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Investigating the cerebellar architecture in psychiatric disorders

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Investigating the Cerebellar Architecture in Psychiatric Disorders

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ABSTRACT

The human cerebellum is one of the earliest brain regions to develop, but continues to grow until late adolescence and adulthood. This makes it especially vulnerable for disorder and disease, specifically affecting neurodevelopmental disorders such as obsessive compulsive disorder (OCD) and autism spectrum disorder (ASD). Cerebello-cortical loops further allow the cerebellum to interact with the neocortex, enabling changes in the cerebellum to influence cortical structures.

This thesis aimed to identify cerebellar and neurocognitive mechanisms modifying (1) typical and (2) abnormal cerebellar growth and brain development. The first aim has been addressed in a series of two studies, exploring plastic changes in cerebellar and cortical functional connectivity (Chapter 2.1), volume, shape and white matter microstructure (Chapter 2.2) in healthy adolescents after learning to drum for 8 weeks. In order to explore distinct and shared cerebellar contributions to abnormal brain development, the role of the cerebellum and brainstem to classify adult and paediatric ASD was studied (Chapter 3.1); the reshaping of cerebellar and brainstem vertex (Chapter 3.2) and neocortical thickness and surface area (Chapter 3.3) with repetitive behaviours and symptom severity was explored in paediatric ASD and OCD. As compulsive and repetitive behaviours are one of the main measurements assessing diagnostic features in psychiatric animal models, distinct brain-behaviour correlates for OCD-phenotypical compulsive and anxious behaviours were studied in context of cerebellar insulin regulation in a mouse model of diabetes mellitus (Chapter 4). The data provided underline the cerebellum's central role in neurocognitive mechanisms and functions important for neurotypical development and functioning. While abnormal cerebellar development co-occurs with neocortical dysfunction and has been connected to a range of childhood disorders, its plastic changes to complex skill learning during adolescence also co-occur with neocortical functional and structural changes. This opens up an opportunity of the cerebellum as a target for possible early intervention for childhood disorders.

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I would also like to declare my sincerest thanks to my friends and family. To quote Chuck Palahniuk: "Nothing of me is original. I am the combined effort of everyone I have ever known." Thus, I would like to express my appreciation for every rare appearance of Basque Judit (as well as the regular ones); the long rides with Flavio lead astray by his 'Swedish lady' GPS system; the crying laughter and oversharing sessions with my L1.12 family (I am looking at you Rong and Sam); my long trips to Oxford visiting most of its pubs and some of its colleges; everyone at the CNS for the many discussions and sanity checks; everyone at St. Thomas, who just accepted that I appear to all of their Christmas parties, events and late night work sessions; the many adventures with my extended TACTICS family that have brought me to places all over Europe and helped me understand the true 'Psychopath Guide to Success'; my extended drumming family for drumming up a great connection and bond I cherish; my Norwegian support system that keeps on 'klikking' (tusen takk, Tore); my new extended family in Providence and the highly suspicious parking lots surrounding them – I am looking forward to taking the show on the road!; all the cerebellum enthusiasts I have ever met and will ever meet; and last but not least my fantastic parents and family - ohne euch wäre es nicht möglich gewesen!

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ABBREVIATIONS

Abbreviation	Meaning
AAL Atlas	Automated Anatomical Labelling Atlas
ACC	Anterior cingulate cortex
ADNI	Alzheimer's Disease Neuroimaging Initiative
ADI-R	Autism Diagnostic Interview - Revised
AKT	Protein kinase B
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
APFC	Anterior prefrontal cortex
ASD	Autism Spectrum Disorder
BBB	Blood-brain-barrier
BC	Basket cells
BDNF	Brain-derived neurotrophic factor
CC	Corpus callosum
CCAS	Cerebellar cognitive affective syndrome
CF	Climbing fibres
CN	Cerebellar nuclei
CNS	Central nervous system
CNTNAP	Contactin-associated protein-like 2
Cre	Creatine
CSF	Cerebrospinal fluid
CT	Cortical thickness
CY-BOCS	Children's Yale-Brown Obsessive Compulsive Scale
DARTEL	Diffeomorphic anatomical registration through exponentiated lie algebra
DCN	Deep cerebellar nuclei
DLPFC	Dorsolateral prefrontal cortex
DM1	Diabetes mellitus type 1
DM2	Diabetes mellitus type 2
DMS	Dorsal medial striatum
DOSS	Different onset, same slope
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion tensor imaging
EGZ	External germinal layer
EHI	Edinburgh Handedness Inventory
EPI	Echo-planar imaging
FA	Fractional anisotropy

FC	Functional connectivity
FDR	False discovery rate
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FSL	FMRIB Software Library
FWE	Family-wise error rate
FXS	Fragile X Syndrome
GABA	Gamma aminobutyric acid
GC	Granule cells
GL	Granule cell layer
Glc	Glucose
Gln	Glutamine
Glu	Glutamate
GM	Grey matter
GO	Golgi cells
GSH	glutathione
HRS	Hi-hat, Ride and Snare
ICD	International Classification of Diseases
ICP	Inferior cerebellar peduncle
ICV	Intracranial volume
IFG	Inferior frontal gyrus
IGF1	Insulin-like growth factor 1
IoPPN	Institute of Psychiatry, Psychology and Neuroscience
IPL	Inferior parietal lobule
IPS	Intraparietal sulcus
KCNQ1	Potassium voltage-gated channel subfamily Q member 1
L	Litre
LPARC	Left paracentral gyrus
LSFG	Left superior frontal gyrus
LU	Lugaro cells
M	Mesencephalon
MAPK	Mitogen-activated protein kinases
mcDESPOT	Multi-component driven equilibrium single pulse observation of T1/T2
MCP	Middle cerebellar peduncle
MD	Mean diffusivity
MIDI	Musical digital interface
ML	Molecular layer
ml+Gly	Sum of myo-Inositol and glycine
mm	Millimetre

mm^2	Square millimetre
MNI	Montreal Neurological Institute
MNI coordinates	Montreal Neurological Institute coordinates
MNS	Mirror neuron system
Mo	Mossy fibre
MOT	Primary motor cortex
MPFC	Medial prefrontal cortex
MP-RAGE	Magnetization prepared rapid gradient-echo
MRI	Magnetic Resonance Imaging
MRS	Magnetic resonance spectroscopy
ms	Milliseconds
MTG	Medial temporal gyrus
MTOR	Mammalian target of rapamycin
MVPA	Multivariate pattern analysis
NAA	N-acetylaspartate
NAcc	Nucleus accumbens
NP	Neuroplasticity
NTZ	Nuclear Transitory Zone
OCD	Obsessive Compulsive Disorder
OFC	Orbitofrontal cortex
PC	Purkinje cells
PCC	Purkinje cell cluster
pcd	Postconceptual days
PCL	Purkinje cell layer
pcw	Postconceptual weeks
Pcp	Purkinje cells precursors
pcy	Postconceptual years
PF	Parallel fibres
PI3K	Phosphatidylinositiol 3-kinase
PRESS	Point resolved spectroscopy
pSTG	Posterior superior temporal gyrus
PTEN	Phosphatase and tensin homolog
RAC1	Ras-related C3 botulinum toxin substrate 1
RBS-R	Repetitive Behaviours Scale - Revised
RL	Rhombic lip
ROI	Region of interest
RPCUN	Right precuneus
rs-FC	Resting state functional connectivity
RSFG	Right superior frontal gyrus
RTT	Rett Syndrome

SA	Surface Area
SC	Stellate cells
SCP	Superior cerebellar peduncle
SD	Standard deviation
SPM	Statistical Parametric Mapping
SPSS	Statistical Package for the Social Sciences
STG	Superior temporal gyrus
SUIT	Spatially unbiased infratentorial template
SVM	Support vector machine
Tau	Taurine
tCho	Total choline
TCV	Total cerebellar volume
TD	Typically developing
TE	Echo time
TH	TALLYHO/JngJ
TI	Time to inversion
TR	Repetition time
TSC	Tuberous Sclerosis
VAPOR	Variable pulse power and optimized relaxation delays
VBM	Voxel-based morphometry
vPMC	Ventral premotor cortex
VZ	Ventricular zone
WM	White matter

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DISSEMINATION

Peer-reviewed articles

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Book chapters

Bruchhage, M. M. K., Bucci, M.-P., Becker, E. B. E. (2018). Cerebellar involvement in autism and ADHD. In: Handbook of Clinical Neurology. Amsterdam: Elsevier.

Conference contributions

Best Trainee oral presentation, oral presentation and traditional poster presentation, ISMRM (2017)

Traditional poster presentation (no oral presentations available), Erice Golgi School "The cerebellum inside out: cells, circuits and functions" (2016)

Traditional poster presentation (no oral presentations available), ISMRM DTI Workshop (2016)

Digital poster presentation, ISMRM (2016)

Oral presentation, BCISMRM (2016)

Traditional poster presentation, KCL Research Student Showcase (2016)

Oral presentation, EU TACTICS steering committee meeting (2015, 2016)

ISMRM abstract reviewer (2016 -)

Invited lectures

Cambridge University (2018)

John's Hopkins University (2018)

UC Davis (2017)

King's College London (2017)

University of Chichester (2016)

University of Southampton (2016)

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'Introduction to Neuroimaging' course, BSc Biological Medicine, King's College London (2016-2017)

Organisation (*main organiser*) of IoPPN Friday Neuroimaging Seminars (2015-2017)

DECLARATION OF THE CANDIDATE'S ROLE

CHAPTER ONE: INTRODUCTION TO THE FIELD

All the work is the candidate's own. The candidate received constructive feedback from her supervisors Dr. Ali Amad, Prof. Christine Ecker and Prof. Steven C. R. Williams.

CHAPTER TWO: THE PLASTIC CEREBELLUM

In chapter 2.1 'Motor Learning induces Plasticity in the Plastic Brain – Drumming Up A Connection', the candidate contributed to the discussion and interpretation of the results with focus on the cerebellar findings. In chapter 2.2 'Give Me A Beat! Drum Training Induces Long-Term Plasticity in the Cerebellum and Connected Cortical Thickness', the candidate extracted, analysed all neuroimaging data and drafted the manuscript. The studies of this chapter have been written in concordance with journal submission guidelines, thus varying slightly in style and length. The candidate received constructive feedback from her supervisors Dr. Ali Amad, Prof. Christine Ecker and Prof. Steven C. R. Williams as well as her co-authors for all manuscripts.

CHAPTER THREE: THE DISORGANISED CEREBELLUM

The candidate performed data acquisition, recruitment and testing of the participants, as well as data extraction and analysis for the TACTICS (adolescent ASD and OCD) cohort contributing to chapter 3.1 'Don't Forget the Cerebellum and Brainstem when Predicting Autism Spectrum Disorder', 3.2. 'The Cerebellum and Brainstem Reshape with Compulsive Behaviour in Autism Spectrum Disorder and Obsessive Compulsive Disorder', and 3.3. 'The Contribution of Repetitive Behaviour to Shared and Distinct Brain-Behaviour Associations in Autism Spectrum Disorder and Obsessive Compulsive Disorder in the Neocortex', as well as and drafting of their manuscripts. However, data analysis in chapter 3.1 was based on freely available Matlab code and machine learning analysis was directed under supervision of Dr. Leon Aksman. The studies of this chapter have been written in concordance with journal submission guidelines, thus varying in style and length. The

candidate received constructive feedback from her supervisors Dr. Ali Amad, Prof. Christine Ecker and Prof. Steven C. R. Williams as well as her co-authors for all manuscripts.

CHAPTER FOUR: THE TRANSLATIONAL CEREBELLUM

For chapter 4 'Converging Evidence Points Towards a Role of Insulin Signalling in Regulating Compulsive Behaviour', the candidate extracted and analysed the MR data, and contributed to drafting the manuscript with focus on the discussion of the neuroimaging findings, methods and results. The candidate received constructive feedback from her supervisors Dr. Ali Amad, Prof. Christine Ecker and Prof. Steven C. R. Williams as well as her co-authors for all manuscripts.

CHAPTER FIVE: DISCUSSION

All the work is the candidate's own. The candidate received constructive feedback from her supervisors Dr. Ali Amad, Prof. Christine Ecker and Prof. Steven C. R. Williams.

CHAPTER ONE: INTRODUCTION TO THE FIELD



The cerebellum and brainstem as demonstrated on the inferior coronal plane here (image used from Gray's Anatomy*)*

I. CEREBELLUM – THE POWER OF THE 'LITTLE BRAIN'

From being described a "neuronal machine" (Eccles et al., 1967) to an 'internal model controller' (Ito, 2008; see chapter I.II), the cerebellum Latin for "little brain" seems central to many of our day to day functions.

Evolutionarily, the cerebellum also seems to be omnipresent, as it can be found in vertebrate animals including fish, reptiles and birds, and even in non-vertebrates such as the octopus ganglia (Hochner et al., 2006). Cerebellar vertebrate comparisons have further revealed an underlying evolutionary pattern within cerebellar neuroanatomy, with the floccular lobe and vermis representing phylogenetically older structures and the cerebellar hemispheres comparably newer regions. Synchronised with neocortical growth patterns, the cerebellar hemispheres are further expanded in mammals and primates, while in non-primates the paraflocculus seems to be enlarged (Ito, 2012). Evidence of the cerebellum's shared development with the neocortex is also reflected in their major anatomical and functional connections. However, cerebellar primate and human expansion was documented to be significantly faster than predicted by the change in neocortex size (**Figure 1**; Barton and Venditti, 2014), deviating from the evolutionary trend of tandem development.

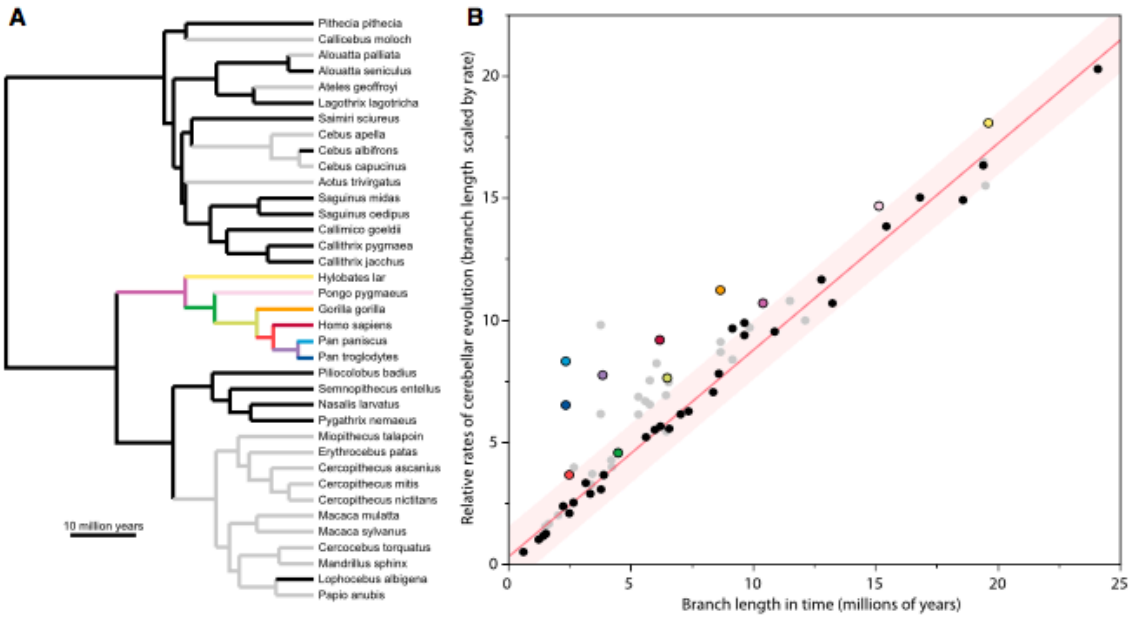


Figure 1. Relative rates of cerebellar evolution in anthropoid primates as a function of time showing (A) the topology of the phylogenetic tree; and (B) relative rates of cerebellar evolution controlling for rates of neocortical evolution on the y axis as a function of branch length on the x axis (image used from Barton and Venditti, 2014). The colour coding in (B) corresponds to the branches displayed in (A), with black and grey circles representing non-ape branches and coloured circles representing ape branches. The regression line with shaded 95% prediction intervals are fitted to non-ape branches, demonstrating increases in relative cerebellum volume.

As a result, the human cerebellum contains four times more neurons than the neocortex (Azevedo et al., 2009), underlining its central role in human neuroanatomy and function. This led Barton and Vendetti (2014) to suggest that natural selection acted disproportionally on cerebellar components during the evolution of humanoids, proposing that cerebellar specialisation contributed to the evolution of humans' advanced technological capacities, possibly in the form of a preadaptation for language. In order to understand the contribution of the human cerebellum to disorder and disease, one has to understand its development, neuroanatomy and connectivity. All three aspects will be reviewed in the following sections.

I.II. CEREBELLAR DEVELOPMENT

The human cerebellum is one of the earliest brain regions to develop (**Figure 2**, Silbereis et al., 2016; Leto et al., 2016), but continues to grow into adulthood (Tiemeier, 2010), making it especially vulnerable for disorder and disease (for example, Wang et al., 2014).

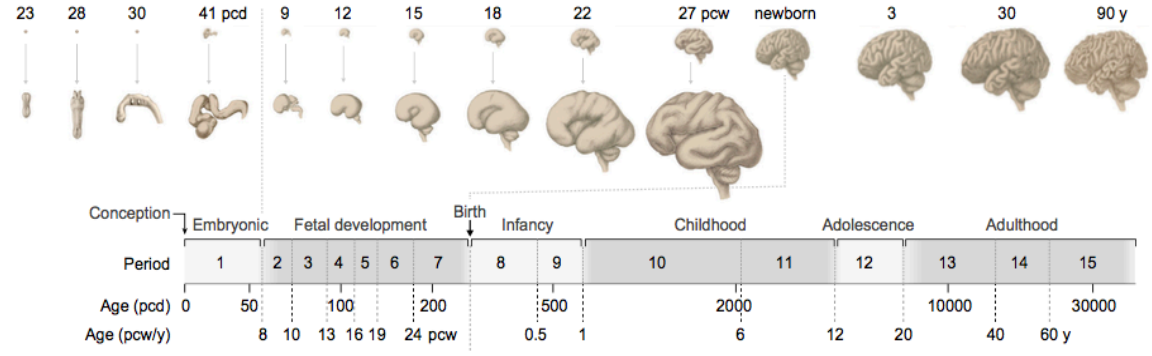


Figure 2. Timeline of key human neurodevelopmental processes (image used from Silbereis et al., 2016). Abbreviations: pcd, postconceptual days; pcw, postconceptual weeks; pcy, postconceptual years

At already week 7 after gestation, the superior cerebellar peduncle starts developing (Barkovich, 2005), a white matter tract connecting the cerebellum in a closed loop connection with the neocortex (Bostan et al., 2013). As a result, cortical afferent and efferent white matter tracts seem to form even if migration of some of the cerebellar cortical components is interrupted. Thus, any cerebellar injury at this developmental stage would still leave it grossly neurotypic in appearance, but possibly result in hypoplasia and affect ongoing neocortical development.

Following this development, the cerebellum begins to differentiate itself at week 9, with its afferent fibres starting to enter the pons and from there spreading throughout the intermediate zone of the prenatal rostral cerebellum. At 16 weeks of gestation, progenitor cells and neurons of the precerebellar nuclei of the lower rhombic lip migrate to the brainstem, where they develop to become the brainstem and deep cerebellar nuclei (Marzban et al., 2014). During this time (weeks 14 to 17), the infratentorial part of the brain (cerebellum and brainstem) contains <5% of the total brain weight but steadily increases, reaching 5% at 18 to 29 weeks and expanding to ≥6.5% by 40 weeks (Guihard-Costa and Larroche, 1990), leading the cerebellum to increase up to 10% of the total brain weight at birth, where it continues at the same growth rate as the rest of the brain (Ellis, 1920). While the overall cerebellum and anterior lobe steadily grow at weeks 15 to 28 leading to a 3.5-fold increase in cerebellar surface (Lemire, 1975), the floccular and posterior lobe decrease (9 to 6% and 80 to 72% respectively (Nowakowska et al., 2014). Different cerebellar regions further develop at different times, with the vermis starting to develop folia by weeks 13 to 14 and completing development by 2 months after birth (Loeser, 1972). After birth, the cerebellum continues to grow until late adolescence and adulthood, expanding laterally primarily from crus I and II in both human and non-human primates (Ito, 2012).

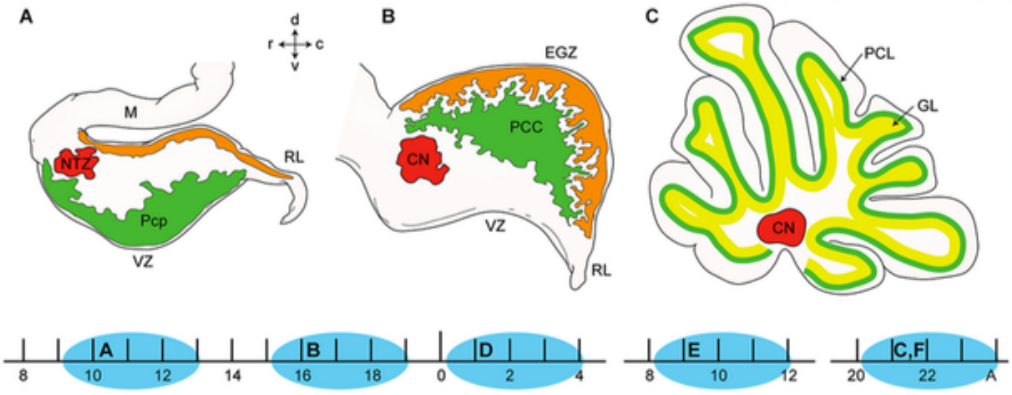


Figure 3. Early human pre- and postnatal development of the cerebellum for (A) days 10-13 after gestation, (B) days 16-17 after gestation and (C) 20 days postnatally (image used from Marzban et al., 2014). Abbreviations: M, mesencephalon; NTZ, nuclear transitory zone; Pcp, Purkinje cells precursor; RL, rhombic lip; EGZ, external germinal layer; PCC, Purkinje cell cluster; CN, cerebellar nuclei; VZ, ventricular zone; PCL, Purkinje cell layer; GL, granule cell layer

Microstructurally, the entire Purkinje cell population is generated before week 13 (**Figure 3**) and arranged in longitudinal clusters in the vermis and paravermis, starting to differentiate only afterwards. Specifically, Purkinje cell bodies are established at 28 weeks in the vermis and 32 weeks in the hemispheres (Friede, 1973). Purkinje cell neurons further rapidly expand in volume between birth and 2 years of age, with a second growth spurt between 7 and 9 years where final size is reached (Tsekhmistrenko, 1999). In contrast to this, granule layer neurons become progressively more clustered, reaching their plateau much later at 14-15 years (Tsekhmistrenko, 2001). This prolonged development further differs considerably from non-primate species (Zecevic and Rakic, 1976), possibly underlining cerebellar contribution to higher cognitive functions that are established during adolescence. Animal and human studies have further shown that these early developmental processes are paralleled by apoptosis (Lavezzi et al., 2006) and synapse elimination (Hashimoto and Kano, 2013), present from 17 weeks after gestation up until at least to 12 months postnatally. However, it remains unclear to what extent apoptosis and synapse elimination are related. Thus, principal cerebellar cytostructure is already set during early development, preceding neurogenesis and axonogenesis during which cerebellar circuits and functions are established and refined (Marzban et al., 2015).

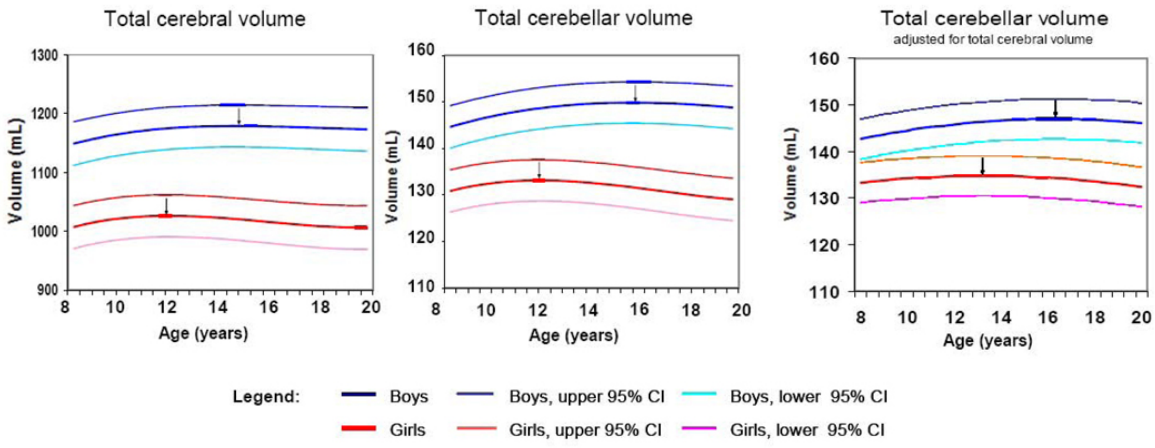


Figure 4. Developmental trajectories for postnatal cerebellar and cerebral development (image used from Tiemeier et al., 2010).

Postnatally, a longitudinal study investigating age ranges 5-24 years revealed that the cerebellum follows an inverted U shape when adjusted for total cerebral volume peaking at age 11.8 years in females and 15.6 years in males (**Figure 4**). Furthermore, cerebellar

volume was 10% to 13% larger in males even after correcting for total brain volume. Furthermore, phylogenetically older regions such as the vermis did not show significant changes during this developmental time, whereas recent regions such as the posterior lobe mature particularly late (Tiemeier et al., 2010). This parallels an evolutionary pattern seen in the neocortex, where the phylogenetically later prefrontal cortex, a structure the lateral posterior cerebellar lobules Crus I and II are reciprocally connected to (Bostan et al., 2013), matures later in development (Fuster, 2002; Gogtay et al., 2004).

I.II. CEREBELLAR ANATOMY

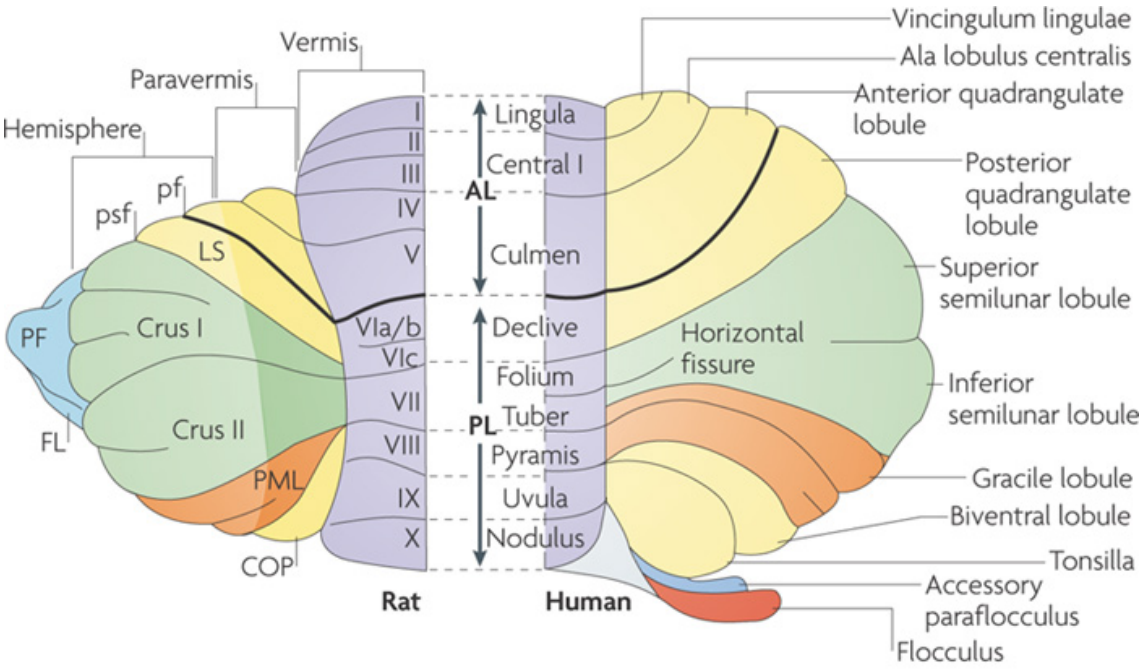


Figure 5. The neuroanatomy of the human and rat cerebellum compared (image used from Apps and Hawkes, 2009).

While Erasistratus (304-250 B.C.) was the first to distinguish the cerebrum from the cerebellum (Malomo et al., 2006), it took until the middle of the twentieth century to fully characterise cerebellar morphology (Jansen and Brodal, 1954; Larsell, 1970). When viewed from above, the human cerebellum appears like a butterfly, with both hemispheres expanding from its middle part, the vermis, like two wings. The primary and posterolateral fissure divide the cerebellar wings further into three parts: the anterior lobe, the posterior lobe, and the flocculondular lobe. Within each lobe, transversely running groves containing folia further subdivide the cerebellum into ten longitudinal

zones, the cerebellar lobules, laid along the vermis (**Figure 5**), a fundamental design common to various vertebrate species (Larsell, 1970). This cerebellar macro- and microstructure is highly conserved throughout species (**Figure 5**; Apps and Hawkes, 2009), making cerebellar findings in rodent models highly relevant for human translation.

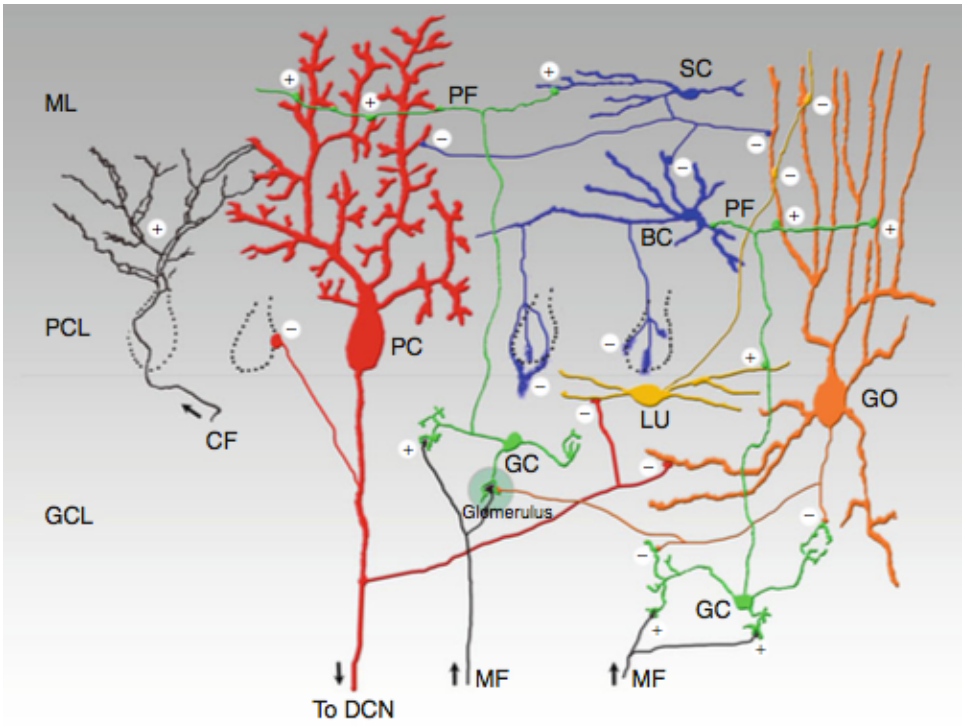


Figure 6. Basic circuitry of the cerebellar cortex (image used from Sassoe-Pognetto and Patrizi, 2013). Abbreviations: ML, molecular layer; PCL, Purkinje cell layer; GCL, granule cell layer; CF, climbing fibres; PF parallel fibres; PC, Purkinje cells; DCN, deep cerebellar nuclei; SC, stellate cells; BC, basket cells; LU, Lugaro cells; GO, Golgi cells; GC granule cells

Within the cerebellar cortex, three distinct layers emerge: the molecular layer containing stellate and basket cells; the Purkinje cell layer including Purkinje and candelabrum cells; and the granular layer containing granule, Golgi, unipolar brush and Lugaro cells (**Figure 6**). Along with some vestibular nuclei, cerebellar nuclei are the sole output of the cerebellum (Ito, 1984; De Zeeuw and Berrebi, 1995; Voogd et al., 1996) with their neurons located within the cerebellar white matter close to the roof of the fourth ventricle. These cerebellar output neurons can further be classified into inhibitory gamma aminobutyric acid (GABA) and excitatory glutamatergic neurons (Hoshino, 1996, 2006; Carletti and Rossi, 2008). GABAergic cells can be found widely distributed in the cerebellum (**Figure 7**) with Purkinje cells and candelabrum cells projecting out of the

cerebellar cortex, interneurons basket and stellate cells in the molecular layer, and Golgi and Lugaro cells in the granular layer. Glutamatergic neurons on the other side include granule cells receiving afferent input (**Figure 7**), unipolar brush cells and excitatory projection neurons (for a review, see Marzban et al., 2015). Thus, cerebellar nuclei can be classified as excitatory glutamatergic neurons projecting to different parts of the brain; inhibitory GABAergic neurons terminating in the inferior olive (Ruigrok, 1997); and inhibitory GABAergic and glycinergic neurons (Uusisaari et al., 2007, 2012).

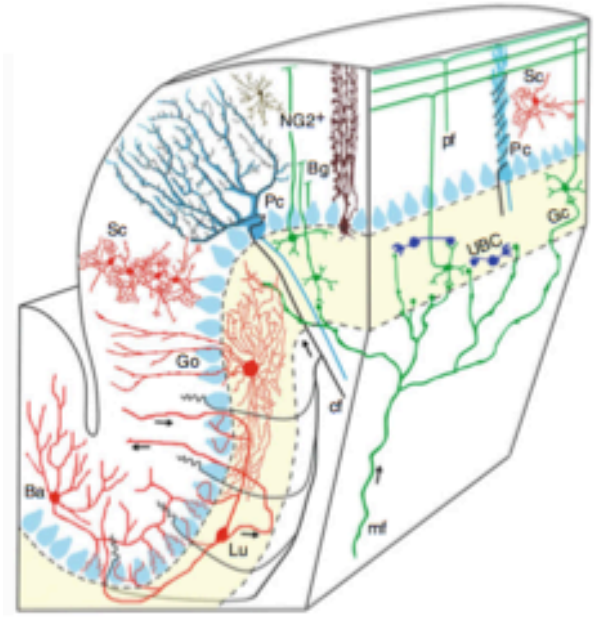


Figure 7. Distribution of inhibitory and excitatory pathways in the cerebellar cortex with the yellow background indicating inhibitory pathways and the outer cerebellar cortical layer indicating excitatory pathways (image used from Barmack and Yakhnitsa, 2013). Abbreviations: Ba, basket cell; Bg, Bergmann astrocyte; Gc, granule cell; Go, Golgi cell; Lu, Lugaro cell, NG2+, glia cell; Pc, Purkinje cell; pf, parallel fiber; Sc, stellate cell; UBC, unipolar brush cell

The cerebellum receives all afferent inputs and efferent outputs through its large white matter tracts – the cerebellar peduncles. Through mossy fibre inputs to the cerebellum, information about the current sensory and motor state of the organism is obtained and distributed across parallel fibre inputs or directly from a single climbing fibre to Purkinje cells. The mossy fibre-Purkinje cell pathway provides the core of cerebellar cortical neuronal circuits, connecting the cerebellar cortex to the cerebellar nuclei by Purkinje cell axons through three distinct ways (Ito, 2012). First, the vermis is connected to the medial (fastigial) nucleus, the intermediate part of the hemisphere to the interpositus nucleus (in humans the emboliform and globose nuclei), the lateral part of the hemispheres to the lateral nucleus (dentate nucleus in primates), and a part of the vermis and the flocculonodular lobe are connected directly to the vestibular and other nuclei in

the medulla oblongata. Second, afferent projections from the inferior olive to seven major zones of the cerebellar cortex. Third, these seven zones consist of specific sets of longitudinal Purkinje cell bands, generated by mitosis dependent on neuronal progenitor cell birth. Climbing fibre afferents for each cerebellar module form a specific subdivision of the contralateral inferior olivary complex, targeting one or more longitudinal zones of Purkinje cells within the cerebellar cortex (Watson et al., 2013). Each Purkinje cell is influenced by two major types of output: direct action of climbing fibres from the inferior olivary complex and indirect action of mossy fibres from a range of sources from the central nervous system via granule-cell parallel fibres and cortical interneurons (Apps and Garwicz, 2005). All afferents to the cerebellum send a direct branch to the cerebellar nuclei, which in turn receive Purkinje cell input essential for monitoring cerebellar out (Marzban et al., 2010, 2015; Hashimoto and Hibi, 2012), and terminate in all three layers of the cerebellar cortex (Jaarsma et al., 1997; Schweighofer et al., 2004). In turn, Purkinje cell axons are the sole output of the cerebellar cortex, which target the cerebellar and vestibular nuclei.

I.III. CEREBELLAR PATHWAYS

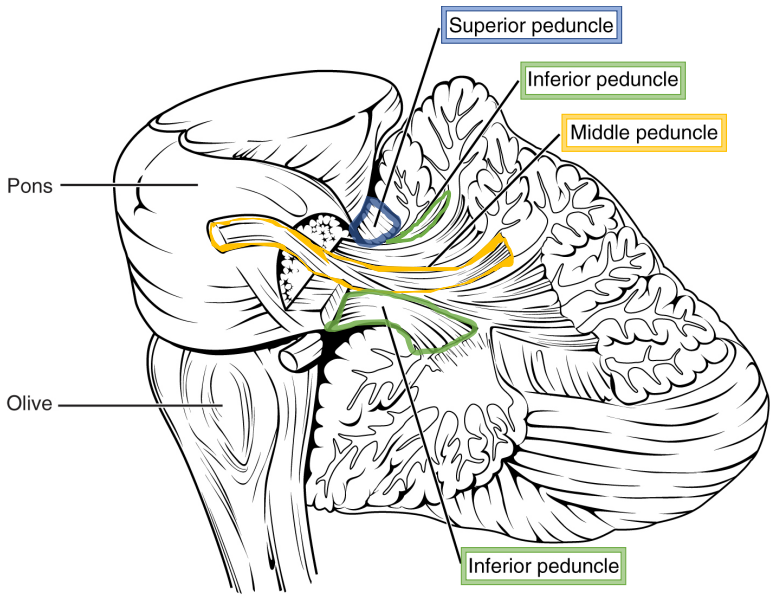


Figure 8. The cerebellar white matter pathways, namely the superior (blue), inferior (green) and middle (yellow) cerebellar peduncle (image used from Gray's Anatomy).

In contrast to the repeating and homogeneous structure of the cerebellar cortex, its connections to other brain regions areas are remarkably manifold. The cerebellum's

broadly distributed system of connections to the neocortex and spinal column is enabled by its three cerebellar peduncles (Figure 8). The inferior cerebellar peduncle (ICP) is a major afferent and efferent pathway, receiving signals both from the spine and olivary nucleus into the cerebellum and sending signals toward the vestibular nuclei. The middle cerebellar peduncle (MCP) is its largest afferent white matter pathway and carries input fibres from the contralateral cerebral cortex via the pontine nuclei across the vermis to the cerebellar cortex. Emerging from deep cerebellar nuclei, the superior cerebellar peduncle (SCP) travels ipsilateral into the dorsal pons, where it crosses at the level of the inferior colliculus to the cerebral cortex via the thalamus (Leitner et al., 2015). Specifically, there are three main pathways allowing flow of information between the cerebral cortex and the cerebellum: the cerebro-olivo-cerebellar pathway terminating in the cerebellar cortex as climbing fibres; the cortico-ponto-cerebellar pathway terminating as mossy fibres; and the cerebello-thalamo-cortico pathway arising from cerebellar nuclei projecting to the cerebral cortex. Converging evidence from monkey tracer studies reveal that the cortico-ponto-cerebellar pathway and the cerebello-thalamo-cortico pathway are interconnected projection pathways (Kelly and Strick, 2003; Bostan et al., 2013), establishing the cortico-ponto-cerebello-thalamo-cortical loop reciprocally connecting the cerebellum with the cerebral cortex.

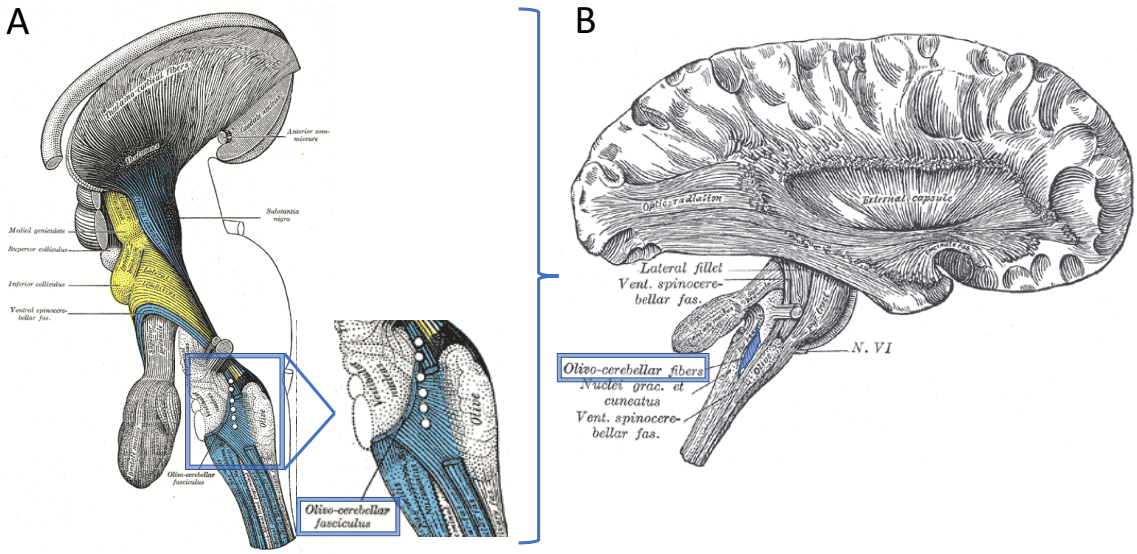


Figure 9. The vestibular nuclei and olivo-cerebellar fasciculus (blue) as demonstrated in a deep dissection of the brainstem (A), and olivo-cerebellar fibres (blue) as shown in their projection towards the cerebral cortex (B) (images used from Gray's Anatomy)

Cerebro-olivo-cerebellar pathways consist of olivo-cerebellar fibres conveying cerebral information via the climbing fibre system to the cerebellar cortex (Figure 9; for a review: Apps, 1999), as well as olivo-cortico-nuclear modules allowing Purkinje cells to project to specific regions of the cerebellar and vestibular nuclei (for a review: Ramnani, 2006; Figure 9). These pathways have been identified through electrophysiological mapping studies by recording climbing fibre responses in the cat evoked in the cerebellum after stimulating different areas of the cerebral cortex (Andersson and Nyquist, 1983). While the predominant projection arises from the motor cortex, numerous polysynaptic climbing fibre projections originate from sensory, motor and association areas of the cerebral cortex and are directed to the paravermal and hemispheral cerebellum (for a review: Allen and Tsukahara, 1974, Apps and Garwicz, 2005).

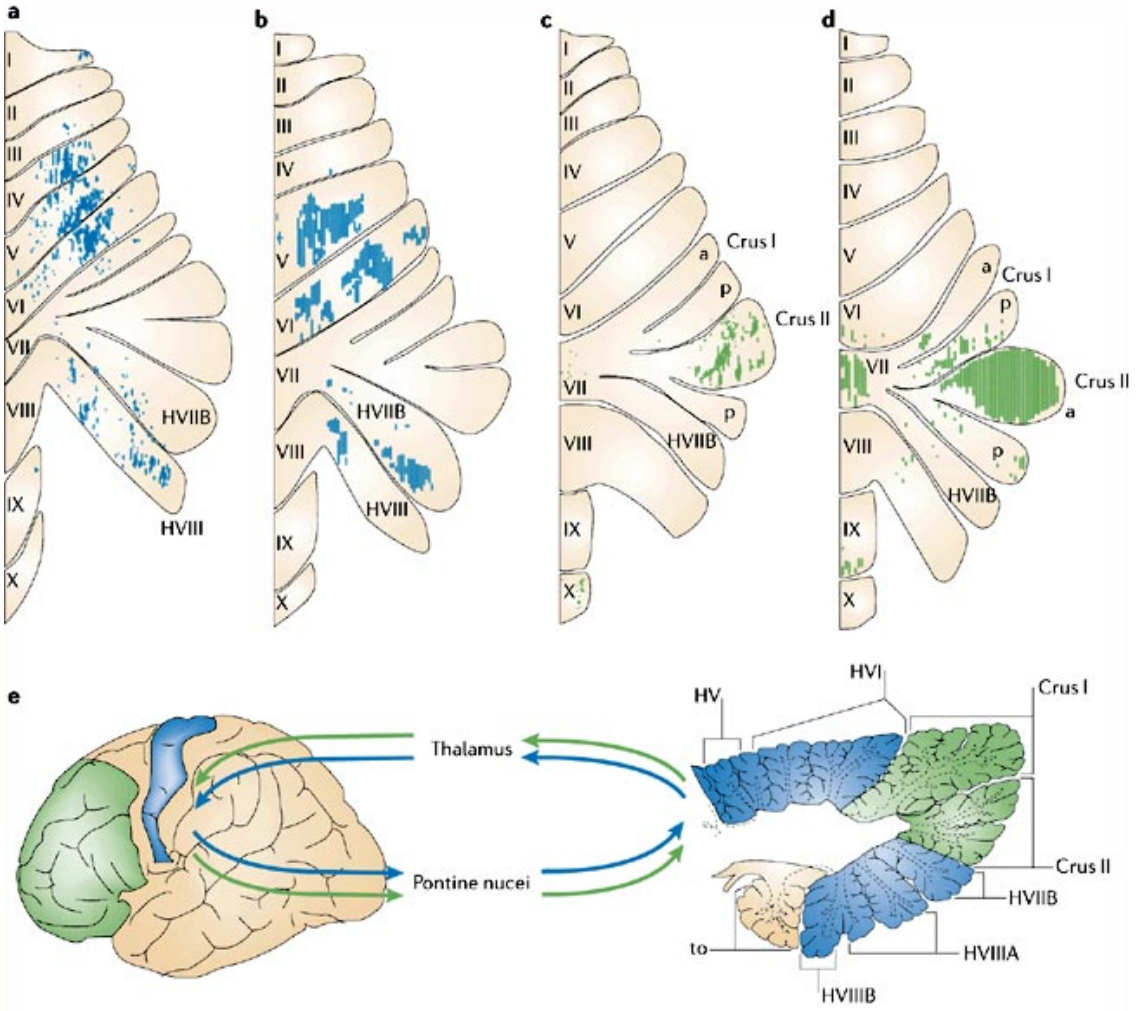


Figure 10. The cortico-ponto-cerebello-thalamo-cortical loop (*image used from Ramnani, 2006).*

In contrast to olivo-cerebellar pathways, the cortico-ponto-cerebello-thalamo loop enables flow of information from the cerebellum to the neocortex and back (Figure 10). Specifically, the cortico-ponto-cerebellar pathway projects to neocortical sensory and motor areas to the cerebellum via the pontine nuclei using corticopontine fibres. Corticopontine fibres descend in the internal capsule and collect ventrally at the base of the midbrain in the cerebral peduncles, constituting the great majority of cerebral peduncle fibres in the human and monkeys. While most pontocerebellar fibres terminate in the contralateral cerebellum, some show an ipsilateral projection. Here, input from visual association area (Broadmann 18) might be involved in regulating whole body movements, while inputs from the suprasylvian area coordinate the independent use of the limbs (Glickstein, 2013), thus suggesting an anatomical-function association not only for the cerebral but also the cerebellar cortex.

In turn, the cerebello-thalamo-cortico projections arise from the contralateral dentate, interpositus nuclei and the fastigial nuclei bilaterally. However, while its anterior regions primarily project to the lateral motor thalamus, its posterior regions project to the medial motor thalamus. From here, they project to the primary motor cortex (for a review see Jones, 2007), supplementary (Rouiller et al., 1994; Sakai et al., 1999) and pre-supplementary motor area (Sakai et al., 2003), the frontal eye field (Broadmann 8) and superior parietal lobule (Schmahmann and Pandya, 1990), as well as to prefrontal areas (Broadman 9 and 46: Middleton and Strick, 2001; Broadmann 45: Contini et al., 2010). Furthermore, monkey rabies tracer studies revealed that the motor cortex and prefrontal cortex are not only cerebellar output targets, but that the regions of the cerebellar cortex receiving input from these neocortical areas are the same as those projecting to it (Kelley and Strick, 2003). Thus, these closed-loop circuits represent a fundamental architectural feature of cerebro-cerebellar interactions, which have led many to speculate on whether these neuroanatomic connections in turn impact cerebellar functioning and connectivity.

I.IV. PLASTICITY

One way how cerebellar networks are able to impact both cerebellar and neocortical functioning and connectivity is through short- and long-term plasticity (Ito, 2012), where experience-dependent adaptive and learning processes act as a salient feature of cerebellar function (Apps and Garwicz, 2005; Miall et al., 2007). Cerebellar learning occurs in two stages, where cerebellar internal reorganisation is followed by plastic changes in the cerebral cortex (D'Angelo, 2014; Li Voti et al., 2014). Due to the vast connections of the cerebellum, the extent of its plastic reorganization as an effect of learning expands to both grey and white matter properties even outside the structure itself (Pecenka et al., 2013; Schlaug, 2001), which can in turn be measured by cortical thickness (Anurova et al., 2015).

In order for the cerebellum to learn for example a new motor movement, it has to create internal predictions for sensory and motor function (Ito, 2012) as well as limb dynamics prior to the actual movement (Ito, 2008). Motor training has been shown to increase posterior cerebellar volume and cortical thickness of parietal and frontal regions (Kelly and Strick, 2003; Szpunar et al., 2007; Chen et al., 2008; Park et al., 2009; Salmi et al., 2010; Bernard et al., 2015; Riedel et al., 2015), and change white matter microstructure

of the inferior cerebellar peduncles (Ramnani and Passingham, 2001; Langheim et al., 2002; Lotze et al., 2003; Drijkoningen et al., 2015). In order to learn efficiently, the volume of structures that no longer need consulting decrease, whereas the ones that do increase. Specifically, motor training in rats increases cerebellar glial volume per Purkinje cell (Drijkoningen et al., 2015; Langheim et al., 2002; Lotze et al., 2003; Ramnani and Passingham, 2001), volume of the molecular layer (Ito, 2012), number of synapses (D'Angelo, 2014; Li Voti et al., 2014) and dendrite of stellate cells (Apps and Garwicz, 2005; Miall et al., 2007). However, cerebellar plastic changes are not restricted only to motor function but also cognitive and emotional cerebellar functions (Schutter, 2013) and can in turn modulate cerebellar connectivity (Amad et al., 2015).

II. CEREBELLAR FUNCTIONS AND CONNECTIVITY

After observing the giraffe's significantly larger cerebellar lobule VI, Bolk (1906) considered that this region might be responsible for the precise control of the animal's impressive neck and shoulder muscles.

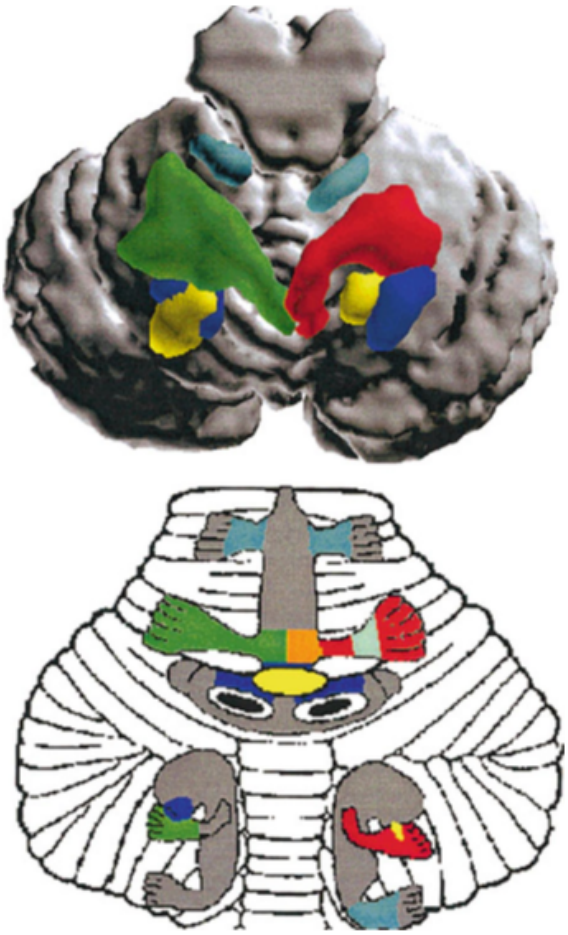


Figure 11. Functional (top) and structural (bottom) cerebellar somatotopic map for sensorimotor control (image used from Stoodley et al., 2013). Colours: blue, feet; green, left hand; red, right hand; yellow, tongue; dark blue, lips; light brown, elbow; grey-green, wrist

Indeed, upside-down somatotopic body maps in the anterior lobe and lobule VIII as previously established in electrophysiological studies (Snider and Eldred, 1951), have been confirmed by functional magnetic resonance imaging (fMRI) studies (**Figure 11**, for an overview, Stoodley et al., 2013). Here, leg and foot sensorimotor representation are localised in lobules II and III (Nitschke et al., 1996), hand representations in lobule V (e. g. Grodd et al., 2001) and orofacial movements in lobule VI (e. g. Dresel et al., 2005). In line with neuroanatomic connections, the ipsilateral site of the cerebellum is active to the body part being moved (for meta-analysis, Stoodley and Schmahmann, 2009). Interestingly, two clusters in the sensorimotor cerebellum are activated during

articulation: lobule IV-VI and VIII, Riecker et al., 2005). These two cerebellar regions are suggested to represent two different networks, the 'preparation' network (lobule IV-VI, pre-SMA, prefrontal cortex) with its activation preceding articulation, and the 'execution' network facilitating the actual movement (lobule VIII, M1, thalamus and striatum).

Results from clinical studies investigating effects of cerebellar damage or degeneration also provide support for cerebellar functional topography and stratification of inter-cerebellar networks. Specifically, damage to the anterior cerebellar lobe and lobules VIII and IX of the posterior cerebellum are more often associated with motor impairment (Konczak et al., 2005; Schoch et al., 2006; Ilg et al., 2008), whereas patients with strokes involving lobule VI experience rather minimal motor impairment (Schmahmann et al., 2009), and injury to posterior cerebellar regions has been connected to cognitive deficits (for a review, Stoodley et al., 2013). These clinical observations are in line with cerebellar neuroanatomic connections, as anterior cerebellar regions have been shown to project to and from sensorimotor areas in the neocortex, whereas the posterior cerebellar lobe is connected with the association cortices. Paralleling these structural differences, a recent meta-analysis using an activation likelihood estimation model (Stoodley and Schmahmann, 2009) revealed a functional division of the cerebellum into the 'sensorimotor cerebellum' (anterior lobe, lobule VI and VIII) and the 'cognitive cerebellum' (vermis of VI and VII, the hemispheres of lobule VI, Crus I/II and VIIb). Furthermore, in patients who show both motor and cognitive symptoms, language and cognitive difficulties can persist after cerebellar motor symptoms have resolved (Marien et al., 1996; Fabbro et al., 2004). This clinical observation has led to the establishment of the cerebellar cognitive affective syndrome (CCAS; Schmahmann and Sherman, 1998), which is defined by deficits in language, spatial processing, working memory and affective regulation after cerebellar posterior lobe damage. Further supporting this, damage to the right cerebellar hemisphere has been shown to result in language difficulties, whereas spatial difficulties can follow left cerebellar hemisphere lesions and damage to the vermis have been associated with social and affective processing deficits (for a review, Stoodley et al., 2013). Interestingly, motor and cognitive deficits after cerebellar damage tend to not be correlated (Gottwald et al., 2003; Stoodley and Schmahmann, 2009), suggesting that higher order deficits as revealed by cognitive tasks are not solely due to motor

dysfunction. However, as not all cerebellar patients show motor deficits, not all patients with cerebellar damage or degeneration display cognitive or affective symptoms (e.g. Richter et al., 2007).

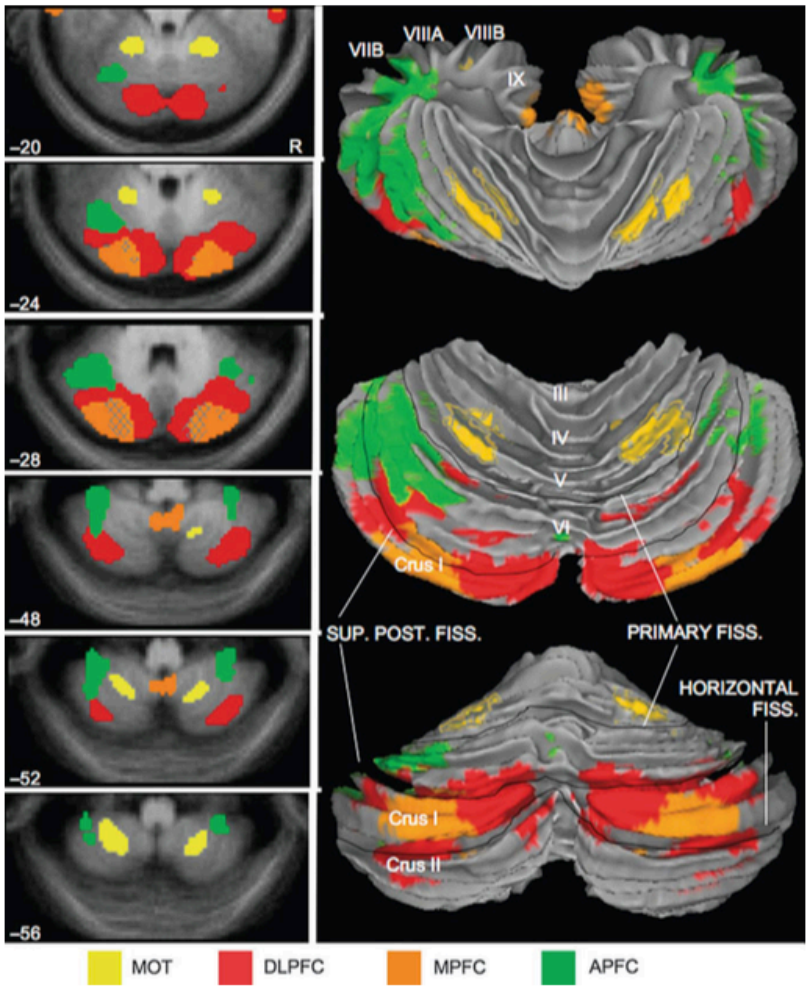


Figure 12. Resting state functional connectivity between the cerebellum and primary motor cortex (MOT, yellow), dorsolateral prefrontal cortex (DLPFC, red), medial prefrontal cortex (MPFC, orange) and the anterior prefrontal cortex (APFC, green) with MOT correlations being present in cerebellar lobule IV-V and prefrontal cortex correlations are present in lobule VI and VII (image used from Krienen and Buckner, 2009).

Functional connectivity resting state analyses also support the existence of different, topographically organised loops with sensorimotor or association cortices (Figure 12, Habas et al., 2009; Krienen and Buckner, 2009; O'Reilly et al., 2010; Buckner et al., 2011). Specifically, lobule VII has been shown to be functionally connected with frontal and parietal cortices, which are not directly involved in sensorimotor processing (O'Reilly et al., 2010), whereas the dorsolateral prefrontal cortex has displayed functional

connectivity with lobule VII including Crus I/II, the medial prefrontal cortex with Crus I, and the anterior prefrontal cortex with lobules VI and Crus I/II (Krienen and Buckner, 2009). Furthermore, posterior cerebellar regions VI, VII and IX correlate with functional networks including the executive control network (particularly Crus I/II), the salience network (lobule VI) and the default network (lobule IX; Habas et al., 2009) and resting state connectivity studies have connected limbic and paralimbic neocortical areas with cerebellar Crus I and II (Habas et al., 2009; Krienen and Buckner, 2009; O'Reilly et al., 2010; Buckner et al., 2011). Cerebellar activation found in studies investigating the neural correlates of pain anticipation, thirst, hunger and smell support the role of the cerebellum in limbic functions (for a review, Blatt et al., 2013). Specifically, the cerebellar vermis was activated during panic, sadness and grief (Reiman et al., 1989; Beauregard et al., 1998; Gündel et al., 2003); whereas autonomic processing (Critchley et al., 2000) and hunger (Evans et al., 2002) activated posterior cerebellar regions (both vermis and hemispheres). This opens up a debate on how these widespread cerebellar functions can be modelled and how cerebellar injury and dysfunction could affect it.

III. CEREBELLAR MODELS

Early lesion studies by Rolando and Flourens (Flourens, 1822) in the nineteenth century revealed the importance of the cerebellum in motor action and movement coordination, leading Flourens to suggest the cerebellum as a seat for motor learning. Since then, studies have shown that the cerebellum expresses a range of different functions in addition to motor action, including language, working memory and emotion regulation (see chapter I.II). This manifold of cerebellar functions combined with its clear topography and consistent microstructure has attracted several researchers to create conceptual frameworks, of which the three most dominant ones will be reviewed here.

III.I. ADAPTIVE FILTER MODEL (AFTER MARR-ALBUS-FUJITA)

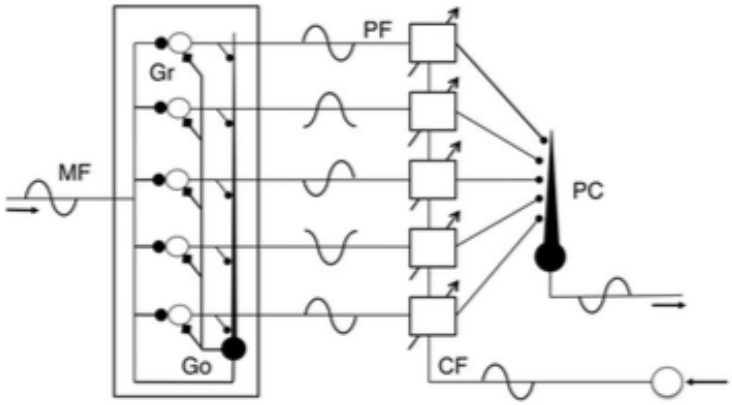


Figure 13. Fujita's adaptive filter model of the cerebellum (image used from Ito, 2012). Abbreviations: MF, mossy fibre; Go, Golgi cell; Gr, granule cell; PF, parallel fibre; CF, climbing fibre; PC, Purkinje cell

One of the most prominent theories trying to explain cerebellar functioning is the Marr-Albus-Fujita adaptive filter model, building on Marr's and Albus' original theories, which were developed nearly a decade earlier. While these early theories managed to explain how the cerebellum discriminates spatial patterns, Fujita's (1982) contribution was to demonstrate how it also discriminates temporal patterns.

After the Marr-Albus-Fujita adaptive filter model, a mossy fibre granule cell – Golgi cell circuit generates a set of mossy fibre input (**Figure 13**). The generated parallel fibre signal outputs are then gathered together through variable synaptic connections. These in turn form a Purkinje cell output converging to the desired response. In addition, climbing fibres encode possible errors and can suppress the Purkinje cell synapses involved in such erroneous performance with the help of long-term depression, refining

the desired response. This way, during rehearsal of an action, cerebellar Purkinje cells learn to recognize contexts and create an anticipatory neural state (Bhanpuri et al., 2013). Once the action has been learned, the occurrence of the context alone is enough to fire the Purkinje cells and cause the next elemental reaction, thus acting as an adaptive filter. This increase in efficiency in turn decreases cerebellar activity in the region used, which enables a resource allocation to other areas (Pecenka et al., 2013; Schlaug, 2001). As a result, an increase in cerebellar activity would induce a decrease in activity in cortical target regions and vice versa (Kelly and Strick, 2003; Szpunar et al., 2007; Chen et al., 2008; Park et al., 2009; Salmi et al., 2010; Bernard et al., 2015; Riedel et al., 2015).

III.II. DYSMETRIA OF THOUGHT THEORY (AFTER SCHMAHMANN)

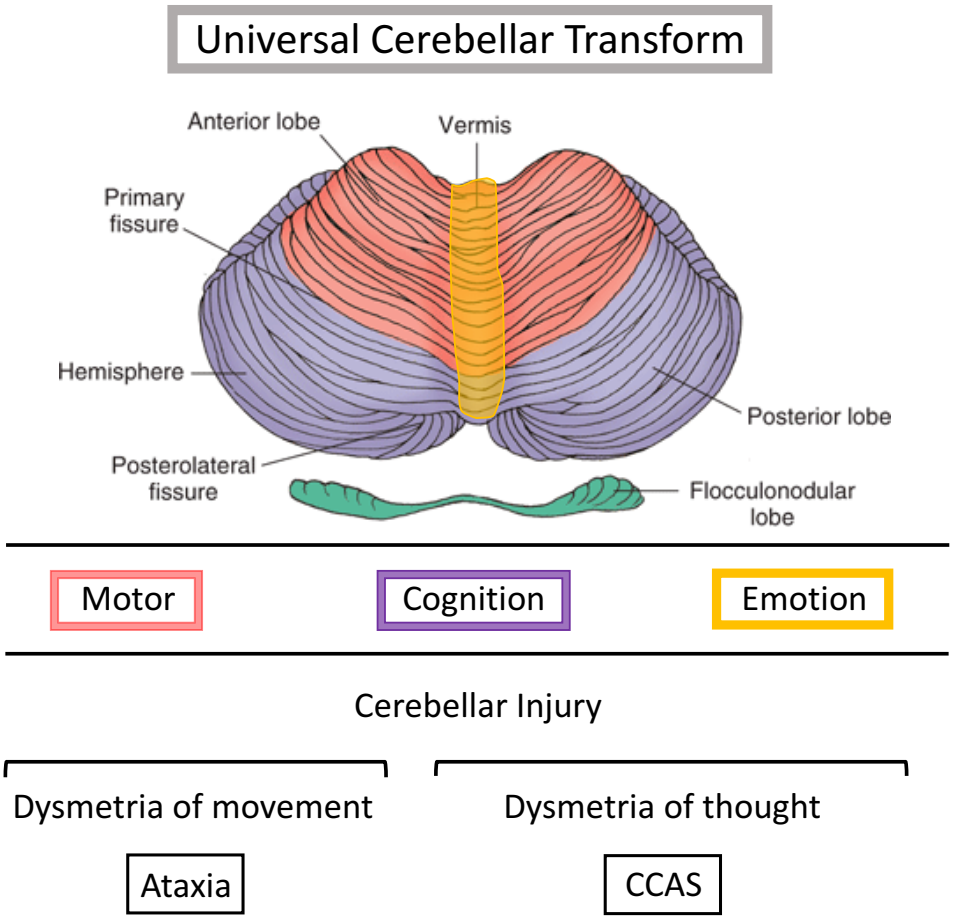


Figure 14. Schmahmann's dysmetria of thought theory with the anterior cerebellar lobe in red, cerebellar vermis in yellow, and posterior lobe in lilac (image adapted from Gray's Anatomy).

Just as the adaptive filter model, the dysmetria of thought theory is based on the uniformity of cerebellar microstructure. However, it does so by focusing on the uniform integration of multiple internal representations, where external stimuli and self-generated responses are integrated implicitly, thus automatic and without conscious awareness. It has been put forward that it does so by using the Universal Cerebellar Transform (Figure 14; Schmahmann and Scherman, 1998; Schmahmann, 2010; Guell et al., 2017), aiming to maintain a balanced homeostatic baseline by smoothing out, modifying and optimizing performance according to context. The modulation permits the cerebellum to harmonise motor, cognitive and affective/ autonomic behaviours, regulating their rate, force, rhythm and accuracy of motor as well as the capacity, consistency and appropriateness of cognitive and emotional processes. As the Universal Cerebellar Transform acts globally, its impairment called dysmetria (a Greek term referring to the lack of coordination or movement) acts equally universal. Following the cerebellar functional division (see chapter I.II), damages to the motor cerebellum manifests as ataxia, whereas lesions to the posterior cognitive cerebellum or the cerebellar vermis affecting the limbic cerebellum result in dysmetria of thought – all symptoms found in the CCAS. Furthermore, the dysmetria of thought theory has been used to explain cerebellar dysfunction in several disorders, ranging from ataxias (Manto and Mariën, 2015) to Alzheimer's disease (Jacobs et al., 2017) and autism spectrum disorder (Phillips et al., 2015).

III.III. THE CEREBELLUM AS AN INTERNAL MODEL CONTROLLER (AFTER ITO)

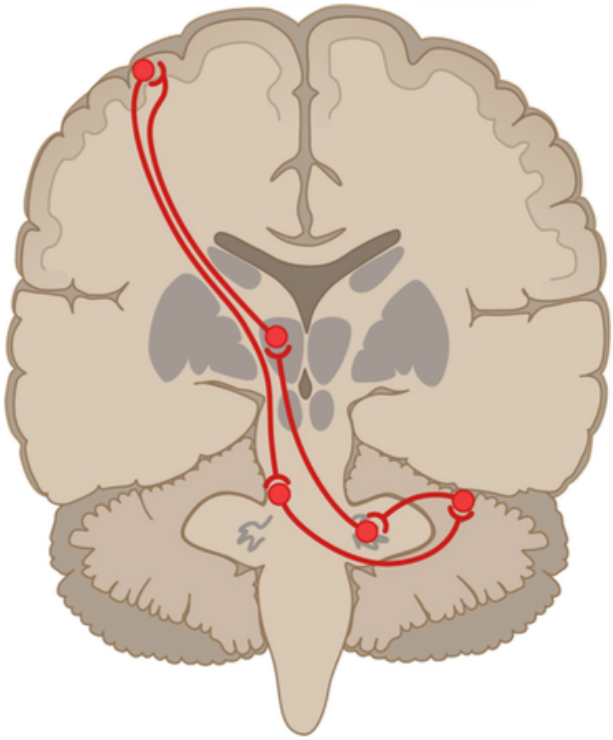


Figure 15. Cerebro-cerebellar circuit with its major inputs and outputs to the neocortex in red (image used from Buckner, 2013). Input projections from the cerebral cortex first synapse on the ipsilateral pons then crossing to the contralateral cerebellar cortex. Output projections first synapse on the dentate and then cross to synapse on the contralateral thalamus and finally project to the cerebral cortex.

While Schmahmann's dysmetria of thought theory focuses on implicit response integration, Ito's internal model controller (Ito, 2008, 2012) aims to integrate explicit mechanisms as well, making use of the cerebellum's many connections with the neocortex (Figure 15). Here, a division is drawn into the cerebellum managing implicit processes, and the cerebral neocortex undertaking explicit processes. This division has been supported by early experiments, where electric stimulation of the cerebral neocortex evoked conscious experiences in humans (Penfield and Perot, 1963), while that of the cerebellar cortex only increased alertness and reduced depression and anxiety, but no conscious experiences (Riklan et al., 1976).

During problem solving, an initial strenuous conscious effort is devoted to the issue and its possible solution. Such an effort can be expressed through repeated thoughts, contemplating the problem and possible solutions, registered as cerebral neocortical mental models. As a reaction, the cerebellum forms an internal model based on the conscious neocortical mental model of these thoughts, so that these could be derived suddenly and without obvious conscious effort if needed. When a stimulus is presented

during active problem solving, it is maintained in the working memory, where it is compared with mental models stored in the temporoparietal cortex. Comparison is also made to cerebellar internal models, which have been copied from mental models and stored in the cerebellum. If the received stimulus matches a mental model or an internal model, the stimulus is consciously recognised to be familiar and the thought process is completed. However, if the stimulus is truly novel, existing models will not be able to recognise it. This creates error signals aiming to (1) suppress conscious familiarity of the mental model, (2) activate the attentional system and in turn the working memory system, searching for stored mental and internal models, and (3) modify cerebellar internal models if errors continue to occur. When the stimulus finds not an identical but close match, the suppression imposed in the first step will be removed in a manner similar to the mechanism of classic conditioning (Schmajuk et al., 1996), resulting in an 'aha' moment. This indicates that the stimulus has been successfully converted and the original stimulus is no longer novel. It has been proposed that this process might be the basis for creativity and innovation (Vandervert et al., 2007), as it builds on existing solutions. However, if the cerebellum as internal model controller is based on dysfunctional mental models, it will result in dysfunctional cerebellar internal models as well. In turn, these dysfunctional internal models will be activated whenever the original stimulus is presented and any built on solutions will be more likely to be dysfunctional as well. This can explain both the persistency and widespread brain abnormalities of psychiatric disorders (Ito, 2008, 2012).

IV. CEREBELLUM IN ABNORMAL DEVELOPMENT

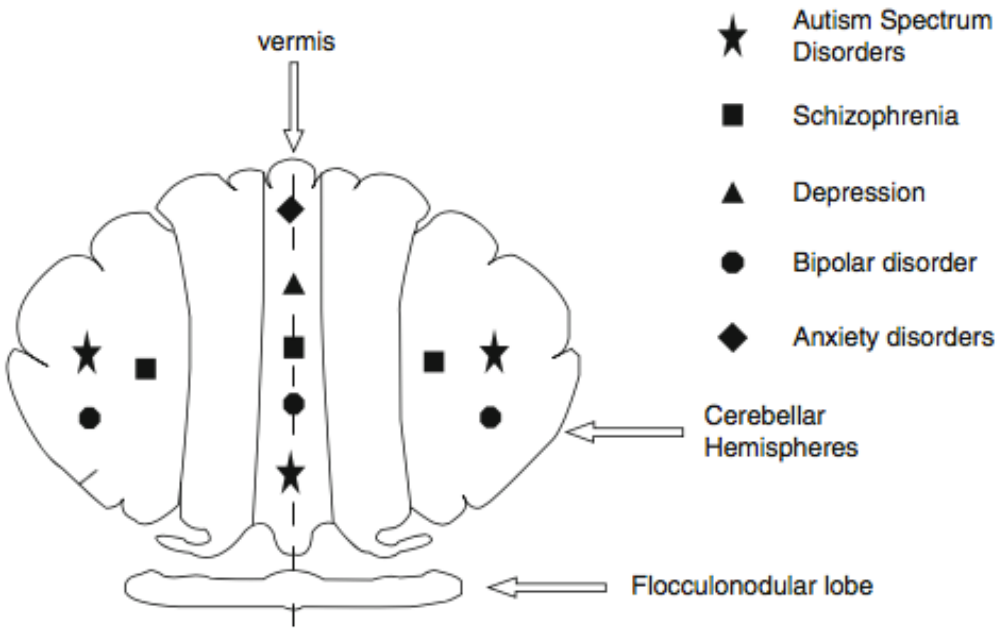


Figure 16. Cerebellar abnormalities in psychopathology (image used from Hoppenbrouwers et al., 2008).

With the cerebellum being one of the earliest structures in the prenatal brain to develop (see chapter I.II), disease processes injure or disrupt the cerebellum specifically in children (Schmahmann, 2004; Tavano et al., 2007) and such disruptions have been associated with poor developmental outcomes (Messerschmidt et al., 2008). As a result, abnormal cerebellar neuroanatomy and dysfunction has been shown in several psychiatric disorders (**Figure 16** as an example of a subset of such). Specifically, neurodevelopmental disorders such as obsessive compulsive disorder and autism spectrum disorder (Catani et al., 2008; Stoodley, 2014, 2015) are affected.

Neurodevelopmental disorders autism spectrum disorder (ASD) and obsessive-compulsive disorder (OCD) share similar patterns of increased repetitive behaviour (Jacob et al., 2009), overlap in clinical symptoms (Boyer and Liénard, 2007; Jacob et al., 2009; Leekam et al., 2011), genetic vulnerability (Jacob et al., 2009; Lionel et al., 2014) and patterns of abnormal brain development (Jacob et al., 2009; Zielinski et al., 2014; Eng et al., 2015). Specifically, genes expressed in the fetal brain (Lionel et al., 2014), and genes regulating neurotransmitters serotonin, glutamate and GABA are disrupted in both disorders (Jacob et al., 2009). These three neurotransmitters are involved in modulating

(serotonin: Oostland and van Hooft, 2013) and regulating cerebellar networks (see chapter I.I) with their disruption affecting cerebellar development and functioning.

IV.I. AUTISM SPECTRUM DISORDER

Autism spectrum disorder is a lifelong neurodevelopmental condition characterised by deficits in social communication and social interaction, as well as increased repetitive behaviours (DSM-5), all functions where cerebellar involvement has been well described (Stoodley and Schmahmann, 2009). ASD affects approximately 1% of the population in the UK (Brugha et al., 2011) and is highly comorbid with disorders associated with cerebellar defects (Becker and Stoodley, 2013) as well as injury to the premature cerebellum (Limperopoulos et al., 2014). While it is highly heritable, ASD is also very heterogeneous and current estimates suggest that up to 1000 risk genes carry *de novo* coding mutations (Willsey and State, 2015). Furthermore, autism-like behaviour is highly present in a number of genetic syndromes including Tuberous Sclerosis (TSC), Rett Syndrome (RTT) and Fragile X Syndrome (FXS).

Histopathologic changes in the cerebellum have been frequently observed in postmortem studies of ASD brains (for review, see Becker and Stoodley, 2013; Hampson and Blatt, 2015). Here, the absence of glial hyperplasia in the ASD cerebellum (Allen, 2005), as well as the widespread dysplasia observed in 62% of studied autistic brains (Wegiel et al., 2010) suggest very early cerebellar developmental deficits in ASD. However, the preservation of basket and stellate cells in the presence of reduced Purkinje cell numbers in some ASD brains would suggest that in some cases Purkinje cells die mostly after normal migration (Whitney et al., 2009), thus indicating possible later cerebellar abnormalities in ASD as well. Furthermore, deep cerebellar nuclear cells to which Purkinje cells project to have been shown to enlarge during childhood and reduce again during adolescence and adulthood (Bauman, 1991). Other cerebellar pathologies in ASD have also been described including a reduction in the number of granule cells and cells in the cerebellar nuclei (Allen, 2005) as well as glial reactivity (Hampson and Blatt, 2015). It remains to be explored whether these are secondary changes caused by the developmental loss of Purkinje cells or independent pathologies.

In addition to cell loss, reduced packing density and a reduction in size of Purkinje cells have been reported in ASD brains (Fatemi et al., 2002; Palmen et al., 2004). A recent

systematic sampling approach revealed that Purkinje cell density was found to be most affected in Crus I and II, an area heavily interconnected with the prefrontal cortex in both males and females, while Purkinje cell density was reduced in lobule X in affected males only, a region associated with vestibular regulation and gaze coordination. Indeed, Purkinje cell density in lobule X was found to correlate with the patient's use of social eye contact (Skefos et al., 2014). These findings suggest that regional Purkinje cell pathology may contribute to selected clinical features of the ASD phenotype.

Studies investigating structural differences in the cerebellum *in vivo* by using magnetic resonance imaging have confirmed abnormal cerebellar development and injury to contribute to the development of ASD. Specifically, disruption of prenatal cerebellar development during specific developmental 'sensitive periods' can lead to key autism features and in turn affect both maturation of cerebellar structures and connected cerebral regions (Wang et al., 2014). Cerebellar injury in preterm infants has been associated with ASD symptom onset, with most severe deficits connected to vermal injury (Limperopoulos et al., 2007). Underdevelopment of the cerebellar vermis (vermal hypoplasia) has been detected in ASD patients as young as two years (Courchesne et al., 2011), as well as in adults diagnosed with infantile autism (Scott et al., 2009). Furthermore, meta-analyses discovered vermal hypoplasia to be restricted to the posterior vermis (lobules VI-VII) in later childhood (Courchesne et al., 2011) and cerebellar undergrowth particularly in the vermis has been associated with increased frontal lobe volume (Carper and Courchesne, 2000) and increased repetitive behaviour in autistic children (Pierce and Courchesne, 2001). In addition to posterior vermal hypoplasia, an anatomic likelihood estimate meta-analysis identified reduced volume of the right Crus I and left VIIIb to be ASD specific across developmental stages (Stoodley, 2014) and reduced right Crus I/II volume was increasingly pronounced with ASD symptom severity in children with autism (D'Mello et al., 2015), paralleling post-mortem findings of reduced Purkinje cell density in lobules Crus I and II (Skefos et al., 2014). In addition, tuberculosis patients with cerebellar lesions exhibited more severe ASD features than those without cerebellar lesions (Eluvathingal et al., 2006) and increased volume of lobules VI-VII has been shown in fragile X syndrome with comorbid ASD, but not without the double diagnosis (Kaufmann et al., 2003).

These cerebellar grey matter differences are accompanied by white matter microstructural alterations of the middle (Shukla et al., 2010; Sivaswamy et al., 2010) and superior cerebellar peduncle (Catani et al., 2008; Sivaswamy et al., 2010), with the latter being associated with decreased motor functioning in children with ASD (Hanaie et al., 2013) and social impairment in adults with Asperger's syndrome (Catani et al., 2008). However, the cerebellar grey and white matter differences found might be part of more global abnormal brain development patterns in ASD characterized by an initial white matter peak quickly followed by an increase of grey matter (Courchesne et al., 2011). This is proposed to be the product of abnormal synaptogenesis and pruning (Bourgeron, 2015), thus affecting not only structure but possibly function of the brain regions involved.

Indeed, functional MRI studies indicate that neuropathological differences in ASD are accompanied by abnormalities in cerebellar functioning and connected circuits. Investigating activation patterns during challenging activities for autistic participants, decreased activity within cerebello-cortical motor networks during finger sequence tapping (Mostofsky et al., 2009), but increased activation of the cerebello-thalamic circuitry during a visually guided saccade task (Takarae et al., 2008) was demonstrated in individuals with ASD when compared to typically developing controls. Further exploring higher cognitive functioning, studies demonstrated reduced cerebello-cortical connectivity related to verb generation (Verly et al., 2014), as well as reduced activation in the Crus I during facial and vocal stimuli processing (Wang et al., 2007) and executive functioning (Solomon et al., 2009). When participants were asked to stay in a restful position with their eyes opened (resting state fMRI) to compare their functional brain connectivity patterns, predominant cerebello-cortical over-connectivity (Khan et al., 2015), and lowered functional connectivity in the left Crus I and right lobule VI consistently across development (Long et al., 2016) were reported.

Together, these findings indicate abnormal local structural and functional abnormalities in ASD of the posterior lateral cerebellar grey matter, cerebello-cortical functional connectivity and white matter microstructure. However, neuroanatomic differences in ASD are not solely expressed in the cerebellum, but also in several cortical structures (for example, Ecker et al., 2013). By using its white matter pathways, the cerebellum

communicates with the cerebral cortex via polysynaptic circuits and closed loop connections (see chapter I.III). This enables separate regions of the cerebellum to be functionally connected to distinct cortical regions, forming a complex somatomotor and association network topography. Atypical cerebellar and cortical anatomy, connectivity and function in ASD are likely to not develop in isolation, but rather are the result of complex cerebello-cortical interactions during development influenced by genetic and environmental factors.

IV.II. OBSESSIVE COMPULSIVE DISORDER

Whereas repetitive, compulsive behaviour is one of the earliest signs of the autism phenotype (Ozonoff et al., 2009), it is also the main characteristic of obsessive-compulsive disorder (OCD). OCD is an impairing condition, associated with a specific set of distressing symptoms incorporating repetitive, intrusive thoughts and distressing, time-consuming rituals. The prevalence rate of OCD varies between 2-3% (Ruscio et al., 2010; Kessler et al., 2012) and OCD has been identified by the World Health Organization as the top 20 causes of years of life lived with disability for 15- to 44-year olds (World Health Organisation, 2003). While both disorders share this phenotypic behaviour, the implication of it can vary. Specifically, repetitive behaviour in OCD is often viewed by the individuals as highly unpleasant and in conflict with the perception of themselves (ego-dystonic; Yadin and Foa, 2009), while individuals with ASD generally don't experience such conflicts and often feel pleased or settled down after showing these behaviours (dystonic; Ozonoff et al., 2009).

While the cortico-striato-thalamo-cortical model of OCD has been the leading model of OCD pathophysiology for the past two decades (Milad and Rauch, 2012), this circuitry has been subjected to controversy (Menzies et al., 2008) as increasing reports implicate outside regions, including the cerebellum. Here, several meta-analyses have demonstrated increased cerebellar volume in adult OCD patients when compared to typically developing controls in both medicated (de Wit et al., 2014; Eng et al., 2015) and unmedicated patients (Pujol et al., 2004). Specifically, a multi-centre mega-analysis study in medicated adult OCD patients demonstrated increased volume of the left anterior cerebellum and bilateral posterior cerebellar vermis (de Wit et al., 2014). However, these findings seem to be inconsistent, with many studies also failing to find differences in the

cerebellum (Radua and Mataix-Cols, 2009; van den Heuvel et al., 2009; Rotge et al., 2009, 2010). In addition, studies investigating cerebellar differences in paediatric OCD are rare, but a recent publication from our group showed increases in the lateral posterior, right anterior and vermis surface in medicated children with OCD when compared to typically developing children, with the same cerebellar regions increasing with OCD symptom severity (Bruchhage et al., 2017), thus adding to previous findings. Interestingly, in unmedicated adults with OCD this association with symptom severity seems to be reversed, as an inversed correlation of cerebellar grey matter volume with disease severity (van den Heuvel et al., 2009) as well as increased cerebellar activation with symptom improvement after successful treatment with cognitive behaviour therapy (Nabeyama et al., 2008) have been shown. This could imply a treatment effect acting on cerebellar structure, underlining its central role in the psychopathology of this disorder.

Thus, these results suggest a link between ASD and OCD on both neuroanatomic and functional abnormalities, with cerebellar dysfunction resulting in dysfunctional behaviour. These shared neuroanatomic cerebellar abnormalities could be related to their shared vulnerability genes (Jacob et al., 2009; Lionel et al., 2014) modulating and regulating cerebellar networks. Specifically, genes expressed in the fetal brain (Lionel et al., 2014) and genes regulating neurotransmitters serotonin, glutamate and GABA are disrupted in both disorders (Jacob et al., 2009). GABA and glutamate make up the main cerebellar output neurons (Hoshino, 1996, 2006; Carletti and Rossi, 2008), with inhibitory GABAergic cells being widely distributed in the cerebellum projecting out of the cerebellar cortex and excitatory glutamatergic neurons receiving afferent input (see Chapter I.II). Additionally, the cerebellar cortex receives beaded fibres containing serotonin, which rather convey a modulatory function than specific information to the cerebellar cortex (Ito, 2012). Such neuromodulation could occur in setting the activity level or operational model of a cerebellar microcomplex to match behavioural demand (Schweighofer et al., 2004) and would in turn influence activity patterns if dysregulated.

However, parallel to ASD, neuroanatomic abnormalities are not limited to the cerebellum but also include neocortical and subcortical brain regions. Interestingly, structures within the cortico-striato-thalamo-cortical model of OCD include the frontal cortex and

thalamus, two structures to which the cerebellum is connected to via closed loop circuits (see chapter I.III).

CHAPTER TWO: THE PLASTIC CEREBELLUM

I. GENERAL INTRODUCTION

In order to investigate the plastic interactions between cerebellar networks and neocortical regions, we designed a study investigating the neuroplastic nature of these cerebello-cortico circuits in neurotypic control participants. For this, half of our novice adolescent participants would perform a demanding multimodal motor training task (drumming), whereas the other half would continue with their usual day-to-day routines. We then continued to study changes in functional cerebello-cortico connectivity (II.II.) and grey and white matter neuroanatomy (II.III.).

II. MOTOR LEARNING INDUCES PLASTICITY IN THE RESTING BRAIN – DRUMMING UP A CONNECTION

II.I. ABSTRACT

Neuroimaging methods have recently been used to investigate plasticity-induced changes in brain structure. However, little is known about the dynamic interactions between different brain regions after extensive coordinated motor learning such as drumming. In this article, we have compared the resting-state functional connectivity (rs-FC) in 15 novice healthy participants before and after a course of drumming (30 minute drumming sessions, 3 days a week for 8 weeks) and 16 age-matched novice comparison participants. To identify brain regions showing significant FC differences before and after drumming, without a priori regions of interest, a multivariate pattern analysis was performed. Drum training was associated with an increased FC between the posterior part of bilateral superior temporal gyri (pSTG) and the rest of the brain (i.e., all other voxels). These regions were then used to perform seed-to-voxel analysis. The pSTG presented an increased FC with the premotor and motor regions, the right parietal lobe and a decreased FC with the cerebellum. Perspectives and the potential for rehabilitation

treatments with exercise-based intervention to overcome impairments due to brain diseases are also discussed.

II.II. INTRODUCTION

Neuroplasticity (NP) is defined as the ability of the nervous system to respond to intrinsic or extrinsic stimuli by reorganizing its structure, function, and connections. Neuroplasticity underlies not only normal development and maturation but also skill learning and memory, as well as the consequences of sensory deprivation or environmental enrichment (Kolb and Muhammad, 2014). Even if NP was initially thought to be limited to critical periods in development, it is now largely accepted to occur throughout the lifespan. In fact, animal studies have identified an enhancement of adult neurogenesis, synaptogenesis, angiogenesis, and the release of neurotrophins as a consequence of learning and/or physical exercise (Hötting and Röder, 2013). Moreover, several famous neuroimaging investigations demonstrate adaptative neuroplastic modifications in the structure (Maguire et al., 2000; Draganski et al., 2004; Driemeyer et al., 2008) and function (Lewis et al., 2009) of the human brain in response to environmental demands in healthy adults (Wan and Schlaug, 2010). Interestingly, few of these neuroimaging studies have investigated neuroplastic modifications following physical activity. These existing studies have mainly focused on cognitive facilitation by cardiovascular exercise in older adults, failing to investigate young participants or different types of physical activity (Voelcker-Rehage and Niemann, 2013).

Over the past decades, musical training has gained increasing interest as a paradigm to study human experience-related NP in the same general model framework (Herholz and Zatorre, 2012). Playing a musical instrument is a highly complex task and requires multimodal skills such as bimanual motor activity dependent on multi-sensory feedback, fine motor skills coupled with metric precision, musical memorization (Wan and Schlaug, 2010), and improvisation (Pinho et al., 2014; Beaty, 2015). These skills involve complex interactions, between sensory and motor systems and high-order cognitive processes, which have to be coordinated at a high degree of synchrony and accuracy (Zatorre et al., 2007).

There is increasing evidence from several different neuroimaging modalities that NP is associated with musical training. By using structural neuroimaging, Gaser and Schlaug reported gray matter volume differences in motor, auditory, and visual–spatial brain regions when comparing professional musicians (keyboard players) with matched groups of amateur musicians and nonmusicians (Gaser and Schlaug, 2003). Cerebral activity patterns associated with musical perception in musicians have also been studied by using functional MRI (fMRI). A significant difference in the degree of activation between musicians and nonmusicians was noted in the temporal regions and for musicians the degree of activation was correlated with the age at which the person had begun musical training (Ohnishi et al., 2001). Functional MRI was also used to explore the brain activations of novices trained to play sequences on a piano keyboard (Lahav et al., 2007; Chen et al., 2012; Herholz et al., 2015) or to listen and imitate different auditory rhythms (Chen et al., 2007). These studies showed complex interactions between auditory and motor brain regions that were associated with musical training.

Interestingly, drumming, which is a coordinative exercise combining musicality, body coordination, cardiovascular exercise, “bilateral” arm, leg movements, and sensorimotor integration processes (De La Rue et al., 2013), has not been studied extensively to date. Only expert drummers have been studied thus far. While Tsai et al. (2012) have shown that the posterior temporal lobes are essential for audio-motor processing, Petrini et al. (2011) showed that expert drummers present a reduced activation bilaterally during an audiovisual task in the cerebellum and the left parahippocampal gyrus, respectively, involved in action–sound representation and audiovisual integration.

Recently, resting-state functional connectivity (rs-FC) analyses have been widely used to investigate neuroplastic modifications. Rs-FC corresponds to the temporally correlated, low-frequency spontaneous fluctuations of blood oxygen level-dependent signals across brain regions that occur when a participant is not performing an explicit task (Fox and Raichle, 2007). Temporal correlations do not appear to be random, as patterns of connectivity have been reliably identified across studies and participant. Moreover, it is now widely accepted that the strength of correlations within and between networks has behavioural relevance (Guerra-Carrillo et al., 2014). Interestingly, it has been proposed that rs-fMRI is an effective measure of plasticity and that rest activity patterns reflect the

history of repeated synchronized activation between brain regions. Thus, the study of rs-FC is particularly adapted to highlight neuroplastic modifications (Buckner and Vincent, 2007; Guerra-Carrillo et al., 2014).

In this article, we have compared the rs-FC in 15 novice healthy participants, by using a fully data-driven approach, before and after a course of drumming with another 16 novice participants who were again evaluated longitudinally but with no intervention. This longitudinal design was used to disentangle cause and effect by investigating the same participants before and after the course of drumming. We hypothesized that participants would exhibit a higher level of functional connectivity (FC) in the sensory and motor systems after drum training during the resting state, which could not be attributed to the MRI session effects (anxiety, novelty of the MRI environment).

II.III. METHODS

Participants

Thirty-one young healthy volunteers (16–19 years) with no prior drumming experience and with no psychiatric or neurological disorders participated in the study after providing written informed consent. The King's College, London, College Research Ethics Committee approved the experimental protocol. Participants were randomly divided into 2 groups: drum group and control group.

Assessment

All participants were attended in to the Institute of Psychiatry Psychology and Neuroscience (IoPPN) at King's College London for 2 scanning sessions. At the first scanning session (t1), the Edinburgh Handedness Inventory Short Form (EHI) for daily activities (e.g., writing, throwing. . .) (Veale, 2014) was used to assess each participant's hand dominance. Responses were coded to generate a Laterality Quotient (scores ranging from −100 to 100), which was then used to classify individuals into left handers (−100 to −61), mixed handers (−60 to 60), and right handers (61 to 100). Moreover, to assess the baseline drumming ability and musical experience, a self-report measure was created. Participants reported their level of skill and length of involvement in drumming, playing of another musical instrument and involvement in dance or singing. Responses for all domains were coded on an ordinal scale (0, no experience; 1, some experience, no

formal instruction; 2, limited formal instruction but not recent; 3, formal instruction, less than 4 years but not current; 4, formal instruction, exams achieved, greater than 5 years involvement and current).

Drumming

Following the first drumming assessment and scanning session the drum group took part in three 30-minute low intensity group drumming sessions per week for 8 weeks with the same drumming tutor. By using the same tutor, we were able to qualitatively screen for signs of innate abilities in all groups and the group sessions allowed for additional motivation of the students. Each session was delivered by a professional drum tutor and comprised 4 integrated parts: (1) a warm up, focused on beating the drum with a relaxed and consistent motion of the drum sticks; (2) snare drum "rudimental" exercises, played on a single drum surface, adopting a "flow sticking" approach to sequences of left and right hands (Queen 2007); (3) coordinated "groove" patterns, incorporating the interplay of bass drum (right foot) and the hi-hat pedal (left foot) with rock/pop back beat ostinato patterns played on the hi-hat or ride cymbal and snare drum, including eighth note (quaver), quarter note (crotchet), sixteenth note (semiquaver), syncopated quarter note and shuffle ostinato patterns (Chaffee, 1980); and (4) performance of learned "grooves" and "fill-ins" to accompany well-known popular music songs. In order to control for motivation and practice effects, the complexity of drumming tuition was increased on a weekly basis in line with participants' demonstration of improved drumming coordination and technique. The control participants were asked to not take part in any musical activities. After the 8 weeks, (t2) participants came back to the IoPPN for a second drumming assessment and scanning session.

All of the drumming was performed on electronic drum sets for both drumming training (HD3, Roland, Nakagawa, Japan) and assessment (TD9, Roland, Nakagawa Japan) with a standard right-handed 5 piece configuration comprising a snare drum, 3 tom-toms, hi-hat, ride cymbal, crash cymbal, bass drum, and hi-hat pedal (played with the feet). Drumming was assessed following a short coaching period on the participants' ability to play a simple 4 quarter note pattern to the song "Green Onions" (Booker T and the MGs, Stax/Atlantic, 1962) and a simple 8 eighth note pattern, consisting of regular eighth note hi-hats with alternating kick and snare on the main beats of the bar, to the song "Billy

Jean" (Michael Jackson, EPIC, 1982). Versions without tempo fluctuations were used, created using the software "Live" (V9.1, Ableton, Berlin). This software analysed the beat points of the original recording and adapted them temporally to ensure that the version of the song heard by the participants was at a constant tempo, with a consistent and precise inter beat interval so errors could be accurately recorded. The songs were played out of a single speaker on one channel, while the underlying beat locations were indicated by recording a click track on a second channel. A standard onset detection algorithm (Bello et al., 2005) was used to determine the audio buffer frame in which the transient of each beat in the click track occurred. A precise onset detection method was then used to find the onset location by iteratively dividing the audio buffer into window segments and determining the window in which the energy change was maximal at successively smaller window sizes. Thereby, a precise sample was specified for each beat location, accurately placed on the transient of each audible click (Robertson, 2014). Timing data were exported from the drum set using the musical digital interface (MIDI) signal. A comparison with a piezo microphone placed on the snare indicated that the recorded MIDI events were a maximum of 4ms from the detected onset using audio-based methods, and generally much closer. Drumming ability was assessed objectively as the percentage of bars of both patterns that were completed during two 2-min periods of data capture (1–3 min of each song). To record a completed bar all elements had to be present in each pattern and within half a beat (250ms for 4 quarter note pattern and 125ms for an 8 eighth note pattern) of the perfect timing. We also evaluated the error in events that should have been synchronous (flamming) as flam error per bar in milliseconds. For each note of the bar, we evaluated the time between the 2 events. When 3 limbs were involved, we evaluated the difference between the first and last event. This was only evaluated for completed bars, as it would be impossible to make a meaningful measurement where the pattern was breaking down or parts were missing. No practice of either song used in the assessment was included in the 8 weeks drumming training. While a drum flam can be a sought-after stylistic feature of a pattern, flams were not part of the stylistically correct performance for the 2 chosen patterns.

MRI Acquisition and Preprocessing

All participants were scanned in a 3T MR scanner (Discovery MR750, General Electric, Milwaukee, WI, USA). All participants underwent an anatomical T_1-weighted MRI using a gradient-echo sequence with the following scan parameters: 196 sagittal slices, time repetition (TR)=7.3ms, time echo (TE)=3ms, time to inversion (TI)=400ms, FA=11 degrees, field of view (FOV)=270mm^2, matrix size=256x256, voxel size=1x1x1mm^3, slice thickness=1.2mm. the rs-functional magnetic resonance imaging (fMRI) was collected using an echo-planar imaging sequence with the following scan parameters: 180 volumes, interleaved ascending slice order, TR=2000ms, TE=30ms, FA=75 degrees, FOV=211mm^2, matrix size=64x64, voxel size=3x3x3mm^3, gap=0.3mm. Four dummy scans were obtained before each fMRI data acquisition to allow for the equilibration of the MRI signal. During acquisition, the participants remained with eyes open attending a cross-hair on the screen in a wakeful resting state. Headphones and earplugs were used to attenuate the acoustic noise of the scanner.

The anatomical and functional data were preprocessed and analysed using Statistical Parametric Mapping (SPM12) and the CONN toolbox Version 14p (http://www.nitrc.org/projects/conn) (Whitfield-Gabrieli and Nieto-Castanon 2012). Functional data were preprocessed using a slice scan time correction, realignment (motion correction), registration to structural images and spatial normalization to Montreal Neurological Institute (MNI) standardized space, smoothing with a Gaussian filter of 5.0 mm spatial full width at half maximum value. A conventional band-pass filter over a low-frequency window of interest (0.008–0.09) was also applied to the resting-state time series. After these preprocessing steps, a CompCor strategy (Behzadi et al., 2007) was implemented, extracting signal to noise from the white matter (WM) and cerebrospinal fluid (CSF) by principal component analysis without affecting intrinsic FC (Chai et al. 2012). These components (WM and CSF) and motion parameters were included in the model and considered as covariates of no-interest.

Voxel-to-voxel Analysis

To identify brain regions showing significant FC differences before and after drumming without having to restrict the analyses to one or several a priori regions of interest (ROI), whole-brain connectivity analysis was performed. In the present study, we employed the "connectome-MVPA" approach (multivariate pattern analysis of whole-brain

connectome) implemented in the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012). In this approach, a low-dimensional multivariate representation characterizing the connectivity pattern between one voxel and the rest of the brain was created for each voxel separately. This representation was defined by performing a principal component analysis characterizing the FC between this voxel and the rest of the brain. The resulting component scores were then stored as functional maps and entered into standard second-level analyses for between-condition tests (i.e., before and after drum training). Finally, an F-test was performed, comparing (for each voxel) the component scores across the 2 conditions. Therefore, the second-level statistical analysis provided a multivariate pattern of correlated voxel clusters associated with drumming. In other words, regions that are significant in the resulting voxel-level analyses indicate condition-related differences in connectivity between those areas and the rest of the brain. More technical details and an example of the use of the connectome-MVPA method can be found in Whitfield-Gabrieli et al. (2016) and in Beaty et al. (2015).

Seed-to-voxel Analyses

To explore which specific aspects of the FC between clusters found in the voxel-to-voxel analysis and the rest of the brain was different between the conditions, a post hoc seed-to-voxel analysis was performed. Therefore, 10 mm spherical ROI based on peak activation clusters from the whole-brain connectivity analysis were extracted and used as seeds to perform seed-to-voxel analysis. Correlations maps were then calculated for each participant, per condition, by extracting the mean signal time course from the seeds and computing Pearson's correlations coefficients with the time course of all other voxels of the brain. Those correlation coefficients were converted to normally distributed z-scores using the Fisher transformation to allow for second-level general linear model analyses. Second-level within group (one sample t-tests) and between group (ANOVA) analyses were performed on the average Z-maps from the new seeds source ROIs. Finally, a paired t-test was performed to examine the change of resting-state connectivity before versus after drum training for all participants.

For confirmatory analysis, seed-to-voxel analyses were applied using anatomically defined ROIs, in order to avoid potential concerns regarding circularity. For this analysis, the posterior superior temporal gyrus (pSTG) was chosen because it is a crucial hub

engaged in sensorimotor integration (Rauschecker and Tian, 2000; Scott et al., 2009), auditory–motor transformations (Warren et al., 2005; Chen et al., 2007) and because it is selectively responsible for action-related sounds (Zatorre et al., 2007; Tsai et al., 2012). Moreover, a recent fMRI meta-analysis of 43 studies on experimental auditory–motor training (i.e., finger tapping), revealed activity in the pSTG (Chauvigné et al., 2014). Two 10 mm spherical ROIs were then created based on the Harvard-Oxford cortical atlas implemented in the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012). The MNI coordinates of left and right pSTG were, respectively, (−62, −29, 3) and (61, −24,1).

As gender and handedness may be a factor which modulates musical training (Koelsch et al., 2003; Klöppel et al., 2007) additional analyses including gender and handedness as covariates were conducted. The same 10 mm spherical ROIs were used as seeds in the control group in order to delineate brain changes which are specific to the drum training.

To precisely identify the brain areas involved, the Harvard-Oxford Cortical and Subcortical Atlas (Desikan et al., 2006; Makris et al., 2006), the AAL Atlas (Tzourio-Mazoyer, et al. 2002), and the Juelich Histological Atlas (Eickhoff et al., 2007) were used. All results were thresholded at a voxel-wise $P < 0.001$ and at the cluster extent $P < 0.01$ FWE corrected. For confirmatory analysis, results were thresholded at a voxel-wise $P < 0.001$ and at the cluster extent $P < 0.05$ FWE corrected, a threshold widely used in confirmatory analysis (Kriegeskorte et al., 2010; Chen et al., 2016).

Correlations with Drum Progression

To evaluate the association between FC and drum progression, correlations (Pearson's coefficients) were performed between the drum progression scores in each participant and the connectivity values (z-scores) of regions showing significant differences after drum training from the seed-to-voxel analysis. Two scores were used. The first score corresponded to the mean number of bars successfully completed (Δ% bars completed) before versus after the drum training (t2 − t1 difference) from "Green onions" and "Billy Jean". The "combined progression score" corresponded to the proportion of bars successfully completed before versus after the drum training (t2 − t1 difference) as the percentage of bars completed (Δ% bars completed) from both songs.

Statistical Analyses

Statistical analyses of demographic data and drum performance were performed using SPSS (SPSS version 18, Chicago, Illinois, USA). Independent sample t-tests or Mann–Whitney rank sum test, and paired t-tests were used to determine baseline differences, and the effects of drum training between and within groups. To test whether there was a significant interaction of group over time, we performed a repeated-measures ANOVA model by specifying "time" as a within-subjects contrast (t1 – t2, i.e. before and after the drum training) followed by a between subject's contrast (drummers vs. controls). Significance was set at $P<0.05$.

II.IV. RESULTS

Participants and Drum Progression

Based on a self-report measure, the drum group (n = 15, 7 men, 8 women, 2 mixed-handed, age 16.8 ± 0.7 years) and the control group (n = 16, 8 men, 8 women, all right-handed, age 17.8 ± 1.4 years) presented no prior drumming experience (all scores ≤2). Moreover, there were no statistical differences between the 2 groups in terms of self-reported other musical instrument ($P = 0.53$) dance and singing experience ($P = 0.31$).

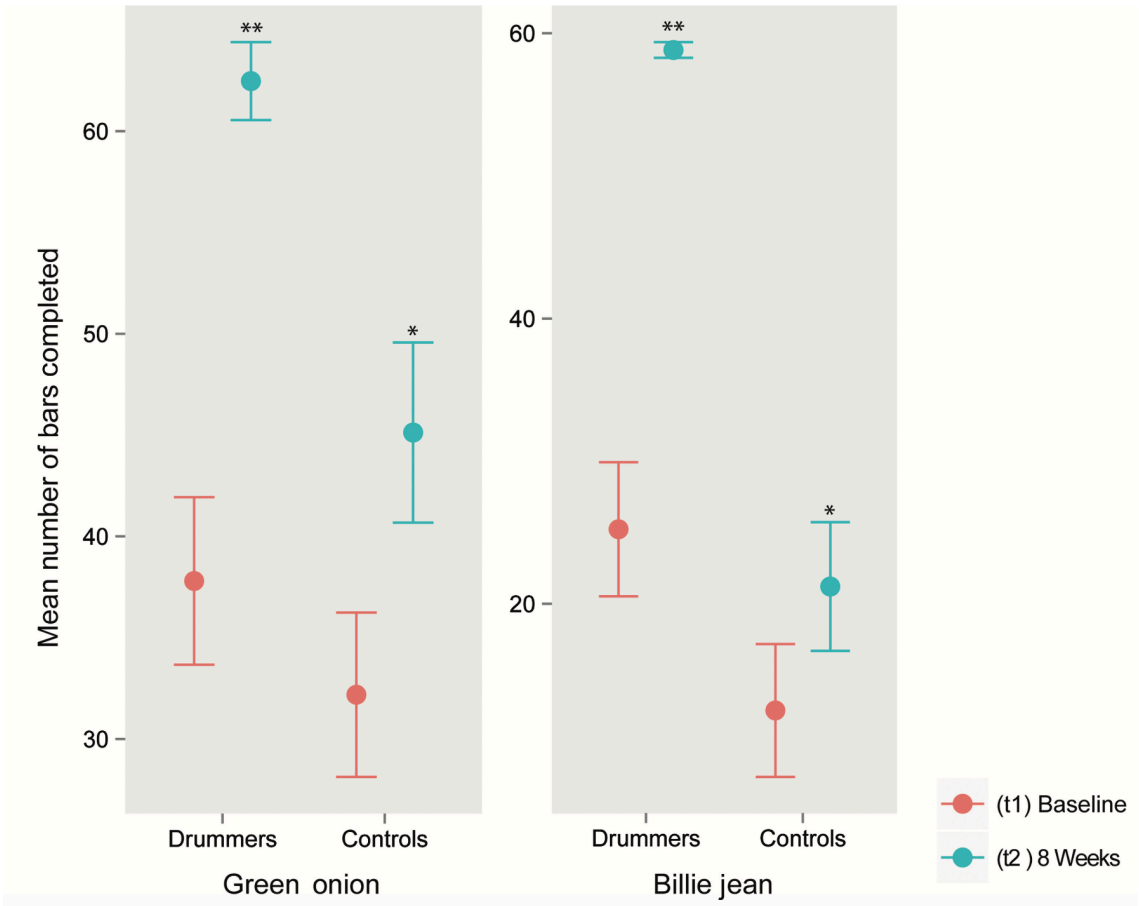
At baseline, the mean number of bars successfully completed for "Green Onions" and "Billie Jean" was not significantly different between the 2 groups (respectively, $P=0.42$ and $P=0.12$) (see **Figure 1**). After 8 weeks, the mean number of bars successfully completed was significantly increased in both groups and for the 2 songs (see **Figure 1**). The mean combined progression score was significantly higher ($P = 0.003$) for the drum group (mean = 0.45 ± 0.29) in comparison with the control group (mean = 0.17 ± 0.19). A before/after video is available for illustration (see https://vimeo.com/141911618).

For "Green Onions", repeated-measures ANOVA revealed no significant effect of group ($F = 3.9$, $P = 0.07$), a significant effect of time ($F = 19.9$, $P = 0.001$) and no group × time interaction ($F = 3.3$, $P = 0.091$). For "Billie Jean", repeated-measures ANOVA revealed a significant effect of group ($F = 26.1$, $P < 0.001$), a significant effect of time ($F = 36.1$, $P < 0.001$) and significant group × time interaction ($F = 21.9$, $P < 0.001$). There was no significant difference in flam error per bar between t1 and t2 in both groups ($P = 0.24$).

Voxel-to-voxel Analysis

The connectome-MVPA analysis revealed 2 symmetric clusters associated with drum training ($P < 0.005$, FWE correction). These clusters consisted of the posterior division of the left and right superior temporal gyri (STG) and middle temporal gyri (MTG) and are described as pSTG throughout the manuscript (see **Table 1** and **Figure 2**).

Figure 1. Mean number of bars successfully completed for "Green Onions" and "Billie Jean" before and after the drum training



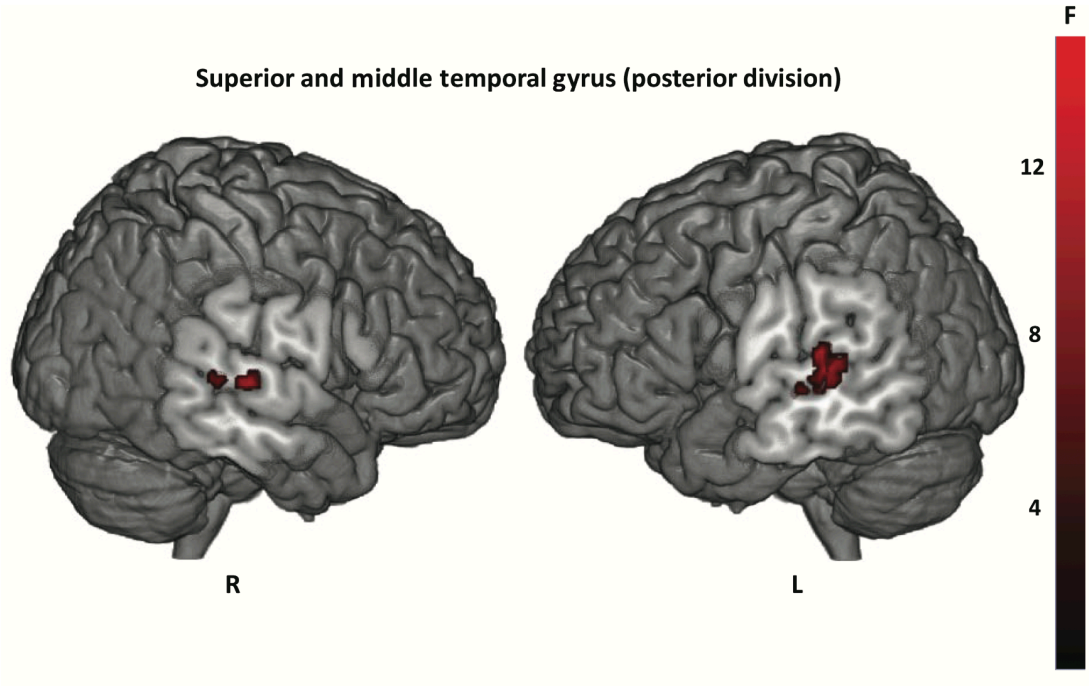
*At baseline, the mean number of bars successfully completed was not significantly different between the two groups for the both songs (respectively P = 0.42 and P = 0.12). After 8 weeks, the mean number of bars successfully completed was significantly increased in both groups and for the 2 songs. Error bars denote the standard deviation. *P < 0.05; **P < 0.001.*

Table 1. Whole-brain connectome-MVPA analysis (voxel-to-voxel analysis)

Cluster size	Side (L/R)	Brain areas	MNI coordinates			F_{max}	Z_{max}	P_{corr} value
			x	y	z			
160	L	Superior Temporal Gyrus (posterior division)	-64	-28	12	15.02	4.78	<0.001
		Middle Temporal Gyrus (posterior division)	-58	-26	-8	9.00	3.72	
49	R	Superior Temporal Gyrus (posterior division)	64	-18	02	11.71	4.25	<0.005
		Middle Temporal Gyrus (posterior division)	70	-28	0	8.80	3.67	

Note: MNI coordinates (x, y, z) represent peaks within a cluster. Cluster size corresponds to spatial extent (i.e., number of voxels). Correction for multiple comparisons was performed using family-wise error correction at the cluster level. L/R, left/right side of the brain.

Figure 2. Results from the voxel-to-voxel analysis showing areas of highest connectivity in the whole brain by comparing before versus after drum training



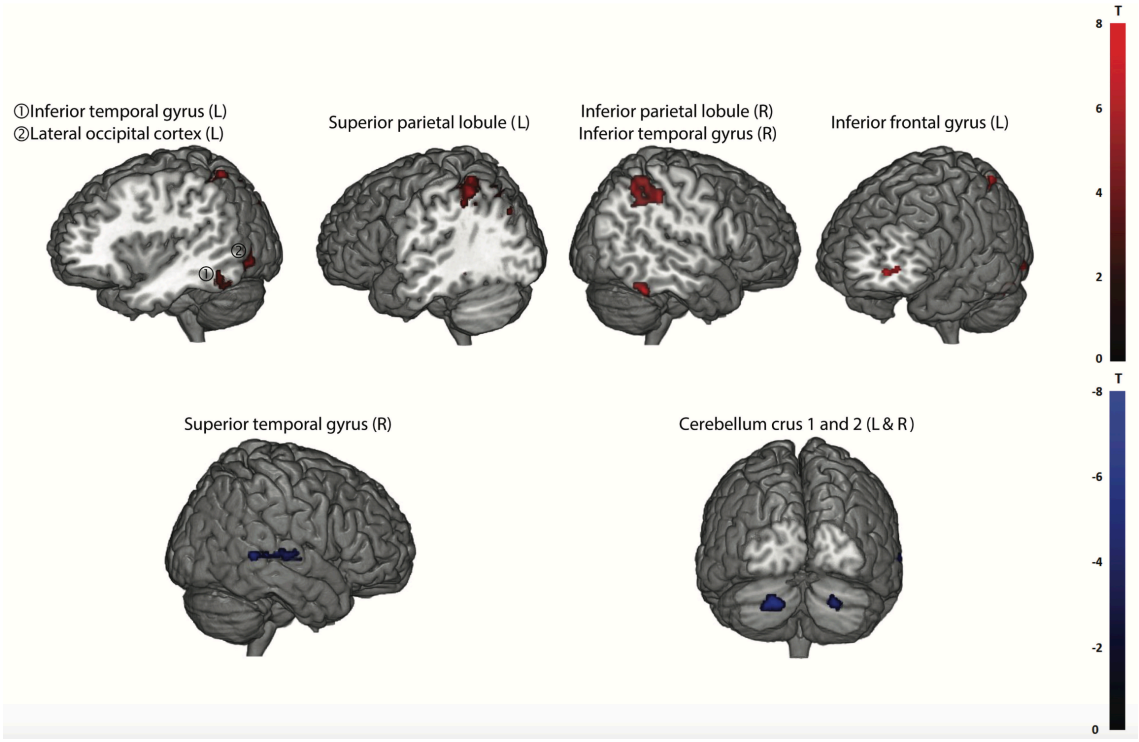
These clusters consisted of the posterior division of the left and right STG and MTG and are described as pSTG. This map is based on F-contrasts thresholded at P < 0.01, FWE corrected. Clusters were rendered on the "ch256" brain template using MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). L/R = left/ right side of the brain.

Table 2. Functional connectivity of the left pSTG

	Cluster size	Brain areas	MNI coordinates				T_{max}	P_{corr} value
			Side (L/R)	x	y	z		
Post > Pre								
Cluster 1	741	Superior parietal lobe	L	-36	-50	50	7.85	<0.0001
		Lateral occipital cortex	L	-24	-60	34	7.54	
Cluster 2	526	Inferior temporal gyrus	L	-50	-46	-18	6.36	<0.0001
Cluster 3	424	Angular gyrus	R	40	-54	52	6.34	<0.0001
		Supramarginal gyrus	R	42	-36	44	6.24	
		Lateral occipital gyrus	R	32	-64	42	5.39	
Cluster 4	103	Inferior temporal gyrus	R	56	-46	-28	8.45	<0.01
Cluster 5	103	Inferior frontal gyrus	L	-30	48	-2	6.43	<0.01
Post < Pre								
Cluster 1	276	Superior temporal gyrus	R	54	-30	4	-6.74	<0.001
		Middle temporal gyrus	R	68	-42	0	-6.54	
Cluster 2	152	Cerebellum crus I and II	L	-18	-76	-36	-6.50	<0.001
Cluster 3	159	Cerebellum crus I and II	R	22	-72	-28	-5.77	<0.001

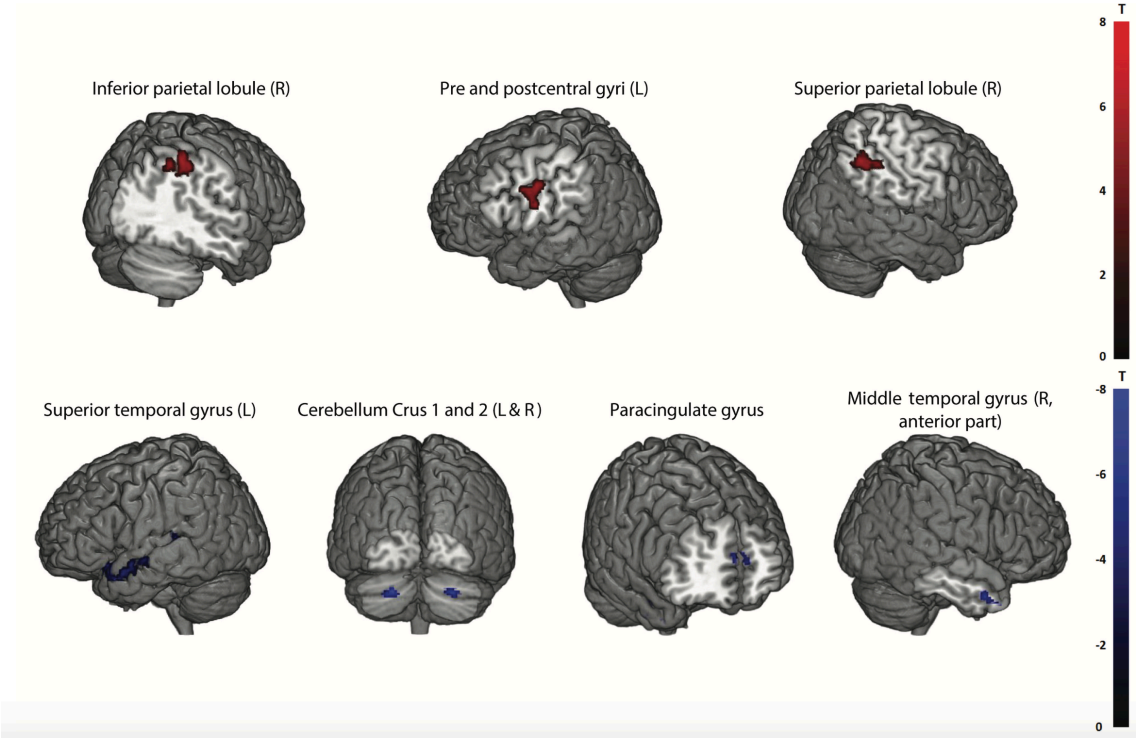
MNI coordinates (x, y, z) represent peaks within a cluster. Cluster size corresponds to spatial extent (i.e., number of voxels). Correction for multiple comparisons was performed using family-wise error correction at the cluster level. L/R, left/ right side of the brain.

Figure 3. Results from seed-to-voxel functional connectivity (FC) of the left pSTG seed (results of voxel-to-voxel analysis)



Higher FC was detected after drum training within left superior parietal lobule, left inferior temporal gyrus, right inferior parietal lobule (angular gyrus and supramarginal gyrus), left and right lateral occipital cortex and left inferior frontal gyrus (upper panel, P < 0.01, FWE corrected). A decreased FC was measured after drum training within right superior and middle temporal gyrus (posterior division) and left and right cerebellum crus (lower panel, P < 0.01, FWE corrected). Clusters were rendered on the "ch256" brain template using MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). L/R = left/ right side of the brain.

Figure 4. Results from seed-to-voxel functional connectivity (FC) of the right pSTG seed (results of voxel-to-voxel analysis)



Higher FC was detected after drum training within right inferior parietal lobule (angular gyrus and superior parietal lobule) and right pre- and postcentral gyrus (upper panel, P < 0.01, FWE corrected). A decreased FC was measured after drum training within left superior and middle temporal gyrus, left temporal pole, left and right cerebellum crus, paracingulate gyrus and the anterior part of the right MTG. Clusters were rendered on the "ch256" brain template using MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). L/R = left/right side of the brain.

Seed-to-voxel Analyses

Group-level seed-to-voxel analyses revealed increased FC after drum training when compared with before between left pSTG and left superior parietal lobule (SPL), left inferior temporal gyrus, right angular gyrus, right supramarginal gyrus, left and right lateral occipital cortex, right inferior temporal gyrus, and left inferior frontal gyrus and a decreased FC between the right STG and MTG (posterior division) and left and right cerebellum crus I and II (see **Figure 3**). Peak spatial coordinates in the MNI space and the corrected P-value (P < 0.01, FWE correction) are reported in **Table 2**.

Group-level seed-to-voxel analyses revealed increased FC after drumming when compared with before between right pSTG and right supramarginal gyrus right angular

gyrus, right SPL and right pre- and postcentral gyrus and a decreased FC with the left STG and MTG (posterior division), left temporal pole, bilateral cerebellum crus I and II, left paracingulate gyrus and right MTG (see **Figure 4**). Peak coordinates in the MNI space and the corrected P-value ($P < 0.01$, FWE correction) are reported in **Table 3**.

The seed-to-voxel analysis was confirmed by adopting new, anatomically defined ROIs, as we observed qualitatively the same patterns of modulated FC involving the same networks (see Supplementary **Tables 1 and 2**).

The supplementary inclusion of gender and handedness as covariates did not change the results as the same clusters were found. No significant FC differences were observed in the control group.

Improvement in Performance and Correlations with Functional Connectivity

Participants with higher improvement in performance had higher right seed-right parietal lobe connectivity scores. Indeed, there was a significant correlation between the improvement in the mean number of bars successfully completed for "Billie Jean", the combined progression score and the right parietal lobe FC (including the right supramarginal gyrus, the right angular gyrus and the right SPL) from the right seed. The correlation coefficients were, $r = 0.60$ ($P= 0.02$) and $r = 0.57$ ($P= 0.03$), respectively (see **Supplementary Figure**). The improvement in performance did not correlate significantly with the FC of the other clusters.

II.V. DISCUSSION

The main goal of this study was to determine whether we could visualize significant differences in the functional networks engaged in drum training in healthy participants by using rs-FC. Drum training was characterized behaviourally in significantly improved performance as assessed by the mean number of bars successfully completed for "Green Onions" and "Billie Jean". Interestingly, after 8 weeks, the mean number of bars successfully completed was significantly increased in both groups and for the 2 songs. This increased drum performance in the age-matched control group can be explained by a learning or familiarization effect since the 2 songs were no longer novel to the participants in both groups. However, the drum group has improved drum performance over and above this simple familiarization effect. Indeed, the progression score was

significantly higher for the drum group in comparison with the control group. Moreover, a significant effect of time (i.e., before and after 8 weeks) has been showed in both groups and for the 2 songs, and a significant group (i.e., drummers vs. controls) × time interaction was showed for "Billie Jean". We were unable to utilize more sophisticated measures of drumming performance (such as timing deviations), because we could only evaluate the timing scores from the data that made up the completed bars, since in this population (of novices) there were periods of no playing and patterns that needed to be stopped as they were playing incorrectly. While timing deviation would be a representative statistic for more experienced drummers, this relies on the subject's ability to play the pattern correctly in order to be meaningful. Drum training was also associated with: (1) an increased FC between the posterior part of bilateral pSTG and the rest of the brain (i.e., all other voxels), (2) an increased FC between pSTG and the premotor and motor regions, (3) an increased FC between pSTG and the right parietal lobe which was correlated with drum performance and (4) a decreased FC between regions of the cerebrum and the cerebellum. Moreover, the seed-to-voxel results were confirmed by adopting anatomically defined ROIs. Finally, these results could not be attributed to the MRI session effects (anxiety, novelty of the MRI environment) or to the normal development (Power et al., 2010; Rubia, 2013), as no significant FC differences were observed in the age-matched control group.

The whole-brain FC underlying drum training was explored and revealed 2 symmetric brain regions located in the pSTG (see **Figure 2**). This result is consistent with previous experiments about the training to play a melody on piano keyboard (Chen et al., 2012), hammering or clapping (Lewis et al., 2005; Galati et al., 2008), and expert drummers (Tsai et al., 2012), which showed that the bilateral pSTG were selectively responsible for action-related sounds. Indeed, the pSTG is a crucial hub of the dorsal auditory streams which is engaged in sensorimotor integration and spatial processing ("how" and "where") (Rauschecker and Tian, 2000; Scott et al., 2009) and therefore plays an essential role in auditory–motor transformations ("do-pathway"), which is essential for music processing (Warren et al., 2005; Zatorre et al., 2007). Interestingly, while using the pSTG as a seed to perform a seed-to-voxel analysis, an increased FC was found between the right pSTG and the left precentral and postcentral gyri including the ventral premotor cortex (vPMC)

and the primary motor cortex. The premotor cortex can compute a variety of sensorimotor transformations that are relevant for music and is involved in the motor prediction and production of complex sequences (Zatorre et al., 2007). Specifically, the left vPMC, which might work in tandem with the pSTG (Hickok and Poeppel, 2007; Tsai et al., 2012), could be associated with the serial sequence prediction and coupling between hearing music and the execution of a motor programme by the motor cortex, that enable the realization of sensory-cued actions (Zatorre et al., 2007). The left and right pSTG seed regions showed an increased FC, respectively, with the left and right SPL, a coupling essential in sensorimotor transformation (Hickok and Poeppel, 2004; Zatorre et al., 2007). The SPL, which is anatomically connected to the posterior temporal lobe (Kamali et al., 2014), is critical for many sensory and cognitive processes, including sensorimotor (Wolpert et al., 1998) and somatosensory integration, motor learning, spatial perception, mental rotation, visuospatial attention, and memory (Wang et al., 2015). Moreover, it has been involved in the perception of bimanual interaction with an external object (Naito et al., 2008) and the storage of movements and kinematics (Seitz et al., 1997). This appears to be very consistent with the present experiment involving drum training. In the music training framework, the SPL has also been suggested to coordinate the complex spatial and timing components of musical performance (Langheim et al., 2002). After drum training, both pSTG were also more functionally connected with the right inferior parietal lobule (IPL), which comprises of the angular and supramarginal gyri, and the intraparietal sulcus (IPS). Interestingly, the SPL and the IPL cluster, which includes the IPS, are part of the dorsal attention network (Petersen and Posner, 2012). The dorsal attention network is involved in the maintenance of spatial priority maps for covert spatial attention, saccade planning, and visual working memory (Vossel et al., 2014). Moreover, our results are consistent with the right hemisphere dominance that has been described for visuospatial attention (de Schotten et al., 2011). Interestingly, these posterior parietal regions also support mental transformations of acoustic or visual information into motor representations (Warren et al., 2005; Zatorre et al., 2007; Herholz et al., 2015). The increased functional coupling between the bilateral pSTG and the right dorsal attention network might be associated with the improvement of the integration

of sensory and motor functions, as the FC of the right parietal lobe cluster was significantly correlated with the improvement in drum performance.

The increased FC between the left pSTG and the inferior frontal gyrus (IFG) corresponds to the ventral auditory streams arising from the primary auditory cortex, and projecting anteriorly from the primary auditory cortex to the IFG along the STG (Scott et al., 2009). This ventral pathway, also known as the what-pathway, is involved in auditory object identification (Leaver and Rauschecker, 2010). It is specialized for invariant auditory object properties, which are time-independent, and less related to motor systems (Zatorre et al., 2007). The left seed was more functionally connected with the left inferior temporal gyrus and the lateral occipital cortex. These are part of the ventral visual pathway involved in human object perception and recognition and in the encoding of spatial relationships between subparts of scenes (Grill-Spector and Weiner, 2014). This increased FC reflects the audio-visual integration, which has been shown to be crucial in multimodal training (Paraskevopoulos et al., 2012).

Interestingly, both seeds also showed a decreased FC with the bilateral cerebellum (crus I and II). A recent meta-analytic connectivity study identified these 2 cerebellar subregions as part of a cerebellar cluster significantly associated with motor learning, working memory, recitation, and repetition as well as music comprehension and production (Riedel et al., 2015). All integral components involved in learning to play the drums. According to the Marr–Albus–Ito theory on motor learning, cerebellar Purkinje cells (PC) learn to recognize contexts during rehearsal of an action, creating an anticipatory neural state (Marr, 1969; Ito, 1970; Albus, 1971). Climbing fibres innervating the PC further encode possible errors, consequently suppressing the PC synapses involved in such erroneous performance with the help of long-term depression. Once the action has been learned, the occurrence of the context alone is enough to fire the PC to cause the next elemental movement. This increase in efficiency in turn decreases cerebellar activity in the region used, which enables a resource allocation to other areas (Petrini et al., 2011). When such movement involves excitatory output to the cerebral cortex, PC form inhibitory synapses with the deep cerebellar nuclei. As a result, an increase in cerebellar activity would induce a decrease in activity in cortical target regions and vice versa (Vahdat et al., 2011). As a result, the cerebellum is able to integrate and

coordinate motor and sensory signals, react quicker and with less error, creating a smooth motor performance. With this increase of efficiency, a decrease of cerebellar activation in regions required for motor learning, music comprehension and production can be expected after drum training.

Both pSTG also showed a decreased FC with the contralateral STG and the right pSTG showed a decreased FC with the ipsilateral anterior part of the MTG.

While the BOLD signal is not sensitive to the inhibitory or excitatory nature of signalling but only its metabolic demands, the decreased FC found in this study could reflect complex interferences between the ipsilateral and contralateral auditory pathways (Pantev et al., 1986) that can be associated with the synaptic inhibition involved in sound localization (Grothe, 2003). In fact, auditory pathways are formed by excitatory and inhibitory neural connections and networks. It is now well-known that music induced lateral inhibition in the human auditory cortex (Pantev et al., 2012), which in turn could reflect the reduced metabolic demand in structures that have successfully learned a task during musical training (i.e. decrease of FC after learning to drum). Moreover, reductions in temporal gyrus activity were reported previously for auditory perceptual pitch training (Jäncke et al., 2001) and discrimination training for micromelodies (Zatorre et al., 2012). The right pSTG was also less functionally connected with more frontal regions, such as the paracingulate gyrus, which can reflect interactions in top-down strategies. The frontal regions may be involved in the control of action plans and in the selection and/or inhibition of action chunks. This is particularly important for musical performance, as chunking is defined as the re-organization or re-grouping of movement sequences into smaller sub-sequences during performance, to facilitate the smooth performance of complex movements and to improve motor memory (Zatorre et al., 2007). Those kinds of patterns involving networks with increased and decreased activations or FC have already been described for motor training (Allison et al., 2000; Voelcker-Rehage and Niemann, 2013) and in the music literature (Lahav et al., 2007; Petrini et al. 2011; Chen et al., 2012; Pinho et al., 2014). They are furthermore thought to be associated with greater automaticity in cognitive processes (Voelcker-Rehage and Niemann, 2013; Beaty, 2015).

Finally, the fact that the adult brain is capable of NP modifications, as demonstrated here, highlights the potential of rehabilitation treatments designed to induce plastic changes to overcome impairments due to brain diseases (Wan and Schlaug, 2010), which may include stroke, traumatic brain injury, and a range of neuropsychiatric disorders (Cramer et al., 2011). Intriguingly, most regions highlighted by our analyses (IPL, SPL, MTG, and STG) are part of the human mirror neurons system (MNS), which is activated when an individual performs an action and when a similar or identical action is passively observed (Molenberghs et al., 2012). From a clinical perspective, our results could be particularly interesting to consider a drum-based intervention in disorders involving a MNS dysfunction such as in autism spectrum disorders (ASD) (Molnar-Szakacs and Heaton, 2012). Drumming with a social partner could then be particularly relevant for individuals with ASD. Previous studies investigating interpersonal body movement synchronization and social processes found that drumming could improve social interaction (Hove and Risen, 2009; Kirschner and Tomasello, 2009; Yun et al., 2012).

By using additional neuroimaging methods, future studies should also investigate further the functional interplay between cortical and cerebellar regions. Here, DTI tractography and/or structural MRI analyses could explore whether the changes in FC are accompanied by structural changes, which would help illuminate the role of the cerebellum in multimodal action learning. Furthermore, the complex interplay between the left–right hemispheres, as showed in our FC results, should be specifically investigated. Indeed, it is now well-known that musical ability is associated with left–right differences in brain structure and function (Schlaug et al., 2001). However, drummers are unique in that they combine both independent and multilimb coordination when playing, which is necessary for the physical multitasking of drumming and which can lead to complex interhemispheric interactions. Adding an active control group participating in nonmusical motor activities would also help distinguish motor action and higher functions involved in music training. Such an active control condition could consist of learning a new physically demanding multilimb motor sport (such as playing basketball). Finally, adolescence is characterized by changes in brain structure and function, particularly in regions of the cortex, notably the frontal, parietal and temporal regions, that are involved in higher-level cognitive processes (e.g., memory), for which capacity may be increased

in adolescence. It has thus been suggested that adolescence might represent a second "window of opportunity" in brain development (Fuhrmann et al., 2015). However, our results cannot be explained by typical growth trajectories, as volume, thickness, and surface area of the temporal and motor cortices are decreasing in adolescence (Østby et al., 2009). Hence, future experimental studies are needed to compare effect of environment manipulation, such as drum training, in child, adolescent, and older adult groups.

In conclusion, this is the first FC study to compare novice healthy participants before and after an extensive coordinated motor learning such as drumming. An agnostic data-driven approach, i.e., resting-state MVPA, was used to examine the FC of all voxels in the brain, independent of a priori anatomical hypotheses. This method allowed us to highlight the central role of the posterior part of the STG in this task. Moreover, by using the MVPA results as seeds to compute a seed-to-voxel analysis, we showed complex patterns of increased and decreased FC associated with drum training that were partly correlated with the improvement in drum performance. Indeed, as musical training has been shown to modulate unimodal as well as multimodal cortical processing, the seed-to-voxel analyses provide important information on the interaction between the cortical areas involved within the whole network supporting the instrumental performance (Pantev and Herholz, 2011). Finally, one can argue that our drum training regimen is a relevant task to highlight the neuroplastic mechanisms involved in motor learning in a naïve population and that drum-based intervention could be relevant to overcome impairments due to brain diseases.

Supplementary Material

Supplementary material can be found at: http://www.cercor. oxfordjournals.org/.

Notes

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II.VI. REFERENCES

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III. GIVE ME A BEAT! DRUM TRAINING INDUCES LONG-TERM PLASTICITY IN THE CEREBELLUM AND CONNECTED CORTICAL THICKNESS

III.I. ABSTRACT

It is unclear to what extent cerebellar networks show long-term plasticity and accompanied changes in cortical structures. Using drumming as a demanding multimodal motor training, we compared cerebellar shape, lobular volume, white matter microstructure and cortical thickness of 15 healthy non-musicians before and after learning to drum, and 16 age matched novice control participants. After 8 weeks of group drumming instruction, 3 x 30 minutes per week, we observed the cerebellum significantly reshaping itself and reorganising its grey (volume increase of left VIIIa, relative decrease of VIIIb and vermis Crus I volume) and white matter microstructure in the inferior cerebellar peduncle. These plastic cerebellar changes were complemented by a reorganisation of cortical thickness (increase in left paracentral, right precuneus and right but not left superior frontal thickness), suggesting an interplay of cerebellar learning with cortical structures enabled through cerebellar pathways.

III.II. INTRODUCTION

Early lesion studies by Rolando and Flourens (Flourens, 1825) in the eighteenth century revealed the importance of the cerebellum in motor action and movement coordination, leading Flourens to suggest the cerebellum as a seat for motor learning. Since then, several studies have shown that intense and prolonged motor activity induces significant changes in cerebellar structures. Specifically, motor training in rodent models increases cerebellar glial volume per purkinje cell (Anderson et al., 1994), volume of the molecular layer (Black et al., 1990), number of synapses (Black et al., 1990) and dendrites of stellate cells (Kleim et al., 1997). In humans, cerebellar networks show long-term plasticity even into adulthood (Imamizu et al., 2000), indicating experience-dependent adaptive and learning processes to be a salient feature of cerebellar function (Hansel et al., 2001).

In order for the cerebellum to use plastic processes for skill development, it has to create internal prediction models prior to actual performance (Bhanpuri et al., 2013). For this,

recent studies suggest that the cerebellum integrates multisensory information in the somatosensory posterior cerebellar lobe to calculate a 'state estimate' in order to accurately plan and optimise actions (Imamizu et al., 2000). Through its broadly distributed system of cortical connections enabled by its three white matter pathways, the cerebellar peduncles, cerebellar plastic changes are able to influence and prime cortical plasticity in connected cortical regions (Popa et al., 2013). Changes in white matter microstructure are indeed often paralleled by alterations in grey matter volume (Sagi et al., 2012; Taubert et al., 2010). Diffusion tensor imaging (DTI) provides measures to study such changes in white matter pathway microstructure, with fractional anisotropy (FA) reflecting anatomical white matter features such as axon calibre and myelination, and mean diffusivity (MD) reflecting membrane permeability amongst others (Jones et al., 2013). Specifically, long-term motor training has been shown to increase white matter FA of the inferior cerebellar peduncles (ICP; Drijkoningen et al., 2015), a pattern that, together with a decrease in white matter MD, has been implicated in plastic changes (Sagi et al., 2012). Furthermore, motor training has been shown to increase posterior cerebellar volume and plastic changes in connected cortical regions such as parietal and frontal regions in humans (Popa et al., 2013) and animals (Lalonde et al., 1990). Moreover, cerebellar inhibition, by using transcranial brain stimulation, has been shown to abolish plasticity in motor and frontal cortices (Popa et al., 2013). Finally, the cerebellum is also capable of not only volume, but also shape changes with learning in humans (Leunissen et al., 2014) and animals (Lerch et al., 2011).

The cerebellum not only plays a role in simple motor learning, but rather expresses a range of different functions, including working memory, response timing, action planning and attentional control (Ito, 2012). In order to take this array of cerebellar functions into account, we chose drumming as a novel and demanding multi-limb training method to challenge the brain to maintain sustained attention, and to integrate somatosensory information. In order to capture several potential aspects of structural reorganisation, we chose to use a variety of multimodal MRI measures and analyses: from structural cerebellar segmentation, DTI, shape analysis to cortical thickness.

Using this holistic approach, we expected that drum training would increase posterior somatosensory cerebellar volume in order to meet the high demands for somatosensory

integration; decreased Crus I volume paralleling previous findings of decreased functional connectivity in this structure in the same group (Amad et al., 2016); and observe changes in cerebellar shape reflecting local cerebellar volume changes. Finally, we expected that these longer term plastic changes in the cerebellum altered white matter microstructure in the ICP, in turn enable plastic changes in connected parietal and prefrontal cortical areas as reflected by increases in cortical thickness.

III.III. METHODS

Participants

The same thirty-one right-handed, young, healthy volunteers (16-19 years) included in Chapter II.I. participated in the study after providing written informed consent. The participants were assigned to two groups: the drum group and the control group (**Table 1**). In order to keep experiences outside the study similar, our volunteers were fellow students matched on age and biological sex. The King's College London College Research Ethics Committee approved the experimental protocol.

Assessment

All participants attended two scanning sessions at the Institute of Psychiatry Psychology and Neuroscience (IoPPN) at King's College London. At the first scanning session (T1) the Edinburgh Handedness Inventory Short Form (Veale, 2014) was used to assess participant hand dominance. To assess the baseline drumming ability and musical experience, a self-report measure was created. Participants were asked to report their level of skill and length of involvement in (i) drumming, (ii) playing of another musical instrument and (iii) involvement in dance or singing in order to provide a measure of drumming and general musical experience to control for skill novelty. Responses for all three domains were coded on an ordinal scale (0, no experience; 1, some experience but no formal instruction; 2, limited formal instruction but not recent; 3, formal instruction of less than 4 years but not current; 4, formal instruction, exams achieved, greater than 5 years involvement and current). Scores for each scale were added to generate a total score from 0-12.

Drumming measures

Following the first drumming assessment and scanning session the drum group took part in three 30-minute low intensity group drumming sessions per week for 8 weeks. Each session was delivered by the same professional drum tutor and comprised of 4 integrated parts: (i) a warm up, focused on beating the drum with a relaxed and consistent motion of the drum sticks; (ii) snare drum 'rudimental' exercises, played on a single drum surface, adopting a 'flow sticking' approach to sequences of left and right hands; (iii) coordinated 'groove' patterns, incorporating the interplay of bass drum (right foot) and the hi-hat pedal (left foot) with rock/pop back beat *ostinato* patterns played on the hi-hat or ride cymbal and snare drum; including eighth note (quaver), quarter note (crotchet), sixteenth note (semiquaver), syncopated quarter note and shuffle continuously repeated rhythm; and (iv) performance of learned 'grooves' and 'fill-ins' to accompany well-known popular music songs. The complexity of drumming tuition was increased on a weekly basis in line with participant's demonstration of improved drumming coordination and technique. The control participants were asked to not take part in any musical activities for the duration of the study. After the 8 weeks (T2) participants came back to the IoPPN for a second drumming assessment and scanning session.

All of the drumming was performed on electronic drum sets for both drumming training (HD3, Roland, Nakagawa, Japan) and assessment (TD9, Roland, Nakagawa Japan). Drumming was assessed following a short coaching period on the participants' ability to play a simple 4 quarter note pattern to the song "Green Onions" (Booker T and the MGs, Stax/Atlantic, 1962) and a simple 8 eighth note pattern, consisting of regular eighth note hi-hats with alternating kick and snare on the main beats of the bar, to the song "Billy Jean" (Michael Jackson, EPIC, 1982). Versions without tempo fluctuations were used, created using the software "Live" (V9.1, Ableton, Berlin), creating a precise sample for each beat location, accurately placed on the transient of each audible click (Robertson, 2014) (see supplementary material for more details). Timing data were exported from the drum set using the musical instrument digital interface (MIDI) signal. A comparison with a piezo microphone placed on the snare indicated that the recorded MIDI events were a maximum of 4ms from the detected onset using audio-based methods, and generally much closer. Drumming ability was assessed objectively as the percentage of

bars of both patterns that were completed during two 2-min periods of data capture (1–3 min of each song). To record a completed bar all elements had to be present in each pattern and within half a beat (250ms for 4 quarter note pattern and 125ms for an 8 eighth note pattern) of the perfect timing. To evaluate the error in events that should have been synchronous (flamming), a flam error per bar was measured (in milliseconds). The time between the two events was measured for each note of the bar. When 3 limbs were involved, the difference between the first and last event was measured. This was only evaluated in completed bars to avoid exaggeration of this error when the pattern was breaking down or incomplete. While a drum flam can be a sought-after stylistic feature of a pattern, flams were not part of the stylistically correct performance for the two chosen patterns.

For both patterns, each limb played a single part of the kit. To perform Green Onions the right hand played ride cymbal, left hand snare drum, right foot kick drum and left foot high-hat pedal. A bar was made up of right hand and right foot striking together followed by right hand, left hand and left foot striking together (repeated to make the four notes of the bar). The Hi-hat, Ride and Snare (HRS) condition required the participants to use their left foot, right hand and left hand. The flam error for this condition was calculated as the time difference (ms) between the first and last action at each point in the bar. The flam error (ms) from the second and third beats of the bar was used as a measure of coordination since this involved the coordination of both hands with the left foot. The differences between the more complex eighth note pattern (Billy Jean) and the less complex quarter note pattern (Green Onions) was used to assess whether the participants had learned to drum, whereas the HRS measure determines how precise their drumming was. No practice of either song used in the assessment was included in the 8 weeks drumming training.

MRI acquisition

All participants were scanned at the Centre for Neuroimaging Sciences, Institute of Psychiatry, London, UK, using a 3-T GE Sigma System (General-Electric, Milwaukee, WI). High-resolution structural T1-weighted volumetric images were acquired with full-head coverage, 196 contiguous slices (1.2 mm slice thickness), a 256 x 256 matrix, and a repetition time/echo time (TR/TE) of 7.3/3ms (FA =11°, FOV = 270 mm^2). Diffusion Tensor

Imaging data was acquired using a spin-echo echo-planar imaging (SE-EPI) sequence providing whole head coverage with the following parameters: TE=78.5ms, TR equivalent to 12 RR interval, FOV=256 x 256, matrix size of 128 x 128, 72 slices with a thickness of 2 mm (no gap) making an isotropic voxel of 2.0 x 2.0 x 2.0 mm. Diffusion weighting was applied along 60 uniformly distributed directions and with a b-value of 1500 s/mm^2. Six non-diffusion weighted volumes were also acquired. The acquisition was gated to the cardiac cycle using a peripheral gating device placed on the participants' forefinger and with a total scan time of approximately 14 minutes. Consistent image quality was ensured by visually inspecting all datasets.

Cerebellar Segmentation

Prior to analysis, each T1 MP-RAGE was visually inspected to ensure inclusion of only minimal movement artefacts. No scans were discarded. Regional volume of the cerebellum was calculated using the SUIT toolbox (http://www.icn.ucl.ac.uk/motorcontrol/imaging/suit.htm) of the SPM12 software (http://www.fil.ion.ucl.ac.uk/spm/). This toolbox provides a high-resolution atlas template of the human cerebellum and brainstem that preserves the anatomical detail of cerebellar structures, as well as dedicated procedures to automatically isolate cerebellar structures from the cerebral cortex and to accurately normalise cerebellar structures to this template. Prior to normalisation, the individually created isolation maps were loaded into FSLView (www.fmrib.ox.ac.uk/fsl) where they were visually inspected against the cropped image and hand corrected if necessary. Using the inverse of the resulting normalisation transform, a parcellation of the cerebellum was obtained, based on the probabilistic magnetic resonance atlas of the human cerebellum (Diedrichsen et al., 2009) provided within the SUIT toolbox. Volumes of interest were then overlaid onto each individual participant's structural scan and inspected to ensure accurate registration. Parallel to whole brain analyses (Sowell et al., 2002), how each lobular volume relates to the rest of the cerebellum throughout development may be conceptualised as each volume's proportion of the total cerebellar volume (TCV). TCV was calculated as the sum of all cerebellar lobules (I-IV, V, VI, Crus I, Crus II, VIIb, VIIIa, VIIIb, IX, X) and regressed out of all lobular volumes. All used cerebellar volumes from hereon were TCV corrected.

Shape Analysis

For shape analysis of the cerebellum, the FSL FIRST software version 5.0.8 (Patenaude et al., 2011) was used. This software performs shape and appearance deformations along the eigenvectors. Shape is modelled using a surface-based point distribution model such that the shape space is constrained to the linear combination of the mean shape and eigenvectors of the vertex coordinates. In addition, to model intensity at the structural boundary, intensities were sampled along the surface normal from the underlying image. Using a linear combination of these eigenvectors, a 3D mesh surface of the cerebellum is deformed from the mean shape for the best registration onto the putamen in the participant's brain. The putamen was used here as the corresponding reference structure. Once this registration was done, the location of each vertex on the surface was recorded and final vertex files were loaded into FSLView, where they were visually inspected. This approach assumes that each vertex corresponds to the same anatomical location across participants. Therefore, the mean shape of, and deviations from the mean, for each participant can be calculated and vertex-by-vertex statistical analyses can be performed to investigate various effects on the shape. The normalised intensities along the surface were sampled and modelled, allowing shape and appearance model being based on multivariate Gaussian assumptions. Shape was then expressed as a mean with modes of variation (principal components).

Cortical Thickness Analysis

The FreeSurfer analysis suite version 5.3.0 (http://surfer.nmr.mgh.harvard.edu/) was used to derive models of the cortical surface in each T1-weighted image. These well-validated and fully automated procedures have been extensively described elsewhere (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 1999; Fischl et al., 2004; Jovicich et al., 2006; Ségonne et al., 2004). In brief, a single filled white matter volume was generated for each hemisphere after intensity normalisation, skull stripping, and image segmentation using a connected components algorithm (Dale et al., 1999). Then, measures of cortical thickness were computed as the closest distance from the grey and white matter boundary to the grey matter and cerebrospinal fluid boundary at each vertex on the tessellated surface. The results were inspected and checked for quality following the ENIGMA protocol (www.enigma.ini.usc.edu).

Diffusion Tensor Imaging

Diffusion data was pre-processed for motion and geometrical distortion correction using ExploreDTI (Leemans et al., 2009). For each participant the b-matrix was then reoriented to provide a more accurate estimate of diffusion tensor orientations (Leemans and Jones, 2009). Diffusion tensor estimation was performed using a non-linear least square fitting method (Jones and Basser, 2004). Fractional Anisotropy (FA) and Mean Diffusivity (MD) maps were generated. Whole brain tractography was performed using all brain voxels with FA ≤ 0.2 as seed region. Streamlines were propagated using an Euler integration (Basser et al., 2000) tractography algorithm with a step size of 0.5mm and with an angular and anisotropy threshold of 45 degrees and FA ≤ 0.2 respectively. Finally, diffusion tensor maps and whole brain tractography were exported to TrackVis version 0.5.2.2 (Wang et al., 2007) for manual dissection and quantification fibre tracts of the cerebellar tracts.

Cerebellar Tract Dissections

To define the cerebellar peduncles, a manual two regions-of-interest (ROI) selection method was used (Catani et al., 2008; Dell'Acqua et al., 2013). First, inclusion and exclusion ROIs were defined on FA maps to define the three cerebellar tracts: the middle cerebellar peduncle (MCP), as well as the left and right ICP and superior cerebellar peduncle (SCP). To extract clean diffusion measurements avoiding crossing and complex white matter regions, each cerebellar peduncle was cut so that only the tract segment between the two ROI was used for quantification. Inclusion ROIs for the ICP were drawn at the medulla oblongata until the level of the dentate; for the MCP a single tract was dissected by selecting the pontine nucleus and before entering the two cerebellar hemispheres; from above the dentate nucleus to the entrance of the tegmentum in the mesencephalon as landmarks for the SCP. Remaining spurious streamlines were removed.

Statistical analyses

Statistical analyses were performed using the SPSS statistics software package (Version 22, IBM, Chicago, Illinois, USA). Independent sample t-tests or Mann-Whitney rank sum test, and paired t-tests were used on group characteristics to determine baseline differences, and the effects of drum training between and within groups. As gender may

be a factor modulating musical training (Koelsch et al., 2003) and possibly cerebellar volume (Llorente et al., 2009), all analyses included gender as a covariate.

In order to assess significant drumming improvement, a mixed model (time x group) ANOVA was run on the performance measures (completed bars for 'Green Onions' and 'Billy Jean', HRS) over time (T1 and T2) between groups with gender as a covariate. Furthermore, a two-tailed Spearman's *Rho* correlation analysis between left VIIIa, VIIIb and vermis Crus I volume and the increase in HRS precision following 8 weeks of drum training (T2) as well as individual ANCOVAs run on cerebellar volumes between groups with gender as a covariate were performed.

To test whether there was a significant interaction of group over time for brain measures, we performed a repeated measures ANOVA model by specifying "time" as a within-participants contrast (T1-T2, i.e. before and after the drum training) followed by a between participants contrast (drummers vs. controls). Significance was set at $p < 0.05$ and results were Bonferroni corrected for multiple comparisons with $p < 0.05$ when applicable.

III.IV. RESULTS

Table 1. Participant demographics displaying participant number, gender and age distribution for both groups

	Drum group			Control group		
	Male	Female	Total	Male	Female	Total
Number	7	8	15	8	8	16
Mean age in years (SD)	17 (.58)	16.6 (.74)	16.8 (.68)	18 (1.41)	17.8 (1.39)	17.9 (1.36)

Based on a self-report measure, the drum group and the control group presented with no prior drumming experience (all scores ≤ 2). Moreover, there were no statistical differences between the two groups in terms of self-reported proficiency in other musical instruments (dance: $p = 0.53$; singing: $p = 0.31$) and both groups were matched on gender and age (female: $p = .063$, male: $p = .105$; **Table 1**). The drumming training had a gender corrected statistically significant effect for time ($F_{(3,24)} = 5.390$, $p = .011$), time x performance ($F_{(3,24)} = 8.292$, $p = .001$) and time x performance x group ($F_{(3,24)} = 3.415$, $p = .034$). A before/after video is available for illustration (see https://vimeo.com/141911618).

Assessing bars completed for 'Green Onions', there was no group x time interaction ($F_{(1,26)} = 3.3$, $p = 0.091$), no main effect of group ($F_{(1,26)} = 3.9$, $p = 0.07$), but a main effect of time ($F_{(1,26)} = 19.9$, $p = 0.001$). Thus, there were no baseline or post-intervention differences between the two groups, with both groups improving their performance after the 8 weeks. For 'Billie Jean', the was a group x time interaction ($F_{(1,26)} = 21.9$, $p < 0.001$), a main effect of group ($F_{(1,26)} = 26.1$, $p < 0.001$), and a main effect of time ($F_{(1,26)} = 36.1$, $p < 0.001$). Follow up analysis showed that the groups were not different from each other at baseline for either song ($p = 0.148$ and $p = 0.123$), but the drum group recorded a higher number of bars than control after the 8 weeks ($p = 0.012$ and $p < 0.001$).

For 'Green Onions', a repeated measures ANOVA on the HRS condition revealed no significant group x time interaction ($F_{(1,26)} = 3.3$, $p = 0.091$) or effect of group ($F_{(1,26)} = 3.9$, $p = 0.07$), significant effect of time ($F_{(1,26)} = 19.9$, $p = 0.001$). For 'Billie Jean', repeated measures ANOVA on the HRS condition revealed a significant group x time interaction

($F_{(1,26)}$ = 21.9, p < 0.001), as well as a significant effect of group ($F_{(1,26)}$ = 26.1, p < 0.001) and time ($F_{(1,26)}$ = 36.1, p < 0.001).

In addition to this, we assessed the participants on their ability to use their left foot, right hand and left hand (HRS) to determine the precision of their drumming. Here, the drum group showed a reduction of error and thus increase in precision of 13.06 % for the HRS condition, while the control group increased in error and thus decreased in precision in -4.63 %.

Changes in regional cerebellar volumes and shape

In the drum group, vermis Crus I decreased significantly (corrected model: $F_{(2,59)}$ = 4.103, p = .039; group: $F_{(1,59)}$ = 9.250, p = .012) and lobule VIIIb (left VIIIb: $F_{(2,59)}$ = 6.368, p = .009; right VIIIb: $F_{(2,59)}$ = 5.199, p = .012), whereas the left VIIIa (corrected model: $F_{(2,59)}$ = 5.980, p = .012) increased in volume (**Table 2**). The increase and decrease pattern of the drum group contrasted with the control group can be seen in **Figure 1** and **Table 2**.

HRS precision following 8 weeks of drum training (T2) was positively correlated with left VIIIa volume (τ = .390, p = .042) after training in the drum group. No significant correlation was evident before drum training (T1).

Vertex-wise analysis of the cerebellum revealed significant local shape differences in the drum group at T2 compared with T1 (p < 0.05, FDR corrected). Here, vector arrows indicate expansions in the posterior lobules including lobule VIII, and a restriction in the lateral cerebellum, including lobules Crus I and II (**supplementary Figure 1**).

Changes in white matter microstructure

Individual ANCOVAs were determined for fractional anisotropy (FA), mean diffusivity (MD) and anisotropic power (AP) in the middle of both hemispheres of the ICP and SCP between groups with gender as a covariate. While FA significantly increased in the right ICP ($F_{(2,59)}$ = 4.466, p = .032) accompanied by decrease in MD ($F_{(2,59)}$ = 4.539, p = .030), the left ICP significantly decreased in FA ($F_{(2,59)}$ = 5.583, p = .012; **Table 2**). Other cerebellar tracts did not show significant differences. All results were Bonferroni corrected for multiple comparisons.

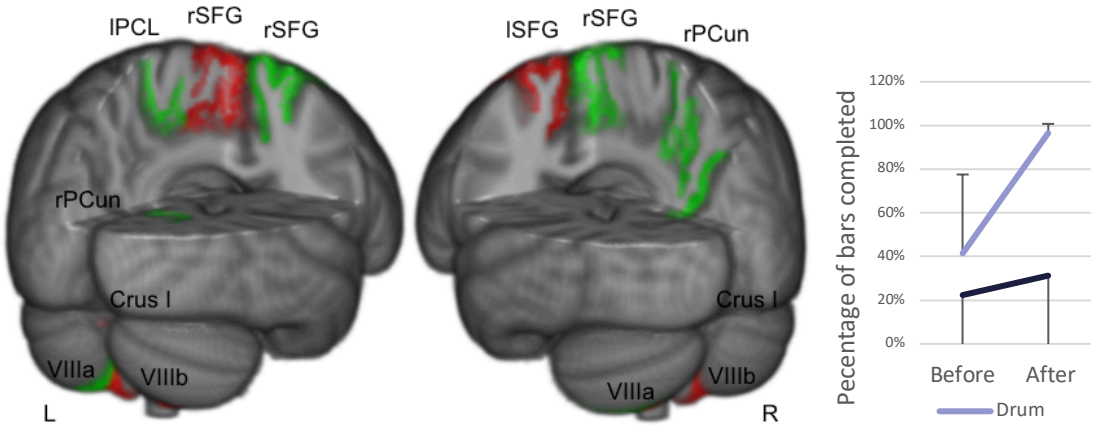
Table 2. Changes in cerebellar lobular volume and diffusion measures as well as cortical thickness after 8 weeks of drum intervention (drum group) and with no intervention (control group)

	Before		After		*p*	*F*
	Control	Drum	Control	Drum		
Cerebellar grey matter						
Vermis Crus I	21 (5.61)	27 (7.18)	23 (5.24)	25 (8.52)	.012*	9.25
Left VIIIa	5743 (10**44**)	5367 (879)	5719 (959)	5402 (863)	.012*	9.16
Left VIIIb	4613 (672)	4454 (672)	4634 (673)	4428 (703)	.003**	11.51
Right VIIIb	4665 (675)	4659 (712)	4641 (670)	4648 (688)	.036*	6.78
Cerebellar white matter						
Left ICP						
FA	56.83 (3.70)	55.97 (3.55)	56.50 (4.48(	55.05 (3.99)	.012*	5.58
MD	0.071 (0.003)	0.073 (0.009)	0.073 (0.005)	0.072 (0.006)	.810	1.34
Right ICP						
FA	58.92 (3.67)	56.74 (2.87)	57.96 (4.38)	57.23 (3.23)	.032*	4.47
MD	0.070 (0.003)	0.072 (0.005)	0.072 (0.004)	0.071 (0.004)	.030*	4.54
Cortical thickness						
LPARC	2.39 (0.17)	2.46 (0.17)	2.40 (0.17)	2.49 (0.16)	.013*	5.10
LSFG	2.83 (0.15)	2.84 (0.18)	2.76 (0.19)	2.87 (0.16)	.049*	3.39
RPCUN	2.57 (0.11)	2.61 (0.13)	2.60 (0.13)	2.65 (0.15)	.025*	4.26
RSFG	2.69 (0.13)	2.74 (0.17)	2.68 (0.15)	2.78 (0.62)	.042*	3.56

Lobular cerebellar volume of vermis Crus I, left VIIIa, left and right VIIIb were corrected for total cerebellar volume (TCV) and all analyses included gender and age as covariates. Significant changes are marked with '' for p≤0.05 and '**' for p≤0.01. TCV corrected cerebellar grey matter is displayed in mm^2, cerebellar white matter and cortical thickness volume in litres. All standard variations (SD) in brackets.*

Abbreviations: ICP, left inferior cerebellar peduncle; MD, mean diffusivity; FA, fractional anisotropy; LPARC, left paracentral gyrus; LSFG, left superior frontal gyrus; RPCUN, right precuneus; RSFG, right superior frontal gyrus

Figure 1. Changes in cerebellar lobule volume and cortical thickness before vs after drum training

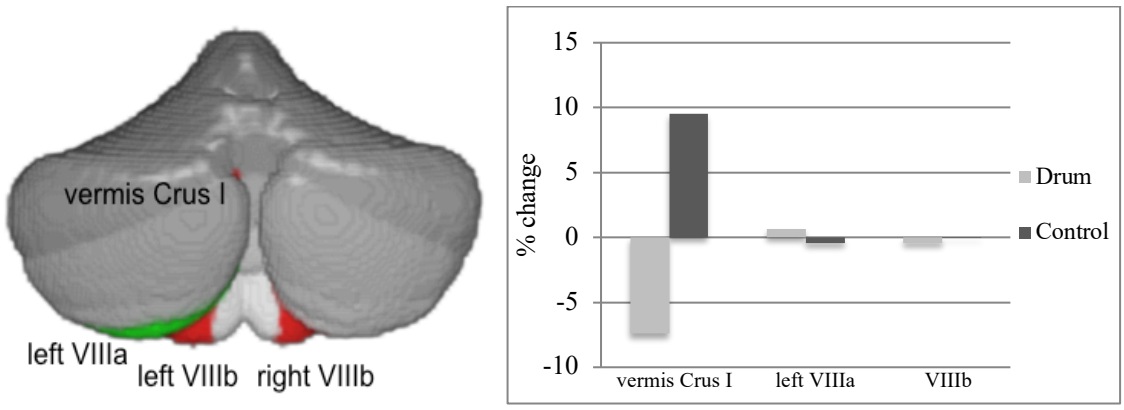


Decreasing cerebellar lobules (vermis Crus I, VIIIb) and areas of cortical thickness (left superior frontal cortex, lSFG) in red. Increasing cerebellar lobules (left VIIIa) and areas of cortical thickness (left paracentral lobule, lPCL; right precuneus, rPCun; and right superior frontal gyrus, rSFG) in green. Percentage of bars completed before and after 8 weeks of drum intervention (drum group) and with no intervention (control group) on the right side.

Changes in cortical thickness

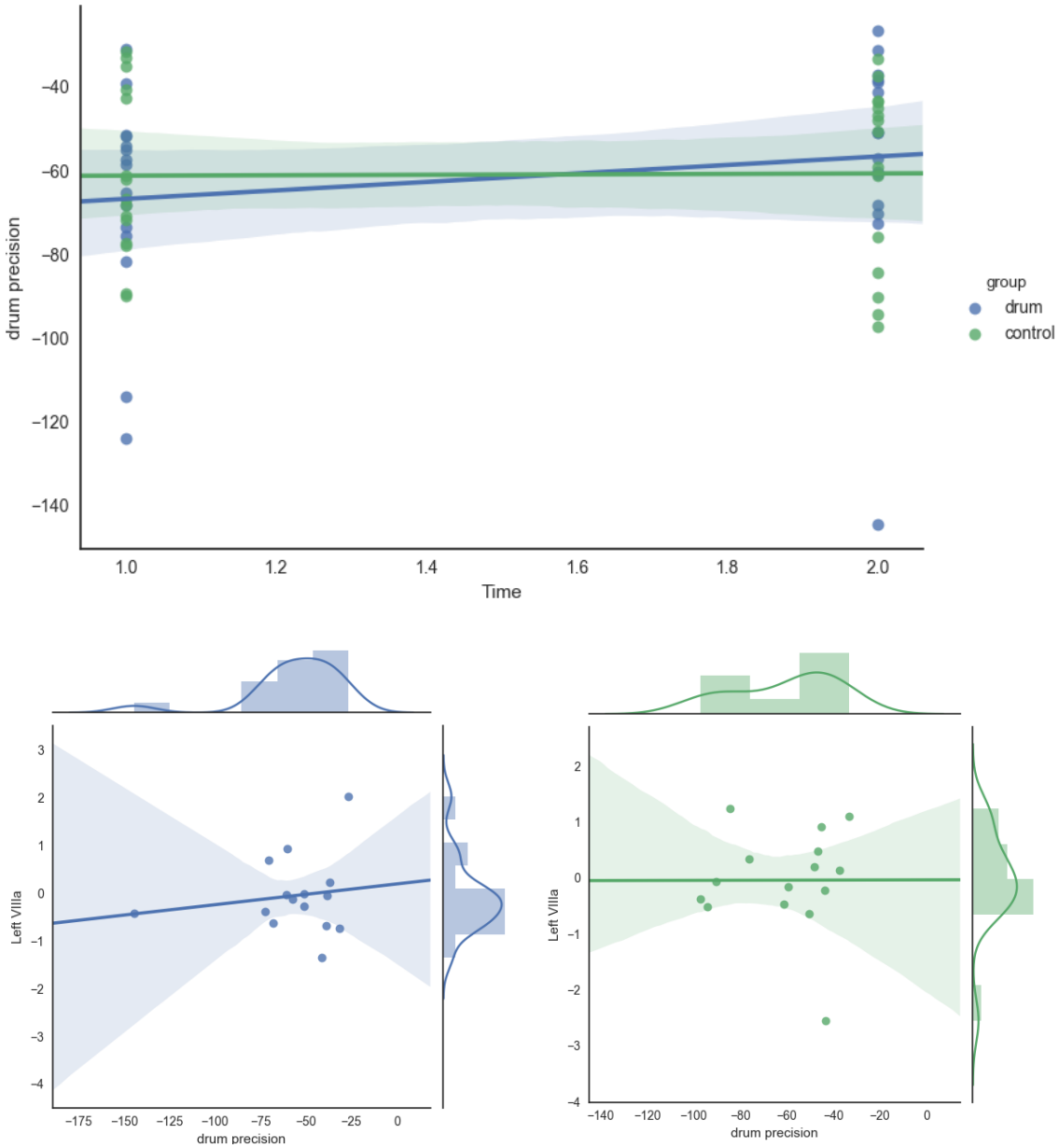
ANCOVAs were calculated for cortical thickness measures between groups with gender as a covariate revealed a significant volume increase of the left paracentral lobule (lPCL $F_{(2,59)}$ = 5.103, p = .026), the right precuneus (rPCun; $F_{(2,59)}$ = 4.264, p = .050), and the right superior frontal gyrus (RSFG; $F_{(2,59)}$ = 3.561, p = .042), but a decrease of the left superior frontal gyrus (LSFG; $F_{(2,59)}$ = 3.385, p = .049) in the drum group (**Table 2**).

Figure 2. Changes in cerebellar volume increase after drumming



On the left, the increased cerebellar volume is shown in green (left VIIIa), and decreased volume in red (vermis Crus I, left and right VIIIb). On the right, the percentage increase of the vermis Crus I, left VIIIa and VIIIb corrected for total cerebellar volume for the drum and control group are shown (vermis Crus I: drum = -7.4 %, control = 9.5 %; left VIIIa: drum = .65 %, control = -.42 %; averaged VIIIb: drum = -.42 %, control = -.03%).

Figure 3. Distribution of drum precision improvement



Distribution of drum precision improvement as measured by the High-hat, Ride and Snare (HRS) condition for the drum (blue) and control (green) group on top and its correlation with left VIIIa volume below for each group at the second scanning time point. Confidence intervals are displayed shadowed, individual values as scatterplots (dots) and regression correlation as a continuous line with kernel density fits for each variable on the top and right side.

III.V. DISCUSSION

In this longitudinal neuroimaging study, we demonstrate that drum training changes cerebellar volume and thickness of connected cortical areas. These findings are in line with previous animal and human studies showing a relationship between motor-skill learning and structural plasticity in the cerebellum (Black et al., 1990; Drijkoningen et al., 2015; Kleim et al., 1997; Taubert et al., 2010). Such plastic changes have been documented to reorganise cerebellar structure first followed by cortical structures (Popa et al., 2013), where for example transcranial magnetic stimulation over the cerebellum has been shown to alter the plastic motor cortex response (Popa et al., 2013). Thus, it could be suggested that the changes observed in our study might indicate a reorganizational mechanism leading to a change of focal brain volume.

Changes in cerebellar volume and shape

It has previously been shown that Lobule VIII integrates somatosensory information and primes cortical plasticity in motor and frontal cortices (Popa et al., 2013). Nevertheless, the specific contribution of each subdivision of this cerebellar lobule remains unclear. A recent meta-analysis of cerebellar functional connectivity clustering the cerebellar lobules by behaviour, revealed that lobule VIIIa together with VIIb comprise a cluster specialised in action and motor execution (Riedel et al., 2015). Clinical and behaviour studies have shown that this cluster is further associated with motor processes requiring perceptive feedback and strong attentional control (Townsend et al., 2013). Drumming requires both of these functions to help drummers to use their arms and legs independently or simultaneously while keeping a steady beat, just as in our HRS condition. Here, participants were required to use their left foot, as well as right and left hand to produce a continuous rhythmic pattern. The increase in VIIIa volume and its positive correlation with error reduction in the HRS condition supports the notion that our training has led to these plastic changes (Figure 3). Unfortunately, the structures VIIIb and vermis Crus I were excluded from Riedel's meta-analysis, thus not allowing for direct comparison. Nevertheless, they are structures shown to be bilaterally activated when a temporal pattern has to be held across a delay period prior to a response. Transcranial magnetic stimulation studies have suggested that the same cerebellar subregions influence temporal error correction (Spencer and Ivry, 2013). In drumming, keeping a

rhythmic pattern in mind before playing it would allow for higher precision playing and staying on the beat. The vermis is further involved in the production of such a timed motor response, particularly when it is novel (Penhune et al., 1998) but reduces its activation when a skill has been learned (Haslinger et al., 2004). We detected a volume reduction of the vermis Crus I paralleling our previous finding of decreased Crus I/ II functional connectivity in the same group (Amad et al., 2016). In addition, while the cerebellar volume of vermis Crus I displayed a 2% stronger increase in the control group than the decrease found in the control group (Figure 2) possibly indicating typical growth patterns as the cerebellum continues to grow until adulthood (Ito et al., 2012), it underlines the strong difference in vermis Crus I volume after drum training between both groups accumulating to 17%. While lobule VIIIb increased in volume, it did so much less than the control group (Figure 1), which could reflect a relative decrease by comparison. The cerebellum uses long term depression in order to automate actions, resulting in a decrease of activation of the lobule to increase efficiency (Haslinger et al., 2004; Petrini et al., 2011).

Thus, the bilateral decrease of VIIIb and vermis Crus I volume after drum training observed here could suggest that temporal error reduction has been successful and temporal motor accuracy has been learned, while the novelty of the action has faded. Intriguingly, the FDR corrected vertex mesh showed a similar pattern of increase and reduction to the one we observed in cerebellar volume, which appeared to be three-dimensional once surface opacity was changed to full transparency (Supplementary Figure 1).

Microstructural white matter changes in the inferior cerebellar peduncle

To further investigate the expansion of cerebellar reorganisation after drum learning, we used diffusion tensor imaging to probe changes in microstructure of the white matter pathways linking the cerebellum to the cortex. One major white matter pathway highly implicated in plastic changes after skill learning is the ICP (Drijkoningen et al., 2015).

While the SCP represents the main efferent cerebellar output, the ICP contains mainly afferent pathways carrying sensory information about the body parts to the cerebellum via fibres from the spinocerebellar tract (Bastian et al., 2004; Naidich et al., 2009). This enables it to mediate and store a spectrum of motor behaviour (Riddle et al., 2009), all

functions highly necessary for successful drumming.

Previous reports have indicated a strong link between changes in structure and diffusion properties, and several studies investigating training effects and brain plasticity have reported increased grey matter volume paired with increased white matter fractional anisotropy (Taubert et al., 2010) and/or with decreased mean diffusivity (Sagi et al., 2012). Fractional anisotropy (FA) is a scalar value that indexes the degree of directional preference in the pattern of water diffusion, while mean diffusivity (MD) assesses the overall rate of water diffusion across the tissue (Jones et al., 2013). Thus, various observations would predict that the landscape of white matter would change after training, showing an increased FA and decreased MD in the relevant white matter pathways (Sagi et al., 2012) and that these changes in diffusion could also be paralleled by changes in cerebellar volume.

Indeed, we were able to detect such an increase in both FA along with a decrease of MD in the right ICP. These changes in diffusion were accompanied by an increase of left VIIIa volume and a decrease in VIIIb and vermis Crus I volume after training. Interestingly, both the VIIIb and the reticulo-cerebellar tract have been implicated in memory storage related to motor control (Spencer and Ivry, 2013) and FA and VIIIb volume showed a similar increase-decrease pattern for both hemispheres after drum training (Table 2). However the exact tissue properties of these white matter microstructure changes cannot be determined, as increases in FA can be attributed to several underlying changes in tissue characteristics including an increase in the extent of myelination (Jones et al., 2013).

Resting-state networks including the default-mode network (Teipel et al., 2010), are structurally interconnected by known white matter tracts, enabling such plastic changes to affect the whole brain. While functional and structural brain organisation are likely to be linked (van den Heuvel et al., 2009), their exact relationship remains unknown (Bullmore and Sporns, 2009). Indeed, the maturation of the cerebellar networks is a dynamic process where changes to one tract are likely to affect the whole circuit, including connected cortical structures, such as the prefrontal and parietal cortex.

Changes in cortical thickness

To play on the beat, drumming requires not only temporal accuracy, but also spatial organisation of movements and strong attentional control. In a PET study with non-musicians trained to play a specific tune on the keyboard, listening to the trained but not equally familiar songs increased activation in the posterior lateral cerebellum, premotor and parietal areas. Furthermore, with practice, the participants increasingly came to depend on internal rather than external cues to time their responses (Ramnani & Passingham, 2001), suggesting the development of an internal model.

Interestingly, we were able to show an increase in posterior cerebellar volume and cortical thickness in two parietal areas – the precuneus (PCun) and paracentral lobule (PCL). The precuneus is specialised in translating visuospatial and temporal information into motor coordinates, especially that of sequential movements with reference to memorised patterns (Cavanna, 2006). It is further activated during music processing (Platel et al., 2003) and can access multiple cerebellar circuits (Cavanna, 2006). Functional and structural network studies have described the precuneus as a network hub between parietal and prefrontal regions (Bullmore and Sporns, 2009). Being part of both the prefrontal and parietal cortex, the paracentral lobule innervates motor and sensory modules of the contralateral extremities and its anterior portion is connected to the cerebellum (Wiesendanger and Wiesendanger, 1985). Furthermore, just as the left VIIIa (Figure 3), a posterior cerebellar region, this structure increased after training. Cortical thickness has been described as a sensitive measure for plastic changes in the brain (Bhanpuri, Okamura, & Bastian, 2014) and the action and motor execution cluster including the VIIIa has been previously shown to be co-activated with the precuneus (Riedel et al., 2015). An increased activation of the precuneus, posterior cerebellum and premotor cortex has further been reported when envisioning future events over the recollection of past events (Szpunar et al., 2007). Thus, this network hub might also contribute to planning future actions.

Besides an increase in PCun and PCL thickness, we were also able to see volume changes in prefrontal regions after drum training, more specifically in the superior frontal gyrus. The SFG is important for working memory and executive processing (Boisgueheneuc et al., 2006). It has been suggested that in musicians, deconstructing and organising a rhythm's temporal structure relates to greater involvement of the prefrontal cortex

mediating working memory (Chen et al., 2008). Indeed, when participants are required to attend to a motor sequence that they can otherwise perform automatically, an increase in prefrontal cortex activity is observed (Jueptner et al., 1997). Furthermore, both prefrontal cortical areas and the left cerebellar VIIIa have been shown to be more engaged in musicians when task requirements are musically relevant in a rhythm task (Chen et al., 2008). These results parallel our findings of increased VIIIa volume and SFG cortical thickness, reflecting the increased need for keeping a steady rhythm in drumming.

It should be noted, that only the right and not the left SFG increased in cortical thickness after drumming. As drumming is a multi-limb activity, drummers are required to use their four limbs independently or simultaneously when they play, leading to complex inter-hemispheric interactions. It is well known that musical ability is reflected in such left-right differences in brain structure and function (Schlaug, 2001), and we were able to display hemispheric differences in functional connectivity in the same group previously (Amad et al., 2016). As all of our participants were right-handed, we propose that the mean diffusivity decrease in the left but not the right ICP paired with the opposite increase-decrease pattern in cortical SFG thickness indicates that left body motor functions have been preferentially strengthened in order to match the high demands of equal velocity needed in drumming.

Furthermore, lateral posterior regions such as the Crus I have been associated with prefrontal cortical networks (Bernard, Orr, & Mittal, 2015), which might suggest a possible connection between the volume decrease of lobule Crus I and LSFG thickness. The changes in cortical thickness that we observed cannot be attributed to ongoing neurodevelopmental processes, as pruning reaches its peak in cortical thickness at age twelve in the parietal and prefrontal lobe (Kostovic and Rakic, 1990). We propose that the increase of cortical thickness reflects an adaptation to the novel demand drumming posits on the brain, namely better temporal accuracy, spatial organisation of movements and strong attentional control enabled by the accumulation of the SFG, PCun and PCL. These structures are further tightly interlinked with each other and the cerebellum, making them an extension of the cerebellar internal model of the drumming rhythms practiced.

Future Studies

Differences in cerebellar volume and functional connectivity have been implicated in several neurodevelopmental disorders including autism spectrum disorder (ASD). While an underlying dysfunctional cerebellar internal model has been proposed for this disorder (Stoodley, 2015), such internal models can be reversed when using a method to match the biased internal model (Bhanpuri et al., 2014). Previous studies investigating interpersonal body movement synchronisation and social processes found that drumming could improve social interaction (Kirschner and Tomasello, 2009) and we recently demonstrated that drum training can alter functional connectivity in regions part of the mirror neuron system (MNS, Amad et al., 2016), a system that is dysfunctional in ASD (Dapretto et al., 2006). Thus, a drum-based intervention in disorders involving MNS and cerebellar dysfunction might be considered to aid treatment. Gaining an understanding of cerebellar development may thus be informative for our understanding of developmental psychopathology, as well as healthy cognitive and motor development (Bernard et al., 2015).

Furthermore, the complex interplay between the left-right hemispheres, as demonstrated by the results from this and our previous study (Amad et al., 2016), should be specifically investigated, for example by using multimodal neuroimaging. Indeed, it is now well known that musical ability is associated with left-right differences in brain structure and function (Schlaug, 2001). However, drummers in particular use their four limbs independently or simultaneously when they play to enable physical multitasking and complex inter-hemispheric interactions. Finally, adding an active control group participating in non-musical motor activities would help distinguish motor action and higher functions involved in music training. Such an active control condition could consist of learning a new physically demanding multi-limb sport (such as playing basketball) or a complex non-rhythmic finger tapping task. To fully understand the extent of the cerebellar learning involved, additional measures that are functionally connected to our results should be applied and more intensive longitudinal scanning comparing both short (e.g. 1hr, 1 day, 3 days, 1 week) and long-term effects (4, 8 and 16 week) is suggested. Such tasks could be tasks for working memory and emotion regulation, as they have previously been implicated in cerebellar function (Ito, 2012).

Conclusion

Drum training reshaped and reorganised the cerebellum, possibly leading to the creation of an internal model of drumming patterns. In order to succumb to the high demands of drumming, we propose that the internal model developed in concordance with cortical structures through cerebellar white matter pathways. This way, the brain is able to maintain sustained attention, multi-limb coordination and integrate signals from multiple sensory modalities in order to achieve optimal drum performance.

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Conflict of interest

The authors declare no conflict of interest.

III.VI. REFERENCES

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IV. GENERAL DISCUSSION

In a succession of two studies, we investigated neuroplastic changes of the brain in neurotypical control participants after learning a demanding multilimb task. After 8 weeks of 3 x 30 minutes per week group drumming instruction, we observed the cerebellum significantly reshaping itself and reorganising its grey and white matter microstructure and functional connectivity, suggesting that internal cerebellar models have been successfully established. These plastic cerebellar changes were complemented by a reorganisation of cortical thickness and functional connectivity, underlining the close connection and development of cerebellar and neocortical structures affecting connected areas in return.

However, such cerebellar models are not always established correctly and underlying dysfunctional cerebellar internal model have been proposed to contribute to the aetiology of neurodevelopmental disorders such as ASD and OCD.

CHAPTER THREE: THE DISORGANISED CEREBELLUM

I. GENERAL INTRODUCTION

While we investigated cerebello-cortico neuroplasticity in neurotypical controls in Chapter two, we were interested to see how cerebello-cortico connections would differ in neurodevelopmental disorders ASD and OCD when compared to neurotypical controls, as differences in cerebellar volume and connected neocortical structures have been highlighted in the literature. Furthermore, while both are distinct disorders, they also share non-diagnosis specific symptomatology in the form of heightened repetitive, compulsive behaviour.

The following array of studies in this chapter aimed at investigating dysfunctional cerebellar internal models by using different neuroimaging methods ranging from machine learning on Jacobian images (II.II.) to shape analysis (II.III.) as well as cortical thickness and surface area (II.IV.) to describe brainstem, cerebellum and neocortical shared and distinct patterns between both neurodevelopmental disorders and neurotypical controls.

II. DON'T FORGET THE CEREBELLUM AND BRAINSTEM WHEN PREDICTING AUTISM SPECTRUM DISORDER

II.I. ABSTRACT

In vivo and post mortem studies have implicated cerebellar and brainstem dysfunction in autism spectrum disorder (ASD), but studies using machine learning driven classification generally exclude both structures. We demonstrate up to 15% higher classification accuracy in childhood and adult ASD driven by a similar ring-like cerebellar pattern when comparing the cerebellum and brainstem as a combined region of interest with the whole brain when both structures were included/excluded.

II.II. INTRODUCTION

Autism spectrum disorder (ASD) is a life-long neurodevelopmental condition affecting 1 in 68 children world-wide (Elsabbagh et al., 2012). It is characterized by repetitive and stereotypic behaviour with known links to cerebellar (D'Mello et al., 2015) and brainstem dysfunction (Rodier, 2002). Both the cerebellum and brainstem develop from the same set of progenitor cells during early prenatal brain development and are involved in sensory integration and emotion regulation (Geva and Feldman, 2008; Buckner, 2013). Accumulating evidence suggests that disruptions during key 'sensitive periods' of shared cerebellum and brainstem development are reflected in neuroanatomic tissue alterations (Skefos et al., 2014). Indeed, reductions in Purkinje cell density and cerebellar hypoplasia are the most consistent post mortem finding to date for ASD (Coleman, 2009; Skefos et al., 2014) with a particular focus on the lateral posterior cerebellar lobe and pons of the brainstem (Hashimoto et al., 1995). Hence, prenatal dysfunctional cerebellar development and/ or damage to the cerebellum as detected by anatomical neuroimaging scans has been identified as a potential in vivo predictor for children developing autistic symptoms (Limperopoulos et al., 2007).

However, to our knowledge no neuroimaging classification approach for ASD using machine learning algorithms has focused on the cerebellum and brainstem, and compared such region of interest classification with the contributions of the neocortex and whole brain to prediction accuracy. Neuroimaging weighted classification maps based on Jacobian images registered to a common template can quantify the amount of enlargement or reduction that each voxel undergoes, possibly informing on deeper tissue properties reflecting tissue changes (Aksman et al., 2016). Thus, this method is able to detect local neuroanatomic tissue alterations driving disease classification.

We chose support vector machine (SVM) learning classification using Jacobian determinant features as an unbiased method to derive images weighted on differences within the cerebellum and brainstem as well as the whole brain between ASD and controls. In order to determine whether these cerebellar tissue alterations were consistently present at different age ranges and thus not due to developmental processes, we performed SVM classification on two independent male ASD cohorts (adult and childhood) of different sizes (140 and 47 participants respectively) and age range (18

to 44 years versus 8 to 12 years), in order to determine the consistency of our findings, following current gold standards for machine learning based classification for ASD (Bone et al., 2015). In order to test the generated SVM classifier, we grouped both ASD cohorts together and tested the generated classifier on the other randomly left unclassified half.

II.III. METHODS

Sample. One hundred and forty participants (70 controls: 70 ASD) aged 18 to 44 years for the adult cohort and 47 participants (23 control: 24 ASD) aged 8 to 12 years for the childhood cohort were included. All participants were male, right-handed, with total IQ≥70, and were free of any major physical or neurological disorders. For the ASD group, a DSM-5 or ICD-10 diagnosis for ASD had to be present.

MRI acquisition. MR data was acquired for each cohort across two sites (adult: King's College London and University of Cambridge; childhood: Donders Institute and Mannheim Central Institute of Mental Health) on 3 Tesla MRI scanners (adult: GE Medical Systems HDx; childhood: Siemens Trio and Siemens Prisma). The volumetric anatomical MRI sequences used were based on standardised ADNI GO (Jack et al., 2010) and DESPOT (Deoni et al., 2008) protocols.

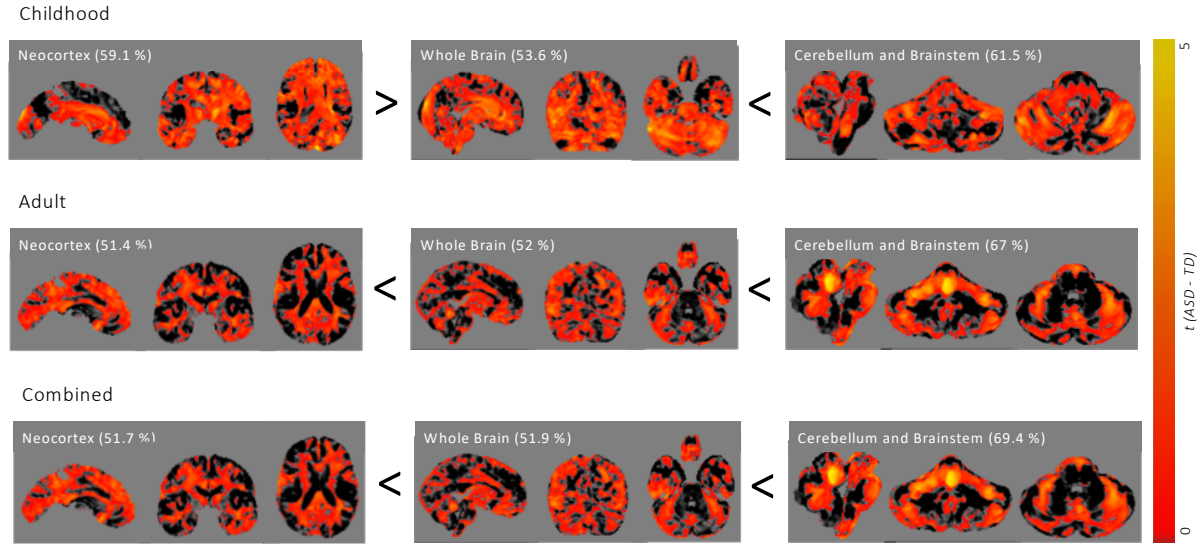
MRI analysis. All anatomical images were first segmented into grey and white matter tissue class images and then registered to a common inter-subject space, by creating a study specific template using SPM12's DARTEL registration procedure for the whole brain (Ashburner et al., 2007) or SUIT (Diedrichsen et al., 2009) for the cerebellum and brainstem. We then used Jacobian determinant images derived from the warping of each subject's grey matter segmentation for the anatomical images to the common template. In order to exclude extracerebral or extracerebellar voxels, we applied a thresholded mask to fit 1) the whole brain including the cerebellum and brainstem, 2) the neocortex excluding the cerebellum and brainstem, or 3) cerebellum and brainstem as a region of interest. Then, we used a linear SVM learning algorithm, implementing the c cost support vector classifier at a fixed value of c=1 throughout all classifications in order to establish high generalisability of the possible results and avoid overfitting. SVM classification occurred in the following steps: 1) while half of each the control and ASD group were randomly used as the training set (known class), 2) the other independent half (unknown

class) was used to test the resulting trained classifier to identify both groups correctly, 3) comparing the estimated class with its true identity. This true identity can be calculated in classification accuracy percentage, containing the proportion of positives (classification sensitivity) and the proportion of true negatives that were correctly identified (classification specificity). Using freely available Matlab code (https://github.com/leonaksman/lpr; Aksman et al., 2016), we created Jacobian weighted images, forward maps, as well as t thresholded maps at p≤.005.

II.IV. RESULTS

The cerebellum and brainstem as a region of interest displayed up to 16% higher classification accuracy when compared with the whole brain when both structures were included/excluded (all results thresholded at p≤.005, **Figure 1**). When the ASD classification algorithm generated by one ASD cohort was used to predict the other, the cerebellum and brainstem together achieved 17% higher classification accuracy. Interestingly, in line with previous findings (e. g. Ecker et al., 2013), the neocortex alone displayed a higher prediction accuracy than the whole brain for both groups, but not when compared with the cerebellum and brainstem as a region of interest.

Figure 1. T maps identifying regional weights driving ASD classification



T maps identifying regional weights driving classification for the childhood (top) and adult sample (middle) as well as for both combined (bottom) displayed for the neocortex, whole brain as well as the cerebellum and brainstem with prediction accuracy in brackets. $T_{0\text{-}5}$ value maps are shown in a red to yellow range, with lighter colour indicating higher t values and thus stronger disorder association.

II.V. DISCUSSION

In both cohorts, visual inspection revealed disease classification to be driven by differences in the lateral cerebellum and brainstem pons, as is demonstrated in the weighted *t* map Jacobian images where stronger positive values indicate a stronger region-disease association (**Figure 1;** Haufe et al., 2014; Aksman et al., 2016). Specifically, a ring-like pattern of larger posterior lateral tissue followed by smaller tissue of the cerebellar cortex driving disease classification was notable in both age groups. The cerebellum grows posterior-laterally throughout its development (Ito, 2012) and post mortem studies of ASD have consistently shown reduced Purkinje cell density in these regions (Skefos et al., 2014) as well as differences in the pons (Hashimoto et al., 1995). The consistency of the ring-like pattern in the posterior lateral cerebellum across both age ranges (**Figure 1**) could suggest a possible structural feature of autism present already in childhood ASD. Previous studies have found abnormal synaptogenesis and pruning during brain development in ASD (Wang et al., 2014), suggesting that the ring-like patterns found might reflect autism specific abnormalities in brain development.

However, it remains unclear whether this pattern corresponds to microtissue changes and should be followed up by post mortem histology. Furthermore, next steps will include integrating our findings with the proven strength of cortical measures as well as their correlation with symptom specific repetitive behaviour to add value to our efforts to classify ASD patients throughout the lifespan.

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III. COMPULSIVE BEHAVIOUR CONTRIBUTES DIFFERENTLY TO CEREBELLUM AND BRAINSTEM SHAPE IN AUTISM SPECTRUM DISORDER AND OBSESSIVE COMPULSIVE DISORDER

III.I. ABSTRACT

Autism spectrum disorder (ASD) and obsessive compulsive disorder (OCD) share genetic vulnerability, display abnormal cerebellum and brainstem volume, and express compulsive behaviour. While accumulating evidence emphasizes regional cerebellum and brainstem dysfunction in ASD, such regional differences have not been investigated in OCD, disenabling possible comparisons between the two disorders. Here, we studied cerebellum and brainstem shape using FSL FIRST, across two paediatric ASD and OCD cohorts in order to identify regional differences and their correlation with compulsive behaviour as well as symptom severity of the disorder. Our results show that compared to controls, brainstem and cerebellum shape differed in both disorders, with a posterior cerebellar thinning in the ASD but enlargement in the OCD group. Brainstem differences were further correlated with the severity of compulsive symptoms across disorders, and correlated with posterior cerebellar enlargement only in the OCD group. Our findings underline the importance of regional cerebellum and brainstem abnormalities in compulsive behaviour in ASD and OCD.

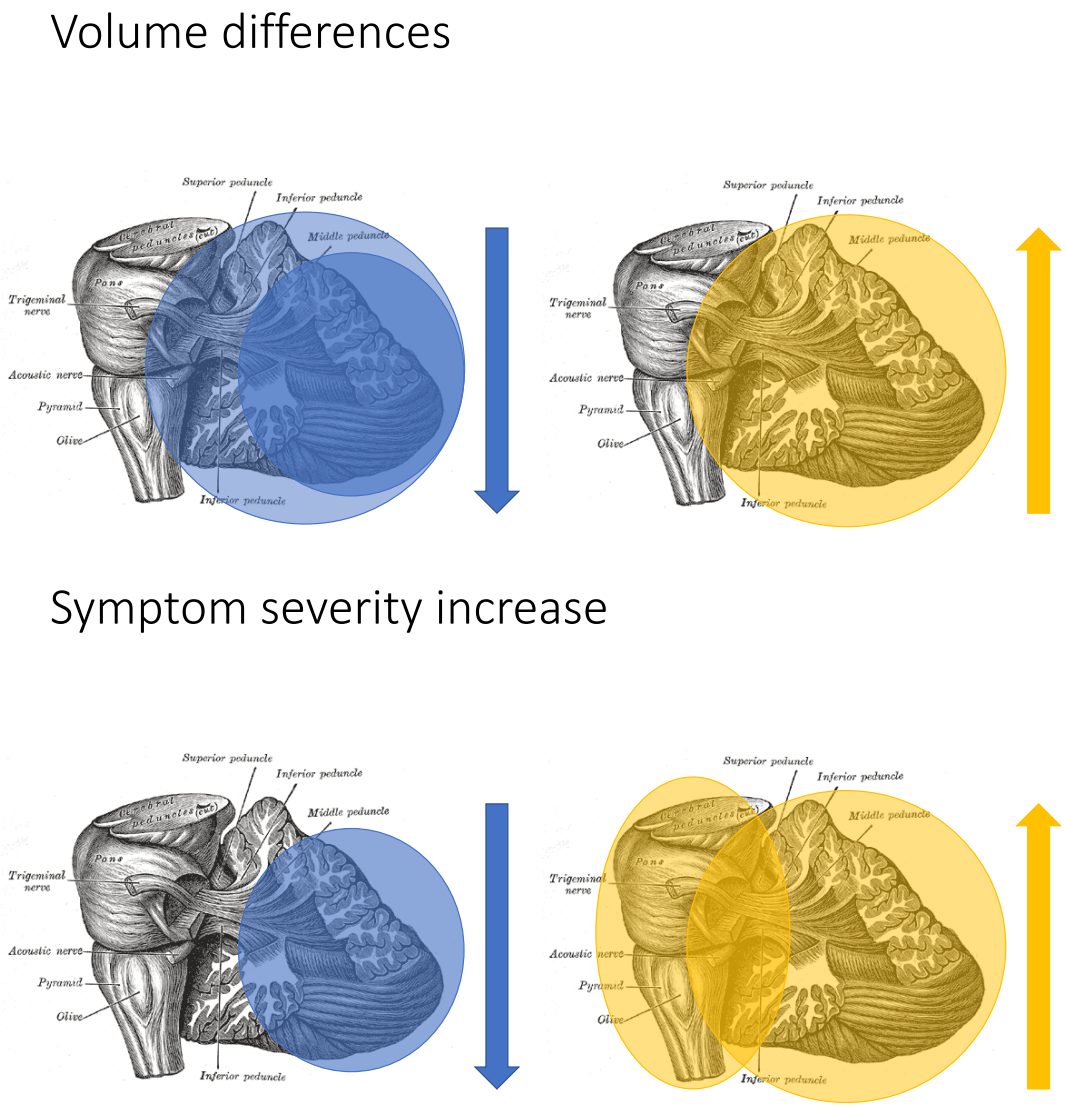
III.II. INTRODUCTION

Neurodevelopmental disorders such as autism spectrum disorder (ASD) and obsessive-compulsive disorder (OCD) share genetic vulnerability (Jacob et al., 2009), patterns of abnormal brain development (Ameis et al., 2016) and overlap in diagnostic symptoms (Jacob et al., 2009). Specifically, the tendency to perform repetitive acts in a ritualised manner (Chamberlain & Menzies, 2009) is one of the earliest signs of the autism phenotype (Leekam et al., 2011) and the main characteristic of OCD (Association, 2000), with lesion studies connecting the cerebellum and brainstem to compulsive behaviour (Figee et al., 2013). During the transition from childhood to adolescence, control over compulsive behaviour is established (Arain et al., 2013), accompanied by ongoing

maturation of brain regions including the cerebellum (Tiemeier et al., 2010) and brainstem (Geva and Feldman, 2008).

Longitudinal studies revealed that during this developmental period, brainstem volume (Jou et al., 2013) and cerebellar vermis volume increase, while total cerebellar volume is lower in ASD (Courchesne et al., 2011), specifically in the posterior cerebellar lobe (Stoodley, 2015). In contrast, overall cerebellar volume is larger in paediatric OCD when compared to controls (Eng et al., 2015). Furthermore, overall symptom severity is positively correlated with cerebellum and brainstem volume in OCD (Zarei et al., 2011), and negatively with posterior cerebellar volume in ASD (Pierce and Courchesne, 2001). Thus, abnormal cerebellum and brainstem development might affect the establishment of compulsive behaviour control, possibly with regional rather than overall differences driving dysfunctional behaviour (as summarised in Figure 1).

Figure 1. Review of cerebellar and brainstem differences between ASD and OCD when compared with neurotypical development



Total cerebellar volume is lower in ASD specifically in the posterior cerebellar lobe (top left), whereas total cerebellar volume is larger in OCD (top right). Symptom severity increases with lower posterior cerebellar volume in ASD (bottom left), while symptom severity increases with total cerebellar and brainstem volume (bottom right).

However, regional cerebellar and brainstem differences in OCD have not been investigated and whether both are comparable across disorders regarding their correlation with compulsive behaviour remains unclear. Furthermore, volume differences only give a summation value and might not detect subtle regional changes. In contrast, shape metrics provide additional group separation information revealing differences in surface morphology, an analysis that has successfully detected local

atrophies revealing disease specific patterns of neuropathology paralleling neurobiological differences in diseases with overlapping symptomatology, such as for example Alzheimer's disease and Lewy Body dementia (Sabattoli et al., 2008). As brainstem alterations and Purkinje cell reductions in the cerebellar cortex have been demonstrated in post mortem analyses (Hashimoto et al., 1995; Coleman, 2009; Skefos et al., 2014), such microstructural abnormalities might also be manifested in cerebellar shape differences similarly to neurodegenerative disorders. As a result, we are investigating cerebellum and brainstem shape across two paediatric ASD and OCD cohorts in order to identify regional differences and their correlation with compulsive behaviour and symptom severity.

III.III. METHODS

Sample: 58 right-handed children DSM-5 (American Psychiatric Association, 2013) diagnosed with ASD (45 male, 13 female), 30 diagnosed with OCD (15 male, 15 female) and 61 age-matched right-handed healthy controls (42 male, 19 female), all aged 8 to 15 years were included. Exclusion criteria for all participants was a history of neurological disorders, any contraindications for MRI, a total IQ<70, as well as comorbidity with the other disorder of interest. No DSM axis I disorders were allowed in any relatives up to two generations back for the control group. Ethical approval for the study was obtained for all sites separately.

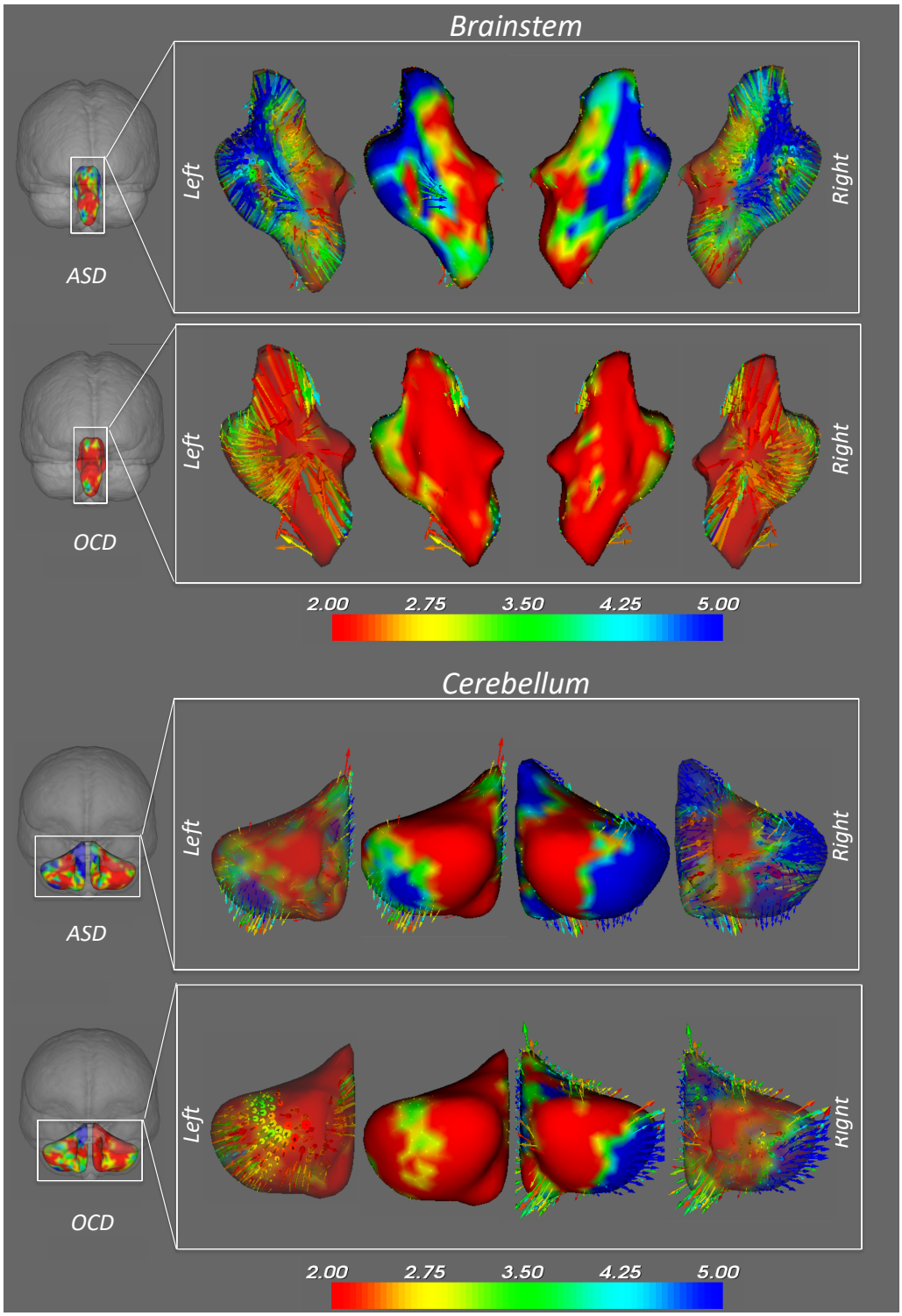
Compulsive Behaviour Measures: Compulsive behaviour was assessed for all groups on a behavioural level using the Repetitive Behaviour Scale Revised (RBS-R; Lam and Aman, 2007) and symptom severity using overall scores on the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS; Scahill et al., 2014) and Autism Diagnostic Interview Revised (ADI-R; Lord et al., 1994) respectively. All measures used were part of a larger test battery and described in detail elsewhere (Naaijen et al., 2016).

MRI acquisition: Structural T1-weighted scanning sequences based on the ADNI GO protocol (Jack et al., 2008) were acquired on comparable 3T scanners at the individual sites (Radboud University Medical Center, Nijmegen, The Netherlands; University Medical Center Utrecht, Utrecht, The Netherlands; King's College London, London, UK; Central Institute of Mental Health, Mannheim, Germany). To assure MR quality and

reliability, 'travelling-head' and phantom data were collected across sites (Naaijen et al., 2016). Each structural scan was visually inspected, and only scans with no or minimal movement artefacts were included in analyses.

Shape Analysis: FSL FIRST version 5.0.8 (Patenaude et al., 2011) was used to perform shape and appearance deformations of the cerebellum and brainstem. For this, shape was modelled using a surface-based point distribution model, constraining shape space to the linear combination of the mean shape and eigenvectors of the vertex coordinates. Vectors indicate the direction of difference, with inward direction indicating smaller/thinner structure, and vice versa. FSL's general linear model was used to create group contrasts comparing each disorder group with the control group, as well as correlation analyses with compulsive behaviour and symptom severity within the disorder group. All analyses were controlled for age, gender and scanning site.

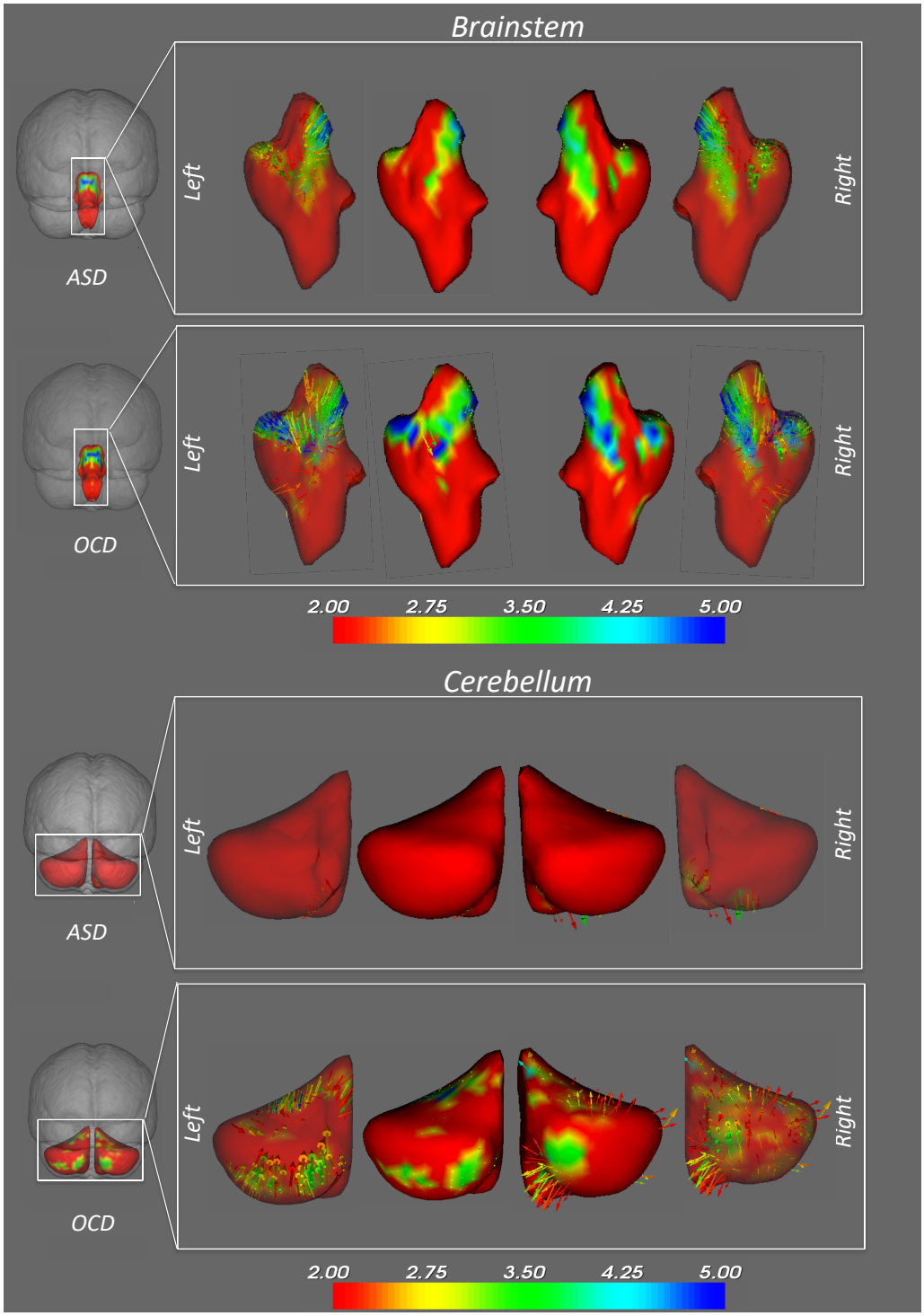
Figure 2. Differences between groups when compared with the typically developing control group



Shape differences of the brainstem and cerebellum are displayed with 50% transparency next to solid surfaces. Colours indicate F values ranging from 2 to 5 with higher values indicating higher statistical significance, and vector arrows showing the direction of reshaping.

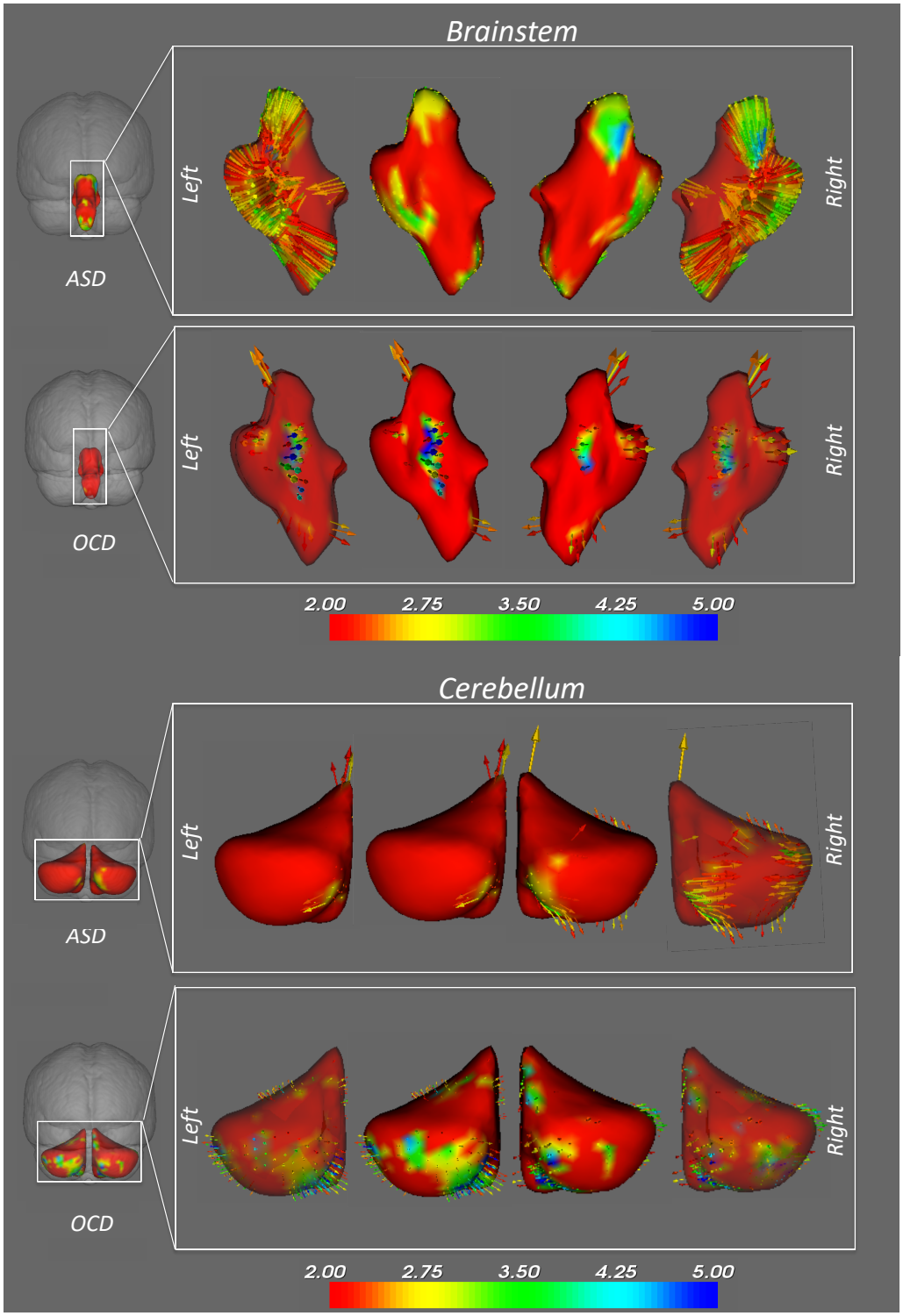
Compared to controls, both disorders showed significant group differences of the cerebellum and brainstem shape, with stronger overall reshaping in the ASD group and anterior as well as lateral posterior reshaping in both groups (Figure 2). Furthermore, the direction of the reshaping differed in the right posterior cerebellum between both patient groups (inward in ASD, outward in OCD; Figure 2). While the anterior brainstem correlated with compulsive behaviour in both groups, larger reshaping of the cerebellum was only shown in the OCD group (Figure 3). Symptom severity was correlated in the brainstem for both patient groups, with more medial reshaping in the OCD but anterior and lateral reshaping in the ASD group (Figure 4).

Figure 3. Correlation analyses for each disorder group with compulsive disorder



Compulsive disorder was assessed by the revised Repetitive Behaviour Scale (RBS-R). Shape differences of the brainstem and cerebellum are displayed with 50% transparency next to solid surfaces. Colours indicate F values ranging from 2 to 5 with higher values indicating higher statistical significance, and vector arrows showing the direction of reshaping.

Figure 4. Correlation analyses for each disorder group with symptom severity



Symptom severity was assessed by the revised Autism Diagnostic Interview (ADI-R) and Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) respectively. Shape differences of the brainstem and cerebellum are displayed with 50% transparency next to solid surfaces. Colours indicate F values ranging from 2 to 5 with higher values indicating higher statistical significance, and vector arrows showing the direction of reshaping.

III.V. DISCUSSION

In this study, we demonstrate differences from controls in brainstem and cerebellum shape in both disorders (**Figure 2**). Brainstem differences were further correlated with compulsive behaviour and symptom severity in both groups, but in the cerebellum only in the OCD group (**Figures 3, 4**). Our results parallel previous findings of higher cerebellar volume in OCD (Eng et al., 2015) and decrease in posterior cerebellar volume in ASD (Stoodley, 2015; see summary in **Figure 1**). However, it has to be noted that in order to use the same control group for both disorder comparisons, our control group was larger than the OCD group. Using a large control group for statistical comparison has been shown to increase probability that the control group will provide an accurate benchmark for statistical comparison and is recommended as a method to increase statistical precision (Riniolo, 1999), as it would be able to reflect a broader range of general population characteristics.

In addition, the mainly posterior cerebellar and anterior lateral brainstem correlated with compulsive behaviour and symptom severity in our OCD cohort could reflect the positive symptom-volume correlation previously shown for both regions (Zarei et al., 2011), and posterior cerebellar thinning the negative symptom-volume correlation in ASD (Pierce and Courchesne, 2001). As these analyses were performed within each disorder group only, these results can be seen as unaffected by the unequal sample size of each group and possible impacts on statistical power. Furthermore, we were able to depict regional differences unrestricted to lobular boundaries and display their vector directions. However, while we were not able to replicate previous findings of brainstem enlargement (Jou et al., 2013) in the ASD group. This might be because we studied both male and female ASD participants, while the study by Jou et al. (2013) only investigated male ASD subjects. While ASD shows a high male prevalence as mirrored by our sample and could enable possible effects of biological sex, all analyses were controlled for age, gender and scanning site.

Our findings underline the importance of regional cerebellum and brainstem dysfunction in ASD and OCD, highlighting the role of the brainstem in compulsive behaviour across both disorders and the cerebellum in paediatric OCD.

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IV. THE CONTRIBUTION OF REPEPTITIVE BEHAVIOUR TO SHARED AND DISTINCT BRAIN-BEHAVIOUR ASSOCIATIONS IN AUTISM SPECTRUM DISORDER AND OBSESSIVE COMPULSIVE DISORDER IN THE NEOCORTEX

IV.I. ABSTRACT

Importance: Autism spectrum disorder (ASD) and obsessive-compulsive disorder (OCD) share genetic vulnerability, abnormal brain development, and diagnostic overlap in repetitive behaviour. However, very little is known about the similarities and differences in cortical architecture between these disorders during early adolescence.

Objective: To determine potential cortical biomarkers across disorders and their dimensional contribution within the disorder group, we built regression models between repetitive behaviour across as well as diagnosis-specific behaviour and measures of cortical thickness and surface area within each disorder.

Design, Setting, and Participants: We acquired structural MRI brain scans of participants with ASD (45), OCD (35) and of 59 typically developing (TD) controls within the age range of 9 to 15 years in a multicentre study across four European sites.

Main Outcomes and Measures: The Revised Repetitive Behaviour Scale (RBS-R) was used to measure across-disorder aspects of repetitive behaviour, the Revised Autism Diagnostic Interview (ADI-R) and Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) to measure symptom severity of ASD and OCD, and FreeSurfer's Qdec program to perform brain vertex-based analyses for cortical thickness and surface area.

Results: Superior parietal surface area increased with ritualistic behaviour within and across both disorders ($p_{corr} \leq .05$). In ASD, autism specific symptoms increased with left superior frontal and left inferior parietal thickness, and compulsive behaviour decreased with left superior frontal and right fusiform surface area in the ASD group only (all $p_{corr} \leq .05$). Obsessive-compulsive symptoms decreased with left and right lateral occipital thickness, as well as left rostral middle and superior frontal (all $p_{corr} \leq .05$) thickness. All results were controlled for total brain measures, age, gender and corrected for medication status and multiple comparisons.

Conclusions and Relevance: Our results confirm ritualistic behaviour as a marker for psychopathology and identify its dimensional association with superior parietal surface area within paediatric ASD and OCD. Distinct diagnosis-specific behaviour was identified for middle frontal and occipital thickness in OCD, and inferior parietal and superior frontal thickness in ASD. The superior frontal cortex also demonstrated a significant association with compulsive behaviour in the ASD but not the OCD group, possibly identifying its unique contribution to the diagnostic expression.

IV.II. INTRODUCTION

With high comorbidity rates and overlap of symptoms across a multitude of neurodevelopmental disorders (Clark et al., 1995), many patients fail to fit seamlessly into specific diagnostic categories (Pittenger et al., 2008). As a result, the traditional categorical view of psychiatric disorders has been challenged and a shift to a more dimensional approach has been suggested (Insel et al., 2010). With the 11th revision of the International Classification of Diseases diagnostic manual fast approaching, the need to determine biomarkers across disorders and their dimensional contribution within disorder groups has increased. Such biomarkers should reflect commonalities in behavioural or cognitive processes associated with discrete deficits in defined neural systems (Robbins et al., 2012).

One umbrella of behaviour that has historically been concerned as a diagnosis-nonspecific marker for neurodevelopmental psychopathology is repetitive behaviour (Lewis and Boldfish, 1998). It refers to a wide range of behaviours including ritualistic and compulsive behaviour that are undertaken often, in an invariant manner and are inappropriate or odd (Turner et al., 1997), often used as a coping mechanism at times of transition (Gesell, 1928; Evans et al., 1997; Boyer and Liénard, 2007). While children typically engage in a significant amount of ritualistic, repetitive, and compulsive-like activity during early development, such behaviour starts to decrease in frequency and severity from ages four on (Evans et al., 1997).

Despite similar patterns of increased repetitive behaviour (Zandt et al., 2007), ASD and OCD share overlap in clinical symptoms (Boyer and Liénard, 2007; Jacob et al., 2009; Leekam et al., 2011), genetic vulnerability (Jacob et al., 2009; Lionel et al., 2014) and

patterns of abnormal brain development (Jacob et al., 2009; Leekam et al., 2011; Lionel et al., 2014). Specifically, genes expressed in the fetal brain (Lionel et al., 2014), and genes regulating neurotransmitters serotonin, glutamate and GABA are disrupted in both disorders (Jacob et al., 2009). These three neurotransmitters influence fronto-parietal (Tapia and Gonzalez, 1978; Di Iorio et al., 2012; Levitt et al., 2014) and fronto-striatal (Buitelaar and Willemsen-Swinckels 2000), brain networks with their disruption affecting brain development and functioning, including fronto-parietal top-down mechanisms (Buschman and Miller, 2007). It has been suggested that activity in fronto-striatal loops is involved in establishing cognitive habits (Graybriel and Rauch, 2000), with an excitatory-inhibitory imbalance of these pathways leading to the emergence of obsessive-compulsive behaviour (Saxena et al., 1998, 2001). Glutamate is one excitatory neurotransmitter that has been implicated to be altered in OCD and ASD (Rajendram et al., 2017; Rojas et al., 2015), albeit the place and direction of these alterations has not always been overlapping across studies or even been absent in ASD (for a review, Rojas, 2015). However, this might reflect the heterogeneity of the disorder and not its contribution to specific types of behavioural contributions, such as repetitive behaviour.

Furthermore, while glutamate is abundant in the human brain, translational imaging studies of ASD revealed consistently reduced glutamate concentrations in both humans and a prenatal rodent model in the cortico-striatal circuitry correlating with ASD symptomatologic behaviour (Horder et al., 2018). In addition, growing evidence is supporting a role for genetic variation of the excitatory neurotransmitter glutamate leading to dysfunctional signalling in repetitive behaviour. Here, a recent OCD animal study using optogenetics to knock-out glutamate regulating SAPAP3 implicating the lateral orbito-fronto-striatal pathway in repetitive behaviour. Furthermore, when this pathway was selectively stimulated via optogenetics, inhibitory signalling was restored and OCD-like and repetitive behaviour prevented (Burguiere et al., 2013). These findings were paralleled and refined by human *in vivo* findings studying disorders with shared repetitive behaviour.

In a recent study using the same cohort as presented here (Naaijen et al., 2015), MR spectroscopy measures of glutamate were higher in the anterior-frontal but not striatal system of the brain in both disorders, suggesting a more region-specific imbalance

affecting frontal areas during this neurodevelopmental stage in both ASD and OCD. In turn, failures of fronto-parietal top-down cognitive control mechanisms can result in compulsive behaviour (Dalley et al., 2011), reflecting aberrant dysregulation of stimulus-response habit learning (Robbins et al., 2012). The tendency to perform repetitive acts in a ritualised manner (Chamberlain et al., 2009) is one of the earliest signs of the autism phenotype (Zandt et al., 2007) and the main characteristic of OCD (American Psychiatric Association, 2013).

Longitudinal neuroimaging studies of ASD have shown cortical thinning in fronto-parietal regions (Zielinski et al., 2014), following an initial cortical expansion during early childhood (Courchesne et al., 2003, 2007; Redcay and Courchesne, 2005; Zielinski et al., 2014). While fronto-temporal thickness in adult ASD shows a positive correlation with total symptom severity and its repetitive subdomain (Ecker et al., 2013) as measured by the revised Autism Diagnostic Interview (ADI-R; Lord et al., 1994), the extent of diagnosis-specific and diagnosis-nonspecific abnormal repetitive behaviour contribute to fronto-parietal thinning in adolescent ASD needs further investigation. A multi-site mega-analysis study of adult OCD also noted frontal, temporal and parietal cortical thinning, with parietal thinning increasing with age and possibly symptom duration (Fouche et al., 2016). OCD symptom severity as measured by the Yale-Brown Obsessive Compulsive Scale (Y-BOCS; Storch et al., 2010) has been negatively correlated with occipital volume in unmedicated adult OCD patients (Kim et al., 2001; Morein-Zamir et al., 2013; Beucke et al., 2013). However, studies of adolescent OCD are few and as a result the nature of diagnosis-specific and nonspecific behaviour contributions to cortical measures remains unclear.

In the present study, we investigate how cortical thickness and surface area differ with diagnosis-nonspecific subtypes of repetitive behaviour (as measured with the revised Repetitive Behaviour Scale, RBS-R; Lam and Aman, 2006) and diagnosis-specific symptom severity (ADI-R and Children's Y-BOCS respectively) in children diagnosed with ASD and OCD compared to typically developing (TD) controls during the transition from childhood to adolescence. Specifically, we determined whether the correlation between cortical measures and subtypes of repetitive behaviour differed between the disorder and the TD control group. This allowed us to localise the specific regional differences in cortical

architecture either prevailing across the disorder groups thus identifying a potential transdiagnostic marker, as well as disorder specific differences reflecting possible intradiagnostic markers. In addition, we investigated how much of this variance would contribute to disorder specific symptom severity within the disorder group, limiting a possible influence of the TD group contrast.

Based on previous evidence, we expected 1) overlapping fronto-parietal regions to emerge as transdiagnostic markers; whereas 2) distinct intradiagnostic markers would reflect previous findings of ASD symptom severity increasing with fronto-temporal thickness and OCD symptom severity decreasing with occipital-parietal thickness.

IV.III. METHODS

Sample

Forty-seven right-handed children diagnosed with ASD, thirty-five diagnosed with OCD and fifty-nine TD controls aged 9 to 15 years matched on age and biological sex were scanned at one of four sites in Europe (Radboud University Medical Center and Donders Institute for Brain, Cognition and Behavior, Nijmegen, The Netherlands; University Medical Center Utrecht, Utrecht, The Netherlands; Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom; Central Institute of Mental Health, Mannheim, Germany). A group breakdown for each scanning site can be found in Figure 1 of the supplementary material. For the ASD and OCD groups, a DSM-5 diagnosis for the representative disorder had to be present and was confirmed by the Diagnostic Interview for Children (DISC; Shaffer et al., 2000), as well as ADI-R (Lord et al., 1994) and CY-BOCS (Scahill et al., 2014) scores for ASD and OCD respectively. In the OCD group, three participants had comorbid diagnoses of anxiety disorders and an additional diagnosis of depression in two cases. Medication status was assessed on the scanning day both for current and previous usage, including starting and possible ending date of treatment and the concordant dosage (**Table 1**).

Exclusion criteria for all participants included a history of neurological disorders, any contraindications for MRI, a total IQ≤70, as well as comorbidity with the other disorder. For the TD comparison group, no DSM axis I disorders were allowed in any relatives up to

two generations back. Ethical approval for the studies was obtained for all sites separately.

Clinical measures

Diagnosis specific symptom severity was assessed using the total CY-BOCS (Scahill et al., 2014) score and total ADI-R (Lord et al., 1994) score alternatively, and diagnosis nonspecific repetitive behaviour subtypes insisting on sameness, limited interests, stereotyped, self-harming, compulsive, and ritualistic behaviour were measured with the revised Repetitive Behaviour Scale, RBS-R (Lam and Aman, 2006) for all three groups. All measures used were part of a larger test battery and described in detail elsewhere (Naaijen et al., 2016).

MRI Acquisition and Analysis

All paediatric cohorts were scanned with contemporary magnetic resonance imaging (MRI) scanners at the respective sites (Siemens Trio and Siemens Prisma, Siemens, Erlangen, Germany; Philips 3T Achieva, Philips Medical Systems, Best, The Netherlands; General Electric MR750, GE Medical Systems, Milwaukee, WI, USA) using a 32-channel (Siemens) or 8-channel head coil (Philips, GE). The neuroanatomical T1-weighted scanning sequences (Table, supplementary material) were based on the ADNI GO protocol described elsewhere (Jack et al., 2008, 2010) and MR quality and reliability across sites was insured by 'travelling-head' and phantom data collected at all sites (Naaijen et al., 2016).

Cortical Thickness and Surface Area

The FreeSurfer analysis suite version 5.8.0 (http://surfer.nmr.mgh.harvard.edu/) was used to derive models of the cortical surface for each 3D, T1-weighted image. These well-validated and fully automated procedures have been extensively described elsewhere (Dale et al., 1999; Fischl et al., 1999, 2000, 2004; Ségonne et al., 2004; Jovicich et al., 2006). The results were inspected and checked for quality following the latest ENIGMA protocol (www.enigma.ini.usc.edu).

Statistical analysis

Independent sample t-tests were used to determine descriptive differences between groups and Pearson correlations to assess correlations between clinical measures using IBM SPSS version 22.

FreeSurfer's Qdec program (www.surfer.nmr.mgh.harvard.edu) was used to perform whole brain vertex-based analyses for cortical thickness (CT) and surface area (SA). CT and SA measures were analysed with regards to inter-individual point-to-point correspondence achieved by the spherical registration at each vertex. Here, a general linear model was applied to compare how diagnosis-nonspecific repetitive behaviour and diagnosis-specific behaviour was expressed differently between and within groups, and a surface map was generated to display the results on an average brain. This was done for the disorder groups and the control group individually and with the disorder groups merged together into a 'repetitive' group to reduce the impact of unequal sample sizes and investigate the consistency of group results. For each measurement of compulsive behaviour and symptom severity, the influence of group and interaction with each measurement on average CT and SA was examined, controlling for age, gender, intracranial volume and scanning site including scanner updates (more information in Figure of supplementary material). We chose a DOSS (different offsets, same slope) design matrix to take brain development into account. DOSS assumes different CT measures in the youngest subjects (different offset) and a similar impact of aging (same slope), thus examining whether a main effect of group exists independent of participant age. Secondary sensitivity analyses were performed to assess interactions between cortical measures and medication status, following MRI study design guidelines for paediatric and clinical populations (Greene et al., 2016).

The spatial CT and SA distribution was smoothed with a circularly symmetric Gaussian kernel of 15 mm full width half maximum to provide normal distribution of the results. To correct for multiple comparisons, pre-cached cluster-wise 1.3 absolute Monte Carlo Null-Z corrections were applied to all analyses, and significance was determined with p-values ≤.05 after correction.

Table 1. Participant Demographics and Total Brain Measures.

	Mean (SD) [Range]		
Variable	ASD	OCD	TD Control
Male : Female % ratio	32 boys : 13 girls	20 boys : 15 girls	39 boys : 20 girls
Age in years	11.28 (1.33) [9-15]	11.46 (1.93) [9-15]	11.0 (1.44) [9-15]
IQ	107 (15.27) [79-140]	105 (15.60) [80-129]	111 (10.86) [85-133]
Diagnostic measures			
ADI-R			
Social	18.56 (5.48)	NA	NA
Communication	14.22 (3.56)	NA	NA
Repetitive behaviour	4.02 (2.71)	NA	NA
CY-BOCS			
obsessions	NA	7.71 (5.66)	NA
compulsions	NA	9.94 (4.59)	NA
Medication status			
Antidepressants	1	8	0
Antipsychotics	4	2	0
Norepinephrine reuptake inhibitors	0	1	0
Potassium channel activator	0	1	0
Stimulants	5	1	0
Compulsive behaviour measures (RBS-R)			
Stereotyped behaviour	3.21 (3.18)	2.43 (2.69)	0.20 (0.74)
Self-harming	1.65 (2.30)	2.11 (3.68)	0.19 (0.54)
Compulsive behaviour	3.40 (4.68)[c]	6.43 (5.41)[c]	0.25 (0.69)
Ritualistic behaviour	4.65 (5.12)	4.43 (4.50)	0.15 (0.41)
Insisting on sameness	9.09 (7.93)[c]	5.71 (6.72)[c]	0.31 (0.88)
Limited interests	3.07 (2.87)[c]	1.26 (1.74)[c]	0.17 (0.38)
Total	25.04 (21.92)	22.34 (20.11)	1.27 (2.33)
Volume, L			
Total intracranial	1.47 (0.14)	1.47 (0.13)	1.45 (0.23)
Total grey matter	0.76 (0.07)	0.76 (0.07)	0.76 (0.07)

Abbreviations: ADI-R, Autism Diagnostic Interview-Revised; CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale; ASD, autism spectrum disorder; OCD, obsessive compulsive disorder; TD, typically developing; NA, not applicable; SD, standard deviation

[a] There were no significant between-group differences in age, IQ, total grey and intracranial volume (2-tailed, p≤.05). The ADI-R values were based on 28 male individuals and 13 female individuals. The CY-BOCS values were based on 21 male and 15 female individuals.

[b] All RBS measures were statistically significant between based on independent t-tests at p≤.05 between the TD control and ASD or OCD group respectively

[c] Statistically significant between the ASD and OCD group respectively based on independent t-tests at p≤.05

[d] Total IQ data was not available for 3 TD control participants, 1 ASD and 1 OCD individual

IV.IV. RESULTS

Descriptives

Participant demographics can be found in **Table 1**. Independent t-tests revealed no differences between groups regarding age, gender, total IQ, total grey matter and intracranial brain volume (all $p>.05$). All RBS measures differed between the control group and the disorder groups (all $p\leq.001$). Between disorder groups, stereotyped behaviour, self-harming, ritualistic behaviour, and total repetitive behaviour did not differ significantly, while compulsive behaviour showed significantly higher values in the OCD and insisting on sameness, and limited interests were significantly higher in the ASD group (all $p\leq.05$; **Table 1**). Clinical measures showed significant correlations with repetitive behaviour (CY-BOCS x RBS-R: $r=.502$, $p=.002$; ADI-R x RBS-R: $r=.384$, $p=.011$).

Associations between Compulsive Behaviour and Cortical Thickness and Surface Area

Superior parietal surface area increased with ritualistic behaviour as assessed with the RBS-R within each disorder (ASD: $log(10)p_{84}=2.11$, OCD: $log(10)p_{75}=2.40$) and when they were grouped together ($log(10)p_{120}=2.36$; **Figure 1a**). Additionally, left superior frontal ($log(10)p_{84}=-2.23$) and right fusiform surface area ($log(10)p_{84}=-2.34$) decreased with compulsive behaviour in the ASD group only (**Figure 1b**).

Associations between diagnosis-specific symptoms and Cortical Thickness and Surface Area

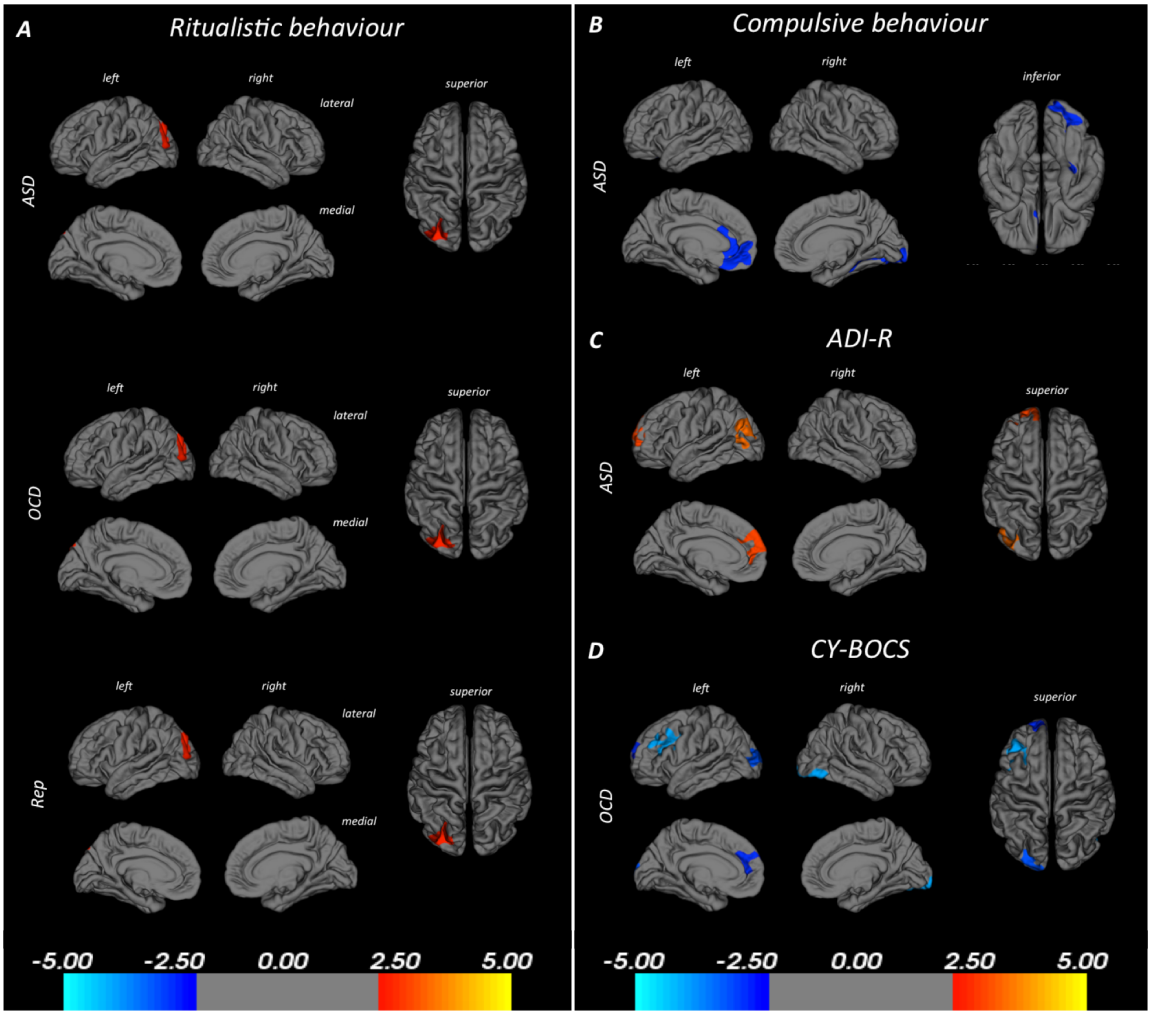
In the ASD group, left superior frontal ($log(10)p_{39}=2.82$) and left inferior parietal ($log(10)p_{39}=3.30$) thickness increased with autism specific symptoms as assessed with the ADI-R (**Figure 1c**); while the left ($log(10)p_{33}=-3.0$) and right ($log(10)p_{33}=-4.0$) lateral occipital, left rostral middle ($log(10)p_{33}=-4.0$) and superior frontal ($log(10)p_{33}=-2.0$) cortex thinned out with increasing symptom severity in the OCD group as assessed with the CY-BOCS (**Figure 1d**).

Sensitivity analyses

To explore possible effects of medication (**Table 1**) and comorbidity, secondary analyses were performed with medication status (medicated vs unmedicated) and comorbidity as a factor, age as a covariate and ICV as nuisance factor, as well as their interaction for each

group. There was no significant main effect of medication status or comorbidity or its interaction with age or ICV.

Figure 1. Transdiagnostic and diagnosis-specific marker regressions with cortical thickness and surface area.



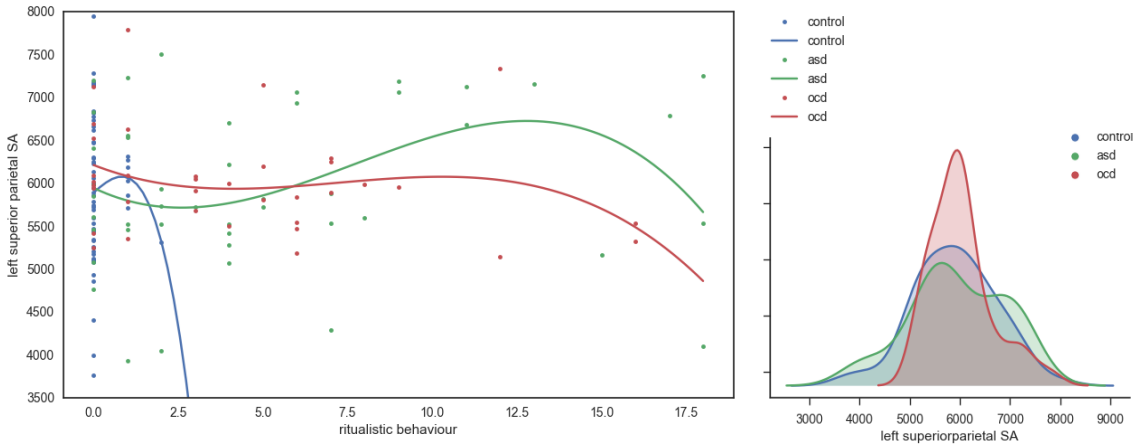
A. Left superior parietal surface area increasing with ritualistic behaviour as measured through the RBS-R for the ASD and OCD group as well as when they are grouped together (Rep) contrasted against the typically developing (TD) control group. B. Decreasing left superior frontal and right fusiform surface area with increasing compulsive behaviour as measured through the RBS-R for the ASD group but no interaction with the OCD group. C. Increasing left superior frontal and inferior parietal cortical thickness with ADI-R values in the ASD group. D. Decreasing left and right lateral occipital and left rostral middle and superior frontal cortical thickness with CY-BOCS values in the OCD group. All results were Monte Carlo Null-Z corrected for multiple comparisons at p≤.05 for positive log(10)p values shown in red and negative values in blue, with lighter colour indicating higher values.

Abbreviations: SA, surface area; CT, cortical thickness; ASD, autism spectrum disorder; OCD, obsessive compulsive disorder; Rep, ASD and OCD grouped together into repetitive disorders

IV.V. DISCUSSION

In our study, we localised a parietal surface area increase with diagnosis-nonspecific ritualistic behaviour in both paediatric disorders. In addition, we discovered distinct occipital and frontal cortical thinning patterns with increasing diagnosis-specific behaviour in our OCD cohort, but parietal and frontal cortical thickness increase with increasing diagnosis-specific behaviour in our ASD cohort. In addition, frontal thickness decreased with increasing diagnosis-nonspecific compulsive behaviour in the ASD but not the OCD group. This overlap in cortical architecture could indicate ritualistic behaviour as a diagnosis-nonspecific marker.

Figure 2. Distribution of ritualistic behaviour as a transdiagnostic marker



Distribution of compulsive behaviour for the ASD (green) and stereotyped behaviour for the OCD group (red) contrasted against the control group (blue). Individual left superior parietal surface area values shown as scatterplots (dots) with the dimensional contribution of ritualistic behaviour displayed as a polynominal order 3 functions (continuous line) on the left, and the normal distribution of the superior parietal surface area for each group on the right.

Ritualistic behaviour

In our study, superior parietal surface area increased with ritualistic behaviour within and across both disorders. The superior parietal cortex plays a role in visuospatial and attentional processing, with meta-analyses (Wager et al., 2004), and lesion studies (Koenigs et al., 2009) demonstrating its importance in manipulating and rearranging information by coding precise kinaesthetic aspects of imitating movements (Iacobini et

al., 1999). Thus, by manipulating the scope of control over an uncertain situation by using repeated movements that can easily be predicted, it serves to regain stability. Dysfunctional sensory processing in children is expressed in stereotypic, rigid and inflexible behaviours (Naaijen et al., 2016) and evidence suggests a relationship between sensory sensitivity and ritualistic behaviour in paediatric OCD (Baranek et al., 1997). The cortical surface area in this region increased dynamically with ritualistic behaviour in both ASD and OCD individually as well when grouped together and compared with the control group (**Figures 1a, 2**).

Based on the modular organisation of neurons into ontogenetic columns perpendicular to the brain surface (Mountcastle, 1997), it is hypothesized that surface area is determined by the number of columns, whereas cortical thickness represents the number of neurons within a column (Turner, 1999). According to the radial unit hypothesis (Rakic, 1995), early proliferation of radial unit progenitor cells in the prenatal brain leads to an increase in the number of ontogenetic columns and thus surface area. Progenitor cells are essential for prenatal brain development (Stiles and Jernigan, 2010) and contribute to cortical neurogenesis (McConnell and Kazonowski, 1991; Frantz and McConnell, 1996; Desai and McConnell, 2000). Both ASD and OCD share genetic vulnerability for disrupted neurogenesis and pruning (Jacob et al., 2009; Lionel et al., 2013), suggesting a possible shared, abnormal early brain development affecting superior parietal surface area in both disorders thus identifying a potential transdiagnostic marker.

Compulsive behaviour

Left superior frontal and right fusiform surface area decreased with compulsive behaviour in the ASD group only (**Figure 1b, 3**). Compulsive behaviour is characterised by a rigid adherence to some rule or mental set (Turner, 1999) and is more expressed in paediatric ASD and OCD when compared to typically developing children (McDougle et al., 1995; Russel et al., 2005). However, there are differences in their expression, as they have been described as less sophisticated in ASD when compared to OCD (Zandt et al., 2007); and their purpose, as compulsive behaviour aims to regulate anxiety in OCD but is associated with sensory seeking in ASD (Boyd et al., 2010).

Activity in fronto-striatal loops is involved in establishing cognitive habits (Menzies et al., 2008), with an excitatory-inhibitory imbalance of these pathways leading to the emergence of obsessive-compulsive behaviour (Saxena et al., 1998, 2001). Lesion studies revealed the left superior frontal cortex to be crucial for monitoring and manipulating working memory and spatially oriented processing (du Boigueheneuc et al., 2006). Functional MRI studies show this structure to be active during sensory seeking inhibition (Goldberg et al., 2004), and impaired sensorimotor gating has been connected to deficits to inhibit repetitive thoughts and actions in ASD (McAinonan et al., 2002; Perry et al., 2007), in turn affecting frontal-executive functioning (Perry et al., 2007). Higher order visual sensory processing appears to be compromised in autism impacting sensorimotor control (Hannant et al., 2016) with studies documenting hypoactivation in the fusiform gyrus during face processing in individuals with ASD (Pierce et al., 2001, 2004; Bölte et al., 2006). Postmortem studies revealed neuronal reductions in the fusiform gyrus in ASD patients (van Kooten et al., 2008) independent of general neuropathological differences and patient age, suggesting early abnormal development affecting this structure. While it can be argued that components of repetitive behaviour as assessed by the RBS-R have been incorporated in the ADI-R (and indeed were significantly correlated in this study), we were specifically interested in repetitive behaviour across both disorder and how it would be reflected in cortical morphology. As a result, the here presented result of a subset of repetitive behaviour correlating with cortical morphology only underlines the importance of this ASD feature.

Symptoms of Autism

Both left superior frontal and inferior parietal thickness increased with symptom severity in the ASD group (Figure 1c). In line with the radial unit hypothesis (Rakic, 1995), cortical thickness increases when surface area decreases, thus complementing our previous finding of decreasing superior frontal surface area with increasing compulsive behaviour. This leads us to speculate for a central role of the left superior frontal gyrus in ASD symptomatology, with increased compulsive behaviour contributing to ASD specific diagnostic measures.

The inferior parietal lobule is part of the mirror neuron (MN) system, a network of brain areas activated during the observation and imitation of an action as well as the actions and experiences of others (Stephan et al., 2012). Structural and functional impairment of the mirror neuron system have been linked to increased ASD symptom severity (Pierce et al., 2001, 2004; Boyd et al., 2010; Hutsler and Zhang, 2010)), and it has been proposed that early dysfunctional development of MN systems triggers a cascade of developmental impairments defining the ASD phenotype (Boyd et al., 2010). During typical development, synapses are generated in the early prenatal brain (Kwan et al., 2012) and reach maximal spine density on prefrontal pyramidal neurons around the age of seven (Petanjek et al., 2011). This process is then followed by a general decline in spine density, indicative of synapse elimination, leaving the bulk of synapses in the adult and adolescent brain dependent of this early development (Silbereis et al., 2016). However, ASD early childhood brain development is characterised by earlier frontal and parietal grey matter peaks (Courchesne et al., 2001), indicating insufficient synapse elimination processes (Stephan et al., 2012). This is supported by post mortem studies demonstrating higher dendritic spine density of Golgi pyramidal cells in the superficial frontal and parietal cortex in individuals with ASD (Hutsler and Zhang, 2010). Thus, the increase of symptom severity with increasing inferior parietal and superior frontal thickness found in our adolescent cohort might reflect a result of such insufficient pruning processes.

Obsessive-Compulsive Symptoms

In the OCD group, the left and right lateral occipital, left rostral middle and superior frontal cortex thinned out with symptom severity (Figure 1d). The lateral occipital cortex is important for activating attention-guided visual and somatosensory modalities (Tallon-Baudry et al., 2005; Larsson et al., 2006). Previous studies demonstrated a negative correlation between lateral occipital cortical thickness and OCD symptom severity in adult unmedicated OCD patients (Beucke et al., 2013), possibly suggesting occipital thinning a consistent feature in OCD.

The rostral middle cortex identifies situations that require additional alertness (Burgess et al., 2005) and reality monitoring of judgements (Simons et al., 2006), whereas the left superior frontal gyrus plays a crucial role in sensorimotor gating (du Boisgueheneuc et

al., 2006). Individuals with OCD misplace special alert to everyday situations, creating a heightened state of anxiety and lost situational control. After the somatic marker hypothesis (Bechara and Damasio, 2005), exposure to particular stimuli or contexts reactivate anxious somatic states, which have been associated with the stimuli through experience. In OCD, unexpected outcomes reactivate these somatic markers leading to excessive behaviour or compulsions (Bechara and Damasio, 2005). This could be reflected in the negative correlation of attention-guided sensory regions we observed in our study, possibly enabling a misinterpretation of the day-to-day situations specific to OCD.

IV.VI. CONCLUSION

We provide evidence for diagnosis-nonspecific ritualistic behaviour as a transdiagnostic marker (**Figures 1a, 2**) and compulsive behaviour as an intradiagnostic marker for ASD (**Figures 1b, 3**), making it possibly a marker of repetitive behaviour within the disorder.
In addition, we identified a frontal-parietal pattern associated with symptom severity, specifically a dynamic relationship with superior frontal thickness within each disorder group (**Figures 1c, d**), possibly suggesting a shared abnormal development in both disorders leading to disruption of these regions. Before disentangling possible comorbidities and finalising the lengthy process of categorising ASD and OCD, we propose a dimensional approach with abnormal ritualistic behaviour as an indicator for both disorders, allowing for early intervention. This would certainly aid the large patient groups failing to fit into specific diagnostic categories (Tasman et al., 2008) and might enable early intervention, in turn mitigating ASD[94] and OCD[95] symptom severity. By including participants under medication, we aim to increase generalisability and clinical significance of our findings, as suggested by guidelines for paediatric and clinical MRI studies (Greene et al., 2016).

Strengths, Limitations and Future studies

While we corrected for site effects and medication, it cannot be excluded that these factors influence our results. However, it can be argued that in a 'real life' scenario different scanner models will be used and that patients are often medicated and asking them to stop or withhold from starting medication would raise several ethical issues.

Furthermore, while we asked whether our participants had any additional known diagnoses, it cannot be excluded that these have not been reported or existed undiagnosed. As the psychometric instruments used for this study are not sensitive to detect the presence of other symptoms such as for example anxiety or ADHD behaviour, such behaviour could occur. One indication of such co-occurrence could be reflected in the reported medication use. However, secondary analyses with medication status and comorbidity revealed no significant main effect. In addition, we acquired CY-BOCS data on a sub group of ASD patients to investigate OCD symptomatology in a subset of our sample (see supplementary Table 1). Furthermore, all participants were met by medical professionals with several years of clinical experience with ASD and often OCD, thus making it unlikely to miss a possible double-diagnosis.

In order to identify how consistent our findings are, collaborative approaches are essential to evaluate our findings with longitudinal testing and behavioural observations such as by using the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2001) in large paediatric cohorts and translational study approaches connecting human and animal study designs. Insights into whether these cortical differences found here are also reflected in connecting white matter, functional connectivity or subcortical volume should also be investigated. As a result, we are planning longitudinal investigations to study the continuing developmental trajectory of compulsive behaviour on various brain measures across ASD and OCD.

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V. GENERAL DISCUSSION

In this chapter, we aimed to investigate shared and distinct brainstem, cerebellar and neocortical patterns of neurodevelopmental disorders ASD and OCD. Indeed, we were able to demonstrate that cerebellum and brainstem shape (III.III.) as well as neocortical thickness and surface area (III.IV.) both show distinct but also overlapping anatomical features, contributing to both disorder specific as well as non-specific symptoms. Furthermore, cerebellar and brainstem contributions demonstrated an up to 17% higher prediction accuracy for classifying ASD, with an overlapping neuroanatomic pattern in both an adult and childhood cohort driving this classification (III.I.). This underlines the importance of cerebello-cortico contributions and possibly underlying dysfunctional internal models to both neurodevelopmental disorders, a topic of research, where often the cerebellum and brainstem are excluded from analysis. Furthermore, our findings of transdiagnostic patterns could stimulate new research investigating commonalities between both disorders and in turn allow for earlier diagnosis and treatment options.

CHAPTER FOUR: THE TRANSLATIONAL CEREBELLUM

I. GENERAL INTRODUCTION

To take our previous findings of transdiagnostic cerebello-cortico compulsivity patterns from Chapter three one step further, we used a diabetes mellitus type II mouse model showing compulsive behaviour in the following chapter to investigate interactions between compulsive behaviour, and white matter, metabolic as well as brain proteomic interactions. Furthermore, this work was based on a previous publication on the genetic landscape of OCD identifying a key role of cerebellar insulin regulation. Using an animal model further allowed us to investigate differences in a more controlled fashion and to follow it up with *post mortem* analyses, making up for the reduced specificity of MRI measures.

II. CONVERGING EVIDENCE POINTS TOWARDS A ROLE OF INSULIN SIGNALING IN REGULATING COMPULSIVE BEHAVIOUR

II.I. ABSTRACT

BACKGROUND: Obsessive–compulsive disorder (OCD) is a neuropsychiatric disorder with childhood onset and is characterized by intrusive thoughts and fears (obsessions) that lead to repetitive behaviours (compulsions). Previously, we identified insulin signalling being associated with OCD and here, we aim to further investigate this link *in vivo.*

METHODS: We studied TALLYHO/JngJ (TH) mice, a model of type 2 diabetes, to 1) assess compulsive and anxious behaviours, 2) determine brain structural connectivity by diffusion tensor imaging (DTI) and neuro-metabolites by 1H magnetic resonance spectroscopy (MRS); and 3) investigate serum and brain protein levels for molecules previously associated with OCD (insulin, IGF1, KCNQ1 and BDNF) in these subjects.

RESULTS: TH mice showed higher compulsivity-like behaviour (reduced spontaneous alternation in the Y-maze) and more anxiety (less time spent in the open arms of the elevated plus maze). In parallel, their brains differed in the white matter microstructure

measures fractional anisotropy (FA) and mean diffusivity (MD) in the midline corpus callosum (higher FA and decreased MD), in myelinated fibres of the dorsomedial striatum (lower FA and MD) and superior cerebellar peduncles (lower FA and MD). MRS revealed increased glucose levels in the dorsomedial striatum and anterior cingulated cortex and also higher glutathione and taurine levels in the latter area in the TH relative to their controls. IGF1 expression was lower in the cerebellum of TH mice but increased in the serum. KCNQ1 expression was higher in the serum.

CONCLUSIONS: Our data provides corroborating evidence for a role of (abnormal) insulin signalling in compulsivity-like behaviour.

II.II. INTRODUCTION

Obsessive-compulsive disorder (OCD) is an often-debilitating condition, characterized by obsessive and/or compulsive behaviours. Obsessive behaviours include recurrent, intrusive, persistent thoughts, impulses and/or ideas that often cause anxiety or distress, and compulsive behaviours are ritualized, stereotypic behaviours or mental acts performed to relieve anxiety or distress associated with the obsessions or according to rigid rules (American Psychiatric Association, 2013). The symptom heterogeneity is reflected in the four factors used to identify symptom dimensions: symmetry, taboo thoughts (aggressive, religious, sexual or somatic), contamination and hoarding (Bloch et al., 2008). Although the exact aetiology of OCD is still unknown, both genetic (reviewed in e.g. Bloch et al., 2013) and environmental factors such as childhood traumatic events (de Silva and Marks, 1999) can contribute to developing the disorder. The neuronal basis of OCD is argued to be an imbalance in the activity of the cortico-striato-thalamo-cortical loop, resulting in hyperactivation of the orbitofrontal-subcortical pathway (Saxena and Rauch, 2000). Imaging studies indeed showed higher activity (both in resting state and task evoked) in the lateral and medial orbitofrontal cortex (OFC) (Menzies et al., 2008; Fitzgerald et al., 2011), the head of the caudate nuclei (Baxter et al., 1988; Whiteside et al., 2004; Menzies et al., 2008; Abramovich et al., 2012) and the anterior cingulate cortex (ACC) (Swedo et al., 1989; Perani et al., 1995; Breiter and Rauch, 1996; Koch et al., 2012) in individuals with OCD. In addition to the cortico-striato-thalamo-cortical loop, the cerebellum is now emerging as a brain region of interest in this disorder (Kim et al., 2001;

Pujol et al., 2004; Nakato et al., 2005). Furthermore, disturbances in the prefrontal cortex networks in OCD patients have been reported to contribute to disrupted cortical-striatal-cerebellar circuits in OCD (Anticevic et al., 2014).

Recently, we have identified that altered central nervous system (CNS) insulin signalling may play a role in OCD (van de Vondervoort et al., 2016). Insulin signalling was found to be the central factor in a molecular landscape based on the two genome-wide association studies of OCD published to date (Stewart et al., 2013; Mattheisenet al., 2015). In this landscape, we identified insulin-related signalling and its downstream PI3K, MTOR and RAC1 pathways as players in the aetiology of the disorder, thus eventually affecting dendritic spine and synapse formation (Lee et al., 2011). This novel finding is supported by the notion that OCD symptomatology is associated with dysregulated peripheral insulin signalling, i.e. diabetes mellitus. More obsessive symptoms were observed in patients with type 1 diabetes mellitus (DM1) (Winocour et al., 1990), and OCD symptoms and the level of glycosylated hemoglobin - a measure of the severity of type 2 diabetes mellitus (DM2) - showed a positive correlation (Kontoangelos et al., 2013). Other disorders in which individuals can present compulsive phenotypes, such as autism spectrum disorder (ASD), are also associated with disturbed peripheral insulin signalling or diabetes mellitus (Freeman et al., 2005; Stern, 2011; Xiang et al., 2015). For instance, insulin signalling may be involved in ASD by regulating PI3K and MTOR pathways via phosphatase and tensin homolog (PTEN) (reviewed in Stern, 2011). Additionally, the prevalence of ASD in children with DM1 is larger (0.9%) than that within the general population (0.34-0.67%) (Freeman et al., 2005), and maternal gestational diabetes mellitus (diagnosed before 26 weeks of pregnancy) is associated with increased risk of ASD in their offspring (hazard ratio of 1.42) (Xiang et al., 2015).

Insulin is well known for regulating blood glucose concentrations in the periphery, but glucose uptake in the CNS is largely independent of insulin. Glucose is transported across the blood-brain-barrier (BBB) by GLUT-1, an insulin-insensitive glucose transporter (Oldendorf, 1971). Likewise, glucose transporters insensitive to insulin are responsible for the majority of the glucose uptake within the CNS (McEwen and Reagan, 2004). However, insulin has additional important and non-metabolic functions in the CNS, including neuronal survival (Valenciano et al., 2006), synaptic and dendritic plasticity (Wan et al.,

1997; Beattie et al., 2000; Chiu et al., 2008), learning and memory (34, 35), and neuronal circuitry formation (Chiu and Cline, 2010). These functions are generally executed via the (PI3k)/AKT and Ras/MAPK cascades (Niswender et al., 2003; Lee et al., 2011), although the PKC/NF-kB pathway is also involved (Liu et al., 2009). There are two sources of insulin in the brain: 1) it can cross the BBB through a saturable mechanism – i.e. at higher plasma insulin levels the increment of CSF insulin level becomes proportionally smaller (Margolis and Altszuler, 1967; Banks et al., 1997) - and thus enter from the periphery (synthesized by the islets of Langerhans in the pancreas) and 2) insulin can be synthesized in the CNS locally (Raizada, 1983; Clarke et al., 1986; Schechter et al., 1988; Devaskar et al., 1993).
In this study, we aimed to elucidate the link between DM2 and compulsivity-like behaviour in the TALLYHO/JngJ (TH) mouse model at the behavioural, anatomical, metabolic and molecular levels. TH mice mimic human DM2, and can develop hyperglycaemia, hyperinsulinemia, hyperlipidaemia, moderate obesity, and enlargement of the islets of Langerhans (Kim et al., 2001, 2006). We used a multimodality approach combining behavioural tests, diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS) and proteomic assays, and we found that disturbed (peripheral and CNS) insulin signalling contributes to the development of compulsivity-like behaviour.

II.III. METHODS

Mice

TH mice and their controls, SWR/J mice (n=9 per strain, male) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and experiments started at 13 weeks of age. The animals were individually housed (Blueline IVC without top, Tecniplast, Buguggiate, Italy) with crushed corncob bedding (The Andersons, Maumee, Ohio, USA), sizzle nesting material (Datesand Ltd., Manchester, UK) and an amber mouse igloo shelter (Datesand Ltd.). Housing was on a reversed day-night cycle (lights on at 20.00h) in a ventilated cabinet with light adjustment kit (Scantainer, Scanbur, Karlslunde, Denmark) and with *ad libitum* food (V1244-703, SSNIFF Spezialdiäten GmbH, Soest, Germany) and autoclaved demineralized water. Experimental procedures were conducted after approval of the Animal Ethical Committee of the Radboud University (project number DEC2014-113).

Behavioural studies

Mice were allowed one week of acclimatization to their new environment and were extensively handled prior to starting the experiments. Experiments were conducted in a room solely lit by an IR lamp (Y-maze and marble burying) or in a brightly lit room (open field and elevated plus maze). The behavioural studies were conducted in the order below, each experiment a few days apart. The order of testing was randomized but after randomization, the animals were tested in the same order during all experiments. The experiments were videotaped using a camera (with IR lens during Y-maze and marble burying) mounted above the maze and Media Recorder software (Noldus, Wageningen, The Netherlands). The activity patterns of the animals were traced using Ethovision XT 9 software (Noldus).

A decrease in spontaneous alternation behaviour in rodents is considered an animal model for perseverative symptoms seen in OCD patients (Yadin et al., 1991). Mice were placed in a Y-maze (Stoelting Co, Wood Dale, IL USA) facing the wall of one of the arms (allocation to start arm was randomized), and allowed to explore freely for five minutes. Distal arm entries were identified using video tracing, and defined as the centre point of the mouse being in the distal 1/3 of the arm. These distal arm entries were then used to manually score spontaneous alternation behaviour. Additionally, an animal going back to the same distal arm after visiting the centre triangle was scored as a repeated arm entry. Fewer spontaneous alternation behaviour and more repeated arm entries are indications of compulsive-like behaviour.

The marble-burying test has been used as a model of compulsive-like and anxiety-like behaviours in mice, with larger numbers of buried marbles reflecting increased compulsive-like behaviour (Njung'e et al., 2013; Gyertyan, 1995). Mice were placed individually in a clean home cage containing 18 glass marbles evenly spaced on 5 cm deep sawdust, without access to food and water for 30 min. The number of marbles that were at least 2/3 buried reflects compulsive/anxiety-like digging behaviour.

Upon introduction in an open field, mice are inclined to explore the peripheral border zone (thigmotaxis) and not the centre zone of the maze. Mice were placed in the centre of a home-built open field (55x55 cm) containing walls (40 cm height) and explored the

maze freely for 30 minutes. A decrease in time spent in the centre zone was used as an indication of anxiety-like behaviour.

The elevated plus maze is used to test anxiety in rodents (Walf and Frye, 2007). In short, animals are placed at the junction of the four arms (two open and two closed arms) of the elevated plus maze (Stoelting Co), facing the open arm and allowed five minutes of free exploration. A decrease in time spent in the open arms and number of entries into the open arms reflects anxiety behaviour. In addition, faecal boli were counted as an indicator of anxiety levels (Seibenhener and Wooten, 2015).

After concluding the behavioural studies, blood was collected via a tail vein puncture and glucose levels were measured using an Accu-Check Aviva hand-held device (Roche Diabetes Care, Almere, The Netherlands).

Magnetic resonance experiments

Magnetic resonance experiments were performed at an 11.7T BioSpec MR system (Bruker BioSpin, Ettlingen, Germany) equipped with an actively shielded gradient set of 600 mT/m. A circular polarized volume resonator and an actively decoupled mouse brain quadrature surface coil were used as transmit and receive coils, respectively, and data was acquired with Paravision 5.1 software. Mice were head-fixed in the magnet using a bite-bar and earplugs and anesthetized by isoflurane. Anaesthesia was induced with 3-4% isoflurane, after which it was maintained at ~2% (1:2 oxygen:air). The respiration rate of the mice was monitored throughout the experiment and the animals' temperature was maintained at 37.5°C using a heated airflow device.

To visualize the brain anatomy, a T1 weighted gradient echo sequence in three orthogonal orientations was used. For diffusion tensor imaging (DTI) experiments, 20 axial slices covering the whole brain were acquired with a T2 weighted spin-echo echo-planar imaging (EPI) sequence (Zerbi et al., 2013). Encoding b-factors of 0 (five b0 images) and 1000s/mm2 were used and diffusion sensitizing gradients were applied along 30 non-collinear directions with the following imaging parameters: TR=7750ms, TE=21.4ms, FOV=20×20mm2, slice thickness=0.5mm, matrix=128×128, Δ=10ms, δ=4ms, number of segments=4, and acquisition time (TA)=18min.

Brain metabolite concentrations were quantified by single-voxel proton MRS using point resolved spectroscopy (PRESS) sequence (TR/TE=2500/11.6ms, 400 acquisitions and TA=17min) with image-guided positioning of voxels of 2.25µL for the right dorsomedial striatum (DMS) and 1.47µL for ACC in both hemispheres (Figure S1). Variable pulse power and optimized relaxation delays (VAPOR) were employed to suppress the water signal. For all spectra, a separate spectrum was acquired without suppressing the water signal, so that it could serve as a reference. For metabolite quantification their signals were fitted by LCModel software (Provencher, 1993). Only signals with a Cramer-Rao lower bound (CRLB)≤20% were included in the quantification. Due to higher Tesla strength, T2 correction was not necessary because of higher quantification precision and short scan time (Li et al., 2012). We estimated the tissue levels of N-acetylaspartate (NAA), creatine (Cre), glutamate (Glu), glutamine (Gln), taurine (Tau), total choline (tCho), sum of myo-Inositol and glycine (mI+Gly), glucose (Glc), GABA and glutathione (GSH).

Euthanasia

Mice were euthanized by cervical dislocation, followed by collection of trunk blood and removal of the brain for proteomic analyses. Blood was immediately centrifuged to obtain serum. Brains and serum were stored at -80°C until further processing.

Proteomics

The prefrontal cortex, the striatum and the cerebellum were dissected from the frozen brains. Mouse brain tissues were weighed and, for each milligram of tissue, 10 µl PBS (P4417-100TAB, Sigma-Aldrich Company Ltd, Dorset, UK) with Protease Inhibitors (88668, ThermoFisher, Waltham, MA, USA) was added. Tissues were lysed on ice using a sonication probe and to each tissue lysate, we added an equal volume of Tissue Extraction Reagent 1 (FNN0071, ThermoFisher) to that of PBS that was then vortexed. Lysates were centrifuged at 13000 rpm for 3 minutes and then aliquoted and stored at -20°C. Serum samples were prepared as per kit protocols. Assays were performed based on the instructions provided in the manual of the analyte kits. KCNQ1, BDNF (ABIN2101758 and ABIN2115886, respectively, from antibodies-online GmbH, Aachen, Germany) and insulin (EMINS, ThermoFisher, Waltham, MA USA) were detected by means of ELISA, whereas

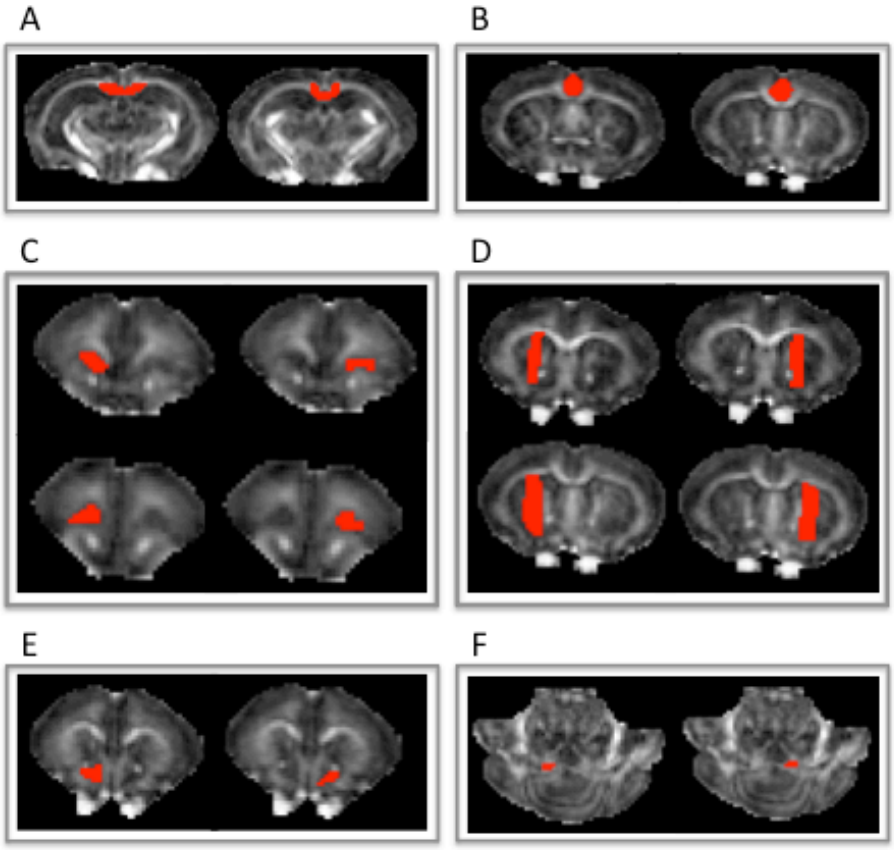
IGF1 was assessed by Luminex Multiplex kit (LXSAMSM-02, Bio-Techne Ltd, Abingdon, UK).

Data analyses

Data from the behaviour studies were analysed using Ethovision XT 9 software (Noldus) for tracing, followed by IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA) for statistical analyses. Significance in the behaviour experiments was tested via independent t-tests or one-way ANOVA. Independent t-tests were also used (after outlier correction) for the proteomics results.

Tissue concentrations of MRS detected metabolites were evaluated relative to the tCr signal as it was found to be unchanged in our models when using water concentrations as references. The DTI analysis was done using ExploreDTI (Leemans et al., 2009) through which fractional anisotropy (FA) and mean diffusivity (MD) maps were generated. Regions of interest (ROIs) were selected based on Allan mouse brain atlas (Lau et al., 2008) and then ImageJ software (Schneider et al., 2012) was used to draw ROIs on two subsequent slices and extract their averaged FA and MD values. Regions of interest were drawn on both white matter and mixed grey and white matter regions (Figure 1). Data is displayed as mean ± SEM with $p<0.05$ as set significance.

Figure 1. Diffusion regions of interest



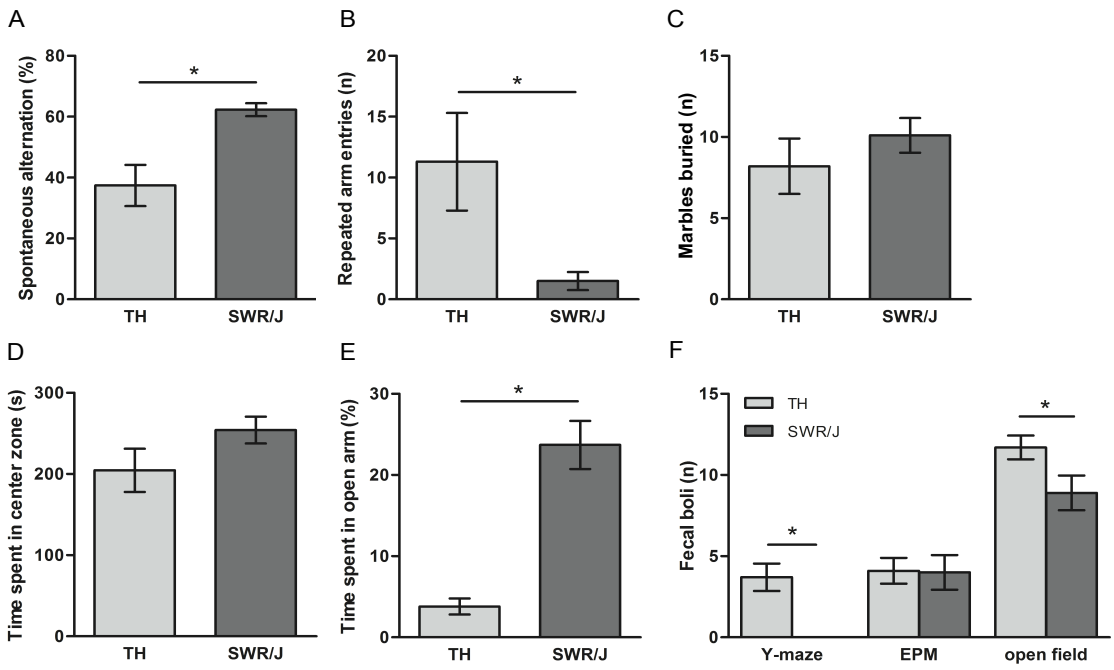
Regions of interest were drawn using the following landmarks: (A) corpus callosum defined as below the restrosplenial and secondary motor area and the above the stratum radiatum; (B) anterior cingulate cortex defined as above the corpus callosum and cingulum bundle, and enclosed by the secondary motor area; (C) the orbitofrontal cortex defined as below the primary and secondary motor area and anterior cingulate cortex, enclosed by the infralimbic agranular insular area and above the olfactory areas; (D) the dorsal medial striatum defined as below the corpus callosum and lateral ventricle, enclosed by the external capsule and endopiriform nucleus and above the nucleus accumbens and medial to the anterior commissure; (E) the nucleus accumbens defined as below the dorsomedial striatum, anterior commissure and nuclei of the stria terminalis, enclosed by the piriform and preoptic area, and above the substantia innominata; and (F) the superior cerebellar peduncles defined as below lobules II and III, and enclosed by the parabrachial nucleus and arbor vitae.

II.IV. RESULTS

TH mice are more perseverative and anxious

TH mice had higher blood glucose levels compared to SWR/J mice (p=0.004), confirming that they show glucose elevations consistent with DM2 (**Figure S2A**). Additionally, their body weight was consistently higher (data not shown). TH mice also demonstrated a significant reduction in locomotion, as quantified in the Y-maze, the open field test and the elevated plus maze, with time spent in the open field was corrected for general activity level (p<0.0001; p=0.004 and p<0.0001, respectively; **Figure S2B**). These findings are in keeping with known literature, showing that the TH mice developed according to expectation (48, 58).

Figure 2. Compulsive behaviour



TH mice show higher compulsive behaviour, as seen in a decrease in the spontaneous alternation behaviour (A) and significantly more repeated arm entries (B). No difference was observed in compulsive marble burying (C). Additionally, TH mice display more anxiety behaviour, namely a trend towards less time spent in the centre zone of the open field. (D) and the open arms of the elevated plus maze (E), and more faecal boli left behind during the y-maze, the open field test and the elevated plus maze (F).

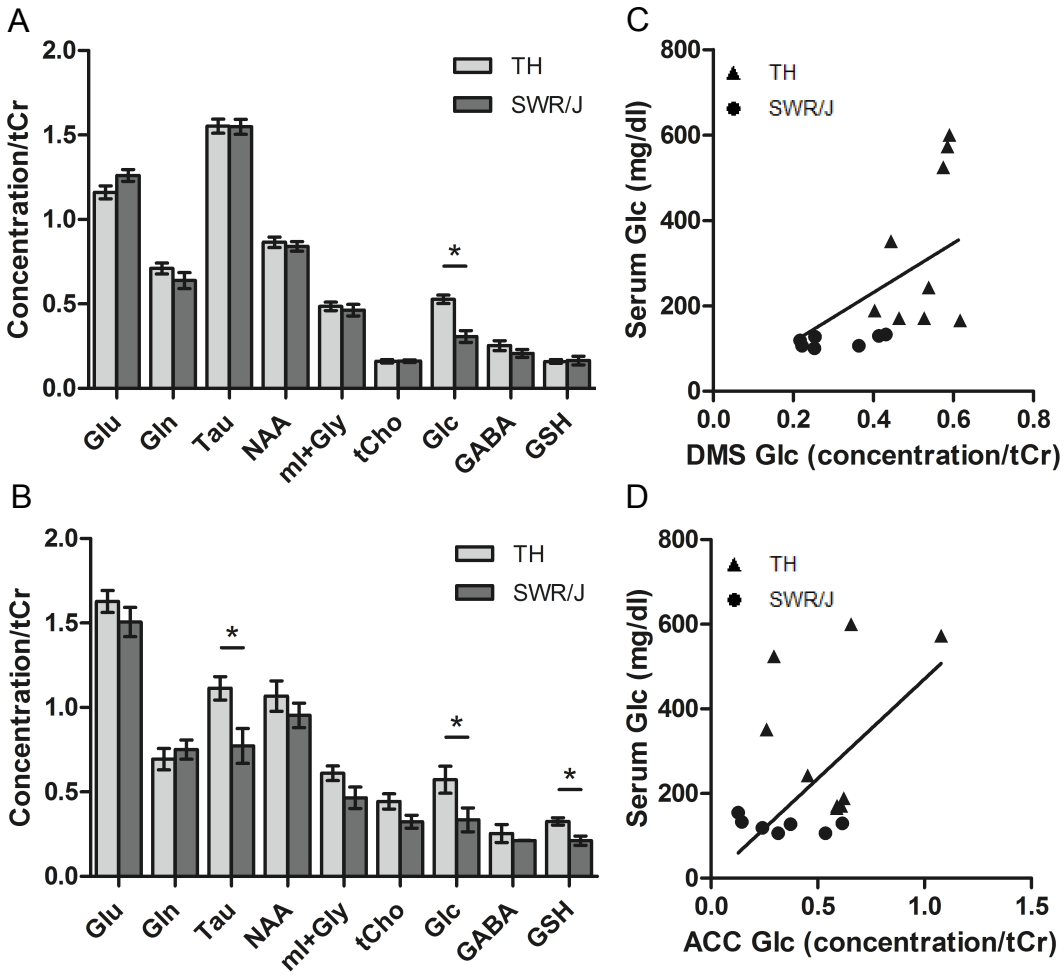
Spontaneous alternation behaviour is an indication of compulsivity-like behaviour (Yadin et al., 1991). When allowed to explore freely, healthy mice usually alternate entering the three arms of the Y-maze. TH mice showed a remarkable reduction in spontaneous alternation behaviour: 37.4(±6.8)% spontaneous alternation versus 62.3(±2.1)% in SWR/J mice (p=0.003; Figure 2A). Additionally, TH mice frequently entered the same arm twice successively, which corresponds to 11.2(±4.0)% and 1.5(±0.7)% repeated arm entries in TH and SWR/J mice, respectively (p=0.029; Figure 2B). No compulsive digging was observed in the marble-burying test (p=0.363; Figure 2C). Our combined findings for the Y-maze suggest that TH mice display more compulsivity-like behaviour.

We also tested whether TH mice exhibit altered anxiety levels. In the open field, there was a trend towards the TH mice spending less time in the centre (p=0.165; Figure 2D). More importantly, TH mice spent less time in the open arms of the elevated plus maze, suggesting that they experienced more anxiety (p=0.028; Figure 2E). This is supported by the number of faecal boli left in the mazes, which was higher in TH mice than in controls in the Y-maze and open field, but not in the elevated plus maze (p=0.001, p=0.048 and p=0.92, respectively; Figure 2F). Taken together, TH mice present both compulsivity-like and anxious phenotypes.

MRS reveals changes in ACC and DMS

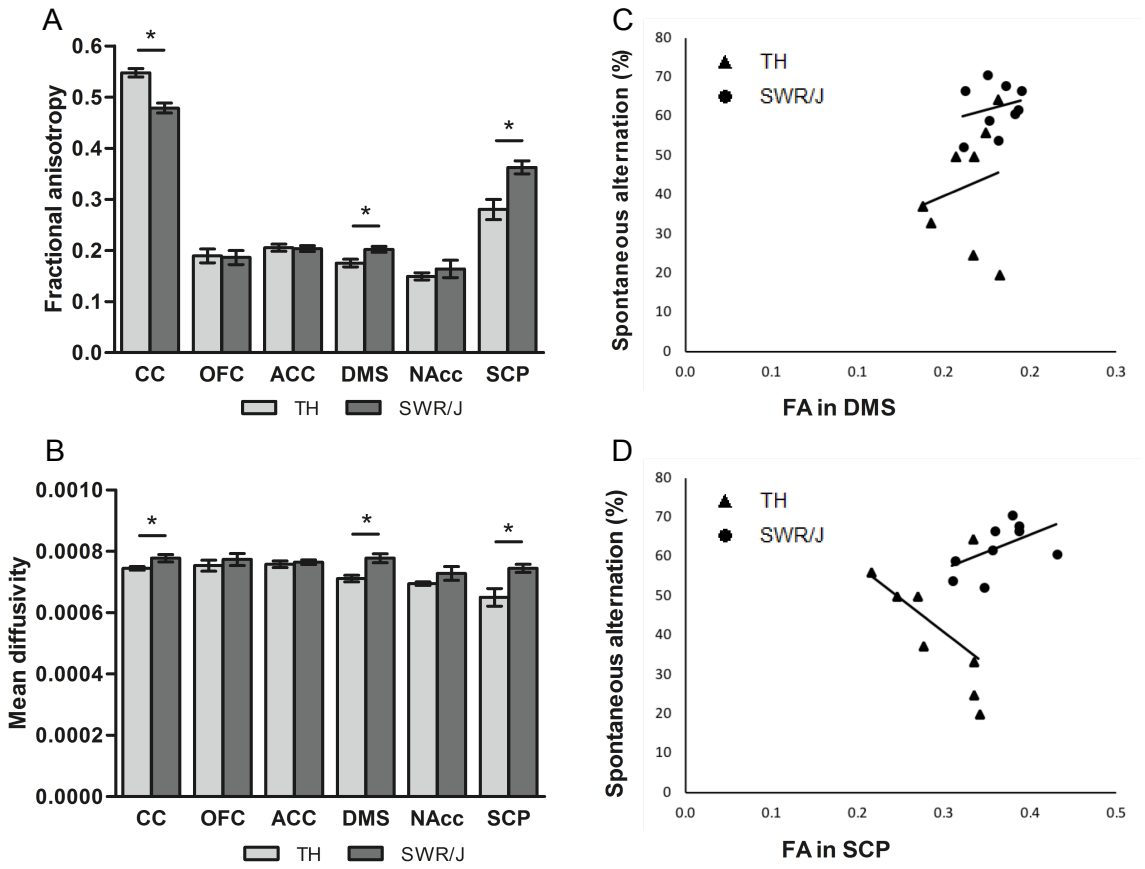
Single-voxel MRS was performed to assess metabolite levels in ACC and DMS of the TH and SWR/J mice. The TH mice had elevated Glc levels in both DMS (p=0.001) (Figure 3A) and ACC (p=0.048) (Figure 3B). Glc levels in the DMS (r=0.664, p=0.005) but not in the ACC (r=0.415, p=0.11) correlated with its concentration in blood (Figures 3C-D). Additionally, GSH (p=0.006) and Tau (p=0.013) levels were specifically higher in the ACC (Figure 3B). No additional differences were detected for the other metabolites.

Figure 3. MRS results



MRS revealed differences in the metabolite content of the DMS (A) and ACC (B). Glc ratios (relative to tCr) were higher in brains of TH mice in both brain regions, whereas Tau and GSH were higher in the ACC only. Blood glucose concentration correlated with DMS Glc concentration (C), but not ACC Glc concentration (D).

Figure 4. DTI results



DTI showed differences in white matter microstructure in brains of TH mice The FA was higher in the CC and lower in the DMS and SCP, whereas the MD was decreased in the three brain regions (A). Compulsivity-like behaviour as shown by the spontaneous alternation is positively correlated to the FA in DMS (B) but differed in the SCP (C).

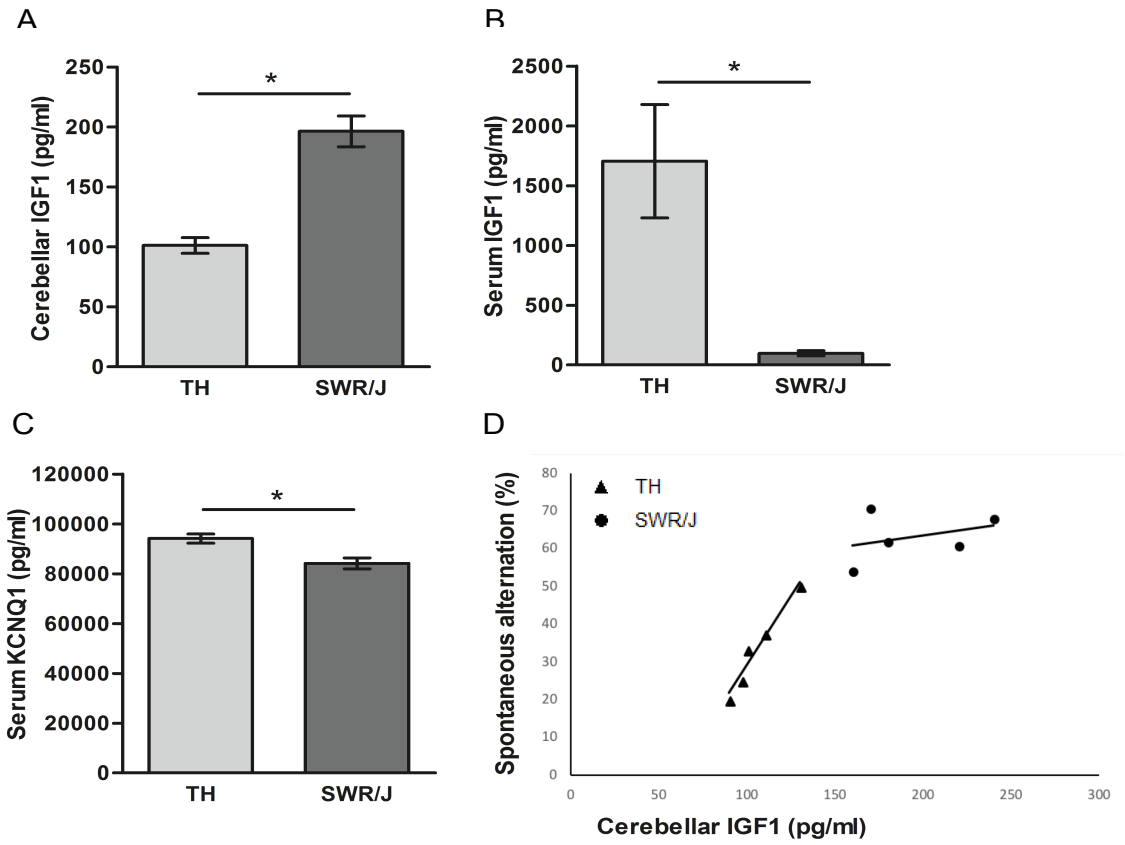
White matter microstructure changes may underlie compulsivity-like behaviour

The FA and MD of the corpus callosum (CC), OFC, ACC, DMS, nucleus accumbens (NAcc) and the superior cerebellar peduncles (SCP) were assessed (**Figure S3**). TH mice had larger FA ($p<0.0001$) and decreased MD ($p=0.024$) in the CC, decreased FA ($p=0.01$) and MD ($p=0.02$) in the DMS, and smaller FA and MD values in the SCP ($p=0.003$ and $p=0.009$, respectively) (**Figure 4A-B**). No differences in FA and MD were detected in the OFC, ACC and NAcc.

Of note, FA in both brain regions positively correlates with spontaneous alternation behaviour when both were grouped together ($r=0.734$, $p=0.001$ and $r=0.548$, $p=0.019$,

respectively), but when grouped separately a diverging directionality for SCP FA values appears (Figures 4C-D). While this might suggest that larger FA only in the DMS is associated with more spontaneous alternation irrespective of the mouse strain, the individual analyses did not reach significance (for DMS TH: r=0.359, p=.544; SWR/J: r=0.235, p=.342; for SCP TH: r=0.149, p=.141; SWR/J: r=0.524, p=.717).

Figure 5. IGF1 concentration



The concentration of IGF1 is reduced in the cerebellum of the TH mice (A), whereas the concentration is higher in their serum (B). Additionally, KCNQ1 levels are higher in the serum of the TH mice (C). Interestingly, cerebellar IGF1 concentrations are correlated to the spontaneous alternation behaviour in the TH and SWR/J mice combined (D).

Levels of insulin-related proteins are altered in both serum and brain

Protein expression was examined for a short list of four molecules that have been associated with OCD before: insulin (van de Vondervoort et al., 2016), insulin-like growth factor 1 (IGF1) (Rosso et al., 2016; van de Vondervoort et al., 2016), potassium voltage-

gated channel subfamily KQT member 1 (KCNQ1) (van de Vondervoort et al., 2016) - that inhibits insulin secretion (Ullrich et al., 2005) - and brain-derived neurotrophic factor (BDNF) (Maina et al., 2010; Taylor, 2011; Fontenelle et al., 2012; van de Vondervoort et al., 2016). Protein expression levels were determined in serum, prefrontal cortex, striatum and cerebellum of TH and SWR/J mice. Protein selection was based on prior findings, in which we constructed a molecular landscape that implicates the regulation of dendritic spine formation through insulin-related signalling in OCD (van de Vondervoort et al., 2016). IGF1 concentrations were decreased in the cerebellum of TH mice ($p=0.0002$, **Figure 5A**), whereas IGF1 concentration was higher in the serum of these mice ($p=0.02$, **Figure 5B**). Additionally, we measured levels of KCNQ1, a potassium channel that inhibits insulin secretion (Yamagata et al., 2011). KCNQ1 was higher in serum of TH mice ($p=0.008$, **Figure 5C**). Insulin and BDNF expression in serum fell below the detection limit, which was also the case for IGF1 levels in striatum. No other expression differences were detected (**Table S1**). Importantly, only cerebellar IGF1 expression correlated with spontaneous alternation (both strains combined: $r=0.931$, $p<0.0001$, TH: SWR/J: ; **Figure 5D**), suggesting that higher cerebellar IGF1 may be associated with more spontaneous alternation behaviour.

II.V. DISCUSSION

Previously, we proposed a role for (CNS) insulin-regulated dendritic spine formation and synaptic plasticity in OCD in a molecular landscape based on data from genome wide association studies (van de Vondervoort et al., 2016). The aim of the current study was to find corroborating evidence for the involvement of insulin signalling in OCD, which was carried out at three levels: behavioural assessment of TH mice (a model for DM2), characterization of their brains via MR imaging and spectroscopy and investigation of prefrontal cortex, striatum, cerebellum and serum of these mice on a molecular level.

On the behavioural level, we showed that TH mice display a compulsivity-like phenotype, and that these mice were more anxious than their control counterparts. These results are in line with previously published findings that anxiety is a well-known symptom of OCD (American Psychiatric Association, 2013). Additionally, patients with DM1 and DM2 display more symptoms of OCD than control subjects (Winocour et al., 1990;

Kontoangelos et al., 2013), and the prevalence of anxiety symptoms in patients with diabetes is higher than in the general population (Collins et al., 2009).

Regarding characterization of the brain, MRS revealed higher glucose levels in both the DMS and ACC, and higher GSH and Tau in the ACC specifically. Insulin resistance inherent to DM2 may have caused higher glucose levels in both examined brain regions. MRS studies in patients with DM2 and a rat model of the same disorder also reported higher glucose levels in various brain regions (Kreis and Ross, 1992; van der Greaaf, 2004; Sinha et al., 2014). This suggests that DM2 has a similar effect on the glucose metabolism of the brain than on the peripheral circulation. However, it is unclear what the relative contribution of this change in glucose metabolism is at the behavioural level. Both GSH and Tau have previously been studied in the context of OCD, although the number of studies is limited. GSH levels in the posterior cingulate cortex were found to be significantly lower in OCD patients versus control subjects (Brennan et al., 2016). Another study showed a reduction in GSH levels in the serum of OCD patients (Orhan et al., 2012). Two studies have investigated GSH levels in animal models of OCD: SAPAP3 knockout mice show a reduction in GSH levels in the striatum (Mintzopoulos et al., 2016), and deer mice that show high levels of stereotypical behaviour have reduced GSH levels in the frontal cortex (but not the striatum; Guldenpfennig et al., 2011).

In the case of Tau, only one study looked at a possible association with OCD: Tau levels in the cerebrospinal fluid of psychotropic drug-naïve OCD patients were compared with matched controls and no differences were found (Bhattacharyya et al., 2009). Thus, more research into this is warranted. Interestingly, GSH and Tau are implicated in insulin-related signalling cascades. Insulin itself stimulates the synthesis of GSH (Kim et al., 2004), while GSH is also involved in the same PI3K/AKT/RAC1 signalling cascaded that are regulated by insulin in the previously publishes OCD landscape (van de Vondervoort et al., 2016; see above). For instance, GSH inhibits the activation of RAC1 (Gonzalez-Santiago et al., 2006) whereas activation of PI3K or AKT regulates GSH synthesis (Kim et al., 2004; Uranga et al., 2013). Further, Tau is an amino acid that stimulates insulin secretion (Batista et al., 2012) and activates components of the insulin-related cascades downstream of PI3K and AKT (Rashid et al., 2013). Importantly, Tau directly increases the levels of GSH (Yang et al., 2013), which at least partially explains why we found higher

concentrations of both metabolites in the brains of TH mice. In summary, our MRS results provide further insights and clues for further research into how insulin regulates OCD-linked behaviour by affecting specific brain regions. However, the relative contribution of the direct metabolic effects - via glucose - and indirect effects of insulin on synaptic plasticity (via the PI3K/AKT/RAC1/GSK3β signalling cascades) needs to be elucidated. While dendritic spine formation and synaptic plasticity concern grey matter components (dendrites and synapses), it is becoming increasingly clear that plasticity occurs beyond the synapse. For example, there is growing evidence from both animal and human studies that activity-dependent regulation of myelin may play a role in learning, complementing grey matter microstructural changes (for a review, Sampaio-Baptista and Johansen-Berg, 2017). Moreover, there is a growing body of evidence pointing towards insulin-related signalling having an effect on white matter microstructure in the brain, which is assessed by diffusion tensor imaging (DTI). For example, insulin resistance in generally healthy middle-aged and older adults is associated with white matter microstructural alterations (Ryu et al., 2014). In young adults, hyperglycemia is also associated with increased white matter hyperintensities (Weinstein et al., 2015). In addition, many studies report an association between DM1 or DM2 and white matter microstructure changes (Kodl et al., 2008; Xiong et al. 2016).

TH mice displayed differences in white matter microstructure in the CC, DMS, and SCP, and the FA of the DMS and SCP correlated with their spontaneous alternation behaviour in the Y-maze. This correlation may indicate that differences in white matter microstructure at least partially underlie the compulsive-like behaviour we observed. Multiple studies (Yoo et al., 2007; Zarei et al., 2011; Li et al., 2011) report higher FA in the CC in OCD patients (reviewed in Bora et al., 2011), but other studies found lower FA in this brain region, both in adult (Garibotto et al., 2010; Bora et al., 2011; Fontenelle et al., 2011; Nakame et al., 2011; Benedetti et al., 2013) and paediatric (Gruner et al., 2012) populations. In this context, drug-naive OCD patients were shown to have lower FA in the CC, the internal capsule and white matter in the area superolateral to the right caudate (Yoo et al., 2007). This increase is FA was no longer observed after 12 weeks of citalopram treatment (Yoo et al., 2007). Few studies have investigated the white matter microstructure in the DMS and/or the SCP of OCD patients, and no consensus has been

reached regarding these differences (Fan et al., 2012; Jayarajan et al., 2012; Rosso et al., 2014). In addition, no DTI studies have been performed that show differences in the CC, DMS or SCP in patients with DM2. Therefore, additional studies are needed to corroborate our findings. Furthermore, it has to be noted, that white and grey matter distinctions are very difficult in the mouse, and only the CC and SCP ROIs were on white matter only, whereas the other ROIs were highly mixed with grey matter (Figure 1). This limits both the general interpretation of such results regarding white matter and cautions the generalisability to human findings.

Possible explanations for such inconsistencies could be various, as for example the use of anaesthesia in animals can influence some metabolites measures even with relatively short exposure (Boretius et al., 2013). Anaesthesia is inevitable in preclinical imaging and an essential methodological factor in extreme stress but is often not necessary for human scanning. While GSH synthesis is severely diminished if DM2 is left uncontrolled as attributed to limited precursor availability (Sekhar et al., 2011), patients with DM2 are usually under drug treatment for the disorder, thus possibly affecting MRS measurements. Finally, while the human CNS follows a pattern of development typical of all mammals, certain neurodevelopmental features are highly derived (for a review, Silbereis et al., 2016), leading to differences in developmental stages between rodents and humans, and as a result to a certain extent brain morphology.

At the molecular level, we found that TH mice have a decreased protein expression of IGF1 in the cerebellum, but larger IGF1 in the serum. The expression of cerebellar IGF1 was positively correlated with their spontaneous alternation behaviour in the Y-maze. Lastly, higher KCNQ1 expression (involved in negatively regulating insulin secretion) was observed in the serum of TH mice. Our findings of different IGF1 and KCNQ1 levels in the cerebellum and/or serum of compulsive TH mice versus their controls support our hypothesis that insulin-related signalling pathways are involved in compulsive behaviour, possibly affecting SCP microstructure. Specifically, the imbalance in peripheral and central IGF1 levels is noteworthy. Recently, IGF1 levels were found to be larger in the serum of OCD patients compared to controls (Rosso et al., 2016), which is in line with the higher serum IGF1 levels we observed in the TH mice. Disturbances in IGF1 levels may be an indication of reduced sensitivity of the tissues to IGF1, so called IGF1 resistance, which

often accompanies insulin resistance in DM2 (Kleinridders, 2016). Insulin/IGF1 resistance blunts the activation of the insulin receptor (IR) and IGF1 receptor (IGF1R) signalling cascades, which would then negatively impact on dendritic spine and synapse formation (Lee et al., 2011).

Interestingly, IGF1 is produced locally in the cerebellum and can act in a paracrine or autocrine fashion there (Croci et al., 2011). IGF1 and IGF1R are known to be abundant in the cerebellum both in rodents and humans (De Keyser et al., 1994; Garcia-Segura et al., 1997). More specifically, IGF1R is present both presynaptically (i.e. in axonal terminals making contact with the soma of Purkinje cells) and postsynaptically, in the dendrites and soma of Purkinje cells of the cerebellar cortex (Garcia-Segura et al., 1997). IGF1 is extremely important during the (pre- and postnatal) development of the cerebellum, as it is essential for normal dendritic growth and Purkinje cell survival (Cheng et al., 2003; Croci et al., 2011) and for regulating synaptic plasticity of the cerebellum in general (Sherrard and Bower, 2003). We propose that a possible mode of action could be that less IGF1 in the cerebellum (of TH mice) results in less stimulation of the IGF1R, which as a consequence impacts on the synaptic plasticity. Taken together, our experimental findings support our hypothesis that insulin-related pathways are involved in OCD aetiology while also supporting an association between white matter integrity and compulsive behaviour. Although it is still not clear what the relative contribution is of (1) the effect of 'spill over' peripheral insulin and (2) locally produced CNS insulin, our data suggest that at least some of the observed behavioural changes are due to the effects on CNS insulin. That being said, further research is required in order to dissociate the peripheral and central insulin effects. For example, the effect on OCD(-like symptoms) could be tested by using pharmacological interventions such as an IGF1 agonist or metformin, a first line treatment of DM2. Moreover, this could open up treatment options for both OCD and DM2 as well as possibly other neurodevelopmental disorders with compulsive behaviour. In addition, mouse models with a conditional/brain region-specific knockdown of IGF1 and/or IGF1R could be created and behaviourally assessed.

II.VI. REFERENCES

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III. GENERAL DISCUSSION

In this chapter we aimed to study interactions between compulsive behaviour and brain anatomy in a DM2 mouse model. Using this model allowed us to demonstrate cerebello-cortico differences in white matter and brain metabolism as well as brain proteomics, with the cerebellum as a key player. Furthermore, by following up MR imaging with *post mortem* proteomic analyses, we were able to demonstrate its mechanistic role of insulin regulation in the CNS associated with compulsive behaviour.

While rodent studies are only able to model human diseases and disorders, the results from this chapter still underline the importance of the cerebellum in identifying transdiagnostic markers of disorders with increased compulsive behaviour - even across species.

CHAPTER FIVE

CHAPTER FIVE: DISCUSSION AND CONCLUSIONS

René Descartes (1644) discussed the search for complex mechanisms underlying the natural world with the clock as a metaphor. Just as a clock can be understood by dismantling and successfully reconstructing it, he argued that the same principle applies when examining any object of the natural world. For Descartes, this natural world included an immaterial mind directly related to the brain, leading him to formulate a modern version of the mind-body problem as pointed by his famous *"Cogito ergo sum"* quote.

In line with this early debate, the results presented in this thesis display findings of the various roles of the cerebellum in both typical and atypical development. Specifically, the mental and physical capacity needed to coordinate multi-limb movements has been shown to change both cerebellar grey matter volume, white matter microstructure and even functional connectivity. Furthermore, repetitive behaviour and obsessions were associated with differences in cerebellar shape and cortical thickness and surface area, underlining the importance of Descartes' early statement *"I think, therefore I am"*.

I. SUMMARY OF THE MAIN FINDINGS

I.I THE PLASTIC CEREBELLUM

In an attempt to dismantle the plastic interactions between cerebellar networks and neocortical regions, we designed a study where half of our novice adolescent participants would perform a demanding multimodal motor training task (drumming), whereas the other half would continue with their usual day-to-day routines. After 8 weeks of group drumming instruction, 3 x 30 minutes per week, we observed the cerebellum significantly reshaping itself and reorganising its grey (volume increase of lobules left VIIIa, relative decrease of VIIIb and vermis Crus I volume) and white matter microstructure in the inferior cerebellar peduncle. These plastic cerebellar changes were complemented by a reorganisation of cortical thickness (increase in left paracentral, right precuneus and right

but not left superior frontal thickness), suggesting an interplay of cerebellar learning with cortical structures enabled through cerebellar pathways (Chapter II.II).

In order to explore the functional dynamic of cerebellar and neocortical connections, we performed an agnostic data-driven approach using resting-state MVPA to examine the functional connectivity of all voxels in the brain without priori regions of interest (Chapter II.I). Here, drum training was associated with bilaterally increased functional connectivity of the posterior superior temporal gyri (pSTG) and the rest of the brain (i.e., all other voxels). These regions were then used to perform seed-to-voxel analysis, resulting in increased functional connectivity of the pSTG with the premotor and motor regions, the right parietal lobe and decreased functional connectivity with the lateral cerebellum (Crus I/II).

CONCLUSION ONE

Cerebellar networks are able to impact both cerebellar and neocortical functioning (Ito, 2012), as is mirrored by our findings of cerebellar internal reorganisation and plastic changes in the cerebral cortex following our learning task. Such global plastic changes in the brain are further not restricted to childhood development but are still ongoing in adolescence and young adulthood, as we demonstrated in Chapter II. Plastic changes in the brain are enabled through cerebellar loops, allowing the cerebellum to interact and shape connectivity with the neocortex to adapt to the new challenges it is facing. This is in line with Ito's model of the cerebellum as an internal controller, where the initial strenuous conscious effort as registered as cerebral neocortical mental models has passed as the initial familiarity with drumming has been completed. The cerebellum then forms an internal model based on the conscious neocortical mental model of these thoughts, so that these could be derived suddenly and without obvious conscious effort if needed and comparison is made to cerebellar internal models, which have been copied from mental models and stored in the cerebellum. Our results support that the stimulus has been successfully converted and the original stimulus is no longer novel. The methods chosen for the studies of Chapter II are designed to reflect such adaptations, as they investigate both functional and structural connectivity, as well as neuroanatomical volume.

Thus, the findings from this thesis help further describe the role of the cerebellum in

complex motor learning and its interaction with the neocortex. Furthermore, this study aims to contribute to understanding the extent of typical plasticity, which in turn would allow to assess atypical development more in detail. Because of the many cerebello-cortical connections and their close neurodevelopment, abnormal cerebellar development or dysfunction can impact both the cerebellum and neocortical structures.

I.II THE DISORGANISED CEREBELLUM

Because cerebellar networks are highly plastic and interconnected with the neocortex, it does not only learn and optimize complex motor tasks but also cognitive functions. As introduced in Chapter I.III, Ito's internal model controller theory (Ito, 2008, 2012) describes the cerebellum to build mental models while integrating explicit mechanisms, in turn making use of its many connections with the neocortex. However, if the cerebellum as internal model controller is based on dysfunctional mental models, it will result in dysfunctional cerebellar internal models too, explaining the persistency and widespread brain abnormalities of psychiatric disorders (Ito, 2008, 2012). Thus, this "neuronal machine" (Eccles et al., 1967) can be altered and disorganised by several pathological contexts. As a result, cerebellar and brainstem dysfunction has been described regularly in ASD and OCD amongst other neurodevelopmental disorders. However, studies tend to exclude both structures. In chapter III, this issue was addressed by investigating the role of the cerebellum and brainstem in ASD and OCD.

In the first study (Chapter III.I), using machine learning, we demonstrated up to 16% higher classification accuracy in childhood and adult ASD when comparing the cerebellum and brainstem as a combined region of interest over the whole brain and neocortex, where both structures were included/excluded. Interestingly, disease classification was driven by a ring-like pattern in the lateral posterior cerebellum and brainstem pons in both cohorts even when both groups were combined.

Following the results from in Chapter III.II and III.III and in line with the scientific consensus about the role of the cerebellum in both neurodevelopmental disorders (Fatemi et al., 2012; Narayanaswamy et al., 2016), we compared adolescents diagnosed with either ASD or OCD with typically developing controls from the same young cohort used in Chapter III.I. Here, we investigated distinct diagnosis-specific and diagnosis-

nonspecific behaviour for both disorders. In the first study, brainstem and cerebellum shape differed in ASD and OCD when compared with the typically developing control group. Interestingly, while anterior as well as lateral posterior reshaping was demonstrated in both groups, the direction of the reshaping differed in the right posterior cerebellum between both patient groups (inward in ASD, outward in OCD), with stronger overall reshaping in the ASD group. Brainstem differences were further correlated with total repetitive behaviour and symptom severity in both patient groups, but repetitive behaviour was correlated with the posterior cerebellum in the OCD group only.

Motivated by the findings of the first study, we decided to investigate the subtypes of repetitive behaviour further in the next study (Chapter III.III). Here, we identified ritualistic behaviour and its dimensional association with superior parietal surface area as a transdiagnostic marker for paediatric ASD and OCD. Distinct diagnosis-specific profiles were identified for increasing OCD symptom severity with middle frontal and occipital thinning in OCD, and increasing ASD phenotypic symptomology with higher inferior parietal and superior frontal thickness. Furthermore, a thinning of the superior frontal and fusiform face area with increasing compulsive behaviour was demonstrated in the ASD but not the OCD group, possibly identifying its unique contribution to the diagnostic expression.

CONCLUSION TWO

As the previous chapter demonstrated, the cerebellum's many connections enable cerebellar networks to also impact subcortical and neocortical functioning and behaviour. However, if the cerebellum is dysfunctional, its impacted connections could affect the rest of the brain. As many neurodevelopmental disorders are highly comorbid with each other and overlap in symptoms, both distinct diagnosis-specific and non-specific brain-behaviour patterns emerge. Specifically, shared and distinct patterns with symptom severity and non-diagnostic repetitive behaviour were detected in the cerebellar and brainstem vertex as well as neocortical thickness and surface area in both OCD and ASD. Distinct patterns in the cerebellum were further of higher predictive value than neocortical and whole brain contributions to classifying ASD, tying cerebellar involvement and behaviour closely to defining elements of the disorder. The results from this chapter

contributes to understanding the extent of the distinct and shared profiles of both disorders, in turn aiming to help fostering advances in diagnosis and treatments.

Interestingly, in all disorders, we were able to demonstrate differences in the posterior lateral cerebellar lobe, a structure interconnected and developing in tandem with the frontal neocortex. Thus, the results from this thesis underline the importance of cerebello-cortical connections and possibly their role in neurodevelopmental disorders.

I.III THE TRANSLATIONAL CEREBELLUM

Diagnostic and behavioural overlap as well as unique genetic contributions have traditionally been tested in genetic, metabolic and/or behavioural mouse models. The omnipresence of the cerebellum across species with its consistent neuroanatomic structure and similar neurodevelopment makes it an ideal brain region to study neurodevelopmental disorders. Building upon our earlier studies investigating overlaps in non-diagnostic behaviours, we used the TALLYHO mouse model originally designed for type 2 diabetes to investigate compulsive and anxious behaviours typically present in OCD, as well as their structural, metabolic and protein level correlates in the brain. Combining MRI, post mortem analyses and behavioural tests, we were able to show more compulsivity-like behaviour and anxiety in the type 2 diabetes model when contrasted with the control mice. These behavioural differences were accompanied by alterations in white matter cerebellar and neocortical microstructure, neocortical glucose, glutathione and taurine levels, as well as higher cerebellar IGF1 and KCNQ1 brain expression.

As has been mentioned in conclusion II, if the cerebellum as internal model controller is based on dysfunctional mental models, it will result in internal models as reflected in aberrant repetitive behaviour. The microstructure of these internal models has been described in Chapter I.III., where after the Marr-Albus-Fujita adaptive filter model, Purkinje cells learn to recognize contexts and create an anticipatory neural state during rehearsal of an action.

Once the action has been learned, the occurrence of the context alone is enough to fire the Purkinje cells and cause the next elemental reaction, thus acting as an adaptive filter. This increase in efficiency in turn decreases cerebellar activity in the region used, which enables a resource allocation to other areas (Pecenka et al., 2013; Schlaug, 2001). As a

result, an increase in cerebellar activity would induce a decrease in activity in cortical target regions and vice versa (Kelly and Strick, 2003; Szpunar et al., 2007; Chen et al., 2008; Park et al., 2009; Salmi et al., 2010; Bernard et al., 2015; Riedel et al., 2015).
In turn, these dysfunctional internal models will be activated whenever the original stimulus is presented and any built on solutions will be more likely to be dysfunctional as well. This can explain both the persistency and widespread brain abnormalities of psychiatric disorders (Ito, 2008, 2012).

CONCLUSION THREE

The highly conserved cerebellar macro- and microstructure across species makes it a great candidate for translational approaches. Paralleling the findings from Chapter III of overlapping non-diagnostic repetitive and compulsive behaviour across disorders as well as diagnosis-specific patterns of OCD being associated with alterations in both the cerebellum and neocortex, neuroimaging was used as a translational method to investigate these links further. By combining neuroimaging with *post mortem* analyses to determine insulin-signalling protein expression in the brain, this study was able to follow up where *in vivo* human assessments come short – directly investigating brain tissue microstructure. As a result, this study provides corroborating evidence for a role of abnormal insulin signalling in compulsivity-like behaviour, with the cerebellum being implicated in insulin-related pathways and their dysregulation, possibly affecting neocortical white matter microstructure and metabolism through its many cerebello-cortical connections. Thus, the results from this study were able to underline the role of the cerebellum in increased compulsive and OCD-like behaviour as established in Chapter III, and additionally expand upon the cerebellum's mechanistic value of such behaviour relating it to abnormal insulin signalling.

II. GENERAL LIMITATIONS

While this thesis has addressed the role of the cerebellum in both typical plasticity and abnormal development, as well as expanded upon its potential role in translational studies, our results have to adhere to several limitations.

For example, to fully understand the extent of complex cerebellar motor learning, more intensive longitudinal scanning comparing both short and long-term effects would be needed. This could in turn illuminate the complex interplay between the left–right hemispheres we found in our drumming task and how it would develop across different windows of plasticity, a finding consistent in musical studies (Schlaug et al., 1995). Adding an active control group participating in nonmusical but physically demanding multilimb motor activities would also help distinguish motor action and higher functions involved in music training. Here, adding tasks assessing higher cognitive functions including working memory and emotion regulation would be of additional value, as they have previously been implicated in cerebellar function (Schutter, 2013). Finally, adding different age groups to compare cerebellar and neocortical plasticity across ages would inform both about age-related plasticity mechanisms in the brain, as well as their developmental interplay.

Paralleling this, in order to fully assess cerebellar atypical development involved in ASD and OCD, a broader age range should also be investigated to explore the possible longitudinal dynamic of our findings and in turn draw more generalised conclusions about the role of the cerebellum in both disorders. Such studies should also include the full spectrum of ASD and OCD symptoms. Such investigations could further shed light on the complexity of each disorder itself, as for example childhood OCD has been declared to be different from adult OCD (Geller et al., 1998), and it has been proposed that the complexity of the autism spectrum is too big to be summarised into one disorder (for example, Betancur, 2011). Furthermore, in order to disentangle the distinct diagnosis-specific and non-specific brain-behaviour patterns of both disorders, it would be important to include individuals diagnosed with both ASD and OCD. Such independent studies should be using collaborative approaches, as they are essential to reproduce previous findings with longitudinal testing in large paediatric cohorts using multimodal analysis methods and be combined or followed up with translational study approaches connecting human and animal study designs.

While neuroimaging is able to study human brain structure and function noninvasively and *in vivo*, significant limitations remain hindering detailed investigation of the physiology and molecular biology of the human brain. As it remains unclear how the

brain-behaviour patterns described in this thesis correspond to microtissue changes, animal studies could be used to replicate findings and allow to be followed up by more detailed *post mortem* tissue analyses. However, modelling human neuropsychiatric disorders with animal models is challenging in itself due to the subjective nature of many key symptoms defining psychiatric disorders, disorder heterogeneity and the early state of implementing neurobiology and genetic findings for diagnosis. For example, it would be possible to assess compulsive behaviour to a certain extent in a rodent model, but not the obsessive thoughts also defining OCD. A further complication is determining how the assessable symptoms in an animal would add up to a recognised human disorder leaving the modelled disorder to an estimate, a seemingly critical issue for the development of therapeutic interventions. Thus, building upon Descartes' early *"I think, therefore I am"* observation, the question of true translatability of such modelled disorders comes to mind.

Regarding specificities in the human studies presented in Chapter III, while we corrected for site effects and medication, it cannot be excluded that these factors influence our results. Nevertheless, it can be argued that in a 'real life' scenario different scanner models will be used and that patients are often medicated. However, asking them to stop or withhold from starting medication would raise several ethical issues.

III. GENERAL STRENGTHS

This leads to the various strengths of the multitude of methods used in this thesis in order to investigate the cerebellar architecture and connected neocortical structures in typical plasticity and neurodevelopmental disorders. Specifically, through six studies, this research explored the cerebellar architecture from a psychological, behavioural, and neurobiological perspective, aiming to understand the underlying mechanisms and cerebellar connections enabling them. This array of studies explored cerebello-cortical connections in both clinical and non-clinical populations, investigated both diagnosis-specific and nonspecific neural and behavioural correlates of ASD and OCD, using adolescent and adult participants, as well as implementing a range of methodologies to assess them. This range included a combination of neuroimaging methods (grey matter volume and shape, Jacobian weighted prediction maps, cortical thickness and surface

area, resting state functional connectivity, and white matter microstructure), neuropsychological tasks, self-reports and animal models. Furthermore, the broad range of inclusion and exclusion criteria employed in all studies allowed for a more representative sample of the individual disorder within their limiting boundaries. Reassuringly, our results appear to be consistent within and across different cohorts despite such limitations and independent of the methods used. This has even been the case where medicated participants were included, such as for the adolescent cohort used in Chapter III.III.

Thus, the comprehensive approach used throughout this thesis enabled this research to address some pressing questions present in psychiatry on how to determine biomarkers across disorders and their dimensional contribution within disorder groups. Such biomarkers should reflect commonalities in behavioural and/or cognitive processes associated with discrete deficits in defined neural systems (Robbins et al., 2012), thus posing a need to integrate a range of modalities. While the studies underlying this thesis aimed to not only identify such biomarkers and use animal models for translation, it also included a case study enabling clinical translation, as this approach mirrors day-to-day clinical practice, where clinicians are faced with individual cases rather than groups of disorders. A better understanding of the pathophysiology and cerbello-cortico connections in psychiatric disorders could help to inform animal models and in turn support treatment development. Especially illnesses with significant disease burden as typically found in neurodevelopmental disorders could benefit greatly from this development. In turn, the results from Chapter II investigating neuroplastic mechanisms could be used to develop a drum-based intervention helping to overcome certain impairments associated with neurodevelopmental disorders such as ASD.

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APPENDICES

Appendix A. Supplementary Material

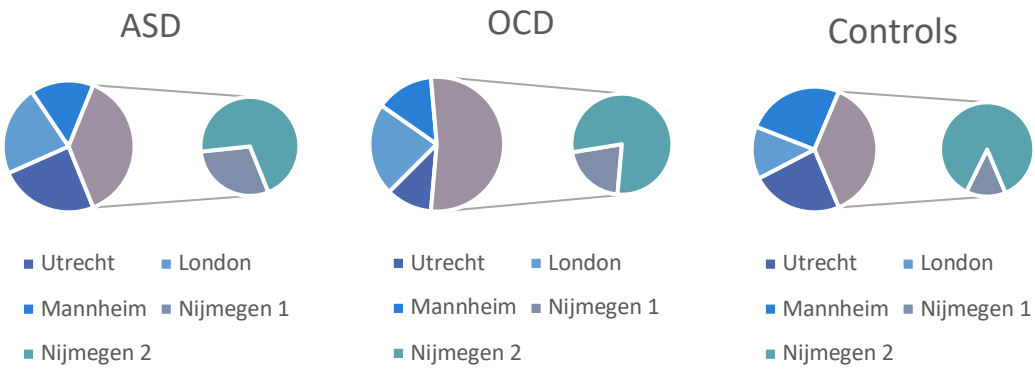
I. THE CONTRIBUTION OF REPEPTITIVE BEHAVIOUR TO SHARED AND DISTINCT BRAIN-BEHAVIOUR ASSOCIATIONS IN AUTISM SPECTRUM DISORDER AND OBSESSIVE COMPULSIVE DISORDER

Table. Neuroanatomical T1-weighted scanning sequences for each site

Site	TR/ TE/ TI (ms)	FA	FoV (mm)	Matrix RL/ AP/ slices	voxel size (mm)	parallel imaging
London	7.31/ 3.02/ 400	11	270	256/256/196	1.1 x 1.1 x 1.2	1.75
Mannheim	2300/ 2.98/900	9	270	212/256/176	1.1 x 1.1 x 1.2	2
Nijmegen	2300/ 2.98/900	9	256	212/256/176	1.0 x 1.0 x 1.2	2
Utrecht	6.8/ 3.10/ 823	9	270	204/252/170	1.1 x 1.1 x 1.2	1.8

TR, repetition time; TE, echo time; FA, flip angle; FoV, field of view; RL, right to left/ coronal; AP, anterior to posterior/ sagittal

Figure. Group breakdown for each scanning site with the site at Nijmegen divided by before (Nijmegen 1) and after (Nijmegen 2) a scanner update



II. CONVERGING EVIDENCE POINTS TOWARDS A ROLE OF INSULIN SIGNALING IN REGULATING COMPULSIVE BEHAVIOUR

Table 1: Protein expression levels (pg/ml) of insulin, IGF1, KCNQ1 and BDNF in the prefrontal cortex, striatum, cerebellum and blood serum

Region of Interest	Insulin			IGF1			KCNQ1			BDNF		
	TH	SWR /J	p	TH	SWR /J	p	TH	SWR/ J	p	TH	SWR /J	p
Prefrontal Cortex	18.1 (3)	18.7 (2)	0.87	46.5 (3)	51.9 (3)	0.26	11306 (436)	11592 (386)	0.64	1614(40)	1578 (41)	0.54
Striatum	25.7 (5)	25.4 (3)	0.96	N.D.	N.D.	N.D.	12305 (571)	11843 (451)	0.53	2320 (75)	2426 (54)	0.26
Cerebe-llum	38.6 (7)	33.4 (2)	0.43	101 (7)	196.5 (13)	<.001	11703 (777)	11319 (337)	0.63	3887 (159)	3937 (90)	0.77
Serum	N.D.	N.D.	N.D.	1704 (474)	97.7 (22)	0.02	94104 (1855)	84168 (2168)	0.008	N.D.	N.D.	N.D.

Data are displayed as mean (SEM). N.D. indicates that the analysis could not be performed for this type of tissue or protein due to limitations in the availability of the tissue.

Figure 1. Representative spectrum and voxel localization in the (A) ACC and (B) DMS.

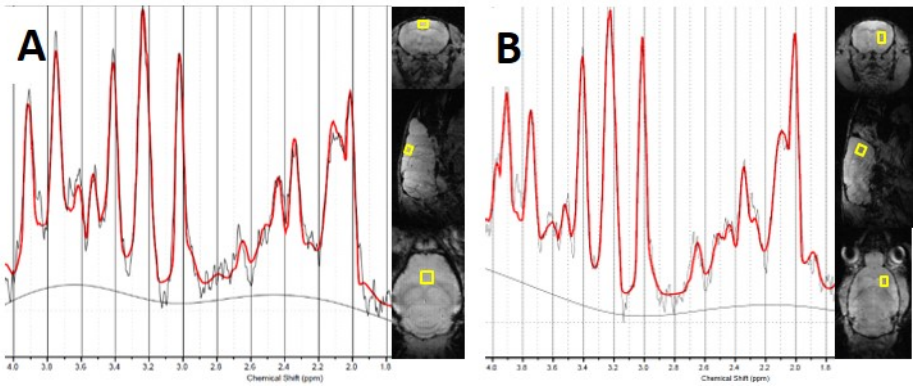
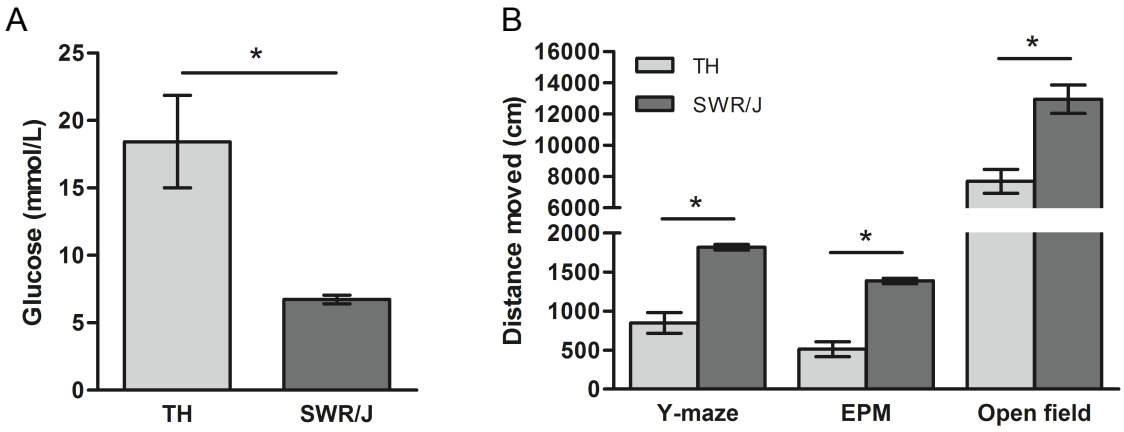
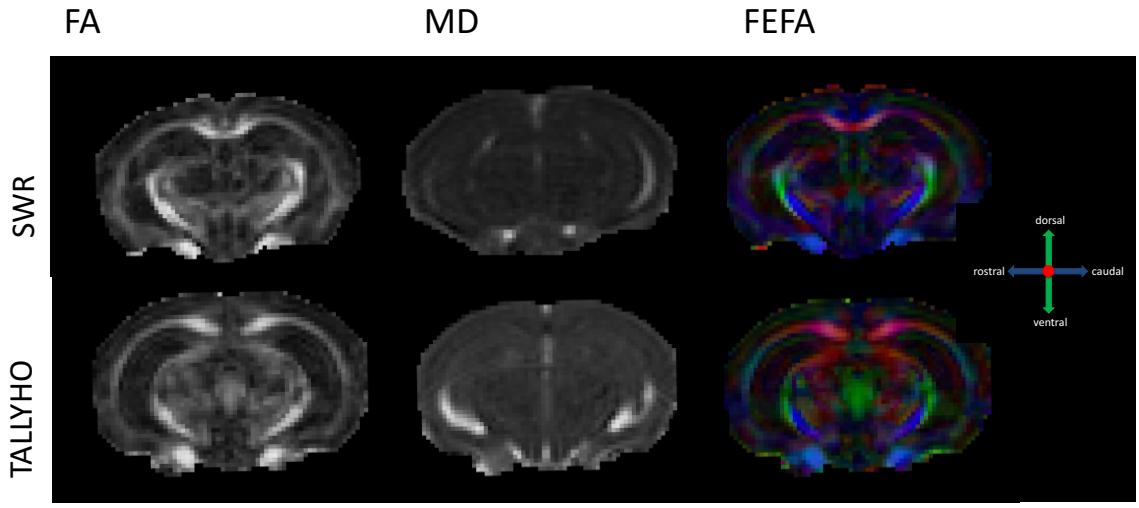


Figure 2. Blood glucose levels



TH mice had higher blood glucose levels compared to their control strain, the SWR/J mice (18.43±3.43 and 6.73±0.32 mmol/L, respectively), indicating that they developed DM2 (A). Additionally, TH mice showed a significant reduction in locomotion observed in three behavioural assays, which corresponds to previous findings (B).

Figure 3. Fractional Anisotropy (FA), Mean Diffusivity (MD) and First Eigenvector Fractional Anisotropy (FEFA) maps



Maps for SWR controls are shown above and TALLYHO mice below. In the FEFA maps, each voxel is given a colour representing the direction of its first eigenvector to display the anisotropy direction/orientation of the brain.

Appendix B. Publications

I. MOTOR LEARNING INDUCES PLASTICITY IN THE RESTING BRAIN – DRUMMING UP A CONNECTION

OXFORD

Cerebral Cortex, March 2017;27:2010–2021

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Original Article

ORIGINAL ARTICLE

Motor Learning Induces Plasticity in the Resting Brain—Drumming Up a Connection

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Abstract

Neuroimaging methods have recently been used to investigate plasticity-induced changes in brain structure. However, little is known about the dynamic interactions between different brain regions after extensive coordinated motor learning such as drumming. In this article, we have compared the resting-state functional connectivity (rs-FC) in 15 novice healthy participants before and after a course of drumming (30-min drumming sessions, 3 days a week for 8 weeks) and 16 age-matched novice comparison participants. To identify brain regions showing significant FC differences before and after drumming, without a priori regions of interest, a multivariate pattern analysis was performed. Drum training was associated with an increased FC between the posterior part of bilateral superior temporal gyri (pSTG) and the rest of the brain (i.e., all other voxels). These regions were then used to perform seed-to-voxel analysis. The pSTG presented an increased FC with the premotor and motor regions, the right parietal lobe and a decreased FC with the cerebellum. Perspectives and the potential for rehabilitation treatments with exercise-based intervention to overcome impairments due to brain diseases are also discussed.

Key words: fMRI, learning, music, neuroplasticity, resting-state

Introduction

Neuroplasticity (NP) is defined as the ability of the nervous system to respond to intrinsic or extrinsic stimuli by reorganizing its structure, function, and connections. Neuroplasticity underlies not only normal development and maturation but also skill learning and memory, as well as the consequences of sensory deprivation or environmental enrichment (Kolb and Muhammad 2014). Even if NP was initially thought to be limited to critical periods in development, it is now largely accepted to occur throughout the lifespan. In fact, animal studies have identified an enhancement of adult neurogenesis, synaptogenesis, angiogenesis, and the release of neurotrophins as a consequence of learning and/or physical exercise (Hötting and Röder 2013). Moreover, several famous neuroimaging investigations demonstrate adaptative neuroplastic modifications in the structure (Maguire et al. 2000; Draganski et al. 2004; Driemeyer et al. 2008) and function (Lewis et al. 2009) of the human brain in response to environmental demands in healthy adults (Wan and Schlaug 2010). Interestingly, few of these neuroimaging studies have investigated neuroplastic modifications following physical activity.

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These existing studies have mainly focused on cognitive facilitation by cardiovascular exercise in older adults, failing to investigate young participants or different types of physical activity (Voelcker-Rehage and Niemann 2013).

Over the past decades, musical training has gained increasing interest as a paradigm to study human experience-related NP in the same general model framework (Herholz and Zatorre 2012). Playing a musical instrument is a highly complex task and requires multimodal skills such as bimanual motor activity dependent on multi-sensory feedback, fine motor skills coupled with metric precision, musical memorization (Wan and Schlaug 2010), and improvization (Pinho et al. 2014; Beaty 2015). These skills involve complex interactions, between sensory and motor systems and high-order cognitive processes, which have to be coordinated at a high degree of synchrony and accuracy (Zatorre et al. 2007).

There is increasing evidence from several different neuroimaging modalities that NP is associated with musical training. By using structural neuroimaging, Gaser and Schlaug reported gray matter volume differences in motor, auditory, and visual–spatial brain regions when comparing professional musicians (keyboard players) with matched groups of amateur musicians and nonmusicians (Gaser and Schlaug 2003). Cerebral activity patterns associated with musical perception in musicians have also been studied by using functional MRI (fMRI). A significant difference in the degree of activation between musicians and nonmusicians was noted in the temporal regions and for musicians the degree of activation was correlated with the age at which the person had begun musical training (Ohnishi et al. 2001). Functional MRI was also used to explore the brain activations of novices trained to play sequences on a piano keyboard (Lahav et al. 2007; Chen et al. 2012; Herholz et al. 2015) or to listen and imitate different auditory rhythms (Chen et al. 2007). These studies showed complex interactions between auditory and motor brain regions that were associated with musical training.

Interestingly, drumming, which is a coordinative exercise combining musicality, body coordination, cardiovascular exercise, "bilateral" arm, leg movements, and sensorimotor integration processes (De La Rue et al. 2013), has not been studied extensively to date. Only expert drummers have been studied thus far. While Tsai et al. (2012) have shown that the posterior temporal lobes are essential for audio-motor processing, Petrini et al. (2011) showed that expert drummers present a reduced activation bilaterally during an audiovisual task in the cerebellum and the left parahippocampal gyrus, respectively, involved in action–sound representation and audiovisual integration.

Recently, resting-state functional connectivity (rs-FC) analyses have been widely used to investigate neuroplastic modifications. Rs-FC corresponds to the temporally correlated, low-frequency spontaneous fluctuations of blood oxygen level-dependent signals across brain regions that occur when a participant is not performing an explicit task (Fox and Raichle 2007). Temporal correlations do not appear to be random, as patterns of connectivity have been reliably identified across studies and participant. Moreover, it is now widely accepted that the strength of correlations within and between networks has behavioral relevance (Guerra-Carrillo et al. 2014). Interestingly, it has been proposed that rs-fMRI is an effective measure of plasticity and that rest activity patterns reflect the history of repeated synchronized activation between brain regions. Thus, the study of rs-FC is particularly adapted to highlight neuroplastic modifications (Buckner and Vincent 2007; Guerra-Carrillo et al. 2014).

In this article, we have compared the rs-FC in 15 novice healthy participants, by using a fully data-driven approach, before and after a course of drumming with another 16 novice participants who were again evaluated longitudinally but with no intervention. This longitudinal design was used to disentangle cause and effect by investigating the same participants before and after the course of drumming. We hypothesized that participants would exhibit a higher level of functional connectivity (FC) in the sensory and motor systems after drum training during the resting state, which could not be attributed to the MRI session effects (anxiety, novelty of the MRI environment).

Material and Methods

Participants

Thirty-one young healthy volunteers (16–19 years) with no prior drumming experience and with no psychiatric or neurological disorders participated in the study after providing written informed consent. In The King's College, London, College Research Ethics Committee approved the experimental protocol. Participants were randomly divided into 2 groups: drum group and control group.

Procedure

Assessment

All participants were attended in to the Institute of Psychiatry Psychology and Neuroscience (IoPPN) at King's College London for 2 scanning sessions. At the first scanning session (t1), the Edinburgh Handedness Inventory Short Form (EHI) for daily activities (e.g., writing, throwing. . .) (Veale 2014) was used to assess each participant's hand dominance. Responses were coded to generate a Laterality Quotient (scores ranging from −100 to 100), which was then used to classify individuals into left handers (−100 to −61), mixed handers (−60 to 60), and right handers (61 to 100). Moreover, to assess the baseline drumming ability and musical experience, a self-report measure was created. Participants reported their level of skill and length of involvement in drumming, playing of another musical instrument and involvement in dance or singing. Responses for all domains were coded on an ordinal scale (0, no experience; 1, some experience, no formal instruction; 2, limited formal instruction but not recent; 3, formal instruction, less than 4 years but not current; 4, formal instruction, exams achieved, greater than 5 years involvement and current).

Drumming

Following the first drumming assessment and scanning session the drum group took part in three 30-minute low intensity group drumming sessions per week for 8 weeks. Each session was delivered by a professional drum tutor and comprised 4 integrated parts: (1) a warm up, focused on beating the drum with a relaxed and consistent motion of the drum sticks; (2) snare drum "rudimental" exercises, played on a single drum surface, adopting a "flow sticking" approach to sequences of left and right hands (Queen 2007); (3) coordinated "groove" patterns, incorporating the interplay of bass drum (right foot) and the hi-hat pedal (left foot) with rock/pop back beat ostinato patterns played on the hi-hat or ride cymbal and snare drum, including eighth note (quaver), quarter note (crotchet), sixteenth note (semiquaver), syncopated quarter note and shuffle ostinato patterns (Chaffee 1980); and (4) performance of learned "grooves" and "fill-ins" to accompany well-known popular music songs. The complexity of drumming tuition was increased on a weekly basis in line with participants' demonstration of improved drumming coordination and technique. The control participants

were asked to not take part in any musical activities. After the 8 weeks, (t2) participants came back to the IoPPN for a second drumming assessment and scanning session.

All of the drumming was performed on electronic drum sets for both drumming training (HD3, Roland, Nakagawa, Japan) and assessment (TD9, Roland, Nakagawa Japan) with a standard right-handed 5 piece configuration comprising a snare drum, 3 tom-toms, hi-hat, ride cymbal, crash cymbal, bass drum, and hi-hat pedal (played with the feet). Drumming was assessed following a short coaching period on the participants' ability to play a simple 4 quarter note pattern to the song "Green Onions" (Booker T and the MGs, Stax/Atlantic, 1962) and a simple 8 eighth note pattern, consisting of regular eighth note hi-hats with alternating kick and snare on the main beats of the bar, to the song "Billy Jean" (Michael Jackson, EPIC, 1982). Versions without tempo fluctuations were used, created using the software "Live" (V9.1, Ableton, Berlin). This software analyzed the beat points of the original recording and adapted them temporally to ensure that the version of the song heard by the participants was at a constant tempo, with a consistent and precise inter beat interval so errors could be accurately recorded. The songs were played out of a single speaker on one channel, while the underlying beat locations were indicated by recording a click track on a second channel. A standard onset detection algorithm (Bello et al. 2005) was used to determine the audio buffer frame in which the transient of each beat in the click track occurred. A precise onset detection method was then used to find the onset location by iteratively dividing the audio buffer into window segments and determining the window in which the energy change was maximal at successively smaller window sizes. Thereby, a precise sample was specified for each beat location, accurately placed on the transient of each audible click (Robertson 2014). Timing data were exported from the drum set using the musical digital interface (MIDI) signal. A comparison with a piezo microphone placed on the snare indicated that the recorded MIDI events were a maximum of 4 ms from the detected onset using audio-based methods, and generally much closer. Drumming ability was assessed objectively as the percentage of bars of both patterns that were completed during two 2-min periods of data capture (1–3 min of each song). To record a completed bar all elements had to be present in each pattern and within half a beat (250 ms for 4 quarter note pattern and 125 ms for an 8 eighth note pattern) of the perfect timing. We also evaluated the error in events that should have been synchronous (flamming) as flam error per bar in milliseconds. For each note of the bar, we evaluated the time between the 2 events. When 3 limbs were involved, we evaluated the difference between the first and last event. This was only evaluated for completed bars, as it would be impossible to make a meaningful measurement where the pattern was breaking down or parts were missing. No practice of either song used in the assessment was included in the 8 weeks drumming training. While a drum flam can be a sought-after stylistic feature of a pattern, flams were not part of the stylistically correct performance for the 2 chosen patterns.

MRI Acquisition and Preprocessing

All participants were scanned in a 3T MR scanner (Discovery MR750, General Electric, Milwaukee, WI, USA). All participants underwent an anatomical T_1-weighted MRI using a gradient-echo sequence with the following scan parameters: 196 sagittal slices, time repetition (TR) = 7.3 ms, time echo (TE) = 3 ms, time to inversion (TI) = 400 ms, FA = 11°, field of view (FOV) = 270 mm², matrix size = 256 × 256, voxel size = 1 × 1 × 1 mm^3, slice thickness = 1.2 mm. The rs-functional magnetic resonance imaging (fMRI) was collected using an echo-planar imaging sequence with the following scan parameters: 180 volumes, interleaved ascending slice order, TR = 2000ms, TE = 30 ms, FA = 75°, FOV = 211 mm², matrix size = 64 × 64, voxel size = 3 × 3 × 3 mm^3, gap = 0.3 mm. Four dummy scans were obtained before each fMRI data acquisition to allow for the equilibration of the MRI signal. During acquisition, the participants remained with eyes open attending a cross-hair on the screen in a wakeful resting state. Headphones and earplugs were used to attenuate the acoustic noise of the scanner.

The anatomical and functional data were preprocessed and analyzed using Statistical Parametric Mapping (SPM12) and the CONN toolbox Version 14p (http://www.nitrc.org/projects/conn) (Whitfield-Gabrieli and Nieto-Castanon 2012). Functional data were preprocessed using a slice scan time correction, realignment (motion correction), registration to structural images and spatial normalization to Montreal Neurological Institute (MNI) standardized space, smoothing with a Gaussian filter of 5.0 mm spatial full width at half maximum value. A conventional band-pass filter over a low-frequency window of interest (0.008–0.09) was also applied to the resting-state time series. After these preprocessing steps, a CompCor strategy (Behzadi et al. 2007) was implemented, extracting signal to noise from the white matter (WM) and cerebrospinal fluid (CSF) by principal component analysis without affecting intrinsic FC (Chai et al. 2012). These components (WM and CSF) and motion parameters were included in the model and considered as covariates of no-interest.

Voxel-to-voxel Analysis

To identify brain regions showing significant FC differences before and after drumming without having to restrict the analyses to one or several a priori regions of interest (ROI), whole-brain connectivity analysis was performed. In the present study, we employed the "connectome-MVPA" approach (multivariate pattern analysis of whole-brain connectome) implemented in the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon 2012). In this approach, a low-dimensional multivariate representation characterizing the connectivity pattern between one voxel and the rest of the brain was created for each voxel separately. This representation was defined by performing a principal component analysis characterizing the FC between this voxel and the rest of the brain. The resulting component scores were then stored as functional maps and entered into standard second-level analyses for between-condition tests (i.e., before and after drum training). Finally, an *F*-test was performed, comparing (for each voxel) the component scores across the 2 conditions. Therefore, the second-level statistical analysis provided a multivariate pattern of correlated voxel clusters associated with drumming. In other words, regions that are significant in the resulting voxel-level analyses indicate condition-related differences in connectivity between those areas and the rest of the brain. More technical details and an example of the use of the connectome-MVPA method can be found in Whitfield-Gabrieli et al. (2015) and in Beaty et al. (2015).

Seed-to-voxel Analyses

To explore which specific aspects of the FC between clusters found in the voxel-to-voxel analysis and the rest of the brain was different between the conditions, a post hoc seed-to-voxel analysis was performed. Therefore, 10 mm spherical ROI based on peak activation clusters from the whole-brain connectivity analysis were extracted and used as seeds to perform seed-to-voxel analysis. Correlations maps were then calculated for each

participant, per condition, by extracting the mean signal time course from the seeds and computing Pearson's correlations coefficients with the time course of all other voxels of the brain. Those correlation coefficients were converted to normally distributed z-scores using the Fisher transformation to allow for second-level general linear model analyses. Second-level within group (one sample *t*-tests) and between group (ANOVA) analyses were performed on the average Z-maps from the new seeds source ROIs. Finally, a paired *t*-test was performed to examine the change of resting-state connectivity before versus after drum training for all participants.

For confirmatory analysis, seed-to-voxel analyses were applied using anatomically defined ROIs, in order to avoid potential concerns regarding circularity. For this analysis, the posterior superior temporal gyrus (pSTG) was chosen because it is a crucial hub engaged in sensorimotor integration (Rauschecker and Tian 2000; Scott et al. 2009), auditory–motor transformations (Warren et al. 2005; Chen et al. 2007) and because it is selectively responsible for action-related sounds (Zatorre et al. 2007; Tsai et al. 2012). Moreover, a recent fMRI meta-analysis of 43 studies on experimental auditory–motor training (i.e., finger tapping), revealed activity in the pSTG (Chauvigné et al. 2014). Two 10 mm spherical ROIs were then created based on the Harvard-Oxford cortical atlas implemented in the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon 2012). The MNI coordinates of left and right pSTG were, respectively, (−62, −29, 3) and (61, −24,1).

As gender and handedness may be a factor which modulates musical training (Koelsch et al. 2003; Klöppel et al. 2007) additional analyses including gender and handedness as covariates were conducted. The same 10 mm spherical ROIs were used as seeds in the control group in order to delineate brain changes which are specific to the drum training. To precisely identify the brain areas involved, the Harvard-Oxford Cortical and Subcortical Atlas (Desikan et al. 2006; Makris et al. 2006), the AAL Atlas (Tzourio-Mazoyer et al. 2002), and the Juelich Histological Atlas (Eickhoff et al. 2007) were used. All results were thresholded at a voxel-wise $P < 0.001$ and at the cluster extent $P < 0.01$ FWE corrected. For confirmatory analysis, results were thresholded at a voxel-wise $P < 0.001$ and at the cluster extent $P < 0.05$ FWE corrected, a threshold widely used in confirmatory analysis (Kriegeskorte et al. 2010; Chen et al. 2015).

Correlations with Drum Progression

To evaluate the association between FC and drum progression, correlations (Pearson's coefficients) were performed between the drum progression scores in each participant and the connectivity values (z-scores) of regions showing significant differences after drum training from the seed-to-voxel analysis. Two scores were used. The first score corresponded to the mean number of bars successfully completed (Δ% bars completed) before versus after the drum training (t2 – t1 difference) from "Green onions" and "Billy Jean". The "combined progression score" corresponded to the proportion of bars successfully completed before versus after the drum training (t2 – t1 difference) as the percentage of bars completed (Δ% bars completed) from both songs.

Statistical Analyses

Statistical analyses of demographic data and drum performance were performed using SPSS (SPSS version 18, Chicago, Illinois, USA). Independent sample *t*-tests or Mann–Whitney rank sum test, and paired *t*-tests were used to determine baseline differences, and the effects of drum training between and within groups. To test whether there was a significant interaction of group over time, we performed a repeated-measures ANOVA model by specifying "time" as a within-subjects contrast (t1 – t2, i.e. before and after the drum training) followed by a between subject's contrast (drummers vs. controls). Significance was set at $P < 0.05$.

Results

Participants and Drum Progression

Based on a self-report measure, the drum group ($n = 15$, 7 men, 8 women, 2 mixed-handed, age 16.8 ± 0.7 years) and the control group ($n = 16$, 8 men, 8 women, all right-handed, age 17.8 ± 1.4 years) presented no prior drumming experience (all scores ≤2). Moreover, there were no statistical differences between the 2 groups in terms of self-reported other musical instrument ($P = 0.53$) dance and singing experience ($P = 0.31$).

At baseline, the mean number of bars successfully completed for "Green Onions" and "Billie Jean" was not significantly different between the 2 groups (respectively, $P = 0.42$ and $P = 0.12$) (see Fig. 1). After 8 weeks, the mean number of bars successfully completed was significantly increased in both groups and for the 2 songs (see Fig. 1). The mean combined progression score was significantly higher ($P = 0.003$) for the drum group (mean = 0.45 ± 0.29) in comparison with the control group (mean = 0.17 ± 0.19). A before/after video is available for illustration (see https://vimeo.com/141911618).

For "Green Onions", repeated-measures ANOVA revealed no significant effect of group ($F = 3.9$, $P = 0.07$), a significant effect of time ($F = 19.9$, $P = 0.001$) and no group × time interaction ($F = 3.3$, $P = 0.091$). For "Billie Jean", repeated-measures ANOVA revealed a significant effect of group ($F = 26.1$, $P < 0.001$), a significant effect of time ($F = 36.1$, $P < 0.001$) and significant group × time interaction ($F = 21.9$, $P < 0.001$). There was no significant difference in flam error per bar between t1 and t2 in both groups ($P = 0.24$).

Voxel-to-voxel Analysis

The connectome-MVPA analysis revealed 2 symmetric clusters associated with drum training ($P < 0.005$, FWE correction). These clusters consisted of the posterior division of the left and right superior temporal gyri (STG) and middle temporal gyri (MTG) and are described as pSTG throughout the manuscript (see Table 1 and Fig. 2).

Seed-to-voxel Analyses

Group-level seed-to-voxel analyses revealed increased FC after drum training when compared with before between left pSTG and left superior parietal lobule (SPL), left inferior temporal gyrus, right angular gyrus, right supramarginal gyrus, left and right lateral occipital cortex, right inferior temporal gyrus, and left inferior frontal gyrus and a decreased FC between the right STG and MTG (posterior division) and left and right cerebellum crus I and II (see Fig. 3). Peak spatial coordinates in the MNI space and the corrected *P*-value ($P < 0.01$, FWE correction) are reported in Table 2.

Group-level seed-to-voxel analyses revealed increased FC after drumming when compared with before between right pSTG and right supramarginal gyrus right angular gyrus, right SPL and right pre- and postcentral gyrus and a decreased FC with the left STG and MTG (posterior division), left temporal pole, bilateral cerebellum crus I and II, left paracingulate gyrus and right MTG (see Fig. 4). Peak coordinates in the MNI space

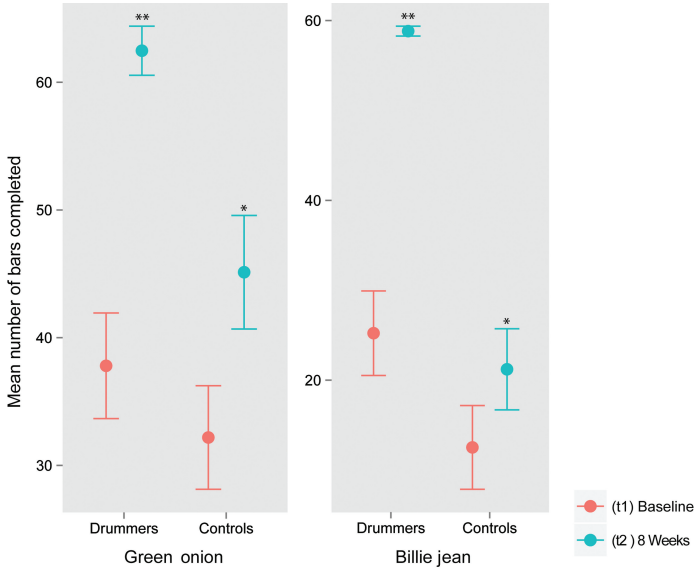


Figure 1. Mean number of bars successfully completed for "Green Onions" and "Billie Jean" before and after the drum training. At baseline, the mean number of bars successfully completed was not significantly different between the two groups for the both songs (respectively $P = 0.42$ and $P = 0.12$). After 8 weeks, the mean number of bars successfully completed was significantly increased in both groups and for the 2 songs. Error bars denote the standard deviation. *$P < 0.05$; **$P < 0.001$.

Table 1 Whole-brain connectome-MVPA analysis (voxel-to-voxel analysis)

Cluster size	Side (L/R)	Brain areas	MNI coordinates			F_{max}	Z_{max}	P_{corr} value
			x	y	z			
160	L	Superior Temporal Gyrus (posterior division)	−64	−28	12	15.02	4.78	<0.001
		Middle Temporal Gyrus (posterior division)	−58	−26	−8	9.00	3.72	
49	R	Superior Temporal Gyrus (posterior division)	64	−18	02	11.71	4.25	<0.005
		Middle Temporal Gyrus (posterior division)	70	−28	0	8.80	3.67	

Note: MNI coordinates (x, y, z) represent peaks within a cluster. Cluster size corresponds to spatial extent (i.e., number of voxels). Correction for multiple comparisons was performed using family-wise error correction at the cluster level.
L/R, left/right side of the brain.

and the corrected P-value ($P < 0.01$, FWE correction) are reported in Table 3.

The seed-to-voxel analysis was confirmed by adopting new, anatomically defined ROIs, as we observed qualitatively the same patterns of modulated FC involving the same networks (see Supplementary Tables 1 and 2).

The supplementary inclusion of gender and handedness as covariates did not change the results as the same clusters were found. No significant FC differences were observed in the control group.

Improvement in Performance and Correlations with Functional Connectivity

Participants with higher improvement in performance had higher right seed-right parietal lobe connectivity scores. Indeed, there was a significant correlation between the improvement in the mean number of bars successfully completed for "Billie Jean", the combined progression score and the right parietal lobe FC (including the right supramarginal gyrus, the right angular gyrus and the right SPL) from the right seed. The correlation coefficients were, $r = 0.60$ ($P = 0.02$) and $r = 0.57$ ($P = 0.03$), respectively (see Supplementary Figure). The improvement in performance did not correlate significantly with the FC of the other clusters.

Discussion

The main goal of this study was to determine whether we could visualize significant differences in the functional networks engaged in drum training in healthy participants by using rs-FC. Drum training was characterized behaviorally in significantly

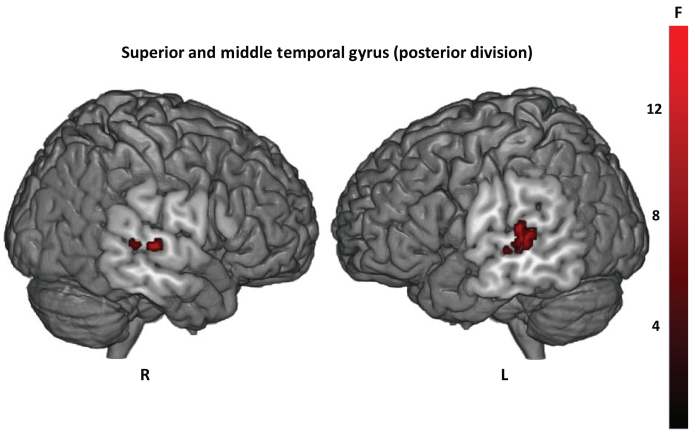


Figure 2. Results from the voxel-to-voxel analysis showing areas of highest connectivity in the whole brain by comparing before versus after drum training. These clusters consisted of the posterior division of the left and right STG and MTG and are described as pSTG. This map is based on F-contrasts thresholded at $P < 0.01$, FWE corrected. Clusters were rendered on the "ch256" brain template using MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). L/R = left/ right side of the brain.

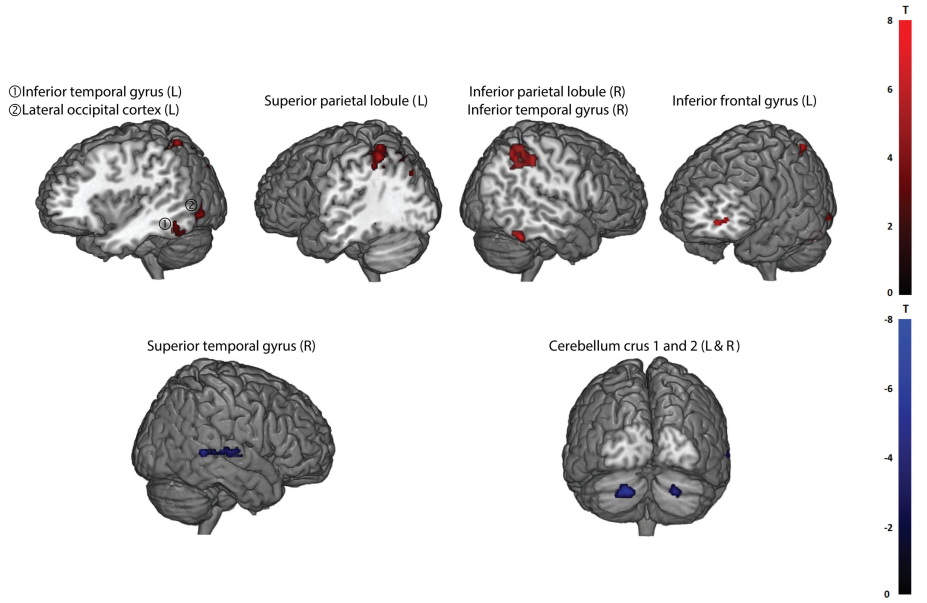


Figure 3. Results from seed-to-voxel functional connectivity (FC) of the left pSTG seed (results of voxel-to-voxel analysis). Higher FC was detected after drum training within left superior parietal lobule, left inferior temporal gyrus, right inferior parietal lobule (angular gyrus and supramarginal gyrus), left and right lateral occipital cortex and left inferior frontal gyrus (upper panel, $P < 0.01$, FWE corrected). A decreased FC was measured after drum training within right superior and middle temporal gyrus (posterior division) and left and right cerebellum crus (lower panel, $P < 0.01$, FWE corrected). Clusters were rendered on the "ch256" brain template using MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). L/R = left/ right side of the brain.

Table 2 Functional connectivity of the left pSTG

	Cluster size	Brain areas	MNI coordinates				T_{max}	P_{corr} value
			Side (L/R)	x	y	z		
Post > Pre								
Cluster 1	741	Superior parietal lobule	L	−36	−50	50	7.85	<0.0001
		Lateral occipital cortex	L	−24	−60	34	7.54	
Cluster 2	526	Inferior temporal gyrus	L	−50	−46	−18	6.36	<0.0001
Cluster 3	424	Angular gyrus	R	40	−54	52	6.34	<0.0001
		Supramarginal gyrus	R	42	−36	44	6.24	
		Lateral occipital cortex	R	32	−64	42	5.93	
Cluster 4	103	Inferior temporal gyrus	R	56	−46	−28	8.45	<0.01
Cluster 5	103	Inferior frontal gyrus	L	−30	48	−2	6.43	<0.01
Post < Pre								
Cluster 1	276	Superior temporal gyrus	R	54	−30	4	−6.74	<0.001
		Middle temporal gyrus	R	68	−42	0	−6.54	
Cluster 2	152	Cerebellum crus I and II	L	−18	−76	−36	−6.50	<0.001
Cluster 3	159	Cerebellum crus I and II	R	22	−72	−28	−5.77	<0.001

MNI coordinates (x, y, z) represent peaks within a cluster. Cluster size corresponds to spatial extent (i.e., number of voxels). Correction for multiple comparisons was performed using family-wise error correction at the cluster level.
L/R, left/ right side of the brain.

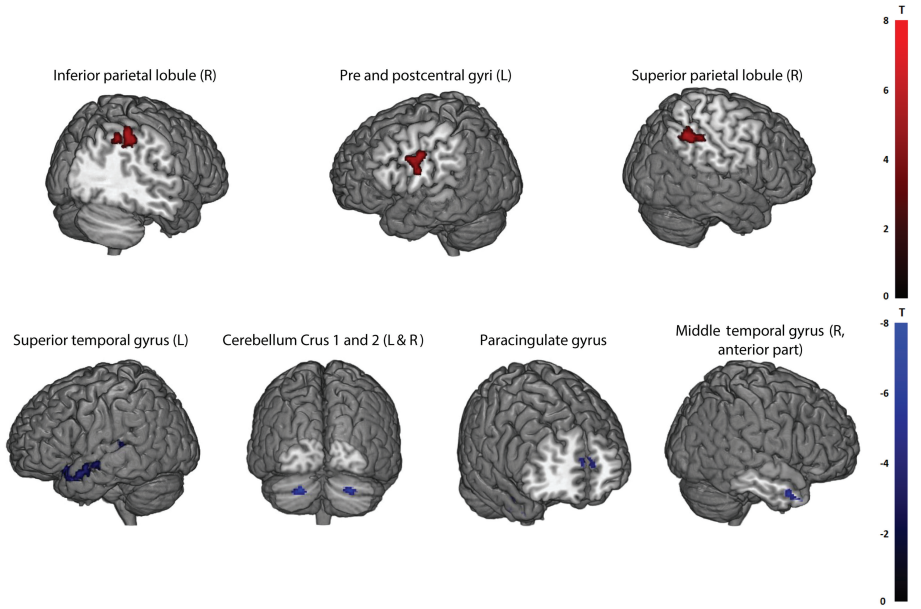


Figure 4. Results from seed-to-voxel functional connectivity (FC) of the right pSTG seed (results of voxel-to-voxel analysis). Higher FC was detected after drum training within right inferior parietal lobule (angular gyrus and superior parietal lobule) and right pre and postcentral gyrus (upper panel, $P < 0.01$, FWE corrected). A decreased FC was measured after drum training within left superior and middle temporal gyrus, left temporal pole, left and right cerebellum crus, paracingulate gyrus and the anterior part of the right MTG. Clusters were rendered on the "ch256" brain template using MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). L/R = left/right side of the brain.

improved performance as assessed by the mean number of bars successfully completed for "Green Onions" and "Billie Jean". Interestingly, after 8 weeks, the mean number of bars successfully completed was significantly increased in both groups and for the 2 songs. This increased drum performance in the age-matched control group can be explained by a learning

Table 3 Functional connectivity of the right pSTG

	Cluster size	Brain areas	Side (L/R)	MNI coordinates			T_{max}	P_{corr} value
				x	y	z		
Post > Pre								
Cluster 1	388	Supramarginal gyrus	R	38	−48	38	6.19	<0.0001
		Angular gyrus	R	42	−54	50	6.09	
		Superior parietal lobule	R	32	−54	44	5.73	
Cluster 2	309	Precentral gyrus	L	−48	−10	32	5.67	<0.0001
		Postcentral gyrus	L	−44	−20	32	5.56	
Post < Pre								
Cluster 1	1010	Middle temporal gyrus	L	−52	−24	−12	−7.64	<0.001
		Temporal pole	L	−50	20	−20	−7.53	
		Superior temporal gyrus	L	−58	−32	−2	−7.19	
Cluster 2	137	Cerebellum crus I and II	L	−20	−76	−34	−8.88	<0.001
Cluster 3	134	Cerebellum crus I and II	R	26	−80	−34	−8.28	0.001
Cluster 4	122	Paracingulate gyrus	L	2	50	14	−6.36	<0.01
Cluster 5	116	Middle temporal gyrus	R	48	4	−28	−7.20	<0.01

Note: MNI coordinates (x, y, z) represent peaks within a cluster. Cluster size corresponds to spatial extent (i.e., number of voxels). Correction for multiple comparisons was performed using family-wise error correction at the cluster level.
L/R, left/ right side of the brain.

or familiarization effect since the 2 songs were no longer novel to the participants in both groups. However, the drum group has improved drum performance over and above this simple familiarization effect. Indeed, the progression score was significantly higher for the drum group in comparison with the control group. Moreover, a significant effect of time (i.e., before and after 8 weeks) has been showed in both groups and for the 2 songs, and a significant group (i.e., drummers vs. controls) × time interaction was showed for "Billie Jean". We were unable to utilize more sophisticated measures of drumming performance (such as timing deviations), because we could only evaluate the timing scores from the data that made up the completed bars, since in this population (of novices) there were periods of no playing and patterns that needed to be stopped as they were playing incorrectly. While timing deviation would be a representative statistic for more experienced drummers, this relies on the subject's ability to play the pattern correctly in order to be meaningful. Drum training was also associated with: (1) an increased FC between the posterior part of bilateral pSTG and the rest of the brain (i.e., all other voxels), (2) an increased FC between pSTG and the premotor and motor regions, (3) an increased FC between pSTG and the right parietal lobe which was correlated with drum performance and (4) a decreased FC between regions of the cerebrum and the cerebellum. Moreover, the seed-to-voxel results were confirmed by adopting anatomically defined ROIs. Finally, these results could not be attributed to the MRI session effects (anxiety, novelty of the MRI environment) or to the normal development (Power et al. 2010; Rubia 2013), as no significant FC differences were observed in the age-matched control group.

The whole-brain FC underlying drum training was explored and revealed 2 symmetric brain regions located in the pSTG (see Fig. 2). This result is consistent with previous experiments about the training to play a melody on piano keyboard (Chen et al. 2012), hammering or clapping (Lewis et al. 2005; Galati et al. 2008), and expert drummers (Tsai et al. 2012), which showed that the bilateral pSTG were selectively responsible for action-related sounds. Indeed, the pSTG is a crucial hub of the dorsal auditory streams which is engaged in sensorimotor integration and spatial processing ("how" and "where") (Rauschecker and Tian 2000; Scott et al. 2009) and therefore plays an essential role in auditory–motor transformations ("do-pathway"), which is essential for music processing (Warren et al. 2005; Zatorre et al. 2007).

Interestingly, while using the pSTG as a seed to perform a seed-to-voxel analysis, an increased FC was found between the right pSTG and the left precentral and postcentral gyri including the ventral premotor cortex (vPMC) and the primary motor cortex. The premotor cortex can compute a variety of sensorimotor transformations that are relevant for music and is involved in the motor prediction and production of complex sequences (Zatorre et al. 2007). Specifically, the left vPMC, which might work in tandem with the pSTG (Hickok and Poeppel 2007; Tsai et al. 2012), could be associated with the serial sequence prediction and coupling between hearing music and the execution of a motor programme by the motor cortex, that enable the realization of sensory-cued actions (Zatorre et al. 2007).

The left and right pSTG seed regions showed an increased FC, respectively, with the left and right SPL, a coupling essential in sensorimotor transformation (Hickok and Poeppel 2004; Zatorre et al. 2007). The SPL, which is anatomically connected to the posterior temporal lobe (Kamali et al. 2014), is critical for many sensory and cognitive processes, including sensorimotor (Wolpert et al. 1998) and somatosensory integration, motor learning, spatial perception, mental rotation, visuospatial attention, and memory (Wang et al. 2015). Moreover, it has been involved in the perception of bimanual interaction with an external object (Naito et al. 2008) and the storage of movements and kinematics (Seitz et al. 1997). This appears to be very consistent with the present experiment involving drum training. In the music training framework, the SPL has also been suggested to coordinate the complex spatial and timing components of musical performance (Langheim et al. 2002). After drum training, both pSTG were also more functionally connected with the right inferior parietal lobule (IPL), which comprises of the angular and supramarginal gyri, and the intraparietal sulcus (IPS). Interestingly, the SPL and the IPL cluster, which includes the IPS, are part of the dorsal attention network (Petersen and Posner 2012). The dorsal attention network is involved in the maintenance of spatial priority maps for covert spatial attention, saccade planning, and visual working memory (Vossel et al. 2014). Moreover, our results are consistent with the right hemisphere dominance that has been described

for visuospatial attention (de Schotten et al. 2011). Interestingly, these posterior parietal regions also support mental transformations of acoustic or visual information into motor representations (Warren et al. 2005; Zatorre et al. 2007; Herholz et al. 2015). The increased functional coupling between the bilateral pSTG and the right dorsal attention network might be associated with the improvement of the integration of sensory and motor functions, as the FC of the right parietal lobe cluster was significantly correlated with the improvement in drum performance.

The increased FC between the left pSTG and the inferior frontal gyrus (IFG) corresponds to the ventral auditory streams arising from the primary auditory cortex, and projecting anteriorly from the primary auditory cortex to the IFG along the STG (Scott et al. 2009). This ventral pathway, also known as the what-pathway, is involved in auditory object identification (Leaver and Rauschecker 2010). It is specialized for invariant auditory object properties, which are time-independent, and less related to motor systems (Zatorre et al. 2007). The left seed was more functionally connected with the left inferior temporal gyrus and the lateral occipital cortex. These are part of the ventral visual pathway involved in human object perception and recognition and in the encoding of spatial relationships between subparts of scenes (Grill-Spector and Weiner 2014). This increased FC reflects the audio-visual integration, which has been shown to be crucial in multimodal training (Paraskevopoulos et al. 2012).

Interestingly, both seeds also showed a decreased FC with the bilateral cerebellum (crus I and II). A recent meta-analytic connectivity study identified these 2 cerebellar subregions as part of a cerebellar cluster significantly associated with motor learning, working memory, recitation, and repetition as well as music comprehension and production (Riedel et al. 2015). All integral components involved in learning to play the drums. According to the Marr–Albus–Ito theory on motor learning, cerebellar Purkinje cells (PC) learn to recognize contexts during rehearsal of an action, creating an anticipatory neural state (Marr 1969; Ito 1970; Albus 1971). Climbing fibers innervating the PC further encode possible errors, consequently suppressing the PC synapses involved in such erroneous performance with the help of long-term depression. Once the action has been learned, the occurrence of the context alone is enough to fire the PC to cause the next elemental movement. This increase in efficiency in turn decreases cerebellar activity in the region used, which enables a resource allocation to other areas (Petrini et al. 2011). When such movement involves excitatory output to the cerebral cortex, PC form inhibitory synapses with the deep cerebellar nuclei. As a result, an increase in cerebellar activity would induce a decrease in activity in cortical target regions and vice versa (Vahdat et al. 2011). As a result, the cerebellum is able to integrate and coordinate motor and sensory signals, react quicker and with less error, creating a smooth motor performance. With this increase of efficiency, a decrease of cerebellar activation in regions required for motor learning, music comprehension and production can be expected after drum training.

Both pSTG also showed a decreased FC with the contralateral STG and the right pSTG showed a decreased FC with the ipsilateral anterior part of the MTG. These decreased FC can reflect complex interferences between the ipsilateral and contralateral auditory pathways (Pantev et al. 1986) that can be associated with the synaptic inhibition involved in sound localization (Grothe 2003). In fact, auditory pathways are formed by excitatory and inhibitory neural connections and networks. It is now well-known that music induced lateral inhibition in the human auditory cortex (Pantev et al. 2012). Moreover, reductions in temporal gyrus activity were reported previously for auditory perceptual pitch training (Jäncke et al. 2001) and discrimination training for micromelodies (Zatorre et al. 2012). The right pSTG was also less functionally connected with more frontal regions, such as the paracingulate gyrus, which can reflect interactions in top-down strategies. The frontal regions may be involved in the control of action plans and in the selection and/or inhibition of action chunks. This is particularly important for musical performance, as chunking is defined as the re-organization or re-grouping of movement sequences into smaller sub-sequences during performance, to facilitate the smooth performance of complex movements and to improve motor memory (Zatorre et al. 2007). Those kinds of patterns involving networks with increased and decreased activations or FC have already been described for motor training (Allison et al. 2000; Voelcker-Rehage and Niemann 2013) and in the music literature (Lahav et al. 2007; Petrini et al. 2011; Chen et al. 2012; Pinho et al. 2014). They are furthermore thought to be associated with greater automaticity in cognitive processes (Voelcker-Rehage and Niemann 2013; Beaty 2015).

Finally, the fact that the adult brain is capable of NP modifications, as demonstrated here, highlights the potential of rehabilitation treatments designed to induce plastic changes to overcome impairments due to brain diseases (Wan and Schlaug 2010), which may include stroke, traumatic brain injury, and a range of neuropsychiatric disorders (Cramer et al. 2011). Intriguingly, most regions highlighted by our analyses (IPL, SPL, MTG, and STG) are part of the human mirror neurons system (MNS), which is activated when an individual performs an action and when a similar or identical action is passively observed (Molenberghs et al. 2012). From a clinical perspective, our results could be particularly interesting to consider a drum-based intervention in disorders involving a MNS dysfunction such as in autism spectrum disorders (ASD) (Molnar-Szakacs and Heaton 2012). Drumming with a social partner could then be particularly relevant for individuals with ASD. Previous studies investigating interpersonal body movement synchronization and social processes found that drumming could improve social interaction (Hove and Risen 2009; Kirschner and Tomasello 2009; Yun et al. 2012).

By using additional neuroimaging methods, future studies should also investigate further the functional interplay between cortical and cerebellar regions. Here, DTI tractography and/or structural MRI analyses could explore whether the changes in FC are accompanied by structural changes, which would help illuminate the role of the cerebellum in multimodal action learning. Furthermore, the complex interplay between the left–right hemispheres, as showed in our FC results, should be specifically investigated. Indeed, it is now well-known that musical ability is associated with left–right differences in brain structure and function (Schlaug et al. 1995). However, drummers are unique in that they combine both independent and multilimb coordination when playing, which is necessary for the physical multitasking of drumming and which can lead to complex interhemispheric interactions. Adding an active control group participating in nonmusical motor activities would also help distinguish motor action and higher functions involved in music training. Such an active control condition could consist of learning a new physically demanding multilimb motor sport (such as playing basketball). Finally, adolescence is characterized by changes in brain structure and function, particularly in regions of the cortex, notably the frontal, parietal and temporal regions, that are involved in higher-level cognitive processes (e.g., memory), for which capacity may be increased in adolescence. It has thus been suggested that adolescence might represent a second

"window of opportunity" in brain development (Fuhrmann et al. 2015). However, our results cannot be explained by typical growth trajectories, as volume, thickness, and surface area of the temporal and motor cortices are decreasing in adolescence (Østby et al. 2009). Hence, future experimental studies are needed to compare effect of environment manipulation, such as drum training, in child, adolescent, and older adult groups.

In conclusion, this is the first FC study to compare novice healthy participants before and after an extensive coordinated motor learning such as drumming. An agnostic data-driven approach, i.e., resting-state MVPA, was used to examine the FC of all voxels in the brain, independent of a priori anatomical hypotheses. This method allowed us to highlight the central role of the posterior part of the STG in this task. Moreover, by using the MVPA results as seeds to compute a seed-to-voxel analysis, we showed complex patterns of increased and decreased FC associated with drum training that were partly correlated with the improvement in drum performance. Indeed, as musical training has been shown to modulate unimodal as well as multimodal cortical processing, the seed-to-voxel analyses provide important information on the interaction between the cortical areas involved within the whole network supporting the instrumental performance (Pantev and Herholz 2011). Finally, one can argue that our drum training regimen is a relevant task to highlight the neuroplastic mechanisms involved in motor learning in a naïve population and that drum-based intervention could be relevant to overcome impairments due to brain diseases.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Notes

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II. THE CEREBELLUM AND BRAINSTEM RESHAPE WITH COMPULSIVE BEHAVIOUR AND SYMPTOM SEVERITY IN AUTISM SPECTRUM DISORDER AND OBSESSIVE COMPULSIVE DISORDER

The Cerebellum and Brainstem Reshape with Compulsive Behaviour and Symptom Severity in Autism Spectrum Disorder and Obsessive Compulsive Disorder

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INTRODUCTION

Neurodevelopmental disorders such as autism spectrum disorder (ASD) and obsessive-compulsive disorder (OCD) share genetic vulnerability (Jacob et al., 2009), patterns of abnormal brain development (Ameis et al., 2016) and overlap in diagnostic symptoms (Jacob et al., 2009). Specifically, the tendency to perform repetitive acts in a ritualised manner (Chamberlain & Menzies, 2009) is one of the earliest signs of the autism phenotype (Leekam et al., 2011) and the main characteristic of OCD (Association, 2000), with lesion studies connecting the cerebellum and brainstem to compulsive behaviour (Figee et al., 2013). During the transition from childhood to adolescence, control over compulsive behaviour is established (Arain et al., 2013), accompanied by ongoing maturation of brain regions including the cerebellum (Tiemeier et al., 2010) and brainstem (Geva & Feldman, 2008).

Longitudinal studies revealed that during this developmental period, brainstem volume (Jou et al., 2013) and cerebellar vermis volume increase, while total cerebellar volume decrease in ASD (Courchesne et al., 2011), specifically in the posterior cerebellar lobe (Stoodley, 2015). In contrast, overall cerebellar volume is increased in paediatric OCD when compared to controls (Eng et al., 2015). Furthermore, overall symptom severity is positively correlated with cerebellum and brainstem volume in OCD (Zarei et al., 2011), and negatively with posterior cerebellar volume in ASD (Pierce & Courchesne, 2001). Thus, abnormal cerebellum and brainstem development might affect the establishment of compulsive behaviour control, possibly with regional rather than overall differences driving dysfunctional behaviour.

However, regional cerebellar and brainstem differences in OCD have not been investigated and whether both are comparable across disorders regarding their correlation with compulsive behaviour remains unclear. Furthermore, volume differences only give a summation value and might not detect subtle regional changes. Here, we are investigating cerebellum and brainstem shape across two paediatric ASD and OCD cohorts in order to identify regional differences and their correlation with compulsive behaviour and symptom severity.

METHODS

Sample: 58 right-handed children DSM-IV-TR (American Psychiatric Association, 2000) diagnosed with ASD (45 male, 13 female), 30 diagnosed with OCD (15 male, 15 female) and 61 age-matched right-handed healthy controls (42 male, 19 female), all aged 8 to 15 years were included. Exclusion criteria for all participants was a history of neurological disorders, any contraindications for MRI, a total IQ<70, as well as comorbidity with the other disorder of interest. No DSM axis I disorders were allowed in any relatives up to two generations back for the control group. Ethical approval for the study was obtained for all sites separately.

Compulsive Behaviour Measures: Compulsive behaviour was assessed for all groups on a behavioural level using the Repetitive Behaviour Scale Revised (RBS-R; Lam & Aman, 2007) and symptom severity using overall scores on the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS; Scahill et al., 2014) and Autism Diagnostic Interview Revised (ADI-R; Lord et al., 1994) respectively. All measures used were part of a larger test battery and described in detail elsewhere (Naaijen et al., 2016).

MRI acquisition: Structural T1-weighted scanning sequences based on the ADNI GO protocol (Jack et al., 2008) were acquired on comparable 3T scanners at the individual sites (Radboud University Medical Center, Nijmegen, The Netherlands; University Medical Center Utrecht, Utrecht, The Netherlands; King's College London, London, UK; Central Institute of Mental Health, Mannheim,

Germany). To assure MR quality and reliability, 'travelling-head' and phantom data were collected across sites (Naaijen et al., 2016). Each structural scan was visually inspected, and only scans with no or minimal movement artefacts were included in analyses. Shape Analysis: FSL FIRST version 5.0.8 (Patenaude et al., 2011) was used to perform shape and appearance deformations of the cerebellum and brainstem. For this, shape was modelled using a surface-based point distribution model, constraining shape space to the linear combination of the mean shape and eigenvectors of the vertex coordinates. Vectors indicate the direction of difference, with inward direction indicating smaller/thinner structure, and vice versa. FSL's25 general linear model was used to create group contrasts, as well as correlation analyses with compulsive behaviour and symptom severity. All analyses were controlled for age, gender and scanning site.

RESULTS

Compared to controls, both disorders showed significant group differences of the cerebellum and brainstem shape, with stronger overall reshaping in the ASD group and anterior as well as lateral posterior reshaping in both groups (Figure 1). Furthermore, the direction of the reshaping differed in the right posterior cerebellum between both patient groups (inward in ASD, outward in OCD; Figure 1). While the anterior brainstem correlated with compulsive behaviour in both groups, larger reshaping of the cerebellum was only shown in the OCD group (Figure 2). Symptom severity was correlated in the brainstem for both patient groups, with more medial reshaping in the OCD but anterior and lateral reshaping in the ASD group (Figure 3).

DISCUSSION

In this study, we demonstrate differences from controls in brainstem and cerebellum shape in both disorders (Figure 1). Brainstem differences were further correlated with compulsive behaviour and symptom severity in both groups, but in the cerebellum only in the OCD group (Figures 2, 3).

Our results parallel previous findings of cerebellar volume increase in OCD (Eng et al., 2015) and decrease in posterior cerebellar volume in ASD (Stoodley, 2015). In addition, the mainly posterior cerebellar and anterior lateral brainstem correlated with compulsive behaviour and symptom severity in our OCD cohort could reflect the positive symptom-volume correlation previously shown for both regions (Zarei et al., 2011), and posterior cerebellar thinning the negative symptom-volume correlation in ASD (Pierce & Courchesne, 2001). Furthermore, we were able to depict regional differences unrestricted to lobular boundaries and display their vector directions. However, while we were not able to replicate previous findings of brainstem expansion (Jou et al., 2013) in the ASD group, this might be because we studied both male and female ASD participants, while the study by Jou et al. (2013) only investigated male ASD subjects.

Our findings underline the importance of regional cerebellum and brainstem dysfunction in ASD and OCD, highlighting the role of the brainstem in compulsive behaviour across both disorders and the cerebellum in paediatric OCD.

Figure 1

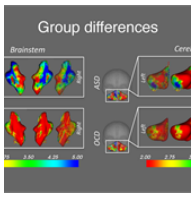


Figure 2

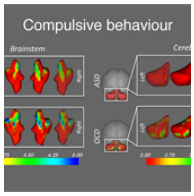
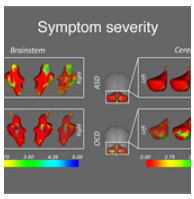


Figure 3



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Appendix C. Questionnaires

I. REPETITIVE BEHAVIOUR SCALE – REVISED

REPETITIVE BEHAVIOR SCALE – Revised (RBS-R)

Name: _____ **ID#**: _____

Gender: female male **Date of Birth**: ___ / ___ / _____ **Today's Date:** ___ / ___ / _____

Informant's Name: _____

Instructions:
Please rate this person's behavior by reading each of the items listed and then choosing the score that best describes how much of a problem the item is for the person. Be sure to read and score all items listed. Make your ratings based on your observations and interactions with the person over the last month. Use the definitions in the box given below to score each item.

0 = behavior does not occur
1 = behavior occurs and is a mild problem
2 = behavior occurs and is a moderate problem
3 = behavior occurs and is a severe problem

When deciding on a score for each item, consider: (a) how frequently the behavior occurs (e.g. weekly versus hourly), (b) how difficult it is to interrupt the behavior (e.g. can be easily redirected versus becomes distressed if interrupted) and (c) how much the behavior interferes with ongoing events (e.g. easy to ignore versus very disruptive).

I. Stereotyped Behavior Subscale

(DEFINITION: apparently purposeless movements or actions that are repeated in a similar manner)

1	WHOLE BODY (Body rocking, Body swaying)	0	1	2	3
2	HEAD (Rolls head, Nods head, Turns head)	0	1	2	3
3	HAND/FINGER (Flaps hands, Wiggles or flicks fingers, Claps hands, Waves or shakes hand or arm)	0	1	2	3
4	LOCOMOTION (Turns in circles, Whirls, Jumps, Bounces)	0	1	2	3
5	OBJECT USAGE (Spins or twirls objects, Twiddles or slaps or throws objects, Lets objects fall out of hands)	0	1	2	3
6	SENSORY (Covers eyes, Looks closely or gazes at hands or objects, Covers ears, Smells or sniffs items, Rubs surfaces)	0	1	2	3

0 = behavior does not occur 1 = behavior occurs and is a mild problem 2 = behavior occurs and is a moderate problem 3 = behavior occurs and is a severe problem

II. Self-Injurious Behavior Subscale

(DEFINITION: movement or actions that have the potential to cause redness, bruising, or other injury to the body, and that are repeated in a similar manner)

7	HITS SELF WITH BODY PART (Hits or slaps head, face, or other body area)	0	1	2	3
8	HITS SELF AGAINST SURFACE OR OBJECT (Hits or bangs head or other body part on table, floor or other surface)	0	1	2	3
9	HITS SELF WITH OBJECT (Hits or bangs head or other body area with objects)	0	1	2	3
10	BITES SELF (Bites hand, wrist, arm, lips or tongue)	0	1	2	3
11	PULLS (Pulls hair or skin)	0	1	2	3
12	RUBS OR SCRATCHES SELF (Rubs or scratches marks on arms, leg, face or torso)	0	1	2	3
13	INSERTS FINGER OR OBJECT (Eye-poking, Ear-poking)	0	1	2	3
14	SKIN PICKING (Picks at skin on face, hands, arms, legs or torso)	0	1	2	3

III. Compulsive Behavior Subscale

(DEFINITION: behavior that is repeated and is performed according to a rule, or involves things being done "just so")

15	ARRANGING / ORDERING (Arranges certain objects in a particular pattern or place; Need for things to be even or symmetrical)	0	1	2	3
16	COMPLETENESS (Must have doors opened or closed; Takes all items out of a container or area)	0	1	2	3
17	WASHING / CLEANING (Excessively cleans certain body parts; Picks at lint or loose threads)	0	1	2	3
18	CHECKING (Repeatedly checks doors, windows, drawers, appliances, clocks, locks, etc.)	0	1	2	3
19	COUNTING (Counts items or objects; Counts to a certain number or in a certain way)	0	1	2	3
20	HOARDING/SAVING (Collects, hoards or hides specific items)	0	1	2	3
21	REPEATING (Need to repeat routine events; In / out door, up / down from chair, clothing on/off)	0	1	2	3
22	TOUCH / TAP (Need to touch, tap, or rub items, surfaces, or people)	0	1	2	3

0 = behavior does not occur 1 = behavior occurs and is a mild problem 2 = behavior occurs and is a moderate problem 3 = behavior occurs and is a severe problem

IV. Ritualistic Behavior Subscale

(DEFINITION: performing activities of daily living in a similar manner)

23	EATING / MEALTIME (Strongly prefers/insists on eating/drinking only certain things; Eats or drinks items in a set order; Insists that meal related items are arranged in a certain way)	0	1	2	3
24	SLEEPING / BEDTIME (Insists on certain pre-bedtime routines; Arranges items in room "just so" prior to bedtime; Insists that certain items be present with him/her during sleep; Insists that another person be present prior to or during sleep)	0	1	2	3
25	SELF-CARE – BATHROOM AND DRESSING (Insists on specific order of activities or tasks related to using the bathroom, to washing, showering, bathing or dressing; Arranges items in a certain way in the bathroom or insists that bathroom items not be moved; Insists on wearing certain clothing items)	0	1	2	3
26	TRAVEL / TRANSPORTATION (Insists on taking certain routes/paths; Must sit in specific location in vehicles; Insists that certain items be present during travel, e.g., toy or material; Insists on seeing or touching certain things or places during travel such as a sign or store)	0	1	2	3
27	PLAY / LEISURE (Insists on certain play activities; Follows a rigid routine during play / leisure; Insists that certain items be present/available during play/leisure; Insists that other persons do certain things during play)	0	1	2	3
28	COMMUNICATION / SOCIAL INTERACTIONS (Repeats same topic(s) during social interactions; Repetitive questioning; Insists on certain topics of conversation; Insists that others say certain things or respond in certain ways during interactions)	0	1	2	3

V. Sameness Behavior Subscale

(DEFINITION: (resistance to change, insisting that things stay the same)

29	Insists that things remain in the same place(s) (e.g. toys, supplies, furniture, pictures, etc.)	0	1	2	3
30	Objects to visiting new places	0	1	2	3
31	Becomes upset if interrupted in what he/she is doing	0	1	2	3
32	Insists on walking in a particular pattern (e.g., straight line)	0	1	2	3
33	Insists on sitting at the same place	0	1	2	3
34	Dislikes changes in appearance or behavior of the people around him/her	0	1	2	3
35	Insists on using a particular door	0	1	2	3
36	Likes the same CD, tape, record or piece of music played continually; Likes same movie / video or part of movie / video	0	1	2	3
37	Resists changing activities; Difficulty with transitions	0	1	2	3
38	Insists on same routine, household, school or work schedule everyday	0	1	2	3
39	Insists that specific things take place at specific times	0	1	2	3

0 = behavior does not occur 1 = behavior occurs and is a mild problem 2 = behavior occurs and is a moderate problem 3 = behavior occurs and is a severe problem

VI. Restricted Behavior Subscale

(DEFINITION: Limited range of focus, interest or activity)

40	Fascination, preoccupation with one subject or activity (e.g., trains, computers, weather, dinosaurs)	0	1	2	3
41	Strongly attached to one specific object	0	1	2	3
42	Preoccupation with part(s) of object rather than the whole object (e.g., buttons on clothes, wheels on toy cars)	0	1	2	3
43	Fascination, preoccupation with movement / things that move (e.g., fans, clocks)	0	1	2	3

Scoring Summary:

1. Number of subscale items endorsed: number of items in a subscale rated 1, 2, or 3
2. Total subscale score: sum of the ratings for all of the items in a subscale
3. Overall number of items endorsed: sum of the "Number of subscale items endorsed"
4. Overall score: sum of the "Total subscale scores"

Subscale	Number of subscale items endorsed	Total subscale score
I. Stereotyped Behavior		
II. Self-injurious Behavior		
III. Compulsive Behavior		
IV. Ritualistic Behavior		
V. Sameness Behavior		
VI. Restricted Behavior		

Overall number of items endorsed	Overall Score

References for RBS-R:
Bodfish, J.W., Symons, F.J., Parker, D.E., & Lewis, M.H. (2000). Varieties of repetitive behavior in autism. Journal of Autism and Developmental Disabilities, 30, 237-243.
Bodfish, J.W., Symons, F.J., Lewis, M.H. (1999). The Repetitive Behavior Scale. Western Carolina Center Research Reports.

CY-BOCS Symptom Checklist

Children's Yale-Brown Obsessive Compulsive Scale

Administering the CY-BOCS Symptom Checklist and CY-BOCS Severity Ratings

1. Establish the diagnosis of obsessive compulsive disorder.
2. Using the CY-BOCS Symptom Checklist (below), ascertain current and past symptoms.
3. Next, administer the 10 item severity ratings (other form) to assess the severity of the OCD during the last week.
4. Re-administer the CY-BOCS Severity Rating Scale to monitor progress.

Patient _____ Date _____

CY-BOCS Symptom Checklist

Children's Yale-Brown Obsessive Compulsive Scale

CY-BOCS Obsessions Checklist

Check all symptoms that apply (Items marked "*" may or may not be OCD Phenomena)

Current	Past	Contamination Obsessions
☐	☐	Concern with dirt, germs, certain illnesses (e.g., AIDS)
☐	☐	Concerns or disgust with bodily waste or secretions (e.g. urine, feces, saliva)
☐	☐	Excessive concern with environmental contaminants (e.g., asbestos, radiation, toxic waste)
☐	☐	Excessive concern with household items (e.g., cleaners, solvents)
☐	☐	Excessive concern about animals / insects
☐	☐	Excessively bothered by sticky substances or residues
☐	☐	Concerned will get ill because of contaminant
☐	☐	Concerned will get others ill by spreading contaminant (aggressive)
☐	☐	No concern with consequences of contamination other than how it might feel *
☐	☐	Other (describe) _____
		Aggressive Obsessions
☐	☐	Fear might harm self
☐	☐	Fear might harm others
☐	☐	Fear harm will come to self
☐	☐	Fear harm will come to others (maybe because of something child did or did not do)
☐	☐	Violent or horrific images
☐	☐	Fear of blurting out obscenities or insults
☐	☐	Fear of doing something embarrassing *
☐	☐	Fear will act on unwanted impulses (e.g., to stab a family member)
☐	☐	Fear will steal things
☐	☐	Fear will be responsible for something else terrible happening (e.g. ,fire, burglary, flood)
☐	☐	Other (describe) _____

Current	Past	Sexual Obsessions
☐	☐	Forbidden or perverse sexual thoughts, images, impulses
☐	☐	Content involves homosexuality
☐	☐	Sexual behavior towards others (aggressive)
☐	☐	Other (describe) _____
		Hoarding / Saving Obsessions
☐	☐	Fear of losing things
☐	☐	Other (describe) _____
		Magical Thoughts / Superstitious Obsessions
☐	☐	Lucky / unlucky numbers, colors, words
☐	☐	Other (describe) _____
		Somatic Obsessions
☐	☐	Excessive concern with illness or disease *
☐	☐	Excessive concern with body part or aspect of appearance (e.g. dysmorphophobia) *
☐	☐	Other (describe) _____
		Religious Obsessions
☐	☐	Excessive concern or fear of offending religious objects
☐	☐	Excessive concern with right / wrong morally
☐	☐	Other (describe) _____
		Miscellaneous Obsessions
☐	☐	The need to know or remember
☐	☐	Fear of saying certain things
☐	☐	Fear of not saying just the right thing
☐	☐	Intrusive (non-violent) images
☐	☐	Intrusive sounds, words, music or numbers
☐	☐	Other (describe) _____

Target Symptom List for Obsessions

OBSESSIONS (describe, listing by order of severity, with #1 being the most sever, #2 second most severe, etc):

1. _____
2. _____
3. _____
4. _____

CY-BOCS Symptom Checklist

Children's Yale-Brown Obsessive Compulsive Scale

CY-BOCS Compulsions Checklist

Check all symptoms that apply (Items marked "*" may or may not be OCD Phenomena)

Current	Past	Washing / Cleaning Compulsions
☐	☐	Excessive or ritualized hand washing
☐	☐	Excessive or ritualized showering, bathing, tooth brushing, grooming, toilet routine
☐	☐	Excessive cleaning of items, such as personal clothes or important objects
☐	☐	Other measures to prevent or remove contact with contaminants
☐	☐	Other (describe) _____
		Checking Compulsions
☐	☐	Checking locks, toys, school books / items, etc.
☐	☐	Checking associated with getting washed, dressed, or undressed
☐	☐	Checking that did not / will not harm others
☐	☐	Checking that did not / will not harm self
☐	☐	Checking that nothing terrible did / will happen
☐	☐	Checking that did not make mistake
☐	☐	Checking tied to somatic obsessions
☐	☐	Other (describe) _____
		Repeating Rituals
☐	☐	Rereading, erasing, or rewriting
☐	☐	Need to repeat activities (e.g. in / out of doorway, up / down from chair)
☐	☐	Other (describe) _____
		Counting Compulsions
☐	☐	Objects, certain numbers, words, etc.
☐	☐	Other (describe) _____
		Ordering / Arranging
☐	☐	Need for symmetry / evening up (e.g. lining items up a certain way or arranging personal items in specific patterns)
☐	☐	Other (describe) _____

Current	Past	Hoarding / Saving Compulsions
		Distinguish from hobbies and concern with objects of monetary or sentimental value.
☐	☐	Difficulty throwing things away, saving bits of paper, string, etc.
☐	☐	Other (describe) _____
		Excessive Games / Superstitious Behaviors
☐	☐	Distinguish from age appropriate magical games (e.g. array of behavior, such as sleeping over certain spots on a floor, touching an object / self certain number of times as a routine game to avoid something bad from happening
☐	☐	Other (describe) _____
		Rituals Involving Other Persons
☐	☐	The need to involve another person (usually a parent) in ritual (e.g. asking a parent to repeatedly answer the same question, making mother perform certain mealtime rituals involving specific utensils) *
☐	☐	Other (describe) _____
		Miscellaneous Compulsions
☐	☐	Mental rituals other than checking / counting
☐	☐	Need to tell, ask or confess
☐	☐	Measures (not checking) to prevent :
☐	☐	harm to self
☐	☐	harm to others
☐	☐	terrible consequences
☐	☐	Ritualized eating behaviors *
☐	☐	Excessive list making *
☐	☐	Need to touch, tap, rub *
☐	☐	Need to do things (e.g. touch or arrange until it feels just right) *
☐	☐	Rituals involving blinking or staring *
☐	☐	Trichotillomania (hair-pulling)
☐	☐	Other self-damaging or self-mutilating behaviors *
☐	☐	Other (describe) _____

CY-BOCS Symptom Checklist

Children's Yale-Brown Obsessive Compulsive Scale

Target Symptom List for Compulsions

COMPULSIONS (describe, listing by order of severity, with #1 being the most sever, #2 second most severe, etc):

1. _____
2. _____
3. _____
4. _____

CY-BOCS Severity Ratings

Children's Yale-Brown Obsessive Compulsive Scale

Administering the CY-BOCS Symptom Checklist and CY-BOCS Severity Ratings

1. Establish the diagnosis of obsessive compulsive disorder.
2. Using the CY-BOCS Symptom Checklist (other form), ascertain current and past symptoms.
3. Next, administer the 10-item severity ratings (below) to assess the severity of the OCD during the last week.
4. Readminister the CY-BOCS Severity Rating Scale to monitor progress.

Patient_____

Date 1st Report_____ Date This Report _____

Obsession Rating Scale (circle appropriate score)

Note: Scores should reflect the composite effect of all the patient's obsessive compulsive symptoms.
Rate the average occurrence of each item during the prior week up to and including the time of interview.

QUESTIONS ON OBSESSIONS (ITEMS 1-5) *"I AM NOW GOING TO ASK YOU QUESTIONS ABOUT THE THOUGHTS YOU CANNOT STOP THINKING ABOUT."*
(Review for the informant(s) the Target Symptoms and refer to them while asking questions 1-5).

1. Time Occupied by Obsessive Thoughts
[Be sure to exclude ruminations and preoccupations which, unlike obsessions, are ego-syntonic and rational (but exaggerated)]

	None	Mild	Moderate	Severe	Extreme
		less than 1 hr/day or occasional intrusion	1 to 3 hrs/day or frequent intrusion	greater than 3 and up to 8 hrs/day or very frequent intrusion	greater than 8 hrs/day or near constant intrusion
Score	0	1	2	3	4

2. Interference Due to Obsessive Thoughts
- How much do these thoughts get in the way of school or doing things with friends?
- Is there anything that you don't do because of them? (If currently not in school, determine how much performance would be affected if patient were in school)

	None	Mild	Moderate	Severe	Extreme
		slight interference with social or school activities, but overall performance not impaired	definite interference with social or school performance, but still manageable	causes substantial impairment in social or school performance	incapacitating
Score	0	1	2	3	4

3. Distress Associated with Obsessive Thoughts

	None	Mild	Moderate	Severe	Extreme
		infrequent, and not too disturbing	frequent, and disturbing, but still manageable	very frequent, and very disturbing	near constant, and disabling distress/frustration
Score	0	1	2	3	4

4. Resistance Against Obsessions
- How hard do you try to stop the thoughts or ignore them? (Only rate effort made to resist, not success or failure in actually controlling the obsessions. If the obsessions are minimal, the patient may not feel the need to resist them. In such cases, a rating of "0" should be given.)

	None	Mild	Moderate	Severe	Extreme
	makes an effort to always resist, or symptoms so minimal doesn't need to actively resist	tries to resist most of the time	makes some effort to resist	yields to all obsessions without attempting to control them, but does so with some reluctance	completely and willingly yields to all obsessions
Score	0	1	2	3	4

5. Degree of Control Over Obsessive Thoughts

	Complete Control	Much Control	Moderate Control	Little Control	No Control
		usually able to stop or divert obsessions with some effort and concentration	sometimes able to stop or divert obsessions	rarely successful in stopping obsessions, can only divert attention with difficulty	experienced as completely involuntary, rarely able to even momentarily divert thinking
Score	0	1	2	3	4

Obsession subtotal (add items 1-5) _____

QUESTIONS ON COMPULSIONS (ITEMS 6-10) *"I AM NOW GOING TO ASK YOU QUESTIONS ABOUT THE HABITS YOU CAN'T STOP"*
(Review for the informant(s) the Target Symptoms and refer to them while asking questions 6-10)

6. Time Spent Performing Compulsive Behaviors

	None	Mild less than 1 hr/day	Moderate 1 to 3 hrs/day	Severe greater than 3 & up to 8 hrs/day	Extreme greater than 8 hrs/day
Score	0	1	2	3	4

7. Interference Due to Compulsive Behaviors
- How much do these habits get in the way of school or doing things with friends?
- Is there anything you don't do because of them? (If currently not in school, determine how much performance would be affected if patient were in school.)

	None	Mild slight interference with social or school activities, but overall performance not impaired	Moderate definite interference with social or school performance, but still manageable	Severe causes substantial impairment in social or school performance	Extreme incapacitating
Score	0	1	2	3	4

8. Distress Associated with Compulsive Behavior
- How would you feel if prevented from carrying out your habits? How upset would you become?

	None	Mild only slightly anxious if compulsions prevented	Moderate anxiety would mount but remain manageable if compulsions prevented	Severe prominent and very disturbing increase in anxiety if compulsions interrupted	Extreme incapacitating anxiety from any intervention aimed at modifying activity
Score	0	1	2	3	4

9. Resistance Against Compulsions
- How much do you try to fight the habits? (Only rate effort made to resist, not success or failure in actually controlling the compulsions.)

	None makes an effort to always resist, or symptoms so minimal doesn't need to actively resist	Mild tries to resist most of the time	Moderate makes some effort to resist	Severe yields to all obsessions without attempting to control them, but does so with some reluctance	Extreme completely and willingly yields to all obsessions
Score	0	1	2	3	4

10. Degree of Control Over Compulsive Thoughts
- How strong is the feeling that you have to carry out the habit(s)?
- When you try to fight them, what happens?

	Complete Control	Much Control experiences pressure to perform the behavior, but usually able to exercise voluntary control over it	Moderate Control moderate control, strong pressure to perform behavior, can control it only with difficulty	Little Control little control, very strong drive to perform behavior, must be carried to completion, can only delay with difficulty	No Control no control, drive to perform behavior experienced as completely involuntary and overpowering, rarely able to delay activity [even momentarily]
Score	0	1	2	3	4

Compulsion subtotal (add items 6-10) _____

CY-BOCS total (add items 1-10) ☐

Total CY-BOCS score: range of severity for patients who have both obsessions and compulsions

0-7 Subclinical
8-15 Mild
16-23 Moderate
24-31 Severe
32-40 Extreme

Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) utilized with permission from Wayne K. Goodman, MD © 1986.

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WPS Edition

Interview Protocol

ADI-R Autism Diagnostic Interview-Revised

Ann Le Couteur, M.B.B.S., Catherine Lord, Ph.D., Michael Rutter, M.D., F.R.S.

SUBJECT

Name/ID Number: _____

Date of Birth: _____ Chronological Age: _____ Gender: ☐ Male ☐ Female

INFORMANT

Name: _____

Relation to Subject: _____

INTERVIEWER

Name: _____

School/Clinic: _____ Date of Interview: _____

This Interview Protocol should be used in conjunction with the *WPS Edition ADI-R Manual* (WPS No. W-382B)

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BACKGROUND

FAMILY MEMBERS/FAMILY MEDICAL AND SOCIAL HISTORY

NOTE THROUGHOUT ANY DISCREPANCIES BETWEEN INFORMANT'S DESCRIPTION AND OBSERVER'S KNOWLEDGE FROM OTHER SOURCES, AND SUMMARIZE AT END OF INTERVIEW. ASK QUESTIONS AS APPROPRIATE FOR RELATIONSHIP BETWEEN INFORMANT AND SUBJECT.

To begin, perhaps you could give me an idea of who's who in [subject]**'s family.**

Does s/he have any brothers or sisters? Could you tell me their names and ages? Do all of them have the same birth parents? Are any of them adopted or fostered? *(If either parent previously married)* **Are any from a previous marriage? Does anyone else live in** [subject]**'s home?**

Have any of the brothers or sisters been delayed in their development? or had any special problems in development for which treatment was sought?

Did either of [subject]**'s parents have any developmental difficulties (or late walking or talking)? or special problems in development for which treatment was given? Is there anyone in** [subject]**'s extended family who has difficulties similar to those of** [subject]**?**

Names of Siblings	Date of Birth	Age	Sex	Relationship to Subject (biological, foster, adoptive, half sibling)	Developmental Difficulties
1.					
2.					
3.					
4.					
5.					
6.					

Biological mother: _____

Biological father: _____

Caregiving mother (if not birth mother): _____

Caregiving father (if not biological father): _____

SUBJECT'S EDUCATION (SCHOOL AND PRESCHOOL)

THE PURPOSE OF THESE QUESTIONS IS TO PROVIDE A FRAME OF REFERENCE FOR THE ITEMS THAT FOLLOW.

Now I'd like to ask about what sort of programs, playgroups, and schools [subject] **has attended.** Was this a regular playgroup or school? How long did s/he attend? Did s/he need any special help/remedial help? Did s/he have any special problems with reading or spelling? (GO THROUGH SCHOOLS AS APPROPRIATE FOR AGE AND OBTAIN DETAILS OF ATTAINMENTS IF APPROPRIATE.) What has s/he done since leaving school?

School	Type	Dates attended	Additional help required
1.			
2.			
3.			
4.			
5.			

PERSONALIZED TIMING

Later on in the interview, I am going to need to ask you to focus particularly on the 12-month period between [subject's] **fourth and fifth birthdays. What is going to be the easiest way for you to think about that age period?** You've just mentioned that [subject] was attending [school] at that time; is that right? Where were you living then? (GO THROUGH OTHER POSSIBLE PERSONALIZED TIMINGS, SUCH AS THE BIRTH OF SIBLINGS, MOVES OF HOUSE, EITHER PARENT STARTING OR FINISHING JOBS, FAMILY DEATHS, ETC.)

DIAGNOSIS *(no coding needed here)*

Did anyone ever say that [subject] **had a medical problem or give you a medical diagnosis for her/him? What about hearing?** (GET DETAILS AND WRITE BELOW.)

MEDICATION *(no coding needed here)*

Does [subject] **take any regular pills or medicines now?** (GET DETAILS AND WRITE BELOW.)

INTRODUCTORY QUESTIONS

THE PURPOSE OF THESE FIRST QUESTIONS IS TO PROVIDE A FRAME OF REFERENCE FOR THE ITEMS BELOW.

I'd like to start off by just getting a general picture of [subject]**. Let me just briefly ask you some questions and then we can then come back to some things in more detail once I have some sense of what** [subject] **is like. Can you tell me a little about** [subject]**?** How would you describe [subject's] behavior to me if I had to pick her/him out in a group of other children/young people the same age? What kinds of things does s/he do when left to her/his own devices? When is s/he at her/his best? What about the most difficult? **What is** [subject]'s **language like?**

1. CURRENT CONCERNS *(no coding needed here)*

Do you have any concerns about [subject]**'s behavior or development now? What are they?**
(OBTAIN DESCRIPTION AND NOTE BELOW.)

EARLY DEVELOPMENT

Can we now go back to talk about [subject]**'s early years?**

Onset of Symptoms

2. AGE (IN MONTHS) WHEN PARENTS FIRST NOTICED THAT SOMETHING WAS NOT QUITE RIGHT IN LANGUAGE, RELATIONSHIPS, OR BEHAVIOR

First, I'd like to talk about [subject]**'s early development.**

How old was [subject] **when you first wondered if there might be something not quite right with her/his development?**

Code age in months, or choose one of the below codes. Try to code an actual age rather than 996, etc. Note: If parents express age in weeks, code to nearest month. If an age range is given (e.g., 3-4 months), take **midpoint** and round up to nearest month. As far as possible, try to code an actual age rather than 996, etc.

991 = parents not concerned, although child was referred by professional

992 = parents have been worried since birth (e.g., if baby premature or very ill at birth)

996 = can't recall, but before 3 years

997 = can't recall, but 3 years or later

998 = N/A

999= N/K or not asked

3. FIRST SYMPTOMS TO AROUSE PARENTAL CONCERN *(no coding needed here)*

What was it that gave you concern at that time?
(ELICIT DETAILS OF SYMPTOMS FIRST CAUSING PARENTAL CONCERN AND NOTE BELOW.)

4. ONSET AS PERCEIVED WITH HINDSIGHT

THE PURPOSE OF THIS ITEM IS TO RECORD THE EARLIEST POINT IN THE CHILD'S DEVELOPMENT THAT ANYTHING UNUSUAL MAY HAVE OCCURRED, ACCORDING TO THE INFORMANT'S BEST JUDGMENT WITH HINDSIGHT.

Looking back with hindsight, when do you think s/he first showed any problems or difficulties in development or behavior?

Do you think that *everything* was alright before then?

Code informant's judgment. ☐

0 = problems present in first 12 months

1 = problems not present before first birthday, but were noted before second birthday

2 = problems not present before second birthday, but were noted at or before third birthday

3 = problems not present before third birthday, but were noted at or before fourth birthday

4 = problems not present before fourth birthday, but were noted at or before fifth birthday

5 = problems not present before fifth birthday, but were noted at or before sixth birthday

6 = problems not present before sixth birthday, but were noted at a later date

(Specify:_____)

7 = child always "different," but differences were not perceived by parents as any kind of abnormality

8 = no problems were noted by parents

9 = N/K or not asked

Motor Milestones

5. FIRST WALKED UNAIDED

What about walking?

At what age did [subject] **walk without holding on?**

(IF NOT WALKING BY 18 MONTHS – OR IF APPARENTLY DELAYED OR DEVIANT – ASK ABOUT OTHER MOTOR MILESTONES, SUCH AS AGE WHEN FIRST SAT UNAIDED ON A FLAT SURFACE. WRITE DETAILS BELOW.)

Note: Remember to take midpoint and round up to the nearest month. As far as possible, try to code actual age rather than using 996, etc.

(Code in months, normal < 18 months.)

995 = still not reached

996 = N/K, but apparently normal

997 = N/K, but apparently delayed

998 = N/A

999 = N/K or not asked

Toilet Training

PROBE FOR THE POSSIBILITY THAT EACH SET OF HABITS MAY HAVE BEEN SUBSEQUENTLY LOST AND RELEARNED. NOTE AGES FOR BOTH, BUT CODE RELEARNING ONLY. DO NOT CODE ISOLATED ACCIDENTS WITH AN UNDERSTANDABLE EXPLANATION, (E.G., SUBJECT UNWELL, HAD A HIGH TEMPERATURE, RESPONDING TO A CHANGE OF ENVIRONMENT, OR IN ACUTE DISTRESS.)

How has toilet training gone?

6. ACQUISITION OF BLADDER CONTROL: DAYTIME

Is [subject] **dry during the day?**

How old was s/he when this was first achieved?

When was s/he first dry for 12 months without accidents?

Code the age (in months) of last daytime bladder accident **before** clear 12-month period; Exclude soiling accidents. Code months when milestone first reached or choose one of the below codes.

993	= successfully toilet trained for a period of 12 months, but has relapsed and now regularly wets
994	= never achieved continence
995	= still not reached, i.e., continent for period of less than 12 months
996	= N/K, but apparently normal
997	= N/K, but apparently delayed
998	= N/A
999	= N/K or not asked

7. ACQUISITION OF BLADDER CONTROL: NIGHTTIME

Is [subject] **dry at night?**

How old was s/he when s/he first remained dry at night?

When was s/he first dry for 12 months without an accident?

Code the age (in months) of last nighttime bladder accident **before** clear 12-month period; or choose one of the below codes. Exclude soiling accidents. Code months when milestone first reached.

993	= successfully toilet trained for a period of 12 months, but has relapsed and now regularly wets
994	= never achieved continence
995	= still not reached, i.e., continent for period of less than 12 months
996	= N/K, but apparently normal
997	= N/K, but apparently delayed
998	= N/A
999	= N/K or not asked

8. ACQUISITION OF BOWEL CONTROL

Does [subject] **soil himself/herself at all (with her/his bowel movements)?**

How old was s/he when s/he first got full control of her/his bowels?

When was s/he first continent for 12 months without an accident?

Code the age (in months) when continence achieved (i.e., after clear 12-month period following last bowel accident); or choose one of the below codes. Code accidents involving soiling or both wetting and soiling.

993	= achieved bowel control for a period of 12 months, but has relapsed and now regularly soils
994	= never achieved continence
995	= still not reached, i.e., continent for period of less than 12 months
996	= N/K, but apparently normal
997	= N/K, but apparently delayed
998	= N/A
999	= N/K or not asked

ACQUISITION AND LOSS OF LANGUAGE/OTHER SKILLS

Now I'd like to talk about [subject]**'s language development and the kinds of things children do before they learn to talk.**

What is [subject]**'s language like now? Has s/he learned to talk yet?**

(ADAPT INITIAL PROBES TO WHAT IS ALREADY KNOWN ABOUT SUBJECT'S LEVEL OF LANGUAGE, AND OBTAIN DESCRIPTIONS TO AID THE WORDING OF LATER QUESTIONS.)

9. AGE OF FIRST SINGLE WORDS

"MEANINGFULLY"' REFERS TO WORDS USED REPEATEDLY AND CONSISTENTLY FOR THE PURPOSE OF COMMUNICATION WITH REFERENCE TO A PARTICULAR CONCEPT, OBJECT, OR EVENT. DO NOT CODE "MOMMY" AND "DADDY"; INCLUDE ANY OTHER **SPONTANEOUS**, PHONOLOGICALLY CONSISTENT SOUNDS THAT APPROXIMATE REAL WORDS IN LANGUAGE OF FAMILY AND ARE USED REPEATEDLY WITH MEANING.

How old was s/he when s/he first used words meaningfully, apart from "mama" and "dada"?

What were her/his first words?

How did s/he show that s/he knew their meaning? (GET EXAMPLES.)

Did [subject] ever use these words to refer to anything else or as sounds that didn't seem to have any specific meaning?

Code age (in months) first spoke single words; or choose one of the below codes (normal < 24 months). ☐☐☐

993 = had some words, then lost and not yet regained

994 = milestone not reached

996 = N/K, but apparently normal

997 = N/K, but apparently delayed

999 = N/K or not asked

10. AGE OF FIRST PHRASES (IF EVER USED)

FOR THE PURPOSES OF THIS CODE, A PHRASE MUST CONSIST OF TWO WORDS, ONE OF WHICH MUST BE A VERB. DO NOT CODE ATTRIBUTE-NOUN COMBINATIONS OR ECHOLALIC SPEECH OR PHRASES THAT MIGHT HAVE BEEN LEARNED AS A SINGLE WORD TO CONVEY A SINGLE MEANING (E.G., "SEE YOU" [MEANING GOOD-BYE]). NOTE THAT THIS DEFINITION DIFFERS FROM WHAT IS REGARDED AS VERBAL FOR ITEM 30, "OVERALL LEVEL OF LANGUAGE."

How old was s/he when s/he first said something that involved putting words together meaningfully (i.e., using two- or three-word phrases)?

What did s/he say?

What about phrases including a verb? (GET EXAMPLES.)

Code age (in months) first used phrases; or choose one of the below codes (normal < 33 months). | | |

993 = had some phrases, then lost; not yet regained

994 = milestone not reached

996 = N/K, but apparently normal

997 = N/K, but apparently delayed

999 = N/K or not asked

Loss of Language Skills (Items 11–19)

THIS ITEM IS TO DETERMINE WHETHER, ONCE THE SUBJECT HAS DEVELOPED COMMUNICATIVE LANGUAGE, THERE WAS A DEFINITE PERIOD OF LOSS OF SKILLS THAT LASTED AT LEAST 3 MONTHS. USE THE FOLLOWING DEFINITIONS:

LANGUAGE BEFORE LOSS: COMMUNICATIVE USE OF AT LEAST FIVE DIFFERENT WORDS (OTHER THAN "MAMA" AND "DADA") ON A DAILY BASIS FOR AT LEAST 3 MONTHS.

LANGUAGE LOSS: LOSS FOR AT LEAST 3 MONTHS OF A LANGUAGE SKILL PREVIOUSLY ESTABLISHED, AS SPECIFIED ABOVE.

Were you ever concerned that [subject] **might have lost language skills during the first years of her/his life?**

Was there ever a time that s/he stopped speaking for some months after having learned to talk?

(IF YES) How much language did s/he have before stopping? Was s/he using at least five different words (other than "mama" or "dada") on a daily basis for as long as 3 months?

11. LOSS OF LANGUAGE SKILLS AFTER ACQUISITION

0 = No
1 = Yes

EVER ☐

If answer is yes, proceed to next question (Item 12).
If answer is no, proceed to item 20 (Page 21).

12. LEVEL OF COMMUNICATIVE LANGUAGE BEFORE LOSS

How much language did [subject] **have before losing it?**

What was s/he able to say before the change occurred?

(PROBE FOR NUMBER OF MEANINGFUL WORDS, EXTENT OF SPONTANEOUS USAGE, AND LEVEL OF COMMUNICATIVE USE.)

EVER ☐

0 = daily, spontaneous, and meaningful speech used communicatively, with at least five different words used at some point before change (and any of the other skills listed below)

1 = occasional and/or fewer than five words used spontaneously and communicatively (alone or in combination with imitative abilities)

2 = produced speech or sounds upon request (may or may not have also spontaneously imitated)

3 = spontaneous imitations of vocalization (without ever having any completely spontaneous speech), with no elicited imitation or spontaneous communicative speech

8 = no change or loss

9 = N/K or not asked

Type of Language Skills Lost (Items 13–16)

FOR THE FOLLOWING ITEMS, "LOSS" REFERS TO ABILITIES THAT THE SUBJECT HAD AND THEN LOST FOR AT LEAST 3 MONTHS

What aspects of language did [subject] **lose?**
Did s/he stop using meaningful words spontaneously?
Did s/he stop using words to communicate with other people?
What about use of grammar?
Was her/his pronunciation of words affected?

0 = no definite loss
1 = probable loss of specified skill
2 = definite loss of specified skill
8 = insufficient language to show change specified
9 = N/K or not asked

13. LOSS OF SPONTANEOUS USE OF AT LEAST FIVE MEANINGFUL WORDS — EVER ☐

14. LOSS OF COMMUNICATIVE INTENT — EVER ☐

15. LOSS OF SYNTACTICAL SKILLS (GRAMMAR) — EVER ☐

16. LOSS OF ARTICULATION (PRONUNCIATION) — EVER ☐

17. AGE WHEN MAIN LOSS OF LANGUAGE SKILLS FIRST APPARENT

How old was [subject] **when s/he first began to lose her/his language?**

Code age in months, or choose one of the following:

998 = no loss

999 = N/K or not asked

☐☐☐

18. ASSOCIATION OF LOSS OF LANGUAGE WITH PHYSICAL ILLNESS

Did [subject] **have any serious physical illness at the time that s/he began to lose language?**
(IF YES, OBTAIN DETAILS.)

0 = loss of skills, but no possibly relevant association with a definite physical illness

1 = loss associated with a definite illness (e.g., high fever with ear infection), but no clear evidence of meningeal or encephalitic involvement

2 = loss associated with impaired consciousness/epileptic attacks or other definite evidence of meningeal or encephalitic involvement

8 = no loss of skills

9 = N/K or not asked

EVER ☐

19. DURATION OF LOSS OF LANGUAGE SKILLS

How long was it before language began to come back?

How long was it before s/he reached the level s/he had been at before the loss took place?

Code in months the time from start of loss to time when previous level regained; or choose one of the following:

993 = loss still present without recovery of language functions

994 = progressive deterioration continuing

998 = no loss

999 = N/K or not asked

☐☐☐

General Loss of Skills (Items 20–28)

20. LOSS OF SKILLS (FOR AT LEAST 3 MONTHS)

SKILL LOSS: A SKILL THAT HAD BEEN ESTABLISHED AND USED ON A DAILY BASIS FOR AT LEAST 3 MONTHS, AND WAS THEN LOST SUBSTANTIALLY OR COMPLETELY FOR AT LEAST 3 MONTHS. DO **NOT** INCLUDE: A LOSS OF BLADDER OR BOWEL CONTROL IF NOT PART OF A MORE GENERAL LOSS OF SKILLS; OR VARIATIONS IN USE OF SKILLS AT TIMES OF WORSENING BEHAVIOR IF SUBJECT CLEARLY RECOVERS (I.E., IF "LOSS" IS PART OF A MORE GENERAL PATTERN OF UPS AND DOWNS). LOSS MUST BE CONSISTENT OVER A PERIOD OF AT LEAST 3 MONTHS.

We've just talked about possible loss of language skills. I'd like to go back now to ask about possible losses in other skills. Has there ever been a period when [subject] **seemed to get markedly worse or dropped further behind in her/his development?**

When was this?

What skills did [subject] **lose?**

(IF LOSS OF LANGUAGE SKILLS) **Did** [subject] **lose these other skills at the same time as losing language?**

EVER

0 = no consistent loss of skills (although behavior may vary at times) ☐

1 = probable loss of skill, but of a degree that falls short of specified criteria

2 = account of definite loss of skills over a period of time

9 = N/K or not asked

If loss, proceed to next question (Item 21).

If no loss of skills, code "0," "8," or "998" for Items 20 to 28 and then skip to Item 29 (Page 24).

Type of Skills Lost (Items 21–25)

What skills did [subject] **lose?**
Did it affect her/his ability to look after her/himself?
Did it affect her/his play?
What about coordination? walking? ability to grip or hold objects?
What about using the bathroom? school-type skills?
(IF LOSS OF LANGUAGE SKILLS) Did [subject] lose these other skills at the same time as losing language?

Code "0" if none, "1" if possible loss, "2" if definite loss

21. PURPOSIVE HAND MOVEMENTS
(ABILITY TO GRIP/HOLD OBJECTS) — EVER ☐

22. MOTOR SKILLS
(POSTURE, GAIT, COORDINATION) — EVER ☐

23. SELF-HELP SKILLS
(FEEDING, DRESS, USING THE BATHROOM ETC.) — EVER ☐

24. CONSTRUCTIVE OR IMAGINATIVE PLAY
(PUZZLES, GAMES, MAKE-BELIEVE ETC.) — EVER ☐

25. SOCIAL ENGAGEMENT AND RESPONSIVENESS
(SOCIAL RELATEDNESS, INTEREST, AND INVOLVEMENT) — EVER ☐

26. AGE WHEN MAIN LOSS OF SKILL FIRST APPARENT

How old was s/he when the loss of this skill first became apparent?

Code age in months, or choose one of the following:

998 = no loss at either "1" or "2" level of skills

999 = N/K or not asked

☐☐☐

27. ASSOCIATION OF LOSS OF SKILLS WITH PHYSICAL ILLNESS

Did [subject] **have any serious physical illness at the time this change occurred?** (IF YES, OBTAIN DETAILS.)

0 = loss of skills, but no possibly relevant association with a definite physical illness

1 = loss associated with definite illness (e.g., high fever with ear infection), but no clear evidence of meningeal or encephalitic involvement

2 = loss associated with impaired consciousness/epileptic attacks or other definite evidence of meningeal or encephalitic involvement

8 = no loss of skills

9 = N/K or not asked

EVER ☐

28. DURATION OF LOSS OF SKILLS

How long was it before [subject]**'s skills began to come back?**

How long was it before s/he reached the level s/he had been at before the loss took place?

Code in months the time from start of loss to time previous level regained; or choose one of the following:

993 = loss still present

994 = progressive deterioration continuing

998 = no loss

999 = N/K or not asked

☐☐☐

LANGUAGE AND COMMUNICATION FUNCTIONING

Now let's come back to [subject]**'s language and communication. Let me begin with what her/his understanding of language is like now.**

29. COMPREHENSION OF SIMPLE LANGUAGE

THIS IS A SUMMARY CODE THAT DEALS WITH THE SUBJECT'S UNDERSTANDING OF SPOKEN LANGUAGE WHEN THERE IS AN ABSENCE OF OTHER CUES SUCH AS THOSE THAT MIGHT BE PROVIDED BY GESTURE, POINTING, OR CONTEXT.

How much language do you think [subject] **understands if you don't gesture?**

What about when s/he can't tell from the situation what is going to happen? For example, can you send her/him into another room to get something like her/his shoes or blanket?

What about your purse or a book?

Could you ask her/him to put them somewhere, other than the usual place?

Could s/he deliver a simple message?

Could s/he follow an instruction with an "if" and a "then"?

Does s/he understand if you say "no" without gesturing or raising your voice?

How about "yes" or "okay"?

How about names of favorite foods or toys or people in your family?

Do you think s/he understands 10 words? 50?

What about at age 4 to 5?

0 = in response to a request can usually perform an unexpected action with an unexpected object; or could place an object, other than something to be used by self (such as boots or a toy), in an unexpected location in a different room ("Put the keys on the kitchen table")

1 = in response to a request can usually get an object, other than something for self or something highly contextualized, from another room ("Get the keys from the kitchen table"), but usually cannot carry out a new action on this object or put it in a "new" place

2 = understands many words (more than 50), including "yes" and names of familiar people, toys, or foods, but does not meet criteria for "0" or "1"

3 = understands fewer than 50 words, but some comprehension of "no" and names of a few favorite objects, foods, or people, or words within familiar routines

4 = little or no comprehension of words, even in context

8 = N/A (e.g., deaf)

9 = N/K or not asked

Current ☐

Most abnormal 4.0–5.0 ☐

30. OVERALL LEVEL OF LANGUAGE

THIS IS A SUMMARY CODE CONCERNING WHETHER SUBJECT USES AT LEAST THREE WORD PHRASES; THIS INCLUDES SPONTANEOUS SPEECH OR ECHOED OR STEREOTYPED SPEECH IF THEY ARE USED FUNCTIONALLY.

How much speech does [subject] **have now?**

Does s/he have phrases with at least three words that s/he uses every day?

Do they ever include verbs?

Do other people understand? If not, does s/he have single words that are used on a daily basis? How many?

CURRENT ☐

0 = functional use of spontaneous, echoed, or stereotyped language that, on a daily basis, involves phrases of three words or more that at least sometimes include a verb and are comprehensible to other people

1 = no functional use of three-word phrases in spontaneous, echoed, or stereotyped speech, but uses speech on a daily basis with at least five different words in the last month

2 = fewer than five words total or speech not used on a daily basis

If code "1" or "2," and subject never had speech at level "0" (or had lost it by age 4 to 5 years), ask next question (Item 31), then skip to Item 42 (Page 37). Code "8" for all categories in Items 32 to 41 (Pages 27 to 36).

If code "1" or "2," but did have speech at level "0" at age 4 to 5 years, do not skip Items 32 to 41. Code these "8" for "current" and as appropriate for 4 to 5 years or "ever."

31. USE OF OTHER'S BODY TO COMMUNICATE

THE EMPHASIS IS ON THE ABNORMAL USE OF ANOTHER PERSON AS A KIND OF EXTENSION OF THE SUBJECT'S ARM OR BODY. FOR EXAMPLE, THE USE OF **ANOTHER** PERSON'S HAND TO POINT, TOUCH AN OBJECT, OR PERFORM A TASK SUCH AS TURNING A DOOR KNOB TO OPEN A DOOR, UNSCREWING A BOTTLE TOP, OR MANIPULATING A ZIPPER OR BUTTONS. THIS BEHAVIOR WILL PROBABLY TAKE PLACE WITHOUT ANY PRIOR ATTEMPT TO COMMUNICATE THE NEED OR REQUEST, BY MEANS OF USING OTHER SOUNDS OR GESTURES. THUS, THE PHYSICAL CONTACT IS NOT TO INITIATE A SOCIAL APPROACH, BUT RATHER TO FACILITATE THE COMPLETION OF THE TASK.

Now let me focus on the time when [subject] **had very little speech.**

How did s/he let you know s/he wanted something then?

Did s/he ever show you what s/he wanted by taking your hand or wrist or some other part of your body?

What exactly did s/he do?

What did s/he do when you were brought to the object wanted?

Did s/he ever use your hand as if it were a tool or an extension of her/his own arm (such as pointing with your hand or getting your hand to turn a door knob)?

Did s/he look at you when doing this?

Did s/he combine taking your hand with trying to communicate with sounds or words?

When did s/he do this?

Did s/he try to communicate first by sounds or gesture?

Does s/he ever take your hand or wrist like this now? (GET DETAILS SUFFICIENT FOR CODING.)

0 = no use of other's body to communicate, except in situations where other strategies have not worked (e.g., when parent conversing with someone and subject cannot get her/his attention), or when taking someone's hand to lead them places

1 = occasional placement of other's hand on objects or use of other's hand as a tool or to point, but some combination with other modes of communication

2 = occasional placement of other's hand or use of other's hand as a tool or to demonstrate "for" the subject without integration with other modes of communication

3 = regular use of other's hand as a tool or to gesture "for" the subject

8 = little or no spontaneous communication

9 = N/K or not asked

CURRENT ☐

EVER ☐

Items 32 to 41 apply only to verbal subjects

32. ARTICULATION/PRONUNCIATION

CODE ONLY FOR SUBJECTS AGED 4 YEARS OR OLDER. ARTICULATION REFERS TO THE ENUNCIATION OF THE SOUNDS OF LANGUAGE.

Let me now come back to [subject]**'s talking now.**

What is her/his pronunciation like?

Are there any words or sounds s/he doesn't get quite right? What are they?

Do other people understand her/him easily?

What about people outside the family?

What was her/his articulation like when s/he reached her/his fifth birthday?

What errors did s/he make at age 5? (NOTE EXAMPLES.)

Could a stranger understand her/him at age 5? (GET DETAILS OF DIFFICULTIES WITH ARTICULATION.)

0 = understood by anybody (i.e., clear enunciation of most sounds, but may include a few consonant omissions or substitutions)

1 = understood better by family than others because of difficulty with some sounds, but mostly comprehensible to strangers at first encounter

2 = definite articulation difficulties such that some words are very difficult for strangers to understand until they get to know her/him

3 = strangers find speech almost impossible to understand or parents have significant difficulties understanding because of articulation

8 = N/A; no speech or subject below age 4.0 years

9 = N/K or not asked

CURRENT (4.0 or older) ☐

AT 5.0 YEARS ☐

33. STEREOTYPED UTTERANCES AND DELAYED ECHOLALIA

THESE ARE DEFINED AS THE NONHALLUCINATORY USE OF REPETITIVE SPEECH PATTERNS THAT ARE CLEARLY ODD IN TERMS OF EITHER STEREOTYPED CONTENT, OR THEIR NONSOCIAL USAGE, OR BOTH. THESE INCLUDE PHRASES THAT ARE INTERSPERSED INTO MORE NORMAL SPEECH; SELF-COMMENTARY ON THE PERSON'S OWN ACTIONS; A REPETITIVE RERUN OF EMOTIVE OR UPSETTING INTERCHANGES; OR ROUTINIZED PHRASES USED OUT OF APPROPRIATE CONTEXT. DO NOT INCLUDE THE REPETITIONS THAT OFTEN OCCUR WITH NORMALLY DEVELOPING CHILDREN AS PART OF PLAY WHEN PHRASE SPEECH IS JUST BECOMING WELL ESTABLISHED, OR FOR REASSURANCE.

Has s/he ever tended to use rather odd phrases or say the same thing over and over in almost exactly the same way? That is, either phrases s/he has heard other people use or ones s/he has made up (e.g., "It's bad to bite your wrist"; "Does this look like a traffic light?"; "Say it's alright now").

Does s/he tend to talk to her/himself in this way when doing something on her/his own, or when upset about something that has happened during the day?

Does s/he use the phrase appropriately or not to mean anything in particular or as part of a conversation with her/himself?

Can you give me some examples?

What about when s/he was younger?

Does s/he ever give a running commentary on what s/he is doing?

Did s/he ever use odd phrases more often than s/he does now?

0 = rarely or never uses stereotyped phrases

1 = speech tends/tended to be more repetitive than most subjects at the same level of complexity, but not stereotyped in an odd or unusual way; or occasional stereotyped utterances, but consistent productive language as well

2 = often uses/used stereotyped utterances with productive language as well

3 = phrases are almost exclusively stereotyped utterances

8 = N/A; no phrases (code "1" or "2" on Item 30)

9 = N/K or not asked

CURRENT ☐

EVER ☐

34. SOCIAL VERBALIZATION/CHAT

THE EMPHASIS IN DEFINING SOCIAL VERBALIZATION/CHAT IS ON WHETHER OR NOT SPEECH IS USED JUST TO BE FRIENDLY OR SOCIAL RATHER THAN TO EXPRESS NEEDS OR GIVE SOME INFORMATION. THE FOCUS OF THIS QUESTION IS SOCIAL APPROACH, NOT SOCIAL RECIPROCITY, WHICH IS DEALT WITH IN ITEM 35.

When people talk, sometimes it is to get something or find out about something, but sometimes the purpose seems mainly just to be with someone – sort of "small talk" – such as saying "It never stops raining," "The telephone is ringing again!" or "You are very busy today." Would [subject] **ever talk with you just to participate in some form of language interchange?**

What about when s/he was younger (but using phrases)?

0 = verbalizes or chats with clear social quality of talking to be friendly or to express interest, rather than to make needs known — CURRENT ☐

1 = some **social** use of speech in response to caregiver or to get attention with no other obvious motivation, but limited in frequency or range of contexts — EVER (when verbal) ☐

2 = uses some speech to alert caregiver to immediate needs or wants, but little or no purely social use of verbalization

8 = N/A (code "1" or "2" on Item 30)

9 = N/K or not asked

35. RECIPROCAL CONVERSATION (WITHIN SUBJECT'S LEVEL OF LANGUAGE)

THE EMPHASIS OF THIS ITEM IS ON THE ABILITY TO FACILITATE THE FLOW OF CONVERSATION (I.E., TO BUILD ON THE **OTHER PERSON'S** RESPONSES RATHER THAN THE SUBJECT'S ABILITY TO TALK/CHAT)

Can you have a conversation with [subject]**? That is, if you say something to her/him, without asking a direct question, what will** [subject] **usually do?**

Will s/he say something?

Will s/he ever ask you a question or build on what **you** have said in such a way that s/he adds something new to what you have said, so that the conversation will continue? In other words, will s/he converse to-and-fro on topics that you have introduced?

Can s/he also bring up appropriate topics?

What about when s/he was younger (but using phrases)?

0 = conversation flows, including both offering information and building on other person's response in a manner that leads to ongoing dialogue

1 = occasional reciprocal conversation, but less frequent than normal or limited in flexibility or topics

2 = little or no reciprocal conversation; others find it difficult to **build** a conversation even if there is apparent positive or social talk by subject; subject fails to follow anyone else's conversation topic; may ask or answer questions but not as part of a single interchange

3 = very little spontaneous speech

8 = N/A (code "1" or "2" on Item 30)

9 = N/K or not asked

CURRENT ☐

EVER (when verbal) ☐

36. INAPPROPRIATE QUESTIONS OR STATEMENTS

THE FOCUS IS ON SOCIALLY INAPPROPRIATE UTTERANCES THAT REFLECT A LACK OF UNDERSTANDING OR A DISREGARD OF THE SOCIAL IMPACT OF SUCH COMMENTS. THESE MAY CONSIST OF UTTERANCES THAT ARE INTRINSICALLY ODD (E.G., "HOW TALL WAS MR. BROWN WHEN HE WAS 2?"), OR UTTERANCES THAT ARE INAPPROPRIATE BECAUSE OF THEIR PERSONAL NATURE OR IN RELATION TO THE SITUATION. REPETITION MAY CONTRIBUTE TO THE ODDNESS BUT IT IS NOT SUFFICIENT IN ITSELF.

Are there times when [subject] **uses socially inappropriate questions or statements?**
For example, does s/he regularly ask personal questions or make personal comments at awkward times?
(GET EXAMPLES)

Was this ever a problem in the past?

0 = no or very rare use of questions/statements inappropriate to conversation or setting

1 = use of some questions/statements regardless of situation; questions or statements are slightly inappropriate and may be repetitious, but are not usually very odd or highly embarrassing

2 = frequent use of questions/statements that are odd or obviously inappropriate to the situation

8 = N/A (code "1" or "2" on Item 30)

9 = N/K or not asked

CURRENT ☐

EVER ☐

37. PRONOMINAL REVERSAL

THE EMPHASIS IS ON THE CONSISTENT ABNORMAL CONFUSION OF PRONOUNS BETWEEN FIRST AND SECOND OR THIRD PERSON. IT DOES NOT INCLUDE "I/ME" CONFUSION AS THIS IS OFTEN A SUBCULTURALLY ACCEPTABLE USAGE.

Has [subject] **ever got her/his personal pronouns the wrong way round? For example, has [subject] ever mixed up "you" and "I"?**

What about saying "he" or "she" instead of "I"? For example, saying "*You* want a drink" instead of "*I* want a drink" or "He is hungry" instead of "I am hungry." (NOTE EXAMPLES.)

(IF YES) When using "you" or "s/he" instead of "I," *how* does s/he say it? For example, does her/his statement have the intonation of a question?

What about when [subject] **was younger?**

0 = no confusion between 1st and 2nd or 3rd person after phrase speech with pronoun use established

1 = refers/referred to self by name instead of "I" after phrase speech established, but no persistent "you/s/he-I" confusion

2 = "you/I" or "s/he/I" confusion after phrase speech established, but "you" or "s/he" not used with intonation of a question

3 = pronominal confusion when asking a question in which "you" or "s/he" is used for "I"

7 = other types of pronominal confusion (other than "I/me"), such as "he/you"

8 = N/A (code "1" or "2" on Item 30 or no pronouns used)

9 = N/K or not asked

CURRENT ☐

EVER ☐

38. NEOLOGISMS/IDIOSYNCRATIC LANGUAGE

NEOLOGISMS MUST BE NONWORDS AND OBVIOUSLY PECULIAR (E.G., "PLIN" FOR A FREE-FALLING PIECE OF PAPER OR FABRIC, OR "MASHUDA" FOR TRIANGLES.) IDIOSYNCRATIC LANGUAGE REFERS TO REAL WORDS AND/OR PHRASES USED OR COMBINED BY THE SUBJECT IN A WAY THAT S/HE COULD NOT HAVE HEARD. THESE ARE USED TO CONVEY **SPECIFIC** MEANINGS; THEY *DO NOT* INCLUDE CONVENTIONAL METAPHORS. DIFFERENTIATE UNUSUAL OR TRULY IDIOSYNCRATIC USAGES FROM ORDINARY CHILDISH REFERENCES TO OBJECTS ACCORDING TO THEIR FUNCTION OR AS PART OF A SHARED GAME OR JOKE.

Does s/he ever use words that s/he seems to have invented or made up?

Does [subject] **ever put things in odd, indirect ways, or have idiosyncratic ways of saying things, such as saying "hot rain" for "steam" or referring to her/his grandmother by her age?**

Would s/he ever take this one step further and refer to other women as "55"? Can you give me some examples?

Did s/he ever use these sorts of odd words or phrases in the past?
(GET EXAMPLES AND PROMPT AS NECESSARY.)

0 = no use of neologisms or idiosyncratic language

1 = occasional use of neologisms and/or idiosyncratic words and phrases used consistently over a period of time

2 = regular use of neologisms and/or idiosyncratic ways of saying things, including generalization of unusual term to reference beyond the example that may have fostered the initial idiosyncratic word or phrase

8 = N/A (code "1" or "2" on Item 30)

9 = N/K or not asked

CURRENT ☐

EVER ☐

39. VERBAL RITUALS

WHEN DECIDING WHETHER VERBAL RITUALS ARE PRESENT, FOCUS ON THE DEGREE OF PREDICTABILITY OF THE CONTEXT AND SEQUENCE, AS WELL AS ON THE COMPULSIVE QUALITY OF THE SPEECH. THE EMPHASIS IS ON THE FIXED SEQUENCES OF UTTERANCES THAT ARE SAID AS IF THE SUBJECT FEELS PRESSURE TO COMPLETE THEM IN A PARTICULAR ORDER. THE SUBJECT IS IMPOSING AN ORDER ON WHAT S/HE SAYS AND MAY, IN ADDITION, POSE SIMILAR RESTRICTIONS ON THE VERBAL RESPONSES/UTTERANCES OF OTHERS. EXCLUDE VERBAL RITUALS THAT OCCUR **SOLELY** AS PART OF A BEDTIME ROUTINE.

Does s/he ever say the same thing over and over in exactly the same way or insist on you saying the same things over and over again?

Does s/he ever keep saying the same thing until you reply in a certain way?

What happens if you interrupt her/him or refuse to comply?

Was this ever a problem in the past? (GET DETAILS.)

0 = no verbal rituals

1 = tendency to say things in ritualized way or to require others to do so, but no indication that this is compulsive, and readily stops if asked to do so

2 = subject has to say one or more things in a special way; rituals may intrude on family life, may involve other family members and some distress at interruption, or may cause some disturbance or minor reorganization of family life that can be tolerated by most families

3 = as for "2," but with marked difficulty to control and marked intrusiveness on family life; family members involved to a degree that causes definite social impairment, disruption, or prevention of some family activities; serious distress at any attempted interruption

8 = N/A (code "1" or "2" on Item 30)

9 = N/K or not asked

CURRENT ☐

EVER ☐

40. INTONATION/VOLUME/RHYTHM/RATE

THIS ITEM REFERS TO UNUSUAL QUALITIES OF PROSODY OR THE PARALINGUISTIC SOUND OF THE SUBJECT'S SPEECH AS EVIDENCED IN INTONATION, VOLUME, RHYTHM, OR RATE. DO NOT CODE USE OF COLLOQUIAL PHRASES OR INVECTIVES HERE.

Is there anything unusual about the way [subject] **speaks? That is, is her/his speech of normal volume or is it consistently too loud or too quiet?**

What about the rate and rhythm of her/his speech?

What about the intonation or pitch?

Does s/he ever repeat whole sentences or monologues in exactly the same tone of voice in which s/he first heard them? (GET DETAILS.)

What about in the past?

0 = normal, appropriately varying intonation, reasonable volume, and normal rate of speech, with regular rhythm coordinated with breathing — CURRENT ☐

1 = speech that shows one or other of the abnormalities listed under "2," but not obviously peculiar and no interference with intelligibility

2 = speech that is clearly abnormal in terms of any or all of the following: (a) odd intonation or inappropriate pitch and stress; (b) markedly flat and toneless or mechanical speech; (c) consistently abnormal volume that lacks modulation; (d) inappropriate, poorly modulated rate or rhythm (either unusually slow or halting or unusually rapid or jerky) to a degree that creates **some** interference with intelligibility — EVER ☐

3 = speech that is frequently obviously peculiar or difficult to understand because of abnormalities of type specified under "2"

7 = stutter or stammer; abnormal intonation due to severe hearing impairment; or intonation abnormality due to neurological disorder causing motor dysfunction

8 = N/A (code "1" or "2" on Item 30)

9 = N/K or not asked

41. CURRENT COMMUNICATIVE SPEECH

THIS ITEM REFERS TO SEMANTIC AND GRAMMATICAL COMPLEXITY WITHIN A SENTENCE IN NONECHOED UTTERANCES. THIS IS A SUMMARY CODE TO ASSESS HOW WELL THE SUBJECT USES HER/HIS LANGUAGE TO COMMUNICATE.

How does [subject] **now use the words s/he has?**

(IT IS OFTEN HELPFUL TO ASK PARENTS/INFORMANTS TO RECALL A PARTICULAR CONVERSATION WITH THE SUBJECT, SUCH AS ONE TAKING PLACE ON THE WAY TO THE APPOINTMENT OR DURING A RECENT MEAL.)

In what sort of situation does s/he talk the most?

Does s/he call you by name or use words to direct your attention? (GET EXAMPLES OF COMMUNICATIVE USE OF WORDS.)

Does s/he ever tell you about things that are not present (e.g., about something that happened a while ago or something s/he is looking forward to)?

What about when s/he was 5 years of age?

0 = speech, at whatever level attained, used frequently and communicatively in a variety of contexts, including some reference to events not present (Do not include requests here) — CURRENT ☐

1 = some communicative use of words (i.e., words used regularly to communicate, with or without an abnormal element), but somewhat restricted in frequency or contexts — AT 5.0 YEARS ☐

2 = some spontaneous words and/or echolalic language, but with limited communicative use

3 = little or no communicative language (including exclusively non-communicative echolalia), though subject has some language

8 = N/A (code "1" or "2" on Item 30, or under 5 years of age for 5.0 year coding)

9 = N/K or not asked

Items 42 onwards apply to both verbal and nonverbal subjects.

42. POINTING TO EXPRESS INTEREST

THIS ITEM IS STRICTLY CONCERNED WITH UNPROMPTED POINTING THAT IS USED AS A SPONTANEOUS COMMUNICATION TO EXPRESS INTEREST OR TO SHOW SOMETHING AT A DISTANCE, RATHER THAN AS A MEANS OF OBTAINING SOME OBJECT. POINTING MUST BE SOCIAL AND IT MUST BE INITIATED BY THE SUBJECT. FURTHERMORE, THE POINTING MUST BE AT OBJECTS THAT ARE WITHIN SIGHT BUT THAT ARE SOME DISTANCE AWAY. POINTING AT BOOKS, OR POINTING AS A LEARNED RESPONSE TO QUESTIONS, IS EXPLICITLY EXCLUDED FROM THIS ITEM. ALSO EXCLUDED IS POINTING THAT IS USED AS A MEANS OF OBTAINING SOME OBJECT. IN ORDER FOR THE POINTING TO BE CODED "0" IT MUST INVOLVE COORDINATED EYE GAZE WITH THE OTHER PERSON, AS DESCRIBED BELOW.

Does [subject] **ever spontaneously point at things around her/him?**

With a finger or sort of a hand extended, like reaching?

In what circumstances?

Does s/he ever point at things at a distance, such as out a window at home or in a car or bus?

If I wanted to get you to look at something, I might first look at it, then look at you, then point and look at the object again, then look back at you to see if you understood. Can [subject] do this?

What about when s/he was 4 to 5 years old?

0 = spontaneously points at objects at a distance with finger to express interest, using coordinated eye gaze in order to communicate

1 = makes some limited attempt to express interest by pointing, but with limited flexibility and/or lack of coordination (e.g., uses arm or points with finger, but without consistent coordination with eye gaze)

2 = little or no spontaneous unprompted attempts to point to express interest in objects at a distance

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

43. NODDING

THIS ITEM IS INTENDED TO DETERMINE IF THE SUBJECT CURRENTLY USES OR HAS EVER USED THE CONVENTIONAL GESTURE OF HEAD NODDING TO COMMUNICATE "YES." NODDING SHOULD HAVE OCCURRED IN SEVERAL DIFFERENT SITUATIONS BUT MAY HAVE DECREASED IN FREQUENCY AS THE SUBJECT LEARNED TO SPEAK.

Does [subject] **nod her/his head to mean "yes"?**

What about when s/he was 4 to 5 years old?
(GET DETAILS.)

0 = consistently nods spontaneously
1 = sometimes nods spontaneously
2 = never nods spontaneously
8 = N/A
9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

44. HEAD SHAKING

THIS ITEM IS INTENDED TO DETERMINE IF THE SUBJECT CURRENTLY USES OR HAS EVER USED THE CONVENTIONAL GESTURE OF HEAD SHAKING TO COMMUNICATE "NO." HEAD SHAKING SHOULD HAVE OCCURRED IN SEVERAL DIFFERENT SITUATIONS, BUT MAY HAVE DECREASED IN FREQUENCY AS THE SUBJECT LEARNED TO SPEAK.

Does [subject] **shake her/his head to mean "no"?**

What about when s/he was 4 to 5 years old?
(GET DETAILS.)

0 = consistently shakes head spontaneously
1 = sometimes shakes head spontaneously
2 = never shakes head spontaneously
8 = N/A
9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

45. CONVENTIONAL/INSTRUMENTAL GESTURES

INSTRUMENTAL GESTURES ARE SPONTANEOUS, CULTURALLY APPROPRIATE, DELIBERATE HAND OR ARM MOVEMENTS THAT CONVEY A MESSAGE BY THEIR FORM AS SOCIAL SIGNALS. EXCLUDE PURELY EMOTIONAL SIGNALS (SUCH AS HANDS TO FACE IN EMBARRASSMENT OR SHRINKING WITH FEAR); DEMONSTRATIONS; AND INSTANCES OF TOUCHING OR PULLING SOMEONE TO GAIN THEIR ATTENTION OR SHOW THEM SOMETHING. ALSO EXCLUDE MANNERISMS SUCH AS TOUCHING THE FACE OR SCRATCHING. ALL GESTURES MUST BE/HAVE BEEN USED OVER A PERIOD OF 3 OR MORE MONTHS TO BE CODED. OFTEN IT IS USEFUL IN HELPING INFORMANTS TO REMEMBER GESTURES TO FOCUS ON HOW THE SUBJECT DIRECTED HIS/HER ATTENTION OR USED GESTURE WHEN OTHER MODES OF COMMUNICATION WERE NOT CLEAR OR SUCCESSFUL.

Does [subject] **wave good-bye?**

When does this happen?

Does s/he ever use other common gestures, such as blowing a kiss, clapping for a job well done, putting a finger to her/his lips to mean "be quiet," or shaking a finger for "bad"?

Does s/he ever use gestures, other than pointing or holding arms up to be lifted, to let you know what s/he wants?

Does s/he use gestures when s/he is trying to get you to help her/him or to get your attention (for example, beckoning to someone, or putting out a hand with palm extended to ask that you give her/him something)?

What about when s/he was 4 to 5 years old?

Note: Subjects who have been taught signing and who use instrumental signs only in the teaching context should be coded "2." However, if the taught signs are used spontaneously with some variety and creativity for instrumental purposes, code "0" or "1" as appropriate.

0 = appropriate and spontaneous use of a variety of conventional or instrumental gestures

1 = spontaneous use of conventional or instrumental gestures, but limited in range or contexts

2 = inconsistent spontaneous use, or use of elicited or well-rehearsed simple conventional or instrumental gestures only

3 = no use of conventional or instrumental gestures

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

46. ATTENTION TO VOICE

For subjects aged 5.0 years or older, probe only for the 4.0- to 5.0-year period.

THE FOCUS IS ON WHETHER THE SUBJECT SHOWS AN ORIENTING RESPONSE WHEN SPOKEN TO AND NOT WHETHER S/HE COMPLIES WITH WHAT IS SAID. THE ORIENTING RESPONSE SHOULD CONSIST OF AN AUTOMATIC LOOKING TO THE SOUND, TOGETHER WITH AN APPROPRIATE FACIAL EXPRESSION. THIS SHOULD OCCUR WITHOUT THE NEED FOR EXTRA STEPS SUCH AS CALLING THE SUBJECT'S NAME OR STANDING VERY CLOSE TO HIM/HER.

If you come into a room and start talking to [subject] **without calling her/his name, what does s/he do? I mean when you say something neutral rather than when you're trying to get her/him to do something. Does s/he look up and pay attention to you?**

How does s/he respond? How about to other people?

Do you need to say her/his name or catch her/his eye first or could you just say something that s/he might not even be that interested in, such as "Oh no, it's raining," or "My goodness, what a lot of toys!"

What did [subject] **do when s/he was 4 to 5 years old?** (GET DETAILS.)

0 = usually looks up and pays attention when spoken to in a positive manner in contexts **other than** to do something that s/he may not want to do

1 = does not consistently appear to pay attention (e.g., might look up briefly, but little sustained attention), but sometimes responds to what is said or responds on occasion only to firm, loud voice

2 = usually does not look up or pay attention when spoken to, and does not respond to what is said; **or** responds to her/his name only or when her/his attention is caught very deliberately

3 = rarely responds, although hearing normal

8 = N/A

9 = N/K or not asked

CURRENT (UNDER 5.0) ☐

MOST ABNORMAL 4.0–5.0 ☐

47. SPONTANEOUS IMITATION OF ACTIONS

For subject's aged 10.0 years or older, probe only for the 4.0- to 5.0-year period.

THE EMPHASIS IS ON THE SPONTANEOUS IMITATION OF A VARIED RANGE OF NONTAUGHT, INDIVIDUAL BEHAVIORS, ACTIONS, OR CHARACTERISTICS OF ANOTHER PERSON. EXCLUDE IMITATION OF TV/FILM CHARACTERS.

Does [subject] **imitate you or other people in the family?**

How about when you are *not* trying to get her/him to do so?

Does s/he copy something you have done, but using a "pretend" object (such as mowing the lawn with some toy vehicle)?

Is the imitation only at the time you are doing whatever it is, or does the copying form part of her/his play at *other* times?

How varied are the things s/he imitates? Does the imitation ever involve some *personal* characteristic, such as the way you walk or gesture or the way you hold something? (GET EXAMPLES.)

What about when s/he was 4 to 5 years old?

Do not code elicited or vocal imitation here.

0 = spontaneous imitation of a varied range of non-taught actions, at least some of which are incorporated into play outside the context of the observed behavior of the imitated person

1 = some indication of spontaneous imitation that goes beyond copying a frequent use of an object, but not of sufficient flexibility or number to meet the criteria for "0"

2 = spontaneous imitation limited to a few familiar routines that are not incorporated into play; including frequent appropriate use of an object probably learned through imitation (e.g., mowing lawn with a toy mower)

3 = very rare or no spontaneous imitation

8 = N/A

9 = N/K or not asked

CURRENT (UNDER 10.0) ☐

MOST ABNORMAL 4.0–5.0 ☐

48. IMAGINATIVE PLAY

For subjects aged 10.0 years or older, probe only for the 4.0- to 5.0-year period.

IMAGINATION IS DEFINED AS PRETEND PLAY THAT IMPLIES THE FORMATION OF MENTAL IMAGES OF THINGS NOT PRESENT. THE FOCUS HERE IS ON THE CHILD'S CREATIVE AND VARIED USE OF ACTIONS OR OBJECTS IN PLAY TO REPRESENT HER/HIS OWN IDEAS.

Does [subject] **play any pretend games?**

Does s/he play with toy tea sets or dolls or action figures or cars? (GET EXAMPLES.)

Does s/he drink the tea/push the car/kiss the stuffed animal?

Has s/he ever given the doll a drink or the action figure a ride in the car?

Has s/he ever used the doll/action figure as the initiator–so that the doll pours and serves the tea or the action figure walks to the car and gets in it?

Does s/he ever talk to her/his dolls or animals? Does s/he ever make them talk or make noises?

Does this type of play vary from day to day?

Has s/he ever made up a sort of story or sequence (e.g., with the toy cars racing each other, being parked in a garage, or going to Granny's house)?

What about at age 4 to 5? (GET EXAMPLES.)

0 = variety of pretend play, including use of dolls/animals/toys as self-initiating agents

1 = some pretend play, including actions directed to dolls or cars, etc., but limited in variety or frequency

2 = occasional, spontaneous pretend actions or highly repetitive pretend play (which may be frequent), or only play that has been taught by others

3 = no pretend play

8 = N/A

9 = N/K or not asked

CURRENT (UNDER 10.0) ☐

MOST ABNORMAL 4.0–5.0 ☐

49. IMAGINATIVE PLAY WITH PEERS

For subjects aged 10.0 years or older, probe only for the 4.0- to 5.0-year period.

THE FOCUS HERE IS ON THE SPONTANEOUS, CREATIVE SHARING OF IMAGINATION AMONG CHILDREN, INCORPORATING BOTH THE SUBJECT'S IDEAS AND THOSE OF OTHER CHILDREN. THE LEVEL OF IMAGINATION MAY BE SIMPLE SO LONG AS IT IS SOCIALLY INTERACTIVE, SPONTANEOUS, AND VARIED. IF THE SUBJECT'S ONLY PLAY IS WITH SIBLINGS, BE PARTICULARLY CAREFUL TO DIFFERENTIATE WELL-PRACTISED ROUTINES FROM SPONTANEOUS, FLEXIBLE PLAY. ALSO DIFFERENTIATE PLAY THAT IS HIGHLY STRUCTURED "FOR" THE SUBJECT BY THE SIBLING FROM PLAY IN WHICH S/HE SHOWS SOME INITIATIVE.

Does [subject] **ever play imaginative games with someone else?**

Do they seem to understand what each other is pretending? How can you tell? Can you give me an example?

Does [subject] ever take the lead in this play? Or does s/he mostly follow the other person's ideas?

What about at age 4 to 5?

0 = imaginative, cooperative play with other children in which the subject both takes the lead and follows another child in spontaneous, pretend activities

1 = some participation in pretend play with another child, but not truly reciprocal and/or pretending is very limited in variety

2 = some play with other children, but little or no pretending

3 = no play with other children or no pretend play even on own

8 = N/A

9 = N/K or not asked

CURRENT (OVER 4.0 AND UNDER 10.0) ☐

MOST ABNORMAL 4.0–5.0 ☐

SOCIAL DEVELOPMENT AND PLAY

Thank you. That has given me a clear idea about her/his communication and imaginative play. Now can we talk about how s/he got on with people when s/he was little?

50. DIRECT GAZE

INCLUDES BOTH THE SUBJECT'S USE OF DIRECT EYE GAZE TO COMMUNICATE AND HER/HIS RESPONSE TO OTHERS' ATTEMPTS TO CATCH HER/HIS EYE.

For subjects UNDER 5.0 years:

Does [subject] **look you directly in the face when doing things with you or talking with you?**

Can you catch her/his eye?

Does s/he sometimes watch you as you walk into the room?

Does s/he look back and forth to your face as other children would?

What about with others?

0 = normal reciprocal direct gaze used to communicate across a range of situations and people

1 = definite direct gaze, but only of brief duration or not consistent during social interactions

2 = uncertain/occasional direct gaze, or gaze rarely used during social interactions

3 = unusual or odd use of gaze

8 = N/A

9 = N/K or not asked

CURRENT (UNDER 5.0) ☐

MOST ABNORMAL 4.0–5.0 ☐

For subjects OVER 5.0 years:

When [subject] **was 4 to 5 years of age, did s/he look at you directly in the face when doing things with you or talking with you?**

Could you catch her/his eye?

Did s/he sometimes watch you as you walked into the room?

Would s/he have looked back and forth to your face as other children would?

What about with others?

51. SOCIAL SMILING

DEFINED AS SPONTANEOUS SMILING DIRECTED AT A VARIETY OF PEOPLE, INCLUDING SMILING BACK AT SOMEONE SMILING AT HER/HIM, SMILING DURING AN APPROACH, AND SMILING IN RESPONSE TO WHAT SOMEONE DOES OR SAYS TO HER/HIM.

When [subject] **is approaching someone to get her/him to do something or to talk to her/him, does** [subject] **smile in greeting?**

What about when s/he sees you for the first time when you've been out? Or when meeting someone s/he knows?

If s/he is not smiling first, what does s/he do if someone else smiles at her/him? Or when someone says something nice to her/him?

What about at age 4 to 5 years old?

0 = regularly predictable, reciprocal, social smiles in response to the smiles of a variety of people besides parent/caregiver

1 = some evidence of reciprocal social smiling, but not sufficient to code "0"

2 = some evidence of smiling while looking at people, but generally not reciprocal; code here if smiles only to parent/caregiver, smiles only upon request, or smiles in odd situations or odd ways

3 = little or no smiling at people, though may smile at other things

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

52. SHOWING AND DIRECTING ATTENTION

THE PURPOSE OF THIS ITEM IS TO DETERMINE WHETHER, HOW, AND IN WHAT CIRCUMSTANCES THE SUBJECT DIRECTS OTHERS' ATTENTION TO TOYS OR OBJECTS IN WHICH S/HE IS INTERESTED. THE FOCUS IS ON SPONTANEOUS DIRECTING OF ATTENTION PURELY TO SHARE INTEREST.

Does s/he ever show you things that interest her/him? For example, would s/he bring a new toy for you to see or call your attention to something s/he is playing with or making? What sorts of things are these?

Does this ever happen for things that aren't part of her/his special interests and aren't things s/he needs you for?

What about when [subject] **was 4 to 5 years old?**

0 = regular showing of objects by bringing things to parent/caregiver and directing her/his attention, with no manifest motive other than sharing

1 = possible showing as described above, but not sufficiently frequent or of purely communicative quality to meet criteria for "0"

2 = some bringing things to parent/caregiver and/or showing, but associated with preoccupations, food, or need for help

3 = rare or no social approaches of this type

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

53. OFFERING TO SHARE

THIS ITEM CONCERNS UNPROMPTED, NONROUTINE OFFERS TO SHARE A RANGE OF DIFFERENT OBJECTS WITH OTHER PEOPLE.

Does [subject] **ever offer to share things, that is, food or toys or favorite objects, with you?**

How about with other children?

Does s/he do this on her/his own or do you need to suggest it?

How often would this happen?

What about when [subject] **was 4 to 5 years old?**
(BE SURE TO DIFFERENTIATE CLEAR, SPONTANEOUS OFFERS TO SHARE FROM RESPONSES TO PROMPTING AND RELINQUISHING THINGS IF ANOTHER CHILD TRIES TO TAKE THEM. PROBES FOR OLDER CHILDREN OR ADULTS COULD INCLUDE SHARING A PEN, PENCIL, CRAYONS, NAPKINS, SPACE ON A BENCH OR COUCH, A BLANKET; OR GETTING A CUP OF TEA OR A DRINK.)

0 = frequent, spontaneous, and varied offers to share different sorts of objects (e.g., toys, comforters) with other people

1 = some spontaneous offers to share, but limited in number of contexts or frequency (must be more than food)

2 = will sometimes share if requested, but not spontaneously, or spontaneous sharing of food only

3 = no sharing

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

54. SEEKING TO SHARE HER/HIS ENJOYMENT WITH OTHERS

THE AIM OF THIS ITEM IS TO DETERMINE WHETHER THE SUBJECT ATTEMPTS TO SHARE HER/HIS ENJOYMENT OF THINGS THAT GIVE HER/HIM PLEASURE WITH OTHERS, WITH NO OTHER APPARENT MOTIVE OTHER THAN SHARING.

What kinds of things might make [subject] **excited and happy?**

How does s/he show these feelings?

Does [subject] **ever seem to want you to share in her/his enjoyment of something?**

Has s/he tried to share these feelings with you?

For example, if s/he has built something or sees something s/he particularly likes, will s/he let you know about her/his excitement by smiling or talking or making noises?

What about when [subject] **was 4 to 5 years old?**

0 = frequent attempts across a variety of contexts to direct several other people's attention to things that s/he enjoys or has done well (must be with more than one parent)

1 = some attempts to share enjoyment, but limited in number or variety or spontaneity, or lacking clear quality of shared pleasure

2 = few or no attempts to share enjoyment

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

55. OFFERING COMFORT

DEFINED AS A SPONTANEOUS UNPROMPTED GESTURE, TOUCH, VOCALIZATION, OR OFFER OF AN OBJECT (E.G., BLANKET). IT ALSO INCLUDES CHANGE IN FACIAL EXPRESSION DIRECTED TO SOMEONE WHO IS SAD, ILL, OR HURT, IN AN ATTEMPT TO HELP HER/HIM FEEL BETTER.

Does [subject] **ever try to comfort you if you are sad, hurt, or ill?**

What does s/he do if you are crying or if you have hurt yourself?

Would her/his facial expression change as s/he does this?

What about with her/his brother or sister?

Does s/he show comfort in more than one situation?

Do people have to show that they are upset in an exaggerated fashion to elicit comfort?

What about when s/he was 4 to 5 years old?

Code only if the subject spontaneously initiates offers of comfort.

0 = flexibly and spontaneously offers comfort in a range of circumstances and ways, for example, by gesture, touching, or vocalization, or offers of objects (e.g., blanket); must include change in facial expression

1 = has partial response (e.g., stands nearby and looks concerned) or indirect physical approach (e.g., comes to sit in lap, but with no clear attempt to comfort); or offers comfort in response to exaggerated expression (e.g., to pretend crying) or in one routine situation (e.g., to baby sister when she's hungry)

2 = rarely offers comfort or only in odd ways

3 = never offers comfort to others

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

56. QUALITY OF SOCIAL OVERTURES

THE FOCUS OF THIS ITEM IS ON THE **QUALITY** OF SOCIAL INTENTIONALITY WHEN SEEKING HELP, NOT ON THE NUMBER OF CONTEXTS IN WHICH SUCH APPROACHES OCCUR. CODE "0" ONLY IF SUBJECT CONSISTENTLY, DURING HIGHLY MOTIVATED APPROACHES (SUCH AS ASKING FOR HELP), MAKES SOME SORT OF VOCALIZATION THAT IS INTEGRATED WITH HER/HIS OTHER BEHAVIORS, INCLUDING EYE GAZE, AND DIRECTS HER/HIS ATTENTION TO THE OBJECT AND THE OTHER PERSON. CODE **TYPICAL**, RATHER THAN BEST, MOTIVATED OVERTURES.

When s/he wants something or wants help, how does s/he try to get your attention?

Does s/he point, give objects to you, or come and get you when s/he needs help?

Does s/he look at the object or you?

Does s/he ever use gestures or movements with sounds or words to get your attention?

If you don't understand at first, what does [subject] do?

Does s/he look at you and then talk or make a sound?

What about when s/he was 4 to 5 years old? (GET EXAMPLES.)

Does s/he show interest in other people or any other activities?

How does s/he show her/his interest, or get other people's attention? How often would s/he do this?

Code according to **majority** of overtures rather than best ones.

- 0 = consistently uses coordinated eye gaze with accompanying vocalization in typical situations when motivated to communicate
- 1 = may use eye gaze or vocalization, but these are weakly integrated
- 2 = rarely shows well-coordinated, focused social intentionality involving eye gaze or vocalization, or shows this in odd ways
- 3 = shows no coordination of eye gaze and vocalization
- 8 = N/A
- 9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

57. RANGE OF FACIAL EXPRESSIONS USED TO COMMUNICATE

THE FOCUS HERE IS ON FACIAL EXPRESSIONS USED TO COMMUNICATE, NOT JUST THOSE ASSOCIATED WITH THE EXPERIENCE OF EMOTIONS. A NORMAL RANGE OF EMOTIONS, EVEN IN A VERY YOUNG CHILD, WOULD BE EXPECTED TO INCLUDE SEVERAL MORE SUBTLE FACIAL EXPRESSIONS USED COMMUNICATIVELY, INCLUDING SURPRISE, GUILT, DISGUST, INTEREST, AMUSEMENT, AND EMBARRASSMENT, AS WELL AS JOY, ANGER, FEAR, AND PAIN.

Does [subject] **show a normal range of facial expression? For example, does s/he frown or pout or look embarrassed as well as laugh or cry?**

Can s/he look guilty...or surprised...or amused?

Can you tell by her/his face when s/he is afraid or disgusted?

Does s/he have the same range of facial expressions as other children?

What about when s/he was 4 to 5 years old?
(GET EXAMPLES.)

0 = full range of facial expression

1 = somewhat limited facial expression; may be rather stilted, exaggerated, in manner

2 = markedly limited range of facial expressions or tendency to have just one facial expression (e.g., happy) for all circumstances

3 = facial expression showing little or no indication of emotion of any kind

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

58. INAPPROPRIATE FACIAL EXPRESSIONS

INAPPROPRIATE FACIAL EXPRESSIONS ARE THOSE THAT INDICATE EMOTIONS INCONGRUENT WITH THE SITUATION, SUCH AS LAUGHING WHEN SOMEONE IS UPSET OR HURT, OR LAUGHING OR CRYING FOR NO DISCERNABLE REASON.

Does [subject]**'s facial expression usually seem appropriate to the particular situation as far as you can tell?**

Does s/he ever laugh or smile in situations that do not seem funny to most people or when you do not understand what it is s/he finds amusing?

Did this ever occur in the past? (NOTE EXAMPLES.)

0 = facial expressions almost always appropriate to mood, situation, and context

1 = facial expressions slightly or occasionally inappropriate or odd

2 = facial expressions obviously inappropriate in several different situations (SPECIFY)

8 = N/A (almost no variation in facial expression, appropriate or inappropriate, as in coding of "3" in Item 57)

9 = N/K or not asked

CURRENT ☐

EVER ☐

59. APPROPRIATENESS OF SOCIAL RESPONSES

THE FOCUS OF THIS ITEM IS ON HOW THE SUBJECT RESPONDS WHEN ADULTS OTHER THAN PARENTS ATTEMPT TO INTERACT WITH HER/HIM IN EVERYDAY, BUT NONROUTINE SITUATIONS.

Now can we turn to how [subject] **responds to what other people say or do?**

Does s/he consistently respond to the approaches of others in familiar situations?

How does s/he respond if a friend of yours whom s/he doesn't know well approaches and speaks to her/him?

What about someone s/he really likes?

How does s/he respond if someone unfamiliar (such as at church or in a shop) appropriately talks to her/him or tries to attract her/his attention?

Does s/he look directly at her/him?

Does s/he smile or show pleasure?

Would s/he show other reactions, such as interest or tentativeness? (GET EXAMPLES. PROBE TO DETERMINE CONSISTENCY AND CIRCUMSTANCES. IF THE CHILD SOUNDS SHY, SEEK FURTHER EXAMPLES OF PEOPLE WHO ARE MORE FAMILIAR.)

What about when s/he was 4 to 5 years of age?

0 = appropriate response to overtures by familiar and unfamiliar adults

1 = some clear positive responses and interactions, but not consistent

2 = responds to parents/caregiver and others in familiar settings, but responses are stereotyped, inappropriate, or very limited

3 = little or no interest in or response to people, except parents/caregiver or very familiar significant others

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

Now I'd like to talk about the way [subject] **plays and the kinds of things s/he is interested in.**

FAVORITE ACTIVITIES/TOYS

If [subject] **could choose anything s/he likes to do, what would be her/his favorite activities? How about favorite toys or any other kinds of objects?** (ASK FOR INFORMATION ABOUT THE MAIN ACTIVITIES AND TOYS, AND RECORD THEM INTO THE SEPARATE COLUMNS BELOW.)

Favorite Activities	Favorite Toys/Objects

60. INITIATION OF APPROPRIATE ACTIVITIES

THIS ITEM CONCERNS HOW THE SUBJECT SPONTANEOUSLY KEEPS HIMSELF/HERSELF OCCUPIED AND INVOLVED IN A RANGE OF NONODD AND NONREPETITIVE ACTIVITIES WHEN NOT SUPERVISED OR DIRECTED.

How good is [subject] **at organizing her/his own play or activities without your help?**

That is, does s/he find things to do without your directing her/him?

What kind of things does s/he do if left to her/his own devices? (GET EXAMPLES.)

What about when s/he was 4 to 5 years old?

0 = spontaneously takes up a range of appropriate play activities, without prompting or organization by other people

1 = spontaneously initiates only a limited range of appropriate activities

2 = engages in passive, but otherwise appropriate, activity, such as watching TV or listening to the radio

3 = engages in no active or constructive play, or engages only in repetitive activities or motor stereotypes

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

ITEMS 61 TO 64:
For children under 4.0 years, only the "current" coding is applicable.
For those aged 10.0 years or older, code only "most abnormal 4.0–5.0 years."

61. IMITATIVE SOCIAL PLAY

THE FOCUS OF THIS ITEM IS ON THE CHILD'S RECIPROCAL PARTICIPATION AS BOTH LEADER AND FOLLOWER IN EARLY SOCIAL GAMES THAT REQUIRE IMITATION AND COORDINATION OF SIMPLE ACTIONS. DO NOT COUNT BALL GAMES.

As a young child, did [subject] **enter into the spirit of social games such as "Here We Go 'Round the Mulberry Bush" or "Ring A Ring O'Roses?**

That is, did s/he spontaneously join in and try to initiate the various actions?

What about teasing games such as "I'm going to get you!" or having your fingers walking toward her/him?

What about with other familiar adults?

How did s/he join in the game?

Can s/he play peek-a-boo? How do you play it?

How about pat-a-cake? Simon says?

What about at age 4 to 5?

Code play with an adult or another child.

0 = normal social play, including clear evidence that the child initiates and responds to simple infant social games and can take both parts

1 = some to-and-fro (i.e., reciprocal) social play, but limited in amount, duration, or contexts in which shown (e.g., only plays peekaboo or pat-a-cake with parents/caregiver)

2 = little to-and-fro social play (e.g., plays peekaboo or pat-a-cake in a limited way only, but not reciprocal)

3 = no evidence of to-and-fro social play

8 = N/A

9 = N/K or not asked

CURRENT (UNDER 10.0) ☐

MOST ABNORMAL 4.0–5.0 ☐

62. INTEREST IN CHILDREN

For subjects aged 10.0 years or older, probe only for the 4.0- to 5.0-year period.

THE FOCUS HERE IS ON THE SUBJECT'S INTEREST IN WATCHING AND INTERACTING WITH OTHER CHILDREN OF THE SAME AGE.

What does [subject] **think about other children of approximately the same age whom s/he does not know? Is s/he interested in them?**

What does s/he do when another child comes to your house or s/he sees a child in another familiar situation (e.g. church, playgroup)?

What about when [subject] **was 4 to 5 years old?**

Code in relation to children of approximately the same age whom the subject does not know. Do not code interest in babies here.

- 0 = often watches other children and sometimes makes a clear effort to approach them or get their attention
- 1 = usually watches other children or indicates interest in them to parent/caregiver in some way (e.g., by pointing, vocalizing, or trying to imitate what they are doing, but no attempt to seek them out); or approaches other children without trying to get their attention
- 2 = occasionally watches other children, but almost never tries to approach them, or to direct parent's/caregiver's attention to them, or copy them
- 3 = shows no, or almost no, interest in other children
- 8 = N/A
- 9 = N/K or not asked

CURRENT (UNDER 10.0) ☐

MOST ABNORMAL 4.0–5.0 ☐

63. RESPONSE TO APPROACHES OF OTHER CHILDREN

For subjects aged 10.0 years or older, probe only for the 4.0- to 5.0-year period.

THE AIM HERE IS TO DETERMINE HOW THE SUBJECT RESPONDS WHEN OTHER CHILDREN APPROACH HER/HIM AND WHETHER THIS RESPONSE CONSTITUTES AN EFFORT TO KEEP AN INTERACTION GOING.

What about if another child approaches her/him?

Does s/he behave differently with [sibling] or with a child s/he has seen many times before?

Does the other child's age make a difference?

Does s/he ever actively avoid other children?

What about at age 4 to 5?

Code in relation to peers and older children; do not include responses to babies.

0 = generally responsive to other children's approaches, although may be hesitant initially if other children are too rough or intrusive; sometimes makes a clear effort to keep an interaction going with a child other than a sibling by gesturing, vocalizing, offering an object, etc.

1 = sometimes responsive to other children's approaches, but response is limited, somewhat unpredictable, or only to a sibling or a very familiar child

2 = rarely or never responds to the approach of even a familiar child (although may show interest in nonapproaching children or babies)

3 = consistently and persistently avoids approaches of other children

8 = N/A

9 = N/K or not asked

CURRENT (UNDER 10.0) ☐

MOST ABNORMAL 4.0–5.0 ☐

64. GROUP PLAY WITH PEERS

For subjects aged 10.0 years or older, probe only for the 4.0- to 5.0-year period.

THE FOCUS IS ON THE SUBJECT'S PARTICIPATION IN GROUPS OF OTHER CHILDREN IN SPONTANEOUS GAMES OR ACTIVITIES. COOPERATION MUST INVOLVE THE SUBJECT ATTENDING TO HER/HIS PEERS AND MODIFYING HER/HIS BEHAVIOR IN A WAY THAT CLEARLY DEMONSTRATES SPONTANEOUS, FLEXIBLE, INTERACTIVE PLAY. CHASING AND BALL GAMES SHOULD BE INCLUDED ONLY IF SPONTANEOUS, FLEXIBLE, AND INTERACTIVE. NOTE PREVIOUS COMMENTS REGARDING CARE IN INTERPRETING PLAY WITH SIBLINGS.

How does [subject] **play with others of her/his age when there are more than two together?**

What is their play like?

Is [subject] different with children or others outside your immediate family?

Does [subject] play cooperatively in games that need some form of joining in–such as musical games or hide-and-seek or ball games? (GIVE EXAMPLES AS APPROPRIATE FOR MENTAL AGE LEVEL.)

Would s/he initiate such games?
Or actively seek to join in?

Can s/he take different parts in these games (like being chased or doing the chasing, or hiding and looking for the other person)?

What about when [subject] **was 4 to 5 years old?**

0 = actively seeks and plays cooperatively in several different groups (three or more subjects) in a variety of activities or situations

1 = some cooperative play, but of insufficient initiative, flexibility, frequency, or variety to code "0"

2 = enjoys "parallel" active play (such as jumping in turn on a trampoline or falling down with others during Ring A Ring O'Roses), but little or no cooperative play

3 = seeks no play that involves participation in groups of other children, though may chase or play catch

8 = N/A

9 = N/K or not asked

CURRENT (OVER 4.0 AND UNDER 10.0) ☐

MOST ABNORMAL 4.0–5.0 ☐

65. FRIENDSHIPS

For subjects aged 5.0 years and older only.

FOR THE PURPOSE OF THIS ITEM, FRIENDSHIP IS DEFINED AS A SELECTIVE, RECIPROCAL RELATIONSHIP BETWEEN TWO PERSONS OF APPROXIMATELY THE SAME AGE WHO SEEK EACH OTHER'S COMPANY AND SHARE ACTIVITIES AND INTERESTS.

Does s/he have any particular friends or a best friend?
In what way does s/he show that they are her/his friends?

Do you know the names of any of her/his friends?

Does s/he see any of them outside of school, like around the neighborhood or in another social setting (e.g., clubs)?

Does s/he ever go out with them such as to the cinema/theatre/concerts? Do they share interests? (PROBE AS APPROPRIATE AND NOTE EXAMPLES.)

Are her/his relationships with others normal? (IF NOT) In what way are they abnormal? (FOCUS ON SUBJECT'S DEVELOPMENTAL LEVEL– I.E., MENTAL AGE, NOT CHRONOLOGICAL AGE.)

Was it the same in the past, or did s/he have fewer/more friends when s/he was younger?

0 = one or more relationships with person **in approximately own age group** with whom subject shares non-stereotyped activities of personal variety; whom subject sees outside prearranged group (such as club); and with whom there is definite reciprocity and mutual responsiveness

1 = one or more relationships that involve some personal shared activities outside a prearranged situation, with some initiative taken by subject, but limited in terms of restricted interests (e.g., model railways) or less than normal responsiveness/reciprocity

2 = people with whom subject has some kind of personal relationship involving seeking of contact, but **only** in group situation (such as club, church, etc.) or in school or at work

3 = no **peer** relationships that involve selectivity and sharing

8 = N/A (subject is outside the specified age range, or there has been an **unusually serious** lack of opportunity for peer contact)

9 = N/K or not asked

CURRENT (5.0 OR OLDER) ☐

MOST ABNORMAL 10.0–15.0 ☐

66. SOCIAL DISINHIBITION

For subjects aged 4.0 or older only.

SOCIAL DISINHIBITION REFERS TO BEHAVIOR THAT IS NOT APPROPRIATELY MODULATED ACCORDING TO THE SOCIAL EXPECTATIONS IN THE SUBJECT'S SOCIOCULTURAL ENVIRONMENT. SUCH DISINHIBITION MAY ARISE FROM A VARIETY OF CAUSES, BUT THE AIM HERE IS TO ASK ABOUT THAT WHICH ARISES FROM A LACK OF AWARENESS OF SOCIAL CUES. CODE INAPPROPRIATE QUESTIONS OR STATEMENTS IN ITEM 36, NOT HERE.

As they grow up, children ordinarily learn that they need to behave differently in different social situations. For example, they are usually more shy or reserved with people that they do not know very well or in certain situations such as church.

Does [subject] **vary in her/his behavior according to whom s/he is with or where s/he is?**

Is s/he ever cheeky or rude, or even inappropriately friendly to strangers?

Does s/he seem aware of social cues or social rules?

Is s/he more socially naive than other children/people (that is, unable to understand what one should say or do in particular social situations)?

Does s/he ever approach or touch strangers inappropriately?

What does s/he do if you visit a friend's home? (GET EXAMPLES.)

Was this ever a problem (after [subject] **turned 4), in a way that it would not have been for other children that age?**

All codings should be as judged by interviewer on basis of descriptions obtained and **not** on informant's inference.

0 = normal social inhibition

1 = occasional cheekiness or disinhibition more than others at same developmental level, but not to the extent of embarrassment: somewhat socially naive or imperceptive for developmental level

2 = definite lack of appreciation of social cues, contexts, or requirements: definitely lacks normal social inhibitions and sometimes behaves in socially embarrassing ways; fails to modulate behavior according to social context

3 = marked social disinhibition: appears unaware of social cues and social requirements so that behavior frequently embarrassing or inappropriate

8 = N/A

9 = N/K or not asked

CURRENT (4.0 AND ABOVE) ☐

MOST ABNORMAL 4.0–5.0 ☐

INTERESTS AND BEHAVIORS

FOR ITEMS 67 TO 78, "INTERFERENCE WITH" REFERS TO DIFFICULTIES FOR THE FAMILY, AND "SOCIAL IMPAIRMENT" REFERS TO SUBJECT'S OWN LIMITATION OR HANDICAP AS A RESULT OF THE AMOUNT OF TIME SPENT IN ABNORMAL ACTIVITY. ALL BEHAVIORS MUST HAVE OCCURRED OVER A 3-MONTH PERIOD TO BE CODED. IT IS IMPORTANT TO ENSURE THAT EXAMPLES FOR "CURRENT" AND "EVER" CODINGS ARE OBTAINED, WHERE SPECIFIED.

CODING INFORMATION FOR ITEMS 67 TO 78:

Code 2 involves some disturbance or reorganization of family life that can be tolerated by most families OR involves some limited interference with subject participating in other activities.

Code 3 requires major disruption or prevention of some family activities OR disruption or prevention of activities by the subject.

67. UNUSUAL PREOCCUPATIONS

AN UNUSUAL PREOCCUPATION IS DEFINED AS AN INTEREST THAT IS ODD OR PECULIAR IN QUALITY—THAT IS UNUSUAL IN ITS INTENSITY AND LACK OF SOCIAL FEATURES—AND WHICH IS REPETITIVE OR STEREOTYPED IN ONE OR MORE OF ITS FEATURES OR ELEMENTS.

Does [subject] **have any unusual or peculiar interests– I mean ones that preoccupy her/him even when the focus of interest is not physically present and might seem odd to other people? For example, is s/he unusually interested in things like metal objects, lights, street signs, or toilets?**

How much does s/he talk about them?

Does this interest influence how s/he behaves?
How long has it lasted?

Does this interfere with her/his other activities or with family life?

Are there things that you do differently as a family because of this interest?

How much of a problem is it for the family?

Was there ever anything like this in the past?

Unusual preoccupations must have gone on for at least 3 months to be coded.

CURRENT ☐

0 = no unusual preoccupations

1 = unusual preoccupation that does not interfere with significant activities of family life or does not cause social impairment of the subject

EVER ☐

2 = definite, repetitive preoccupation that intrudes into family life, but does not disrupt it significantly; or definite, repetitive preoccupation that does not cause substantial interference with social functioning, but which does constrain or intrude upon subject's other activities

3 = definite preoccupation that causes substantial interference or social impairment and severely limits the subject's other activities

9 = N/K or not asked

68. CIRCUMSCRIBED INTERESTS

For subjects aged 3.0 years or older only.

A CIRCUMSCRIBED INTEREST IS DEFINED AS A PURSUIT THAT DIFFERS FROM ORDINARY HOBBIES IN ITS INTENSITY; ITS CIRCUMSCRIBED NATURE (I.E., IT MAY INVOLVE A HIGH LEVEL OF EXPERTISE, BUT THIS REMAINS UNUSUALLY FOCUSED AND HAS NOT DEVELOPED INTO A BROADER CONTEXT OF KNOWLEDGE); ITS NONSOCIAL QUALITY (IT MAY BE SHARED WITH ANOTHER INDIVIDUAL WITH A SIMILAR CIRCUMSCRIBED INTEREST BUT NOT AS PART OF A SPECIALIZED CLUB OR ASSOCIATION); AND ITS RELATIVE NONPROGRESSION OR DEVELOPMENT OVER TIME (THAT IS, THE INTEREST PERSISTS, BUT DOES NOT FORM THE BASIS OF A BUILDING UP OF SHARED OR USED EXPERTISE). IT DIFFERS FROM AN "UNUSUAL PREOCCUPATION"(ITEM 67) IN THAT IT LACKS PECULIAR OR ODD CONTENT. CIRCUMSCRIBED INTERESTS ARE UNUSUAL IN THEIR QUALITIES (AS ABOVE) BUT NOT IN THEIR CONTENT.

Does [subject] **have any special hobbies/interests that are unusual in their intensity?**

How long has s/he had this interest?

In what way is it unusual?

Has it developed or changed at all over time?

Does s/he share the interest with other people? In what way?

Does it (the interest) seem at all encompassing in its intensity?

What happens if you interrupt her/him?

Does it interfere with her/his doing things?

Have there been any special interests in the past? (GET DETAILS.)

Circumscribed interests must have gone on for at least 3 months to be coded.

0 = no circumscribed interests

1 = special interest of unusual degree, but not definitely intrusive into or constraining of the subject's or family's other activities

2 = definite circumscribed interest that does not cause substantial interference with social functioning, but which does constrain or intrude upon subject's or family's other activities

3 = definite circumscribed interest that causes clear-cut social impairment

8 = N/A (age below 3.0 years)

9 = N/K or not asked

CURRENT (3.0 OR OLDER) ☐

EVER (3.0 OR OLDER) ☐

69. REPETITIVE USE OF OBJECTS OR INTEREST IN PARTS OF OBJECTS

THIS ITEM INVOLVES ACTIONS OF A STEREOTYPED OR REPETITIVE NATURE THAT ARE NONFUNCTIONAL AND THAT INVOLVE A FOCUS EITHER ON PARTS OF OBJECTS OR ON A USAGE OF AN OBJECT THAT IS CLEARLY SEPARATE FROM THAT WHICH IS ORDINARILY ACCEPTED.

How does s/he play with her/his toys or things around the house? (GET EXAMPLES.)

Will s/he play with the whole toy or does s/he seem to be more interested in a certain part of the toy (e.g., spinning the wheels of a car or opening and shutting its door), rather than using it as it was intended?

Are there particular kinds of objects s/he really likes?

Does s/he ever collect or gather certain sorts of objects?

What does s/he do with them?

Does s/he ever line things up or do the same thing over and over with them, such as drop things from the same distance?

Do these activities change over time or are they exactly the same?

Has s/he ever used objects in these ways in the past?

Repetitive use of objects or interests in parts of objects must have gone on for at least 3 months to be coded.

CURRENT ☐

EVER ☐

0 = little or no repetitive use of objects

1 = some repetitive use of objects (e.g., shaking strings or spinning things), or interest in parts (e.g., turning wheels and dials) or very specific types of objects (e.g., collecting bits of paper), in conjunction with several other activities but not causing social impairment

2 = play linked to highly stereotypic use of objects or attention to specific parts or types of objects, but which does not constrain or intrude upon subject's other activities

3 = play linked to highly stereotypic use of objects to an extent that prevents or seriously interferes with other activities

7 = an interest in "infant" toys, such as music boxes or rattles, but play is with a variety of objects and not in a highly stereotypic fashion

8 = N/A (no play with objects)

9 = N/K or not asked

70. COMPULSIONS/RITUALS

THE EMPHASIS OF THIS ITEM IS ON FIXED SEQUENCES THAT ARE PERFORMED AS IF THE SUBJECT FEELS PRESSURE TO COMPLETE THEM IN A PARTICULAR ORDER. **COMPULSIONS** MAY ALSO INCLUDE HAVING TO PLACE PARTICULAR OBJECTS IN EXACT POSITIONS OR RELATIONSHIPS IN SPACE, SUCH AS OPENING ALL DOORS TO A CERTAIN ANGLE OR TURNING ALL LIGHTS OFF. A COMPULSION WITH LIGHTS DIFFERS FROM REPETITIVE USE OF OBJECTS (CODED IN ITEM 69) IN THAT THE SUBJECT INSISTS THAT SEVERAL LIGHTS MUST REMAIN OFF, RATHER THAN CARRYING OUT A REPETITIVE ACTION OF FLICKING LIGHTS OFF AND ON.

RITUALS DIFFER FROM DIFFICULTIES WITH CHANGES AS DESCRIBED IN ITEM 74 IN THAT THEY HAVE SEQUENCE; IN ADDITION, IN A RITUAL OR COMPULSION THE SUBJECT IS IMPOSING AN ORDER ON EVENTS, RATHER THAN RESPONDING TO A PERCEIVED CHANGE. THUS, A SUBJECT WHO NEEDS TO LAY HER/HIS NAPKIN OUT FLAT AND PLACE HER/HIS SPOON ON IT BEFORE S/HE WILL EAT, COULD BE CODED AS HAVING A RITUAL, WHEREAS A SUBJECT WHO IS UPSET IF S/HE IS GIVEN A DIFFERENT NAPKIN WOULD BE CODED ONLY UNDER "DIFFICULTIES WITH MINOR CHANGES IN SUBJECT'S OWN ROUTINES OR PERSONAL ENVIRONMENT" (ITEM 74).

BEDTIME ROUTINES ARE EXPLICITLY EXCLUDED FROM THIS ITEM BECAUSE THEY OCCUR SO COMMONLY IN NORMALLY DEVELOPING CHILDREN.

Are there things that [subject] **seems to have to do in a very particular way or order; that is, rituals that s/he has to do or has to have you do?**

Like touching particular things or putting things in special places before going on to do something else?

How does s/he react if s/he is unable to complete the whole sequence of her/his activity or is disrupted during the course of her/his actions? (GET DETAILS AND EXAMPLES.)

Was this ever a problem in the past? (PROBE AS APPROPRIATE, USING PROMPTS OR A BRIEF DEMONSTRATION IF NECESSARY.)

Compulsions/rituals must have gone on for at least 3 months to be coded.

CURRENT ☐

0 = no compulsions/rituals

1 = some activities with unusually fixed sequences, but no activity that appears compulsive in quality

EVER ☐

2 = one or more activities that subject has to perform in a special way; subject appears to be under pressure or becomes anxious if activity disrupted, or family goes to unusual lengths to avoid interrupting ritual or to make sure subject is forewarned if it is necessary to interrupt her/him; compulsive quality present, but little interference with family life or social impairment

3 = one or more activities that subject has to perform in a special way; subject appears to be under **marked** pressure or becomes extremely anxious or distressed if activity disrupted; degree of compulsive quality intrudes upon family life or causes definite social impairment to subject

9 = N/K or not asked

71. UNUSUAL SENSORY INTERESTS

UNUSUAL SENSORY INTERESTS ARE DEFINED AS UNUSUALLY STRONG SEEKING OF STIMULATIONS FROM THE BASIC SENSATIONS OF SIGHT, TOUCH, SOUND, TASTE, OR SMELL THAT ARE DISSOCIATED FROM MEANING. THE FOCUS IS ON THE EXTENT TO WHICH THE ABNORMAL INTEREST DISTURBS OR REPLACES "NORMAL USE" OF THE OBJECT.

Does s/he seem particularly interested in the sight, feel, sound, taste, or smell of things or people? For example, does s/he tend to sniff toys, objects, or people inappropriately?

Or is s/he unusually concerned with the feel or texture of things?

Or does s/he tend to peer at or look at things for long periods of time?

Or does s/he touch things to her/his lips or tongue to see how they feel?

How long has s/he been interested in this? (GET EXAMPLES, SPECIFYING THE SENSE AND CIRCUMSTANCES WHERE SHOWN.)

Has there ever been a time when s/he showed an unusual interest in sensations?

Unusual sensory interests must have gone on for at least 3 months to be coded.

0 = has no unusual sensory interests

1 = shows one or two unusual interests regularly

2 = has unusual sensory interest that takes up a major amount of time or prevents or limits alternative use of that material in its ordinary function

9 = N/K or not asked

CURRENT ☐

EVER ☐

72. UNDUE GENERAL SENSITIVITY TO NOISE

THE FOCUS NEEDS TO BE THE PREDICTABLE, GENERALLY INCREASED SENSITIVITY TO EVERYDAY SOUNDS, SUCH AS HOUSEHOLD APPLIANCES OR TRAFFIC, RATHER THAN A REACTION TO A SUDDEN, HARSH, OR UNEXPECTED NOISE SUCH AS THUNDER OR A LOUDSPEAKER. DO NOT INCLUDE IDIOSYNCRATIC RESPONSES TO HIGHLY SPECIFIC SOUNDS (THESE ARE COVERED BY ITEM 73).

Has s/he ever seemed oversensitive to noise?

Has s/he ever deliberately and regularly put her/his hands over her/his ears in response to ordinary sounds?

Does s/he do this now?

To what kinds of sounds?

Have you ever had to adjust what you do because [subject] was so upset by noises?

To code for general sensitivity to noise, more than one occurrence is required, however clearly it is remembered (must have gone on for at least 3 months to be coded).

CURRENT ☐

EVER ☐

0 = no general sensitivity to noise

1 = slight sensitivity to noise: somewhat sensitive to loud sounds such as the vacuum cleaner, motorbikes, or other appliances

2 = definite sensitivity to noises that are not distressing to most other people, the sensitivity being accompanied by a clear behavioral change (such as avoidance, hands over ears, or crying)

3 = definite sensitivity to noises to the extent that subject's distress/disturbance in relation to certain noises interferes with family or household routines

9 = N/K or not asked

73. ABNORMAL, IDIOSYNCRATIC, NEGATIVE RESPONSE TO SPECIFIC SENSORY STIMULI

TO MEET THE CRITERIA FOR CODING, THE SUBJECT'S RESPONSE MUST BE PREDICTABLE; BE SPECIFIC TO SOME IDENTIFIABLE AND PARTICULAR SENSORY STIMULUS (OR GROUP OF STIMULI); BE IDIOSYNCRATIC; AND INVOLVE SOME FORM OF NEGATIVE, EMOTIONAL REACTION OTHER THAN FEAR (OFTEN IT INVOLVES ANGER OR MARKED IRRITATION). THUS, GENERAL DISTRESS IN RELATION TO VERY LOUD NOISES IS EXCLUDED, AS ARE NEGATIVE REACTIONS TO ENVIRONMENTAL CHANGE (SEE ITEMS 72, 74, AND 75).

Does [subject] **ever get unusually upset or irritated by particular sounds such as people coughing or a baby crying?** (TAKE CARE TO DIFFERENTIATE FROM A FEAR REACTION.)

What does s/he do?

How does s/he show that s/he is upset?

Do you think s/he is afraid or is it more like anger or irritation?

Is it just one particular sort of sound?

Does [subject] **ever react in an unusual, but predictable, way to other sensations (such as tastes or smells or the sight or feel of things)? For example, does s/he react to the sight of something like earrings or men with beards?**

How long has this gone on?

Was this ever a problem in the past? (GET EXAMPLES.)

Abnormal, idiosyncratic, negative response to specific sensory stimuli must have gone on for at least 3 months to be coded.

CURRENT ☐

EVER ☐

0 = no predictable, abnormal, idiosyncratic, negative response to specific sensory stimuli

1 = predictable, abnormal, idiosyncratic, negative response to one or more specific stimuli, but reaction mild or controllable so does not give rise to avoidance or to any interference with ordinary life

2 = predictable, abnormal, idiosyncratic, negative response to one or more specific stimuli; some intrusion into ordinary activities so there are occasional tantrums/disturbances or attempts by family to avoid subject being exposed to specific stimulus; however, no substantial interference with general pattern of family life

3 = predictable, abnormal, idiosyncratic, negative response to one or more specific stimuli that causes substantial interference with family life or totally, or almost totally, prevents some activity

9 = N/K or not asked

74. DIFFICULTIES WITH MINOR CHANGES IN SUBJECT'S OWN ROUTINES OR PERSONAL ENVIRONMENT

THIS ITEM CONCERNS MARKED NEGATIVE REACTIONS TO A VARIETY OF MINOR CHANGES IN HOW, WHERE, OR WHEN THE SUBJECT CARRIES OUT DAILY ACTIVITIES. THESE CHANGES MUST BE MINOR. DO NOT INCLUDE MOVING HOUSE, CHANGING SCHOOL, OR ANY OTHER MAJOR TRANSITION THAT WOULD BE EXPECTED TO AFFECT ANY SUBJECT. THE EMPHASIS FOR THIS ITEM IS ON AN UNUSUAL DEGREE OF UPSET OR INSISTENCE ON MAINTAINING THE ORIGINAL CONDITION IF A MINOR ASPECT OF THE **SUBJECT'S** ROUTINE IS CHANGED.

Is [subject] **bothered by minor changes in her/his routine? Or in the way her/his personal things are arranged?**
For example, does it bother her/him to switch from one pair of mittens or gloves to another or from winter to summer clothing (e.g., long sleeves to short sleeves)?

How about changes in schedule?

Does it make a difference to [subject] if, for instance, you bathe her/him or s/he takes a bath 15 minutes earlier or later than usual, or s/he gets dressed before breakfast or after, if this broke her/his routine? What does happen?

Do *minor* changes in eating routines, such as where the salt and pepper are on the table or where food is placed on her/his plate, cause any difficulty?

Was this ever a problem in the past?
(PROBE FOR DETAILS AND NOTE EXAMPLES.)

Difficulties with minor changes in subject's own routines or personal environment must have gone on for at least 3 months to be coded.

CURRENT ☐

EVER ☐

0 = no difficulties with minor changes in own subject's own routines

1 = unusually negative reaction to minor changes in subject's own routines, but with no serious distress and little or no interference in family life

2 = definite, unusual reactions to minor changes in subject's own routines, causing resistance or distress/or family going to unusual lengths to avoid changing minor aspects of subject's routines or to prepare subject for minor changes; but no substantial interference in family life

3 = definite, unusual, and marked resistance to minor changes in subject's own routines, with substantial interference with or impairment of family activities

9 = N/K or not asked

75. RESISTANCE TO TRIVIAL CHANGES IN THE ENVIRONMENT *(NOT DIRECTLY AFFECTING THE SUBJECT)*

THIS ITEM CONCERNS THE SUBJECT'S MARKED DIFFICULTY WITH MINOR OR TRIVIAL CHANGES IN ASPECTS OF THE ENVIRONMENT THAT HAVE NO DIRECT EFFECT ON HER/HIM, FOR EXAMPLE, THE POSITION OF ORNAMENTS, THE ORIENTATION OF THE TELEPHONE, OR CLOTHES WORN BY PEOPLE OTHER THAN SUBJECT. THE EMPHASIS IS ON THE **SUBJECT'S** UNUSUALLY NEGATIVE REACTION TO THESE TRIVIAL CHANGES.

How does [subject] **react to changes about the house, or to changes in small details of her/his environment or surroundings?** For example, how does s/he react to a change in someone else's daily routine, how the furniture is arranged, or what you wear (e.g., if you wear glasses or a hat)?

Does s/he get distressed?

What about when s/he was younger?

Was this ever a problem in the past?

(IF THIS IS/WAS A PROBLEM, PROBE FOR DETAILS AND NOTE EXAMPLES.)

Resistance to trivial changes in the environment must have gone on for at least 3 months to be coded.

CURRENT ☐

EVER ☐

0 = none

1 = unusually negative reaction to trivial changes in the environment, but with no serious distress and little or no interference in family life

2 = definite, unusual reactions to trivial changes in the environment, causing marked distress or causing family to go to unusual lengths to avoid trivial changes in the environment or to prepare subject for such trivial changes; but no substantial interference in family life

3 = definite, unusual, and marked resistance to trivial changes in the environment, with substantial interference with or impairment of family activities

9 = N/K or not asked

76. UNUSUAL ATTACHMENT TO OBJECTS

AN ATTACHMENT IS DEFINED AS AN UNUSUAL INTEREST AND DEPENDENCE ON A PARTICULAR OBJECT THAT THE SUBJECT CARRIES AROUND WITH HER/HIM. THE FOCUS HERE IS ON ATTACHMENTS TO UNUSUAL OBJECTS, I.E., NOT THE SOFT, CUDDLY BLANKETS OR STUFFED TOYS USED BY MOST CHILDREN. THE STRENGTH OF THE ATTACHMENT IS DETERMINED BY HOW DIFFICULT IT IS FOR THE SUBJECT TO SEPARATE FROM THE OBJECT AND WHETHER ITS POSSESSION INTRUDES INTO THE SUBJECT'S OR FAMILY'S LIFE. THE BEHAVIOR OF AN UNUSUAL ATTACHMENT MUST HAVE LASTED 3 MONTHS, BUT THIS MAY OR MAY NOT HAVE INVOLVED THE SAME OBJECT THROUGHOUT.

Does [subject] **have anything to which s/he is particularly attached and that s/he likes to carry around with her/him?**

What is it like?

Is it something like a teddy or blanket, or is it something more unusual, like a piece of pipe, a clothes peg, or a stone? (GET EXAMPLES.)

What does s/he do with it?

If asked to put it down, will s/he do so?

Does s/he take it to bed?

What happens if it is taken away or if it gets mislaid?

What about when s/he was younger?

Has s/he ever been particularly attached to anything?

Unusual attachments to objects must have gone on for at least 3 months to be coded.

CURRENT ☐

EVER ☐

0 = no attachment or attachment only to cuddly object used as comforter

1 = some attachment to slightly unusual object, such as piece of paper or soft brush, or several similar interchangeable objects, but puts down if asked to do so and can tolerate separation from it; no interference with activities

2 = attachment to an unusual object causes significant distress on separation or causes caregivers to try to ensure object always readily available for subject because of anticipated distress; occasional interference with activities

3 = unusual attachment so intrusive that it prevents many everyday activities

6 = attachment to soother/comforter or blanket or other usual object beyond age 5 or with such intensity that interferes with social functioning or activities (if has also had an unusual attachment, code that instead)

7 = series of short-lasting (1–3 days) attachments to unusual objects or groups of objects, replaced by new attachment to different kind of unusual object also for short time

9 = N/K or not asked

77. HAND AND FINGER MANNERISMS

HAND AND FINGER MANNERISMS OF THE TYPE COVERED BY THIS ITEM TYPICALLY INVOLVE RAPID, VOLUNTARY, REPETITIOUS MOVEMENTS OF THE FINGERS AND HANDS, OFTEN BUT NOT ALWAYS WITHIN THE LINE OF THE SUBJECT'S VISION. DO **NOT** INCLUDE NAIL BITING, HAIR TWISTING, OR THUMB SUCKING. CLAPPING IS **NOT** A HAND MANNERISM, NOR ARE THE NONSPECIFIC OVERFLOW MOVEMENTS SEEN IN INFANTS AND TODDLERS WHEN THEY ARE EXCITED. IF HAND AND FINGER MANNERISMS ONLY OCCUR DURING WHOLE BODY MOVEMENTS, CODE ON ITEM 78 ONLY. DO NOT INCLUDE ROCKING IN EITHER CODE.

Does [subject] **have any mannerisms or odd ways of moving her/his hands or fingers? Such as twisting or flicking her/his fingers in front of her/his eyes?**

Do they interfere with getting [subject] to do other things? In what way?

What happens if you try to get her/him to stop?

Are there any particular circumstances in which s/he does this more than in others? (GET DETAILS.)

Did s/he ever show any of these types of mannerisms or odd movements in the past? (NOTE EXAMPLES.)

Hand and finger mannerisms must have gone on for at least 3 months to be coded.

CURRENT ☐

0 = no hand and finger mannerisms

1 = occasional hand and finger mannerisms only or type not as clearly specified as for rating of "2"

EVER ☐

2 = definite, frequent hand mannerisms or finger flicking/twisting, but no interference with other activities or distress if interrupted

3 = marked mannerisms of type specified; associated with social impairment or distress when interrupted or infrequent interruption due to others' concern about subject's reaction

8 = N/A (e.g., physically disabled)

9 = N/K or not asked

78. OTHER COMPLEX MANNERISMS OR STEREOTYPED BODY MOVEMENTS *(DO NOT INCLUDE ISOLATED ROCKING)*

THE FOCUS HERE IS ON COMPLEX, STEREOTYPIC, VOLUNTARY, WHOLE-BODY MOVEMENTS, SUCH AS ARM WAVING WHILE ROCKING UP ONTO TIPTOES.

Does [subject] **have any complicated movements of her/his whole body, such as spinning or repeatedly bouncing up and down?**

Do they interfere at all with getting [subject] to do other things? In what way?

What happens if you try to get her/him to stop? (GET DETAILS.)

In the past, did s/he have any of these movements? (NOTE EXAMPLES. ISOLATED ROCKING IS EXCLUDED BUT COMPLICATED MANNERISMS THAT INVOLVE ROCKING ONLY AS ONE COMPONENT MAY BE APPLICABLE.)

Complex mannerisms or stereotyped body movements must have lasted 3 months to be coded.

CURRENT ☐

EVER ☐

0 = no complex mannerisms or stereotyped body movements

1 = only occasional complex mannerisms or stereotyped body movements

2 = definite, frequent other mannerisms or stereotypies, but will stop without distress if interrupted

3 = marked mannerisms associated with social impairment

8 = N/A

9 = N/K or not asked

79. MIDLINE HAND MOVEMENTS

THESE MOVEMENTS ARE THOSE THAT OCCUR IN THE MIDDLE OF THE SUBJECT'S BODY AND USUALLY INVOLVE BOTH HANDS MOVING IN SIMILAR WAYS IN A HAND WRINGING OR HAND WASHING MOVEMENT.

Does s/he have any particular ways of moving her/his hands in front of her/his body, for example, hand wringing or turning the hands from side to side together as if washing them?

Midline hand movements must have lasted 3 months to be coded.

0 = no midline hand movements

1 = only occasional midline hand movements or type not as clearly specified as for a rating of "2"

2 = definite abnormal wringing hand movements mainly in the midline

8 = N/A

9 = N/K or not asked

CURRENT ☐

EVER ☐

GENERAL BEHAVIORS

80. GAIT

THE FOCUS IS ON UNUSUAL WAYS OF WALKING–PARTICULARLY TIPTOEING OR BOUNCING–THAT ARE NOT CLEARLY ASSOCIATED WITH PHYSICAL HANDICAP.

Is there anything unusual about the way [subject] **walks** (e.g., bouncing, exaggeration of toe-heel, up on toes)**?** (GET DESCRIPTION.)

Do you think other people notice it?

Has there ever been anything unusual?

How did s/he walk when s/he was 4 to 5 years old?

Do not code broad-based, immature or clumsy gait.

0 = normal gait

1 = somewhat unusual gait

2 = definitely odd gait (e.g., toe-walking or abnormal bouncing)

3 = gait sufficiently odd to be noticed by others outside family or teachers

7 = gait apparently due to a neurological condition or to severe developmental motor impairment

8 = N/A

9 = N/K or not asked

CURRENT ☐

MOST ABNORMAL 4.0–5.0 ☐

81. AGGRESSION TOWARD CAREGIVERS OR FAMILY MEMBERS

THIS ITEM IS CONCERNED WITH EPISODES OF AGGRESSION WITHIN THE FAMILY, INCLUDING WITH CAREGIVERS, OF SUFFICIENT SEVERITY AND/OR FREQUENCY TO CONSTITUTE A SIGNIFICANT CAUSE FOR CONCERN.

Have there been times when [subject] **has been aggressive toward other people within the family (or other caregivers)?**

Has s/he ever hit or bitten anyone?

What about when s/he was younger? (GET DETAILS OF CIRCUMSTANCES AND OF AGGRESSIVE ACTS.)

What does s/he do?

What are the circumstances?

What seems to start the aggression?

Has s/he ever really hurt someone? What happened?

Has s/he ever used any kind of implement like a stick or a knife?

How long do the aggressive episodes last (a few minutes or several hours)? How often do they occur?

0 = no aggression or only rare episodes; rare aggressiveness, not a significant problem

1 = mild aggressiveness only (threatening without physical contact; or behavior that might represent just unduly rough play **or** momentary, provoked lashing out)

2 = definite physical aggression involving hitting or biting but no use of implements

3 = violence that involves the use of implements

9 = N/K or not asked

CURRENT ☐

EVER ☐

82. AGGRESSION TOWARD NONCAREGIVERS OR NONFAMILY MEMBERS

THIS ITEM CONSTITUTES A DIRECT PARALLEL TO ITEM 81 AND IS DIFFERENT ONLY IS SO FAR AS THE AGGRESSION IS DIRECTED TO INDIVIDUALS WHO ARE NOT CAREGIVERS OR MEMBERS OF THE FAMILY. IT INCLUDES AGGRESSION TOWARD BOTH PEERS AND ADULTS.

What about aggression toward people outside the family, as at school or in shops or on buses?

Has s/he ever behaved in ways that might lead other people to think that s/he was going to be aggressive?

Has there ever been a concern that s/he might harm or hurt other people?

What about when s/he was younger?
(GET DETAILS OF CIRCUMSTANCES AND OF AGGRESSIVE ACTS.)

What does s/he do?

What are the circumstances?

What seems to start the aggression?

Has s/he ever really hurt someone? What happened?

Has s/he ever used any kind of implement like a stick or knife?

How long did/do the aggressive episodes last (a few minutes or several hours)?

How often did/do they occur?

0 = aggression rare, not a significant problem

1 = mild aggressiveness only (threatening without physical contact; or behavior that might represent just unduly rough play **or** momentary provoked lashing out)

2 = definite physical aggression involving hitting or biting but no use of implements

3 = violence that involves the use of implements

9 = N/K or not asked

CURRENT ☐

EVER ☐

83. SELF-INJURY

SELF-INJURY IS A DELIBERATE SELF-DIRECTED AGGRESSIVE ACT (E.G., BITING THE WRIST, BANGING THE HEAD) THAT RESULTS IN TISSUE DAMAGE AND OCCURS OVER A PERIOD OF AT LEAST 3 MONTHS.

Does [subject] ever injure himself/herself deliberately, such as by biting her/his arm or banging her/his head or anything else like this? (GET DETAILS.)

Was this ever a problem in the past?

Self-injury must occur over a period of 3 months to be coded.

0 = no self-injury

1 = slight self-injury (e.g., occasionally bites own hand/arm when annoyed, pulls hair, or slaps face); no substantial tissue damage

2 = self-injury definitely present (e.g., actual bruises or calluses, repeated head banging, hair pulling, biting associated with definite tissue damage; do not count picking of spots)

3 = definite self-injury with serious damage (e.g., skull fracture, eye injury, etc.)

9 = N/K or not asked

CURRENT ☐

EVER ☐

84. HYPERVENTILATION

HYPERVENTILATION INVOLVES EPISODES OF RAPID, DEEP, REPETITIVE BREATHING IN SITUATIONS OTHER THAN THOSE THAT ELICIT PANIC.

Does [subject] **ever breathe in deeply with repeated rapid breaths?**

Does s/he ever sound as if s/he is gasping for air over and over within a period of a few seconds?

0 = no hyperventilating
1 = occasional hyperventilating
2 = frequent hyperventilating
9 = N/K or not asked

CURRENT ☐

EVER ☐

85. FAINTS/FITS/BLACKOUTS

THE FOCUS IS ON EPISODES INVOLVING AN UNEXPLAINED CHANGE IN LEVEL OF CONSCIOUSNESS WITH OR WITHOUT FALLING OR JERKING MOVEMENTS OF THE LIMBS.

Has [subject] **ever fainted or had a fit/seizure/convulsion?**

Has s/he ever had medicine to control fits?

(IF YES, PROBE FOR FURTHER DETAILS ABOUT THE "EPISODES," INCLUDING AGE OF ONSET, FREQUENCY, A CLEAR DESCRIPTION, AND WHETHER THEY REQUIRED INVESTIGATION AND TREATMENT, INCLUDING PAST AND CURRENT MEDICATION AND/OR HOSPITAL ADMISSION.)

0 = no attacks — CURRENT ☐

1 = history of attacks that might be epileptic, but diagnosis not established

2 = definite diagnosis of epilepsy

7 = febrile convulsions only, with no continuing daily medication outside the period of fever — EVER ☐

9 = N/K or not asked

86. AGE WHEN ABNORMALITY FIRST EVIDENT

IF IT IS ALREADY CLEAR THAT BEHAVIOR ABNORMAL BY AGE 3 YEARS, QUESTION ONLY ON EARLIER AGES IN ORDER TO ASSESS PROBABLE TIME THAT ABNORMALITIES FIRST EVIDENT. IF ACCOUNT SO FAR SUGGESTS SUBJECT NORMAL UP TO 3 YEARS, FOCUS FIRST ON AGE 3 YEARS IN ORDER TO DETERMINE IF DEVELOPMENT DEFINITELY NORMAL AT THAT AGE, AND THEN EXPLORE EARLIER AGES. THIS CODING IS MADE ON THE INTERVIWER'S JUDGEMENT USING ALL AVAILABLE INFORMATION FROM THE INTERVIEW.

When we started talking about [subject], I asked you when you thought that s/he first showed any difficulties in development or behavior. You said that you thought [day/month/year]. (REFER BACK TO ITEM 2) **I'd like now just to check back on those early years.**

Could you tell what [subject] **was like about the time of her/his third birthday?**

What was her/his play like?

What toys did s/he play with?

Any pretend games?

How was her/his talking then?

What about looking after herself/himself? Feeding? Toileting? Dressing?

What were her/his relationships with other children like?

Working back again just to check: what about at age 1 year and at age 2 years?

0 = development in the first 3 years of life clearly normal in quality and within normal limits for social, adaptational, language, self-help, and motor milestones; no behavioral problems of a type that might indicate developmental delay or deviance

1 = development possibly within normal limits during first 3 years, but uncertainty because of either the quality of behavior or the level of skills

2 = development probably abnormal by the age of 3 years, as indicated by developmental delay or deviance, but not of a degree or type that is definitely incompatible with normality

3 = development definitely abnormal in the first 3 years but quality of behavior/social relationships/ communications not unambiguously autistic at that age

4 = development definitely abnormal in the first 3 years and quality of behavior/social relationships/ communications strongly indicative of autism at that age

9 = N/K or not asked

☐

87. INTERVIEWER'S JUDGMENT ON AGE WHEN DEVELOPMENTAL ABNORMALITIES PROBABLY FIRST MANIFEST

Code in months

Special Isolated Skills (Items 88–93)

For Items 88 to 93, probe as appropriate to the subject's level of functioning and get details of level and pattern of skills, as well as extent that skill involves meaning and interpretation and can be applied in day-to-day situations. Describe in detail. Code for "current" and "ever."

Does [subject] **have any unusually marked special skills? Are there any things that s/he seems to be *very good* at, either currently or at any time in the past?** (GET DETAILS AND EXAMPLES.) **Are these skills related to one of her/his special interests or unusual preoccupations?**

Is [subject] particularly good with shapes–as in puzzles or jigsaws? Has this *ever* been a particular ability?

What about her/his memory? Was it ever exceptional?

Does s/he have particular musical skills? How about in the past?

Is s/he unusually good at drawing? Was s/he in the past?

How about reading? In the past?

What about computations? In the past?

Throughout this section, the focus should be on a particular skill or ability. Once a decision has been made about the presence or absence of a skill, the next assessment needs to be in relation to how this skill compares with the subject's overall level of functioning and how this would compare with the general population. For example, a child with mental retardation who could multiply three-figure numbers in his/her head but could not apply this skill would get a code of "2." For Item 93 "computational ability," If s/he could apply the skill in real-life situations, s/he would get a code of "7." If his/her computational skill was average by population norms, but well above his/her mental age, this would be coded "1."

Code special skills on next page.

SPECIAL ISOLATED SKILLS

0 = no outstanding skills/knowledge in relation to overall level of ability, whether high or low

1 = isolated skill/knowledge that is definitely out of keeping with subject's general level of ability, but not above general population norms

2 = isolated skill/knowledge that is definitely above the subject's general level of ability and above the general population's normal level, but is not used functionally or meaningfully to any marked extent (e.g., a preschool child who can read without comprehension or a calendrical calculator would be coded here)

7 = isolated skill/knowledge that is above the subject's general level and above the normal population's level of ability and is used meaningfully (i.e., genuine talent or ability used adaptively, such as performing music for others' enjoyment or participating in age-appropriate children's hobbies such as model-building or computer programming); subject is recognized by peers as having exceptional skill

8 = N/A (e.g., reading in a nonverbal subject)

9 = N/K or not asked

	CURRENT	EVER
88. VISUOSPATIAL ABILITY (IN PUZZLES, JIGSAWS, SHAPES, PATTERNS, ETC.)	☐	☐
89. MEMORY SKILL (ACCURATE MEMORY FOR DETAIL, AS OF DATES OR TIMETABLES)	☐	☐
90. MUSICAL ABILITY (RECOGNITION, COMPOSITION, ABSOLUTE PITCH, OR PERFORMANCE)	☐	☐
91. DRAWING SKILL (UNUSUALLY SKILLED USE OF PERSPECTIVE OR CREATIVE APPROACH)	☐	☐
92. READING ABILITY (E.G., EARLY SIGHT READING)	☐	☐
93. COMPUTATIONAL ABILITY (E.G., MENTAL ARITHMETIC)	☐	☐

CONCLUDING COMMENTS (NO CODING REQUIRED)

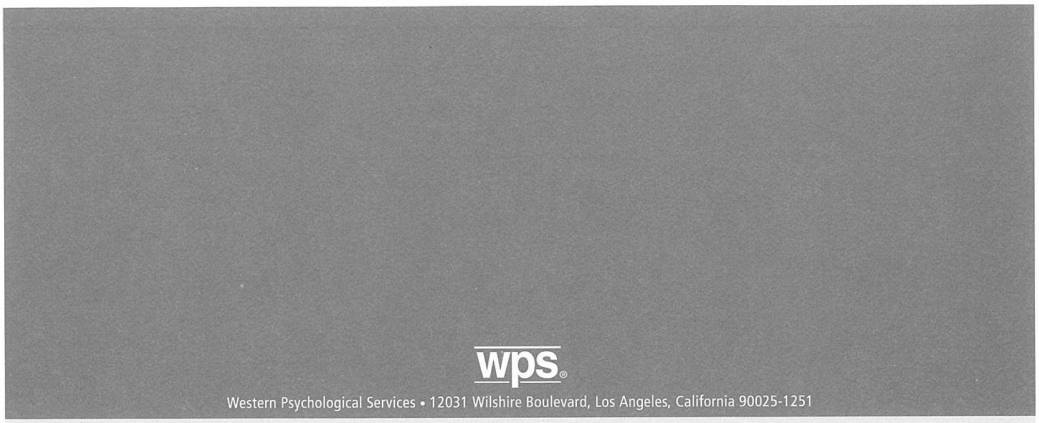
OVERALL ASSESSMENT

Are there any other aspects of [subject]**'s behavior that particularly concern you?** (PROBE ONLY IF POSSIBLY RELEVANT TO ANY OF SPECIFIED CODINGS OR TO DIFFERENTIAL DIAGNOSIS OF AUTISM.)

Is there anything else you would like to tell me about that we haven't covered?

INTERVIEWER'S IMPRESSIONS AND CIRCUMSTANCES OF INTERVIEW
(DESCRIBE. NOTE WHETHER AUDIO/VIDEO RECORDING MADE.)

SUMMARY OF DISCREPANCIES BETWEEN INFORMANT DESCRIPTION AND OBSERVER INFORMATION


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ADI-R Autism Diagnostic Interview-Revised

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Comprehensive Algorithm Form

Subject

Name of Subject/ID:

Date of Birth: / / Chronological Age: Gender: Female Male

Respondent

Name:

Relation to Subject:

Clinician

Name:

School/Clinic:

Date of Interview: / /

Mark the algorithm used.

Current Behavior Algorithm

- ☐ 2 Years, 0 Months to 3 Years, 11 Months
- ☐ 4 Years, 0 Months to 9 Years, 11 Months
- ☐ 10 Years, 0 Months or Older (Verbal Subjects only; Item 30 = 0)

Diagnostic Algorithm

- ☐ 2 Years, 0 Months to 3 Years, 11 Months
- ☐ 4 Years, 0 Months or Older

Item Code to Algorithm Score Conversion

Code	*0*	*1*	*2*	*3*	*7*	*8*	*9*
Score	0	1	2	2	0	0	0

Score Summary

										Diagnostic Algorithm cutoffs*
A1		+ A2		+ A3		+ A4		=	Total A	cutoff = 10
B1		+ B4		+ B2(V)		+ B3(V)		=	Verbal Total B	cutoff = 8
B1		+ B4						=	Nonverbal Total B	cutoff = 7
C1		+ C2		+ C3		+ C4		=	Total C	cutoff = 3
									Total D	cutoff = 1

*Cutoffs are for Diagnostic Algorithms only.
The Current Behavior Algorithms do not have cutoffs.

Additional copies of this form (W-382E) may be purchased from WPS. Please contact us at 800-648-8857, Fax 310-478-7838, or www.wpspublish.com.

382E

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A: Qualitative Abnormalities in Reciprocal Social Interaction

Copy each code into the box for algorithm type and subject age level.



A1: Failure to use nonverbal behaviors to regulate social interaction

Convert to Scores

50 Direct Gaze (Code only if under 5)

51 Social Smiling

57 Range of Facial Expressions Used to Communicate

Total A1

A2: Failure to develop peer relationships

49 Imaginative Play With Peers

62 Interest in Children

63 Response to Approaches of Other Children

64 Group Play With Peers
(score if 4.0 to 9.11 years)

OR *(score either 64 or 65, depending on age of subject)*

65 Friendships
(score if 10.0 years or older) (Most Abnormal 10.0–15.0)

Total A2

A3: Lack of shared enjoyment

52 Showing and Directing Attention

53 Offering to Share

54 Seeking to Share Enjoyment With Others

Total A3

A4: Lack of socioemotional reciprocity

31 Use of Other's Body to Communicate (ever) (ever)

55 Offering Comfort

56 Quality of Social Overtures

58 Inappropriate Facial Expressions (ever) (ever)

59 Appropriateness of Social Responses

Total A4

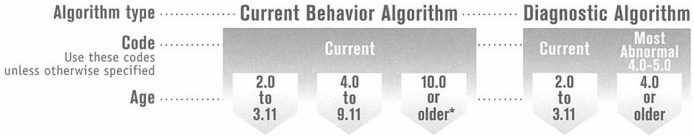
*Current Behavior Algorithm for 10.0 or older should be scored only for verbal subjects (Item 30 = 0). For this age group, no Current Behavior Algorithm is available for nonverbal subjects (Item 30 = 1 or 2).

Total A
A1 + A2 + A3 + A4
(cutoff = 10, diagnostic only)

B: Qualitative Abnormalities in Communication

- Score B1, B4, B2(V), and B3(V) for verbal subjects (Item 30 = 0).
- Score only B1 and B4 for nonverbal subjects (Item 30 = 1 or 2).

Copy each code into the box for algorithm type and subject age level.



B1: Lack of, or delay in, spoken language and failure to compensate through gesture

Convert to Scores

42 Pointing to Express Interest

43 Nodding

44 Head Shaking

45 Conventional/Instrumental Gestures

Total B1

B4: Lack of varied spontaneous make-believe or social imitative play

(B4 is not scored on Current Behavior Algorithm for 10.0 or older.)

47 Spontaneous Imitation of Actions

48 Imaginative Play

61 Imitative Social Play

Total B4

VERBAL SUBJECTS ONLY

B2(V): Relative failure to initiate or sustain conversational interchange

34 Social Verbalization/Chat — ever

35 Reciprocal Conversation — ever

Total B2(V)

B3(V): Stereotyped, repetitive, or idiosyncratic speech

33 Stereotyped Utterances and Delayed Echolalia — ever, ever

36 Inappropriate Questions or Statements — ever, ever

37 Pronominal Reversal — ever, ever

38 Neologisms/Idiosyncratic Language — ever, ever

Total B3(V)

Verbal Subjects

Total B(V)
B1 + B4 + B2(V) + B3(V)
(cutoff = 8, diagnostic only)

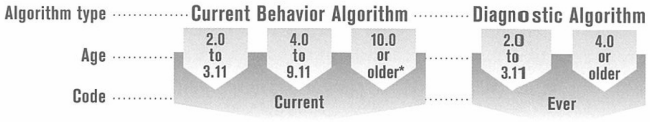
Nonverbal Subjects

Total B(NV)
B1 + B4
(cutoff = 7, diagnostic only)

*Current Behavior Algorithm for 10.0 or older should be scored only for verbal subjects (Item 30 = 0). For this age group, no Current Behavior Algorithm is available for nonverbal subjects (Item 30 = 1 or 2).

C: Restricted, Repetitive, and Stereotyped Patterns of Behavior

Copy each code into the box for algorithm type and subject age level.



C1: Encompassing preoccupation or circumscribed pattern of interest

Convert to Scores

67 Unusual Preoccupations .....

68 Circumscribed Interests *(score if 3 years or older)* .....

Total C1

C2: Apparently compulsive adherence to nonfunctional routines or rituals

39 Verbal Rituals *(score only if Item 30 = 0)* .....

70 Compulsions/Rituals .....

Total C2

C3: Stereotyped and repetitive motor mannerisms

77 Hand and Finger Mannerisms .....

78 Other Complex Mannerisms or Stereotyped Body Movements .....

Record the higher of the two scores

Total C3

C4: Preoccupation with parts of objects or nonfunctional elements of material

69 Repetitive Use of Objects or Interest in Parts of Objects .....

71 Unusual Sensory Interests .....

Record the higher of the two scores

Total C4

*Current Behavior Algorithm for 10.0 or older should be scored only for verbal subjects (Item 30 = 0). For this age group, no Current Behavior Algorithm is available for nonverbal subjects (Item 30 = 1 or 2).

Total C
C1 + C2 + C3 + C4
(cutoff = 3, diagnostic only)

D: Abnormality of Development Evident at or Before 36 Months

Diagnostic Algorithms only.

		Code	Score
2	Age Parents First Noticed *(if <36 months, score 1)*		
9	Age of First Single Words *(if >24 months, score 1)*		
10	Age of First Phrases *(if >33 months, score 1)*		
86	Age When Abnormality First Evident *(if coded 3 or 4, score 1)*		
87	Interviewer's Judgment on Age When Abnormalities First Manifest *(if <36 months, score 1)*		

Total D
(cutoff = 1, diagnostic only)