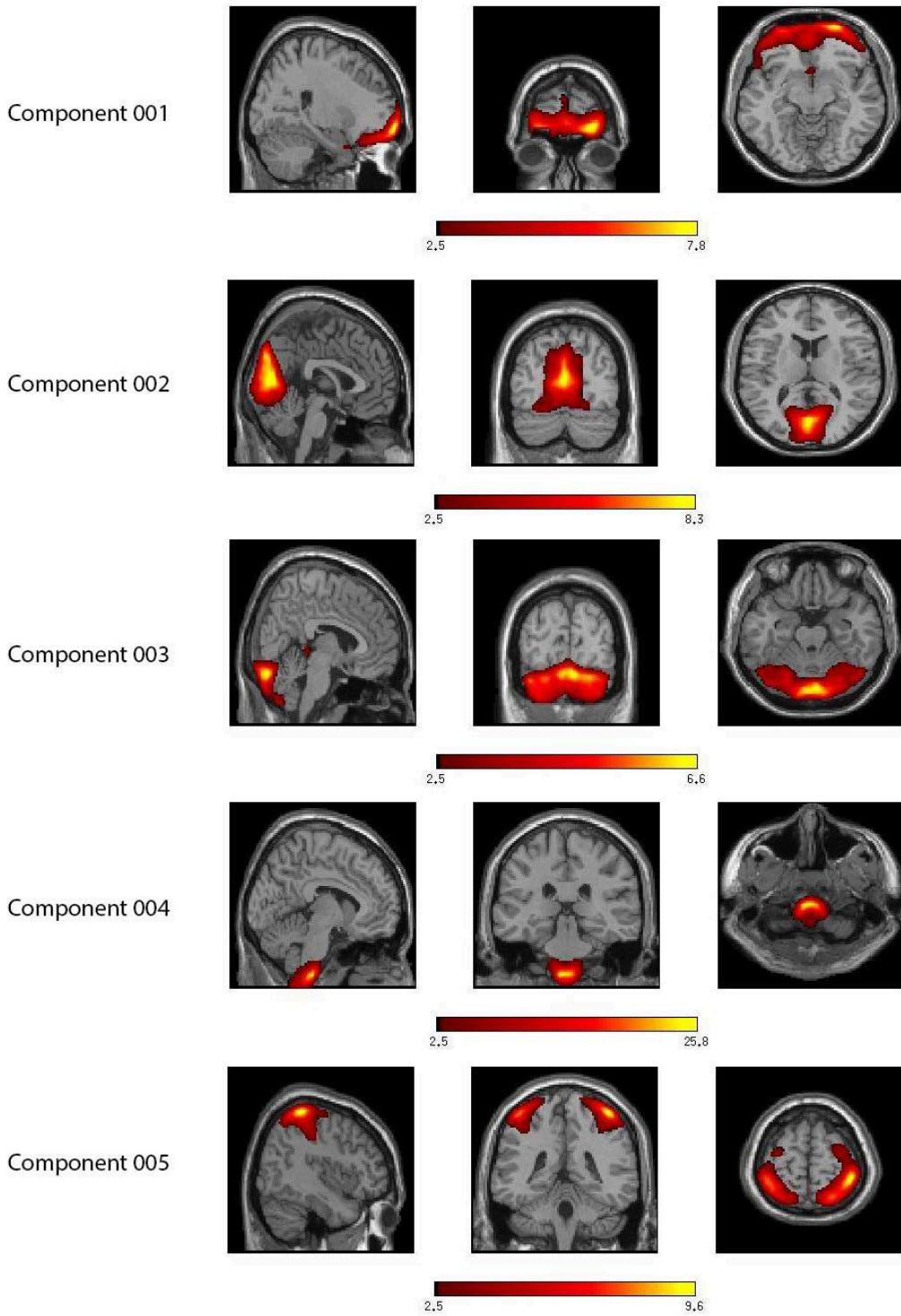
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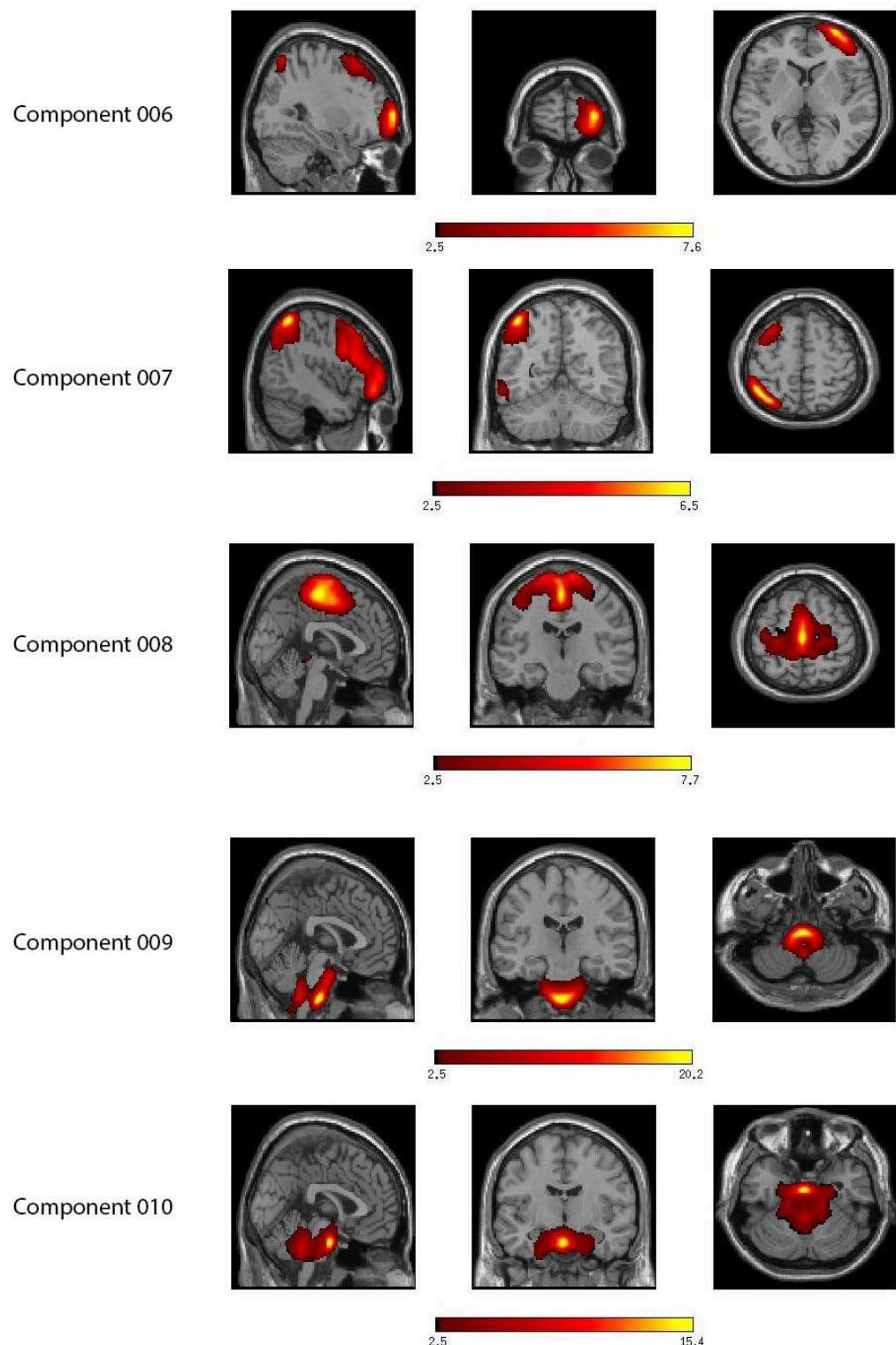
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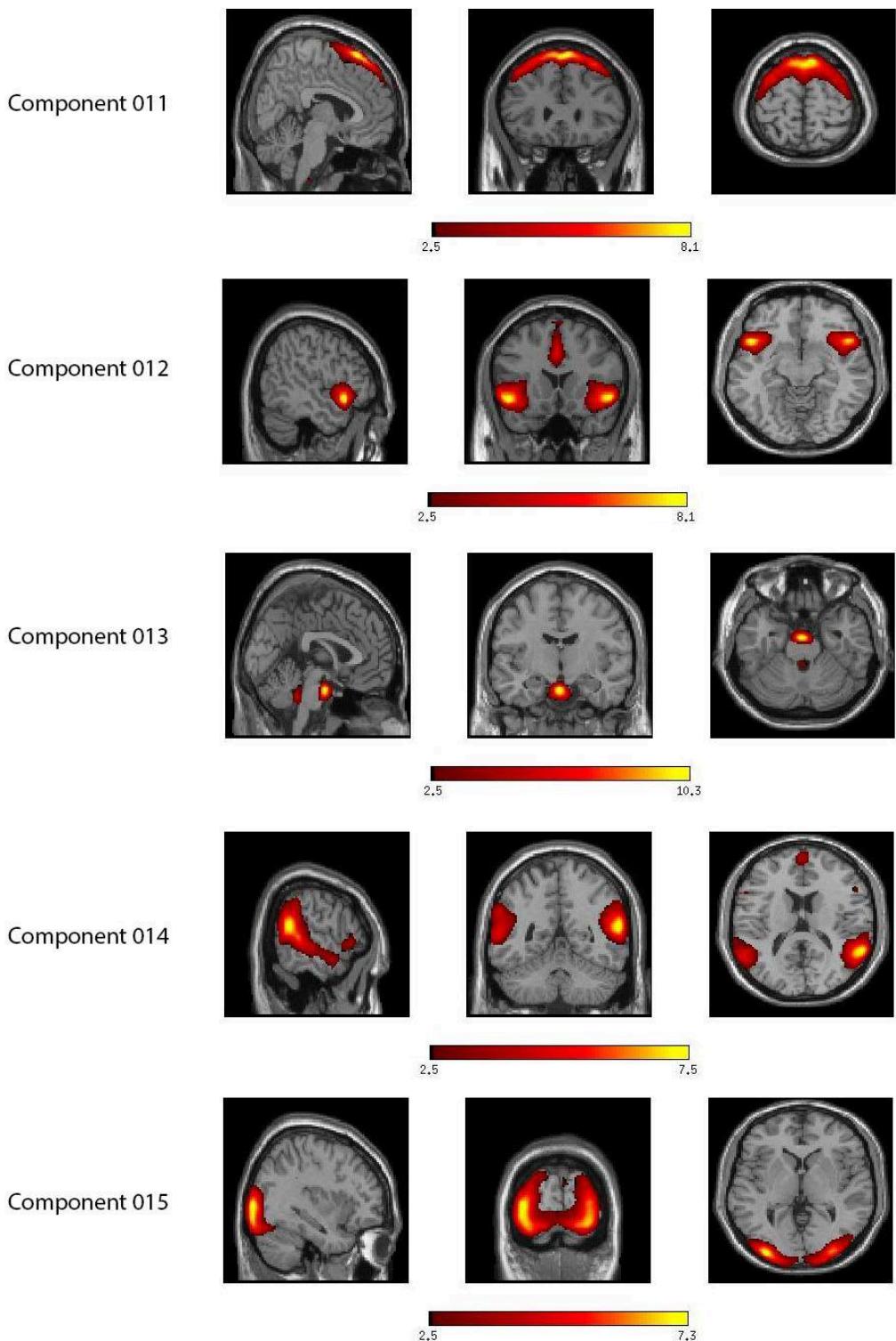
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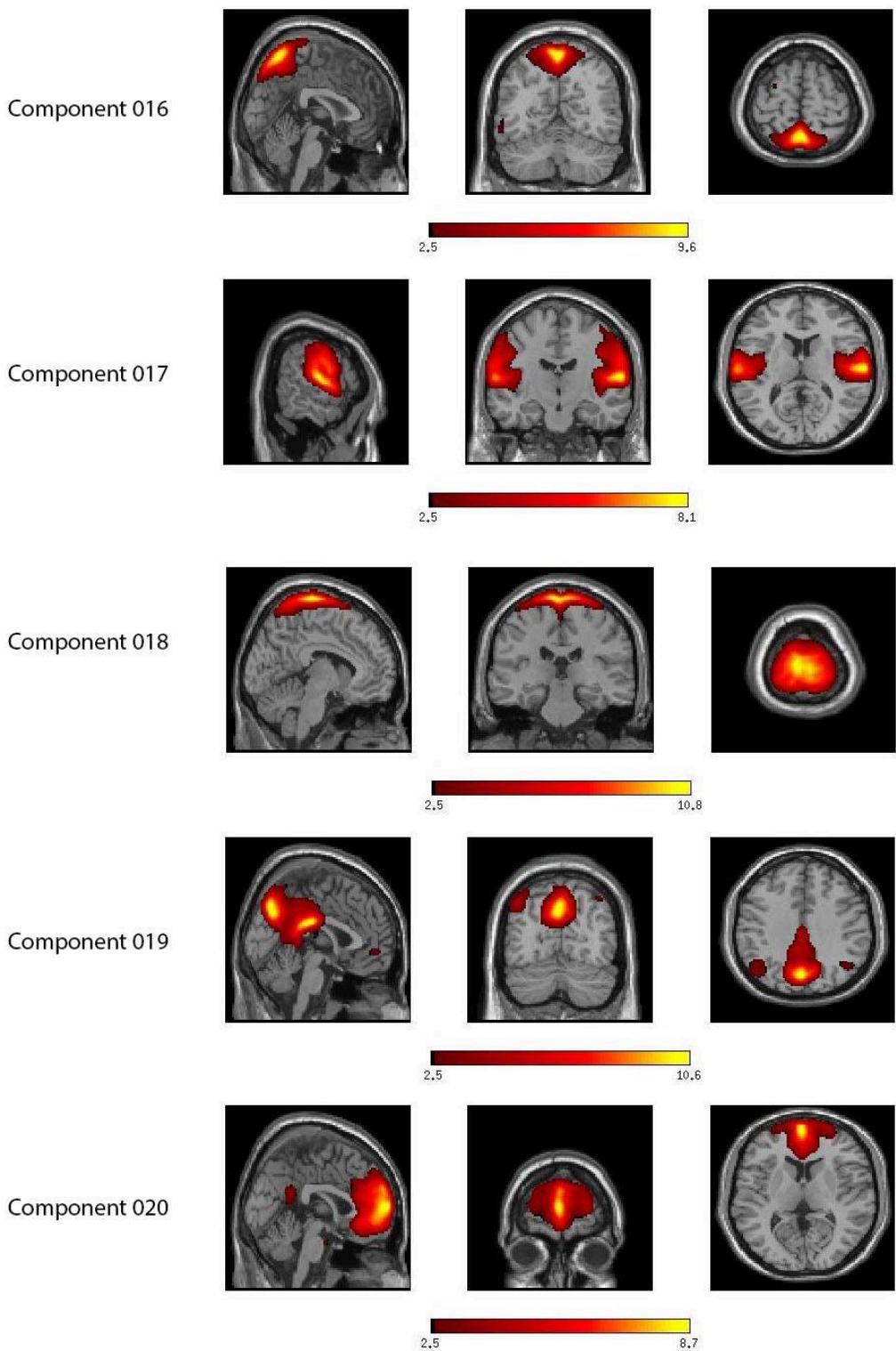
Appendix 1

Overview of the 20 independent components of our functional data.



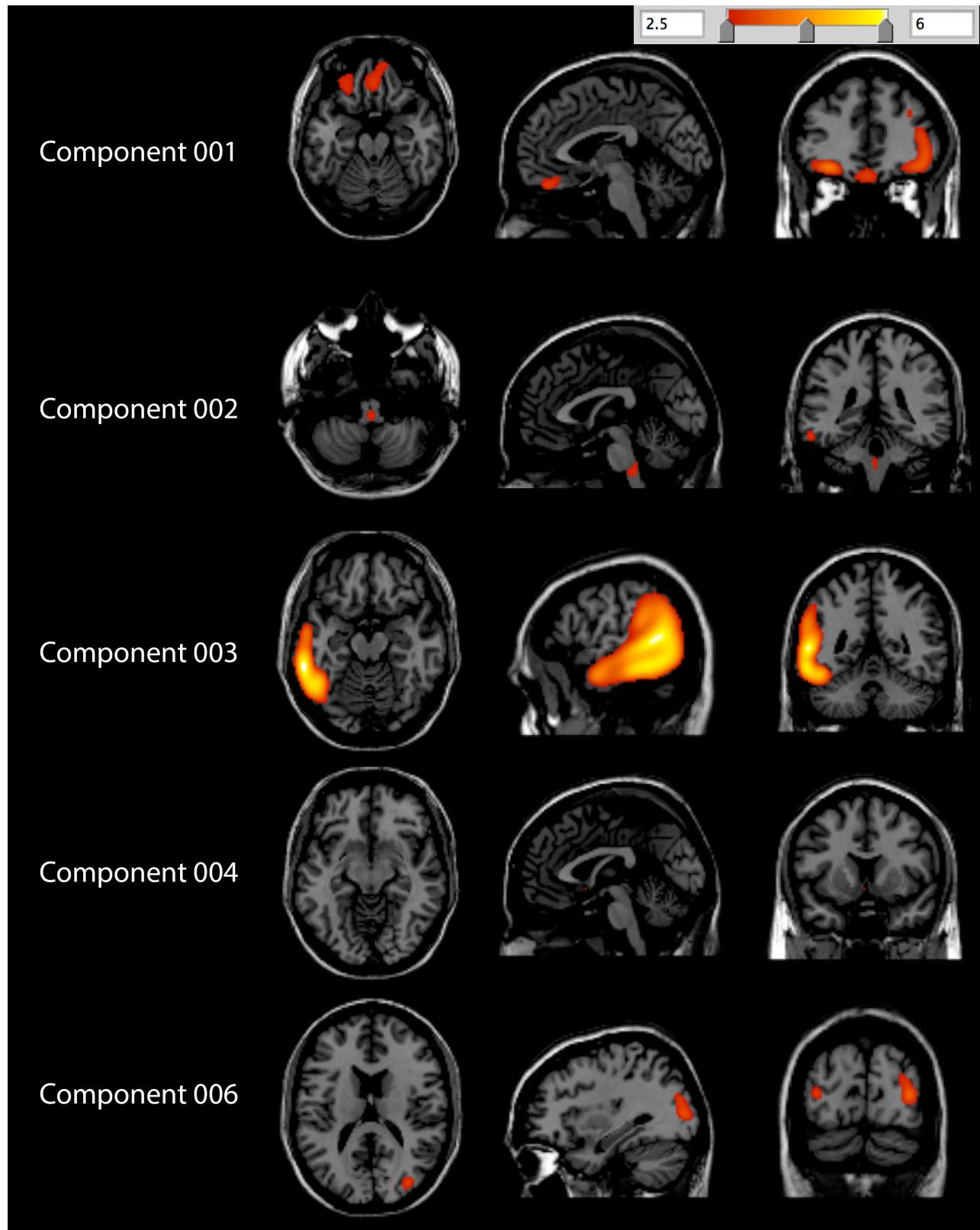


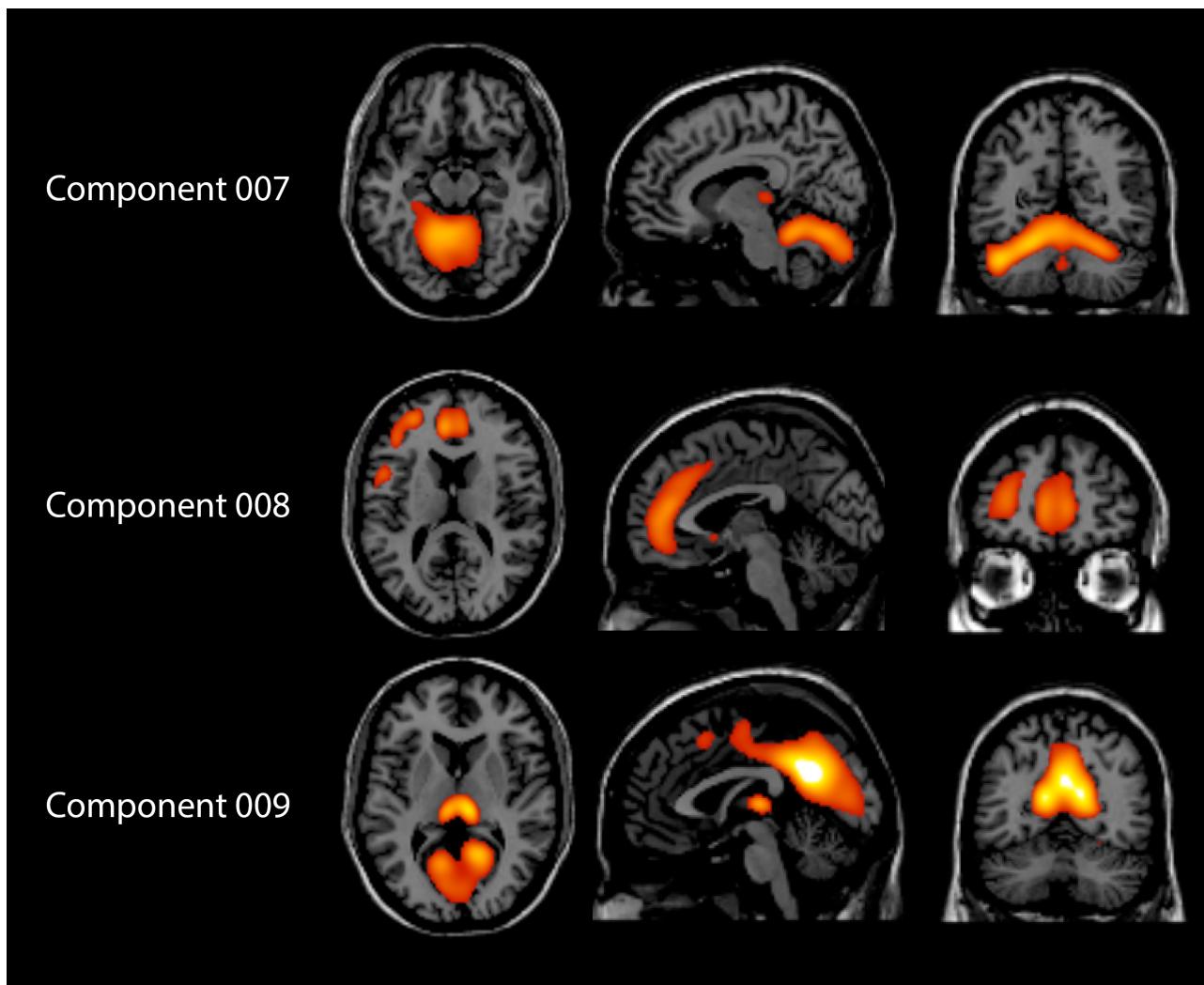




Appendix 2

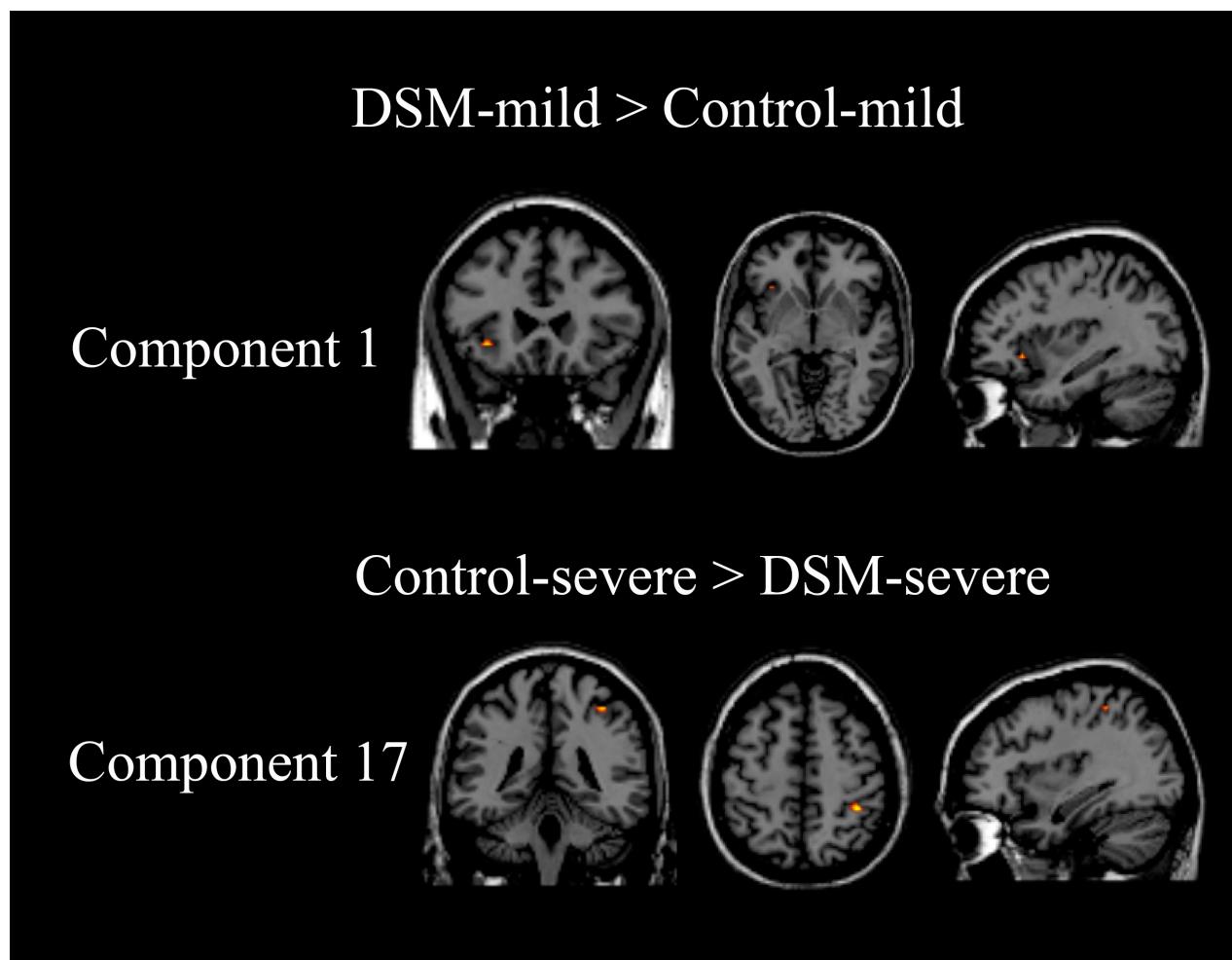
Overview of the 9 components of our anatomical data. Note that component 5 did not survive $Z>2.5$ thresholding and is therefore not included in the overview.





Appendix 3

Contrast maps of functional component 1 and 17. Only functional components 1 and 17 showed differences across our four groups. Contrasts resulting from the ANOVA of these components are shown. Coordinates of component 1: $x = -33$, $y = 24$, $z = -6$; peak t-value = 6.04 in the left anterior insula (voxel size = 1) which is part of the dorsal DMN (component 1). Coordinates of component 17: $x = 39$, $y = -42$, $z = 54$; peak t-value = 6.20 in the right superior parietal lobule (voxel size = 3) within the auditory network (component 17).



Appendix 4

Layout of SBM component 8 (Frontal – Cingulate network). Only our eight' component shows differences in the loading coefficient between our control groups and the ASD groups. Regions included: Inferior and Middle Frontal Gyrus, the Precentral Sulcus, the Sub-Gyral, Anterior Cingulate, the Medial Frontal Gyrus, the Superior Frontal Gyrus, the Postcentral Gyrus and the Cingulate Gyrus (see Table 2).

