

The neurobiological underpinnings of developmental stuttering

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St Anne's College
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*A thesis submitted for the degree of
Doctor of Philosophy*

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The aim of this thesis was to investigate the neural underpinnings of persistent developmental stuttering. We explored neural systems important for speech-motor integration and focused on subcortical control systems: the basal ganglia and cerebellum. A secondary aim of this work was to distinguish effects related to general traits of the disorder from those reflecting specific states of stuttered speech. To address these aims we used a variety of neuroimaging methodologies as well as an extensive neuropsychological and empirical test battery. Our examination of neural pathway microstructure using diffusion-tensor imaging replicated previous findings of widespread disorganisation of white matter in people who stutter. This disruption included all major white matter pathways leading in and out of the cerebellum. In our second, third, and fourth studies we examined functional activity at rest and during different types of speech. The brain networks used by people who stutter and controls largely overlapped. The brain regions that distinguished general traits and specific states of stuttering were somewhat task-specific. Subcortical activation in the basal ganglia and cerebellum was related to the frequency of dysfluent speech in the scanner. In our final study we examined performance on a variety of classical tasks of motor learning. We observed evidence of delayed learning in response to changes in environmental feedback in the stuttering group relative to controls. Within people who stutter, subgroups who differ according to heritability of the disorder may also differ in the balance of dopamine in the basal ganglia. Overall, we concluded that cerebellar alterations contribute to the general trait of stuttering, while basal ganglia disruption may reflect specific effects within stuttering. Our work supports a broader role of the subcortical system in speech production, generally.

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Dedicated to:
the first female scientist
I ever knew
who instilled in me
a love for discovery
a respect for nature
and a sense of duty to the world.

To my mother: Vickie Connally

In memory of:
the first man
I ever knew
who always had my back
who told me I could be
or do anything in the world
and who meant it.

To my father: Lavagia Jim Connally

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Statement of Originality

I am the sole author of this thesis. To the best of my knowledge, the contents of this thesis do not infringe on any copyright or other rights including the proprietary rights to ideas of other people. My supervisor, Prof Kate E. Watkins made intellectual contributions including editing of each chapter. Specific contributions to other chapters are acknowledged below.

Chapter 2. Subject recruitment and data collection were completed by Prof. Kate E. Watkins. Dr. Patricia Gough assisted with data collection. We are grateful to Dr. Alina Jurcoane for useful discussions of tractography and our colleagues at FMRIB, particularly Drs. Saad Jbabdi, Stamatios Sotiropoulos, and Prof. Steve Smith for analysis support. We thank Ned Jenkinson for useful comments on the manuscript and anatomy. This chapter resulted in a publication with several co-authors who also made contributions, in particular to interpretation of results:

Connally, E.L., Ward, D., Howell, P., Watkins, K.E. (2014). Disrupted white matter in language and motor tracts in developmental stuttering. *Brain and Language*. 131, 25-35.

Chapters 3, 4, and 5. My neuroimaging studies were part of a collaboration with Dr. David Ward, and data were collected at the University of Reading. The protocol was largely designed by Prof. Kate E. Watkins. Some of the subject recruitment and data collection were completed by members of the University of Readings neuroimaging centre, predominately Dr. Christos Pliatskias. Transcripts of speech in the scanner and resulting fluency coding was completed by three different independent raters: Rowan Boyles, Sarah Finnegan, and Jess Bretherton-Furness. Advice in model design for chapter four was provided by Prof Mark Jenkinson, Dr. Matthew Webster, and Dr. Anderson Winkler. Drs Winkler and Webster also assisted with computer scripts for a portion of one analysis. Results of these studies have been presented publicly with input from Dr. David Ward and Dr. Christos Pliatsikas.

Chapter 6 Dr. Jennifer Chesters and Nia Carson assisted with subject recruitment for this study. Bethann Markall assisted with stuttering

severity assessment through transcribing a portion of the video interviews. This study consisted of a battery of tasks, some of which were designed by other laboratories:

- Implicit Sequence Learning: Stimuli were provided by Dr. Charlie Stagg, assistance with experimental coding and piloting was provided by Dr. Jack Rogers and Rowan Boyles, members of Prof Watkins laboratory.
- Visuomotor adaptation: Stimuli, experimental design, administration scripts, and analysis scripts were provided by Dr. Ned Jenkinson and Dr. Muriel T. N. Panouillres, who also checked some of data for errors and providing training in the data analysis pipeline.
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Abstract

The aim of this thesis was to investigate the neural underpinnings of persistent developmental stuttering. We explored neural systems important for speech-motor integration and focused on subcortical control systems: the basal ganglia and cerebellum. A secondary aim of this work was to distinguish effects related to general traits of the disorder from those reflecting specific states of stuttered speech. To address these aims we used a variety of neuroimaging methodologies as well as an extensive neuropsychological and empirical test battery. Our examination of neural pathway microstructure using diffusion-tensor imaging replicated previous findings of widespread disorganisation of white matter in people who stutter. This disruption included all major white matter pathways leading in and out of the cerebellum. In our second, third, and fourth studies we examined functional activity at rest and during different types of speech. The brain networks used by people who stutter and controls largely overlapped. The brain regions that distinguished general traits and specific states of stuttering were somewhat task-specific. Subcortical activation in the basal ganglia and cerebellum was related to the frequency of dysfluent speech in the scanner. In our final study we examined performance on a variety of classical tasks of motor learning. We observed evidence of delayed learning in response to changes in environmental feedback in the stuttering group relative to controls. Within people who stutter, subgroups who differ according to heritability of the disorder may also differ in the balance of dopamine in the basal ganglia. Overall, we concluded that cerebellar alterations contribute to the general trait of stuttering, while basal ganglia disruption may reflect specific effects within stuttering. Our work supports a broader role of the subcortical system in speech production, generally.

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Chapter 1

Introduction

It's probably not as bad as other people's, but obviously it's the worst thing in my world. — A 21-year-old female describes her experience of stuttering

Stuttering is documented in almost all languages and cultures [242]. Still, we do not know what causes stuttering in children, nor what maintains it into adulthood. Further, several meta-analyses have been conducted to consolidate the correlates of developmental stuttering using both functional [40][42][28] and structural [173] literatures. Given the documented interindividual variability in stuttering (*e.g.* [262]), the failure of these meta-analyses to converge on specific abnormalities within the speech motor system is not entirely unexpected. Functional neuroimaging studies in particular diverge with respect to specific locations and directions of cortical disruptions, the role of subcortical system in stuttering, and whether differences reflect disruption or compensation [40][42][28]. The aim of this thesis is to identify the neural underpinnings of persistent developmental stuttering. We are particularly interested in clarifying what role, if any, the subcortical system (basal ganglia and cerebellum) plays in persistent developmental stuttering.

1.1 What is stuttering?

Stuttering is characterised by interruptions to the smooth flow of speech. Symptoms include: sound, syllable or monosyllabic word repetitions; sound prolongations; or blocking that typically occur at the beginning of utterances (Figure 1.1A). In many cases stuttering coincides with facial tension and increased muscle movement, known as concomitant behaviours. Concomitant behaviours include grimacing, muscle twitches, and tremor, and have been likened to facial tics as seen in disorders like Tourette's syndrome [155][264][229] and are theorised to reflect intensity of dysfluent states [5]. Standard measures of severity typically quantify frequency

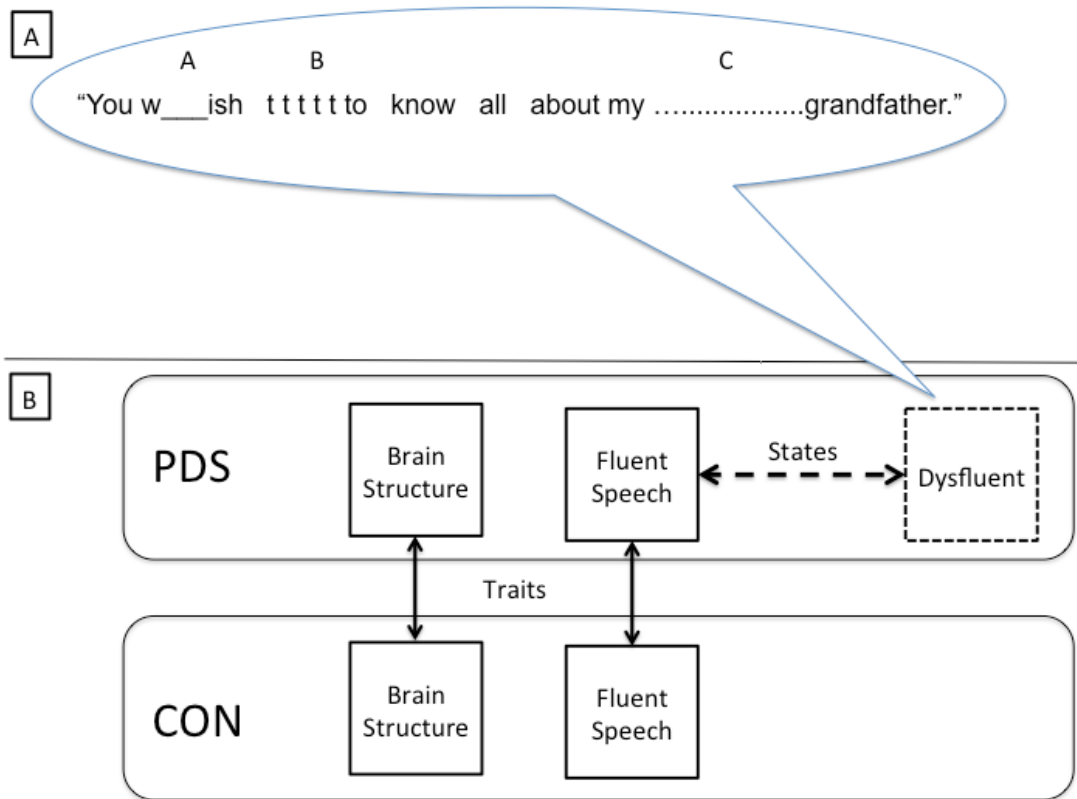
and duration of dysfluent speech states and presence or absence of concomitant behaviours [203].

Developmental stuttering is a disorder that emerges gradually during early childhood with no known cause. Up to one in ten children stutter by age four [201], however progressively fewer children who stutter continue to do so as they age. The rate of spontaneous recovery is 6% before age 4 [201], over 50% by age 12 [112], and 80% before adulthood [11][158]. By puberty, therefore, at least half of developmental stuttering cases are reclassified as recovered. The relatively small subgroup of children who continue to stutter into adulthood (1 in 100, [86]) have a syndrome known as persistent developmental stuttering (PDS).

One approach to PDS research is distinguishing between what causes symptoms of dysfluent speech states and what maintains general traits of the PDS syndrome ([242], Figure 1.1B). The presence or absence of stuttering symptoms defines specific states of speech as either dysfluent or fluent, respectively. Studies that isolate general traits of PDS relative to fluent speakers reflect not only causes but also consequences of dysfluent states in PDS. Because dysfluent states are rare (*e.g.* [177][249]), difficult to predict with 100% accuracy [70], only occur during speech, and remit in most children who stutter [11], the majority of relevant research informs general traits of PDS in adults. The emergence of structural traits across speech development provides a context for tracking which brain-behaviour links are disrupted, and when, in PDS.

Throughout this thesis the term “disruption” is used to describe differences relative to control groups observed in stuttering populations. The formal definition of a disruption derives from disrupt: “to break apart or rupture, to throw into disorder, or to interrupt the normal course or unity of” [163]. When used in this thesis, it is intended as meaning the second and third definitions, *i.e.* associated with a disorder, such as an “interruption” or a “difference” between a patient group and controls, and not as indicating something is specifically broken apart or ruptured. Our intent was to avoid inappropriate use of terms like “impairment” or “abnormality” that have established clinical thresholds (usually 2 standard deviations above or below the mean of standardization groups). Frequently in stuttering research these terms are used to describe subtle effects reported in the literature that could, in theory, be compensation and not impairment, or reflect normal variation (not abnormalities). The definition of disruption allows for an “interruption to the normal course or unity” but does not coincide to statistical thresholds and is therefore preferred as a descriptive term for observed effects.

Figure 1.1: The complexity of stuttering: symptoms, states, and traits.



A. Excerpt from the grandfather reading passage illustrating the symptoms observed during stuttering states: A) prolongations; B) repetitions, and C) blocks. B: Illustration of the dissociation between general traits (vertical arrows) shared by people with persistent developmental stuttering (PDS) that are comparable to traits of fluent controls (CON) and specific speech states of fluent or dysfluent speech (horizontal arrow) within PDS.

1.2 Speech development and stuttering emergence

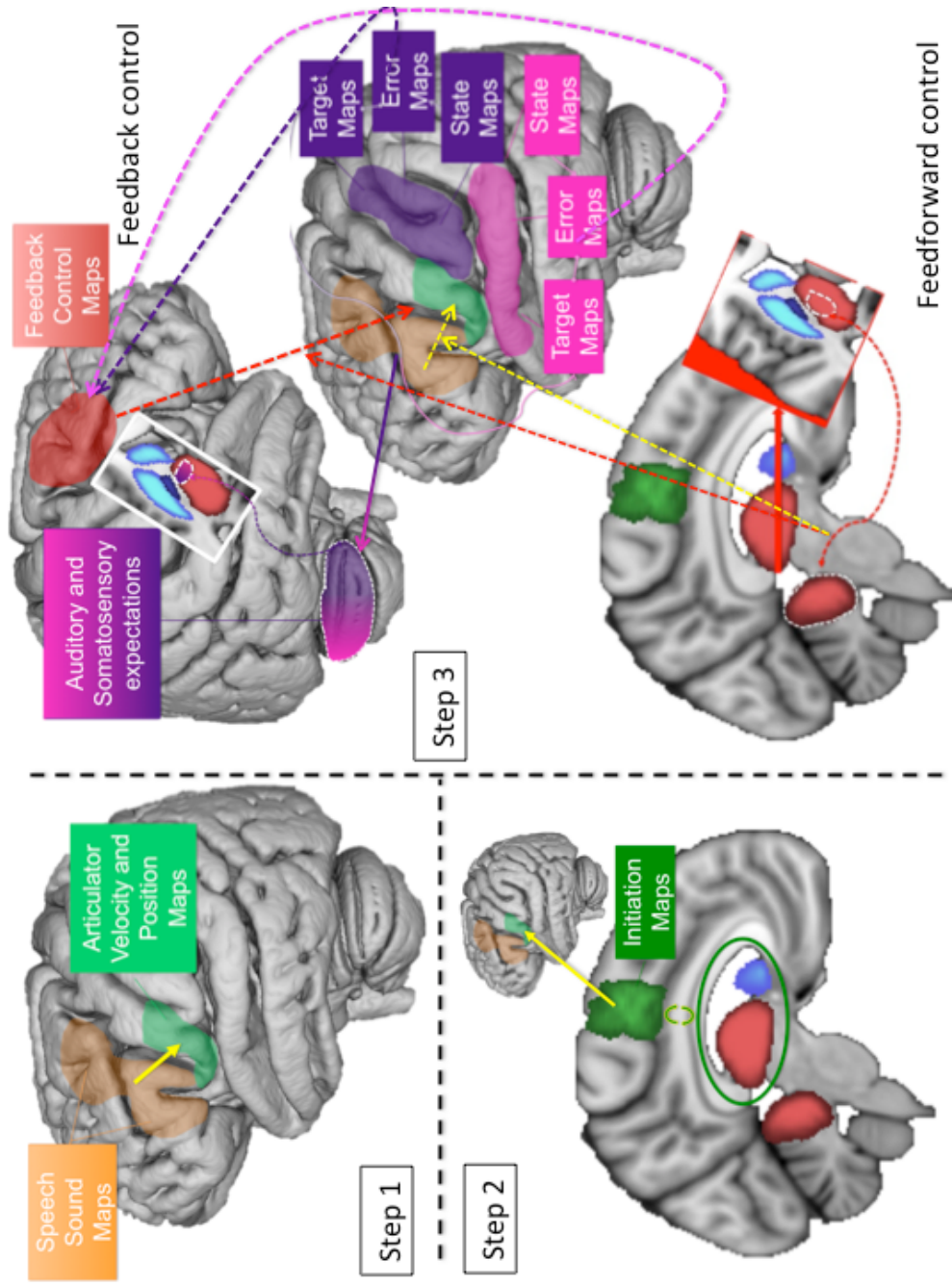
Speech is a procedural skill that requires three basic steps: 1) translating thoughts into motor plans; 2) executing motor plans in sequence; and 3) monitoring the output of these plans [106], Figure 1.2). According to theory, prefrontal cortex translates thoughts into motor plans [184]. In humans this process utilizes speech sound maps, thought to be stored in nearby inferior frontal gyrus [35] and feedforward propagation of articulatory representations ([89], p2872) to the action cells in motor cortex. Execution of motor plans in a sequence requires coordination of basal ganglia nuclei with medial premotor regions and of course the motor cortex signals delivered to the articulators via corticobulbar tracts. Monitoring of procedural movement requires coordination of the lateral and medial frontal, primary sensory

and motor cortices with the cerebellar and posterior parietal cortices that integrate sensory feedback [182]. Overall, the speech process is facilitated by several white matter pathways, which myelinate early in life [196] and continue to mature through adulthood [144].

See Figure 1.2 for an illustration of the circuits that are necessary for the three steps of speech production, according to a popular theory, called the DIVA model [106]:

- Step 1: Speech sound maps in left hemisphere inferior frontal gyrus and ventral premotor cortex (orange areas) project translations of thoughts into action via the articulator velocity and position maps in mouth motor cortex (green areas).
- Step 2: The initiation maps in the SMA (green) are part of a circuit including subcortical grey matter (putamen, caudate nucleus, globus pallidus; blue areas, and thalamus; red) that executes sequential movements via cues to action maps in mouth motor cortex [58].
- Step 3: Monitoring of speech output involves two control subsystems, a slow feedback control system, and a rapid feedforward system. The feedback control system (top and middle) relies on auditory and somatosensory feedback, which is processed by the state maps in respective cortices (pink and purple). Sensory expectations of action plans are delivered from speech sound maps in frontal cortex to lateral cerebellum - ventral anterior thalamic nucleus loops, as well as target maps in relevant sensory cortices. The target maps, together with state maps in the sensory cortices, update local error maps which then tune action representations via feedback control maps in right hemisphere ventral premotor cortex (top, red). The right ventral premotor cortex then projects directly to the mouth motor cortex as well as via a medial cerebellar- ventrolateral thalamus loop (bottom, red dashed arrows) to deliver the feedback commands. The second system is a feedforward control system (middle and bottom, yellow arrows) which is very rapid, and reliant mostly on efferent copies directly from ventral premotor cortex to motor cortex, as well as via the medial cerebellar - ventrolateral thalamus loop (red; bottom) [106].

Figure 1.2: An illustration of the circuits that underly the DIVA model [106]



1.2.1 Speech sequence skills coincide with stuttering onset

Before age four, children have mastered speech sounds, are just starting to build sentences [141], and roughly 95% of PDS case emerge. Many cases emerge with only the speech symptom, but one in three present with concomitant behaviours [34]. Near the age of onset, childhood stuttering is associated with disruptions to the white matter pathways in the brain, including a critical component of speech-motor infrastructure: the premotor-subcortical loop [55]. The connections between this loop and regions critical for sensory processing, including auditory cortex [55] and the cerebellum [51] are also disrupted in early childhood stuttering.

The premotor-subcortical loop is theorized to execute motor plans in sequence: According to a specific model of speech production, the DIVA model, the premotor cortex builds and thereafter releases components of speech [106], while the basal ganglia is thought to function as gate circuits that signal this release by estimating the likelihood of success of motor plans [58]. If the putamen and SMA circuit facilitates the timely release of sequence plans [35], weakening of this link in childhood stuttering would be consistent with theories of improper plan execution [114] and impaired basal ganglia function [4] in PDS. Further, disruption of the connections between the subcortical-premotor circuit and regions critical for sensory processing support a theory of altered sensory integration in stuttering [160], which accounts for higher variability in coordinated movement timings observed in childhood stuttering [179] that persist into adulthood [269] and abnormal speech processing by the auditory cortex observed in preschoolers who stutter, which are also largely maintained in adult PDS [208].

1.2.2 Feedforward control circuits are disrupted in childhood

Before age nine, fluent children show coordinated activation of two resting state networks in a left-lateralised circuit during expressive language tasks [255]. The new resting state networks include: 1) a premotor-expansion to the sensorimotor network available at birth [272]; and 2) a separable network extending from frontal operculum throughout the inferior frontal gyrus and anterior insular cortices [272], which might/could eventually facilitate rapid “efferent copy” mismatch mapping in speech [106]. At this age in children who stutter, white matter disorganisation extends to left language and major motor pathways, bilaterally, including pathways subserving left hemisphere mouth motor region [52]. Several stuttering-related reductions in grey matter volume also surface at this time: 1) in lateral and medial premotor cortices, bilaterally ([17][52]); 2) in the left putamen and the forceps minor, bilaterally [17] ; 3) and in the right hemisphere caudate nucleus [93] and inferior frontal cortex [170].

1.2.3 Disorganisation is maintained into adulthood

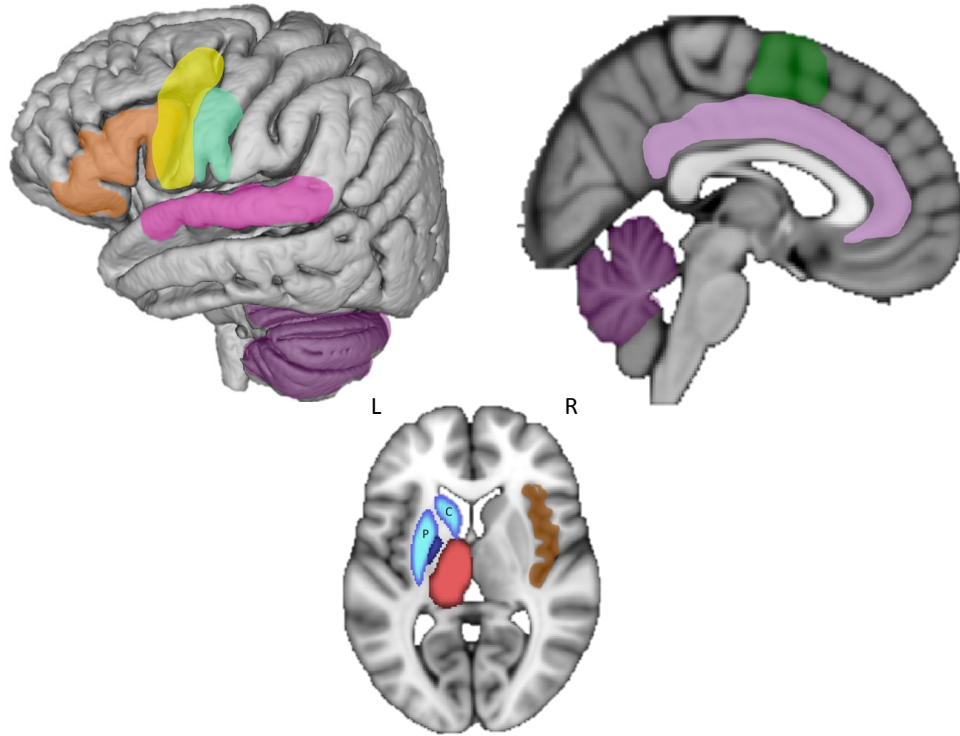
By adolescence, single-symptom PDS profiles are nonexistent [34] [242] and corresponding structural disruptions are numerous, but sometimes inconsistent across studies. Differences in grey-matter volume or cortical thickness in PDS are rare and varied. Reported perisylvian abnormalities ([90][65]) failed to replicate in a much larger, independent sample [102]. Reduced volume of the left caudate nucleus in adult PDS relative to controls [223] is a reversal of findings in children [93]. Bilateral reductions in the size of portions of the inferior frontal cortex observed in middle childhood ([17][52]) are left-lateralised in adults ([152][137]) and associated with alterations in resting state functional connectivity [152], white matter connectivity [137], and worsened stuttering severity [137]. However, differences in inferior frontal cortex size are not always significant in PDS [223].

While grey matter abnormalities are less consistent, white matter disruptions are well replicated in adolescents [249], and adults ([220]. What appears to be a circuit-specific disconnection between subcortical control and premotor planning regions at stuttering onset [55] develops into disorganised sensorimotor connectivity even after complete symptom recovery [52]. By early adulthood, disorganisation of white matter pathways is no longer limited to the subcortical-premotor-auditory circuits but is instead observed bilaterally throughout the speech motor system, as are accompanying functional abnormalities, regardless of speech conditions or fluency [249]. White matter disorganisation further corresponds to altered connectivity at rest with other components of the sensorimotor system [53] and increased stuttering severity [44]. Widespread structural disorganisation in adult PDS is reflected in several other general traits of stuttering, including network-level disruptions of task and rest-related functional activity and behavioural abnormalities in non-speech tasks that rely on shared speech-motor system pathways.

1.3 Underpinnings of functional traits

In adults with PDS, widespread disruption within the speech-motor system has been documented using positron emission tomography (PET) and blood oxygen level dependent functional magnetic resonance imaging (BOLD fMRI) for over thirty years (Table 1.1). Each region of the speech-motor system is implicated in at least ten independent reports of PDS (Figure 1.3). Further, not some, but all of the major components of the speech-motor network show altered activation in the same stuttering individuals in over half of published reports, which is largely consistent with conclusions drawn following three meta-analyses of stuttering research ([40][42][28]). The specific locations and roles of disruptions vary from report to report.

Figure 1.3: Widespread disruption is reported in stuttering



A selection of speech-motor regions showing functional activation differences in PDS relative to fluent controls in at least ten independent reports: Lateral left-hemisphere cortex: Inferior frontal gyrus (orange), ventral premotor cortex (yellow), primary mouth motor cortex (sea green), auditory cortex (pink), and cerebellum* (purple). Medial surface: SMA (green), cerebellar vermis (purple). 3) Axial slice of subcortical grey matter: striatum (light blue, P = putamen, C = caudate nucleus), globus pallidus (dark blue), thalamus (red) and insula (brown). The left hemisphere is illustrated here for convenience, however, the cerebellum is implicated in almost all studies of PDS, including all four cerebellar lobes and the medial surfaces.

Table 1.1: A summary of functional neuroimaging reports reviewed for this thesis.

Study	modality	PDS N	CON N	Age	gender	handedness	premotor cortex	primary motor	primary auditory	insular cortex	thalamus	cerebellum	cingulate gyrus	basal ganglia caudate nucleus putamen globus pallidus caudate nucleus
Wu 1995	PET FDG	4	4	20-54	M/F	right			X			X	X	basal ganglia caudate nucleus
Fox 1996	PET 15-O	10	10	21-55	M	right	X	X	X	X	X	X	X	putamen
Braun 1997	PET 15-O	18	20	23-51	M/F	right	X	X	X	X	X	X	X	globus pallidus caudate nucleus
Wu 1997	PET FDOPA	3	6	21 - 69	M	right								
De Nil 2000	PET 15-O	10	10	20-45	M	right	X	X		X	X	X		putamen, caudate nucleus caudate nucleus
Ingham 2000	PET 15-O	4	4	28-50	M	right	X	X		X	X	X		
Fox 2000	PET 15-O	10	10	21-55	M	right	X	X		X	X	X		
De Nil 2001	PET 15-O	13	10	20-35	M	-						X		
Neumaan 2003	fMRI	16	16	26-41	M	1 left	X	X	X	X		X	X	putamen, globus pallidus
De Nil 2003	PET 15-O	13	10	19-40	M	right	X	X	X	X	X	X	X	putamen, caudate nucleus, globus pallidus
Preibisch 2003	fMRI	16	16	18-51	M	5 left	X	X	X	X	X	X	X	
Ingham 2004	PET 15-O	10	10	25 - 75	F	right	X	X	X	X	X	X		putamen, caudate nucleus, globus pallidus
Neumaan 2005	fMRI	9	0	24-41	M	1 left	X	X	X	X	X	X	X	
De Nil 2008	fMRI	15	15	21 - 48	M	right	X	X	X	X	X	X		
Watkins 2008	fMRI/DTI	12	10	14 - 27	M/F	1 left	X	X	X	X	X	X		putamen caudate nucleus
Giraud 2008	fMRI	16	0	18-48	M	2 left							X	
Kell 2009	fMRI/DTI	26	13	16-65	M	mixed	X	X	X	X	X	X		
Chang 2009	fMRI	20	20	23 - 46	M/F	right		X	X	X	X	X		putamen
Lu 2009	fMRI	10	9	20-29	M/F	right	X	X	X	X	X	X		putamen
Salek 2009	fMRI	8	10	20-53	M/F	right	X							
Lu 2010	fMRI	12	12	20-29	M	right	X	X	X	X		X		putamen
Chang 2011	fMRI/DTI	23	23	33, 35	M/F	right	X	X	X	X	X	X	X	caudate nucleus globus pallidus
Toyomura 2011	fMRI	12	12	18-55	M/F	1 left	X	X	X	X	X	X	X	putamen, caudate nucleus, globus pallidus
Ingham 2012	PET 15-O	18	12	20-67	M	right	X	X	X	X	X	X		putamen
Howell 2012	fMRI	9	9	20-29	M/F	right	X	X				X		
Lu 2012	fMRI	28	13	22-35	M	right	X					X		putamen, globus pallidus
Jiang 2012	fMRI	20	0	17-38	M	right	X	X				X		
Xuan 2012	fMRI	44	46	17-37	M	right	X	X				X		
Wymbs 2013	fMRI	4	0	19-25	M	right	X	X	X	X		X	X	putamen, caudate nucleus, globus pallidus
Chang 2013	fMRI/DTI	20	26	3 - 9	M/F	right	X	X	X	X	X	X	X	
den Ouden 2014	fMRI	1	1	46-73	M	left	X	X	X	X		X	X	putamen, globus pallidus
Toyomura 2015	fMRI	10	10	20 - 33	M	right	X	X	X		X	X		putamen, caudate nucleus, globus pallidus
Sitek 2016	fMRI/DTI	20	19	18-47	M/F	right	X	X	X		X	X		putamen, caudate nucleus
Chang 2016	fMRI	17	17	6 - 11	M/F	-	X		X			X		
Yang 2016	fMRI	16	18	21 - 35	M/F	right	X	X				X	X	

1.3.1 Network representations during tasks

Eight different studies have investigated contrasts of speech relative to “rest” in right-handed individuals, which resulted in a general consensus of increased activation of premotor, primary motor, and cerebellar cortices in the presence of decreased auditory cortex activation for adult PDS relative to controls ([95][39][122][53][123][262][231][210]). In almost as many studies, however, activation of premotor, primary motor, and auditory cortices is positively correlated, such that increases in one of these regions are associated with increases in the others ([175][177][68][137]) and accordingly, decreases are associated with decreases ([54][151][249]). Cerebellar abnormalities are common to almost all reports, however, in some cases co-activation is positively correlated with relevant cortex ([137][68][54][151]) whereas in others, cerebellar signal change is in the opposite direction of cortical abnormalities ([249][175][177]). The differences between speaking conditions are exaggerated in PDS relative to fluent controls [123], and alterations in PDS are somewhat task dependent [54].

During perception, and planning of speech and non speech sounds [54], nonsense speech repetition [53], and silent semantic decision making [194], speech-motor system activations are generally decreased in PDS. On the other hand, speech and non-speech production tasks both show increased recruitment of speech-motor regions in PDS [54]. In the presence of altered white matter organisation, both “hyper” and “hypo” activation patterns are observed in the same individuals during nonsense speech repetition [53], as well as during speech under different types of feedback [249]. The separable planning and execution phases of speech are associated with bidirectional abnormalities not only in BOLD fMRI [151], but also in MEG [206]. The role of abnormalities as disruptive or compensatory is rather unclear, given the low frequency of dysfluent states in the scanning environment.

Stuttering rates (typically percentage syllables stuttered) range from less than 1% [137], to less than 1.5% [177], to 3% [231] and almost never more than 10% ([175][100]). Since most speech is fluent in the scanner, over activation in PDS is typically considered compensatory. For example, increased recruitment of right inferior frontal cortex, not present children who stutter [52], is thought to be compensatory in adulthood ([39][94][175]). Increased cerebellar recruitment is likewise interpreted as compensatory [40], even though cerebellar anomalies are the single most common alteration in the stuttering literature: not only in multiple imaging modalities, PET [68], fMRI (*e.g.* [249], and perfusion [71]); but also under varied conditions including speech related activation (*e.g.* [95][39]), non-speech tasks (*e.g.* [53][231]), and periods of rest (*e.g.* [71][210][152][267]). Without knowing which alterations correspond specifically to dysfluent speech, it is difficult to interpret differences in stuttering beyond a general vulnerability of the speech-motor system.

1.3.2 Network representations at rest

Resting state fMRI (rfMRI) allows measurement of functional traits of PDS without the confound of different speech states. Patterns of correlated activity at rest reflect network connectivity. Differences in connectivity in PDS have been observed throughout the speech-motor system, and findings are generally inconsistent (see Table 1.1). Alterations to patterns of correlated activity at rest have been observed using PET imaging (FDOPA, [261] O-15, [123]), and rfMRI ([263][267][210][152]). Most often, connectivity is altered within networks including primary sensorimotor cortices and subcortical structures including the basal ganglia [267], SMA [152][263], and cerebellum [210][267][152].

Altered connectivity at rest occurs between the cerebellum and multiple resting state networks, and also between lobules within the cerebellum [267]. Specifically, intracerebellar connectivity is disrupted between lobules most connected to the motor system and those associated with higher level cognitive function [267]. Cerebellar co-activation is also related to stuttering severity within the resting motor network and “default mode network” [267] as well as the cerebellar-midbrain-thalamic circuit [210]. Further, altered cerebellar connectivity within the sensorimotor network at rest normalises following treatment [152]. Overall, functional neuroimaging reports of PDS corroborate disruption throughout the speech motor network including the cerebellum, which supports several theories of stuttering that further predict corresponding behavioural consequences outside the speech domain in PDS (*e.g.* [160][4][220][249]).

1.3.3 Behavioural traits suggest motor disruption

Behavioural differences in PDS may not be limited to sequential movements in the speech domain. Some early work reported difficulty with basic syllable production in stuttering, though findings are not consistent across all studies (pgs 185-188, [34]). Further, difficulty with nonsense syllable production that is reported in adults who stutter [218] could be evidence of a generalized initiation problem not specific to learned grammatical speech or sequential movement. Additionally, increased task demands may be selectively difficult for people who stutter, as evidenced by error prone repetitive sequence tapping [250] slower bimanual tapping [251], and disrupted sequence learning under dual performance conditions [218]. Such non-speech motor disruptions are consistent with several theories of stuttering including the disconnection hypothesis [220], a basal ganglia hypothesis [4], altered sensory-integration [160], impaired internal timing mechanisms [84], and the general motor theory that PDS is caused by an inherent deficit in motor skills [171][241][155].

Potential differences in motor function in stuttering generalise beyond dysfluent speech states and the facial muscles [159]. Movements of the jaw and flexion of

specific finger muscles were delayed in one study [159], manual sequence learning reaction times were variable in another, [215] and gains with sequence tapping practice were reduced in PDS relative to fluent controls in a different report [217]. When reaction times are equivalent, responses are still more variable in stuttering, not only in motor timing [159], but also in sensory processing [149] and nonsense word sequence training [16]. Unstable motor skills would be exacerbated by the rapid demands of speech [171] and are further supported by variable excitability of motor cortex in PDS, as measured by transcranial magnetic stimulation (TMS) [173]. Because these reports have not been independently replicated, and have small sample sizes (which is typical of stuttering research), we should take care not to over interpret these findings.

Variations in cortical excitability in PDS are linked to the physical proximity of the muscle of interest to mouth motor cortex. Reduced tongue motor cortex excitability is thought to reflect an orofacial motor deficit of “restricted range of neuronal dynamics at rest” in PDS (p 63, [173]). Nearby cortex facilitating the first dorsal interosseous finger muscle [140][189], also shows reduced excitability in PDS [43]. The abductor digiti muscle, located furthest from mouth motor cortex [140][189], however, is associated with increased resting and action thresholds [221], particularly in the left hemisphere [6], and increased variability in excitatory potential of this muscle in PDS [219]. Either a disconnection hypothesis [220] or abnormal sensory integration hypothesis [160] can account for excitability differences: the reduction of motor thresholds is consistent with loss of sensory input, whereas increased thresholds are consistent with loss of motor output [221]. A dopamine imbalance view can also account for the differences, however, as fluctuations in striatal dopamine can either reduce or increase excitability of motor cortex, depending on the specific type of dopamine and location of imbalance (see [4] for discussion of findings in dystonia).

Resolving dual explanations for motor behaviour disruptions in stuttering is relatively straightforward if one looks past explicit motor sequencing tasks to more classic motor learning skills. Visuomotor adaptation uses sensory feedback to update internal motor maps and is associated primarily with cerebellar, but not dopaminergic dysfunction [78]. Visuomotor adaptation performance can inform the sensory-integration hypothesis of stuttering, which is supported by evidence of decreased auditory activation in stuttering [239], altered motor-to-sensory priming in auditory cortex [206], enhanced fluency with a variety of alterations to sensory feedback during speech [39], and disrupted connectivity underlying map storage in inferior frontal cortex and the cerebellum [249]. Reinforcement learning paradigms, on the other hand, show sensitivity to changes in extracellular dopamine [96], which is not abundant in the cerebellum. Performance on tasks of statistical inference can address the dopamine hypothesis of stuttering to determine whether PDS

reflects “abnormal elevations of cerebral dopamine” (p 92, [157]); altered striatal metabolism [260]; or imbalance in the basal ganglia-cortical circuit [4]. It is unclear whether the speech motor system disruptions hypothesised to underly non-speech behavioural differences also underpin dysfluent states in PDS.

1.4 Underpinnings of specific speech states

Direct comparisons of dysfluent to fluent speech states within an individual with PDS are rare. Of over thirty published neuroimaging studies in PDS (Table 1.1), only four directly compare speech that was confirmed as dysfluent to fluent states within individuals ([222][134][262][70]). Across these studies, speech-related activity recruited the same fundamental network regardless of fluency, consistent with studies indicating similarities in activation are also far greater than differences in trait research comparing PDS to fluent controls [95] [123]. To a certain extent, relationships between neural activation and stuttering severity or treatment gains can supplement the dearth of direct examinations of dysfluent speech. However, in some studies stuttering severity is simply defined as the percentage of syllables stuttered (*e.g.* [100]), whereas in other studies a standardized instrument is used that calculates a composite score using stuttering frequency, duration of stuttered events, and subjective severity of concomitant behaviours (Stuttering Severity Instrument, [203], *e.g.* [204]). The common theme in assessments of stuttering severity is a proclivity to more frequent dysfluency [28], which may involve different circuits to those underlying the presence or absence of dysfluent speech, respectively.

1.4.1 Speech states within individuals

The specific directions and locations of state related alterations in task-related functional brain activity differed widely from individual to individual, even within studies [262]. Specific studies can loosely be grouped according to those showing reduced recruitment of language dominant inferior frontal cortex during dysfluent speech [70][222], those studies showing increased recruitment of the speech-motor network during dysfluent speech [262][222], and a single study reporting both these patterns of alterations and suggesting they underlie different specific stuttering symptoms [134].

1.4.1.1 Reduced recruitment of inferior frontal activity during dysfluent states

Case studies are exciting for attempts to capture subject-specific fluency measures in the scanner, but as a general rule, the results are difficult to generalise to the

larger population. One case involved a 73 year-old left-handed male who exhibited atypical language dominance to a self-generated word list [70]. His stimuli were chosen according to likelihood to evoke different speech states [70]. He was fairly, but not 100% accurate: 10% of his “fluent” list evoked dysfluent states and one of his “dysfluent” words was spoken fluently [70]. Activation during dysfluent states (n=52 trials) was directly compared to fluent states (n=44 trials). With the exception of increased motor cortex recruitment, dysfluent utterances were largely associated with decreases in the functional brain network associated with fluent speech: namely medial and inferior frontal cortex.

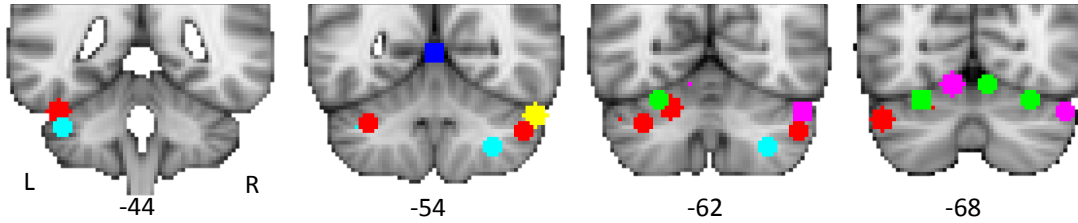
In a single MEG case study, speech “blocks” were preceded by reductions in left inferior frontal cortex activity for up to 800 ms post-stimulus onset relative to fluent speech [222]. Additionally, prior to vocalisation, between 300 and 600 ms post-stimulus onset, activity increased in sensorimotor, auditory, and inferior posterior parietal cortices, bilaterally and throughout the right hemisphere inferior frontal premotor cortex for blocked relative to fluent speech [222]. Widespread overactivation during blocking was interpreted as compensation otherwise unnecessary during fluent speech, which successfully recruited the left inferior frontal cortex. Decreased recruitment of left inferior frontal regions is consistent with the “disconnection” hypothesis of stuttering that argues white matter disorganisation interferes with signals to and from this region [220] [249] [173].

1.4.1.2 Increased recruitment of speech-motor network and cerebellar cortex in dysfluent speech

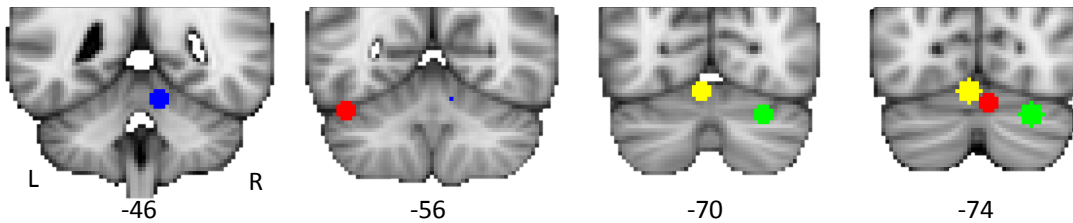
In a small sample of four right handed adult males, BOLD activation increased throughout the classic speech-motor network for dysfluent relative to fluent states across all individuals [262]. In this design, participants read words aloud and experimenters later selected two subsets of stimuli (n=12 each), specific to each individual, confirmed to be fluent or dysfluent [262]. Individual variability in the specific location of alterations was very high in this study. The one exception to this was the over activation of left lobule IV of the cerebellum in all four subjects for dysfluent relative to fluent items (Figure 1.4, top). Overactivation of this region is commonly interpreted as a compensatory trait stuttering [40] [42] [28]. Because lobule IV is selectively activated during tasks of visuomotor adaptation [29], disruption in this region would strongly support views of altered sensory-motor integration in PDS [160].

Figure 1.4: Cerebellar state effects reported in stuttering

Cerebellar activation increases during dysfluent states



Cerebellar activation normalizes following intervention



Cerebellar alterations in PDS are widespread during dysfluent states (top) as well as within individuals in response to treatment (bottom). There is very little overlap across studies. Top: Locations of state effects observed in the cerebellum in PDS (MNI space Y axis coordinate). The majority of peaks observed in individuals with PDS reflect increased activation for dysfluent relative to fluent speech states, organized by individual subjects in [262]: 1= red, 2 = green, 3 = dark blue, 4 = pink. The one exception is a single peak for which fluent speech increased cerebellar activation over dysfluent in a single subject (shown in yellow). Group effects are shown in bright blue and indicate increased activation for less typical dysfluent speech symptoms relative to more typical symptoms as well as fluent speech [134]. Bottom. Locations showing treatment effects in cerebellar activation within PDS following: 8 weeks rhythm practice (blue, [231]), 3 weeks fluency shaping therapy (speech= red, semantic decision task = green, [175]), and short term speech training influencing resting state functional connectivity strength (yellow, [152]).

1.4.1.3 Alterations in inferior frontal and subcortical activity during dysfluent speech may be symptom-specific

Different stuttering symptoms show separable activation patterns according to how “typical” they are [134]. “More” typical symptoms - prolongations, repetitions, and blocks- increased recruitment of left inferior frontal and precuneus cortex compared to other symptoms and fluent speech. Less typical symptoms - multiple character (phrase) repetitions and pauses between characters - increased recruitment of the putamen and cerebellum, bilaterally, and right globus pallidus more so than the “more” typical symptoms and fluent speech. In speech-motor regions, the magnitude of brain activity related to fluent states was reliably in between the extreme highs

and lows associated with dysfluent symptoms [134]. The dissociation of symptoms was consistent enough to survive pattern classification training and testing, suggesting that within the same networks and individuals, different types of symptoms are associated with either excessive subcortical or excessive cortical activation in PDS [134]. The presence of this seemingly counterintuitive “bidirectional” altered activation in stuttering is consistent with several other reports [249] and theories of dopaminergic system imbalance [4].

1.4.2 Frequency of dysfluent states within individuals

Two indirect ways of assessing state effects within stuttering individuals are to compare natural speech to short-term fluency enhancement conditions or to compare natural speech before and after therapy. These methods allow us to look for neural correlates of changes in speech dysfluency, or in the proclivity to frequent dysfluent states. Across these sorts of studies, the basal ganglia and cerebellum are frequently implicated.

1.4.2.1 Basal ganglia

Dysfluent states occur primarily at the beginning of speech attempts, which is thought to reflect inability to initiate the components of the speech sequence [4]. This deficit is alleviated by the “rhythm effect” in PDS: a consistent characteristic of fluency enhancement techniques is the presence of an external timing cue [4]. The simple act of listening to a metronome normalises altered caudate nucleus activation in PDS [231]. Longer term interventions normalised reductions in basal ganglia activity in the caudate nucleus following 8 weeks of metronome-based training [231] and in both the putamen immediately and in the red-nucleus portion of the midbrain two years after another form of speech therapy [175]. The latter - a 3-week intensive fluency shaping course - attenuated the relationship between frequency of dysfluent states and altered reading-related activation in the striatum in several samples ([175][100][137]).

1.4.2.2 Cerebellum

Altered sensory feedback is another mechanism shared by several interventions in PDS: metronome-paced speech [231]; delayed feedback [204] [249]; reading in unison or choral speech [95]; and frequency-shifted feedback [249]. Adjustments to feedback would involve the cerebellar circuits. Accordingly, cerebellar anomalies in PDS normalize following interventions, reflected in: 1) speech-related activation following 8 weeks of metronome training [231]; 2) both reading and semantic-decision task activation following 3-weeks of intensive fluency shaping [175]; and resting state

functional connectivity within the sensorimotor system following one week of a word repetition intervention [152] (Figure 1.4, bottom). The commonly reported cerebellar over activation [40], also decreases gradually following an intensive fluency treatment and maintenance program [67], but does not always attenuate following therapy [137]. Within PDS subgroups, activation is negatively correlated with stuttering severity in a larger network including the cerebellum, substantia nigra, insula, and inferior frontal cortex [175].

1.4.2.3 Speech-motor cortex

Cortical portions of the speech motor network show some relationship to fluency gains, as well. Absent or decreased auditory cortex activity in PDS [40] is increased bilaterally in the short term by altered auditory feedback such as formant shifting [26] and in the long term by fluency shaping therapy [175][176]. Fluency shaping therapy does not always attenuate abnormal activation of auditory cortex [137], however, nor does metronome based intervention [231]. Both metronome based [231] and fluency-shaping therapies [176] normalise altered motor cortex activation, however the relationship between rolandic operculum activity and stuttering severity is not altered by treatment [137]. Generally, the relationship between frequency of dysfluent states and activation in primary cortices support a sensory-integration hypothesis of stuttering [160].

Overall, the subcortical systems (basal ganglia and cerebellum) are implicated in direct comparisons of speech states as well proclivity to more frequent dysfluent speech states. As is the case in much of the stuttering literature, the specific locations and directions of the disruptions vary across studies. An important final consideration is the need to clarify sources of heterogeneity (*e.g.* age, severity, handedness, sex, heritability) and their roles in underpinning state and trait effects within PDS.

1.5 The heterogeneity of stuttering

An inherent challenge in PDS research is the heterogeneity of the population. In addition to the progression of symptoms and associated neural markers with age, severity of these symptoms can vary from day to day within individuals. Sinistrality, or left handedness, is sometimes increased in populations of children who stutter (25% relative to 14% [49]), which is thought to reflect abnormal cerebral dominance in PDS [232], supported by altered lateralisation of functional activity during speech processing [208]. Altered speech dominance is not common, even in sinistral individuals (less than 33%, [31]), and PDS occurs in only 1% of the total population [86]. If we use these base rates (25%, 33%, and 1%) we can calculate

the likelihood that a person who stutters will be sinistral, and show altered cerebral dominance (.0008) At most, therefore, eight of every ten thousand people will fall into this subgroup of stuttering. This proportion is simply not representative of the additional 92 people in the same sample of 10,000 who could have PDS. On the other hand, up to 80% of the same sample could be male, and 70% could have a familial risk to PDS, making sex and heritability more reasonable causal risk factors for consideration.

1.5.1 The sex ratio in stuttering

Right-handed males, the largest “homogenous” subgroup of PDS, are often the sole focus of research. At stuttering onset, males show a slightly higher risk of stuttering than females (1.8 male to 1 female, [49]). In adults, the gender disparity increases to anywhere from 2:1 [162] to almost 4:1 [33][86], presumably due to a higher recovery rate among females [265]. In individuals related to other people who stutter (familial stuttering) the male to female ratio is typically lower than 2.5 [127][81]. In PDS without relatives who stutter (idiopathic stuttering), however, the gender disparity is anywhere from 3.5 [7] to over 4.5 times higher than in familial stuttering [81]. The frequently reported “4 to 1” gender imbalance in stuttering [264] is equivalent to averaging across familial and idiopathic subgroups, potentially masking a critical interaction between hormonal and heritable risk in PDS.

1.5.2 Heritability of stuttering

Risk of stuttering at any point in life is three time more likely in families with first-degree relatives who stutter than in the wider population [138] [8]. Therefore familial stuttering is more common than idiopathic stuttering, with some estimates of heritability around 70% ([192][118][7][86]). Recovered stuttering is also more likely in families with members who recover [266]. Heritability is not limited to PDS: having family or personal history increases risk of acquired stuttering, as well [105].

Genetic markers of stuttering are linked to multiple loci on at least 8 different chromosomes ([80][200][85][209][202][108]). Chromosomal linkage and gene expression is somewhat specific to ancestral descent, with different loci implicated in European lineage [227], south Asian samples [80], North American groups and a Brazilian sample [108]. FOXP2 has high expression in the basal ganglia relative to cerebral cortex, brain stem, and cerebellum [108] and impairs song learning in songbirds when knocked out [107]. Because FOXP2 expression is not more common in familial stuttering than in fluent controls [108], random mutations in this gene could play a role in idiopathic stuttering via disruption of speech rhythm learning.

1.5.3 Stuttering subgroups

Given the differences in sex ratios and genetic associations, familial and idiopathic stuttering likely represent different aetiological subgroups. Speech symptoms largely overlap between subgroups, as do the consequences of stuttering such as anxiety [7], social isolation, or efforts at conscious control of speech [201]. Classic motor learning tasks can tap function of different circuits of the network necessary for speech production without confounding interpretation with shared symptoms. For example, if the DIVA model of speech holds, then this procedural skill requires translating thoughts into plans, sequential execution, and monitoring (Figure 2) that relies on brain regions shared with the motor system. We can tax motor planning, sequential execution, and feedback based learning without ever requiring people to speak. In this way, we have the potential to determine how many, if any, of these steps are disrupted outside of the speech domain. If so, we also have the potential to dissociate subgroups of PDS based on whether they share or have different disruptions to the specific functions required for speech

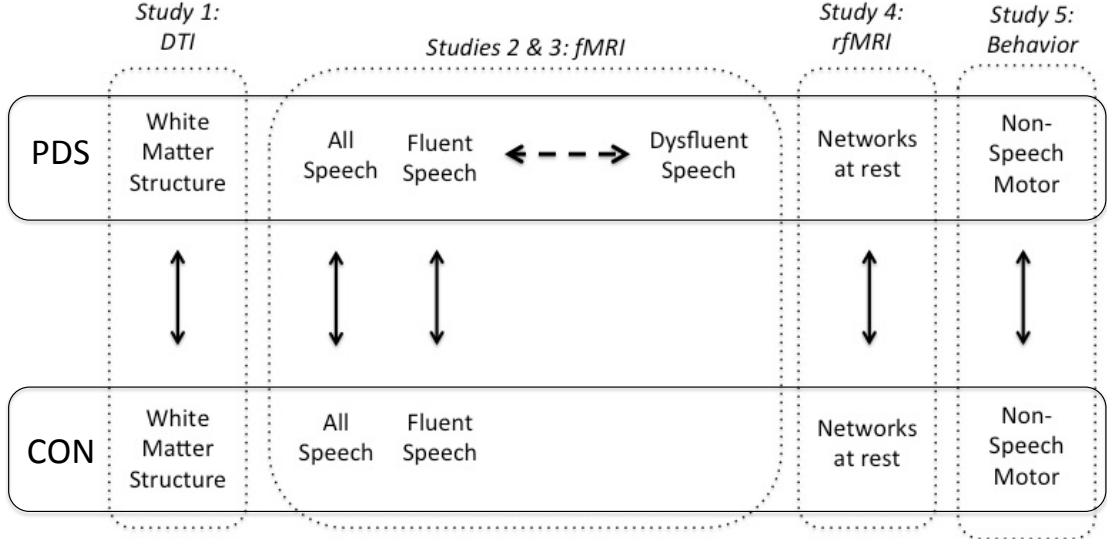
1.6 The scope of this thesis

The primary aim of this thesis is to identify the neural underpinnings of PDS (Figure 1.5). We address this question through first identifying general traits of stuttering, and then speculating about specific underpinnings of the dysfluent state. The model system for this research is the speech motor network, with specific focus on the subcortical control units of the brain: the basal ganglia and cerebellum. A secondary aim is to document sources of variability in PDS. Finally, the broader impact of this thesis is the potential to better understand the neural circuitry that facilitates smooth speech production.

1.6.1 Study 1: White matter structure

We used diffusion weighted imaging (DWI) to replicate and extend findings of disrupted white matter organisation in the speech-motor system in PDS. We examined grey matter and white matter structure within the cerebellum and the major pathways connecting the cerebellum to the rest of the brain. The majority of this chapter was published in *Brain and Language* [59]. Our findings of widespread disorganisation underlying the speech-motor system, in particular disruption to all three sets of cerebellar peduncles, are consistent with the theory that sensory feedback is impaired in PDS [160], but not with the widely accepted interpretation that functional over activation in the cerebellum in PDS is compensatory. This work used a relatively large cohort of PDS and fluent control individuals.

Figure 1.5: The scope of this thesis



Italics refers to specific study and methods employed. Effects reflecting general traits in comparison to fluent controls are indicated in the boxes by vertical arrows and state effects within PDS by a horizontal arrow

1.6.2 Study 2: All Speech

We used sparse-sampling BOLD fMRI activations during picture description and sentence reading to identify patterns of activation that differed between PDS and fluent controls in a new cohort of individuals, which had very little overlap with the samples used in Study 1. We failed to replicate classic meta-analytic findings in stuttering that identified “neural signatures” thought to exist regardless of task demands or specific states of fluency [40].

1.6.3 Study 3: Speech states vs general traits

We used the same dataset obtained for Study 2 to isolate indicators of state and trait effects in PDS.

1.6.3.1 Trait effects

The direct comparison of fluent speech was achieved by statistically removing the effects related to dysfluent utterances from the analysis in each individual. A general trait of cortical overactivation of speech-motor components was observed to facilitate fluent picture description in stuttering. This finding is consistent with previous observations of increased recruitment of these structures [40], and could reflect

a general inefficiency in the speech motor-control system [171]. However, fluent sentence reading was associated with decreased activation in stuttering, suggesting that differences in speech-related activity differ according to task demands.

1.6.3.2 Stuttering subgroups

Within people who stutter, we used the frequency of dysfluent states to examine underpinnings of the proclivity to stuttering within people who share general traits of PDS. We observed increased recruitment of left hemisphere lobule VI of the cerebellum, as well as basal ganglia structures, including the putamen and caudate nucleus, bilaterally, in a PDS subgroup who was “mostly fluent” relative to a group that was “somewhat dysfluent”. This finding is consistent with reports of basal ganglia activity levels (i) responding to treatment gains [231]; (ii) differing between recovered and persistent PDS, [116][52]; (iii) correlating with stuttering severity measures [100][123].

1.6.3.3 State effects

We examined BOLD contrasts between dysfluent and fluent utterances within individuals who stutter during a picture description task. During dysfluent relative to fluent picture description, we observed decreased recruitment of the caudate nucleus specifically and generally reduced levels of activity across a variety of speech-motor regions. During dysfluent relative to fluent sentence reading, however, we observed increased levels of activity in the putamen bilaterally as well as lateral and medial premotor cortices. This study provides the first direct evidence that basal ganglia activity is altered during dysfluent speech states in a group of PDS and that the specific locations and directions of differences relative to baseline differ according to task demands.

1.6.4 Study 4: Networks at rest

We used resting state fMRI collected in the same dataset as used for studies 2 and 3 to isolate traits of stuttering that reflect functional connectivity without complications of compensatory activity in real-time. We tested the hypothesis that disruptions to functional connectivity of premotor cortex and the cerebellum are a general trait of stuttering. We observed disruption in several components that overlap with the speech-motor network, particularly involving premotor and cerebellar connections, which is consistent with previous findings [152][263][267][210]. Rest-related cerebellar activation was also related to stuttering severity in several components.

1.6.5 Study 5:Non-speech motor skills

We compiled a battery of motor tasks that in combination address several competing hypotheses in PDS. In a new cohort of PDS and controls that had minimal overlap with the other studies in this thesis, we found no evidence of general motor deficits in stuttering [171]. We observed a specific disruption in visuomotor adaptation performance in PDS relative to fluent controls, supporting the altered sensory integration theory of stuttering [160] and cerebellar disruption in PDS. We found no effect of sex and handedness on motor performance in PDS, but did find that family history was related to reinforcement learning performance. In particular, the dissociation between familial and idiopathic subgroups of PDS suggests that the specific direction of the dopaminergic imbalance in stuttering [4] could be related to heritability.

1.6.6 Theoretical contribution

Overall, the studies in this thesis use three separate cohorts of stuttering and fluent individuals. We addressed several competing theories of PDS. In particular, our work uncovered evidence for subcortical underpinnings to persistent developmental stuttering that had strong theoretical support implicating the dopaminergic system [4] but little empirical evidence. Further, our findings were consistent with the theory of altered sensory-motor integration in stuttering [160], though we hypothesize that the locus of this disruption is in the cerebellum and not primary sensory cortices. Finally, the findings broaden our understanding of how disruption to components in the speech motor system can interfere with the mechanisms of fluent speech production.

Chapter 2

Disrupted white matter in people who stutter

Summary: White matter tracts connecting areas involved in speech and motor control were examined using diffusion-weighted imaging in a sample of people who stutter ($n=29$) who were heterogeneous with respect to age, sex, handedness and stuttering severity. We replicated previous findings that showed disorganisation in white matter underlying ventral premotor cortex, cerebral peduncles and posterior corpus callosum in people who stutter relative to controls. Tractography analyses additionally revealed significant white matter disorganisation in the arcuate fasciculus bilaterally and the left corticospinal tract; and significantly reduced connectivity within the left corticobulbar tract in people who stutter. Regions-of-interest analyses revealed white matter disorganisation in people who stutter in the three pairs of cerebellar peduncles that carry the afferent and efferent fibers of the cerebellum. Within the group of people who stutter, the higher the stuttering severity index, the more disorganised the white matter underlying left angular gyrus, but the greater the white matter connectivity in the left corticobulbar tract. Also, in people who stutter, handedness and age predicted the organisation of the corticospinal tract and peduncles, respectively. Further studies are needed to determine which of these white matter differences relate to the neural basis of stuttering and which reflect experience-dependent plasticity.

2.1 Introduction

Persistent developmental stuttering arises in early childhood, remits before puberty in around 80% of cases, and persists to adulthood in 1% of the population [11]. Developmental stuttering is a disorder of speech fluency marked by involuntary sound repetition and prolongation, articulation cessation (blocks), and sometimes

accompanied by marked oromotor disturbance likened to facial tics in disorders of motor control (*e.g.* Tourette’s syndrome) [155][264]. Though the symptoms of stuttering can sometimes be quite severe, there are usually no associated brain lesions, neuropathology, or impairment in other areas of cognition. As such, people with persistent developmental stuttering (PDS) comprise a unique population with focal dysfluency for exploring the neural underpinnings of speech production. Furthermore, the high rate of recovery in PDS during childhood [158] offers an opportunity to investigate mechanisms underlying neuronal plasticity and spontaneous recovery.

2.1.1 Brain function in stuttering

With the availability of non-invasive brain-imaging methods, the number of studies of brain structure and function in PDS has rapidly increased. Even so, the precise neural basis of stuttering remains elusive and its ontogenesis is still poorly understood. Hypotheses regarding incomplete cerebral dominance [232], altered basal ganglia activity [4], and abnormalities in predicting the sensory consequences of motor speech [174][160] have all been considered as explanations of stuttering. Support from functional imaging studies for these theories is mixed, however.

For example, a meta-analysis of eight early functional imaging studies [40] described right hemisphere over-activity in PDS, which is consistent with altered cerebral dominance in this population. The same analysis found an “absence” of activity in auditory cortex bilaterally and overactivity in the cerebellum, lending support to the idea that sensorimotor integration is abnormal in stuttering [178]. The meta-analysis did not reveal abnormal function in the basal ganglia of PDS, however. The numerous functional imaging studies published since 2005 have not helped to clarify the picture, replicating in part previous findings but on the whole failing to identify a clear set of functional correlates of stuttering [54][100][148]. Studies of functional connectivity during task and at rest show promise in studies of stuttering [53][154][263] and could be sensitive to changes in speech networks due to treatment and recovery[152].

2.1.2 Brain structure in stuttering

Analysis of brain structure in PDS has also produced varied results: reduced cerebral asymmetry and altered sulcal anatomy have been reported but not replicated [170][52][65][92][90]. Similarly, grey matter volume is described as increased in several brain areas in adults who stutter relative to controls [18][65][126] but decreased in children who stutter [52]. Also, the volume of the rostral half of the corpus callosum was assessed to be increased in adults who stutter relative to controls [57] but there were no differences among groups of boys who persisted in or recovered from stuttering and those who were normally fluent [56]. The lack of

a consensus picture across functional imaging studies may relate to the sensitivity of these methods to functional differences in PDS that are due to differences in the speech act itself (including, but not only, dysfluent speech). Structural image analysis is uninfluenced by these dynamic differences but both methodologies are also sensitive to differences in PDS that reflect the etiology (possibly genetic) of the disorder as well as the brain’s plastic response to it. A major challenge in stuttering research is sifting through these findings in an attempt to determine what sorts of anomalies indicate plasticity and which are more likely to reflect congenital disorganisation or disconnection.

Against this background of varied findings of differences in brain function and structure in PDS is the small set of studies that show an abnormality affecting the white matter underlying left ventral sensorimotor cortex [52][64][137][220][249]. These reports used diffusion-weighted imaging to measure white matter organisation in PDS. The microstructure of white matter is quantitatively assessed using fractional anisotropy (FA), a ratio of diffusion of water along the principle diffusion direction (longitudinal diffusivity, L1) to that along the two orthogonal axes (radial diffusivity, RD). Barriers to diffusion, such as the structure of white matter fiber tracts and their layers of myelin, impede radial diffusivity [20]. The principal diffusion direction is often used, therefore, to infer the trajectory of large well-aligned fiber bundles and FA is used to measure white matter organization. Low FA might reflect disorganisation of white matter tracts, because longitudinal diffusivity is reduced, for example in areas of crossing fibers, or because radial diffusivity is increased, perhaps due to a disruption in the normal myelin distribution or formation.

In PDS, reduced FA in white matter was associated with increased radial diffusivity [64], which can be interpreted as indicative of fewer barriers to diffusion across the fiber tracts. An increase in radial diffusivity in PDS possibly reflects abnormal myelin development. The finding of reduced FA in the white matter underlying left ventral sensorimotor cortex of PDS across several studies is reassuring, particularly as the studies differed in the number of diffusion directions acquired, analysis approaches and in the demographics of the populations studied. The effect is especially interesting given that PDS showed decreased activity during speech production (in functional MRI) in the same region they showed reduced FA [249]. It should be noted, however, that at least one report found elevated FA in white matter close to this region in PDS [137].

2.1.3 Variability in stuttering

Several other questions regarding the nature of the white matter abnormalities in stuttering remain. Not least of these, is whether structural differences reflect a possible cause of the disorder, perhaps due to genetic risk factors, or are a

consequence of life-long stuttering behaviour. Longitudinal analyses are required to address this question, but the persistence of the abnormality in children who recovered from stuttering [52] would seem to rule out the idea that this particular white matter difference reflects experience-dependent plastic change in response to stuttered speech. Other questions relate to the relationships between white matter differences and sex, handedness, age and stuttering severity in PDS. Sex and handedness have been shown to influence sulcal anatomy and grey matter volume in language areas [193] in healthy controls, but the degree to which stuttering might impact the influence of those factors is still undetermined. The factors of sex and handedness are often considered to be confounds contributing to the lack of clear structural and functional correlates of stuttering. Consequently, studies have aimed to address this by focussing analyses on the largest homogeneous subpopulation of stutterers, usually right-handed males who stutter [65][64].

2.1.4 The aims of the current study

The primary aim of the current study was to replicate previous findings in developmental stuttering. A secondary goal was to extend our knowledge by evaluating the relationship between white matter differences in people who stutter and factors such as age, sex, handedness and stuttering severity. To achieve these aims we investigated a large and heterogeneous sample of PDS and examined separately how the factors contributing to this heterogeneity affect the white matter differences detected. We considered age, sex, handedness and stuttering severity in these analyses. We also used probabilistic tractography and regions-of-interest (ROI) analyses to focus on specific pathways involved in speech and motor control.

2.2 Methods

2.2.1 Participants

Twenty-nine PDS (mean age 22.6 years; range 14 to 42 years; eight females; 21 males) and 37 fluent controls (CON; mean age 24.3 years; range 14 to 45 years; 14 females; 23 males) were scanned using diffusion-weighted imaging. The groups were well matched for age and proportion of males to females. Three of the 21 males and four of the eight female PDS were left-handed; there were two left-handed males in the CON group. The PDS group ranged in stuttering severity, as assessed by two independent raters using the Stuttering Severity Instrument-3, SSI-3, [203] from mild to severe (mean 22.9; range 8 to 38.5). An SSI-3 score was not available for one right-handed male participant whose stuttering was estimated as mild relative to those in the cohort. The data from some of the group were used in a previous

report [249] but further data have been added from both PDS and fluent-speaking controls.

2.2.2 Imaging

Structural and diffusion-weighted MRI images of the whole brain were acquired using a 1.5T Siemens Sonata clinical imaging system. High-resolution T1-weighted images (1-mm isotropic voxels, FLASH sequence, TR=12ms, TE=5.65 ms, flip angle = 190°) were obtained in each participant and coregistered to the MNI152 template. Two sets of echo-planar images were acquired (53 x 2.5 mm axial slices, in-plane resolution 2.5mm²). Each set included three non-diffusion-weighted and 60 diffusion-weighted images acquired with a b -value of 1000 s/mm² uniformly distributed across 60 gradient directions. Diffusion data were preprocessed using the FMRIB Diffusion Toolbox (FDT v2.0; <http://www.fmrib.ox.ac.uk/fsl>); [24][25]. Images were corrected for eddy currents and head motion by affine registration to non-diffusion volumes. Data were averaged across the two sets to improve signal to noise ratio. Images of FA, longitudinal (or axial) diffusivity along the primary diffusion direction (L1) and average radial diffusivity (RD) across the two axes perpendicular to L1 were created. Diffusion images were registered, using boundary-based registration [103], first to each participant's T1-weighted image and then to the MNI152 standard space brain for group comparisons. Probabilistic tractography was run using the BedpostX and ProbtrackX functions in FDT.

2.2.3 Analysis

2.2.3.1 Cerebral white matter

Tract-based spatial statistics. Voxel-wise statistical analysis of the FA data was performed using Tract-Based Spatial Statistics (TBSS) [213]. TBSS registers the diffusion images non-linearly to an in-house target image obtained from a high-resolution average of 58 healthy participants aligned to standard space. The average of all data was reduced to a 1-mm thick white matter skeleton that should align with the center of white matter tracts common across participants. For each participant's non-linearly registered FA image, the highest value of FA nearest to the skeleton was projected onto the skeleton for analysis. A t -test was performed at each voxel in the skeleton. Permutation testing was performed using the Randomise function in FSL (10,000 permutations) [256] and statistical inference was based on an uncorrected voxel-based threshold $p < .005$, with an extent threshold of at least 10 voxels (there were no significant differences in FA between PDS and CON when fully corrected for multiple comparisons). FA was compared between the groups of PDS and CON while the effects of the confound regressors age, sex,

and handedness were accounted for through inclusion as covariates in the model. Significant group differences in FA were further explored by analysis of the L1 and RD values that contribute to the calculation of FA; planned group comparisons of these values are reported at $p < .05$ uncorrected. The correlation between FA and SSI-3 was also tested in regions showing significant group differences. Again, these exploratory analyses are reported at $p < .05$ uncorrected.

Tractography. Probabilistic tractography was used to identify the corticospinal tract (CST) and arcuate fasciculus (AF) in each hemisphere and for each participant. This analysis used previously described methods for defining seed and target masks in standard space [99]. For the AF, a seed mask was placed in a single coronal slice ($Y = -38$) at the arc of the tract and two target masks were placed along that tract, one coronal mask in the white matter anterior to the seed mask, and another axial mask ventral to the seed mask. Exclusion masks, including one for the midline, were used to remove any branches extending outside of the AF and termination masks were located immediately outside the target masks. The number of probabilistic streamlines per pathway, resulting from the tractography, were thresholded in each participant in order to isolate the AF more confidently; only voxels through which at least 500 streamlines (out of a possible 5000 per voxel generated from the seed to the target masks) passed were retained. See Figure 2.2A for placement of AF masks and resulting tract. The CST seed mask was located in the pons on an axial slice at $Z = -22$ with an axial target mask in dorsal white matter of the same hemisphere underlying the motor cortex at the level of $Z = 26$. Termination masks included the area superior to and surrounding the target mask and the whole brain inferior to the seed mask. An exclusion mask ran through the midline. The pathways resulting from the tractography were thresholded in each participant to remove spurious connections; only voxels through which at least 20 streamlines passed were retained [99]. See Figure 2.2B for placement of CST masks and resulting tracts.

The CST tracked as above comprises mostly fibers from the dorsal corona radiata travelling in the middle part of the posterior limb of the internal capsule to the pons. For comparison, the corticobulbar tract (CBT) fibers were isolated so that the hypothesis that they are selectively involved in the pathology underlying stuttering could be tested. Probabilistic tractography between the seed mask placed in the pons (axial slice at $Z = -22$ as above) and two new target masks placed in the most dorsal aspect of the internal capsule were used. The two target masks comprised 9 voxels each (3×3 square) and they were drawn in the posterior limb of the internal capsule on each participant's FA modulated primary diffusion direction images (first eigenvector), in which the orientation of fibers can be inferred from the color (red for left-right, blue for superior-inferior and green for anterior-posterior).

The targets were drawn in the same axial slice, identified as a cross-section through the basal ganglia in which both genu and splenium of the corpus callosum were visible and the internal capsule was at its broadest [36].

The new target that was used to isolate the CBT was located close to the genu of the posterior limb of the internal capsule comprising mostly fibers with a superior–inferior orientation (blue color). The target used to isolate the CST was positioned just posterior and lateral to the CBT target in the middle portion of the posterior limb of the internal capsule (also comprising fibers with a superior–inferior orientation). Termination masks covered the brain tissue ventral to the seed mask in the pons (as for CST above) and the whole axial slice above the two target masks (see Figure 2.3). Tracking the CBT from cortex to pons would require traversing the fibers orientated rostro–caudally that comprise the superior longitudinal fasciculus (SLF). These crossing-fibers may confound measurements made in the CBT. By restricting the analysis to the ventral portion of the tracts, we avoided the potential confound introduced by tracking across the SLF. The pathways resulting from this tractography were thresholded at a minimum of 20 streamlines (as above).

Total volume, mean FA, and the number of streamlines (total number of tracts generated from the seed mask that reached the target masks) were measured for each probabilistic tract. Data were compared using analysis of variance (ANOVA), with a between-subjects factor of group (PDS vs. CON) and a within-subjects factor of hemisphere (left vs. right). For the tract data in the posterior limb of the internal capsule, another within-subjects factor of tract (CBT vs. CST) was added. The contribution of age, sex, handedness and stuttering severity (SSI–3 score) to the measures that showed significant differences from CON in PDS were evaluated using step-wise linear regression.

2.2.3.2 Region of interest: The cerebellum

The cerebellum was excluded from the TBSS analysis partly because the cerebellar peduncles are slender and not always robustly detected by the TBSS skeleton (presumably due to differences in registration). Also, it is not possible to dissociate specific tracts within the cerebellar white matter using the skeleton approach in TBSS. Instead, the organisation of cerebellar white matter was evaluated using ROIs for the peduncles, as well as the segmented white matter of each cerebellar lobe and the vermis.

Cerebellar lobes. Within the cerebellum, white matter volume and mean FA was calculated for each of five ROIs: the vermis and the anterior and posterior lobes, bilaterally. These ROIs were drawn on the T1-weighted structural images in MNI152 standard space. The posterior vermis was identified using probability maps (thresholded at 20%) previously published [73] and included as an atlas in

the FSLView software. The anatomical boundaries for the anterior lobe vermis are unclear; therefore, data from this region were excluded by removing voxels in the midsagittal plane ($X=0$) for the anterior lobe ROIs. The four lobes were drawn using the midline and primary fissure as boundaries between hemispheres and anterior and posterior ROIs, respectively, and once drawn the vermis mask was subtracted from the masks of the lobes to yield five masks with no overlapping voxels. Only the posterior vermis ROI contained voxels in the midline. These five ROIs were transformed from standard space to the participant's structural image. White matter voxels in these ROIs were identified using the partial volume estimates, which were produced by the segmentation procedure used during boundary-based registration to standard space. Voxels that had a partial volume estimate of > 0.99 (*i.e.* were completely white matter) were retained. Within the white matter estimates, the mean FA across these voxels for each ROI was calculated. Group differences in mean FA were assessed using two repeated measures ANOVAs (2×5) with a single between subject factor of group (PWS vs. CON) and a within-subject factor of ROI (5: left and right anterior and posterior lobes and vermis).

Cerebellar peduncles. In order to better isolate the afferent and efferent pathways of the cerebellum, one ROI was placed in each of the cerebellar peduncles of each participant, bilaterally, for a total of six ROIs per participant. Masks were drawn using FA-modulated primary diffusion direction images (color coded) to identify regions in the superior (SCP, 10 voxels), middle (MCP, 18 voxels) and inferior (ICP, 8 voxels) connections between the brainstem and the cerebellum [139]. In essence, this involved placing the ROIs in the center of these peduncles. The SCP is visible on sagittal slices close to the midline as the more dorsal connection from the posterior margin of the brainstem to the cerebellum travelling in an anterior-posterior/superior-inferior direction. The SCP ROI comprised 10 voxels in the middle of the peduncle in up to three contiguous sagittal slices. The MCP is the largest peduncle and clearly identified on an axial slice at about the level of the mid pons as large tracts extending in the anterior-posterior direction bilaterally with obvious laterally-crossing pontine fibers between those extensions anteriorly. ROIs were drawn on two contiguous coronal slices (9 voxels each) located immediately posterior to the ICP, which is visible ventral to the MCP on coronal slices. ICP ROIs were always inferior to the SCP, anterior to the MCP, and were identified on a single sagittal slice as steep inferior-superior extensions. ICP ROIs were drawn on two contiguous coronal slices (4 voxels each) such that the entire ROI could be seen in a single sagittal slice. See Figure 2.4 for placement of cerebellar peduncle ROIs in a representative subject. FA was extracted from these images and subjected to ANOVA with the between-subject factor of group (PDS vs. CON) and two within-subject factors of peduncle (SCP vs. MCP vs. ICP) and hemisphere (left

vs. right). Significant differences in FA involving group were further explored for these small ROIs by analysis of the underlying L1 and RD values that contribute to FA.

Cerebellar white matter measures that showed significant group differences were explored further with step-wise linear regression to evaluate their relationships with age, sex, handedness and stuttering severity (SSI-3 score) in PDS.

2.3 Results

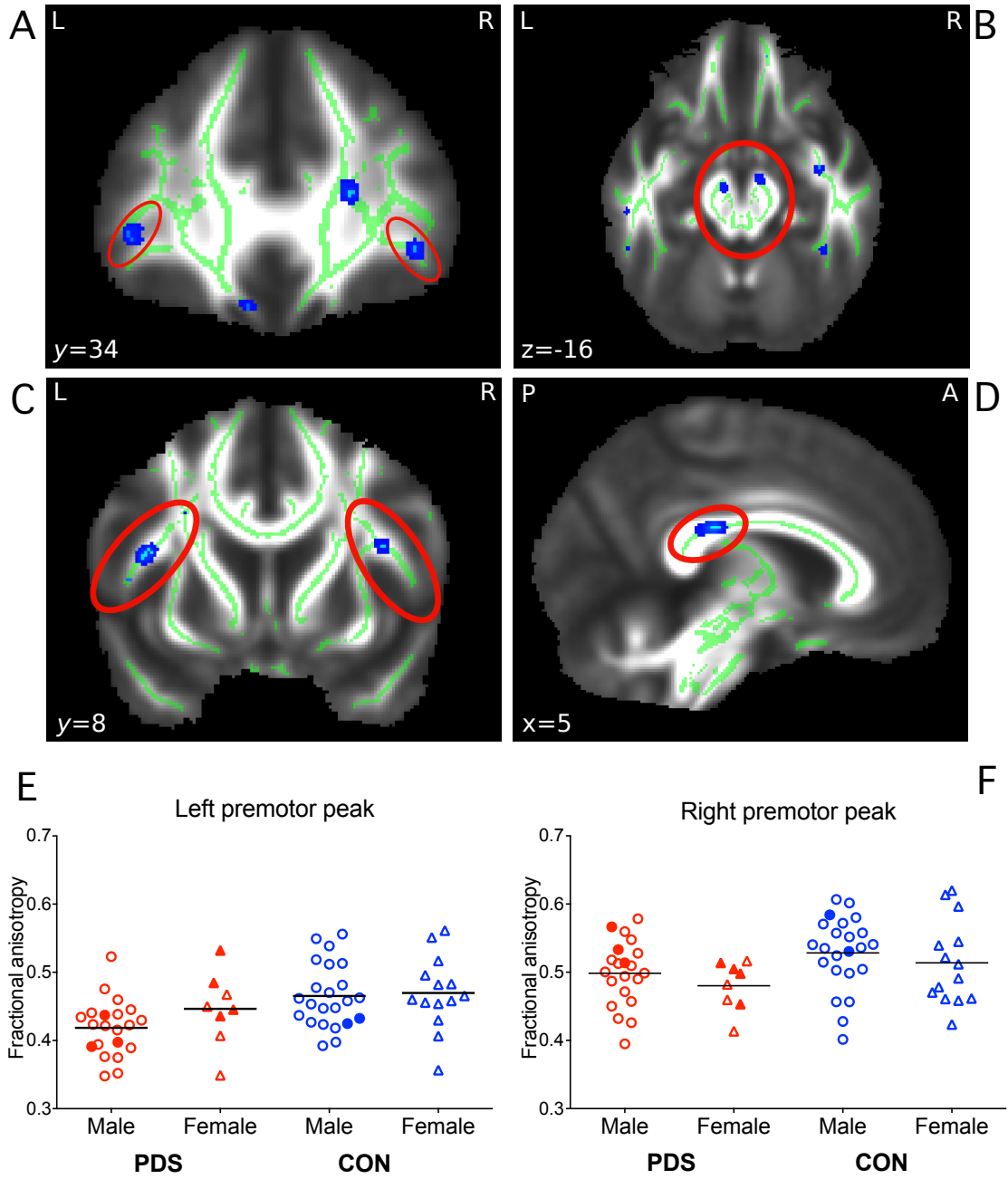
2.3.1 Cerebral white matter

2.3.1.1 Tract-based spatial statistics

The PDS and CON groups did not differ significantly in mean FA across all cerebral voxels and all voxels in the skeleton (cerebral FA: CON $0.225 \pm .008$, PDS $0.222 \pm .009$; skeletal FA: CON $0.438 \pm .015$, PDS $0.434 \pm .016$). There were no significant group differences in the TBSS analyses following correction for multiple comparisons using threshold-free cluster enhancement. Therefore, group differences in FA are reported at an uncorrected threshold of $p < .005$ when they extended for at least 10 contiguous voxels. Differences that survived the $p < .005$ threshold that comprised fewer than 10 voxels are also reported if they were in areas contralateral to a homologous region in the other hemisphere that survived the extent threshold of ≥ 10 voxels. At this uncorrected threshold, several regions showed significant differences between the two groups and many occurred bilaterally (the significance of these pairs is $p < .0052$; see Table 2.1). PDS had significantly lower FA than CON: (i) bilaterally in white matter underlying the superior frontal gyrus, anterior inferior frontal gyrus, ventral premotor cortex, angular gyrus, lateral occipital cortex, forceps major, optic radiations and the cerebral peduncles; (ii) in the body of the corpus callosum; (iii) in multiple areas in the left hemisphere including the white matter underlying the middle frontal gyrus and posterior middle temporal gyrus, the anterior corona radiata and superior longitudinal fasciculus; and (iv) in the right ventral temporal and occipital white matter (see Figure 2.1). PDS had significantly higher FA than CON in the white matter underlying the middle frontal gyrus and dorsal precentral gyrus bilaterally and the left temporal pole.

RD and L1 were examined in order to understand better the microstructure underlying FA differences. Across all these areas, when the PDS group had lower FA than CON, they also had lower diffusivity along the longitudinal axis of the tract (L1), whereas in those regions where PDS had higher FA than CON, they also had higher L1. RD averaged across the two directions orthogonal to the principal diffusion direction was significantly higher in PDS than CON in the following regions in which FA was lower in PDS than CON: angular gyrus and optic

Figure 2.1: Tract based spatial statistics analysis of white matter.



Blue areas indicate regions in which PDS had significantly reduced FA relative to CON. The map of the p -value was thresholded at $p < .005$ and the remaining voxels thickened for visualization purposes. The underlying brain image is the mean FA across all participants with the white matter skeleton overlaid in green. R, right; L, left. A. Coronal slice at 34 mm in front of the anterior commissure. Anterior inferior frontal white matter areas are encircled in red. B. Axial slice at 16 mm below the bicommissural plane. Cerebral peduncles are encircled in red. C. Coronal slice at 8 mm in front of the anterior commissure. White matter areas underlying the ventral premotor cortex are encircled in red. D. Sagittal slice through the right hemisphere at 5 mm from the midline. The posterior corpus callosum is encircled in red. E. Graphs showing individual data for FA in the white matter underlying left and right ventral premotor cortex. Red circles male PDS; Red triangles female PDS; Blue circles male CON; Blue triangles female CON. Filled left handers; unfilled right handers. The horizontal line indicates the group mean.

radiations bilaterally, the body of the corpus callosum, the left cerebral peduncle, right superior frontal gyrus and fusiform gyrus (see Table 2.1). RD was significantly lower in PDS than CON in the following regions in which FA was higher in PDS than CON: dorsal precentral gyrus bilaterally and right middle frontal gyrus (see Table 2.1).

Correlations between FA and stuttering severity were explored within the PDS group. FA in the left angular gyrus was lowest in participants with the highest SSI-3 (negative correlation; $r=-0.41$, $p = .03$; Figure 2.5A).

Table 2.1: White matter regions showing significant differences between people who stutter and controls in the tract-based spatial statistics analysis.

White Matter Region	X	Y	Z	p FA PDS < CON	# voxels	p RD PDS > CON
L superior frontal gyrus	-10	50	34	0.0005	16	
R superior frontal gyrus	10	50	34	0.0005	4*	0.011
L inferior frontal gyrus (anterior)	-31	38	5	0.0013	11	
	-40	35	0	0.001	10	
R inferior frontal gyrus (anterior)	32	37	7	0.0023	2*	
	39	33	-4	0.0008	8	
L middle frontal gyrus	-35	11	43	0.0006	10	
L ventral premotor	-41	8	16	0.0012	11	
R ventral premotor	38	8	19	0.0041	3*	
L anterior corona radiata	-27	4	30	0.0013	11	
L cerebral peduncle	-8	-15	-19	0.0003	11	0.016
R cerebral peduncle	9	-11	-15	0.0036	3*	
Body corpus callosum (posterior part)	5	-24	24	0.0005	11	0.022
L superior longitudinal fasciculus (mid)	-37	-25	30	0.0011	27	
R angular gyrus	41	-52	42	0.0001	18	0.01
L angular gyrus	-42	-51	43	0.0005	5*	0.022
L middle temporal gyrus (posterior)	-42	-59	11	0.0001	12	
R fusiform gyrus (mid)	37	-45	-12	0.0001	25	.001#
L forceps major	-23	-43	25	0.0005	36	
R forceps major	32	-58	-10	0.0004	10	
	29	-61	15	0.0003	11	
L optic radiation	-36	-52	6	0.0005	15	0.025
	-29	-71	12	0.0007	19	
R optic radiation	39	-54	3	0.0017	2*	
	25	-78	3	0.0003	56	0.005
R lateral occipital	37	-76	-6	0.0001	12	
L lateral occipital	-33	-77	-1	0.0004	23	
R ventral occipital	15	-82	-5	0.0001	11	
				p FA PDS > CON		p RD PDS < CON
R middle frontal gyrus (anterior)	33	37	22	0.0002	22	0.025
L middle frontal gyrus (mid)	-32	19	34	0.0003	10	
L temporal pole	-37	5	-30	0.0003	13	
L dorsal precentral gyrus	-15	-20	58	0.0006	25	.0025+
R dorsal precentral gyrus	15	-17	59	0.0018	4*	.0008+

* Peaks with fewer than 10 voxels bilateral to white matter regions passing threshold. + PDS > CON in RD where PDS > CON in FA. # PDS < CON in RD where PDS < CON in FA

2.3.1.2 Tractography

The arcuate fasciculus (AF) and corticospinal tract (CST) were successfully tracked bilaterally in all participants using probabilistic tractography. The mean FA for the total tract, the tract volume and the number of streamlines in the PDS and CON groups were compared for these anatomically-defined tracts using ANOVA.

For the AF, PDS had significantly lower FA relative to the CON group across both hemispheres ($F(1,64)=5.04$, $p = .028$) (Figure 2.2A). The PDS and CON groups did not differ in volume of the AF or in the number of streamlines. There was a significantly higher number of streamlines in the left compared to the right hemisphere across both groups ($F(1,64)=8.09$, $p = .006$). Age, sex, handedness and SSI-3 score did not significantly predict FA in the left or right AF or the average FA across the two sides in PDS.

For the CST, PDS had lower FA on the left compared with the right ($t(28)=4.18$, $p < .001$), whereas there was no difference in FA between hemispheres in CON (group x hemisphere interaction: $F(1, 64)=7.06$, $p = .010$; see Figure 2.2B). PDS and CON groups did not differ in the volume of the CST or in the number of streamlines. Age, sex, handedness and SSI-3 score did not significantly predict FA in the left or right CST separately in PDS. Handedness, however, was a significant predictor of the difference in FA between the two hemispheres in PDS (model fit: $F(1,26)=4.52$, $p = .043$; $r=0.39$, $p = .022$). In right-handers who stutter, FA tends to be higher in the right CST than the left, whereas in the small number of left-handers who stutter the average difference between FA in the two hemispheres was negligible (see Figure 2.5B).

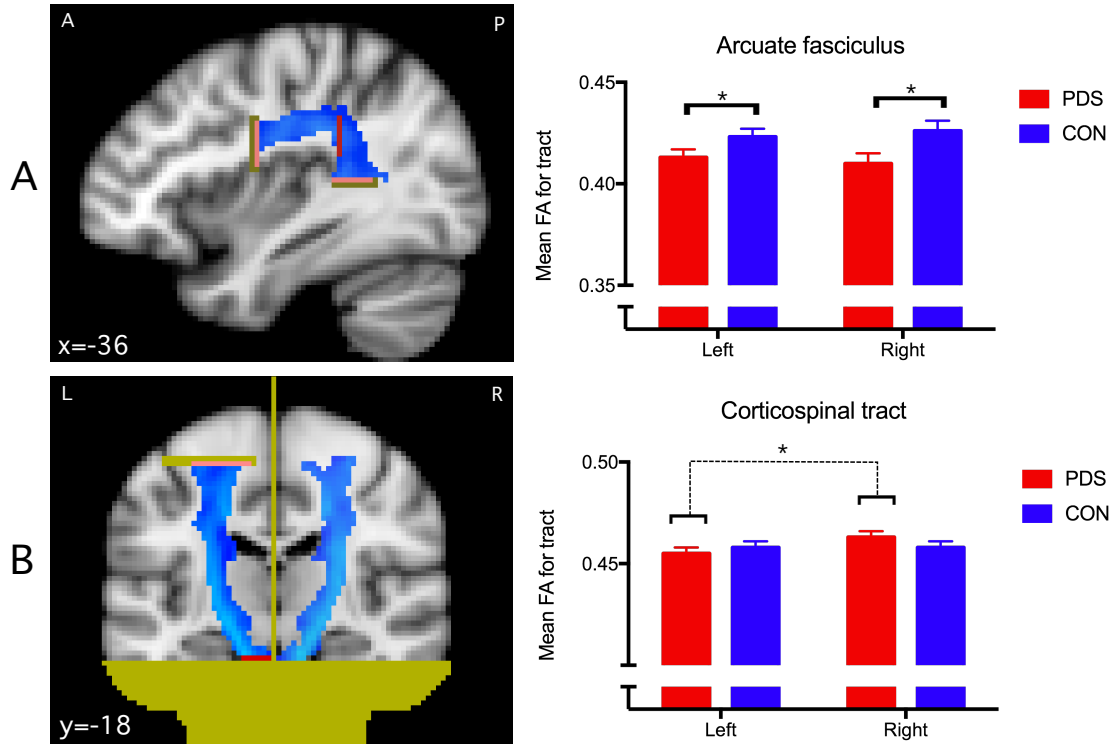
2.3.1.3 Posterior limb of internal capsule

The CBT and CST from the posterior limb of the internal capsule to the cerebral peduncles were successfully tracked bilaterally using probabilistic tractography in all participants. For these tracts, the mean FA for the total tract, tract volume and number of streamlines were compared between the PDS and CON groups.

Analysis of the mean FA of these tracts revealed that there were hemispheric differences that were different for the two tracts in the two groups (group x tract x hemisphere interaction: $F(1,64)=5.00$, $p = .029$). Separate analysis for the left and right hemispheres revealed that this interaction was due to higher FA in PDS relative to CON in the right CBT with no differences in the right CST (group x tract interaction: $F(1,61)=4.13$, $p = .046$) and no group differences in the left CBT and CST. Age, sex, handedness and SSI-3 score did not significantly predict FA in the right CBT in PDS.

A similar pattern was seen for the analysis of tract volume (significant group x tract x hemisphere interaction: $F(1,64)=6.23$, $p = .015$). This interaction was

Figure 2.2: Probabilistic tractography of arcuate fasciculus and corticospinal tracts.

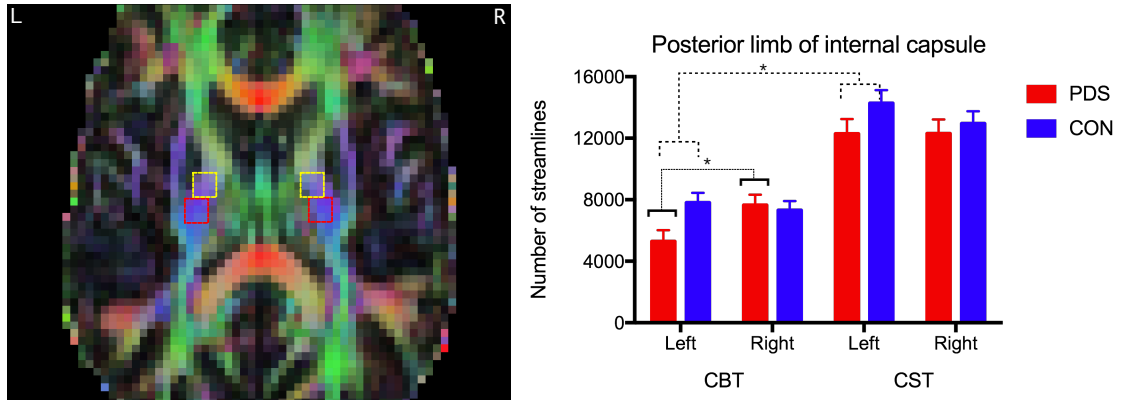


Underlying brain image is the MNI152 template T1-weighted image in standard space. Blue areas are the average of the thresholded tracts across all participants after running probabilistic tractography between the seed masks (red) and the target masks (pink). Termination and exclusion masks (see text for details) are also shown (yellow areas). A: anterior; P: posterior; L: left; R: Right. Bars on graphs show means for groups of PDS (red) and CON (blue). Error bars standard error of the mean. * significant difference. A. Arcuate fasciculus shown on sagittal slice through the left hemisphere 36 mm from the midline. Significantly reduced FA bilaterally in PDS relative to CON. B. Corticospinal tract shown on coronal slice at 18 mm posterior to the anterior commissure. FA is significantly lower in the left hemisphere compared to the right in PDS.

due to a significant reduction in volume of the CST in PDS relative to CON in the left hemisphere ($t(64)=2.24$, $p = .029$) but not the right; the left CBT volume in PDS was also reduced but this difference was not quite significant ($t(64)=1.87$, $p = .066$). Age, sex, handedness and SSI-3 score did not significantly predict the volume of the left CST in PDS.

There were significantly fewer streamlines in PDS relative to CON in the left hemisphere in both the CBT and the CST (group x hemisphere interaction: $F(1,64)=4.678$, $p = .034$). In the PDS group, there were significantly fewer streamlines in the left compared with the right CBT ($p = .001$), but the left and right CST did not differ; there was no difference between hemispheres for either tract in the CON group (see Figure 2.3). SSI-3 score was the only significant predictor of the number of streamlines in the left CBT in PDS (model fit: $F(1,26)=5.34$, $p = .029$; $r=0.41$, $p = .015$); age, sex and handedness did not improve the model fit. This positive relationship indicates that the number of streamlines in the left CBT is highest in those who have the highest SSI-3. As the direction of this relationship was unexpected, we further explored whether SSI-3 score related to the number of streamlines in the right CBT (this was also a positive relationship and close to significance, $p = .089$) or the difference in the number of streamlines between the two hemispheres ($p = .09$).

Figure 2.3: Probabilistic tractography in the posterior limb of the internal capsule.



Underlying brain image is a diffusion image showing the principal diffusion direction at each voxel modulated by the underlying FA (bright areas high FA). Red—diffusion in the left–right axis; green—diffusion in the anterior–posterior axis; blue—diffusion in the superior–inferior axis. Squares outlined show the voxels selected for the target masks in probabilistic tracking of the corticobulbar (CBT; yellow) and corticospinal (CST; red) tracts. L left; R Right. Bars on graphs show mean number of streamlines in each tract for groups of PDS (red) and CON (blue). Error bars standard error of the mean. * significant difference.

2.3.2 Cerebellar white matter

2.3.2.1 Cerebellar lobes

FA was compared between groups for the white matter of the left and right, anterior and posterior cerebellar lobes and the vermis. Mean FA did not differ between PDS and CON groups in any of these areas.

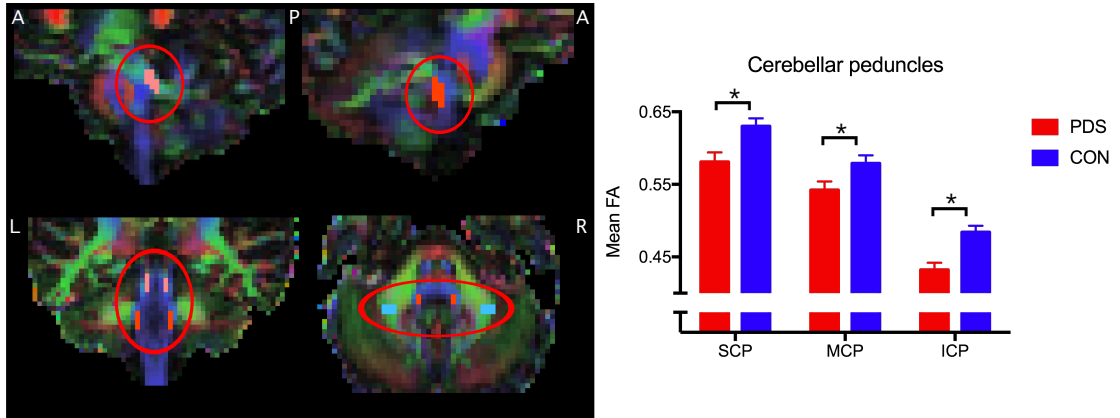
2.3.2.2 Cerebellar peduncles

PDS had significantly lower FA than CON in each pair of cerebellar peduncles ($F(1,64)=21.55$, $p < .001$; see Figure 2.4). For both groups, FA was significantly lower in the left peduncle compared to the right ($F(1,64)=7.97$, $p = .006$) and in the inferior cerebellar peduncles relative to the middle cerebellar peduncles, which were, in turn, significantly lower than the superior cerebellar peduncles ($F(2,64)=100.6$, $p < .001$). This pattern did not differ between groups or hemispheres. There were no differences between PDS and CON groups in L1 and RD in any of the peduncles. Age was the only significant predictor of the average FA in the inferior cerebellar peduncles in PDS (model fit: $F(1,26)=9.31$, $p = .005$; $r=0.51$, $p = .003$); sex, handedness and SSI-3 did not improve the model fit. This positive relationship indicates that FA increases with increasing age in the PDS group, only: The CON group did not show a significant relationship between age and FA in the inferior cerebellar peduncles (Figure 2.5C).

2.4 Discussion

Our objective was to use diffusion tensor imaging to examine for the neural correlates of developmental stuttering in the white matter tracts connecting speech and motor areas of the brain. The large sample studied comprised right- and left-handed male and female PDS and normally fluent controls who ranged in age from 14 to 45 years. The contributions made by these potential confounds to the white matter differences observed in PDS were examined. The main findings were that PDS have relatively disorganised white matter microstructure (lower FA): (i) in a large number of areas throughout the cerebrum, often occurring in homologous regions in both hemispheres; (ii) in the arcuate fasciculus bilaterally; (iii) in the left corticospinal tract; and (iv) in the cerebellar peduncles. In addition, the connectivity of the left corticobulbar tract (as indexed by the number of streamlines) was significantly reduced in PDS. The more severe the dysfluency in the PDS group, the greater the reduction in white matter organisation in the left angular gyrus but the greater the connectivity in the corticobulbar tract. In PDS, right-handedness predicted a

Figure 2.4: Cerebellar peduncle region of interest analysis.



Left underlying brain images are slices through a diffusion image in a single participant (see legend to Figure 3 for details). The superior cerebellar peduncle (SCP) masks are shown in pink, the middle cerebellar peduncle (MCP) masks are shown in light blue and the inferior cerebellar peduncle (ICP) masks are shown in red. L-left; R-Right; A-anterior; P-posterior. Bars on graphs show mean FA for each region of interest in each peduncle for groups of PDS (red) and CON (blue). Error bars-standard error of the mean. * significant difference.

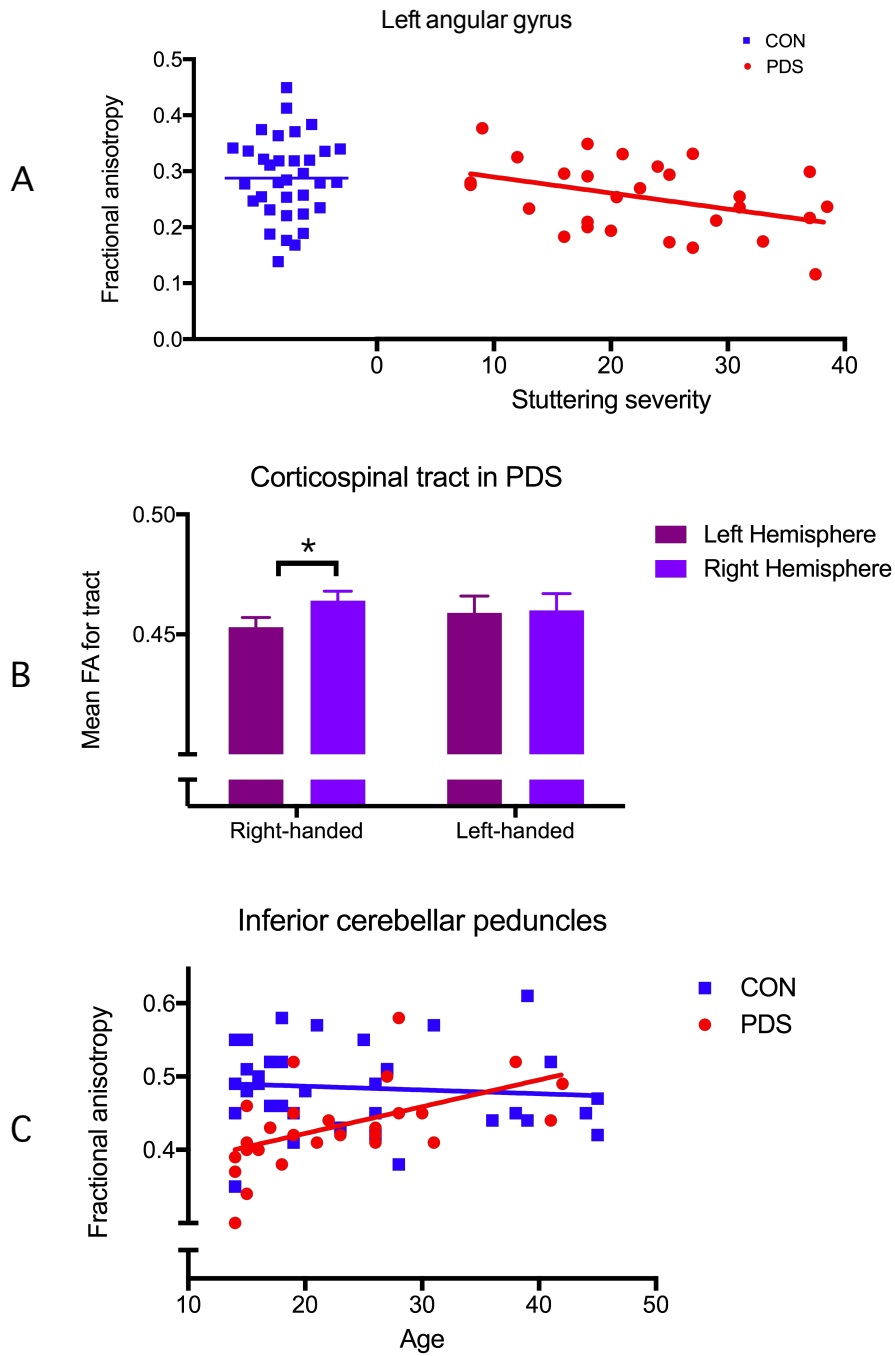
greater rightward asymmetry in the white matter organisation of the corticospinal tract and age predicted increased organisation in the inferior cerebellar peduncle.

2.4.1 Relation to previous reports

The current analysis using tract-based spatial statistics replicated corresponding findings from a study conducted by Watkins and colleagues [249], which was based on data from the younger half (14–27 years) of the dataset reported here. It is not surprising, therefore, that decreased FA was found again bilaterally in the white matter underlying the ventral premotor cortex, an area that the previous study also found to be functionally hypoactive in the left hemisphere during speech production in PDS. As mentioned in the introduction, white matter abnormalities in the left ventral sensorimotor (peri-Sylvian or opercular) cortex have been described in a group of ten male and five female adult PDS (age range 18–44 years; one left-hander) [220], in a group of 15 right-handed male children who persisted in or recovered from stuttering aged 9–12 years [52], and in a group of 13 right-handed male PDS (mean age 31 years; [64]). In contrast with this last study, however, we did not find any difference in radial diffusivity in this region in PDS.

Increased white matter disorganisation (*i.e.* lower FA) in the region of the left ventral sensorimotor cortex could reflect disruption to one or more of several pathways in this area that are known to play a role in speech production. The

Figure 2.5: Variability in white matter within stuttering.



A. The relationship between FA in the white matter underlying the left angular gyrus and SSI-3 is shown for PDS in red (line=regression line) and the scatterplot of data in CON in blue (line=mean). B. The difference between left- and right-handed PDS in the FA in the left (dark purple) and right (light purple) CST. Error bars=standard error of the mean. *-significant difference. C. The relationship between age and FA in the inferior cerebellar peduncle in PDS (red) and CON (blue). Lines are regression lines.

largest candidate pathway in this region is the superior longitudinal fasciculus, which connects posterior temporal cortex, inferior parietal cortex and lateral prefrontal cortex [46][188] and is important for integration of auditory and motor signals necessary for fluent speech. This tract contains fibres from the arcuate fasciculus [47][188], traditionally “the language” pathway [98], lesions of which were thought to cause conduction aphasia (but see [109]). This region also contains small, local, cortico–cortico “u–fibres” that connect the ventral premotor cortex with the posterior part of inferior frontal cortex (Broca’s area) and the primary motor representations of the articulators. The idea that these small local connections are disrupted is consistent with reports that in PDS the timing and sequence of activation is abnormal between the areas involved in planning speech and the areas involved in executing speech [206]. The ventral sensorimotor region also contains fibres that originate from the projection neurons from primary motor cortex to the pons carried in the corticobulbar tract. Microstructural differences in this tract could interrupt the normal information flow required for fluency and thereby be related to the speech dysfluency characteristic of stuttered speech. Our tractography analysis aimed to specifically evaluate the organisation of the arcuate fasciculus and corticobulbar tracts in PDS and revealed anomalies in both pathways (see below for discussion).

There are other similarities between the findings of the current tract–based spatial statistics analysis and those of previous studies using this method. In the study of right–handed boys who stutter [52], two peaks in the left superior longitudinal fasciculus (–48 –27 25 and –40 –51 40) were described with reduced FA in children who stutter relative to fluent controls that are very closely located to the peaks identified in the current study. Here (see Table 2.1), these peaks were described as left SLF (mid portion at –37 –25 30) and left angular gyrus (–42 –51 43). This latter peak also showed a significant positive correlation with SSI–3 in our analysis: the higher the score on the SSI–3, the lower the FA in this location in PDS. A study of right–handed male PDS [64] revealed reduced FA in an extensive cluster in the body of the corpus callosum. Consistent with this previous finding, we also report reduced FA and increased radial diffusivity in PDS in the posterior portion of the body of the corpus callosum (see Figure 2.1).

2.4.2 Interpreting white matter disorganisation

The organisation of white matter is quantified by the ratio of longitudinal to radial diffusion (FA) in the tensor model that is fit to water diffusion measurements made in each voxel of the diffusion image. Areas showing lower FA in PDS compared to controls are described as showing reduced white matter “integrity”. It is important to note that low FA could reflect differences in several features of the underlying microstructure, such as myelin and axonal membrane organisation

and/or the packing density and caliber of axons [20]. Consequently, low FA might affect the speed and quality of signal transmission between brain areas and the synchronization of signals across them. The areas of the cerebral white matter showing low FA in PDS were further explored by comparing radial and longitudinal diffusivities in the same regions. In all areas where FA was low in PDS, longitudinal diffusivity was also low. Radial diffusivity was higher in PDS than in controls only in a small number of regions with low FA, notably the body of the corpus callosum, where it is thought to reflect abnormal myelination of the fibers in this region [64]. In areas where longitudinal diffusivity was low but radial diffusivity was not different in PDS relative to controls, we can speculate that it is fiber organization rather than myelin that is disrupted. However, such reverse inference should be applied cautiously.

2.4.2.1 The relevance of finding abnormalities bilaterally

The growing consensus towards replicable findings in diffusion-weighted imaging studies of developmental stuttering is encouraging and points to the greater sensitivity of this method compared with morphometric analyses of cortical grey matter. Nevertheless, there were also some striking differences between the current study and other findings. The most notable difference was the frequency with which anomalies were observed in white matter tracts in both hemispheres that were in almost symmetrically homologous brain areas. Only a minority of the white matter differences occurred unilaterally. Based on the uncorrected statistical threshold of $p < .005$ that was used, a false positive result would occur in 1/200 voxels examined. We attempted to mitigate this false positive rate by requiring the clusters that were reported to show significant differences to have at least 10 neighboring voxels that also survived this statistical threshold (this is a stringent extent threshold, particularly as the skeleton is only one millimeter thick, which means that clusters can only extend along the skeleton). Voxels in the hemisphere contralateral to these small clusters were examined bilaterally to search for homologous anomalies. The likelihood of two voxels occurring by chance in homologous regions in both hemispheres is very small. We propose that the presence of these low probability differences bilaterally reflects an early, possibly genetic aetiology.

Structural abnormalities that occur bilaterally are typical of developmental disorders, particularly those with a suspected or known genetic etiology (*e.g.* verbal apraxia, [27]; specific language impairment, [12]; schizophrenia, [75]). It seems plausible that a genetic risk factor would have effects on similar structures in each hemisphere rather than acting unilaterally. Congenital disruption would, therefore, suggest that these abnormalities are potentially causal factors in the speech disorder rather than occurring as a consequence of it. Myelination of left language areas typically occurs later than other areas, at 35 months of age [196],

preceding but close to the typical onset of developmental stuttering. It could be that this late myelination phase further exaggerates the effects of a pre-existing difference in organization of white matter tracts in children who stutter. Reduced white matter organisation in both hemispheres would also explain the lack of successful reorganization of function between hemispheres, which is typically seen in focal unilateral lesions acquired congenitally or in childhood [15][243]. The possibility that some of the structural differences observed (particularly the unilateral ones) are a consequence of maladaptive functional reorganization cannot be ruled out [152]. Interesting questions remain about plasticity and recovery during childhood and more frequent recovery in female children [122]. There is a pressing need for longitudinal studies from early in development to address the mechanisms of recovery from stuttering [115].

2.4.3 Implication of cerebral tract differences

We used targeted analyses of probabilistic tractography to dissect out specific anatomical pathways in individual participants so that the white matter tracts connecting areas involved in motor control and language could be explored further. We were careful to track within white matter rather than into the cortex, where cortical differences [65][91] could contribute to the estimate of FA and add variance to connectivity measures. We specifically selected the arcuate fasciculus, corticospinal and corticobulbar tracts and cerebellar peduncles for analysis, as measurements in these tracts are highly reproducible using our standardized methods (see [99]) and these are likely candidates for disruption in developmental stuttering.

The arcuate fasciculus is one of several major tracts comprising the superior longitudinal fasciculus (see above). The standardized tractography analysis successfully dissected the arcuate fibers by placing a seed mask in the middle of this white matter bundle and retaining the tracts that connected it to two target masks located anteriorly in premotor white matter and caudally in posterior temporal white matter. Although the volume of these tracts did not differ between PDS and CON in either hemisphere, the organisation of the tracts (as indexed by FA) was reduced in PDS. The disorganisation in PDS was seen in both hemispheres. However, in the left hemisphere the resulting disruption would likely impair the communication between auditory areas in the posterior temporal cortex and motor areas in the frontal lobes, and these interactions are necessary for fluent speech production [199]. A similar level of anomaly in the right hemisphere might mean that any recovery of function mediated by reorganization to the right hemisphere homologues of speech areas would be suboptimal too.

Dissecting pathways that contribute to speech-motor function separately from those with more general motor function has potential to clarify the picture of white matter abnormalities in PDS. The corticobulbar tract carries the axons of

upper motor neurons from the ventral part of the motor cortex via the genu of the internal capsule to the pons where it innervates the brainstem nuclei of the cranial nerves that supply the muscles involved in articulation and phonation. The organisation of this tract from the most dorsal part of the capsule, where fibers funnel in from the lateral convexity and travel via the posterior limb to the pons, was examined. Data from this tract were compared to data from the same portion of the corticospinal tract that runs in the posterior limb alongside the corticobulbar tract. The connectivity of the left corticobulbar tract was weaker in PDS relative to controls and relative to its right hemisphere counterpart. Connectivity strength was quantified using the number of streamlines in the tract, which is a difficult metric to interpret (see [135]).

Nevertheless, the difference between hemispheres in the PDS group and between the PDS and CON groups is strongly suggestive of a selective impairment in PDS. As only the left tract was affected, this might reflect persistent dysfunction in the left-lateralized motor control of speech. There was, however, an unexpected inverse relationship between stuttering severity and reduced connectivity as measured by number of streamlines: those with greatest dysfluency had the highest number of streamlines in the left corticobulbar tract. One possible interpretation of this apparently contradictory result is that those PDS with the weakest connectivity in the left corticobulbar tract (*i.e.* the greatest structural abnormality) have been forced to reorganize speech motor function to, or recruit involvement of the right tract. Hypothetically, successful reorganization would have resulted in greater fluency and, therefore, lower scores on the SSI-3; reorganization would not have occurred in those individuals with milder abnormality of the left tract. This hypothesis predicts a negative relationship between stuttering severity and the connectivity of the right corticobulbar tract (more streamlines, the lower the severity rating). Our analysis did not support this hypothesis, however, as the relationship was positive and not quite significant.

The corticobulbar and corticospinal tracts continue from the internal capsule to the brainstem or spinal cord via the crus cerebri in the cerebral peduncles. The tract-based spatial statistics analysis revealed bilateral reductions in FA in the cerebral peduncles (see Table 2.1 and Figure 2.1). Acquired lesions in this area affecting the pons and midbrain can cause neurogenic stuttering (*e.g.* [13][74]). Together our results for the cerebral peduncles and the posterior limb of the internal capsule strongly implicate impairment in the descending corticobulbar or cortico-pontine pathways in developmental stuttering.

2.4.4 Implications of cerebellar differences

Functional imaging studies have reported overactivity of the cerebellar vermis in developmental stuttering during speech production (see [40]). It has been proposed

that the cerebellum integrates sensory afference and motor efference to build forward models that predict the consequences of motor acts [165] [258]. As such, abnormal activity in the cerebellum in PDS may be indicative of suboptimal internal models during speech [160]. The organisation of the major white matter fiber bundles, namely the cerebellar peduncles connecting the cerebellum to the rest of the brain, as well as the white matter within the cerebellar lobes and vermis were investigated. There were no significant differences in the white matter in body of the cerebellum but all three peduncles showed disorganisation bilaterally in PDS.

The cerebellum receives the majority of its afferent input through the middle cerebellar peduncle, the majority of which originates in the contralateral pons. The pons in turn receives direct and collateral input from the majority of the cortical mantle including upper motor neurons conveying motor commands via the corticobulbar and corticospinal tracts. Copies of these signals are conveyed to the posterior lobe of the cerebellum along with sensory input from the periphery via proprioceptive and kinesthetic inputs carried mainly in the inferior cerebellar peduncle. Information from the cerebellum is then fed back to the contralateral motor cortex from the cerebellar nuclei via the superior cerebellar peduncle, through the ventrolateral nucleus of the thalamus. One theoretical function of these cerebellar loops is that the feedback from the cerebellum allows the system to adjust ongoing movements and correct errors. It is unclear in stuttering whether there is a primary disruption of the signals to the cerebellum from the motor cortex about the motor commands or the inputs fed back from the periphery or whether it is the integration of these signals that is impaired [160]. In any case, the current analysis shows that all possible pathways via the three peduncles have abnormal microstructure that might reduce the quality or subtly affect the timing of signals in these important feedback loops. In particular, the timing of planning and execution signals is thought to contribute to stuttered speech (*e.g.* EXPLAN model, [111]).

2.4.5 The effects of sex, handedness and age

The potential effects of sex and handedness on neuroanatomical development are not yet well understood. Instead of limiting our sample to a specific gender or handedness, we attempted to evaluate their contribution to the differences we observed in white matter. The small numbers of subgroups of left-handed males and left- or right-handed females who stutter in our study meant it was not possible to examine the interaction between sex and handedness. Also, data were available in only two left-handed controls making between group comparisons impossible. Nevertheless, our exploratory analysis revealed an interesting difference between left- and right-handers who stutter in the asymmetry of the cortico-spinal tract. The right-handed PDS showed a rightward asymmetry whereas the left-handed PDS showed symmetry similar to the pattern seen for this tract in controls. The

structural brain differences between males and females who stutter and the effect of handedness within each group requires further evaluation with larger numbers.

The age range of our PDS and control groups was well matched. Within the PDS there was a significant prediction of white matter organisation in the inferior cerebellar peduncles by age. This relationship was not observed in the controls (see Figure 2.5C). As age and years of stuttering are strongly related in persistent stuttering, this increase in white matter organisation with age might reflect experience-dependent plasticity that is unique to the stuttering group. Longitudinal studies are ideal for testing for this effect further.

2.4.6 Conclusion

A comprehensive analysis of a sizeable sample of PDS replicated previously reported reductions in white matter organisation and extended them using a more targeted approach to tracts of interest. The general picture remains complicated but several interesting associations between white matter differences, speech dysfluency, handedness, and age warrant further study.

Chapter 3

Speech-related activity in people who stutter

Summary: People who stutter show structural and functional abnormalities of the speech and motor system relative to fluent controls. However, it is unclear whether these abnormalities are consistent across tasks. We used functional MRI during two different speech tasks to assess difference between people who stutter and fluent controls. Generally, we observed that group effects were dependent on task demands, and differences between tasks were exaggerated in the stuttering group. In particular, relatively exaggerated increases in activity in the basal ganglia, premotor, and primary motor regions were observed in our stuttering group in response to changing task demands for generating speech content. Finally, we were unable to replicate previous reports of “neural signatures” of stuttering revealed by meta-analysis [40]. Overall our data emphasise the overlap between speech networks in stuttering and fluent adults, and the task-related differences that may contribute to lack of consensus across previous imaging studies.

3.1 Introduction

Stuttered speech can contain repetitions of sounds, or inability to initiate sounds and often occurs simultaneously with facial-motor behaviours similar to tics seen in patients with movement disorders. People who stutter do not typically have other gross movement difficulties nor do they exhibit other cognitive deficits. Components of the speech and motor systems are thus considered the most likely candidates for anomalous development in people who stutter. As there are no post-mortem studies of stuttering, currently, the bulk of our knowledge about disrupted neural systems in stuttering comes from two sources: structural magnetic resonance neuroimaging (MRI), including diffusion weighted imaging (DWI), and functional neuroimaging

(fMRI, positron emission tomography (PET), and resting state fMRI).

3.1.1 Structural abnormalities in stuttering

Structural imaging gives some evidence for disrupted grey matter development in the speech and motor systems in the form of abnormal auditory cortex gyrification [90], reduced thickness in speech-motor planning regions [152], and alterations to volume in left basal ganglia [154][223]. Some groups have shown anomalous white matter development in portions of the corpus callosum [57][59][65]. A consistent finding across other diffusion studies show disorganised white matter underlying speech-motor planning regions [220] that also show disrupted function [249]; relatively disorganised white matter in major speech and motor pathways including the superior longitudinal fasciculus and all three pairs of cerebellar peduncles [59] (See Chapter 2).

3.1.2 Functional abnormalities in stuttering

Functional imaging literature likewise supports disruption in the speech and motor systems, in event-related and resting-state studies. A meta-analysis of overt speech in stuttering compared activation maps in people who stutter and in fluent controls in eight early neuroimaging studies, six of which used PET imaging [40]. The authors concluded that people who stutter and fluent controls largely recruit the same brain regions during speech, though a diagnosis of stuttering was related to increased activation in lateral speech-motor regions and other components of the motor system including the supplementary motor area (SMA), cingulate cortex, and the vermis of cerebellum. The meta-analysis identified three “neural signatures” of stuttering: 1) over-activation of the right frontal operculum, anterior insula, or both; 2) over-activation of the cerebellar vermis; and 3) an “absence” of activity in auditory cortex [40]. Treatment studies combined with fMRI to evaluate outcome due to therapy also implicate the frontal operculum, which is resistant to interventions both during tasks [175] and at rest [152]. Abnormal cerebellar activity appears to be related to treatment both during speech tasks and at rest in those same studies.

Though notably not a conclusion in the meta-analysis, abnormal basal ganglia activity is possible in stuttering, particularly as other core motor system components including cerebellum and SMA show altered activation consistently across studies. It is unlikely that activity of any kind in core components of the motor network would occur independent of corresponding signals from hub that excites and inhibits virtually all neural processes relating to intentional movement and sensory feedback processing. Empirical support for basal ganglia involvement in stuttering is not conclusive, however: dopamine blockers are only sometimes successful in treating

stuttering (for review see [157]); the early imaging studies of increased dopamine uptake in the basal ganglia components (*e.g.* [261]) have not been replicated in independent samples with more appropriate control groups; and increased activity within individuals subsequent to treatment [175], compared to fluent controls during speech-related fMRI [249] or during periods of rest [55][267][152] is reported in only a portion of studies (see Table 1, Introduction). Further, correlations between basal ganglia activity and stuttering severity or constellations of symptoms should be considered indirect evidence, at best [100][134]. There is strong theoretical corroboration for central involvement of the motor control system in stuttering, still, there is a lack of continuity across functional imaging studies implicating specific basal ganglia structures or the direction of functional abnormalities (over vs. under-activity) relative to controls.

3.1.3 The aim of the current study

The primary aim of this study is to explore the influence of stuttering on overt speech-related brain activity in adults with persistent developmental stuttering (adults who stutter, PDS) and fluent controls (CON). We used two tasks to elicit overt speech: 1) sentence reading and 2) picture description. We selected these conditions because traditionally stuttering is only elicited through more complex, longer utterances [40]. Additionally, the conditions should be well matched for speech production generally, as they place similar demands on the articulatory system. Previous studies have used sentence reading (*e.g.* [249]) and described differences in brain activity in people who stutter in several regions, including some of those identified as neural signatures. The broader utility of these neural signatures in other contexts is questionable, however, as illustrated by a PET study under two conditions: oral reading and self-generated free monologue [123]. In fact, the presence of neural signatures was only partially supported, and only at lowered statistical thresholds. Further, the networks engaged across overt-speech tasks, in both people who stutter and fluent controls, were virtually identical, however, regions showing activation differences between tasks were exaggerated in the stuttering group [123]. Our study is the first to investigate fMRI activation during self-generated speech sequences using picture description in stuttering (as opposed to picture naming [151][117]).

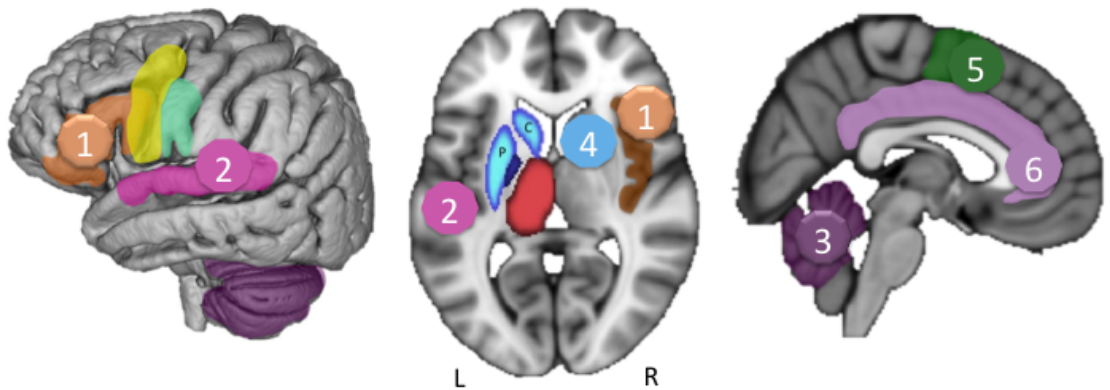
We expected recruitment of additional speech-motor planning regions in the picture description task, which has a spontaneous speech component involving planning of both speech content and articulatory movement. Furthermore, we significantly increased the amount of speech, mostly fluent, produced in the scanner from that obtained in previous studies. By effectively doubling the amount of speech and including the picture description condition, we hoped to be able to capture utterances that were both fluent and dysfluent and compare the related

patterns of activity (see next chapter). The contrasts of interest include each condition relative to baseline and the picture description condition relative to sentence reading.

We made the following predictions regarding the differences between groups:

- Across conditions we expected over-activity in right inferior frontal cortex, under-activity in auditory cortex, and over-activity in the cerebellum in PDS relative to CON, consistent with the reported “neural signatures” of stuttering (Figure 3.1; 1,2,3, respectively).
- We predicted that the contrast between conditions (picture description > sentence reading) would be greater in PDS than in CON in primary speech-motor planning regions (preSMA and medial prefrontal cortex; Figure 3.1: 4,5, respectively) and in regions supplying internal cuing about speech initiation to the medial frontal cortex (*i.e.* striato-pallidal-thalamic outputs) of the basal ganglia: Figure 3.1 5).

Figure 3.1: Functional disruptions in stuttering



Regions in which we predicted differences between fluent controls (CON) and adults with persistent developmental stuttering (PDS) based on the “neural signatures” of stuttering: 1) frontal operculum/insular cortex; 2) auditory cortex; and 3) cerebellar vermis. Components expected to be taxed by the spontaneous speech component of our tasks: 4) basal ganglia (C=caudate nucleus, P=putamen); 5) pre-supplementary motor area; and 6) anterior cingulate cortex.

3.2 Methods

3.2.1 Participants

The dataset used in this study was the same as that used in Chapters 4 and 5, which examined speech-related activation and rest-related activation in stuttering individuals and fluent controls, respectively. Seventeen adults with persistent developmental stuttering (PDS: 13M:4F; aged 19-54 years; 3 left-handers) and 17 age- and sex-matched fluent controls (CON: 13M:4F; aged 19-53 years, avg. 32.3y; 3 left-handers) were scanned using functional MRI. No controls had a history or diagnosis of learning or speech disorders. All participants gave informed consent to their participation in the research in a protocol approved by University of Reading's ethics committee. Stuttering ranged in severity from very mild to very severe as assessed using the Stuttering Severity Instrument [203] (SSI, versions III and IV, range 10-46, average 24.7, s.d. 10.9).

3.2.2 Data acquisition

Functional MRI data were obtained at the University of Reading's Centre for Integrative Neuroscience and Neurodynamics using a 3-T Siemens Trio scanner with a 12-channel head coil. Whole-head T2*-weighted echo-planar images (TE=30ms) were acquired every 9s with a silent delay of 7s (*i.e.* sparse sampling) and comprised 2-s acquisition of 32 4-mm axial slices (in-plane resolution 3 mm x 3 mm). A '+' appeared in the middle of the screen during the 2-s acquisition period (Figure 3.2).

During the 7-s silent delay between measurements, subjects saw a stimulus via scanner-compatible goggles that was either a picture with a descriptive sentence below it, a picture with no text, or a '+' in the middle of the screen. Subjects were instructed to read the sentences aloud (Sentence Reading condition) or to overtly describe the pictures (Picture Description condition) and were explicitly told to stop speaking when the crosshair appeared so that there would be no speech-related movement of the head during data collection. Prior to the scan the task was explained to subjects who were allowed to practice outside the scanner. For each of the conditions and the baseline condition, 40 volumes of data were acquired for a total of 120 volumes per run (18 min); the order of conditions was fixed and pseudorandom. Two runs were acquired in each subject, yielding 80 volumes of each condition (Sentence Reading, Picture Description, and Baseline). The picture description condition requires planning in both speech content and motor movement. The sentence reading condition involves articulatory system coordination without the need to generate speech content, but does not allow PDS to use word-substitution strategies to avoid dysfluencies. Speech was recorded using an MRI-compatible microphone. These recordings were later checked for task

compliance. Sentences were marked as normal or dysfluent.

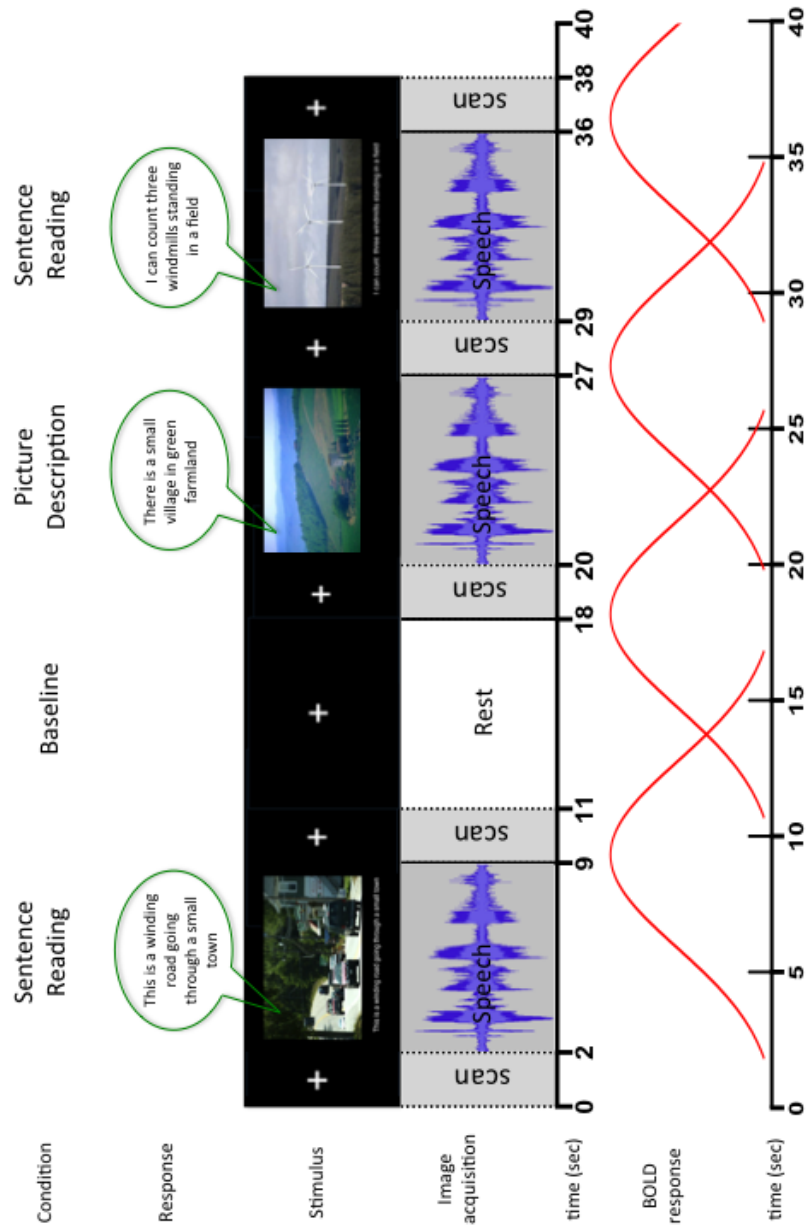
3.2.3 Image analysis: Whole brain

3.2.3.1 Preprocessing

The functional images were analyzed using the FMRIB Software Library (FSL 5.0.6; <http://www.fmrib.ox.ac.uk/fsl>, [132]). Standard motion correction, and individual volumes that were motion outliers were included as separate regressors at the first level for each subject. Excessive motion (*i.e.* $> 4\text{mm}$) was observed towards the end of a single scan session in one PDS and one CON, and these volumes were removed from the time series (*i.e.* the runs were truncated by 29 volumes (PDS) and 20 volumes (CON) within a single 120-volume run). The remaining data were analyzed in the same way for all participants. Each dataset was unwarped using a fieldmap and PRELUDE and FUGUE software running in FSL and spatially smoothed with an 8-mm full-width-at-half-maximum smoothing kernel. A temporal high-pass filter with a cutoff of 150 seconds was used to remove low-frequency fluctuations in the signal. Two further regressors were used in the first-level analysis to remove residual image artefacts due to physiological changes. These regressors were the mean time-courses extracted from preprocessed data from 4-mm radius spheres in areas where task-related activity was not expected, one was placed within cerebrospinal fluid of the anterior lateral ventricle (standard space coordinates 2, 10, 8) and the other within white matter in the dorsal posterior frontal lobe (-26, -22, 28) [145]. Images were registered using boundary-based registration [103] to the individual subject's T1-weighted structural image (1 mm³ voxels; TR = 2020 ms, TE = 2.9 ms, flip angle = 90°), which in turn was registered using FNIRT (FMRIB's nonlinear registration tool) to the MNI-152 template.

For individual subjects, statistical maps were generated to show patterns of activation during each condition relative to baseline and between the Picture Description and the Sentence Reading conditions. Contrast masking was used at the first level to examine differences between conditions only in regions where positive activation ($Z > 0$) occurs in response to the stimuli in each condition separately. The data for the two runs in each subject were combined using a fixed-effects analysis. Group averages and contrasts between groups were analyzed using FMRIB's Local Analysis of Mixed Effects stage 1 [259]. Contrast masking was used for the group comparisons to show only regions in which both groups had positive activity ($Z > 0$) for each contrast of interest overall. Because PDS and CON were well-matched on age, sex, and handedness, we did not model these variables for inclusion in whole-brain group comparisons.

Figure 3.2: Task design and scanner acquisition method



Two conditions: Read (Sentence Reading) and Speech (Picture Description) were used to assess speech-related brain activity. Sparse sampling catches the middle of peak activation (BOLD response) which would occur 4-6 seconds after speech is initiated and remain until that long after speech stops.

3.2.4 Image analysis: Regions of interest

We had a-priori expectations of anomalous activity in PDS in those areas identified as the “neural signatures of stuttering” [40]. We therefore explored activity in regions of interest (ROIs) selected from a seminal metaanalysis to assist with interpretation of whole brain effects. We extracted average percent signal change data from the fixed-effects parameter estimates for each individual, averaged across scan session.

We created spheres (8mm radius) around atlas-transformed standard-space coordinates of peaks reported in the meta-analysis [40]. These coordinates were transformed into MNI space suitable for use with FSL tools using GingerAle freeware [82]: 1) first to MNI space from Talairach, using a Brett transformation, 2) then back into Talairach space using the inverse of that transformation, and 3) finally back into MNI space using a Lancaster transformation [142][143]. MNI coordinates for ROIs were the right frontal operculum (44, 11, 7), right cerebellar vermis (2, -44, -14), and auditory cortex, bilaterally ((-)50, -3, -3).

Group differences in signal change were assessed using repeated-measures ANOVAs with a between-subject factor of group (PDS vs. CON) and within-subject factors of condition (sentence reading vs picture description), and ROI (Auditory cortex vs Frontal operculum vs Vermis). Because PDS and CON were well-matched on age, sex, and handedness, we did not model these variables for inclusion. Significant group effects were explored using independent *t*-tests, and pairwise *t*-tests were used to explore significant within-subject effects, all with the threshold of $p < .05$ following Bonferroni correction to adjust for multiple comparisons.

3.3 Results

3.3.1 Behavioural results

We predicted the picture description condition would place additional demands on linguistic and motor planning and self-initiated speech processes that could be disrupted in PDS. We expected the conditions to be otherwise well matched for articulatory efforts and overt speech execution. PDS stuttered in the scanner, on average, 20 times out of 160 utterances. CON showed some dysfluencies, but many fewer (2 of 160). Stuttering frequency was equivalent across conditions. Results reported in this chapter contain all speech events.

3.3.2 Whole brain results

We first compared all overt speech-related activity in PDS and CON. The data were analyzed by comparing each of the speaking conditions (Picture Description and Sentence Reading) with the baseline (crosshair) for the two subject groups. During both conditions all groups had similar patterns of activation, with increased activity in the expected networks including ventral sensorimotor cortex, superior temporal cortex, motor cortex, premotor regions, basal ganglia, cerebellum, preSMA, and occipital cortex (see Figure 3.3). The patterns of activation for each condition were then examined for group differences and compared between PDS and CON (Figure 3.4). Finally, the interaction of condition by group was modeled by comparing groups using the contrasts of Picture Description with Sentence Reading (Figure 3.5).

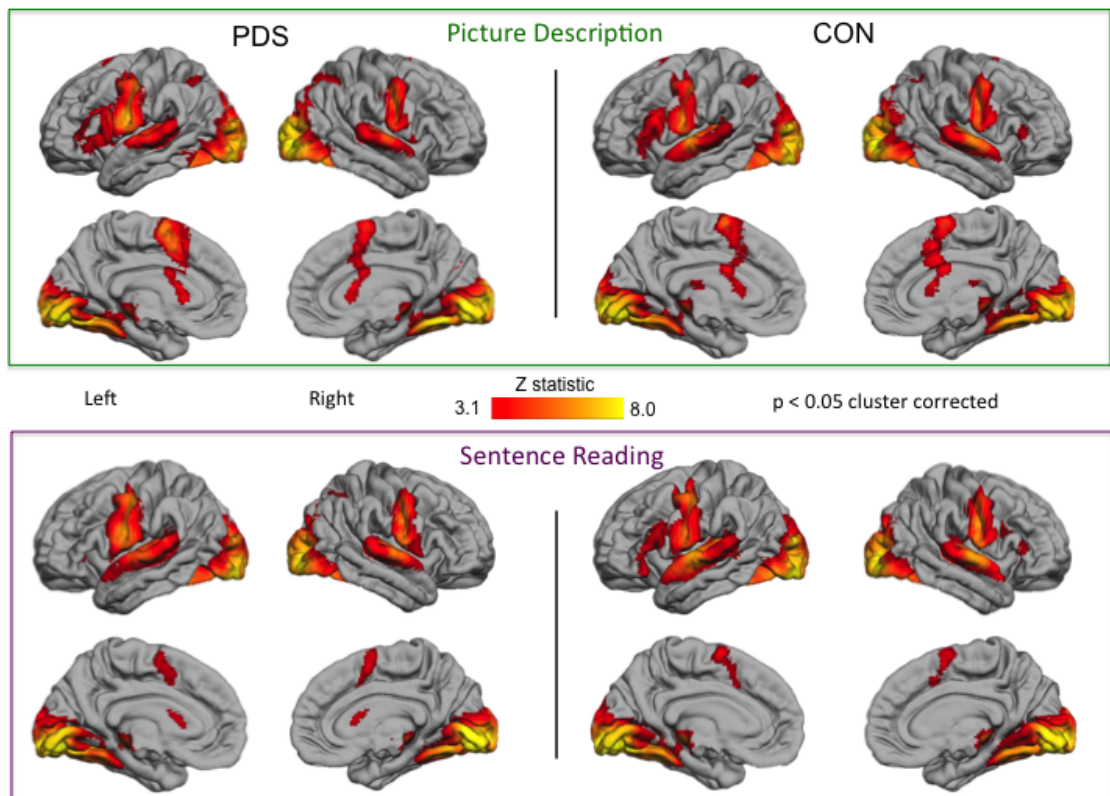
For the group averages, data were thresholded at a cluster-forming threshold of $Z > 3.1$ and an extent threshold of $p < 0.05$ corrected for multiple comparisons. The analysis did not reveal any significant group differences at these thresholds corrected for multiple comparisons across the whole brain. Comparisons between groups are therefore reported at an uncorrected threshold of $p < 0.01$, with an additional constraint that the cluster size was at least 30 voxels for peaks in the grey matter of motor- or language-related areas. Group differences at this threshold in the frontal pole/orbital regions, inferior temporal cortex, or occipital cortex are reported but not discussed.

3.3.2.1 Picture description compared to baseline

Both groups activated the expected network of areas involved in overt speech production, namely bilateral posterior superior temporal cortex, sensorimotor cortex at about the level of the face representation, SMA and preSMA, and left lateralized posterior IFG. Extensive medial and lateral occipital cortex activity was seen due to the presentation of the picture stimulus for description (Figure 3.3).

Examination of these group averages shows the PDS and CON to be strikingly similar in terms of the speech networks activated. The PDS show more extensive activation of the left IFG, lateral and medial premotor cortex relative to the controls. Direct contrasts between the groups revealed that these cortical regions on the lateral and medial surface were significantly more active in PDS compared to CON, but only at an uncorrected threshold of $p < .01$ and extent ≥ 30 voxels (see Figure 3.4, Table 3.1). Additionally, at the same statistical threshold, the head of the caudate nucleus and putamen in the left hemisphere were more active in PDS compared to controls. Furthermore, PDS had significantly less activity than CON in the right IFG and superior temporal gyrus, in left posterior STG (including auditory cortex), and left posterior lobe of the cerebellum (Table 3.1, Figure 3.4).

Figure 3.3: Overt speech-related activity in adults who stutter (PDS), and controls (CON).



The same pattern was observed in both groups: the expected network of auditory cortex in both hemispheres, motor cortex, pre-supplementary motor area, basal ganglia, and frontal speech-motor regions was observed in both conditions

Table 3.1: Regions where there were differences between groups in activity during Picture Description vs. baseline.

Brain Region	# voxels	Z statistic	X	Y	Z
PDS > CON					
Left caudate (head)	34	2.99	-18	16	10
Left putamen	42	2.6	-20	10	-4
Left preSMA	341	3.65	-10	6	48
Left cingulate gyrus		3.33	-12	6	38
Left inferior frontal junction	78	2.81	-52	2	18
pars opercularis					
Left lingual gyrus	36	3.38	-28	-58	-2
Right dorso lateral occipital cortex	137	3.1	28	-68	22
Left ventrolateral occipital cortex	53	3.1	-52	-70	-20
Right occipital pole	88	3.54	42	-94	0
PDS < CON					
Right inferior frontal gyrus	30	2.75	52	32	8
pars triangularis					
Right superior temporal gyrus	61	3.02	64	0	-4
Left auditory cortex	88	2.93	-56	-12	2
Heschl's Gyrus					
Left superior temporal gyrus (ant)		2.61	-58	-10	-8
Left posterior lobe of the cerebellum (crus I)	133	2.99	-46	-52	-30

Location of the highest peak in a cluster is given: voxelwise, $p < .01$, *uncorrected* with ≥ 30 voxel extent. Selected sub-peaks within the large clusters are also described. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space.

3.3.2.2 Sentence reading compared to baseline

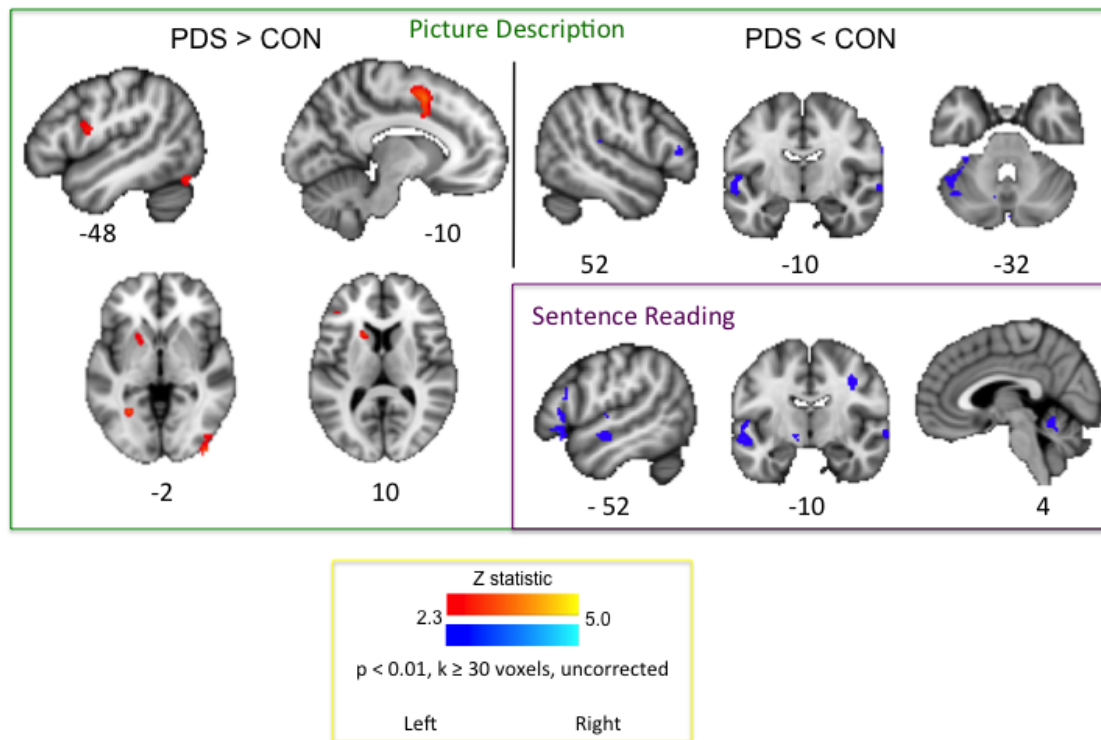
Both groups activated the expected network of areas during sentence reading, namely bilateral posterior superior temporal cortex, sensorimotor cortex at about the level of the face representation, SMA and preSMA, and left lateralized posterior IFG. Extensive medial and lateral occipital cortex activity was seen due to the presentation of the picture stimulus (Figure 3.3). Examination of these group averages shows PDS and CON to be strikingly similar in terms of the reading networks activated. The PDS show relatively less activation of the left IFG and lateral premotor cortex relative to the CON.

Direct contrasts between the groups revealed that these cortical regions on the lateral surface were more active in CON compared to PDS, but only at an uncorrected threshold of $p < .01$ and extent ≥ 30 voxels (see Table 3.2, Figure 3.4). PDS showed relatively less activation for the sentence reading compared to CON occurring bilaterally in several speech regions including inferior frontal cortex (pars opercularis, pars triangularis) and superior temporal cortex. In addition, PDS also showed several right hemisphere reductions in activity relative to CON in the insular cortex, parietal operculum, motor cortex, the cerebellar vermis, and posterior lobe of the cerebellum. As predicted, the left auditory cortex was also underactive in PDS relative to CON.

3.3.2.3 Picture description versus sentence reading

As expected, both groups showed greater activity for the picture description condition than the sentence reading condition in the major components of the classic motor-planning network, namely medial pre-motor cortex and preSMA, and subcortical grey matter localized in the head of the caudate nucleus and extending into putamen and thalamus on the left (Figure 3.5). Direct contrasts between the groups revealed that the difference between conditions was most pronounced in PDS, as would be expected given the patterns observed in each condition relative to baseline, but only at an uncorrected threshold of $p < .01$ and extent ≥ 30 voxels (see Table 3.3, Figure 3.5). PDS showed a greater difference in activation between conditions relative to CON in the left hemisphere in one extensive cortical cluster in the posterior IFG extending to preSMA; and one extensive cluster in the subcortical gray matter localized in the head of the caudate nucleus. A single cluster showed greater contrast between conditions for CON relative to PDS in the left anterior lobe of the cerebellum at this lowered threshold.

Figure 3.4: Group activation differences during tasks conditions in adults who stutter (PDS) compared to controls (CON).



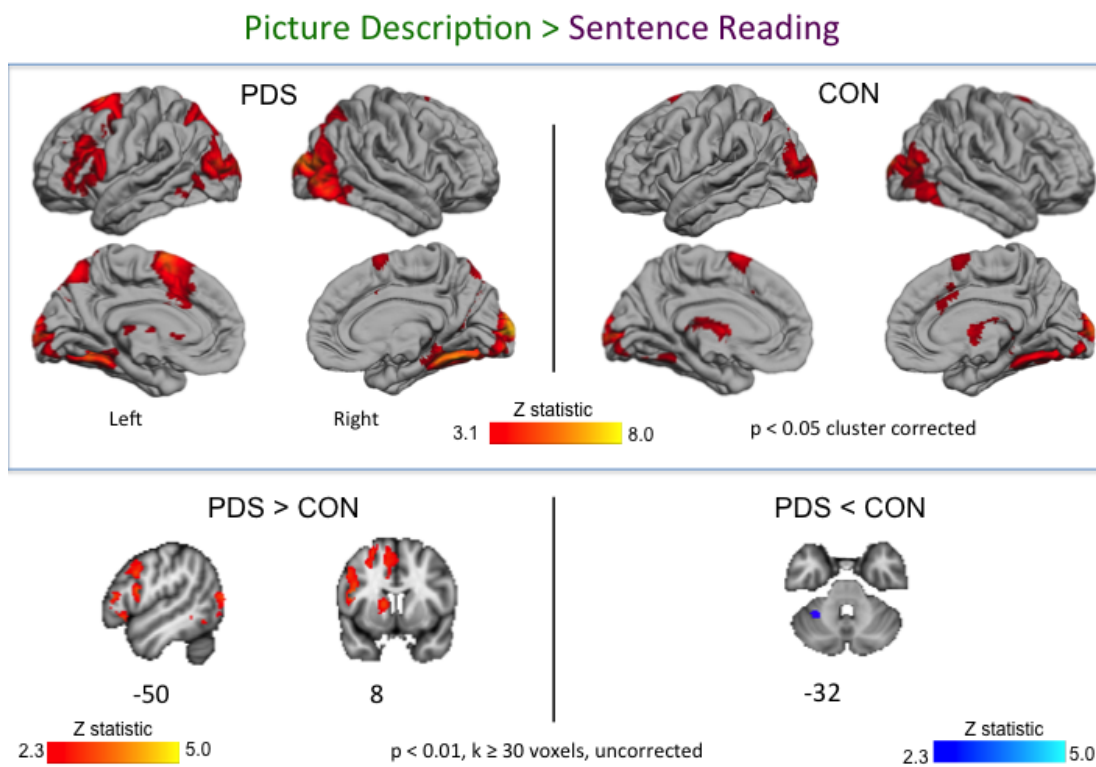
PDS show increased activity relative to CON only in the picture description condition, which has an additional spontaneous speech component. This increased activity in PDS is localized to preSMA, left ventral premotor cortex, and the basal ganglia. Decreased activity in PDS relative to CON was observed in the left hemisphere cerebellum as well as superior temporal regions, bilaterally, and right hemisphere inferior frontal cortex. During sentence reading there were no regions for which PDS had increased activity relative to CON, however relatively decreased activity in the PDS group was seen in inferior frontal gyri occurring bilaterally, in the right hemisphere insular and opercular cortices and cerebellum and the left hemisphere auditory cortex.

Table 3.2: Regions where there were differences between groups in activity during Sentence Reading vs. baseline.

Brain Region	# voxels	Z statistic	X	Y	Z
PDS < CON					
Left frontal pole	105	3.5	-46	26	-10
pars orbitalis					
Right inferior frontal gyrus	33	3.01	54	34	8
pars triangularis					
Right inferior frontal gyrus	64	3.66	44	20	20
pars opercularis					
Left inferior frontal gyrus	216	3.03	-44	20	22
pars opercularis					
Left inferior frontal gyrus		2.74	-58	20	4
pars triangularis					
Right motor cortex	64	3.14	36	-8	40
Left superior temporal gyrus	251	3.02	-56	-10	-10
Left auditory cortex		2.8	-56	-12	2
Heschl's Gyrus					
Left temporal pole		2.73	-64	4	-6
Right insular cortex	76	3.04	40	-24	20
Right parietal operculum		2.72	46	-26	20
Right anterior vermis	69	2.6	6	-52	-18
Right posterior lobe of the cerebellum (crus I)	45	3.13	16	-86	-26
Left ventrolateral occipital cortex	38	2.68	-34	-86	8
Right dorsolateral occipital cortex	56	3.41	40	-88	20

Location of the highest peak in a cluster is given: voxelwise, $p < .01$, *uncorrected* with ≥ 30 voxel extent. Selected sub-peaks within the large clusters are also described. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space.

Figure 3.5: Group activation differences during picture description compared to sentence reading in adults who stutter (PDS) and controls (CON).



Group averages at the top illustrate an exaggeration of the patterns observed in tasks, that is overactivity during the picture description condition in speech-motor planning regions in PDS.

Table 3.3: Regions where there were differences between groups in activity during Picture Description vs. Sentence Reading

Brain Region	# voxels	Z statistic	X	Y	Z
PDS > CON					
Left inferior frontal gyrus	2779	4.09	-44	36	4
pars triangularis		3.95	-42	32	6
pars opercularis		3.86	-48	10	18
ventral premotor cortex		3.83	-44	12	34
superior frontal gyrus		3.76	-24	14	56
preSMA		3.25	-4	4	52
Left dorsal striatum (caudate nucleus)	297	3.35	-12	12	8
putamen		2.5	-20	0	6
thalamus		2.5	-12	-6	4
Left supramarginal gyrus	53	2.96	-32	-42	38
Right temporal occipital fusiform cortex	510	3.42	28	-54	-16
lingual gyrus		3.35	22	-40	-10
parahippocampal gyrus		3.03	30	-40	-12
Left temporal occipital fusiform cortex	671	4.21	-28	-58	-8
lingual gyrus		3.09	-20	-42	-12
parahippocampal gyrus		3.21	-30	-40	-12
Precuneus	655	3.44	8	-70	54
Left occipital	230	3.46	-54	-78	12
Left occipital	37	2.71	-8	-64	72
Right occipital	292	3.64	44	-84	-4
Right occipital	33	2.74	46	-68	6
Right occipital pole	226	3.16	12	-96	8
Right occipital pole	62	2.9	34	-94	16
PDS < CON					
Left anterior lobe of the cerebellum (lobule VI)	37	2.67	-32	-50	-32

Location of the highest peak in a cluster is given: voxelwise, $p < .01$, *uncorrected* with ≥ 30 voxel extent. Selected sub-peaks within the large clusters are also described. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space.

3.3.3 Regions of interest results

In the 3 (region) X 2 (condition) X 2 (group) repeated measures ANOVA, the assumption of sphericity was not met, therefore Wilks' Lambda-corrected values are reported. There was no main effect of group, and the 3-way interaction between condition, region, and group was not significant. Though the patterns among regional means appeared to differ between groups, even uncorrected independent *t*-tests did not reveal any significant differences between groups for any of the possible ROI combinations or pairs probed (Figure 3.6).

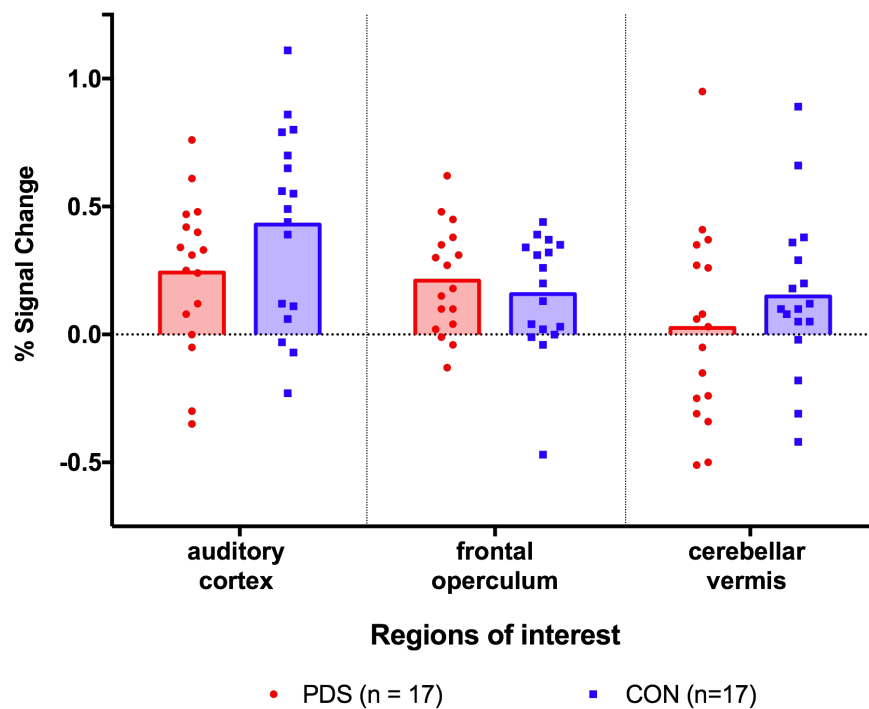
There was a significant main effect of region, ($F(2,31)=5.618, p = .008$), that interacted with condition, ($F(2,31)=8.898, p = .001$) and group ($F(2,31)=3.770, p = .034$), such that within CON, the auditory cortex was more active than both other ROIs across conditions, however, within PDS, activation did not differ between regions. During sentence reading, the auditory cortex was relatively overactive compared to both other ROIs ($p < 0.001$), which did not differ ($p = .073$). During picture description, however, the % signal change was equivalent across regions. The frontal operculum did not show a change in activation between conditions for either group. In the auditory cortex, percent signal change was higher during sentence reading than during picture description ($p = .007$). The opposite pattern was observed in the vermis ($p = 0.001$).

3.4 Discussion

Our aim was to explore the influence of stuttering on overt-speech related brain activity. We expected to confirm involvement of the neural signatures of stuttering [40], however, our primary finding was that PDS and CON recruit the same networks, to similar degrees, during overt-speech production, regardless of condition. In the whole brain analysis we observed some group differences, but only at small effect sizes that did not survive correction for multiple comparisons. We report these results at an uncorrected threshold and attempt to limit false positives by imposing an arbitrary cluster extent threshold that is consistent with the literature. At this lowered threshold, we observed a general task-related pattern across regions in PDS relative to CON: under-activity during sentence reading and over-activity during picture description. Specifically, relative to CON at lowered thresholds, PDS showed:

1. Decreased activity in auditory cortex and right inferior frontal gyrus in both conditions.
2. Primarily right-lateralized decreases during sentence reading and left-lateralized decreases during picture description in the posterior cerebellar lobes.

Figure 3.6: “Neural signatures” of anomalous activity in stuttering were not confirmed



Even at liberal thresholds, there were no differences between groups in any region. Scatterplots have a bar indicating the group average to illustrate spread of percent BOLD signal change in these regions, averaged across condition. As was the case in the whole brain analysis, activation within these regions does not distinguish PDS from CON.

3. A general shift from relatively decreased activity during sentence reading to relatively increased activity during picture description in the left posterior IFG.
4. Increased activity during picture description in left-lateralized speech-motor planning network, including lateral and medial premotor regions (posterior IFG, preSMA) and subcortical motor control structures (caudate nucleus, putamen).

3.4.1 Similarity is a signature of stuttering

Our findings are consistent with previous work (*e.g.* [123]) emphasising the similarities in the overt-speech network activated by PDS and CON and questioning the appropriateness of attempts to isolate a consistent set of “neural signatures”, given the subtle effect sizes most commonly reported. Overall, our investigation of fMRI activation during self-generated speech sequences was consistent with two studies using PET imaging during similar utterances [39] [123]. In particular our findings of increased recruitment of premotor and motor regions in stuttering relative to fluent controls during self-formulated speech replicated previous work using similar paradigms [39][123]. Our general findings of decreased activation in sentence reading are also somewhat consistent with the literature [249], though reductions in stuttering are most common during single-word production [178][54][153][151][117]. Our primary finding was that activation patterns during speech are more similar than different between adults who stutter and fluent adults. Further, the direction of differences was somewhat specific to the tasks selected for our study, as were similarities to the literature. It is key to identify not only the factors that contribute to our inability to detect valid differences between groups, if they exist, but also those factors that maximize valid similarities.

In any fMRI experiment, the first logical question to ask is: what are people actually doing during the scan? The scanning environment often interferes with normal speech patterns, and in particular, induces fluency in PDS. PDS and CON recruit the same networks in most speech tasks in the scanner, and this could be because the two groups are largely doing the same thing, that is, they are both speaking fluently. In fact, many studies selectively attempt to induce fluency in order to compare groups. Perhaps we must observe dysfluency in real time to properly isolate the functional correlates of stuttering.

Our task design, with long, complex, and sometimes self-generated utterances, was intended to increase the chance of dysfluency in the scanner. To a certain extent we were successful in that effort. Although some of our PDS were almost completely fluent in the scanner, over half were somewhat dysfluent. In the next chapter we take a multi-level approach to exploring the influence of fluent speech

as a potential factor maximizing similarities in overt-speech related activity in PDS and CON. We will first observe activation patterns during fluent events only, then compare activity between PDS who are mostly fluent and those who are mostly dysfluent, and finally, within the dysfluent PDS, compare fluent to dysfluent utterances directly. In this way we hope to isolate activation related specifically to the dysfluent state, as well as to the group trait of “dysfluent” during fluent speech only.

3.4.2 Variability is a signature of stuttering

As a field we should reexamine the plausibility of a “neural signatures” view of stuttering [40]. In the whole brain analysis, we did observe the predicted decrease in auditory cortex activity in PDS in both conditions, but only at lowered thresholds. Again, in the whole brain analysis, we did see abnormal activity in the right IFG and cerebellum, but these activations were in the opposite direction of those predicted: PDS show reduced activity, not increased activity, in these regions in our study.

In general, PDS showed under-activity relative to CON in cerebellar regions in both conditions and in the contrast between conditions. The spatial location of these differences, however, was particularly variable within our study: the right posterior lobe of the cerebellum and cerebellar vermis were underactive only during sentence reading; the left posterior lobe of the cerebellum was underactive only during picture description; and the left anterior lobe of the cerebellum was underactive in the contrast between conditions. The cerebellum is the hub for almost all command processes integrating primary systems in the brain. For this reason, it is likely that subjects recruited cerebellar resources for a variety of processes necessary to complete these tasks, including speech-motor planning and visual processing. Contrary to our predictions, there were no cerebellar over-activations in PDS relative to CON in any of the contrasts of interest. The lateralized under-activity within the cerebellum is partially consistent with contralateral cortical reductions in activity in PDS, however, a clear pattern of network-level changes is beyond the scope of this analysis. Future work should examine cerebellar functions specifically within PDS with data-driven exploration of functional divisions of the lobes in order to clarify the nature of this disruption.

Though partially supported by the whole brain analysis, evidence for neural signatures of stuttering was not confirmed in a more focused approach. In the regions of interest analysis, using peaks derived from the seminal metaanalysis [40], signal change was equivalent in PDS and CON in all regions, including the auditory cortex. Peaks derived from the literature were localized anterior and medial to the peaks showing group differences in the whole-brain analysis in this study. In this way, it is clear that methodological variability limits the utility of such specific, directional regional predictions.

Differences in methods (study design, tasks demands), subject variables (age, gender, handedness, family history), and presentation of the stutter (rate, severity, therapy history) all likely contribute to the variability in previous reports of stuttering. The fact remains, however, that even in well-matched samples such as the one recruited for this study, or gender and handedness restricted samples, the effect sizes of activation differences are small at best. It could be the case that we simply do not have power to detect very real differences between PDS and CON. Rather, it could be the case that we have the power, but the differences are actually that subtle, that refined, that complex, and a more focused yet integrated approach is necessary.

3.4.3 Speaking conditions matter in stuttering

Our complex task design was also intended to selectively tax components of the speech-motor system critical for motor planning and initiation, processes thought to be disrupted in stuttering. We selected sentence reading and picture description as conditions well-matched for speech production generally, as they place similar demands on the articulatory system. These conditions differ critically, however, in the demands they place on the planning system. In the sentence reading condition, subjects did not need to generate content for speech, but instead simply read a descriptive sentence. In this case the sentence acted as both the cue and the content for speech. In the picture description condition, however, utterances were self-generated, and though a picture stimulus was provided as a cue for content, the need to plan content and then internally cue speech would necessitate recruitment of additional structures, namely the medial and lateral premotor cortices and subcortical regions.

Though the additional network recruited by the picture description condition was largely similar in both groups, consistent with our prediction, the contrast between conditions (picture description > sentence reading) was exaggerated in PDS relative to CON. Further, this contrast was an exacerbation of the patterns of abnormal activity observed in each condition individually relative to baseline. In both tasks we observed abnormal activity, albeit at lowered thresholds, but the direction of the differences and the dysfunction they could reflect was dependent on task. During the sentence reading task, in which both content and cue for speech was provided, PDS were largely underactive relative to CON. During the picture description condition, however, the need for planning speech content and internally cuing speech initiation led to a pattern of over-activation in PDS relative to CON, observed in primary speech-motor planning regions (preSMA and medial prefrontal cortex) and in subcortical structures supplying internal cuing about speech initiation to those planning regions, which is again consistent with similar designs using PET imaging [123].

Traditionally, over-activity in one group in fMRI is viewed as excitation, facilitation, or compensation, whereas under-activity is most often interpreted as dysfunction. This oversimplification of the relationship between the BOLD signal, its spread and magnitude, and the corresponding neuronal functions is not applicable in the current study. On the one hand, increased fMRI activation could represent an excitatory effort to recruit regions for completing a task, or to take it one step further, to complete a task successfully. In this case we could interpret our findings of over-activation in PDS during picture description as compensatory: PDS must recruit the same regions to a greater extent than CON to accomplish the same thing. On the other hand, increased activation could represent an inability to suppress or attenuate signal following adaptation, it could represent an inefficient, less focused information processing loop, or even a more fundamental imbalance of excitatory and inhibitory signals. In this case, reduced activation would reflect facilitation, and observed over-activation could be interpreted as potentially dysfunctional.

The requirements of the picture description tasks likewise recruited subcortical structures, primarily the caudate nucleus and the putamen. The brain is an incredibly complex functional circuit, with no regions acting in isolation. Even, and especially, in the subcortical structures, where signal input is sometimes unidirectional (*e.g.* signals come into the thalamus and go out to the cortex), the signals themselves can serve as both excitatory and inhibitory depending on their origins and destinations. These structures are important for feedback processing and are critical for generating internal cues for movement. In both CON and PDS, these subcortical structures showed a sharp increase in percent signal change with the requirements of the picture description condition relative to the sentence reading condition, suggesting similar recruitment to meet tasks demands.

The subcortical signal was exaggerated and left-lateralized in PDS for picture description only. This task-specific over-recruitment in PDS could suggest a crucial role of the subcortical system in spontaneous speech production in stuttering. Perhaps this over-activation reflects disorganised or excess input from cortical structures or excitatory signals contributing to other over-activations in the speech-motor planning system. It could also reflect disorganised or excessive inhibitory signals contributing to, or ultimately originating from, the under-activations in right inferior frontal gyrus and left cerebellum. We could be observing compensatory activity, an effort to meet increased task demands. These structures have long been implicated in stuttering, though empirical evidence of their involvement is not consistent enough to paint a clear picture of just how, where, or when the basal ganglia are disrupted (or supportive) in stuttering.

3.4.4 Conclusion

The similarities between adults who stutter and controls in the networks recruited for overt-speech are striking. Group differences are only apparent at very low thresholds, increasing the chance of false positive findings. That being said, our results are in agreement with previous reports that emphasise the variability in abnormal speech-related activity in stuttering (*e.g.* [262]). We observed general patterns of abnormality that changed with task demands, which were also consistent with previous work [123]. These abnormalities could potentially reflect processes related to the presence or absence of a timing cue for the initiation of speech, to the demands of generating speech content versus reading a set script, or both. The degree to which this altered activity is maladaptive or compensatory, or some combination of the two is an area for serious theoretical and empirical investigation. Determining the degree to which these abnormalities are related to dysfluency states, or are rather some subtle correlate or consequence of fluent speech production reflecting traits of people who stutter more generally, is especially critical.

Chapter 4

Indicators of state and trait effects in people who stutter

Summary: People who stutter show structural and functional abnormalities of the speech and motor system relative to fluent controls. However, it is unclear which abnormalities reflect general traits of the disorder and which are related to the specific states of dysfluent speech. We used functional MRI during two different speech tasks to identify structures that might indicate (i) general traits of fluent speech (ii) effects related to frequency of dysfluent speech states and (iii) within subjects comparing fluent to dysfluent states. Generally, we observed that both state and trait effects are somewhat dependent on task demands. Further, abnormal activity in the basal ganglia was observed during both fluent and dysfluent speech in different task conditions. Finally, the frequency of dysfluent speech states was related to basal ganglia and cerebellar activity in both tasks. Overall our data emphasise the complexity of state and trait effects in stuttering that may contribute to lack of consensus across previous imaging studies.

4.1 Introduction

No one is completely fluent all of the time. A person who stutters, likewise, is not completely dysfluent. In fact, people who stutter can predict situations or specific sounds that are more or less problematic. Many report the same problematic situations, such as speaking on a telephone, public speaking, feeling pressure to speak, and the beginnings of words or sentences. These situations are more likely to result in stuttered speech. Stuttering **states** are these specific acts of dysfluent speech. Stuttering **traits** are commonalities among people who stutter. In other words, what distinguishes stuttered speech from fluent speech is a stuttering state. What distinguishes people who stutter from people who are fluent is a stuttering

trait. Distinguishing between **traits** of stuttering, *i.e.* “commonalities among people who stutter”; and **states** of stuttering, *i.e.* “disruptions related to the act of stuttering” is critical in unraveling the underpinnings of this complex developmental disorder of speech fluency.

4.1.1 Speech motor system disruption in stuttering

disorganisation of white matter is a structural trait of developmental stuttering. The structural disruption was first thought to be localized to a critical speech–motor pathway underlying the left central opercular cortex [220]. Further studies showed underlying white matter disruption extended into ventral premotor cortex, bilaterally, and corresponded to abnormalities in functional activation of nearby grey matter [249]. We observed a more diffuse pattern of white matter disorganisation, with many disruptions occurring bilaterally [59] (See Chapter 2). White matter was disorganised in all four cerebral lobes, in the cerebral and cerebellar peduncles, and within the posterior body of the corpus callosum. Notably, descending motor pathways (corticobulbar tracts) were disrupted in the left hemisphere compared to the right within people who stutter. Fluent controls showed no differences between hemispheres in these pathways. In stuttering, white matter pathways are disrupted throughout the brain and are especially pronounced in the left hemisphere speech–motor pathways.

Functional neuroimaging studies of stuttering also show diffuse disruption in language and motor networks. A seminal meta-analysis summarized findings as three “neural signatures of stuttering”: overactivity in the right inferior frontal cortex and anterior insula; underactivity in the auditory cortex; and overactivity in the cerebellar vermis [40]. These traits emerged in spite of variability of neuroimaging method, tasks used, and fluency state.

4.1.2 Attempts to distinguish state and trait effects

Two more recent meta-analyses replicated the originally-reported neural correlates, and attempted to dissociate state and trait effects. One meta-analysis suggests that the cortical correlates of stuttering are traits, *i.e.* increased activation of right hemisphere insula, inferior frontal gyrus, premotor cortex and rolandic operculum, and decreased activation of left hemisphere “larynx” motor cortex, whereas decreased recruitment of auditory cortex and increased recruitment of the cerebellum and supplementary motor area (SMA) are considered state effects [28]. The other meta-analysis extended “neural signatures” of stuttering relative to the original study [40] to include traits effects of increased activation of the SMA, right hemisphere insula and motor cortex, but re-categorized decreased auditory cortex activation as a “state effect” alongside increased cerebellar activations [42].

All together, patterns of abnormal activity in lateral cortical regions for speech production (premotor and language cortices) are consistent traits of stuttering, whereas medial premotor (SMA) activations have been categorized as both state and trait effects.

Some state-related influences of stuttering are commonly associated with the subcortical systems: both recent meta-analyses classify abnormal activity in the cerebellar vermis as a state effect [28][42]. However, the classification of subcortical regions of the dopaminergic system is not agreed upon, as some consider them trait [42], and others state, effects [28]. Within subjects, both the basal ganglia and cerebellum are associated with lower magnitude of signal change during “more typical” relative to “less typical” stuttering symptoms [134], which could suggest a symptom-specific role of these regions in stuttering states. The cerebellum shows disruption during dysfluent speech within-subjects [262]. Further, cerebellar activity normalizes following treatment [67][152], but so does basal ganglia activity [231]. Also, speech-related activation in the basal ganglia is correlated with stuttering severity measures in several studies [123][100]. In people who stutter, mostly fluent speech is associated with increased activity relative to controls in the dopaminergic system, specifically in the midbrain at the level of the substantia nigra and red nucleus [249]. Generally, many studies implicate some portion of the subcortical system in stuttering, but the specific roles of basal ganglia and cerebellar structures are still not agreed upon.

The somewhat “dual” classifications of basal ganglia structures and SMA as influencing both trait and state effects are in agreement with a hypothesis that dopamine imbalance is involved in stuttering states, and that the resulting disruptions specifically impact the putamen-SMA circuit, which leads to the stuttering trait [4]. While none of the three meta-analyses in stuttering implicate the putamen, this circuit in particular has shown disruption in several studies. The functional connectivity of the brain at rest is disrupted between these two regions near the age of onset in stuttering, as are the connections between that circuit and auditory cortex [55], as well as the cerebellum [51]. Disruption of this circuit during scans taken at rest persists into adulthood in stuttering males [263]. Computational simulations of stuttering states are also consistent with this hypothesis, and suggest two mechanisms could cause disruption to the putamen-SMA circuit in particular: 1) an imbalance in dopamine; or 2) disorganised white matter [58]. The critical question remains: is this circuit disrupted during dysfluent speech states, specifically?

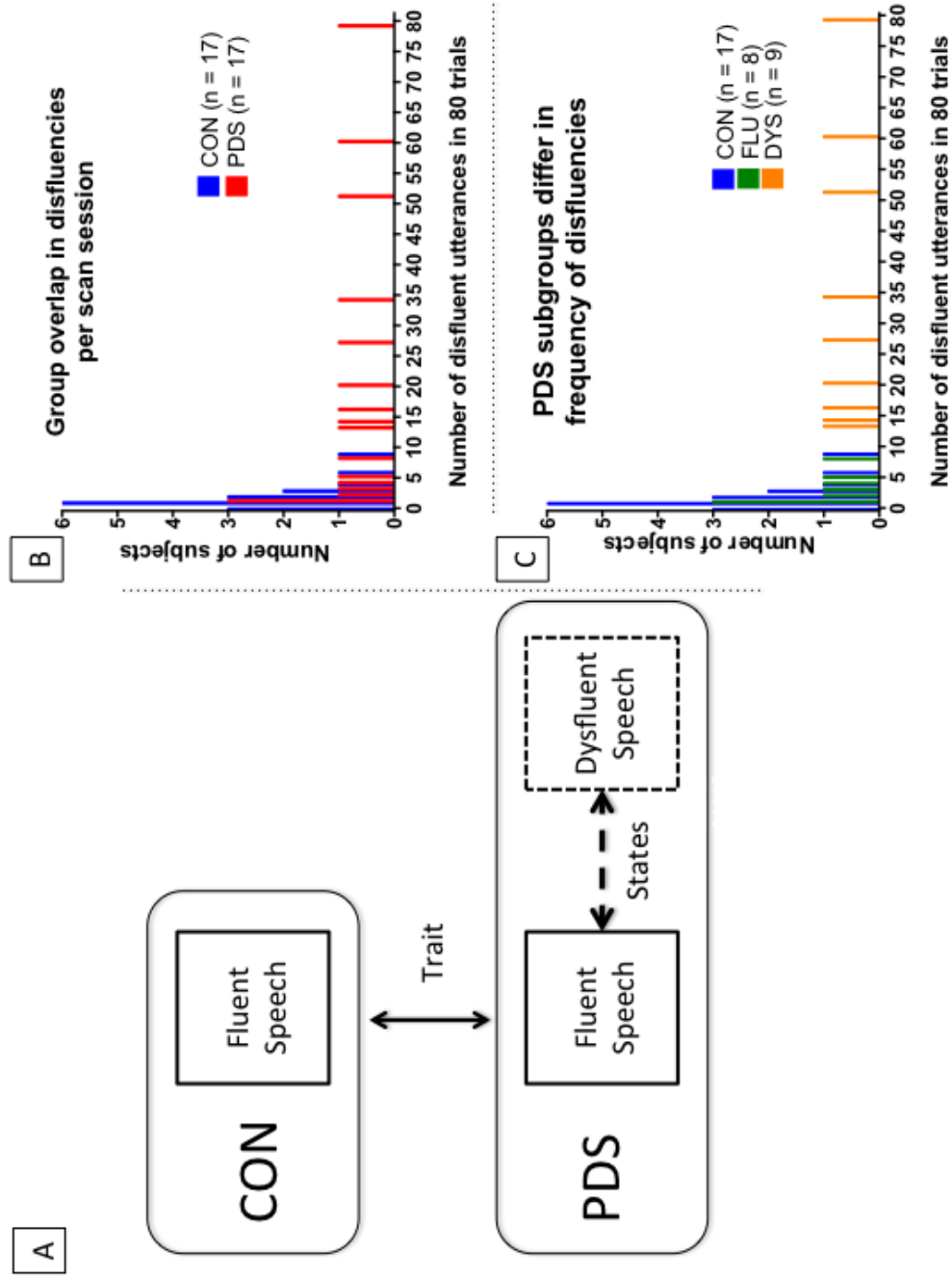
4.1.3 The aims of the current study

Here, we used a whole-brain approach to explore speech-related activity as reflecting either general traits of stuttering or specific states of stuttered speech. We defined

trait and state effects in stuttering as follows (Figure 4.1):

- **General traits:** Activity during fluent–speech production in adults who stutter (PDS) that differs from that in controls (CON).
- **State-based subgroups:** We divided our sample of PDS, who all share the stuttering trait, into two subgroups: one subgroup contained individuals who were mostly fluent (FLU) during scanning (< 10 dysfluent utterances per run) and the other those who were somewhat dysfluent (DYS) (> 10 dysfluent utterances per run). This subgroup approach examined general proclivity to stuttering in the scanner, but does not directly compare fluent to dysfluent states within individuals.
- **State effects in individuals:** Within individuals in the DYS subgroup, we compared fluent to dysfluent utterances to explore potential indicators of stuttering state in people.

Figure 4.1: Our approach to distinguishing state and trait effects



A) We used fluent speech to isolate a general trait (vertical arrow) shared by adults who stutter (PDS) relative to fluent controls (CON). Within PDS specific speech states of fluent and dysfluent speech (horizontal arrow) can be compared. B) In our study, about half of the PDS group overlapped with CON in the number of dysfluent utterances per scan, so we created two PDS subgroups using a data-driven threshold of 10 dysfluent utterances per scan session. C) The resulting subgroups were mostly fluent (FLU) and somewhat dysfluent (DYS) and were compared directly.

4.2 Methods

4.2.1 Participants

The dataset used in this study was the same as that used in Chapters 3 and 5, which examined speech-related activation and rest-related activation in stuttering individuals and fluent controls, respectively. Seventeen adults with persistent developmental stuttering (PDS: 13M:4F; aged 19-54 years; 3 left-handers) and 17 age- and sex-matched fluent controls (CON: 13M:4F; aged 19-53 years, avg. 32.3y; 3 left-handers) were scanned using functional MRI. No controls had a history or diagnosis of learning or speech disorders. All participants gave informed consent to their participation in the research in a protocol approved by University of Reading's ethics committee. Stuttering ranged in severity from very mild to very severe as assessed using the Stuttering Severity Instrument (SSI, versions III and IV, [203] range 10-46, average 24.7, s.d. 10.9). Within PDS, state-based subgroups were also well-matched on stuttering severity (SSI total score), age, and education (Table 4.1).

Table 4.1: Sample summary.

		CON	PDS	FLU	DYS
Total n	[females]	17[4]	17[4]	8[3]	9[1]
Left handed	[females]	3[0]	3[1]	2[1]	1[0]
Age in years	Avg (sd)	32.4 (11.4)	31.4 (11.0)	31.75(14.4)	31.11(7.8)
Range		19 - 53	19 - 54		
Education in years	Avg (sd)	17.29 (1.8)	16.35 (1.8)	16.25(1.7)	16.44(1.9)
Stuttering severity	Avg (sd)		24.7 (10.9)	20.5 (10.6)	28.4 (10.3)

Groups were well-matched on age, education, handedness and gender. State-based subgroups were not significantly different in age, education, or stuttering severity.

4.2.2 Data acquisition

Functional MRI data were obtained at the University of Reading's Centre for Integrative Neuroscience and Neurodynamics using a 3T Siemens Trio scanner with a 12 channel head coil. Wholehead T2*-weighted echoplanar images (TE=30ms) were acquired every 9s with a silent delay of 7s (*i.e.* sparse sampling) and comprised 2s acquisition of 32 4mm axial slices (in-plane resolution 3 mm x 3 mm). A '+' appeared in the middle of the screen during the 2s acquisition period (Figure 4.2).

During the 7s silent delay between measurements, subjects saw a stimulus via scanner compatible goggles that was either a picture with a descriptive sentence below it, a picture with no text, or a '+' in the middle of the screen. Subjects

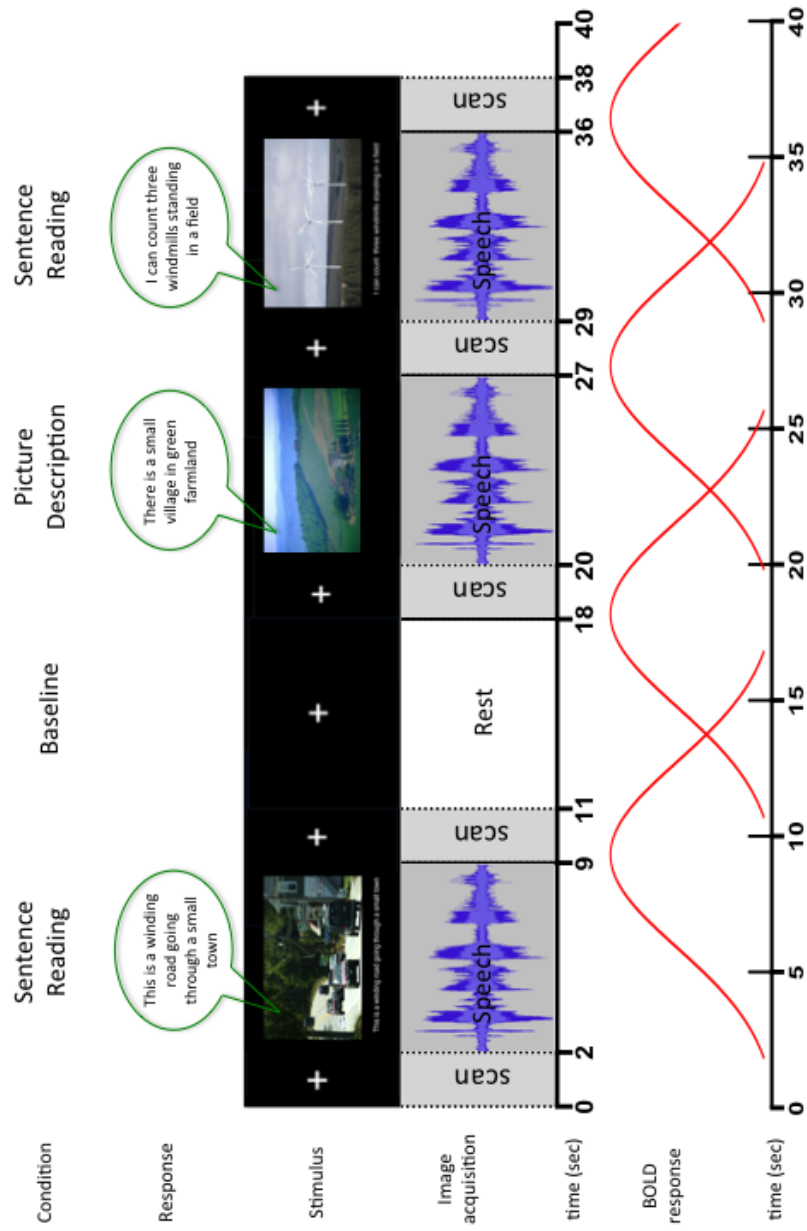
were instructed to read the sentences aloud (Sentence Reading condition) or to overtly describe the pictures (Picture Description condition) and were explicitly told to stop speaking when the crosshair appeared so that there would be no speech related movement of the head during data collection. Prior to the scan the task was explained to subjects who were allowed to practice outside the scanner. For each of the conditions and the baseline condition, 40 volumes of data were acquired for a total of 120 volumes (18 min); the order of conditions was fixed and pseudorandom. Two runs were acquired in each subject, yielding 80 volumes of each condition (Sentence Reading, Picture Description, and Baseline).

4.2.3 Speech conditions

We used two tasks to elicit overt speech: 1) sentence reading and 2) picture description. The picture description condition requires planning in both speech content and motor movement. The sentence reading condition involves articulatory system coordination without the need to generate speech content, but does not allow PDS to use word-substitution strategies to avoid dysfluencies.

Speech was recorded using an MRI-compatible microphone. Each subject produced a total of 160 task-related utterances in the scanner. A native British-speaker was blinded to participant group and asked to rate recorded utterances as either dysfluent or fluent. In this case a “dysfluent” utterance would include any sort of disruption, including interjections or normal speech errors that a non-stuttering person could produce. In some cases, these dysfluent utterances would also correspond to “dysfluent” speech states, which are typically associated with abnormal fluency, as observed in stuttering. The rater was instructed to mark sentences as dysfluent if any disrupted speech occurred during the entire utterance. A broad categorization of any sentence with disrupted speech flow as abnormal allows us to have a better understanding of the underlying distribution of dysfluencies in normal speech in PDS and CON, without requiring they take the form of characteristic stuttering symptoms.

Figure 4.2: Task design and scanner acquisition method



Two conditions: Read (Sentence Reading) and Speech (Picture Description) were used to assess speech-related brain activity. Sparse sampling catches the middle of peak activation (BOLD response) which would occur 4-6 seconds after speech is initiated and remain until that long after speech stops.

4.2.4 Image analysis

4.2.4.1 Preprocessing

The functional images were analyzed using the FMRIB Software Library (FSL 5.0.6; <http://www.fmrib.ox.ac.uk/fsl>, [132]). Standard motion correction, and individual volumes that were motion outliers were included as separate regressors at the first level for each subject. Excessive motion (*i.e.* $> 4\text{mm}$) was observed towards the end of a single scan session in one PDS and one CON, and these volumes were removed from the time series (*i.e.* the runs were truncated by 29 volumes (PDS) and 20 volumes (CON) within a single 120 volume run). The remaining data were analyzed in the same way for all participants. Each dataset was unwarped using a fieldmap and PRELUDE and FUGUE software running in FSL and spatially smoothed with an 8mm full-width-at-half-maximum smoothing kernel. A temporal high-pass filter with a cutoff of 150 seconds was used to remove low-frequency fluctuations in the signal. Two further regressors were used in the first-level analysis to remove residual image artefacts due to physiological changes. These regressors were the mean time-courses extracted from preprocessed data from 4-mm radius spheres in areas where task-related activity was not expected, one was placed within cerebrospinal fluid of the anterior lateral ventricle (standard space coordinates 2, 10, 8) and the other within white matter in the dorsal posterior frontal lobe (−26, −22, 28) [145]. Images were registered using boundary-based registration [103] to the individual subject's T1-weighted structural image (1 mm³ voxels; TR = 2020 ms, TE = 2.9 ms, flip angle = 90°), which in turn was registered using FNIRT (FMRIB's nonlinear registration tool) to the MNI-152 template.

4.2.4.2 Modeling of trait and state effects

General traits: We defined stuttering traits as abnormal activity (*i.e.* differences to CON) in PDS observed during fluent speech. For individual subjects, for each scanner run, statistical maps were generated to show patterns of activation during each condition relative to baseline. Each condition was modeled using a separate regressor. To examine these trait-based effects, for all subjects (including CON), we added a behavioural regressor at the first-level coding for dysfluent utterances. This regressor removed the residual effect of dysfluent utterances, thereby allowing us to isolate fluent speech.

We modeled activation changes for each condition relative to baseline. In a second-level analysis, to combine data from multiple runs, we averaged statistical maps from the first level for each subject using a fixed-effects analysis. At the final level, group averages and contrasts were analyzed using FMRIB's Local Analysis of Mixed Effects stage 1 [259]. Contrast masking was used for the group comparisons to eliminate negative activity ($Z > 0$) in either group.

State-based subgroups: We divided our PDS sample into subgroups of individuals who were mostly fluent (FLU, $n=8$) or somewhat dysfluent (DYS, $n=9$) based on a cutoff of 10 dysfluent utterances per scan session. The cutoff was based on the CON group’s distribution of the number of utterances that were dysfluent on average per run (see Figure 1). In other words, we set a cutoff independent of speaking condition, yet still indicative of general proclivity toward more frequent dysfluencies throughout the scan session. For this analysis, we did not use an additional regressor for dysfluent utterances, but otherwise all aspects of the first two levels were identical to the general trait pipeline described above. At the highest level we compared subgroups of PDS, rather than PDS to CON.

State effects: Within the DYS subgroup, we explored activity related specifically to the two types of speech coded: fluent and dysfluent. We set a minimum of 10 fluent or 10 dysfluent utterances per scan to explore the activation related to specific states of fluency. Therefore, one DYS participant who had only a single completely fluent utterance across both scans, was excluded from this analysis. Detailed coding by a second rater confirmed that dysfluent speech within the DYS group was consistent with dysfluencies typical of stuttering. We therefore refer to this analysis as comparing dysfluent and fluent states, which are approximated by comparison of dysfluent to fluent utterances. For this analysis we coded four variables at the first level so that we could examine activity related to fluent and dysfluent states within the Picture Description and Sentence Reading conditions, respectively. At the second level, as described above, we averaged the two scanner runs for each subject. Finally, at the highest level instead of comparing groups of subjects, we compare groups of utterances based on state (dysfluent vs fluent) within each condition separately.

4.3 Results

4.3.1 Behavioural results

Using the cutoff of 10 dysfluent utterances per scan run (not per condition, Figure 4), we segregated our PDS group into 2 subgroups: fluent (FLU) and dysfluent (DYS). Below we report results of a single Kruskal–Wallis test using family-wise error correction ($p < 0.05$, $n = 10$) to adjust for multiple comparisons. There were no significant differences between the sentence reading and picture description condition in terms of the frequency of dysfluent utterances in any group nor subgroup (Table 4.2).

Table 4.2: Summary of speech fluency in the scanner.

Participants	Condition	Mean (<i>SD</i>)	Min	Max
CON (<i>n</i> =17)	Sentence Reading	1.59 (1.9)	0	6
	Picture Description	3.18 (3.6)	0	12
PDS (<i>n</i> =17)	Sentence Reading	20.18 (23.7)	0	80
	Picture Description	20.24 (23.9)	1	79
FLU (<i>n</i> =8)	Sentence Reading	4.25 (4.7)	0	14
	Picture Description	2.5 (1.2)	1	12
DYS (<i>n</i> =9)	Sentence Reading	34.2 (25)	4	80
	Picture Description	36 (23.5)	9	79

Mean and standard deviations for number of dysfluent sentences during the speech conditions for PDS, CON, and the 2 subgroups of PDS: FLU and DYS

4.3.1.1 General traits

PDS had significantly more dysfluent utterances than CON in the sentence reading condition ($p = .003$), but this difference was only marginally significant in the picture description condition ($p = .058$).

4.3.1.2 State-based subgroups

As expected (because they were selected on this basis), the DYS subgroup was significantly less fluent than the FLU subgroup in both sentence reading ($p = .029$) and picture description ($p = .014$). The FLU subgroup did not differ from CON in either condition. These subgroups did not differ significantly in stuttering severity (SSI total score), age, or education (Table 1).

4.3.2 Neuroimaging results: General traits

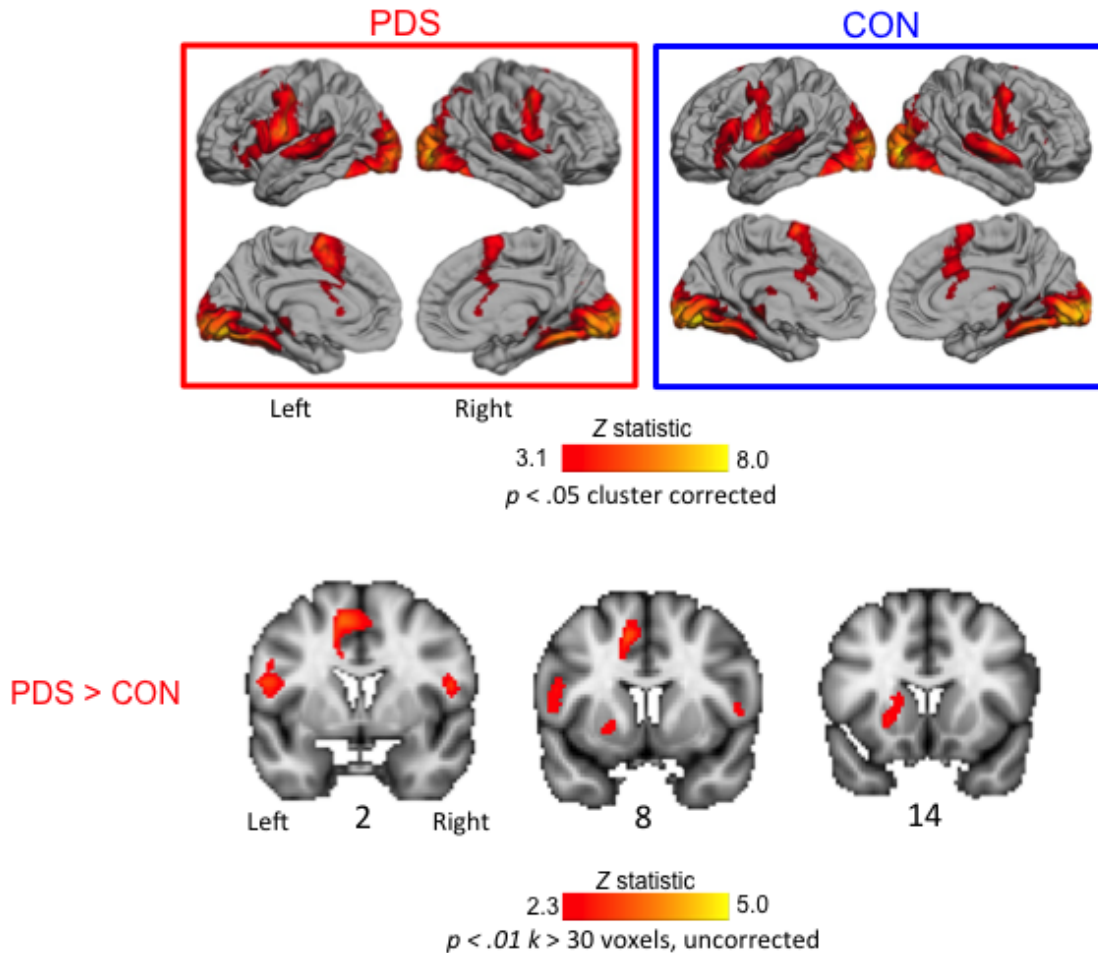
We compared activity during fluent speech in PDS and CON groups. The data were analyzed by contrasting each of the speaking conditions (Picture Description and Sentence Reading) with the baseline (crosshair). The patterns of activation for each of these contrasts were examined first for each group separately (group averages), and then the differences between the PDS and CON groups were examined. In both conditions, both groups activated the expected network of areas, namely posterior superior temporal cortex, sensorimotor cortex, SMA and preSMA, bilaterally, and left posterior inferior frontal gyrus (IFG). In addition both groups showed extensive medial and lateral occipital cortex activity. For the group averages, data were thresholded at a cluster forming threshold of $Z > 3.1$ and a cluster significance threshold of $P = 0.05$ for correcting for multiple comparisons. The comparison between groups did not reveal any significant differences at this corrected threshold. Comparisons between groups are therefore reported at an uncorrected threshold of $p < .01$ ($Z > 2.3$), with an additional constraint that the cluster size was at least 30 voxels and the peaks were located in motor or language related areas. Group differences at this threshold in the frontal pole and frontal orbital regions, inferior temporal cortex, or occipital cortex are reported in tables, but not discussed.

4.3.2.1 Picture description

Overall, PDS showed a left lateralized increase in activity relative to CON during fluent picture description relative to baseline (Figure 4.3). Increases included an extensive cluster with peaks in left hemisphere ventral premotor cortex extending into inferior frontal gyrus, another in the preSMA extending into the SMA, and a subcortical cluster with a peak in the left putamen extending into caudate nucleus. Notably, a single cluster in the right ventral premotor cortex showed increased

activity in PDS relative to CON in this condition (Figure 4.3, Table 4.3). There were no areas where PDS had decreased activity relative to CON.

Figure 4.3: Trait effects during fluent picture description



Adults who stutter (PDS) had relatively increased recruitment of left hemisphere premotor regions and subcortical grey matter compared to controls (CON) during fluent description of pictures. There were no areas where PDS < CON. Coordinates in MNI space Y axis.

4.3.2.2 Sentence reading

Differences between groups were observed in both directions in this condition. PDS showed increased activation relative to CON in localized clusters in posterior superior temporal gyrus, bilaterally, in right central opercular motor cortex, and in left central sulcus (Figure 4.4, Table 4.4). PDS showed decreased activation relative to CON in anterior superior temporal sulcus on the left and the right

Table 4.3: Regions where there were differences between groups in fluent speech related activity during Picture Description vs. baseline.

Brain Region	# voxels	Z statistic	X	Y	Z
PDS > CON					
Left putamen	105	2.84	-22	12	-4
Left motor cortex*	205	3.32	-50	4	18
Left preSMA	360	3.62	-10	2	52
Right motor cortex*	36	3.02	52	0	16
Left white matter underlying somatosensory cortex	204	3.58	-36	-20	32
Right dorsolateral occipital cortex	376	3.18	30	-60	28
Left ventrolateral occipital cortex	128	3.53	-50	-74	-16

Location of the highest peak in a cluster is given: voxelwise, $p < .01$, uncorrected with ≥ 30 voxel extent. Selected subpeaks within the large clusters are also described. There were no areas where PDS < CON for Picture Description. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space.

inferior frontal sulcus/gyrus at the levels of pars opercularis and pars triangularis. At a reduced voxel threshold, *i.e.* 19 voxels at $Z > 2.3$, a cluster appeared in the left inferior frontal gyrus at the level of pars opercularis, suggesting that decreased inferior frontal activation in PDS relative to CON occurred bilaterally (Figure 4.4).

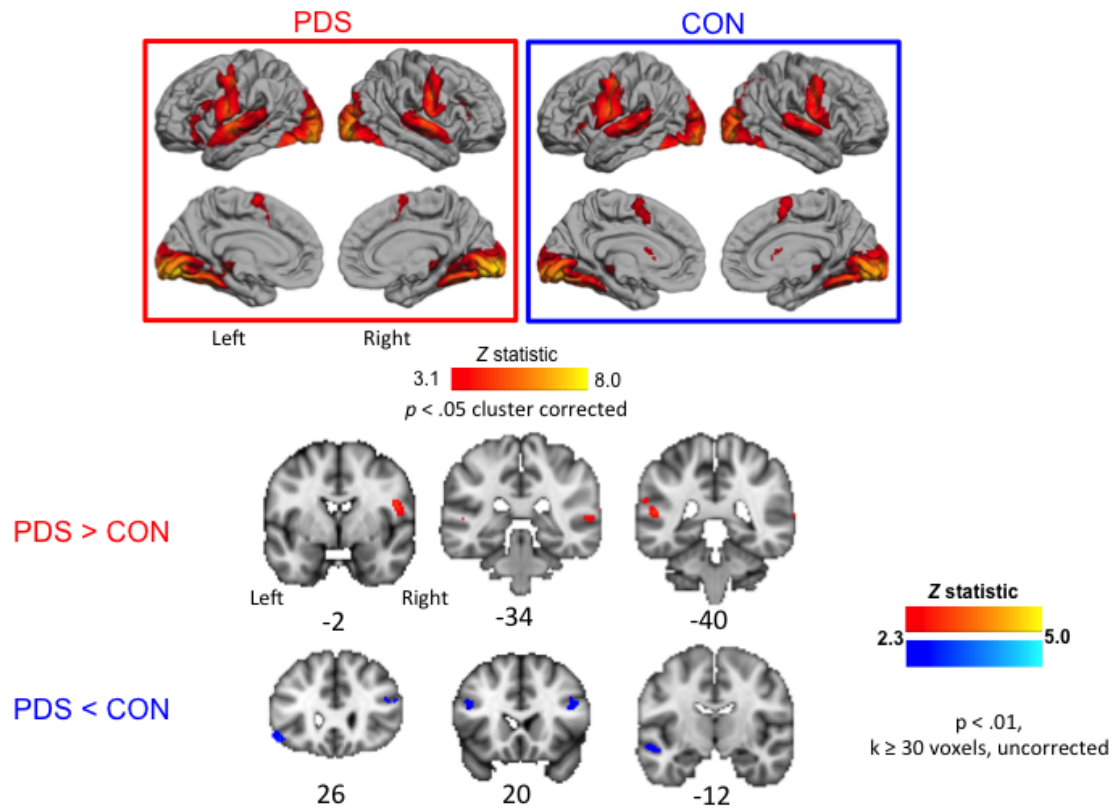
4.3.2.3 Summary of trait effects

The speech networks recruited by PDS and CON during fluent speech largely overlapped. The general pattern during picture description was additional recruitment of left-lateralized speech-motor regions in PDS, namely the ventral motor cortex, preSMA, and putamen. Differences during sentence reading were more mixed, with increased activation of superior temporal regions and decreased recruitment of inferior frontal cortex both occurring bilaterally in PDS relative to CON. In summary, our results suggest that the general traits of stuttering related to fluent speech may differ depending on task demands and underpin the need to consider the neural correlates of specific speech conditions separately in stuttering.

4.3.3 Neuroimaging results: State-based subgroups

Following the general trait analysis, we divided our PDS group into two subgroups based on a data-driven fluency cutoff. The resulting subgroups of PDS were mostly fluent (FLU, $n=8$), and somewhat dysfluent (DYS, $n=9$). This analysis allowed us to isolate activations related to proclivity to dysfluent states within a group of people who share the trait of stuttering but do not differ in stuttering severity.

Figure 4.4: Trait effects during fluent sentence reading.



PDS had relatively increased recruitment of right hemisphere central opercular motor cortex and posterior superior temporal gyrus bilaterally. Relative to CON, PDS showed reduced activation in right hemisphere orbito frontal cortex as well as anterior superior temporal sulcus, and in the inferior frontal sulcus, at the level of pars opercularis, bilaterally. Coordinates in MNI space Y axis.

Table 4.4: Regions where there were differences between groups in fluent speech related activity during Sentence Reading vs. baseline.

Brain Region	# voxels	Z statistic	X	Y	Z
PDS > CON					
Left genu of corpus callosum	65	3.3	-18	28	4
Right central opercular cortex	108	3.45	52	0	18
Left central sulcus	176	3.23	-36	-18	34
Right posterior superior temporal gyrus*	30	2.73	60	-34	6
Left posterior superior temporal gyrus*	34	3.3	-52	-40	12
Right dorsolateral occipital cortex	161	3.02	24	-68	46
Left ventrolateral occipital cortex	73	3	-50	-74	-16
Left lingual gyrus	34	2.72	-4	-82	-14
Left dorsolateral occipital cortex	44	2.96	-18	-84	24
PDS < CON					
Left frontal orbital cortex	54	3.2	-48	26	-12
Right inferior frontal gyrus (pars opercularis)*	56	3.08	46	22	24
Right inferior frontal gyrus (pars triangularis)		2.6	52	26	22
Left temporal pole	41	2.91	-64	6	-4
Left superior temporal sulcus	71	2.71	-52	-10	-12
Left intracalcarine cortex	62	3.01	-18	-56	8
Left occipital fusiform	35	3.22	-16	-88	-12
Left inferior frontal gyrus (pars opercularis)*	19	2.56	-44	20	26

Location of the highest peak in a cluster is given: voxelwise, $p < .01$, uncorrected with ≥ 30 voxel extent. Selected subpeaks within the large clusters are also described. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space.

4.3.3.1 Whole brain analysis

The data were analyzed by contrasting each of the speaking conditions (Picture Description and Sentence Reading) with the baseline (crosshair) then comparing these contrasts between the FLU and DYS subgroups. For the subgroup whole-brain analysis, we used a cluster-forming threshold of $Z > 3.1$ and a cluster significance threshold of $P = 0.05$ for correcting for multiple comparisons. The analysis did not reveal any significant subgroup differences at these thresholds. We therefore reported subgroup comparisons at an uncorrected threshold of $p < 0.01$ ($Z > 2.3$), with an additional constraint that the cluster size was at least 30 voxels and the peaks were located in the grey matter of motor- or language-related areas. Subgroup differences at this threshold outside of speech, motor, or language-related areas in the dorsal auditory processing stream (frontal pole/orbital regions, inferior temporal cortex or occipital cortex) are reported in tables but not discussed.

Both subgroups activated the expected network of areas involved in overt speech production, namely bilateral posterior superior temporal cortex, sensorimotor cortex at about the level of the face representation, SMA and preSMA, and left posterior IFG. Direct contrasts between the subgroups revealed that the FLU group showed greater activation relative to DYS in each condition relative to baseline, but only at the uncorrected threshold of $p < 0.01$ and extent $k \geq 30$ voxels (Figure 4.5).

Overall, the pattern of differences between subgroups was fairly consistent across speech conditions. In both conditions relative to baseline, FLU showed greater activation compared to DYS in subcortical grey matter, located symmetrically in both hemispheres (Figure 4.5, Table 4.5). These clusters were extensive, with peaks in the putamen that extended into the thalamus in the picture description condition and into the external globus pallidus in the sentence reading condition. Subgroup differences were less symmetrical in the sentence reading condition: the right hemisphere peaks were primarily in globus pallidus, but clusters extended into other nuclei, whereas left hemisphere peaks were in the putamen, and clusters extended into local maxima in the external globus pallidus. In addition, the FLU subgroup showed greater recruitment of the left medial posterior lobe of the cerebellum (lobule VI) relative to DYS in both conditions. In the sentence reading condition, this cluster extended into the cerebellar vermis. In both conditions, portions of the right central operculum showed increased activation for the FLU relative to DYS subgroups. In the picture description condition, this cluster was located on the lateral surface and was symmetrical to a cluster in the superior temporal gyrus across the Sylvian fissure. In the sentence reading condition, the cluster in the central opercular cortex was located on the inferior surface and extended into the insular cortex. There were no regions showing higher activation in DYS than FLU in any areas in either condition relative to baseline.

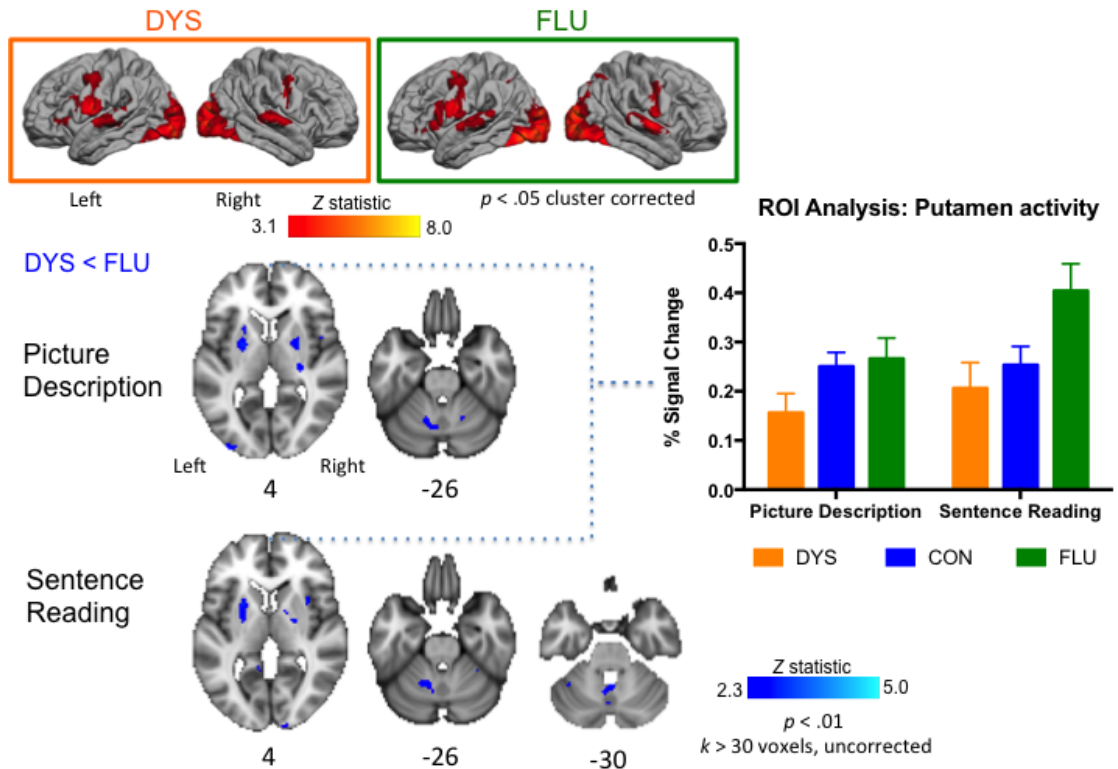


Figure 4.5: Subcortical activity distinguished stuttering subgroups in both conditions

The expected network of auditory cortex in both hemispheres, motor cortex, pre-supplementary motor area, basal ganglia, and frontal speech-motor regions was observed in both conditions. The top row of images here shows average subgroup activation during the picture description condition. The second row shows subgroup contrasts in the picture description condition. The bottom row shows subgroup contrasts from the sentence reading condition. There were no areas where $DYS > FLU$ in either condition. Coordinates in MNI space Z axis. Left is left. The call out shows results from our ROI analysis of the putamen in each condition, averaged across hemispheres. In both conditions, $FLU > DYS$ but CON did not differ from either PDS subgroup.

Table 4.5: Regions where there were differences between PDS subgroups (FLU > DYS)

Brain Region	# voxels	Z statistic	X	Y	Z
Picture Description					
Left superior frontal gyrus/ white matter	238	4.13	-18	0	50
Left putamen *	210	3.23	-24	-2	2
Right putamen *	203	2.81	26	-2	4
Right central operculum	32	2.66	58	-6	16
Right superior temporal gyrus	39	3.44	64	-8	-2
Left lingual gyrus	94	3.01	-14	-44	-4
Right temporal occipital fusiform cortex	39	3.03	38	-50	-22
Left temporal occipital fusiform cortex	64	3.17	-46	-54	-18
Left posterior lobe of cerebellum (lobule VI)	58	2.68	-14	-58	-24
Right lateral occipital cortex	48	2.89	30	-62	58
Left lateral occipital cortex	263	3.49	-22	-66	34
Right lateral occipital cortex	221	2.93	26	-66	30
Left lateral occipital cortex	446	3.84	-42	-74	-8
Left inferior occipital pole	45	3.54	-2	-92	-10
Right superior occipital pole	198	4.13	12	-96	28
Left superior occipital pole	154	4.07	-34	-96	14
Sentence Reading					
Right insula/ central opercular cortex	63	3.83	36	10	10
Left putamen	200	3.09	-24	8	4
Left globus pallidus (external)		2.61	-26	-8	0
Right globus pallidus (internal)	274	3.52	12	-6	-8
Right globus pallidus (external)		3.34	22	-6	-2
Right temporal occipital fusiform	34	2.77	36	-36	-18
Left lingual gyrus	35	2.91	-14	-44	-4
Left posterior lobe of the cerebellum (lobule VI)	92	2.72	-16	-56	-26
Vermis		2.5	0	-54	-30
Right dorsolateral occipital cortex	30	2.66	24	-66	60
Left dorsal occipital cortex	139	3.8	-26	-68	32
Left occipital fusiform cortex	33	2.74	-28	-70	-10
Left inferiolateral occipital cortex	117	3.07	-42	-72	-12
Right dorsal occipital cortex	419	3.73	30	-76	42
Left occipital pole	150	4.56	-28	-94	20
Right occipital pole	244	5.2	10	-96	26

Location of the highest peak in a cluster is given: voxelwise, $p < 0.01$, uncorrected with ≥ 30 voxel extent. Selected sub peaks within the large clusters are also described. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space.

4.3.3.2 Regions of interest

In order to further probe these subgroup effects, we explored percent signal change in activity in the basal ganglia using a regions-of-interest (ROI) analysis. Basal ganglia ROIs were identified in each participant (PDS and CON) using the FSL tool FIRST, an automated tool for parcellating the basal ganglia and limbic system structures [185]. Subject-specific masks were extracted from subcortical structures in both hemispheres, and were transformed to standard space. We selected only the masks of the putamen for this analysis.

We averaged percent signal change across the putamen masks during both speech conditions in all participants. We also computed grey matter volume for the putamen in each hemisphere. We included CON as a reference group specifically to determine whether our subgroups differed from CON in putamen activity, as well. Percent signal change was measured during all utterances, *i.e.* both dysfluent and fluent speech epochs and was extracted from fixed-level analyses to yield an estimate of average activation change for each condition across both scan runs.

ROI analysis. We probed the state-based subgroup effect using a single repeated-measures ANOVA (3x2x2) with a between-subject factor of subgroup (FLU vs. DYS vs. CON), two within-subject factors: hemisphere (left vs. right) and condition (picture description, sentence reading). Significant group effects were explored using post-hoc *t*-tests, and pairwise *t*-tests were used to explore significant within-subject effects. All pairwise results are reported at the threshold of $p < 0.05$, Bonferroni corrected. Within PDS we computed correlations between stuttering severity and both average percent signal change and average grey matter volume for the putamen in each hemisphere.

Results. The main effect of subgroup was significant ($F(2,31)=3.534$, $p=.041$) for the percent signal change in the putamen ROI, and followed a general pattern of FLU > DYS > CON in both hemispheres and both conditions (Figure 4.5). Pairwise comparisons suggest that this effect was primarily driven by a significant difference between FLU and DYS subgroups ($p=.037$), while neither subgroup differed significantly from the CON group independently. The main effect of hemisphere (left > right) was significant for both conditions ($F(1,31)=10.387$, $p=.003$). The main effect of condition was also significant (sentence reading > picture description) for both hemispheres ($F(1,31)=6.225$, $p=.018$). There were no significant interactions between hemisphere, subgroup and/or condition. There were no significant interactions or main effects of subgroup with respect to putamen volume, which did not differ between subgroups in either hemisphere. Stuttering severity was not significantly correlated with any of the putamen measures.

4.3.3.3 Summary of subgroup effects

The FLU subgroup showed greater activity than DYS subgroup within the basal ganglia, bilaterally, as well as left-lateralized lobule VI of the cerebellum within the same overlapping speech network in both conditions. An ROI analysis of putamen activity supported these whole-brain findings: the FLU subgroup showed greater activity than DYS subgroup in both conditions, bilaterally, but neither subgroup differed from CON. One interpretation of these differences is that PDS achieve the fluent state more successfully by overactivating these regions, or conversely, that the DYS subgroup are more dysfluent because they fail to activate these regions.

4.3.4 Neuroimaging results: State effects

We directly compared dysfluent to fluent states within individuals in the DYS group in each condition. Note one participant was excluded, as all but one sentence contained dysfluent speech, thus the group size for this analysis was eight. Examination of averages shows that all utterances activated the expected network of areas involved in overt speech production to a similar extent.

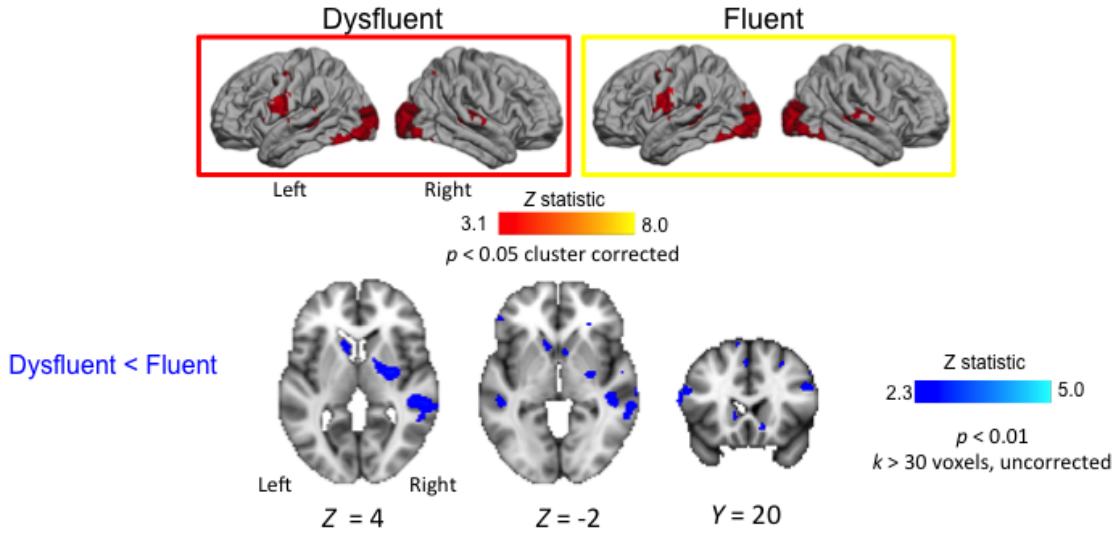
The data were analyzed by modeling speaking conditions (Fluent Picture Description, Dysfluent Picture Description, Fluent Sentence Reading, Dysfluent Sentence Reading). The contrast between fluent and dysfluent utterances within each condition are reported. For the whole-brain analysis, we used a cluster-forming threshold of $Z > 3.1$ and a cluster significance threshold of $P = 0.05$ for correcting for multiple comparisons. The analysis did not reveal any significant differences between fluent and dysfluent states at these thresholds. We therefore reported comparisons at an uncorrected threshold of $p < 0.01$, with an additional constraint that the cluster size was at least 30 voxels and the peaks were located in the grey matter of motor-or language-related areas. Differences at this threshold in regions outside of the dorsal auditory processing stream (frontal pole/orbital regions, inferior temporal cortex or occipital cortex) are reported in tables but not discussed.

4.3.4.1 Picture description

The overall pattern was decreased activity throughout the expected language and motor networks for dysfluent relative to fluent items in this condition (Figure 4.6, Table 4.6). In particular, dysfluent items were associated with decreased activation relative to fluent utterances in inferior frontal sulcus/gyrus (pars opercularis), occurring bilaterally, the right hemisphere cluster extending anteriorly and inferiorly into frontal gyrus, pars triangularis. Dysfluent items also showed decreased activation relative to fluent items in several regions associated with the dopaminergic system,

including the head of the caudate nucleus and brainstem on the left, and the right hemisphere putamen, external globus pallidus, and nucleus accumbens. Finally, dysfluent items also showed decreased activation relative to fluent items in two large clusters covering extensive portions of the anterior and posterior superior temporal sulcus.

Figure 4.6: State effects during picture description.



Within our DYS subgroup ($N=8$) we separated fluent from dysfluent items in order to isolate fluent and dysfluent speech state effects, respectively. Generally both speech states recruited the expected network of auditory cortex in both hemispheres, motor cortex, pre-supplementary motor area, basal ganglia, and frontal speech-motor regions in both conditions. The top row of images here shows average speech state activation during the picture description condition. The second row shows contrasts between speech states within the DYS group. There were no areas where dysfluent speech states showed increased activation relative to fluent in the picture description condition. Coordinates in MNI space.

4.3.4.2 Sentence reading

In direct contrast to the pattern we observed during picture description, the overall pattern for the sentence reading condition was greater activation for dysfluent items relative to fluent items throughout the speech-motor network. Higher activity was observed to occur for dysfluent items bilaterally throughout the cortical regions associated with speech, including the IFG extending to anterior insula and ventral premotor cortex, the primary motor and somatosensory cortices, and the preSMA. Increased activation was also observed in the putamen and brainstem at the level of the pons, occurring bilaterally in both cases (Figure 4.7, Table 4.7). The increased

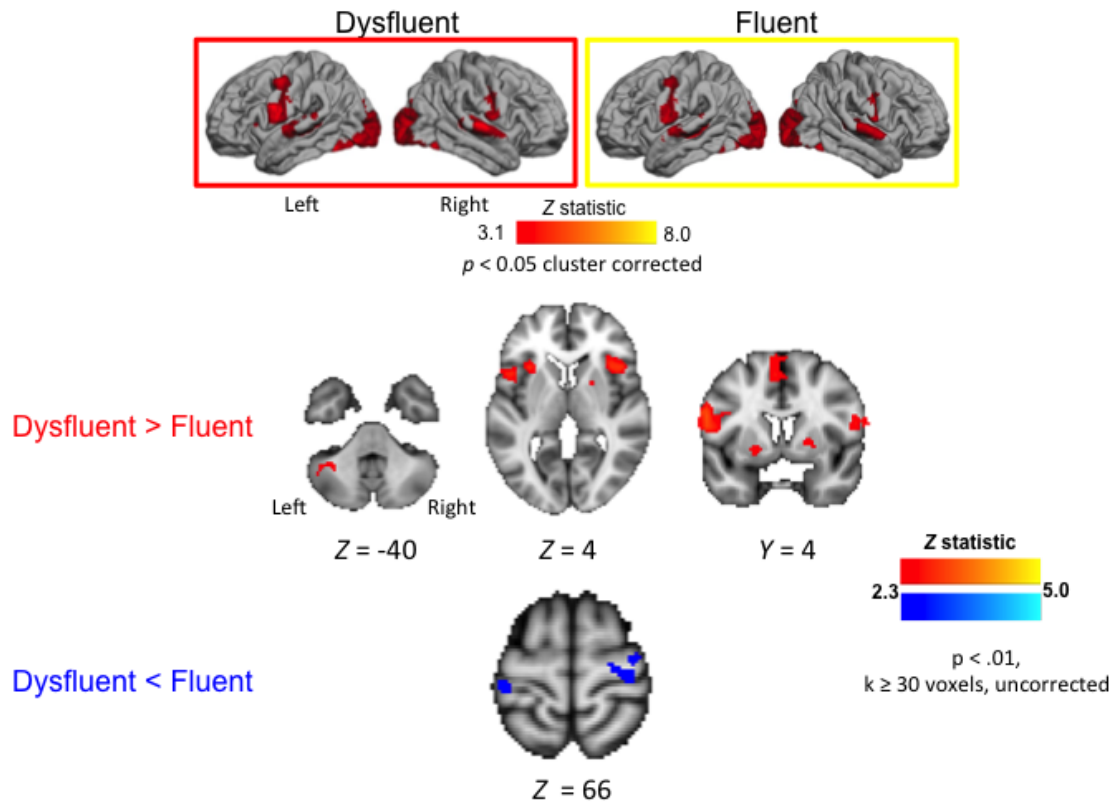
Table 4.6: Regions where there were differences between fluent and dysfluent items within individuals during Picture Description vs. baseline.

Brain Region	# voxels	Z statistic	X	Y	Z
Dysfluent < fluent					
Right frontal pole	37	2.59	26	54	14
Left frontal pole	40	2.69	-16	50	36
Right inferior frontal gyrus, pars triangularis	916	2.99	52	34	16
Right precentral sulcus		2.85	44	4	50
Right middle frontal gyrus		2.78	44	14	30
Right inferior frontal gyrus, pars opercularis*		2.76	52	24	26
Left paracingulate cortex	201	2.87	-4	28	40
Left inferior frontal gyrus, pars opercularis*	99	2.67	-60	22	14
Left caudate nucleus*	140	2.9	-10	16	2
Right nucleus accumbens/ caudate nucleus*	75	2.74	10	16	-10
Left superior temporal sulcus, anterior*	59	2.61	-52	6	-18
Left middle frontal gyrus	146	2.9	-32	2	56
Left precentral gyrus	54	2.5	-48	2	26
Right superior temporal sulcus, anterior*	778	3.06	56	-4	-8
Right amygdala	193	2.74	32	-4	-16
Right hippocampus		2.73	32	-22	-18
Right putamen	229	2.95	30	-8	4
Right globus pallidus (external)		2.46	16	4	0
Left brainstem	89	2.6	-10	-26	-40
Right postcentral gyrus	33	2.55	44	-28	46
Left superior temporal gyrus, posterior*	35	2.67	-50	-34	-2
Right superior temporal gyrus, posterior*	443	2.99	62	-36	2
Right middle temporal gyrus		2.5	60	-46	-6
Right temporal occipital fusiform cortex	168	2.79	40	-58	-14
Right lateral occipital	203	2.68	20	-68	50
Left precuneus	128	2.89	-8	-72	52

Location of the highest peak in a cluster is given: voxelwise, $p < 0.01$, uncorrected with ≥ 30 voxel extent. Selected sub-peaks within the large clusters are also described. There were no regions for which dysfluent items > fluent items. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space.

activation for dysfluent relative to fluent items also surfaced in the left hemisphere auditory cortex extending into planum temporale and the posterior lobe of the cerebellum (crus I). Fluent items in the sentence reading condition showed relatively little increased activation compared to dysfluent items with the exception of along the central sulcus, with peaks in sensorimotor cortex occurring bilaterally (Figure 4.7, Table 4.7).

Figure 4.7: State effects during sentence reading



Within our DYS subgroup (N=8) we separated fluent from dysfluent items in order to isolate fluent and dysfluent speech state effects, respectively. Generally both speech states recruited the expected network of auditory cortex in both hemispheres, motor cortex, pre-supplementary motor area, basal ganglia, and frontal speech-motor regions in both conditions. The top row of images here shows average speech state activation during the sentence reading condition. The networks recruited largely overlapped between the two speech states. The second row shows contrasts between speech states where dysfluent speech states showed increased activation relative to fluent. The bottom row shows the areas where dysfluent speech states showed decreased activation relative to fluent in the sentence reading condition. Coordinates in MNI space.

Table 4.7: Regions where there were differences between fluent and dysfluent items within individuals during Sentence Reading vs. baseline.

Brain Region	# voxels	Z statistic	X	Y	Z
Dysfluent < fluent					
Left frontal pole	122	3.01	-14	64	-6
Right inferior frontal gyrus, pars opercularis	362	3.2	46	18	2
Right insular cortex		3.1	38	20	6
Left preSMA	212	2.99	-4	6	64
Left putamen*	41	2.67	-20	4	-4
Right putamen*	24	2.59	22	6	2
Left premotor and sensorimotor cortex*	1988	3.48	-58	2	22
Left precentral gyrus		3.24	-52	-4	38
Left postcentral gyrus		3.15	-54	-18	26
Left insular cortex		3.01	-32	18	4
Left inferior frontal gyrus, pars opercularis		2.98	-48	14	6
Right postcentral gyrus	242	2.78	66	-4	22
Right precentral gyrus*		2.75	58	4	16
Right inferior frontal gyrus, pars opercularis		2.55	52	10	16
Right precentral gyrus	130	2.7	50	-4	36
Left brain stem (pons)*	103	2.77	-2	-22	-30
Right brainstem (pons)*	119	2.61	14	-32	-34
Left auditory cortex (planum temporale)	63	2.73	-64	-34	16
Left posterior lobe of cerebellum (crus I)	42	2.54	-32	-54	-38
Dysfluent > fluent					
Right motor cortex	330	2.79	36	-22	56
Right sensory cortex*		2.64	36	-20	66
Left sensory cortex*	32	2.59	-40	-26	64
Left occipital pole	42	2.73	-10	-94	16

Location of the highest peak in a cluster is given: voxelwise, $p < .01$, uncorrected with ≥ 30 voxel extent. Selected sub-peaks within the large clusters are also described. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space.

4.3.4.3 Summary of state effects

Similar to what we observed in our trait analysis examining fluent speech states only, within this more dysfluent PDS subgroup, differences between speech states are task-dependent. Within the picture description condition we observed focal increased activation of ventral premotor cortex, superior temporal cortex, and subcortical gray matter occurring bilaterally during fluent relative to dysfluent states. We did not observe any regions of increased activity during dysfluent picture description.

During sentence reading, on the other hand dysfluent states resulted in widespread increases in activation throughout the speech network including lateral inferior frontal gyrus and ventral premotor, motor, and somatosensory cortices, occurring bilaterally, focal clusters in the putamen and brainstem at the level of the pons, bilaterally, and left-hemisphere posterior auditory cortex and posterior cerebellar lobe (crus 1). Focal increases were observed for fluent relative to dysfluent states in the sentence reading condition only in the superior somatosensory cortices, bilaterally.

4.4 Discussion

Our findings indicate that patterns of activity related to general traits of stuttering differ according to task demands. Further, the patterns of activity that are associated with specific states of speech fluency in stuttering also differ according to task demands. Patterns related to relative frequency of dysfluent speech that distinguished our PDS subgroups, however, were consistent across tasks. We isolated the following indicators of trait and state effects of stuttering using speech-related activity (Figure 4.8):

- **General traits:** During fluent picture description, adults who stutter tended to show greater activity than controls in a shared network typical of self-initiated speech. Overactivity of the left putamen and preSMA occurred with increased motor cortex activity appearing bilaterally in adults who stutter relative to controls during fluent picture description. The only region that showed increased activation during fluent speech in both conditions in adults who stutter relative to fluent controls was the right hemisphere motor cortex (at the level of central operculum). During fluent sentence reading, the inferior frontal cortex showed decreased activation, bilaterally, in adults who stutter relative to fluent controls.
- **State-based subgroups:** We observed overactivation of the dorsal striatum, occurring bilaterally, and the left hemisphere cerebellar lobule VI in our fluent

subgroup relative to the dysfluent subgroup in both conditions. The increased activity in the putamen was further confirmed by an ROI analysis. Activity in this part of the striatum was larger in the fluent subgroup relative to the dysfluent subgroup but neither stuttering subgroup differed from fluent controls. Further, activity in the putamen was not related to stuttering severity.

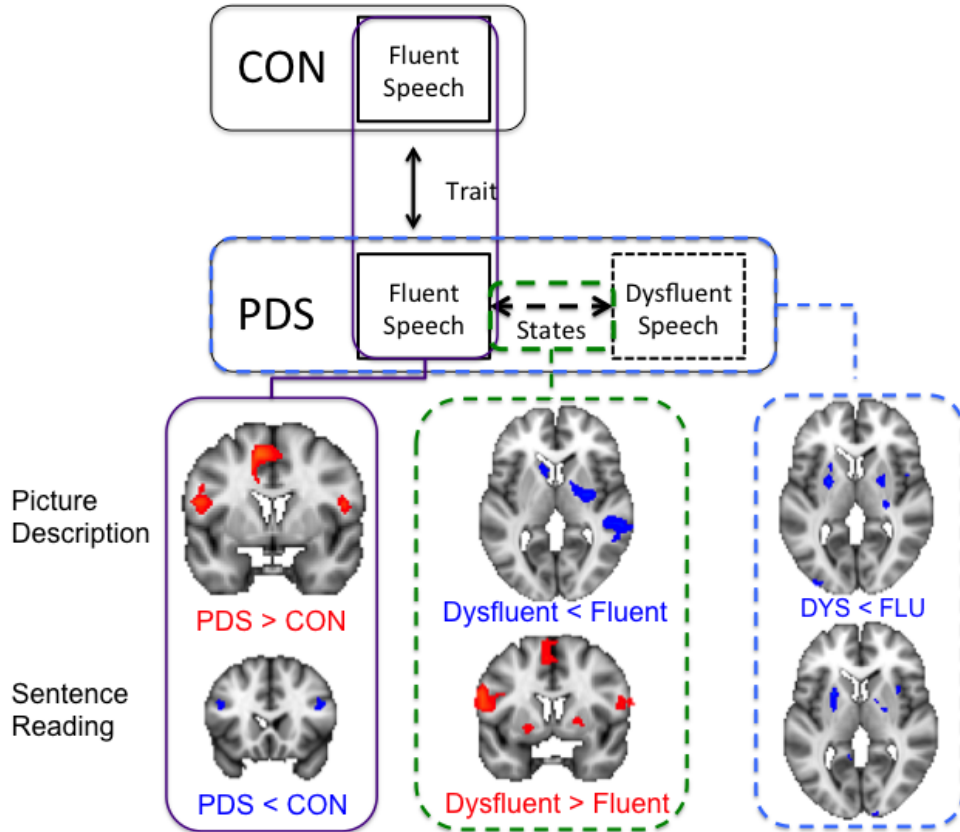
- **State effects:** Striatum underactivity was related to the dysfluent speech state during picture description, while overactivity of the putamen as well as insular and premotor cortices was associated with the dysfluent state during sentence reading. Within the dysfluent subgroup, we observed increased activation of the left caudate nucleus and right putamen, as well as ventral premotor cortex for fluent relative to dysfluent speech states in the picture description condition. During sentence reading we observed a generally opposite pattern: increased activation for dysfluent relative to fluent speech states, in the anterior insular cortex, motor cortex, brainstem and putamen occurring bilaterally. Superior sensory cortices showed a bilaterally distributed reduction in activity for dysfluent relative to fluent speech states during sentence reading.

4.4.1 Cortical nodes of the speech network reflected trait effects

The primary observation in this study was of overlapping networks between adults who stutter and fluent controls during fluent speech. Further, the networks activated during fluent and dysfluent speech states were also largely overlapping. These findings are largely consistent with previous reports using PET imaging which confirmed that networks utilized for speech production overlap much more so than they differ between adults who stutter and fluent controls [123]. Ours was the first study investigating fMRI activation during self-generated speech sequences (as opposed to naming pictures with single words or reading sentences) in adults who stutter, though two studies have used positron emission tomography (PET) imaging with narrative [39] and monologue [123] conditions that were self-formulated. Our results are largely similar to what was reported in those studies, in particular that adults who stutter show increased recruitment of premotor and motor regions relative to fluent controls during self-formulated speech [39][123].

Our general findings of increased activation in adults who stutter are less consistent with two previous fMRI studies that elicited spontaneous speech using picture naming in Native Mandarin speakers, which revealed decreased activation and decreased strength of connectivity in adults who stutter relative to fluent

Figure 4.8: Summary of trait and state effects observed in this study



Purple Box: Comparisons reflecting trait effects that distinguished fluent speech in PDS from that in CON were dependent on the speech task. In picture description (top) PDS generally showed increased recruitment of the premotor regions of the speech network, whereas in sentence reading the PDS showed less activity in inferior frontal cortex anterior to the ventral premotor region that was overactive in picture description (bottom). Green Box: In comparisons of speech states we also observed an influence of task demands: during picture description dysfluent states showed decreased activation relative to fluent states in the basal ganglia nuclei as well as superior temporal regions and frontal premotor regions (top), whereas during sentence reading activation during dysfluent speech states was increased relative to fluent sentence reading in premotor regions that were over-recruited during fluent speech in PDS relative to CON (bottom). Blue box: In our contrast between subgroups, the results were similar across conditions in that the DYS subgroup showed consistently reduced activation relative to the FLU subgroup, particularly in the basal ganglia nuclei and cerebellum in both conditions. Left hemisphere is the left side of the image.

controls, generally [151][117]. In further contrast to previous reports that emphasise right-lateralized overactivity in people who stutter, [40], in both conditions, trait differences between our groups were primarily bilaterally-distributed within the speech network. Finally, our observations during sentence reading, of increased central opercular activation in adults who stutter, are largely consistent with most of the literature. The exceptions are studies that combined or contrasted natural speech and fluency enhancement conditions [249][122] and studies that examined activation during single-word production [178][54][153][151][117][70]. Overall, trait differences between adults who stutter and fluent adults were specific to the tasks examined in our study, as were similarities to the literature. Finally, with the exception of a single cluster in the right central operculum, trait effects did not overlap for the two conditions.

A single cluster of overactivity emerged in the right central operculum extending into ventral premotor cortex in adults who stutter relative to fluent controls during fluent speech, which is consistent with reports from meta-analyses [40][28][42]. Generally, this trait has been interpreted as compensatory [175], however, that could be premature, as activity in this region was increased during dysfluent sentence reading relative to fluent sentence reading within stuttering subjects. The interpretation of right-hemisphere overactivity as compensatory is often based on the assumption that increased recruitment develops in response to reduced integrity of the arcuate fasciculus [59] and/or the ventral prefrontal white matter in stuttering [249], however these structural differences are bilaterally distributed. Still, one proposed mechanism of this compensation is consistent with the need to adjust for a periodic mismatch between predicted and actual signals from auditory cortex to Broca's region, which could be overcome by compensatory activation in the right hemisphere homologue. In turn, this additional state-related activation could contribute to a general pattern of overactivation in the left hemisphere and restoration of appropriate speech-motor signals along with a normalization of feed-forward suppression in auditory cortex [100]. Though we did not observe evidence of this suppression effect, the effect in the right central operculum was related specifically to fluent speech and not to task demands.

4.4.2 Medial premotor regions reflected state and trait effects

Seemingly contradictory state and trait effects predicted changes in activity in the preSMA, both of which were sensitive to task demands. Increased recruitment of the preSMA was associated with fluent picture description in adults who stutter relative to fluent controls, but the region did not differ during fluent sentence reading. Within the dysfluent stuttering subgroup, preSMA activation was increased during

dysfluent relative to fluent speech states in the sentence reading condition, but not during picture description. The preSMA and SMA are functionally connected to the basal ganglia circuits and thought to be involved in the preparation and execution of movements, respectively [62]. Accordingly, across a large-scale review of brain regions implicated in speech, the functional dissociation between preSMA and SMA proper was summarized as generative or planning vs articulation-based, respectively [195]. This distinction is partially based on a bulk of work suggesting a preparatory role in speech production for the preSMA, including selection of speech content (*e.g.* words) as well as grammatical structure [3][233][234][235].

Damage to the preSMA/SMA complex is associated with acquired stuttering, including initial-sound disfluency in the form of prolongations, repetitions, and pauses without concomitant behaviours [2], whereas more focal damage specific to the SMA induced severe dysfluency that resulted in repetition of almost all speech sounds along with concomitant behaviours [240]. A lesion disconnecting the posterior part of the SMA from primary motor cortex initially induced mutism, which resolved to “frequent and prolonged pausing, false starts, and repetitions” including whole-word repetitions and difficulty initiating speech, which worsened with increasing utterance length, especially during spontaneous speech without external stimuli [271]. Overall, depending on the precise location, lesions to the preSMA/SMA complex can induce a variety of stuttering-like symptoms, which almost always includes difficulty initiating speech, and repetitions.

Activity in the preSMA/SMA complex is both positively and negatively correlated with stuttering frequency within people who stutter [123]. Our results are generally consistent with the conclusion that overactivity of the SMA complex has been linked to both state and trait stuttering [28]. Further, this complex appears to show some variability in state effects during word reading within individuals who stutter [262]. We conclude that SMA activation is likely related to the demands of speech production generally, and could be related to monitoring processes in stuttering specifically. The interaction between speaking conditions and stuttering is an area that should be the focus of future work, especially considering that experimental conditions can be associated with different fluency rates within subjects, as we observed in our study.

4.4.3 Subcortical activity reflected state effects

We confirmed that subcortical activity reflects state effects within stuttering individuals, as well as between subgroups of individuals who differ in the frequency of dysfluent states. In the sentence reading condition, the putamen in particular showed increased activation, occurring bilaterally, during dysfluent speech relative to fluent speech, as did a cluster in the left posterior lobe of the cerebellum, located in the crus I subregion. In combination with increased activation of the preSMA

during dysfluent sentence reading, these subcortical findings somewhat support the theory of disrupted basal ganglia activity in stuttering, potentially arising from the putamen–SMA motor execution loop [4]. However, these state effects were completely task-dependent. In the picture description condition, dysfluent states were associated with decreased activation of the basal ganglia nuclei relative to fluent speech states, in a different part of the dorsal striatum, the caudate nucleus. Increased recruitment of the left caudate nucleus was also observed during fluent picture description relative to fluent controls. The increase of caudate nucleus involvement could reflect efforts to force movement into the next portion of a speech sequence by increasing signal in loops with inferior frontal regions [106] to override a delay in the putamen–SMA loop [58]. The net effect of this increase would be associated increases in inferior frontal activity for fluent speech, as we also observed in our study during picture description.

In our study, cerebellar differences during states surfaced in the posterior lobe of the cerebellum at the level of the crus I, a region tightly connected with prefrontal activity [181] and implicated in higher level functions like working memory [226]. This region is part of the network critical to the accurate predictions of dynamic motion [182] which develops abnormally in disease and is the first to deteriorate with age [76]. The effect could be related to the increased self-monitoring during dysfluent reading, as it only surfaced during the sentence reading condition when participants were explicitly required not to substitute words (a common strategy) to avoid dysfluencies. Increased activation of the cerebellum has been previously reported during dysfluent speech states in sentence reading, though those were localized to lobule VI [262], which was associated with subgroup differences in our study.

Our subgroups share the stuttering trait with one another, and differed in the frequency of dysfluent speech in the scanner but did not differ in stuttering severity as assessed outside the scanner. During both conditions these groups differed in the extent to which they activated the basal ganglia nuclei and the cerebellum. Specifically, increased recruitment of lobule VI of the cerebellum was associated with decreased stuttering frequency in the scanner. The differences indicated increased activation of the medial portion of the posterior lobe of the cerebellum (lobule VI) in the mostly fluent subgroup in both speaking conditions relative to the more dysfluent subgroup. This region did not show any differences in direct comparisons of state, nor in our trait comparisons. The specific localization of the cerebellar subgroup effect was near somatomotor representations for the tongue that are theorized to contain copies of cortical maps [41]. These maps are thought to be utilized in both feedback monitoring and feedforward models for speech production [106].

We also observed differential recruitment of basal ganglia activity by our

subgroups. Our study replicated previous findings of increased activation in the putamen corresponding to group-wise change in stuttering state, which has been shown in scans pre- and post-intervention [100] and is specifically related to treatment success [124]. The putamen can play both inhibitory and excitatory roles through connections (via the pallidum and thalamus) with the SMA and substantia nigra for the planning of volitional movements. In our study, the recruitment of the putamen could be successful self-initiated attempts to increase cues in a putamen-SMA loop that shows disruptions in simulated stuttering [58] and is thought to hold the core disruption in theories of developmental stuttering [4].

There are several plausible explanations for the subgroup effects we observed. First, the increased cerebellar and putamen activity could reflect a proclivity to compensation for defects in the internal timing networks through recruitment of the external system, which would include left cerebellum and its connected right ventral premotor cortex, also observed in our results. In this case we would speculate that the differences reflect ability to utilize rhythmic noise in the scanning environment, as these subgroups did not differ in stuttering severity outside of the scanner, only in frequency of dysfluent states within the machine. This is also somewhat consistent with findings that the putamen and cerebellum differ in their response to self and externally stimulated speech in fMRI [231]. Second, the difference could reflect a use of sensorimotor map copies by the fluent subgroup via cerebellar-motor loops to compensate for deficient efferent copies supplied to the speech cortex [40] and corresponding adjustments to internal gating by the putamen with regard to movement success prediction [58]. Third, the under-recruitment of this cerebellar region by the dysfluent subgroup could lead to a delay in integration of sensorimotor information necessary for smooth speech flow [160] and/or the under-recruitment of the putamen in the dysfluent subgroup could reflect the hypothesized core disruption in stuttering [4], and over-recruitment of the other regions observed in the fluent subgroup could indicate compensation for either or both of those disruptions.

An alternative explanation of these observed increases in striatal activity in the fluent subgroup is as follows: fluent speech is more rewarding for people who stutter. The “expectation of success” that would result in reward (*e.g.* boost of dopamine) is processed in the striatum [182]. The models necessary for planning dynamic action, such as the coordination required for speech execution, utilize both the putamen and the cerebellum [182]. Expectation of reward would be specific to fluent speech activation and increase levels relative to activity for expected failure or motor execution alone. Distinguishing among the various conflicting explanations for these subcortical findings is an exciting area for future research focus.

4.4.4 Variability in states is a trait of stuttering

We confirmed the general observation that activation patterns during dysfluent speech states are variable from subject to subject in stuttering individuals [262] and expanded this to include variability dependent on task demands. There are practical concerns in averaging this variability across large scale activation studies, including masking of interesting and meaningful effects [5]. These concerns are particularly relevant during large-scale meta-analyses. A more fine grained approach like ours attempts to describe that variability in terms of contributing factors from a trait, or overall diagnosis, as well as those predicted by specific speech states and their frequency.

Our work is not without limitations, however. First and foremost, the definitions of state-based subgroups were arbitrarily decided, and a more parametric approach could represent the natural fluency continuum that likely exists in stuttering. However, this approach is correlational in nature, and does not necessarily make best use of fMRI, where power is gained through direct contrasts of BOLD signal. Research that includes sampling from the continuum could be critical in stuttering, but would need larger sample sizes than were available for this work. Further, we make a presumption that the activation following a speech event is the meaningful activity in stuttering, and it could be the case that an error signal detected before trials gives us a more reasonable causal indicator. Use of real-time fMRI, or sampling from epochs prior to speech may lead to more legitimate speculations about compensatory activity in the absence of clear disrupted activity. Designs of this nature could also distinguish the results observed in our study, which mostly appear to be compensatory and related to fluent speech.

All of our results are reported at uncorrected statistical values. To a certain extent, we believe these values are meaningful because the effects we observed very closely replicate previous reports in stuttering at the same thresholds [123]. In fact, many published effects in stuttering are subtle and do not survive stringent statistical correction for multiple comparison. In all likelihood, the variability in stuttering contributes to the “small” effect sizes, therefore we feel that more liberal statistical thresholds should be explored and applied in stuttering generally. The replication of these findings, as well as the relative effect sizes are more meaningful than single findings at strict thresholds. Exploratory thresholding allows a more comprehensive documentation of the potential contributions to variability in stuttering, including the differences in task demands, state and trait effects, and the relative frequency in the scanner, as observed here. Our opinion remains that replication, and not statistical significance, should be the test of which of these findings are ultimately meaningful.

4.4.5 Conclusion

The primary finding of this paper is that both state and trait effects in stuttering are related to task demands. Further, we observed that frequency of dysfluent states within stuttering was related to both basal ganglia and cerebellar activation. In general, these observations suggest that increased recruitment of subcortical control regions is associated with fewer dysfluencies in the scanning environment. Our findings support efforts to document and model variability within stuttering, specifically in dissociating indicators of state and trait influences of this disorder.

Chapter 5

Spontaneous brain activity in people who stutter

Summary: Stuttering is associated with alterations to spontaneous brain activity near the age of onset in childhood [55] that persist into adulthood [263][152][210][267]. Such alterations include differences in measures thought to reflect functional connectivity among regions of speech-motor system thought to control movement timing, initiation, and coordination, *i.e.* the basal ganglia, the premotor cortices, and the cerebellum. In this study we used a data-driven approach to comparing spontaneous brain activation in stuttering with a group of well-matched controls. We replicated previous findings of differences in metrics of connectivity at rest in medial premotor cortices and the cerebellum, in particular. These differences were reflected in increased correlation strength between a number of regions and specific networks of correlated activity in our stuttering group relative to our control group. We further replicated findings that rest-related connectivity of the cerebellum was correlated to stuttering severity. Our work emphasises the importance of using data-driven approaches in stuttering research.

5.1 Introduction

The neuroimaging literature on stuttering suggests widespread disruption to the speech-motor network including premotor, auditory, and primary motor cortices, and subcortical grey matter including the basal ganglia and cerebellum (for meta-analyses see [42][28]). Functional differences have been associated with relative disorganisation in nearby underlying white matter in children [55], adolescents, and adults who stutter [249][137]. Though specific locations of group differences are fairly widespread, they are contained primarily within shared networks between people who stutter and fluent controls, such that both the networks utilized for

speech and those present at rest overlap much more than they differ between these groups [123].

Examination of functional magnetic resonance imaging blood oxygen dependent activation at rest (rfMRI) allows us to probe the connectivity of networks, which are represented in patterns of correlated activity in the absence of tasks. This approach avoids common task-related complications, for example, motion artefact from speech movements. Further, in stuttering, the presence of dysfluent speech states and compensatory efforts have no equivalent counterpart in fluent controls, making interpretation of direct comparisons somewhat untenable. The confounds imposed by direct contrasts are not part of rfMRI, because rather than define signal as task-related relative to baseline, the goal is to better understand the composition of the baseline.

Connections between the more basic resting networks available at birth [272] can be modified with experience such that nodes are dropped, added, or recombined into new systems. In adulthood, many networks that reliably co-activate at rest across samples resemble task-based networks [211]. One particular network of correlated activity at rest, termed the “default mode network” [197] was identified because of reliable negative signal relative to baseline during tasks. Task-based signal reductions are often interpreted as necessary disengagement of regions for reallocation of resources to task-relevant cortex. Abnormal rfMRI activation in stuttering could therefore suggest problematic connectivity in engaging the necessary networks for speech, disengaging unnecessary regions, or both.

5.1.1 Previous reports of rest-related alterations in stuttering

In contrast to the amount of task-related literature, there are only a few resting-state studies of stuttering. To a certain extent, the areas that are implicated as abnormal in stuttering depend on the analyses conducted, and specifically, the network of interest. Near the age of onset, alterations to subcortical-premotor connectivity extend to auditory cortex in a study focusing on “speech” regions [55], and to the cerebellum, bilaterally, in a different study focusing on the “rhythm network” [51]. Both of these studies involved the same sample of children who stutter and compared them to fluent peers. In adults who stutter, though alterations to the premotor-subcortical circuit have been observed using both positron emission tomography (PET) imaging (FDOPA, [261]; O-15, [123]), and rfMRI ([263][267]), the specific direction of these disruptions in terms of over- or under-activity is somewhat dependent on the methods chosen.

In a study that examined global signal change not specific to connectivity within a single network, reduced amplitude of supplementary motor area (SMA)

activation was reported in stuttering relative to controls [263]. When medial-premotor activation is examined with regards to specific networks, stuttering is associated with: 1) both increases and decreases within a network defined by speech-related activity within subjects [123]; 2) increased coupling with spatial maps similar to the “speech” networks at rest [152]; 3) increased connectivity to spatial maps resembling the “sensorimotor” networks at rest [263], and 4) altered connectivity within the “basal- ganglia-thalamocortical network” [55][267]. Two different publications reported altered “SMA” connectivity in stuttering adults but in fact the network abnormalities were localized in the preSMA proper, anterior to Y=0 coordinate [267][263]. Resting PET activity in the preSMA is increased in stuttering relative to controls and SMA activation is decreased in the network that is activated during speech in the same subjects [123]. Though the preSMA and SMA are both involved in movement timing and coordination, these regions differ in their cortical and subcortical connections. Therefore, it is critical to clarify the location and direction of medial premotor connectivity disruptions in stuttering.

The cerebellum is another region which shows altered connectivity in stuttering in multiple resting state networks [152][267][210]. Stuttering severity is also related to resting-state correlations between subregions of the cerebellum and medial premotor, inferior frontal, posterior parietal, and sensorimotor cortices [267] as well as cerebellar connections with frontal medial cortex [210]. Successful speech therapy resolves alterations in strength of connectivity between the cerebellum and portions of the “speech network” that are observed in stuttering groups relative to controls before treatment [152]. Connectivity within the cerebellum, between lobules typically associated with motor and higher cognitive function, is also altered in stuttering [267]. These disruptions to connectivity in the cerebellum at rest should challenge the common interpretation of task-related overactivity as compensatory ([40][42][28]), yet surprisingly are reported as confirmation of compensation [210].

5.1.2 Methodological considerations for resting state research

In the field of stuttering research, studies have used one or both of two common approaches to resting-state analyses: seed-based, or network-based. In a seed-based approach, regions of interest are either a-priori defined, usually using anatomical methods to select a region (*e.g.* [55][267][210]), or selected because they exhibit group differences in a whole-brain global signal analysis (*e.g.* [263]). Correlation strength of rest-related activity is calculated between the average signal within these seeds and other voxels throughout the brain. In the network approaches previously used in stuttering, correlated activity in the whole brain was examined,

sometimes using independent component analysis (ICA) at the subject level [152], and sometimes using a combination of filtering techniques [55]. Specific networks were selected because of spatial similarity to task-related networks [123], or similarity to typically occurring resting state networks [152][55]. The primary difference between the seed-based connectivity analysis and a network-based approach is that the former builds networks around a specific region or regions, whereas the latter defines networks as combinations of specific regions, regardless of other networks in which those regions may participate.

Another network-based approach is more data-driven, and therefore more objective, than previous work in stuttering and utilizes ICA at both the subject level and the group level: Multivariate Exploratory Linear Optimized Decomposition (MELODIC, [22]). A group-level ICA, called a temporal concatenation, extracts networks from the dataset according to shared spatial and temporal properties within the study sample, and independence of components from one another. This approach is used to identify maps of resting state networks common to a group or groups of individuals (*e.g.* [211][88][214]). Through a process called dual-regression [88], individual maps of the identified networks can be extracted and compared between groups (*e.g.* stuttering and fluent controls) to reveal differences in these networks. Further, the relationship between continuous variables and voxels contributing to the network can be assessed within each component. Overall, a group ICA allows examination of effects, both between stuttering and fluent controls, and related to stuttering severity within group, across several networks, which can determine if the differences previously reported are indeed specific to the networks selected, and, further, if any other networks of correlated activity are disrupted in stuttering.

5.1.3 The current study

Here we modeled spontaneous brain activity in adults who stutter and well-matched controls using ICA. We then used dual regression [88] to compare groups across all components extracted from our sample. We examined the relationship to stuttering severity within these same components. We predicted that the medial premotor cortex, subcortical nuclei, auditory cortices, and cerebellum would show disrupted connectivity in our stuttering group relative to controls. We predicted that cerebellar connectivity at rest would be related to stuttering severity.

5.2 Methods

5.2.1 Participants

The dataset used in this study was the same as that used in Chapters 3 and 4, which examined speech-related activation in stuttering individuals and fluent controls. Seventeen adults with persistent developmental stuttering (PDS: 13M:4F; aged 19-54 years; avg. 31.4 years, 4 left-handers) and 17 age- and sex-matched fluent controls (CON: 13M:4F; aged 19-53 years, avg. 32.4y; 4 left-handers) were scanned (Table 5.1). No controls had a history of learning or speech disorders. All participants gave informed consent to their participation in the research in a protocol approved by the University of Reading’s ethics committee. Stuttering ranged in severity from very mild to very severe as assessed using the Stuttering Severity Instrument (SSI, versions III and IV, [203], range 10 - 46, average 24.7, s.d. 10.9).

Table 5.1: Sample summary

		CON	PDS
Total n	[females]	17[4]	17[4]
Left-handed	[females]	3[0]	3[1]
Age in years	Avg (sd)	32.4 (11.4)	31.4 (11.0)
Range		19 - 53	19 - 54
Education in years	Avg (sd)	17.29 (1.8)	16.35 (1.8)
Subject=level ICAs			
# components	Avg (sd)	28.6 (4.1)	35.5 (7.2)
# noise components	Avg (sd)	14.5 (4.1)	18.5 (5.5)
Signal / noise	Avg (sd)	1.1 (.47)	.99 (.38)

Groups were well matched on age, education, handedness and gender. Group differences in the number of components extracted, and the total number of noise components removed from analyses were significant, but the ratio of signal to noise components did not differ between groups.

5.2.2 Data acquisition

All data were obtained at the University of Reading’s Centre for Integrative Neuroscience and Neurodynamics using a 3-T Siemens Trio scanner with a 12-channel head coil. Whole-brain functional MRI scans were taken of the brain at rest using T2*-weighted echo-planar images (TR=3000ms, TE=30ms, flip angle = 90°, field of view = 192mm x 192mm x 168 mm in 56 axial slices, voxel dimensions

= 3mm³, 200 volumes, acquisition time = 10:11 mins). Throughout the scan, the following text appeared in the middle of the screen: “Please take a rest for a few minutes. The experiment will start soon.” Additionally, participants were verbally instructed by experimenters to try not to fall asleep and to keep eyes open. A high-resolution 3D T1-weighted structural scan was acquired using a MPRAGE sequence (TR = 2020ms, TE=2.9ms, flip angle = 90°, field of view = 256mm x 256mm x 176 axial slices, voxel dimensions = 1 mm³, acquisition time = 4:34 mins). Resting state sequences were taken prior to the structural image and any tasks completed in the scanner. Following the structural scan a single complex fieldmap image was collected (TR=488ms, TE = 4.92/7.38ms, flip angle = 60°, field of view = 192mm x 192 mm x 148 mm in 37 slices, voxel dimensions = 3mm x 3mm x 4mm).

5.2.3 Image analysis

Data were analyzed using FMRIB Software Library v 5.0.9 (FSL; <http://www.fmrib.ox.ac.uk/fsl>, [132]). For each subject, structural images were processed with an optimized tool for segmentation and brain extraction (FSL.anat). Fieldmaps images were processed using `fsl_preparefieldmap` and brain extraction (BET; [212]). Functional data were processed at the subject-level using FMRI Expert Analysis Tool, v6.00 (FEAT). Standard preprocessing was implemented using head motion correction (MCFLIRT [131]); BET [212], grand-mean intensity normalisation, high-pass temporal filtering (100s), and spatial smoothing (Gaussian kernel FWHM 6 mm); *B0* unwarping was conducted using the fieldmap images [128][130]; and automatic dimensionality estimation was completed using the FSL tool for independent components analysis: Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC v 3.14 [23]). All fMRI volumes were aligned to the individual’s structural scan using brain-boundary-registration (BBR, [103]), implemented using FMRIB’s Linear Image Registration Tool (FLIRT, [129][131]) and were registered to 2mm MNI standard space using FMRIB’s Nonlinear Image Registration Tool (FNIRT, [9][10]).

All subject –level functional components were labeled manually using standard guidelines to identify characteristics of noise and signal in spatial maps, times series, and power spectra [104]. We used FMRIB’s FSL regression filtering tool (`fsl_regfilt`) to remove components that resembled structured noise and leave any components that plausibly contained signal alongside noise in the data. Cleaned data were temporally concatenated across subjects to create a single 4D group ICA image constrained to 25 components using MELODIC v 3.14 [119][23][22]. Dual regression was then used to probe for differences in resting state representations between group using FSL tool `dual_regression` [88].

Dual regression decomposed the 4D group ICA image into a 4D image for

each component containing subject-specific information. Dual regression uses two different linear regression steps to achieve this decomposition: 1) regression of spatial information from the group-ICA maps to isolate temporal dynamics for each component and subject; and then 2) regression of subject-specific temporal information against the dataset to isolate subject-specific spatial maps [88]. In the final step of dual regression, all components of interest were tested for differences between PDS and CON using FSL’s nonparametric permutation tool (RANDOMISE [256]): 500 permutations, Threshold-free cluster enhancement (TFCE) corrected to $p < .05$ within each component). We included age as a covariate of non-interest because of known effects on resting state networks [66]. Having no a-priori ability to predict how typical resting state networks would be represented across components, which of those representations would show group differences, or in what direction, we probed all 25 components for group differences at a Bonferonni-adjusted p value for additional comparisons $(.05/25 \times 2) = .001$.

In order to probe for the effects of stuttering severity on component representations, we re-ran the above analysis with an additional variable added: stuttering severity instrument (SSI) scores for the PDS group only. We wanted to restrict our analysis of interest to severity-specific effects and therefore modelled group differences and the impact of age as effects of noninterest. We used a permutation tool to determine significant bidirectional relationships between voxels within components and these scores (RANDOMISE, [256]: 500 permutations, TFCE and FWE corrected to $p < .05$ within each component). We probed all 25 components for significant relationships (positive or negative) with stuttering severity at a Bonferonni-adjusted p value for additional comparisons $(.05/25 \times 2) = .001$.

5.3 Results

5.3.1 Single subject extraction and denoising

We used automatic-dimensional ICA to extract independent spatial-temporal components from each of 34 individual subjects. Across our sample an average of 32 components were extracted per subject, with significantly more components extracted for PDS individuals than CON ($F(1,32) = 11.751$, $p = .002$, $r = .52$, Table 5.1). On average, 16.5 components from each individual were confirmed as noise and removed, with significantly more components identified as noise for PDS individuals relative to CON ($F(1,32) = 5.955$, $p = .02$, $r = .40$). The proportion of total retained components to removed components (signal / noise) did not differ between groups ($F < 1$). Within the group of PDS, stuttering severity was not significantly related to age, education, the number of components extracted, the number of components removed, or the signal/noise ratio.

5.3.2 Group level component identification

We extracted a total of 25 group-level components from a temporal composite of the individual subjects' cleaned resting state datasets. Group level components were labeled as either signal or noise, according to standards used for automatic classification [205]. Spatial patterns of typical resting state networks [22][211] and temporal functional modes [214] were used as a reference for confirming signal components. A total of five group components reflected structured noise: one white matter component, one motion artefact component, and three cardiac artefact components which collectively accounted for 16.14% of explained variance in the analysis [205]. The remaining 20 components were considered to be meaningful functional signals.

5.3.3 Group differences in signal components

Significant differences between groups were identified using a statistical threshold of $p < .05$, TFCE corrected within each component for nine signal components; in all components there was significantly greater coupling of activity in PDS relative to CON. These components collectively accounted for 36.72% of explained variance in the analysis, while the eleven components showing no group differences accounted for 47.14% of explained variance. It should be noted, however, that none of these group differences survived the adjusted $p < .001$ threshold, which corrects for the 25 components extracted. We have grouped results according to the anatomical areas showing alterations in stuttering relative to the normal activation patterns in fluent controls: 1) medial premotor cortex; 2) cerebellar cortex; 3) auditory and lateral premotor cortices; 4) orbitofrontal cortex; and 5) parietal and occipital cortices. When possible, we have labeled components according to their spatial similarity to resting state networks (RSNs) [22][211] and/or temporal functional modes (TFMs) [214] previously described in the literature, for ease of reference.

5.3.3.1 Medial premotor cortex

Based on previous findings of altered medial premotor functional connectivity in stuttering, we predicted abnormalities in components involving the preSMA/SMA regions. Accordingly, we confirmed such abnormalities, occurring bilaterally, in two separate components: 1) A sensorimotor network involving abnormal SMA connectivity; and 2) a frontoparietal network involving abnormal preSMA connectivity.

Sensorimotor RSN. This component's activation pattern included primary sensory and motor cortices in distribution similar to previous reports and was labeled as the sensorimotor RSN [22][211]. In this component, PDS had significantly

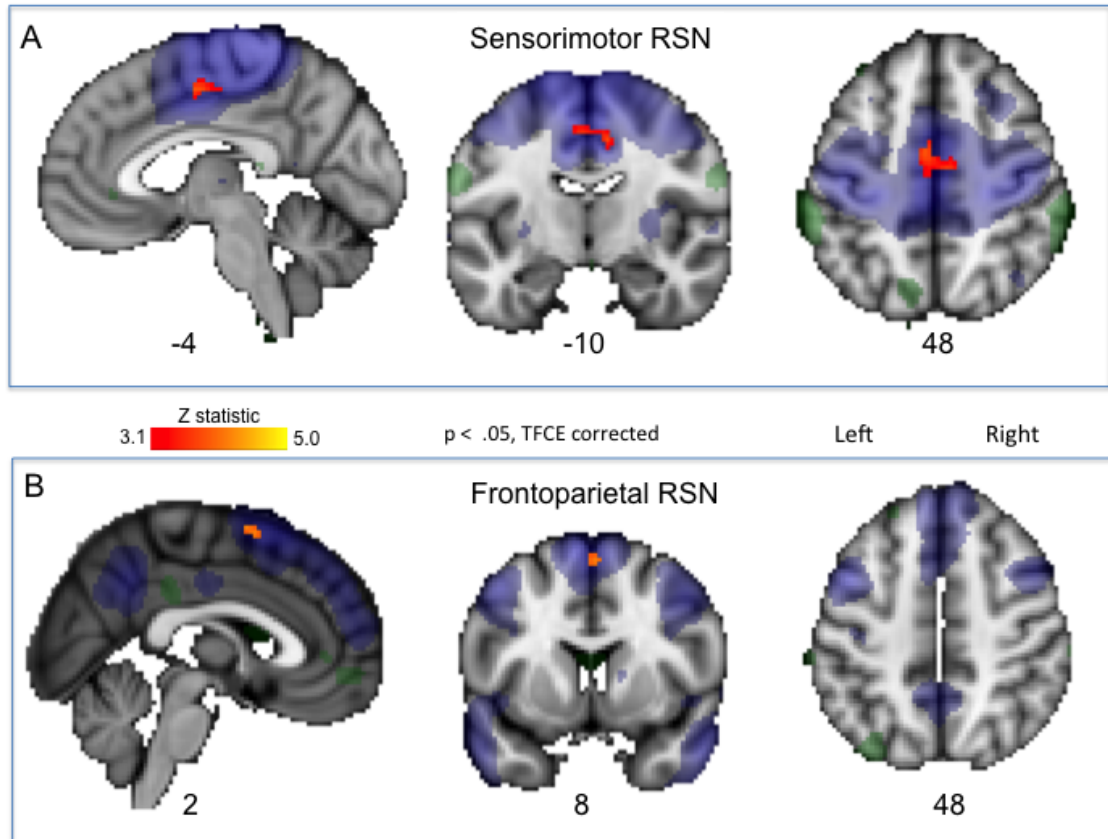
higher correlated activity relative to CON in a single large cluster with local peaks in SMA proper in the right and left hemispheres extending into cingulate cortex (Figure 5.1A, Table 5.2). This RSN of the group-ICA accounted for 4.26% of the explained variance and showed correlated activity in three primary cortices: auditory, sensory, and motor. Clusters with positively correlated activity occurred predominantly bilaterally and included (i) dorsal pre and post-central cortex extending into medial premotor cortex, (ii) superior temporal cortex (including Heschl’s gyrus and Planum Temporale) extending into posterior insular cortex and subcortical grey matter, and (iii) antero-medial and postero-lateral cerebellar cortices. Clusters with negatively correlated activity in this component included anterior cingulate cortex, orbitofrontal cortex, ventral premotor cortex, ventral motor cortex, the tail of the caudate nucleus, posterior somatosensory cortex, and lateral occipital cortex, all again occurring bilaterally.

Frontoparietal RSN. This component accounted for 4.17% of the explained variance and showed correlated activity in a spatial pattern consistent with two typical resting state networks that often appear lateralized to each of the hemispheres, labeled “frontoparietal” RSNs when previously described [211]. In this component, PDS had significantly higher correlated activity relative to CON in a single cluster with peaks occurring bilaterally in preSMA (Figure 5.1B, Table 5.2). In this RSN, clusters with positively correlated activity were observed bilaterally, including (i) a large medial-frontal cluster extending from the frontal pole into SMA proper, (ii) orbitofrontal cortex extending laterally through inferior frontal cortex to primary motor cortex, (iii) posterior cingulate cortex, (iv) middle temporal cortex extending into parietal and lateral occipital cortices, (v) precuneus, and (vi) lateral cerebellar cortices in both lobes. Clusters with negatively correlated activity again were observed bilaterally in this component and included two clusters in the cingulate cortex, the orbitofrontal cortex, the frontal poles, and somatosensory cortex.

5.3.3.2 Cerebellar cortex

We predicted altered cerebellar connectivity at rest in stuttering based on several previous reports. We replicated findings of altered cerebellar connectivity in stuttering, reflected in increased coupling of cerebellar voxels to activity in a single component resembling the “cerebellar resting state network” [211]. Group differences in this component were primarily located in the crus I/II sub-regions of the left hemisphere cerebellum, though a single voxel in the right hemisphere crus II was also significant (Figure 5.2, Table 5.3). All group differences were increased correlation of these voxels to the larger component in PDS relative to CON. The cerebellar component accounted for 3.44% of the explained variance in the group ICA and included positively correlated clusters occurring extensively

Figure 5.1: Abnormal connectivity of medial premotor regions in stuttering.



A. Sensorimotor RSN: The SMA showed increased coupling within this component in PDS. B. Frontoparietal RSN: The preSMA showed increased coupling with these regions in PDS relative to CON. Group contrast shown in red-yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected; spatial map for components thresholded at $2.3 < z < 8$ in blue for positive and green for negative correlations. Coordinates in MNI-152 standard space.

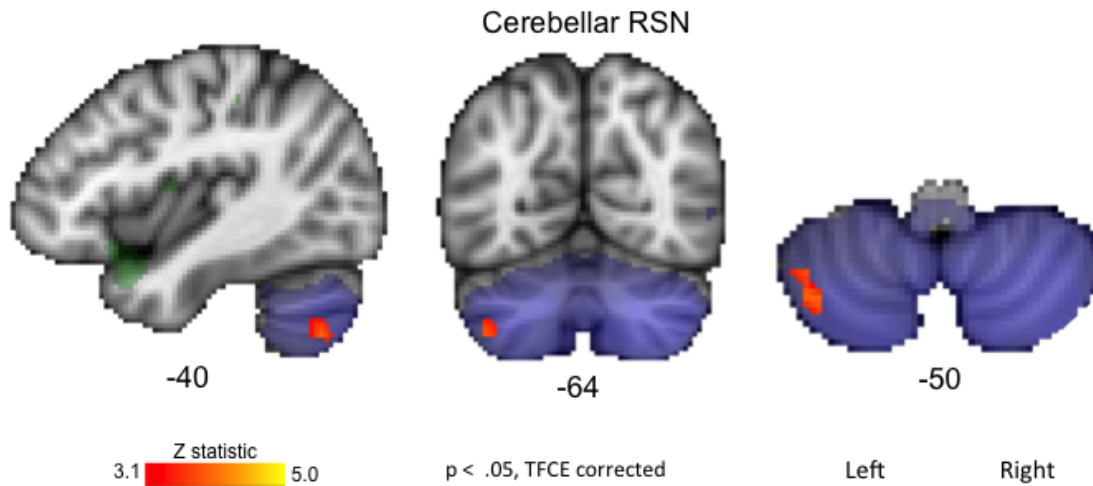
Table 5.2: Regions showing altered connectivity in PDS relative to CON in medial premotor cortices

Brain Region	# voxels	Z statistic	X	Y	Z
Sensorimotor RSN					
Right SMA	86	4.04	10	-8	44
Left SMA		3.89	-4	-4	48
Frontoparietal RSN					
Right preSMA	21	4.18	2	8	62
Left preSMA		4.13	-6	12	60

Location of the highest peak in a cluster is given: voxelwise, $p < .05$, corrected. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space. There were no regions where $PDS < CON$.

throughout cerebellar cortex, as well as with portions of the striatum and thalamus, all occurring bilaterally. Negatively correlated clusters were found bilaterally in the temporal poles and localized to the left hemisphere posterior cingulate gyrus and the medial surface of the central opercular cortex.

Figure 5.2: Abnormal cerebellar connectivity in stuttering



Increased correlated activity of the cerebellar crus I/II within a cerebellar RSN was observed in PDS relative to CON. Group contrast shown in red–yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected; spatial map for components thresholded at $2.3 < z < 8$ in blue for positive and green for negative correlations. Coordinates in MNI-152 standard space.

Table 5.3: Regions showing increased connectivity in PDS relative to CON in the cerebellar component

Brain Region	# voxels	Z statistic	X	Y	Z
Left crus II	1	3.52	-46	-56	-54
Left crus I	1	3.44	-38	-58	-42
Left crus II	54	4	-40	-66	-50
Right crus I	1	3.87	38	-70	-28

Location of the highest peak in a cluster is given: voxelwise, $p < .05$, corrected. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space. There were no regions where PDS < CON.

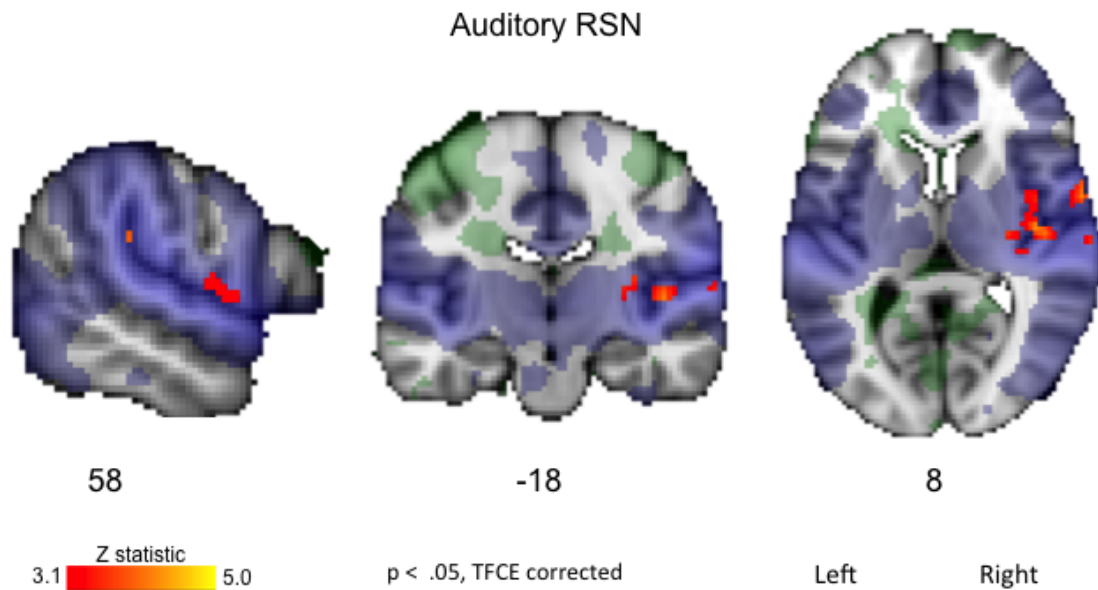
5.3.3.3 Auditory and lateral premotor cortices

Previous studies of stuttering have reported altered connectivity between the speech-motor regions and auditory cortex. Consistent with this, we found abnormalities in PDS relative to controls in two networks that encompass speech-motor areas and auditory cortex bilaterally. The central opercular cortex showed increased coupling in PDS with each of these networks. In the right hemisphere, portions of opercular and posterior superior temporal cortex showed increased correlated activity with an extensive network that encompassed peri-sylvian cortex and subcortical areas bilaterally. In the left hemisphere, a very small portion of central opercular cortex showed increased correlated activity in our PDS group with a network encompassing inferior posterior frontal cortex extending to insular and superior temporal areas.

Auditory RSN. A component which accounted for 4.33% of explained variance was spatially similar to the “auditory system” resting state network previously described [22]. PDS showed increased coupling within this component in a large cluster in the right hemisphere extending through posterior insular cortex and auditory cortex into the striatum, with local peaks in posterior insula, Heschl’s gyrus, central operculum, and the putamen. A second smaller cluster was located posterior-laterally in superior temporal gyrus at the level of Planum Temporale in the right hemisphere, and another small cluster was localized to the parietal operculum, on the surface of the posterior ascending ramus of the Sylvian fissure (Figure 5.3, Table 5.4). In this RSN, positively correlated activity was observed bilaterally and extensively in peri-sylvian regions encompassing primary and association auditory cortices extending subcortically as well as posteriorly into inferior parietal cortex through supramarginal and angular gyri, into lateral occipital lobes. Another cluster of positive correlated activity extended through the entire cingulate cortex into medial premotor regions. Though bilateral, this activity was

more extensive in the right hemisphere, encompassing the right inferior frontal gyrus extending into ventral premotor cortex and primary motor cortex. Negatively correlated activity in this component was left-lateralized and included clusters in i) superior frontal gyrus and SMA, ii) middle frontal gyrus extending into superior primary motor cortex, iii) superior primary motor cortex extending into somatosensory cortex, and iv) a single cluster in the left posterior lobe of the cerebellum in the crus II subregion.

Figure 5.3: Abnormal right opercular and superior temporal connectivity in stuttering



In a peri-sylvian component extending throughout the regions critical for speech and language, bilaterally, we observed increased coupling of right hemisphere auditory cortex including Heschl's gyrus extending into planum temporale and insular cortex in PDS relative to CON. Group contrast shown in red-yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected; spatial map for components thresholded at $2.3 < z < 8$ in blue for positive and green for negative correlations. Coordinates in MNI-152 standard space.

Premotor RSN. We have labeled this network because of extensive correlated activity in ventral motor and premotor cortices observed bilaterally in a component accounting for 3.65% of explained variance. Differences between groups were left-lateralized in the central opercular cortex. Significantly increased coupling with this component in PDS relative to CON was observed in two small clusters (Figure 5.4A, Table 5.4). When the threshold was dropped to $z > 3.1$, uncorrected, for visualization purposes, the cluster extended throughout central opercular cortex into primary auditory cortex (Figure 5.4B). Notably, a second cluster in the planum

temporale, which did not overlap with this component, was also overrepresented in PDS relative to CON at this reduced threshold (Figure 5.4B). When a region not overlapping with the component spatial map shows significant group differences in this sort of analysis, the interpretation is that the connectivity of this region with other portions of the network differs between groups, though the correlation of voxels in this region to the rest of the component is not strong on average across the groups.

In this RSN, positive correlated activity was observed bilaterally in extensive clusters that included lateral premotor cortex, and ventral motor cortex that extended into superior temporal cortex and then subcortically. Additional positive correlations were observed in more focal clusters in the thalamus, the SMA/preSMA junction, and the cerebellum at the level of lobule VI, again all occurring bilaterally. Negatively correlated activity in this component was relatively less extensive, with clusters occurring bilaterally in posterior cingulate gyrus, inferior cerebellum in lobule VIII, and temporal poles as well as unilaterally in left fusiform gyrus, and right superior parietal lobule

Table 5.4: Regions showing increased connectivity in PDS relative to CON in auditory and premotor cortices

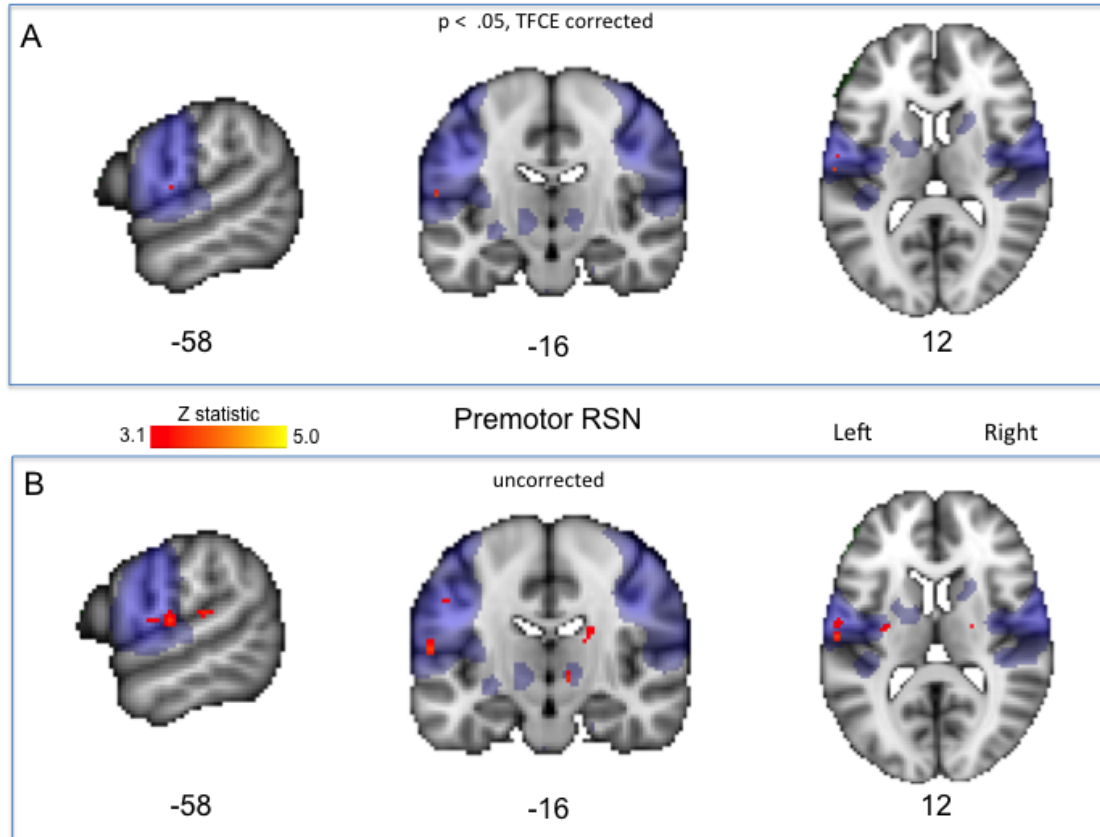
Brain Region	# voxels	Z statistic	X	Y	Z
Auditory RSN					
Right insular cortex	282	4.27	42	-14	8
Right central opercular cortex		4.19	62	0	8
Right Heschl's gyrus		4.26	46	-16	4
Right putamen		3.63	30	-16	8
Right superior temporal gyrus	6	3.52	66	-20	8
Right parietal opercular cortex	2	3.95	58	-30	24
Premotor RSN					
Left Central Opercular Cortex	1	3.2	-58	-8	12
Left Central Opercular Cortex	2	3.72	-60	-16	12

Location of the highest peak in a cluster is given: voxelwise, $p < .05$, corrected. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space. There were no regions where PDS < CON.

5.3.3.4 Orbitofrontal cortex

We observed increased coupling in PDS relative to CON in a medial orbitofrontal component accounting for 3.32% of the explained variance. Group differences in

Figure 5.4: Abnormal left opercular connectivity in stuttering



A. In a ventral premotor component extending through to superior temporal cortices, bilaterally, we observed increased coupling in PDS in left hemisphere central opercular cortex. Group contrast shown in red-yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected. B. We visualized these clusters at lower thresholds, which revealed a cluster in the left planum temporale that was positively correlated with this component in stuttering but not in controls. Group contrast shown in red-yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, uncorrected; spatial map for components thresholded at $2.3 < z < 8$ in blue for positive and green for negative correlations. Coordinates in MNI-152 standard space.

this RSN were several clusters of increased connectivity in PDS relative to CON, all located in the frontal medial and frontal orbital cortices, bilaterally. However, there was one cluster in the right hemisphere body of the caudate nucleus that also showed increased coupling in PDS relative to CON (Figure 5.5, Table 5.5) but this did not overlap with the spatial map for this component. The orbitofrontal RSN was composed of an extensive positively correlated cluster encompassing most of the inferior frontal medial cortex including orbitofrontal gyri, subcallosal gyri, and the frontal poles, as well as the temporal poles and planum polare, in both hemispheres. Left-lateralized positively correlated clusters were observed in the left crus I of the cerebellum, left lateral occipital cortex, and left primary motor and sensory cortices. Negatively correlated clusters in this component included cerebellar cortex at the level of lobule V/VI, the right thalamus, and insular cortex, all occurring bilaterally.

Table 5.5: Regions showing increased connectivity in PDS relative to CON in orbitofrontal cortex

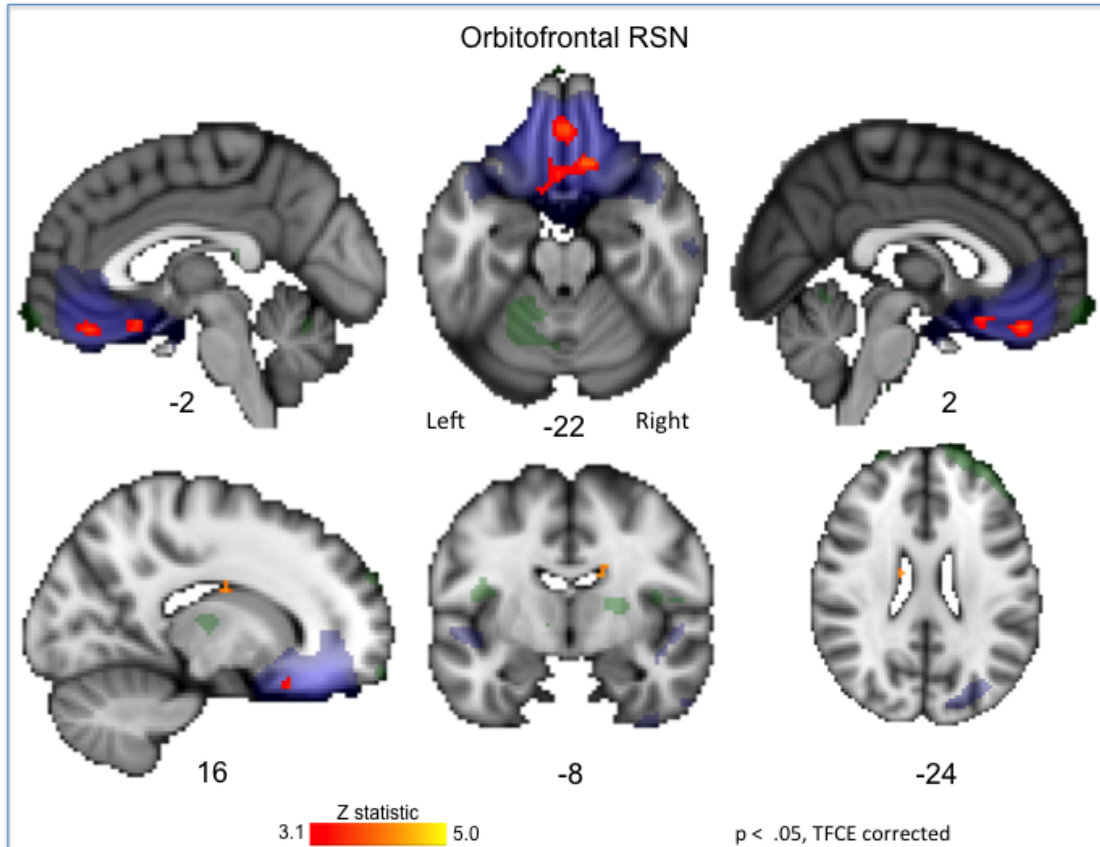
Brain Region	# voxels	Z statistic	X	Y	Z
Left orbitofrontal cortex	21	3.13	-22	26	-30
Right rostral sulcus	450	4.06	12	22	-22
Right gyrus rectus		3.93	2	38	-22
Left gyrus rectus		3.28	-6	22	-22
Left orbital frontal cortex	1	2.68	-30	22	-28
Left temporal pole	59	3.45	-46	16	-18
Left temporal pole	4	3.7	-42	10	-32
Right caudate nucleus (body)	7	4.33	16	-8	24

Location of the highest peak in a cluster is given: voxelwise, $p < .05$, corrected. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space. There were no regions where PDS < CON

5.3.3.5 Parietal and occipital cortices

Previous studies described altered functional connectivity in of the medial parietal cortex, known as the precuneus, in stuttering. Likewise, we observed abnormally increased coupling in PDS relative to CON in medial parietal cortex in three different components. The specific foci did not overlap, suggesting potential functional or anatomical specificity of this region, which is traditionally associated with the “default mode” of the brain [197]. The relevant components were composed of extensive correlated activity in occipital and parietal regions, and were somewhat

Figure 5.5: Abnormal orbitofrontal connectivity in stuttering



In a component encompassing frontal medial cortex bilaterally, we observed increased coupling in PDS relative to CON in several clusters in medial orbitofrontal cortex, occurring bilaterally. In addition we observed a small cluster in the right body of the caudate nucleus that was positively correlated with this component in PDS but not CON. Group contrast shown in red–yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected; spatial map for components thresholded at $2.3 < z < 8$ in blue for positive and green for negative correlations. Coordinates in MNI–152 standard space.

similar to “visual” and “default mode” RSNs previously described [22][211]. However, in general the spatial distributions were much more consistent with reported “temporal functional modes” (TFMs) [214] that allow spatial overlap in data-driven ICA but require temporal independence. The result is what appear to be spatial combinations of the traditional RSNs, as we observed here. Accordingly, we have labeled these components by the temporal functional mode to which they are most comparable.

Temporal functional mode 8. A component which accounted for 4.64% of the explained variance in the group ICA was similar in spatial distribution to “temporal functional mode 8” (TFM) [214], which is thought to represent a combination of visual cortex and dorsal streams that are typically represented in separate resting state networks [211]. Group differences were right-lateralized and included a cluster in the precuneus extending inferiorly into the cuneus and lateral occipital cortex (Figure 5.6A, Table 6). Several smaller clusters were also observed in right lateral occipital cortex, as well as a single cluster localized in the central sulcus, which was just inferior to the component representation in that area, all of which showed increased connectivity within PDS. The correlated activity in TFM 8 consisted of largely bilaterally occurring positive correlations in superior parietal lobule extending posteriorly and inferiorly through lateral occipital cortex. Other positively correlated clusters included, again bilaterally, dorsal primary motor cortices extending into frontal eye fields, the preSMA/SMA junction, and the striatum at the level of the nucleus accumbens. Unilaterally occurring positive correlations were right-lateralized and included central opercular cortex, the putamen, and the central sulcus. Negatively correlated clusters were largely left-lateralized in middle frontal gyrus and somatosensory cortex extending into the superior parietal lobe. Supramarginal gyrus, medial occipital cortex, lingual gyrus, and inferior precuneus all showed negatively correlated activity in this component occurring bilaterally.

Temporal functional mode 13. A component that accounted for 4.44% of the explained variance in the group ICA was somewhat similar in spatial distribution to the posterior portions of “temporal functional mode 13” [214], which overlaps with the “default mode” RSN [211]. In our TFM 13, negatively correlated activation in inferior frontal cortex was less extensive than in the original temporal functional mode, but still present. Three clusters showed increased coupling with this network in PDS relative to CON, two of which were in the right lateral occipital cortex, and the third of which was in the right retrosplenial cortex at the most anterior extent of the parietal-occipital sulcus (Figure 5.6B, Table 6). Positively correlated activity in our TFM 13 was localized to the precuneus cortex, the posterior portion of the cingulate gyrus, the occipital cortex adjacent to the calcarine sulcus, the angular

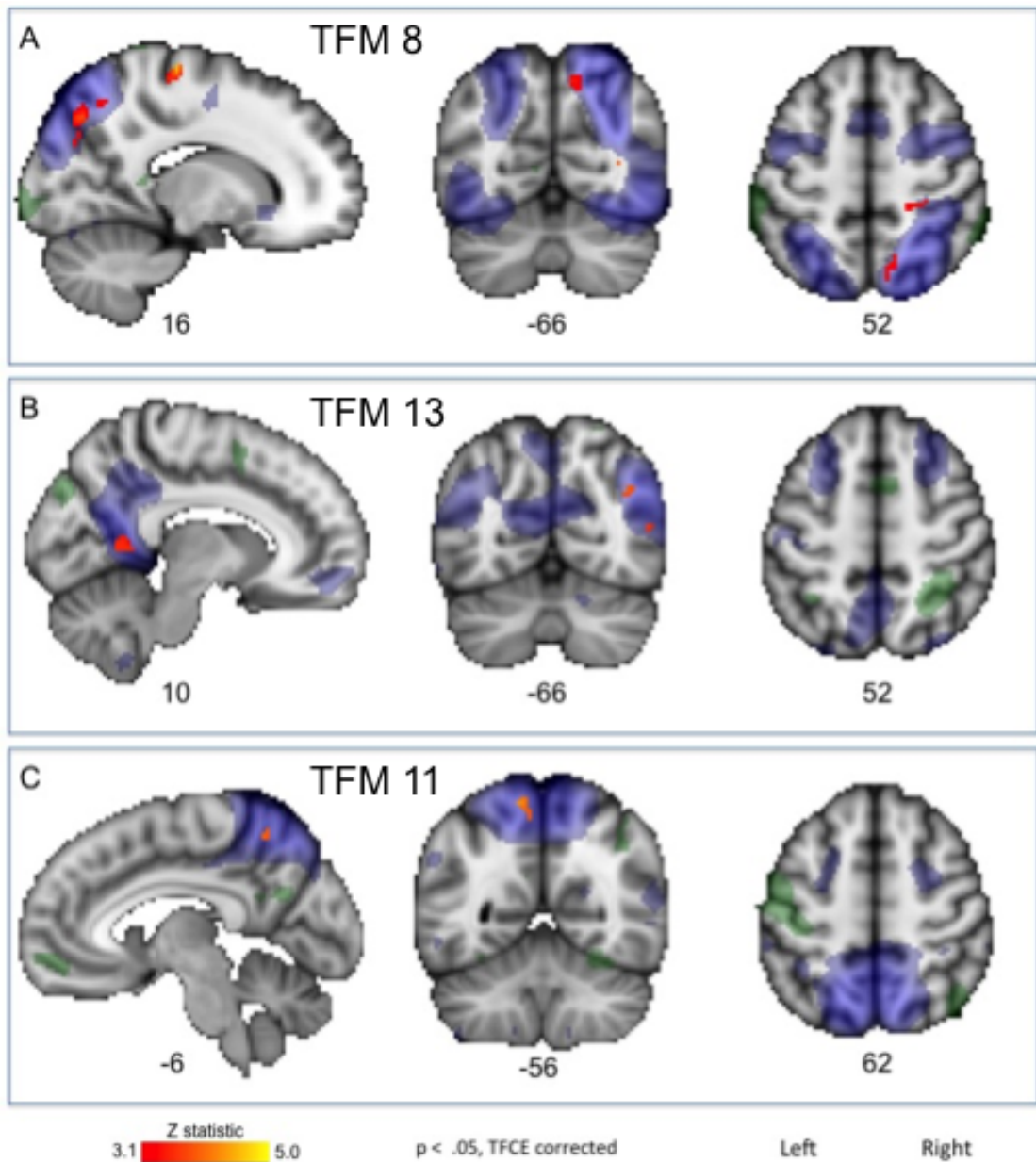
gyrus, parahippocampal gyrus, superior frontal gyrus, and Heschl's gyrus, occurring bilaterally. The left motor cortex and the left crus II of the cerebellum were also positively correlated with this component. Negatively correlated clusters included left-lateralized inferior frontal gyrus at the level of pars triangularis, superior frontal gyrus occurring bilaterally and extending into preSMA, right-lateralized posterior somatosensory cortex extending into supramarginal gyrus, superior parietal lobule occurring bilaterally but much more extensively in the right hemisphere, left hemisphere angular gyrus, cuneus, occurring bilaterally, and the right cerebellar crus II.

Temporal functional mode 11. Another component which overlapped with the “default mode” RSN [211] accounted for 4.47% of the explained variance in the group ICA. The spatial distribution of this component was similar to “temporal functional mode 11”, which is thought to be a combination of the semantic network and the “default mode” RSN [214]. Group differences in this component were isolated to a single cluster in the left hemisphere precuneus cortex which showed increased coupling of activity with this component in PDS relative to CON (Figure 5.6C, Table 6). In TFM 11, positively correlated activity was predominately bilaterally distributed, encompassing the majority of the precuneus cortex, extending inferiorly into posterior cingulate gyrus and laterally into superior parietal lobules. Symmetrical clusters of positively correlated activity were observed in both hemispheres in supramarginal gyri extending into angular gyri lateral occipital cortex, as well as superior frontal gyrus at the level of the frontal eye fields, anterior cingulate gyrus, and the frontal poles. Negatively correlated activity in this component occurred in orbitofrontal cortex extending into paracingulate gyrus, in superior temporal gyri including Heschl's gyrus and planum temporale, fusiform gyrus, inferior lateral occipital cortex, and a small cluster in the inferior precuneal cortex adjacent to the calcarine sulcus, all occurring bilaterally. Unilateral negative correlated activity was observed in a cluster in the left primary motor and sensory cortices at the level of the hand knob, and the right superior lateral occipital cortex.

5.3.4 Stuttering severity

We assessed the relationship between stuttering severity (SSI score) and voxelwise connectivity within each component. Stuttering severity was not significantly related to activation in any components at the adjusted $p < .001$ threshold (controlling for the number of components extracted), so all results are reported at $p < .05$, TFCE corrected within component. A total of eight components contained differences in connectivity related to stuttering severity within our PDS group. In three signal components, significant correlations occurred primarily in single-voxel clusters and/or were located outside of grey matter and are thus not reported. The

Figure 5.6: Abnormal medial parietal connectivity in stuttering



A. TFM 8: This component includes visual cortex, association areas, and dorsal stream regions. Differences between groups were right-lateralized and included precuneus. B. TFM 13: In a component including lateral parietal and occipital activations, and precuneus activity the stuttering group showed right-lateralized increases in correlated activity in this network in lateral occipital clusters as well retrosplenial cortex. C. TFM 11: The third component showed a focal increase in correlated activity within the left hemisphere precuneus in stuttering. Group contrasts shown in red-yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected. The component spatial maps are thresholded at $2.3 < z < 8$ in blue for positive and green for negative correlations. Coordinates in MNI-152 standard space.

Table 5.6: Regions showing increased medial parietal correlated activity in PDS relative to CON

Brain Region	# voxels	Z statistic	X	Y	Z
TFM 8					
Right superior temporal white matter	4	4.3	58	-22	4
Right central sulcus	105	4.52	16	-24	68
Right lateral occipital cortex	28	3.34	30	-58	38
Right lateral occipital white matter	1	3.92	34	-66	8
Right precuneus	290	3.79	16	-72	42
Right lateral occipital cortex	8	3.13	26	-74	14
TFM 13					
Right retrosplenial cortex	39	3.6	12	-52	4
Right lateral occipital cortex	7	3.95	40	-66	30
Right lateral occipital cortex	40	4.24	50	-72	16
TFM 11					
Left precuneus	13	4.29	-12	-56	62

Location of the highest peak in a cluster is given: voxelwise, $p < .05$, corrected. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space. There were no regions where PDS < CON. TFM=Temporal functional mode.

five remaining components contained clusters of reasonable size and anatomically meaningful locations. Three of these components were identified as signal components and also showed significant differences between groups, whereas the other two represent structured noise. We have grouped results according to the general regions showing significant relationships with stuttering severity.

5.3.4.1 Cerebellar cortex

Two of the components that showed greater connectivity in PDS relative to CON also showed clusters of voxels wherein connectivity was significant related to stuttering severity. These voxels were not located within the parts of these networks that showed group differences, however.

In the cerebellar RSN previously described (Figure 5.2) several clusters showed significant positive correlations with stuttering severity, most of them located in the right hemisphere crus I, with one exception in the left hemisphere near the midline at the level of lobule VI, just lateral to the vermis (Figure 5.7A, Table 5.7).

In the TFM 13 component (Figure 5.6B), a significant negative relationship between connectivity in a cluster in the left crus II of the cerebellum and stuttering severity was observed (Figure 5.7B, Table 5.7). This cluster was located outside of the component spatial map, suggesting that stuttering severity is related to the connectivity of this region and other portions of the component, specifically, but the region itself is not strongly connected to regions within the spatial map in the average across groups, including CON. Further, the functional connectivity between this cluster and the spatial component is stronger in those with the mildest stuttering severity (which could suggest a compensatory function).

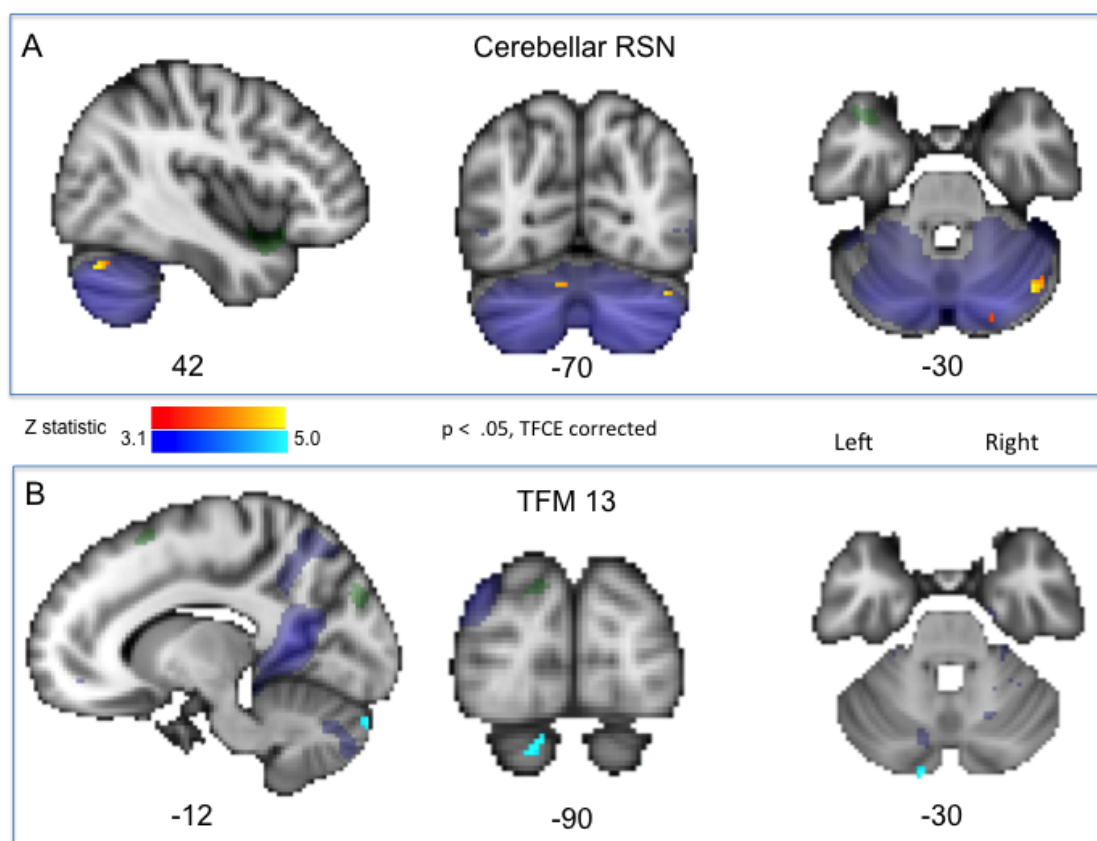
5.3.4.2 Temporal–occipital cortex

Two components contained clusters showing significantly positive relationships with stuttering severity that overlapped with one another. The specific location of this overlap is the right inferior temporal gyrus, extending into temporal–occipital fusiform cortex. The cluster was almost fully contained within the spatial map for the signal component showing this difference, previously described as TFM 8, representing extensive activation of primary and association cortices for visual function (Figure 5.8A, Table 5.8). The second cluster was also positively correlated with stuttering severity, but located outside the spatial component map for a “white matter” noise component [205]. (Figure 5.8B, Table 5.8).

5.3.4.3 Speech–motor cortex

A single noise component, similarly distributed to “cardiac noise” [205] contained a number of clusters showing significantly positive relationships with stuttering

Figure 5.7: Abnormal cerebellar connectivity related to stuttering severity



Significant correlations with stuttering severity surfaced in the cerebellum in two different components. A: Cerebellar RSN: In a component primarily consisting of widespread cerebellar activation stuttering severity was positively correlated to activity in several clusters, shown in red–yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected. B. TFM 13: In a component consisting of parietal activation a single cluster in the left hemisphere crus of the cerebellum showed negative correlations to stuttering severity, shown in blue–light blue $3.1 < z < 5$, masked for voxels at $p < .05$, corrected. Component spatial maps thresholded at $2.3 < z < 8$ in blue for positive and green for negative correlations. Coordinates in MNI–152 standard space.

Table 5.7: Regions showing significantly correlated activity to stuttering severity in the cerebellum

Brain Region	# voxels	Z statistic	X	Y	Z
Cerebellar component					
Right frontal pole	4	5.47	30	66	6
Right crus I/II	3	3.89	50	-66	-42
Right crus I	17	4.93	42	-68	-30
Left lobule VI	3	4.5	-10	-70	-26
Right crus I	3	3.8	22	-84	-30
TFM 13*					
Superior parietal lobule	1	5.14	-36	-48	64
Left crus II	14	5.09	-14	-90	-32

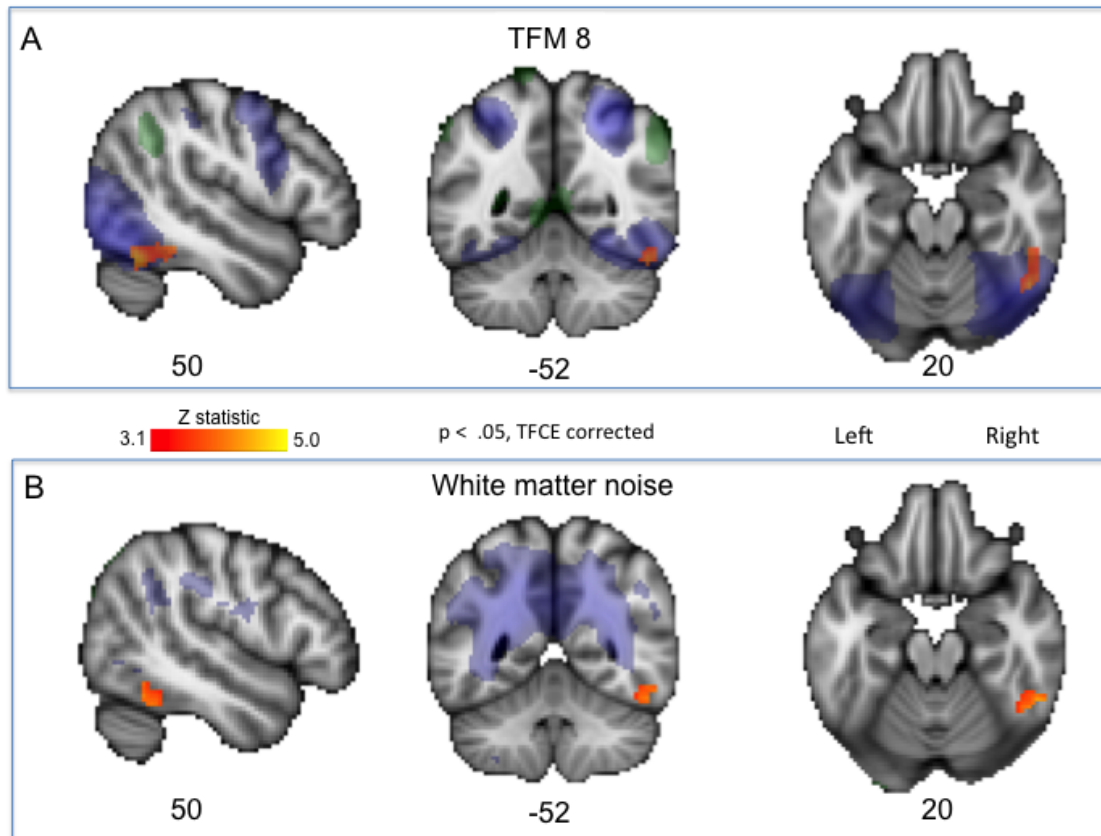
Location of the highest peak in a cluster is given: voxelwise, $p < .05$, corrected. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space. TFM=Temporal functional mode, * Correlations were negative in this component.

Table 5.8: Temporal-occipital regions showing significantly positively correlated activity to stuttering severity

Brain Region	# voxels	Z statistic	X	Y	Z
TFM 8					
Right frontal pole	21	4.88	22	44	30
Left frontal white matter (forceps minor)	5	4.51	-14	28	14
Right inferior temporal gyrus*	136	4.47	50	-56	-24
White matter noise					
Middle temporal gyrus	39	4.75	62	-40	-12
Right inferior temporal gyrus*	109	4.39	54	-48	-22

*Cluster overlaps in these two components. Location of the highest peak in a cluster is given: voxelwise, $p < .05$, corrected. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space. TFM=Temporal functional mode. There were no significantly negative correlations with stuttering severity and these components.

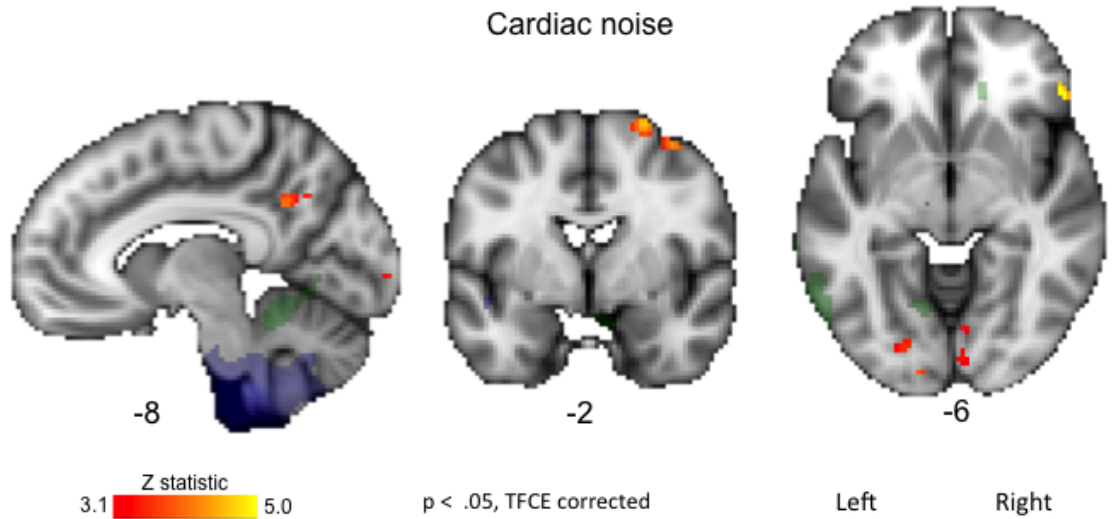
Figure 5.8: Abnormal connectivity of right inferotemporal cortex in relation to stuttering severity



Clusters showing significantly positive correlations to stuttering severity overlap in signal and noise components. A: In TFM 8 right inferior temporal gyrus extending into fusiform cortex was positively correlated with stuttering severity, shown in red–yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected. B: In a component consisting of structured white matter noise, a very similar cluster in the right inferior temporal gyrus showed significant positive correlations to stuttering severity, shown in red–yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected. Component spatial maps thresholded at $2.3 < z < 8$ in blue for positive and green for negative correlations. Coordinates in MNI–152 standard space.

severity located outside of the spatial map. The locations of several of these clusters were in regions with previously reported as functionally disrupted in stuttering (Figure 5.9, Table 5.9). Specifically, a cluster surfaced in right dorsal premotor cortex that extended into primary motor cortex, and another cluster showed significant positive correlations with stuttering severity was located in the right inferior frontal cortex at the level of pars triangularis. Posteriorly, significant correlations with stuttering severity were left-lateralized in the precuneus and angular gyrus. Positive correlations also surfaced in two large clusters in medial visual cortex, occurring bilaterally in the lingual gyrus extending into occipital fusiform gyrus. This component undoubtedly contained the largest number of significant relationships to stuttering severity. The presence of these relationships in a component that is otherwise structured noise could suggest the relationships also reflect noise, or it could suggest a need to further probe the relationship between cardiac noise and stuttering more generally in independent samples.

Figure 5.9: Abnormal connectivity of cardiac noise in relation to stuttering severity



Positive correlations with stuttering severity and cardiac noise activation, shown in red–yellow at $3.1 < z < 5$, masked for voxels at $p < .05$, corrected; Spatial map for component thresholded at $2.3 < z < 8$ in blue for positive and green for negative correlations. Coordinates in MNI-152 standard space.

5.4 Discussion

Our work was the first to use entirely data-driven approaches to examining rest-related alterations in stuttering. To a certain extent, we replicated pre-

Table 5.9: Regions showing significantly positively correlated activity to stuttering severity within a component consisting of structured cardiac noise.

Brain Region	# voxels	Z statistic	X	Y	Z
Right inferior frontal gyrus, pars triangularis	12	5.23	50	32	-6
Superior frontal gyrus	145	4.73	26	0	68
Right motor cortex	4	4.1	42	-8	52
Left supramarginal gyrus	1	4.1	-66	-44	32
Left precuneus	27	4.18	-10	-52	34
out of brain	5	4.34	-64	-52	40
Left angular gyrus	60	4.36	-56	-60	24
Left lateral occipital cortex	68	4.45	-48	-72	24
Right lingual gyrus	131	4.33	10	-78	-12
Left occipital fusiform gyrus	106	4.1	-20	-84	-10

Location of the highest peak in a cluster is given: voxelwise, $p < .05$, corrected. The number of voxels in a cluster is listed along with the peak height and coordinates of the peak location in MNI-152 standard space. TFM=Temporal functional mode. There were no significantly negative correlations with stuttering severity and this component.

vious findings, but we observed that alterations differ across coordinated networks, and are not specific to regions of interest. Furthermore, our findings overwhelmingly suggest increased strength of correlated activity in stuttering relative to fluent controls within several resting state components, with widespread distribution, including: 1) medial premotor cortex, both the SMA and preSMA; 2) the cerebellar cortex; 3) auditory, insular, and central opercular cortices; 4) frontal medial cortex; and 5) the parietal and occipital cortices. Notably, even though we identified a left-lateralised network from our group ICA that resembled the speech/language network seen in previous ICAs, there were no significant differences between people who stutter and fluent controls in the connectivity of this network. We also replicated previous findings of significant relationships between stuttering severity and rest-related activation in the cerebellar cortex. Finally, we observed relationships between stuttering severity and several noise components, though we did not observe differences between groups in those components.

5.4.1 Medial premotor connectivity is altered in stuttering

We observed increased strength of correlated activity between the preSMA and SMA within respective components. The separate involvement of these different portions of medial premotor cortex is consistent with the functional dissociation of preSMA and SMA as involved in higher order planning and execution of motor actions, respectively [190]. Specifically, stuttering-related increases in coupling of SMA proper correlated with coactivation throughout primary sensory and motor

cortices, whereas the increased preSMA connectivity was observed in a component encompassing medial frontal and lateral frontal association cortices. Each of these regions was implicated in previous stuttering research investigating single network connectivity at rest, described as: 1) the “basal–ganglia–thalamocortical network” [55][267]; 2) the “sensorimotor network” [263]; and 3) a network defined by speech tasks within the same subjects [123]. Excessive coupling of activity in the medial premotor cortex at rest is also consistent with increased task–related activity in stuttering across studies [42] that is exacerbated by task demands [123]. Our dissociation of alterations within preSMA from those in the SMA according to network connectivity in stuttering, however, is novel.

Our results showing an increased coupling of SMA activation are localized near other reported differences that were described as attenuation of SMA activation in children [55][51] and adults who stutter [263]; that is, these previous reports found decoupling, or reduced connectivity of the SMA in stuttering groups, whereas we found network-specific increases in regional coupling. Differences in direction of these abnormalities are likely due to methods chosen: while we examined network-specific coupling, studies showing decreased rest–related activity in this region refer to global signal. Increased network-specific SMA coupling was previously described but was mislocalized and in fact represented anomalous preSMA connectivity [263]. It is therefore unclear whether and how the global signal reductions reported in the SMA are reflected in network-level connectivity in stuttering.

In our study, the finding of a preSMA cluster which showed increased activation in stuttering is consistent with findings in eyes-closed rest conditions of speech-PET studies in stuttering [123], as well as reports of altered “SMA” connectivity in stuttering adults, within the “basal ganglia–thalamocortical network” [267] and the “sensorimotor network” [263]. The preSMA is involved in auditory processing of speech tasks, in particular when speech is unclear, and is theorized to be critical for linking auditory information with internal motor representations [147]. Disruption of the connectivity between the preSMA and higher order association areas at rest in stuttering could therefore impact auditory–motor integration in this population.

Because excessive stimulation of either the SMA or preSMA in either hemisphere can result in involuntary bimanual movements [63], our findings have significant theoretical impact. Increased coupling of each region within two separable networks could suggest multiple mechanism by which stuttering occurs. A reduced threshold in the SMA could trigger initiation of actions by connected regions prior to completion of receipt of motor plans, whereas a reduced threshold in the preSMA could release plans prior to completion and could potentially release updates to internal representations of motor plans prior to integration of error information. Accordingly, lesions to the medial wall can result in difficulty making voluntary movements, specifically in the initiation of sequential speech utterances [271]. Over-

all, alterations to SMA/preSMA complex are consistent with the core disruptions of several theories of stuttering [220][160][4][117][58].

5.4.2 Cerebellar connectivity is altered in stuttering

Our study replicated a common finding of altered resting state connectivity of the cerebellum in stuttering [152][210][55][51][267]. In contrast to previous reports of altered connectivity with multiple different cortical regions [152][210][51][267], our observations were specific to intracerebellar connections in stuttering [267]. We did not observe group differences within other networks, in spite of strong cerebellar cortex connectivity within those other components. Specifically, within a component that was largely isolated to the cerebellum, the left cerebellar crus showed increased connectivity in stuttering relative to controls, which is similar to previous reports of disrupted connectivity between cerebellar lobules in stuttering, including the left cerebellar crus I [267]. This region also corresponds to the location of altered connectivity within a network in childhood stuttering that shows accompanying absence of typically-correlated rhythm discrimination performance [51].

The cerebellar crus is interconnected with prefrontal cortices as well as the posterior-parietal cortices [181] that are thought to be involved in integrating different types of predictive information during ongoing interaction with a changing environment [182]. The crus is not typically involved in either statistical or dynamic prediction processing, which relies on contributions from lobules VI and VIII [182] but is rather involved in the feedforward processing of learned “symbolic” cues that guide action [14]. This learning is likely facilitated by the connections between the crus and higher-order association cortices, which is consistent with associated activation patterns during a variety of cognitive tasks [226]. It is difficult to determine which, if any, of the behaviours subserved by the crus are impacted in stuttering using resting-state data alone, as each function could theoretically be important for speech from working memory to sensory processing, to coordination of movements.

Intracerebellar connectivity within the cerebellar component was positively correlated to stuttering severity in the right cerebellar crus I/II and the left lobule VI. This result suggests that stuttering severity worsens with increased coupling of these subregions within this network. Though the right cerebellar crus findings are somewhat in contrast to a negative relationship with SSI scores previously reported in the right cerebellum more generally [210], positive correlations between intracerebellar correlations in left lobule VI and stuttering severity have been previously reported [267]. Further, left lobule VI has also shown responsiveness to fluency enhancement therapy in stuttering [152]. Several studies interpret rest-related cerebellar increases in stuttering as compensatory [210][152][267], as

do reports of over-recruitment during speech tasks in stuttering (*e.g.* [40][42][28]). This common interpretation is inconsistent with the observation that increased cerebellar activation is thought to correspond to incomplete or inefficient learning [79]. Further, lobule VI is specifically recruited during dynamic processing of information about our environment and is related to corresponding predictive success of those processes [182]. Excessive local correlations of this region at rest with other components of the cerebellum might delay receipt of sensory information necessary for integration of environmental information with motor plans, which is consistent with several causal theories of stuttering [220][160].

5.4.3 Auditory connectivity is altered in stuttering

Within a component covering the extent of primary auditory and auditory association cortices, as well as insular cortex, bilaterally, we observed right-lateralized increases in correlated activity in stuttering that extended from insular cortex into Heschl's gyrus, Planum Temporale, central opercular cortex, posterior putamen, and inferior frontal gyrus. In a different component containing an isolated network consisting primarily of basal ganglia, posterior auditory cortex, and primary motor cortex activation, increases in stuttering connectivity were left lateralized to central opercular cortex. Previous work using seed-based analyses have suggested altered connectivity involving these regions, though typically the pattern is decreased connectivity within stuttering [263][55][267] [123]. During speech tasks the right auditory cortex is often, but not always decreased in activation relative to controls (*e.g.* [176][40]), whereas increased recruitment of the right hemisphere inferior frontal cortex is considered compensatory for contralateral underactivation [194]. The central opercular cortex, bilaterally, is underactive in speech tasks in stuttering [249], and corresponding white matter underlying this region is disorganised [220][249]. Increased coupling of these regions collectively during rest could reflect an inherent disorganisation in the timely reallocation of resources that would be necessary for the rapid processing each region completes individually during speech production.

5.4.4 Orbitofrontal connectivity is altered in stuttering

Within a single component encompassing orbitofrontal cortex we observed altered connectivity of orbital frontal regions, occurring bilaterally in stuttering. This result somewhat replicated previous findings of altered orbitofrontal connectivity in stuttering [210]. Further, overt reading in stuttering typically recruits this region to a greater extent than it does in fluent controls [137]. The orbitofrontal region is thought to be critical for processing environmental feedback through its interconnectedness with sensory cortex, somatosensory cortex, premotor cortices, subcortical grey matter, and association areas [83]. Increased activation of this

region in stuttering could suggest an overactive system for feedback monitoring during speech, as well. In our study, within this component an additional region, a portion of the right body of the caudate nucleus, was positively correlated in stuttering but not part of the overall component. The caudate nucleus is typically well connected to higher order association areas and is important for goal-oriented behaviour. It's presence in this component in the stuttering group, but not the controls could suggest unnecessary goal-oriented reward monitoring even during rest, which would be consistent with view of altered dopaminergic function in stuttering [4].

5.4.5 Posterior connectivity is altered in stuttering

We observed increased coupling of subregions of the precuneus in stuttering in two different signal components containing positive correlations with parietal and occipital lobes. Increased connectivity in stuttering has been previously reported within the right hemisphere precuneus to left middle frontal cortex connections [210] and left pars opercularis [55] and within precuneus, bilaterally, to right prefrontal cortex and left frontal pole [263]. Some of the same reports suggest that precuneus activity in stuttering is reduced within the “default mode network” [263], and connections between limbic structures and the precuneus are attenuated, bilaterally, in stuttering [210]. The relationship between precuneus cortex and cerebellar lobule VI at rest has been reported as positively correlated to stuttering severity, [267], however we did not replicate that finding. We instead observed a negative correlation between stuttering severity and left cerebellar crus activation in a component showing increased coupling of right retrosplenial cortex in stuttering relative to controls.

Generally, the precuneus is multi-functional, and well connected to higher order association areas (but not sensory cortices) and subcortical grey matter [48] but it is best known as part of the “default mode network” that disengages during tasks. An over-active precuneus during rest most likely suggests increased effort would be needed to disengage for successful completion of tasks. Such an interpretation is consistent with the meta-analytic result of increased precuneus activation during speech conditions confirmed to contain at least some dysfluent utterances [28], but reduced activation of this region during other speech tasks as well as non-speech tasks [54]. Speech-related activity in the precuneus distinguishes between different stuttering symptoms [134] and is negatively related to stuttering severity following successful treatment [100]. The precuneus is recruited to a greater extent by people who stutter than fluent controls during imagined stuttering [121], which could be a form of self-referential processing, a function thought to depend on the precuneus [48]. Because it is not part of the traditional “speech” network, relevant links to stuttering may be under-reported, as some are reported and not discussed [263].

Though it is clear that the precuneus shows rest-related alterations to connectivity strength in stuttering, the specific direction of these differences could depend on functional subregions and certainly merits further investigation.

In another region outside of the classical speech network, the right inferotemporal-occipital cortex we observed unexpected positive relationships to stuttering severity in a cluster that overlapped across two group components. This cluster showed positive relationships to stuttering severity within a network of positively-correlated lateral inferior occipital cortex and primary motor cortex, alongside negatively-correlated somatosensory cortex. The similar temporal functional mode network is thought to represent a combination of visual streams and lateral visual cortex [214]. This finding is somewhat consistent with findings that the fusiform cortex of both hemispheres is negatively correlated to primary somatosensory cortex activity and stuttering severity [210]. The other component showing the same relationship with stuttering severity was structured noise.

5.4.6 Stuttering severity is related to structured noise

We observed relationships between stuttering severity and several noise components that are somewhat difficult to interpret, especially given we did not observe any differences between our groups in noise components. First, we wish to emphasise that the “measurement” of noise was only possible because of our chosen methodology. If we had limited our analyses to signal components only, these relationships would have been missed. Second, speculating about any relationship within a noise component is difficult given that these components are typically manually removed and/or ignored in the literature. Third, while we do not know with certainty how white matter and cardiac noise relate to cognitive function, we do know that at rest these signals are common and robust, and do not represent the sort of “residual” noise that is unable to be identified in experiments. Fourth, we can rule out a few conclusions with regards to the effects we observed as related to structured noise. Our groups did not differ in the noise components that would reflect motion or movement artefact, nor was there a relationship between stuttering severity and these components. Therefore, we found no evidence that more severe stuttering involves more movement at rest, generally. The question with which we are struggling is, if stuttering severity correlates with structured noise, is that actually meaningful? We think perhaps it could be.

Within the white matter noise component, an extensive cluster showing positive correlation to stuttering severity was in the inferotemporal-occipital cortex and overlapped with a very similar cluster showing the same relationship in a signal component. Very few clusters overlapped across components in our study, we therefore do not think this correspondence is likely due to random chance alone. The fMRI signal in white matter is little understood, but theorized to be important

in disorders of myelination [97]. Stuttering is not officially classified as such a disorder, though white matter disorganisation is the core of two theories of stuttering, one which suggests that impaired myelination in early life contributes to stuttering [64]; and the other, called the “disconnection hypothesis”, which suggests that white matter damage impedes signal transfer from speech–motor cortex [220]. In this thesis we replicated findings of widespread differences in white matter organization in stuttering that are consistent with both these hypotheses [249][59] (See Chapter 2). Finally, the inferotemporal–occipital cortex has been previously associated with speech–related activation differences in stuttering [40]. Generally, our findings suggest that more severe stuttering severity is associated with increased coupling of inferotemporal–occipital cortex activity not only to the temporal functional mode reflecting coordination of several visual networks, but also to the average signal of cerebral white matter.

We also observed significant relationships between stuttering severity and voxels within a component with positively correlated activation primarily in cerebral spinal fluid, and near the major branching of arteries at the circle of willis, termed “cardiac noise” [205]. Several of these positive relationships were located in regions reported previously: 1) stuttering severity was positively related to the strength of right pars triangularis rest–related fMRI connectivity [267]; 2) stuttering frequency was correlated to task–related PET activation in right primary motor cortex, and left lingual gyrus [123]; ; and 3) the coupling of stuttering severity and lingual gyrus in particular is often reported in stuttering research and rarely discussed [121][40][54][123][42]. Early theories of stuttering suggested that breathing technique impacting total oxygen levels available were a causal factor of stuttering [225]. Further, several studies have reported alterations to resting cerebral blood flow specifically related to severity in stuttering [191][71]. If the relationship we observed represents nothing other than an increased coupling of stuttering severity to physiological cardiac signal, we believe that is in and of itself an interesting area for further investigation. We do not wish to speculate further, just to suggest that reporting effects within what is considered “structured noise” in rest–related brain activity may provide useful inroads for further research.

5.4.7 Methodological considerations and future directions

A major strength of this work is the use of data–driven analyses, which provided an opportunity to explore several correlated networks at rest and resulted in unexpected and novel contributions to the stuttering literature. Still, our work is certainly not without limitations. First and foremost, interpretation of resting–state network activity is often difficult because we cannot say with certainty that abnormalities observed at rest have any meaningful relationship to task–related function. Further, a data–driven approach is in many ways more objective, but also less replicable: it

is unlikely to derive exactly the same components in exactly the same configurations across samples. For this reason, it is important to report the anatomical description of automatically derived components, which may resemble canonical resting state networks somewhat [211] but are unlikely to completely overlap with components derived from independent samples.

One way to address the uncertainty of data-driven approaches is to derive components using an ICA, then to compare groups using templates of canonical networks derived from larger samples, which are publically provided [211][214]. The risk of such an approach is that differences between groups could be exaggerated on templates, which are derived entirely from “normal” populations, and could result in significant findings that are either not meaningful, or too numerous to consider carefully. We chose a more conservative option, which would mean that some stuttering-related differences could be absorbed during the decomposition process. In other words, we may have under-estimated stuttering-related differences that in our study are reflected instead in a slight variation in component spatial maps relative to templates of RSNs in the literature. For this reason, a template-based analysis of these data is an obvious next step in our work.

The variability of stuttering is also incredibly important, and differences surface in speech-related activation from one individual to the next [262]. For example, determining whether the group differences observed are common to most or all of the stuttering group could aid with interpretation. Further, the existence of subgroups in stuttering is likely, which could mean that our observations represent an averaging across groups and are not comprehensive. For this reason, a data-driven approach to resting-state patterns within individuals could be an interesting next step, as well.

5.4.8 Conclusions

Overall, our current findings are largely consistent with previous resting state studies of stuttering. In fact, our examination of multiple networks at once meant that our results provide convergence across several studies, which each examined only specific regions or networks of interests. The examination of multiple networks also argues strongly against seed-based or region-specific analyses in stuttering, which ignore the complexity of neural systems and their interconnectedness and potentially miss important correlates of disorders. While the direction of group differences can be difficult to interpret in resting state analyses of this sort, it is clear that even without tasks, patterns in neural activation in stuttering are different to that of fluent controls. Overall the most important result of this work is the support for future researchers to consider more objective, data-driven approaches to examining rest-related activity in stuttering.

Chapter 6

Motor skills in people who stutter

Summary: Speech is a complex motor act and, as such, relies on processes common to other complex motor movements. The neural correlates of these processes are well-established and are related to different extents on dissociable functions subserved by the motor system. Here, we assessed the function of core processes in the motor system using four classic motor learning tasks (6.1 1) Visuomotor Adaptation [167]; 2) Reinforcement Learning [96]; 3) Uni and Bi-Manual Tapping [146]; and 4) Implicit Sequence Learning [77]. We hypothesized that performance on a battery including these tasks could distinguish between several causal theories of stuttering. These theories are summarized below.

Table 6.1: Summary of motor learning tasks

	Visuomotor Adaptation	Reinforcement Learning	Uni and Bi-manual Tapping	Implicit Sequence Learning
Motor processes probed	adaptation learning and retention: sensory/motor information, reaction time,	contingency learning and reward/punishment reinforcement test strategy bias, reaction time	reaction time, adjustment to increasing task demands, bimanual coordination, sequential movement, practice effects for rapid, internally-timed movements	Implicit motor sequence learning and retention, practice effects for single finger-tapping to external stimulus
Motor processes not probed	articulation, self-generated timing, sequential movement, bimanual coordination	articulation, sequential movement, bimanual coordination	articulation, learning	articulation, self-generated timing, bimanual coordination, explicit learning
Associated brain regions	cerebellum	basal ganglia and general dopamine (D1 vs D2) receptor density	motor cortex, supplementary motor area, cerebellum, basal ganglia	motor cortex, supplementary motor area, cerebellum, basal ganglia
Timing	external (computer, 750 ms) paced	internal (self, up to 4 s) paced	internal (self, rapid) paced	external (computer, 1 s trials) paced
Movement design	right hand only: random: 8 targets	right hand only: random/balanced: 2 targets	both hands: single tapping space and 4 item sequential uni and bi-manually	right hand only: random/balanced: 4 targets and 10 item sequential

Four classic motor learning tasks were selected based on the motor processes they probe and the brain regions in the motor system associated with these processes. Collectively, these tasks allowed us to address several theories of stuttering that would influence specific motor processes and result in dissociable behavioural profiles.

6.1 Introduction

6.1.1 Theories of stuttering addressed by task-general deficits

The general motor theory of stuttering [242], more recently termed a “speech motor skills perspective” describes stuttering as arising from a weakened, unstable, and inflexible motor system [171]. This perspective is supported by a general consensus that the timing of motor coordination is variable in children [179] as well as adults who stutter [269]. Increased variability in stuttering has also been observed in lip movements [171], speech sequence learning [16] and non-speech movements of the facial and finger muscles [161]. Further, abnormalities in explicit sequence learning in stuttering, which include exaggerated sensitivity to dual task conditions [218][217], and variable practice effects and reaction times [215], support a more general motor theory [242][155][217][241]. A weakened motor system should result in reduced performance on our task battery, even on simple unimanual tapping tasks, and further destabilization in response to progressively more demanding tasks such as sequence learning, bimanual coordination, reinforcement learning, and visuomotor adaptation.

A more specific hypothesis that is testable across a variety of tasks posits that stuttering is caused by deficient internal timing mechanisms [4][84]. One form of this hypothesis suggests that the internal timing dysfunction is caused by an alteration to rhythmic patterns of brain activation in the beta band, and further argues that the brain’s external timing network is engaged to compensate for this abnormality [84]. Increases in cerebellar and right inferior frontal gyrus activation have been observed across several studies of stuttering [42][28][40] and are interpreted as evidence of compensation by the brain’s external timing network. A behavioural dissociation between internal and external timing networks is easily achieved by contrasting self-paced to computer-paced tasks. According to this theory, external timing cues compensate for internal timing deficits in stuttering, therefore, in our task battery, we would expect normal or enhanced performance on our computer-paced tasks (visuomotor adaptation and implicit sequence learning), and impairment on the self-paced tasks (reinforcement learning, uni- and bi-manual tapping).

According to a leading computational hypothesis of stuttering, sequential, rather than internal or external, movement timing, is most likely to be sensitive to the disruptions that interfere with smooth speech in this population [58]. Specifically, simulations of speech dysfluency demonstrate that a single circuit deficiency is sufficient to produce stuttering symptoms [58]. This circuit is the medial premotor-basal ganglia circuit and can be disrupted by either one or both of two underlying mechanisms involving the direct and indirect pathways through the basal ganglia: 1) dopamine imbalance, which delays activation of the direct pathway motor program

for initiation of the first syllable; or 2) white matter damage that would induce a transmission error in the indirect pathway and impede selection and initiation of the second syllable [58]. Both of the disruptions that successfully simulate stuttering in computational models, imbalance in dopaminergic function [260][4], and white matter disorganization [220][249] have been proposed in theoretical accounts as potential causes of developmental stuttering. Abnormalities in either dopaminergic function or white matter organization can therefore account for findings of altered functional connectivity within the premotor-basal ganglia circuit that are observed in childhood [55] and adulthood stuttering [55][154]. According to this computational account of stuttering, task demands of sequential motor movements are more critical than the source of timing cues. If the disruption is specific to the premotor-basal ganglia circuit in stuttering, we would expect impairment in our task battery on the two tasks requiring sequential movement (implicit sequence learning, uni-and bi-manual tapping), regardless of pacing.

6.1.2 Theories of stuttering addressed by task-specific deficits

A number of other theories predict selective impairment on specific tasks. For example, the “EXPLAN” theory of stuttering describes a deficit of “planning, execution, and the mechanism that interfaces them” [114]. Structural equation models of altered speech-related fMRI activation in stuttering suggest this deficit arises from two parallel disruptions: one impacting the cerebellar circuits and the other impacting the lateral premotor-basal ganglia circuit [154]. Cerebellar activation is abnormal in people who stutter, not only during speech tasks [40] but also at rest [152][267]. Cerebellar abnormalities also normalize with successful therapy [152][67]. According to the EXPLAN theory, both planning and coordinated movement execution are disrupted, a process that would be evident under increased planning and execution demands in the absence of external cues. Accordingly, this theory would predict greater impairment in our task battery on self-paced bi-manual relative to uni-manual tapping in stuttering. The EXPLAN theory would further predict intact performance on implicit sequence learning with computer-pacing that negates the need for self-generated action execution.

An underlying imbalance in the dopaminergic system is also theorized to cause stuttering [4]. Such an impairment would disrupt basal ganglia functions, which rely on a delicate ebb and flow of different types of dopamine receptor activity, but would be unlikely to selectively disrupt the basal ganglia-premotor circuits (though perhaps because of high density of receptors in that circuit disruptions could be magnified). Neuroimaging evidence supporting this theory includes significant relationships between neural activation in basal ganglia nuclei and stuttering severity using functional magnetic resonance imaging (fMRI) [100] and positron emission tomography (PET) imaging [123] as well as disrupted speech-related fMRI

activity located to the substantia nigra, which produces cerebral dopamine [249]. Studies of acquired stuttering also support this theory, in that lesions within the dopaminergic system can either induce [228] or reduce [270] stuttering. Similarly, dopaminergic medication both treats [157] and induces [38] stuttering.

Central to this theory of stuttering is the distinction between imbalance and depletion. Depletion in dopamine could impact virtually any task that required action selection and movement initiation. Imbalance, on the other hand, would selectively impact tasks that are sensitive to dopamine receptor levels, such as reinforcement learning paradigms. Preferential strategy use in a reinforcement learning paradigm changes with shifts in the level of extracellular dopamine within individuals [96]. Furthermore, the binding of specific dopaminergic receptors (D1 and D2) is associated with learning from positive or negative feedback, respectively [60]. The D1 and D2 dopamine receptor subtypes specifically segregate with the two main pathways through the basal ganglia (D1 for direct pathway, and D2 for indirect pathway) [248] and have opposing effects when bound to dopamine. In reinforcement learning, choosing a positive reward is thought to reflect direct pathway function, whereas avoiding punishment is thought to reflect the inhibitory, indirect pathway [96]. Altered use of either strategy in stuttering would address the dopamine hypothesis [4] without taxing articulatory, sequential movement timing, or bimanual coordination functions explicitly.

A “disconnection hypothesis” of stuttering theorizes deficiencies in the premotor-motor circuit occurring subsequent to basal ganglia involvement [220]. The theorized portion of the higher- order motor circuit affected in stuttering provides action cues as well as primary motor modulation of cerebellar signals [237]. Evidence of the hypothesized physical disconnection in the white matter pathways that subserve mouth motor function is well replicated in the literature: people who stutter have disorganized white matter underlying central operculum [220] that corresponds to abnormal functional activity in central opercular cortex [249]. The disconnection is thought to disrupt processes of motor function and result in the altered motor cortex facilitation observed in stuttering [173]. This disconnection hypothesis is also consistent with explanations of contralateral premotor activation as compensatory for left hemisphere disconnections [40][175][176]. In addition to neuroimaging evidence suggesting functional disruption of the cerebellar-thalamic-cortical circuit [51], a higher-level motor disruption accounts for the observation that longer utterances induce more stuttering [245]. According to this theory, we would predict a deficit specific to implicit sequence learning in our study, which requires learning and executing long sequences of movements. However, subcortical damage alone can also impair implicit sequence learning, be it damage to the striatal dopaminergic system bilaterally or dysfunction reflecting the traditional cerebellar syndrome [77]. In both subcortical cases, however, we would expect

performance deficits in other tasks in our battery, as well, such as reinforcement learning or visuomotor adaptation.

Finally, one hypothesis of stuttering suggests the problem lies not within the motor system *per se*, but instead with how motor and sensory information are integrated in the brain [160]. This theory is supported by observations of abnormal motor-to-sensory priming in auditory cortex in stuttering [206], though others find the priming is delayed but otherwise normal [19]. Computational models of speech suggest that integration of sensory feedback into internal maps is performed by the inferior frontal cortex and cerebellum [106], both of which show white matter disorganization underlying functional disruption in stuttering [249]. Further, white matter microstructure is abnormal in all three pairs of white matter pathways connecting the cerebellum to the cortex [59] (See Chapter 2). Finally, parts of the cerebellum are consistently overactivated in studies of stuttering [40], suggesting maladaptive learning, since cerebellar activation tends to decrease with learning success [79]. The specific lobules that are disrupted in stuttering are also selectively activated by visuomotor adaptation tasks [29]. According to the theory of altered sensory-motor integration [160], people who stutter should show impairment specific to processes that require integration of sensory information with internal motor maps. In our task battery, we would predict abnormalities in visuomotor adaptation, in particular to periods of altered sensory feedback. Such a finding would also discount a long-held assumption that cerebellar activity represents compensation in stuttering [40].

6.1.3 The aims of the current study

The primary aim of this study was to isolate which motor system functions are general traits that distinguish adults who stutter from fluent speakers. We compiled a test battery tapping the processes dependent on components of the core motor system that are necessary for smooth speech: 1) Visuomotor Adaptation [167]; 2) Reinforcement Learning [96]; 3) Uni and Bi-Manual Tapping [146]; and 4) Implicit Sequence Learning [77]. We first determined which motor skills are impaired in adults with persistent developmental stuttering (PDS) compared with fluent speakers (CON), thereby reflecting the general trait of stuttering. We then assessed these motor skills within PDS with respect to stuttering severity. Finally, we compared motor performance between subgroups of PDS, who share the stuttering trait, but differ in whether they have a family history of stuttering, which might suggest differences between subgroups in terms of aetiology.

6.2 Methods

6.2.1 Participants

The dataset used for this study protocol had minimal overlap with the other two datasets used in this thesis. We recruited 46 adults who referred themselves in response to an advert. Fluent controls (CON) were selected to match adults with persistent developmental stuttering (PDS) according to age, sex, and handedness. Self-report was used to gather standard psychosocial and demographic information. Nine participants (4 PDS; 5 CON) were excluded for self-reported history of psychiatric, neurological, or other speech disorder diagnoses. We retained 37 participants in our final sample: 19 PDS (ages 20-44 years, 3 females, 2 left-handed males); and 18 CON (ages 19-41 years, 3 females, 2 left-handed males). All participants gave informed consent to their participation using this research protocol, which was approved by University of Oxford's ethics committee. A family tree was used to prompt for information about handedness of relatives, familial stuttering, and to assist with portions of the interview. Hand preference was assessed the Edinburgh Handedness Inventory [180]. The summary of sample demographic information is presented in Table 6.2, along with a summary of family history variables of interest.

6.2.1.1 Family history

Participants were considered to have familial stuttering if any first or second degree relative (parent, sibling, or offspring, grandparent, aunt/uncle, grandchild/half-sibling) ever stuttered, including those who recovered. In addition, participants were asked if they had any cousins who stuttered, though this alone did not constitute familial stuttering. In our PDS sample, two individuals had cousins who stuttered, one of whom also had familial stuttering. In our CON sample, three individuals had cousins who stuttered, but no CON had familial stuttering. In familial stuttering, gender (male or female) and status of the relative who stuttered were also coded (recovered or persistent). If PDS participants did not have a relative who stuttered, they were considered to have idiopathic stuttering, which means any genetic risk is from a de novo mutation that could still be passed on to offspring. Participants were considered positive for familial sinistrality (left-handedness) if at least two family members - including the participant and first or second-degree relatives - were left handed, ambidextrous, or forced to use the right hand.

Table 6.2: Demographic summary

		CON	PDS
Total n	(females)	18(3)	19(3)
Left-handed	(females)	2(0)	2(0)
Age in years	Avg [sd]	28.72 [6.58]	29.74 [7.42]
	Range	19 - 41	20 - 44
Education in years	Avg	16.28	15.47
Family History			
Family Sinistrality		4	4
Familial Stuttering [recovered]		0	11 [6]
Affected relatives	Male Only		7 [4]
	Male and Female		4 [2]
	Female only		0

Groups were well-matched on age, education, handedness, gender, family sinistrality, and cousins who stutter. Family history measures are reported as the total number of participants meeting criteria.

6.2.2 Procedure

We administered a battery of tasks in an attempt to better characterize the motor disruption in stuttering. The battery included assessment of speech fluency and neuropsychological function as well as motor performance. We assessed motor performance using four classic empirical tasks from movement disorders and motor learning research. Each of the four tasks is thought to isolate a different skill necessary for smooth motor function. Likewise, each task is thought to specifically rely on different functions of the motor network.

6.2.2.1 Control tasks

Speech. A speech sample was recorded by video. The sample included two reading passages followed by spontaneous conversation prompted by experimenter questions. We assessed overall severity using the Stuttering Severity Instrument (SSI-IV, [203]). The SSI-IV is composed of subscales for frequency of stuttering events across both types of speaking tasks, duration of the longest stuttering events, and presence and intensity of concomitant behaviours. Possible scores range from zero to 46, with a minimum score of 10 necessary to be considered as having “very mild” stuttering relative to the standardization sample. Only PDS completed portions of the non-recorded interview specific to speech. PDS self-reported situations that worsen or reduce stuttering, age of onset, therapy history, comparisons to other affected family members, and the severity of stuttering and

physical concomitants on the day of testing. Two other measures were taken related to speech symptoms. The first, an adaptation of a tic-scale used in Tourette’s syndrome [244], quantified physical concomitants in more detail than the SSI-IV and allowed us to determine how aware each PWS is of each movement. The second was an assessment of the subjective experience of living with stuttering, which includes measures of anxiety around speaking (OASES, [268]). A summary of speech and psychosocial history measures is presented for the stuttering group at the end of this chapter, Table 6.4.

Neuropsychological function. We administered control tasks selected from standard neuropsychological assessment batteries. Participants completed these tests during the delay period for experimental tasks. We did not use the computer for these measures. Measures of interest included intelligence, processing speed, motor dexterity, executive function, and anxiety. A summary of group performance on neuropsychological measures of interest is presented in Table 6.3.

An abbreviated scale of intelligence (WASI, [254]) was administered to all participants. The digit-symbol subtest was selected from the Wechsler Adult Intelligence Scale-Revised (WAIS-R, [252]) to assess speed of processing for visuo-motor coding using the dominant hand for writing. The Grooved Pegboard [125] test was used to assess fine motor dexterity in both hands in all participants. Executive function was assessed using adaptations of standardized tasks: 1) a shortened digit span task to measure short-term memory capacity [253]; and 2) an in-house adaptation of the Golden Stroop task to measure cognitive interference [101]. The primary measure of interest in the Stroop task is an inhibition ratio, which is the proportion of correct colours named in the interference condition in which the text names a different colour, to the proportion of correct colours named in the colour condition in which rows of Xs are printed in different coloured ink. This ratio is thought to reflect ability to inhibit the response to read [101]. Finally, a standardized measure of trait-anxiety, the Beck Anxiety Inventory (BAI, [21]), was included to probe for severity of 21 common anxiety symptoms not specific to speaking situations. Possible BAI scores range from zero to 63, with scores lower than eight considered “minimal” levels of anxiety, between eight and 15 as “mild”, 16-26 as “moderate” and above 26 as “severe”.

Statistical analysis. We did not anticipate group differences on control tasks. We performed two-tailed Mann-Whitney tests to probe for differences between PDS and CON at a Bonferonni corrected threshold for comparisons across ten measures ($p < .005$). SPSS was used for all statistical analyses [224].

Table 6.3: Summary statistics for neuropsychological control measures

Psychological Measures	CON		PDS	
	Mean	sd	Mean	sd
BAI*	5.36	3.51	11.24	8.24
WASI				
Full Scale IQ	117.39	12.59	113.47	8.21
Verbal IQ	118.28	13.68	116.32	12.13
Performance IQ	112.56	11.78	108.11	7.24
WAIS Digit Symbol				
Symbols coded - errors	66.33	10.95	66.58	11.94
Digit Span				
Total digits forward	7.28	0.83	7.37	1.01
Total digits backward	5.89	1.41	5.68	1.06
Grooved Pegboard				
Right times (s)	68.17	17.43	70.05	11.27
Left time (s)	72.33	13.32	74.84	12.62
Stroop				
Inhibition ratio	0.81	0.12	0.84	0.1

Groups were well-matched. No measures were significantly different at corrected thresholds. Only anxiety measures differed significantly between groups at lowered thresholds (* $p < .05$, uncorrected).

6.2.3 Experimental tasks

The specific methods of our four computerized experimental tasks are described below.

6.2.3.1 Visuomotor adaptation

This classic motor learning paradigm requires visual feedback to update internal models and guide movements. The cerebellum is thought to facilitate this form of motor learning [167].

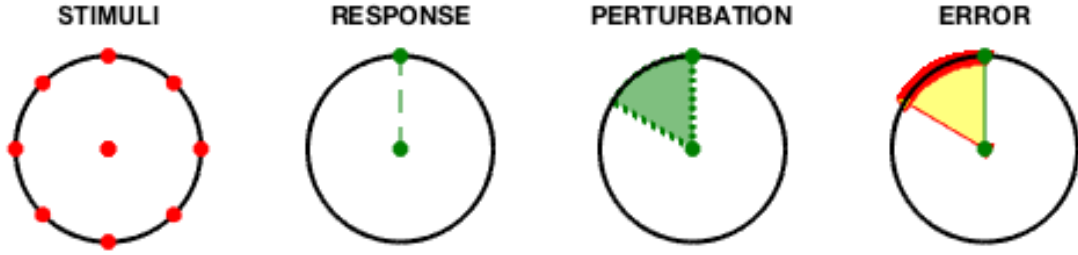
Procedure. Participants used a specialized joystick to move a cursor from the centre of the screen to one of 8 target positions outside and equidistant from the centre, but located at different radial positions around a clock face (0° , 45° , 90° , 135° , 180° , 225° , 270° , 315°). PsychoPy software [186][187] was used to present stimuli at a fixed inter-trial interval of 750ms. Participants were instructed to look only at the screen and use their right hand to move the cursor to the target in one smooth motion without correcting for errors. At the end of each trial, participants were asked to release the joystick, which would return the cursor to the centre position. For all trials we measured movement error, reaction time, movement velocity, and movement duration. All participants were allowed to practice the task before beginning baseline trials.

Stimuli were colored dots on a black background. A red target dot appeared in the centre of the screen then at one of the outer target locations (Figure 6.1: Stimuli). A green dot was controlled by the participant through the use of a joystick (Figure 6.1: Response). This task was designed in-house to present altered visual feedback in the form of an angular rotation of the true response in real time (Figure 6.1: Perturbation). Angular error of the movement error was quantified as the angle between the target position and initial trajectory of the movement towards the target (Figure 6.1: Error).

Our task involved four phases, with a delay between the second and third phases:

1. Baseline ($n=24$ trials). Visual feedback was unaltered. A short period of practice was required to reduce angular error to almost zero.
2. Adaptation ($n=120$ trials). We altered visual feedback so that the movement of the green dot appeared rotated counterclockwise by 60° . In this phase, angular error in the movement trajectory is large initially, then reduces as the participant adapts to the environmental feedback.

Figure 6.1: Visuomotor adaptation trial design



Stimuli: Centre cue and outer target positions. Response: Participants moved a green dot toward the outer target using a joystick. Perturbation: In some phases (adaptation and retention), visual feedback of the response cursor was rotated. Error: Movement error was the difference, in degrees, between target position and initial trajectory

Delay. During the delay period most participants completed several neuropsychological tasks and the bimanual tapping task. The average delay was 51.70 min ($sd= 5.96$, range 31–60 min) and did not differ between groups.

3. Retention ($n=120$ trials). As for the adaptation phase, the visual feedback remained rotated counterclockwise by 60° . In the third phase we measured how well the adapted state is remembered by the motor system. Accuracy is achieved more quickly than in phase 2 and angular error further reduces.
4. Wash-out ($n=120$ trials). Perturbation of visual feedback is removed to allow us to measure the wash-out or extinction of the adaptation response. As expected, participants initially experience an after effect of the adaptation and the movement trajectory of the green dot appears to overshoot the target in the opposite direction (*i.e.* an apparent clockwise rotation).

Analysis. For each participant, we rejected trials with an initial reaction time of less than 150ms (to avoid carryover from the previous trial), or with angular error greater than 90° from target. We excluded six individuals for whom more than 20% of trials were rejected (3 PDS, 3 CON). In the remaining 15 CON and 16 PDS individuals, we averaged responses into bins of 12 consecutive trials each. This averaging resulted in two bins for the baseline phase, and 10 bins for each of the adaptation, retention, and wash-out phases, for each participant.

We sought to address specific questions of interest, namely, do groups differ at baseline, do they respond differently to perturbation, and/or do they learn differently? To answer these questions we used a series of repeated-measures ANCOVAs, (2x2) with one between subjects factor (PDS, CON), and one within subjects factor for response bin. We specifically sought to confirm the expected

learning trajectory, which would be increased movement error during the adaptation phase relative to baseline, reduced error when comparing the first to last bins of the adaptation phase, retention of adapted response in the retention phase, and an overshoot in error movement between the final retention trials and initial wash-out phase trials. To probe for group differences along critical points in this trajectory, we designed ANCOVAs including the following bins:

1. The two baseline bins to determine if groups differ in baseline performance.
2. The first adaptation bin, the last adaptation bin, and the first retention bin to determine whether adaptation is learned and whether learning is retained.
3. The last bin of retention, and the first and last bins of the wash-out stage, to compare the after-effect of the adapted state once the perturbation was removed.

For each of the above three ANCOVAs, we included age and anxiety (BAI total) as covariates to account for variance known to affect motor performance. When Mauchly’s test of Sphericity indicated significantly different variances in ANCOVA cells, greenhouse-geisser correction is reported.

Within PDS, we conducted Pearson’s partial correlations between stuttering severity and average movement error for each bin included in the above ANCOVAs (1st and last baseline, adaptation and retention, and wash-out) while controlling for age and anxiety. All results are reported at $p < .006$, corrected for multiple comparisons using Bonferonni adjustment for the eight correlations conducted. To explore for differences due to familial stuttering, we repeated the ANCOVAs described above within the PDS group only, using a between subject factor of PDS subgroup (familial or idiopathic).

6.2.3.2 Reinforcement learning

In this task participants are trained using visual feedback to learn which stimulus in a pair is more likely to be rewarded. Acquisition of relative hierarchies is then tested through recombining stimuli into novel pairs. Optimal probabilistic selection, that is choosing the most rewarding pair member and avoiding the stimulus associated with the most frequent punishment, is thought to depend on the balance of dopaminergic receptors that facilitate basal ganglia pathways [96]. The direct pathway is facilitated by D1 receptors, which are excitatory, and associated with seeking reward, whereas the indirect pathway is inhibitory, more dependent on D2 receptors [248], and thought to facilitate punishment avoidance [96]. In this study we replicated a probabilistic selection task showing sensitivity to medication-induced changes in extracellular dopamine in Parkinson’s patients [96].

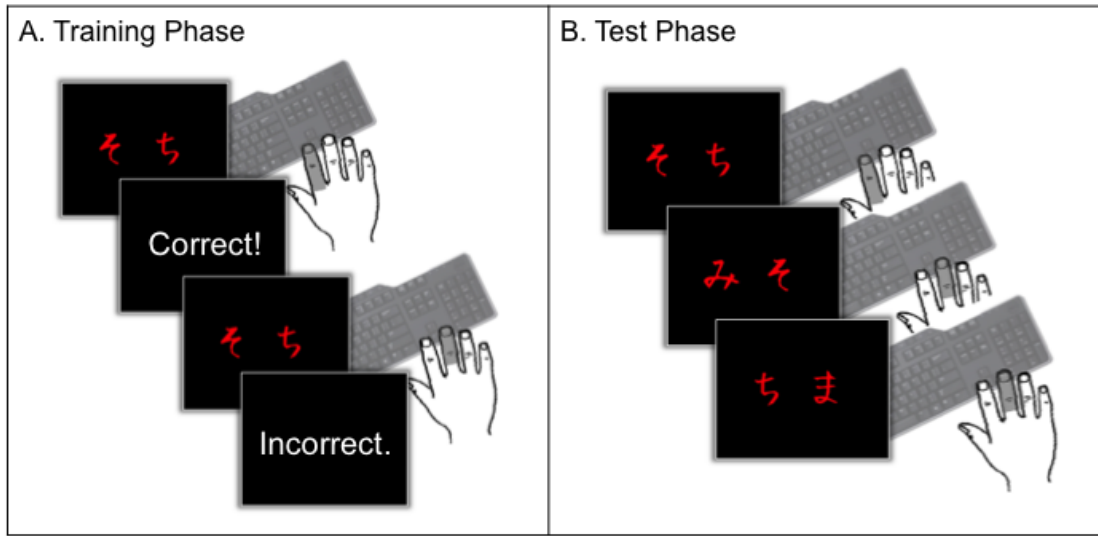
Procedure. We used a self-paced two-alternative forced-choice paradigm to train probabilistic learning of three pairs of characters. In contrast to typical forced choice paradigms, the chance of success was not 50%, but was dependent on pre-determined reward contingencies for each stimulus. Stimuli were red pictures of Hiragana characters, with one member of each pair presented on either side of the centre of a black screen (Figure 2). Stimuli were presented in a random order generated for each participant using Psychopy software [186][187].

We administered the task in two phases, with no delay between them:

1. Training. Participants were instructed to choose which member of the pair they thought was correct. Participants were explicitly reminded that the side of the screen the figure appeared on did not determine whether it was correct. Visual feedback - “Correct!” or “Incorrect” - was given following each response (Figure 6.2A). Participants responded within four seconds or “No response” appeared on the screen. Each member of the pair had a pre-determined probability of being correct, none of which was the usual “50%” associated with two alternative forced choice designs. A minimum of 60 trials (20 per pair) were presented during training, but the exact number of trials differed among participants (max = 600), based on when each achieved the desired minimum accuracy for each pair:
 - AB trials: Stimulus A led to positive feedback for 80% of the trials, whereas B led to positive feedback on 20% of the trials. Therefore, on 80% of trials, selecting B led to negative feedback, whereas selecting A lead to negative feedback on 20% of trials. Participants were trained to 65% accuracy on this pair.
 - CD trials: Probability of positive feedback for stimulus C was 70%; and D, 30%. Because contingencies were slightly closer to chance for this pair, only a 60% accuracy was required to move on to the test phase.
 - EF trials: The likelihood of positive feedback for stimulus E was 60%; and F, 40%. Before participants could move on to the test phase, they were trained to performance to 50% accuracy, which is equivalent to random chance for each decision.
2. Test. Participants were informed that this task was identical to the previous, except no feedback would appear following a decision (Figure 6.2B). They were told to go with their “gut instinct” if unsure. A total of 90 test trials were presented in a random order, counterbalanced for the side of screen on which the correct option appeared. The test phase included two types of pairs:

- Trained: Six repetitions of each trained pair (AB, CD, EF: 18 total trials)
- Recombined: Six repetitions of each novel test pair. Novel pairs were recombinations of training pair members (AC, AD, AE, AF, BC, BD, BE, BF, CE, CF, DE, DF: 72 total trials).

Figure 6.2: Reinforcement learning experimental design



A pair of stimuli appeared in red on a black screen. Participants selected a stimulus by pressing either the right or left arrow key on the keyboard. A. Training phase. Immediately after each selection, feedback was presented in white text on the black screen. B. Testing followed immediately after training. No feedback was given.

Analysis. We were predominately interested in responses that would allow use of one of the two optimal test strategies, described previously as seeking success, (*i.e.* the “carrot”, choosing A items, associated with increased D1 levels, the excitatory pathway) or preventing failure (*i.e.* the “stick”, avoiding B items, associated with increased D2 levels (and/or decreased D1 levels), the inhibitory pathway) [96]. For each participant, we calculated the average accuracy (correct/total) per participant for 30 test pairs containing A stimuli, and 30 test pairs containing B stimuli; representing the “carrot” and “stick” conditions, respectively. For each participant we calculated the average reaction time for all responses for each test strategy (carrot, stick) and for each training pair (AB, CD, EF) during the initial training block ($n = 60$ trials, 20 per pair).

We compared groups using repeated-measures ANCOVAs. To compare strategy at test we used an ANCOVA (2x2) with a single between subject factor of group (PDS, CON) and one within subject factor for accuracy based on type (carrot, stick). While we did not expect main effects, our primary hypothesis tested was that the interaction between group and strategy would be significant, such that PDS show a preference for one type of strategy over the other. To compare reaction time measures, we ran a second ANCOVA (2x5) with a single between subject factor of group (PDS, CON) and one within subject factor for pair type (AB, CD, EF, carrot, stick). For both ANCOVAs, age and anxiety (BAI total) were entered as covariates. When Mauchly's test of Sphericity indicated significantly different variances in ANCOVA cells, greenhouse-geisser correction is reported. All results are reported at uncorrected values ($p < .05$).

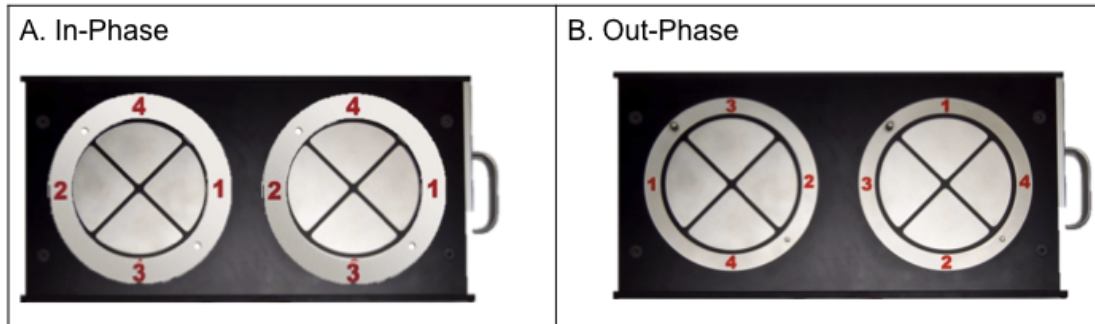
Within PDS, we conducted Pearson's partial correlations between stuttering severity and reinforcement learning response accuracy and reaction times, while controlling for age and anxiety. All results are reported at $p < .05$, corrected for multiple comparisons using Bonferonni adjustment. To explore for differences due to familial stuttering, we repeated the ANCOVAs described above with a between subject factor of PDS subgroup (familial or idiopathic).

6.2.3.3 Uni- and bi-manual tapping

Participants used each hand separately and also coordinated both hands to tap as quickly as possible. This task is facilitated by rapid communication between hemispheres, which relies on medial premotor cortex in particular.

Procedure. A modified bimanual tapping paradigm [146] utilized myTap software to count the number of taps made under time pressure. Participants used a stylus to tap one of four metal plates appearing on a circular ring of the Leonard Tapping Apparatus (Figure 6.3, [146]). A total of ten tapping blocks were completed. Two of the blocks required tapping a single plate with each hand as quickly as possible for 15 seconds (Rapid Dominant and Rapid Nondominant). Four blocks involved sequential tapping "1, 2, 3, 4" as many times as possible for the span of 30 seconds. Each of these sequential blocks was conducted twice. Complexity was increased from initially requiring unimanual tapping with each hand separately (Uni-Dominant and Uni-NonDominant), to requiring bimanual tapping in-phase (Figure 6.3A, In-Phase), and finally bimanual tapping with positions out of phase with one another (Figure 6.3B, Out-Phase). Participants were instructed to stand with their feet together, to hold the other hand behind their back for unimanual tasks, and to avoid speaking or counting out loud during the task. Participants were encouraged to go as fast as possible.

Figure 6.3: Uni- and bimanual tapping experimental design



A. For the In-Phase blocks, segments are labelled so that the two hands move in-phase with one another. B. For the Out-Phase blocks, labels are changed such that the tapping sequence is conducted with each hand moving out of phase to the other.

Analysis. For each participant the total number of correct taps per each of the 10 blocks was counted, and the coefficient of variance in intertap interval (ITI) was calculated for these movements. A total percentage of errors across the entire task was also calculated for each person. Several types of errors were possible: missing the plate, tapping the wrong part of the sequence, repeating a correct tap, tapping out of synch in the bimanual conditions, or tapping in-phase in the out-phase condition.

Our primary analysis of interest was a repeated-measures ANCOVA (2x2x4) assessing differences in total number of taps in each of the sequential blocks. The ANCOVA had a single between subjects factor of group (PDS, CON) and two within subject factors: repetition (1,2) and block (In-Phase, Out-Phase, Uni-Dominant, Uni-Nondominant). To probe for group comparisons in rapid tapping, we ran a repeated measures ANCOVA (2x2) with a single between subjects factor of group (PDS,CON) and a single within subject factor of hand (dominant,nondominant) for total counts in those two blocks. To see if error rates or types differed between groups, we ran a single ANCOVA (2x5) on error rates with a single between subjects factor (PDS, CON) and a single within subjects factor (error type). Finally, we expected bimanual movements to be more variable in PDS, and conducted an ANCOVA (2x2x2) using the coefficients of variance for bimanual block ITI, with a single between subjects factor (PDS, CON), and two within subject factors: repetition (1,2) and block (In-Phase, Out-Phase). Age, and anxiety (BAI total) were used as covariates in all analyses to account for variance known to affect motor performance. When Mauchly's test of Sphericity indicated significantly different variances in ANCOVA cells, greenhouse-geisser correction is reported.

To explore for differences due to familial stuttering, we repeated the four ANCOVAs described above with a between subject factor of PDS subgroup (familial

or idiopathic) in place of the group (PDS, CON) factor. Within PDS, we conducted Pearson’s partial correlations between stuttering severity, the total number of correct movements for each block, the coefficient of variance for correct movements in the bimanual blocks, and the total percentage of errors in this task. In all correlations with stuttering severity, we included age and anxiety as control variables. All results are reported at $p < .05$, corrected for multiple comparisons using Bonferonni adjustment.

6.2.3.4 Implicit sequence learning

Implicit training of a sequence is achieved by cuing movements, one after another in rapid succession, without any indication of sequence beginning or end. This process is thought to rely on higher order motor circuits including the premotor cortex, though cerebellar damage also interferes with this type of procedural learning [77].

Procedure. Participants were required to press one of four buttons on a button box (Cedrus, [50]) using only their right hand (Figure 6.4). The button to be pressed was indicated when the corresponding bar was replaced by an asterisk. Stimuli were presented at one-second intervals in black color on a white computer screen, using Presentation software (www.neurobs.com).

This task was completed in five phases, with a delay separating the 3rd and 4th phases:

1. Baseline phase. All participants completed 60 random trials before training (Figure 6.4).
2. Training phase. A total of three blocks (300 trials) were presented with self-paced rest between blocks. Each block was a set of 10 repetitions of a 10-item sequence being trained (2-1-4-1-3-4-2-3-4-1).

3. Random phase. After initial training, participants completed a single block of 100 trials with random presentation.

During the delay period, the WASI was administered. The average delay period was 44.62 min ($sd= 6.62$ min, range 28-63 min) and did not differ between PDS and CON groups. After the delay, two blocks (200 trials) were presented with self-paced rest between blocks.

4. Retention phase. The first test block included 10 repetitions of the trained sequence (100 trials). Performance on this phase is compared to the initial 100 training trials to determine practice gains and sequence learning.

5. Transfer phase. The second test block contained 100 random trials. Performance on this phase can be compared to the random phase to determine non-sequential learning.

Some participants completed subtests of the WASI following the final transfer phase to avoid prolonged delay during sequence learning trials. At the end of the experimental session all participants were asked if they had noticed a pattern in their tapping, and if so to replicate it. There was no evidence of explicit knowledge of the sequence in any participant. Technical problems were experienced for five participants in this task (1 CON, 4 PDS). For two PDS, no responses were recorded, for one PDS, no responses following the third transfer bin were recorded, and for another PDS no responses following the delay were recorded. For the CON participant, recording stopped during the ninth bin of the retention phase and continued throughout the rest of the task. We included all of the recorded data whenever possible from these data sets up to the point of technical error.

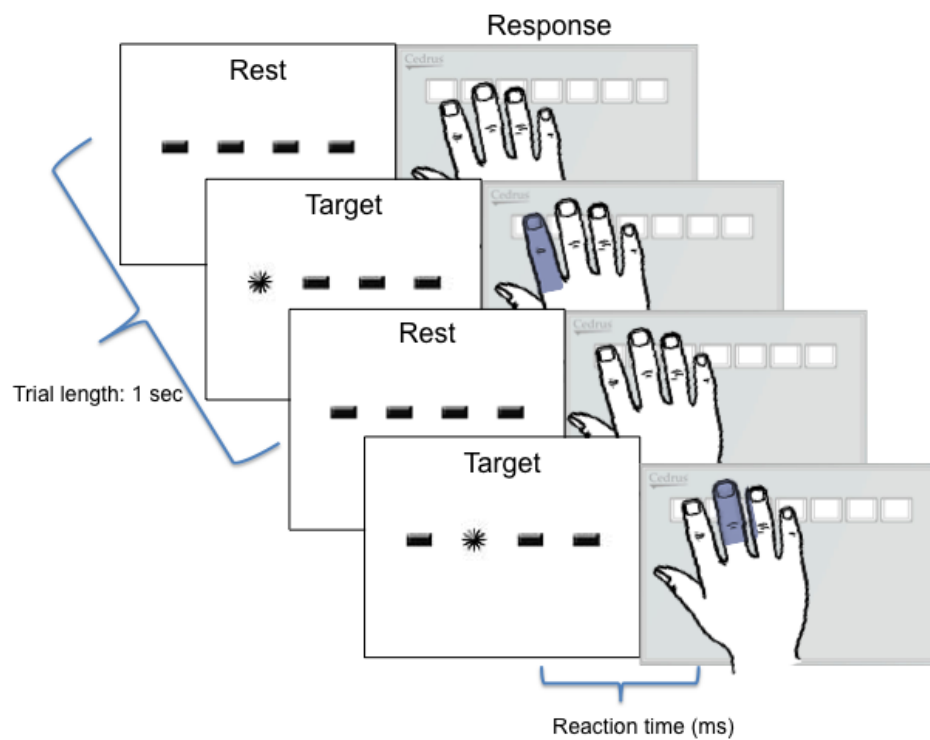
Analysis. For each participant, we measured the reaction time in milliseconds for correct responses. We then averaged reaction time into bins of 20 consecutive trials. For the sequence trials, this meant two repetitions of the sequence per bin. Unexpectedly, age and anxiety were predominately positively correlated to implicit sequence learning reaction time in our CON group, but negatively related to reaction time in our PDS group. Though not all of these relationships were strong or statistically significant, the patterns were clearly in opposite directions for our two groups, which violated the assumptions for our preferred model, the ANCOVA. Given our small sample sizes, the implications of this incidental finding would be entirely speculative and are thus not discussed. We instead used ANOVAs to explore these data.

We used several ANOVAs to assess performance on this task. When Mauchly's test of Sphericity indicated significantly different variances in ANOVA cells, greenhouse-geisser correction is reported.

We sought to address several specific questions with these models:

1. In order to address the question of whether our groups differ in the baseline phase, we conducted a repeated measures ANOVA (2×2) with a between subjects factor of group (PDS, CON) and a within subjects factor of baseline bin (first, last).
2. To determine whether the sequence was learned, and if so, whether groups differ in learning, retention, or consolidation of the sequence, we conducted a repeated measures ANOVA (2×4) with group (PDS, CON) as a between subjects factor and bin (first training, last training, first retention, last retention) as a within subjects factor.

Figure 6.4: Implicit sequence learning experimental design



Trials were computer-paced at one-second intervals. A target movement quickly replaced one of the bars on the rest screen. The measure of interest was the time between the appearance of the target and the button press.

3. In order to determine if nonsequential learning occurred, and if so, whether it differed between groups, we computed a repeated measures ANOVA (2x2x2) with a single between subjects factor of group (PDS, CON), and two within subjects factors of phase (random, transfer) and bin (first, last) within each of those phases.

Within PDS, we conducted Pearson's partial correlations between stuttering severity and average reaction time for each of the bins included in the analyses above, while controlling for age and anxiety. All results are reported at $p < .005$, corrected for multiple comparisons using Bonferroni adjustment for the 10 bins of interest. To explore for differences due to familial stuttering, we repeated the ANOVAs described above with a between subject factor of PDS subgroup (familial or idiopathic). Given the small sample sizes in these subgroups, results are reported at uncorrected values ($p < .05$).

6.3 Results

In addition to traditional reporting parameters, for all significant p values we converted distribution statistics to effect size estimates (r) [87].

6.3.1 Control tasks

Our two groups were well-matched on measures of neuropsychological function (Table 6.3). No comparisons survived adjustment for multiple comparisons. Trait anxiety, as assessed by total score on the BAI, was the only standard measure showing a difference between groups at an uncorrected threshold ($U = 88.0$, $z = -2.533$, $p = .01$, $r = -.42$), such that PDS endorsed higher trait anxiety than did CON. The majority of our sample was below even mild clinical thresholds for the anxiety measure.

6.3.2 Experimental assessment of general trait effects

In the analyses below, covariates for age and anxiety (BAI total) are included in models to control for variance in motor performance due to known factors not of interest to our specific hypotheses.

6.3.2.1 Visuomotor adaptation

Overall, both groups showed the expected learning pattern in this task (Figure 6.5). We conducted several ANCOVAs to probe for group differences using a single between subjects factor (PDS, CON), and a single within subjects factor specific to

our question of interest. In an ANCOVA (2x2) probing our two baseline bins, we confirmed that our groups did not differ in baseline performance, nor was there any significant difference between baseline bins or interaction with group in baseline phase performance.

In an ANCOVA (2x3) that included the first and last bins of the adaptation phase and the first bin of the retention phase, we probed adaptation learning and retention. We observed a significant group difference such that movement error was larger in the PDS group ($F(1,27) = 5.076$, $p = .033$, $r = .40$). Both groups showed the expected decrease in movement error across bins, evidenced by a significant main effect of bin ($F(1,27) = 8.579$, $p = .001$, $r = .49$), that did not significantly interact with group. Pairwise t -tests confirmed that the error was largest in the first adaptation phase, followed by the last adaptation phase, and movement error was also significantly decreased over the delay relative to both adaptation bins, indicating consolidation of adapted response over time (for all, $p < .001$, Bonferroni corrected).

Finally, in an ANCOVA (2x3) designed to assess the after-effect as well as the rate of de-adaptation we compared group (PDS, CON) performance during the last retention bin, the initial wash-out bin, and the end of the wash-out phase. We observed a significant effect of bin, ($F(1,27) = 9.929$, $p < .001$, $r = .52$), such that movement error was the highest during the initial wash-out bin and in the opposite direction of the final retention bin, the final retention bin had lower movement error than the initial wash-out bin but higher movement error than the final wash-out bin, which had a mean movement error near to zero in both groups. These effects were confirmed by Bonferroni-corrected pairwise t -tests ($p < .001$ for each). Groups did not differ in this ANCOVA, nor was the interaction between group and bin significant.

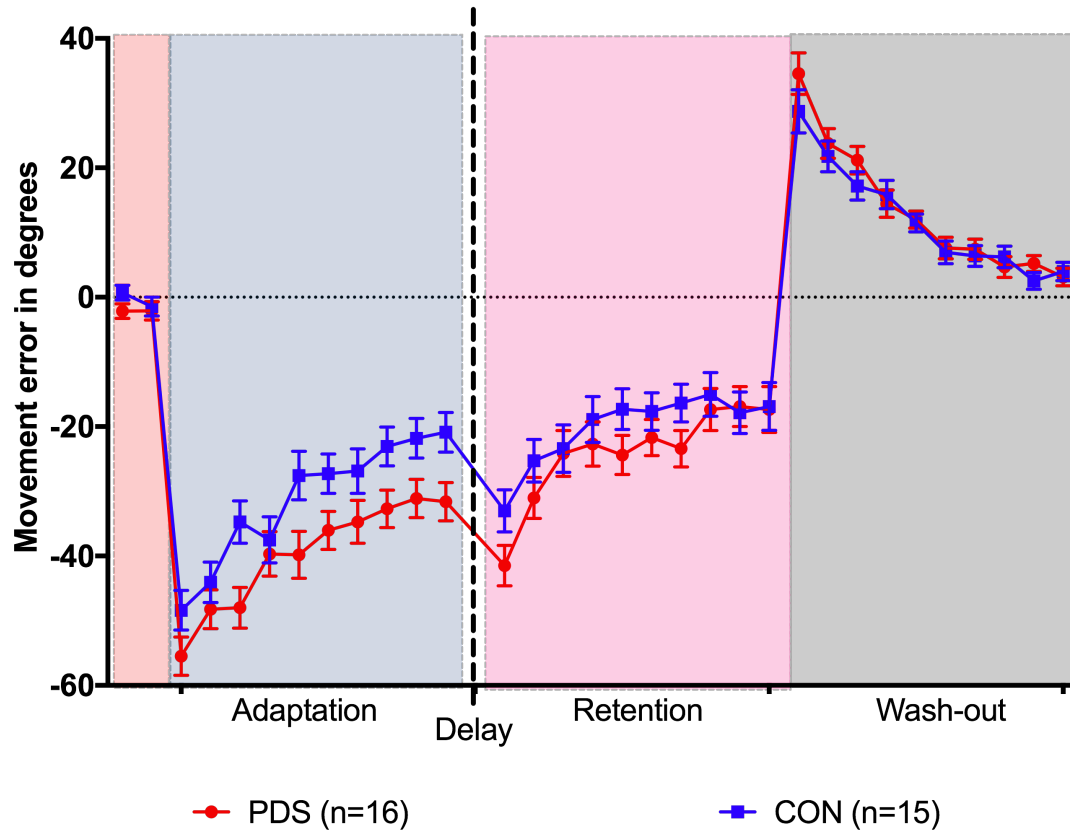
6.3.2.2 Reinforcement learning

We used repeated measures ANCOVAs to compare groups in response strategy test and speed during reinforcement learning and test. Groups did not differ in accuracy for any training pair, nor for either response strategy at test (carrot or stick), $F < 1$. However, throughout the first training phase and for both response strategies at test, PDS were significantly slower than CON ($F(1,33) = 7.688$, $p = .009$, $r = .43$) (Figure 6.6).

6.3.2.3 Uni- and bi-manual tapping

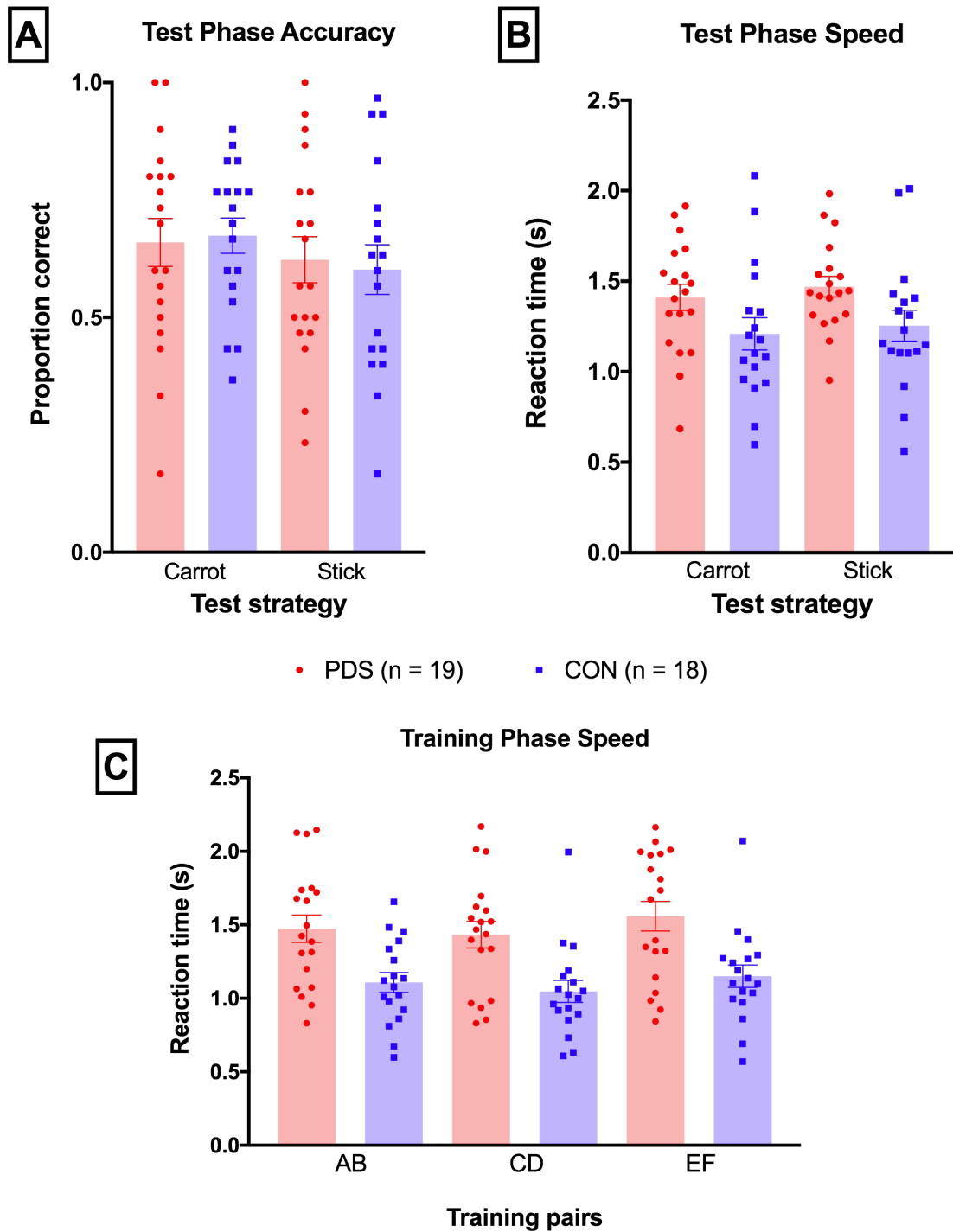
Our primary analysis of interest was a repeated measures ANCOVA (2x2x4) comparing total correct tap count in each repetition (2) of our four sequential blocks (dominant and nondominant unimanual; and in-phase and out-phase bimanual),

Figure 6.5: Visuomotor adaptation is altered in stuttering



Responses are averaged across bins of 12 and graphed in order of presentation with standard error about the mean indicated by bars at each bin. Both groups showed equivalent learning, as evidenced by reduction in movement error over time in the adaptation and retention conditions. However, overall, CON make smaller movement errors than PDS in response to altered feedback. This difference is maintained through the first retention bin but group differences are not significant thereafter.

Figure 6.6: Reinforcement learning performance



A: Groups did not differ in accuracy for either test strategy. B: PDS were consistently slower at test for both strategies. C: PDS were also slower throughout training. Graphs shows scatter plots of individual mean average reaction time for each response type. Bars represent group averages, with standard error about the mean indicated with t-bars.

between groups (PDS, CON). Overall, there were no significant effects of group or interactions with group in this analysis. The expected interaction between block and repetition was significant ($F(1,31) = 4.027, p = .009, r = .34$). Significant main effects included block ($F(1,33) = 11.811, p < .001, r = .51$) and repetition ($F(1,35) = 18.281, p < .001, r = .59$). The main effect of block was in the expected direction for both groups and repetitions: dominant unimanual tapping produced the most correct movements. Next followed the nondominant unimanual tapping, then in-phase bimanual block, and finally the out-phase bimanual block had the fewest total taps. The main effect of repetition was such that the number of taps increased for the second repetition in both groups and all blocks. The interaction between block and repetition amounted to different practice gains in the various blocks, where in-phase bimanual tapping showed an average practice gain of 15 taps between repetitions, out-phase showed an increase of less than six taps, for example. In other words, there was evidence of learning in all blocks, but gains were smallest in the out-phase bimanual blocks.

Our secondary analysis was an ANCOVA (2x2) comparing groups (PDS, CON) on total count in rapid unimanual nonsequential tapping. There were no significant main effects nor interactions between these measures and age, anxiety, or group. We also compared error rates between groups using a ANCOVA (2x5) and did not observe any significant main effects nor interactions between these measures and age, anxiety, or group.

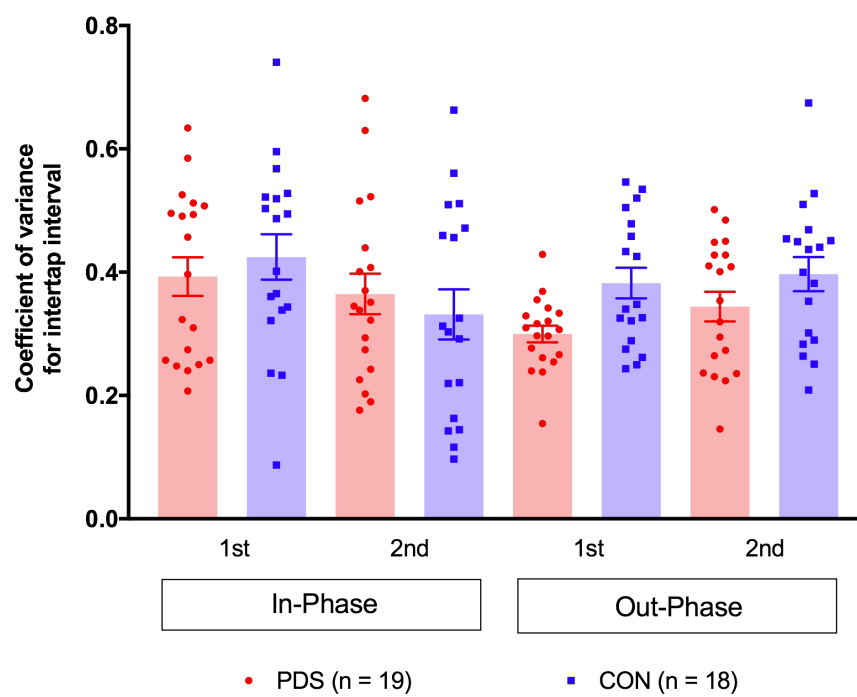
Our final group comparison on this task was using the coefficient of variance for bimanual movements using an ANCOVA (2x2x2). In this analysis no interactions between group (PDS, CON), block (In-Phase, Out-Phase) or repetition were significant. However, the main effect of group was significant such that PDS had lower relative variability in timing of coordinated movements than did CON ($F(1,33) = 4.230, p = .048, r = .34$, Figure 6.7), though this appeared to be driven by the out-phase blocks.

6.3.2.4 Implicit sequence learning

We ran repeated measures ANOVAs to address specific questions about group performance on implicit sequence learning, using response bins of 20 trials each (Figure 6.8).

We conducted a repeated measures ANOVA (2 x 2) with a between subjects factor of group (PDS, CON) and a within subjects factor of baseline bin (first, last) to probe for group differences in baseline performance on this task. We observed a significant interaction between bin and group on this task ($F(1,33) = 5.876, p = .021, r = .39$). The improvement in reaction times with practice across the baseline task was exaggerated in the PDS group relative to CON. The main effect of bin was also significant ($F(1,33) = 10.094, p = .003, r = .48$). Post-hoc comparisons

Figure 6.7: Bimanual tapping is less variable in stuttering



On average, variability of movements was lower in PDS than in CON during bimanual tapping, though this effect was strongest in the out-phase condition.

confirmed the groups did not significantly differ in baseline performance in either bin ($t < 1$).

We conducted a repeated measures ANOVA (2x4) with group (PDS, CON) as a between subjects factor and bin (first training, last training, first retention, last retention) as a within subjects factor to assess overall sequential learning. We observed a significant interaction between bin and group ($F(1,31) = 4.117$, $p = .009$, $r = .34$). Our PDS group was significantly faster than our CON group during the final training bin ($t(1,33) = 2.681$, $p = .012$, uncorrected, $r = .42$) but not during the initial training bin, the initial retention bin, or the final retention bin ($p > .05$). The main effect of bin was also significant ($F(1,31) = 2.978$, $p = .036$, $r = .30$). Pairwise comparisons confirmed that this effect was primarily driven by a significant reduction in reaction time between the initial training bin and the initial retention bin ($p = .03$, Bonferroni corrected). This reduction was observed in both groups. Though our PDS group appeared faster throughout this task, the main effect of group was not significant.

Finally, we computed a repeated measures ANOVA (2x2x2) with a single between subjects factor of group (PDS, CON), and two within subjects factors of phase (random, transfer) and bin (first, last) to probe for differences in nonsequential learning between our groups. We observed a significant main effect of bin in this model, ($F(1,30) = 5.901$, $p = .021$, $r = .41$), which indicated that performance typically worsened within each phase across bins. There were no significant effects of group or phase, or interactions with group or phase. Overall, we did not observe evidence of nonsequential practice gains across these random trials and likely observed evidence of fatigue following sequential training.

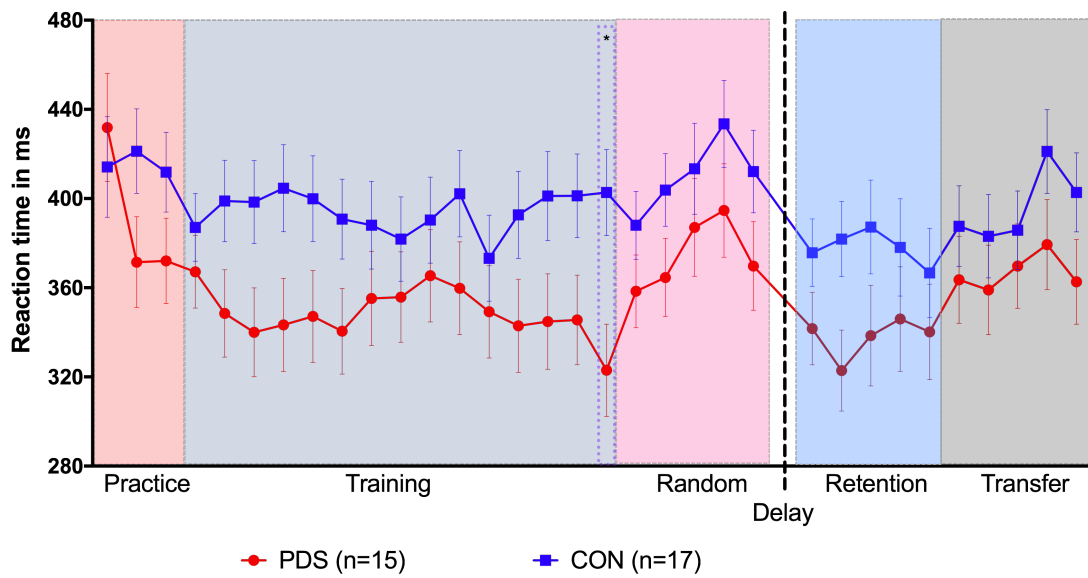
6.3.3 Motor skills and stuttering severity

We examined the relationships between stuttering severity within PDS and experimental task measures using Pearson's partial correlations with age and anxiety (BAI total) as covariates. No correlations with any measures across the four experimental tasks nor with any control tasks passed Bonferroni correction for multiple comparisons.

6.3.4 Experimental assessment of familial stuttering

We repeated the group analyses above to probe for differences between PDS subgroups. All models were identical with the exception of a subgroup factor in exchange for group factor. Within PDS, we did not observe any significant differences between familial and idiopathic subgroups on measures of visuomotor adaptation, or implicit sequence learning.

Figure 6.8: Implicit sequence learning is not impaired in stuttering

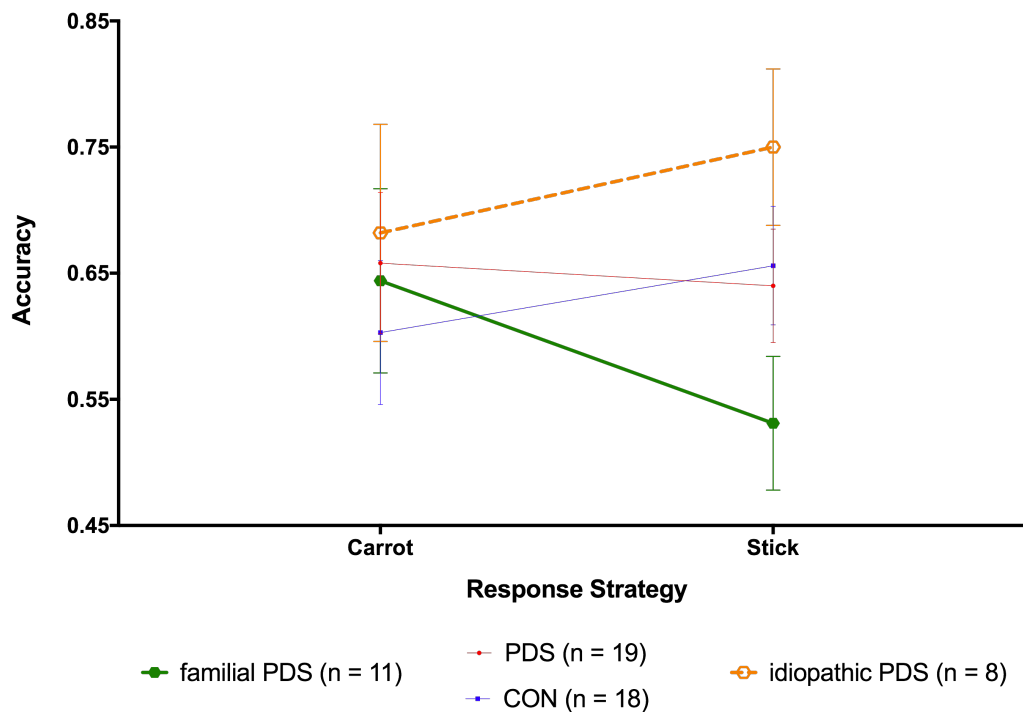


Both groups show similar learning curves overall in this task. Though generally, PDS are faster than CON, only the final training bin showed a significant group difference in average reaction time. Plots show group averages, with t-bars representing standard error about the mean for each group. * indicates significant difference between groups.

6.3.4.1 Reinforcement learning

We used ANCOVA's controlling for age and anxiety to explore differences in reinforcement learning strategy use and reaction time in our PDS subgroups. Our subgroups were significantly different in accuracy, but not reaction time in the reinforcement learning paradigm. Overall, we saw lower performance in this task for familial PDS relative to idiopathic PDS ($F(1,15) = 5.954, p = .028, r = .53$, Figure 6.9). Though the critical theoretical interaction was not statistically significant, the difference between subgroups was driven by performance using the “stick” strategy ($t = 2.664, p = .018, r = .57$).

Figure 6.9: Stuttering subgroups differ in reinforcement learning



Familial subgroups of PDS showed differences in reinforcement learning accuracy, particularly for the “avoid b” test strategy. The CON and PDS group accuracy measures are shown here for reference.

6.3.4.2 Uni- and bi-manual tapping

We used ANCOVA's controlling for age and anxiety to explore differences in total number of correct taps, error rates, and coefficient of variance in intertap interval

between our PDS subgroups (familial, idiopathic). Our primary analysis of interest was a repeated measures ANCOVA (2x2x4) comparing total correct tap count in each sequential block between subgroups. Overall, there were no significant effects of group or interactions with subgroup in this analysis. The expected effects of block and repetition were consistent with the larger group analysis described above.

Our secondary analysis was an ANCOVA (2x2) comparing PDS subgroups (familial, idiopathic) on total count in rapid unimanual nonsequential tapping. A single interaction between hand and subgroup emerged ($F(1,15) = 12.030$, $p = .003$, $r = .68$). The interaction was such that the familial stuttering subgroup clearly showed a dominant hand advantage ($t(1,10) = 4.139$, $p = .002$, $r = .79$, uncorrected), while the idiopathic subgroup did not ($t < 1$). The subgroups also did not significantly differ in total count for either hand (Figure 6.10A).

We also compared error rates between subgroups using an ANCOVA (2x5) and observed a main effect of subgroup in this analysis ($F(1, 15) = 7.740$, $p = .014$, $r = .58$). Our idiopathic subgroup had almost twice the error rate as our familial subgroup across this tapping task, an effect that was driven by relatively large errors in maintaining sequence across tasks, in addition to slipping in-phase during the out-phase bimanual task (Figure 6.10B). We did not observe any significant main effects of error type nor interactions between these measures and age, anxiety, or group.

Our final subgroup comparison on this task was using the coefficient of variance for intertap intervals during bimanual blocks. There were no significant interactions with subgroup or effects that differed from the group analysis reported previously.

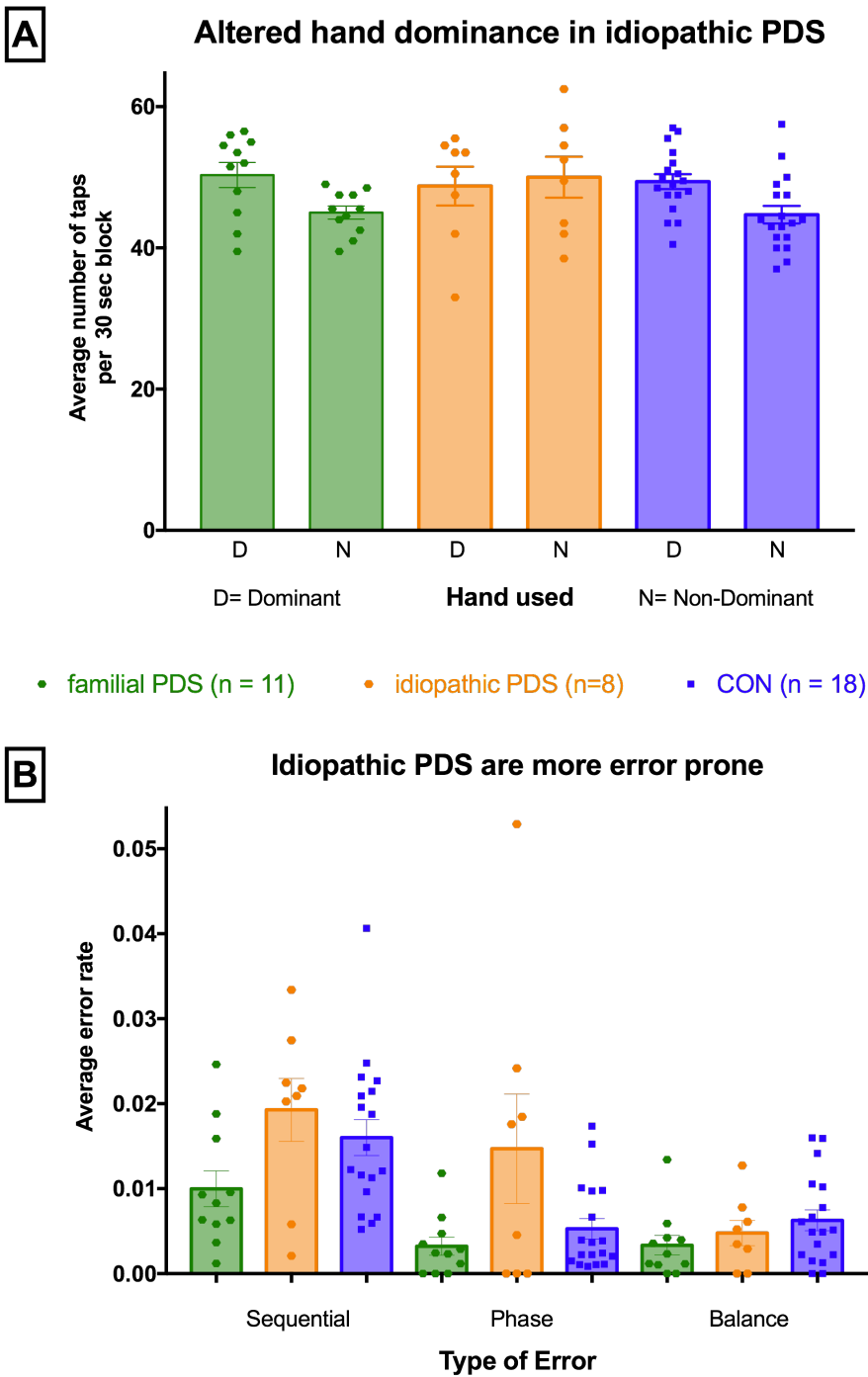
6.4 Discussion

We explored motor system function in a group of adults who stutter and matched fluent controls. We found alterations to specific components of motor learning and evidence supporting different aetiologies in familial and idiopathic stuttering. We did not observe general deficits in motor function or significant relationships between stuttering severity and motor function.

Our results can be summarized as follows:

1. A general trait of stuttering was relatively slowed error reduction during visuomotor adaptation, suggesting an abnormality in the integration of sensory feedback into internal models.
2. We observed slower reinforcement learning reaction times in adults who stutter relative to controls, but we did not observe general slowing in other motor tasks. This general trait suggests that slowed response times in stuttering are task-dependent.

Figure 6.10: Stuttering subgroup tapping performance



A. The familial PDS subgroup showed the typical dominant hand advantage for tapping, whereas the idiopathic subgroup did not. The CON group is shown for reference. B. The Idiopathic subgroup had larger error rates than the familial subgroup across all tapping tasks. This was particularly evident in cases of making the wrong movement in a sequence (Sequential), or making in-phase movements during the out-phase task (Phase) but was not for other error types, including moving out of sync in the bimanual conditions (Balance). The CON group is shown for reference.

3. In two tasks we observed what might be considered improved performance in our stuttering group: faster reaction times during the final training bins of implicit sequence learning, and decreased variability in movement timing during bimanual tapping. These general traits could suggest motivation to perform better on behalf of the stuttering group.
4. Within adults who stutter, we created two subgroups based on family history. Our subgroups not differ in cognitive function, anxiety, or stuttering severity but performed significantly differently on tasks thought to tap basal ganglia function:
 - (a) A familial stuttering subgroup showed impaired reinforcement learning performance when an optimal strategy required inhibiting a response in order to avoid punishment. The dissociation between familial and idiopathic subgroups on use of this test strategy was remarkably similar to differences observed in Parkinson’s patients on and off medication, respectively [96].
 - (b) Our idiopathic subgroup made proportionally more errors throughout a uni and bi-manual task requiring sequential movement execution under time pressure. In particular, the idiopathic subgroup had more difficulty maintaining the required sequence than did our familial subgroup and was more likely to revert to in-phase hand positions during an out of phase task.

6.4.1 Theories addressed by altered sensory feedback use

Our primary research finding was that adults who stutter exhibit a greater initial sensitivity to perturbation and slower error reduction during visuomotor adaptation relative to fluent controls. A general trait of stuttering is therefore an altered use of sensory feedback to update internal models, which is consistent with theories of altered sensory integration in stuttering [160]. Ours is not the first study to report visuomotor tracking differences in stuttering [136], however, our paradigm allowed us to determine that the adaptation phase of motor learning was specifically disrupted. In the baseline, retention, and wash-out phases, performance by people who stutter matched that of controls. Our results therefore do not support the view that stuttering is an overreliance on feedback [160] which would maintain the observed deficit in stuttering into the retention phase. Nor do our results suggest that sensory feedback use is compensatory for limited speech motor skills [171], as sensory feedback use was disrupted, while a variety of other motor skills were intact. Our finding suggests impaired early motor learning in response to

changes in sensory feedback, which supports a specific deficit in the integration of sensorimotor information into internal representations.

The cerebellum is thought to facilitate successful integration of sensory feedback into internal models necessary for movement [32] through error detection and correction [78]. This process involves updating locally stored internal sensorimotor maps [198], forming predictive models for sensorimotor information [230], and dynamic processing of relevant trajectory information [182]. Cerebellar involvement in visuomotor adaptation in particular is well supported by evidence that cerebellar lesions result in uncoordinated movements that completely disrupt adaptation [236]. The use of transcranial magnetic stimulation (TMS) on the lateral cerebellum disrupts visuomotor adaptation performance [166], and the cerebellum responds to visuomotor adaptation paradigms in studies using fMRI [120] in particular lobule IV, which is activated in fMRI and PET imaging studies of this task [29]. These same cerebellar regions are consistently overactivated during speech tasks in stuttering [40][28][42] and are responsive to treatment [152][231]. Finally cerebellar white matter is related to visuomotor adaptation performance [69] including the superior cerebellar peduncle, which is also disorganized in stuttering [59](See Chapter 2).

In the adaptation phase, we observed increased sensitivity to perturbation in stuttering that mirrors effects seen in cerebellar patients [236]. In this way, we observe results consistent with cerebellar dysfunction in a developmental disorder in which sensory feedback influences speech fluency. In contrast to the continued disruption typical of cerebellar patients on this task, our stuttering group reduced error at a rate similar to controls, and retained gains after a delay. This learning was evidenced by identical performance by our groups during the wash-out phase of this task. Though the initial response to perturbation is reminiscent of the sensory deficit associated with cerebellar lesions, the learning pattern in particular is also similar to that seen in patients with Parkinson’s disease off medication [164]. The critical difference between our study and the performance of Parkinson’s patients on this task is that our stuttering group was eventually able to recover from the initial oversensitivity and learn to the same extent as fluent controls, whereas the Parkinson’s group learned at a similar rate but did not recover to the same extent as the control group in that study [164]. The striatum is involved in early-motor learning [78] and adjusting to environmental changes through its connections with the motor cortex and cerebellum that are thought to facilitate dynamic updating of predictive models [182]. Overall our findings suggest an initial oversensitivity to, but eventual ability to adjust to environmental changes that is not indicative of structural damage to the cerebellum or dopaminergic degeneration, but is instead suggestive of more subtle abnormalities in these subcortical circuits.

6.4.2 Theories addressed by slower feedback learning

Movement times were slower in a self-paced reinforcement learning task in stuttering, which could support theories that cognitive load interacts with limited motor skills in this population [245][171]. However, adults who stutter did not show slowing during the perturbation phases of visuomotor adaptation, specifically, nor did they respond differently to increased task demands in the uni- and bi-manual tapping tasks relative to our control group. In this way, the reinforcement learning task, which is thought to rely on dopaminergic function to extract probabilities from experience and later to transfer predictive knowledge to new contexts [60], presents a specific challenge leading to slowed reaction time, but equivalent accuracy for adults who stutter relative to controls. Specifically, striatal dopamine is thought to modulate reinforcement learning through a balance of the two independent pathways, reliant on D1 and D2 signaling to respectively execute (“GO”) and inhibit (“NOGO”) actions. The finding of slowed performance in this task could reflect that traditional speed/accuracy trade-off in decision making: in order to perform well, people who stutter had to take more time on this task.

On the other hand, this result represents a slowing in the use of feedback to update predictive models in stuttering and is consistent with our visuomotor adaptation result. Learning gains from feedback are the same in stuttering, given enough time. Computational simulations of stuttering corroborate two mechanisms that could slow feedback learning: 1) dopamine imbalance, which causes a delay in activating the direct pathway; or 2) white matter damage that would induce a transmission error and thus delay indirect pathway signals [58]. Both the direct and indirect pathways are used in this reinforcement learning task, but the mechanism of disruption is a delay, and not a total loss in signal, so eventually people who stutter are able to make use of the feedback. The general slowing across this task regardless of demands supports a view combining dopaminergic imbalance [260][4] with a “disconnection syndrome” hypothesis [220]. Further, the disruption to the feedback learning system somewhat argues against theories that suggest feedback is compensatory in stuttering [160].

The reinforcement learning paradigm was selected because it allowed us to address the dopamine hypothesis of stuttering. Many variations of this hypothesis have been offered, from “abnormal elevations of cerebral dopamine” (p 92, [157]); to altered striatal metabolism specifically [260]; or rather an imbalance in the basal ganglia-cortical circuit [4] that interferes with internal timing mechanisms [84]. Speech related activity in the dopaminergic system shows both positive and negative correlations with stuttering severity measures [100][123]. Further, increased recruitment of some subcortical structures (globus pallidus) are associated with fluent speech in stuttering [28] and abnormal fMRI activation in the putamen and caudate nucleus show normalization with therapy [231]. Still, we did not find

any evidence of systematic imbalance in this system in all people who stutter. Instead, within our stuttering group we observed evidence of imbalance in two subgroups and the finding suggests the direction of imbalance in dopamine was related to family history of stuttering. We do not wish to overspeculate, given our very small sample size in our subgroup analyses, however, we think these findings should be considered suggestive for directions that merit pursuit in larger samples.

6.4.3 Theories addressed by differences between subgroups

In our two self-paced tasks we observed evidence that merits future research distinguishing between familial and idiopathic subgroups. Our findings tentatively indicate these subgroups might differ in aetiology related to dissociable subcortical deficits, which is somewhat consistent with theorized subtypes of stuttering [4]. First, we observed impaired test performance in familial relative to idiopathic stuttering in a reinforcement learning paradigm, suggesting a disruption to the dopaminergic system, most likely localized to the striatum. Specifically, the performance of familial and idiopathic subgroups on using the optimal test strategy to avoid punishment was similar to previous reports of Parkinson’s patients’ performance on and off medication, respectively [96]. The implication of this difference is that the impaired “NoGo” learning observed in our familial stuttering subgroup is suggestive of excessive dopamine in the system, whereas the enhanced performance of our idiopathic group in avoiding punishment could indicate reduced dopamine levels [60]. The inability to avoid punishment in familial stuttering suggests either an abundance of extracellular D1, which would saturate existing receptors leading to dominance of the direct pathway, or a reduction of D2, eliminating the indirect pathway’s ability to inhibit behaviours. Such an imbalance of striatal dopamine could result in a delay in reaching necessary thresholds to control movements, which could theoretically underly stuttering symptoms [4].

In the uni- and bi-manual tapping task, we observed two alterations to typical performance that were specific to the idiopathic stuttering subgroup. The first finding was an increased error rate in the idiopathic subgroup. Though the error rates were calculated across all tapping tasks, the differences between subgroups were exaggerated specifically for errors that involved making the wrong movement in a sequence. The other errors made by the idiopathic subgroup involved making the wrong movement with only one hand during the bimanual tapping task, which is most frequently in the form of making an in-phase movement when the task is to coordinate out of phase movements. Both of these errors could reflect a “loss of set” or impulsivity behaviour that is consistent with neuropsychological effects of damage to the dopaminergic system. We feel it important to stress that potential for differences in the overall functioning of the dopaminergic system may not necessitate a neurochemical origin. In other words, fluctuations in dopamine could

be achieved through a neuroanatomical or structural anomaly in connectivity that interrupted flow or interfered with receptor function and signal transmission. For this reason we wish not to overspeculate and do not mean to equate “damage to the dopaminergic system” with a neurochemical imbalance or structural difference that is specific to the dopaminergic system. In other words, though we have evidence to suggest a disruption to this system, we make no claims that the cause lies with dopamine levels nor that it has no impact on neurotransmission more generally.

The second finding was an absence of the typical increase in speed associated with use of the dominant relative to nondominant hand. This effect is unlikely to be driven by hand usage, as both our subgroups had a single sinistral individual and two individuals with familial sinistrality. It is possible that idiopathic stuttering reflects a part of the population with incomplete or altered cerebral dominance, which was a long-held theory of stuttering generally [232], but aside from a single case study [70], evidence for this interpretation is lacking. At the very least, the observed difference in lateralization for rapid tapping in our study should encourage more research using these two subgroups.

While most attempts to subgroup stuttering have used symptom-based approaches that do not translate to reliable differences in the laboratory [242], separating familial from idiopathic subgroups may be more useful. These subgroups have the potential for increasing our understanding of the underpinnings of stuttering, because they allow us to resolve potential heterogeneity in the population. It is unclear the degree to which each subgroup, however, is heterogeneous, and this area deserves more investigation with much larger samples. In addition to the differences in underlying aetiology based on heritable risks, we observed potentially dissociable behavioural profiles, which make the distinction critical for future studies. While both subgroups showed disrupted visuomotor adaptation when compared to fluent controls, differences between subgroups on other tasks provide two potential pathways to that disruption. In familial stuttering, the ability to use negative feedback, such as an obvious error, to correct decisions in the future is impaired. In idiopathic stuttering, however, this ability is enhanced when the feedback is verbal and explicit, however when feedback must be extracted from the environment, this subgroup is error prone. In other words, the familial subgroup has difficulty applying feedback, and the idiopathic subgroup has difficulty detecting it. Though speculative and based on small samples, the distinction should warrant more research.

6.4.4 Theories addressed by potential motor strengths

We observed several surprising results during sequential tapping tasks, including faster reaction times during the final training stage of in implicit sequence learning and lower variability in bimanually coordinated tapping in adults who stutter

relative to fluent controls. Specifically, during our implicit learning task, our stuttering group showed unexpected improvements during initial baseline training that was not observed in the fluent controls and faster reaction time during the final portion of sequential training relative to controls. These strengths provide compelling evidence against a selective deficit in the premotor-basal ganglia circuit simulated by computational models of stuttering [58]. Deficits in this circuit would affect sequential action execution, specifically, and we did not observe deficits in either sequential tapping or sequence learning in our stuttering groups. Our inability to detect deficits in planning and execution behaviours is not wholly in accord with the EXPLAN theory of stuttering [114]. The lack of impairment on implicit sequence learning further argues against a specific dysfunction in higher-order motor circuits in stuttering [173]. Performance on the implicit sequence learning task in stuttering was also not suggestive of deficits observed following widespread cerebellar damage or bilateral reductions in dopaminergic distribution to the striatum [77]. However, these findings could indicate a benefit of external timing that is specific to stuttering, which would be consistent with several causal theories of this disorder [4] [84].

Unlike previous work indicating an increased variability in movement timing during bimanual coordination to an external stimulus (metronome), which was reported in stuttering children [179] and adults [269], we observed reduced variability in self-paced bimanual movement timing in our stuttering group. Specifically, in coordinated bimanual tapping both in-phase as well as out of phase, the variability of intertap interval was significantly lower in our stuttering group than in fluent controls (Though this appeared to be driven primarily by out of phase movements). In these same tapping tasks, as well as during implicit sequence learning, we failed to replicate previous claims of generally slower reaction times in people who stutter [217]. Indeed, the general trend of faster reaction times in our implicit sequence learning task in our stuttering group sets up a potential strength in overall movement timing in stuttering: self-paced coordination is less variable, and external pacing facilitates learning. Whether this distinction exists in fluent controls is unclear, and in general should be examined in future research to better understand the mechanism of benefit in external pacing for speech [231]. Our findings in implicit sequence learning suggest a potential benefit from computer-pacing that does not enhance gains in speed in controls and is worth pursuing in further studies. Our findings overall do not support theories of general slowed internal timing mechanisms, or underlying deficits that lead to significant movement delays, or a higher-level permanent disconnection impacting motor signals. We cannot discount more nuanced theories of unstable internal timing mechanisms in stuttering, as would result from imbalance in the dopaminergic system [4].

6.4.5 Limitations and future directions

The battery we administered had many possibilities. We focused on only a few measures within each task that tapped the functions in which we were most interested. Of particular interest is to see whether the tighter intertrial variability observed during the bimanual finger tapping blocks in our stuttering group extends to movements made during other tasks, particularly computer-paced tasks. Though our stuttering group did not differ in tapping errors, we are not able to answer questions about whether they differed in the specific types of errors made across the three learning tasks. An in-depth examination of the types of learning errors made could provide avenues for future research. Further, our stuttering subgroups appear to reflect different behavioural profiles, in particular the idiopathic group was more prone to errors in our tapping task. A more comprehensive examination of errors on the other tasks, as well as separating tapping errors according to blocks is planned for the future to aide with interpretation and determine how generalizable the proclivity to errors is in this subgroup. As mentioned before, because of the limited sample sizes in this study we must be careful not to overspeculate on findings or exhaust the data through multiple comparisons.

Overall, our work supports the view that specific motor learning skills are altered in stuttering, not general skills related to movement execution that would reflect a general deficit in motor function, limited motor ability, gross slowing of reaction time, or widespread deficits in internal timing mechanisms. Specifically, our primary finding is of impairment in early motor learning, *i.e.* adaptation to changes in sensory feedback, which suggests a deficit in the integration of sensorimotor information in stuttering [160]. Further, within stuttering we identified empirical support for using family history as a way to subgroup stuttering samples in the future. Familial and idiopathic groups show dissociable performance on tasks that indicate imbalance in the dopaminergic system, as has been theorized to cause stuttered speech states [4].

Table 6.4: Summary of family and stuttering measures for individuals in our PDS group.

PDS ID	Age in years	sex	handedness	Sinistrality	Stuttering	Familial information			Age of onset	Self-rate	Stuttering severity			
						Recovered	Relative Gender	Affected relatives (recovered)			SSI-IV Score	SSI-IV label	OASES	BAI
1	20	M	R	-	X	X	M	father	0 - 3 years	mild	20	mild	mild/moderate	-
2	27	M	R	-	X	-	M	maternal and paternal grandfathers	0 - 3 years	moderate	21	mild	mild/moderate	-
3	33	M	R	X	X	X	M	maternal uncle	3 - 6 years	moderate	30	moderate	moderate/severe	mild
4	44	M	R	-	X	X	M	father	3 - 6 years	mild	29	moderate	moderate	moderate
5	21	M	R	-	X	-	MF	younger sister, maternal uncle, cousin	3 - 6 years	moderate	39	very severe	moderate/severe	severe
6	25	M	R	-	X	X	M	father	6 - 9 years	mild	27	moderate	moderate	moderate
7	22	M	R	X	X	X	MF	older brother and mother	6 - 9 years	moderate	36	severe	mild/moderate	-
8	20	F	R	-	X	-	MF	maternal half-siblings, great uncle	6 - 9 years	moderate	43	very severe	moderate	-
9	39	M	L	-	X	-	M	twin brother	9+ years	mild	24	mild	moderate	mild
10	39	M	R	-	X	X	MF	2 younger siblings, maternal aunt	don't know	severe	46	very severe	moderate	-
11	35	M	R	-	X	-	M	paternal uncle	don't know	moderate	36	severe	moderate	moderate
12	23	F	R	-	-	-	-	maternal 1st cousin	0 - 3 years	mild	30	moderate	mild/moderate	-
13	30	F	R	-	-	-	-	-	3 - 6 years	moderate	32	severe	mild/moderate	-
14	31	M	R	-	-	-	-	-	3 - 6 years	mild/moderate	15	very mild	moderate	moderate
15	35	M	R	-	-	-	-	-	3 - 6 years	mild	28	moderate / severe	moderate/severe	-
16	37	M	R	-	-	-	-	-	3 - 6 years	mild	25	moderate	moderate	mild
17	22	M	R	-	-	-	-	-	6 - 9 years	mild	12	very mild	moderate	-
18	27	M	L	X	-	-	-	-	6 - 9 years	severe	37	very severe	moderate/severe	mild
19	35	M	R	X	-	-	-	-	6 - 9 years	mild	21	mild	moderate	moderate

M=Male, F=Female, MF=male and female. R= right handed, L=left handed, X = meeting criteria for (sinistrality, familial stuttering, or familial stuttering with recovered relatives (Recovered)).

Chapter 7

Discussion

Summary of main findings: The aim of this thesis was to identify the neurobiological underpinnings of persistent developmental stuttering (PDS). Across five studies using different neuroimaging and neuropsychological methods, we found evidence that reflects disruptions to the subcortical system, in particular the basal ganglia and cerebellum. In comparisons between fluent controls and PDS, which isolated general traits of the disorder, the cerebellar circuits showed evidence of disruption in all studies with one exception: contrasts that were specific to fluent speech-related activation. Altered cerebellar function in stuttering provides a potential alternate mechanism supporting the theory of abnormal sensorimotor integration in this population [160]. We also identified neural correlates of specific stuttering states and differences between subgroups of people who stutter that collectively support a view of altered basal ganglia function in stuttering [4].

Overall our findings suggest that disruptions which influence specific stuttering states may differ from those associated with general traits of the disorder. Across studies our examination of variability within stuttering generally suggests the need for better efforts to identify sources of heterogeneity in future samples within this population. The confirmation of simultaneous basal ganglia and cerebellar abnormalities observed across studies in this work supports the view that reciprocal interactions between these subcortical structures is part of a larger integrated system subserving a variety of functions [45]. Further, we might conclude that subcortical systems have a critical role in speech production beyond gross motor coordination, most likely involving the timely integration of sensory information into internal models [106].

7.1 General trait effects in stuttering

The key findings with respect to general traits that distinguish people who stutter from fluent controls can be loosely categorised into two types of results: 1) Replications of widespread, but subtle disruptions, to brain microstructure and functional activation throughout the speech-motor system; and 2) Focal cerebellar disruption across multiple methods.

7.1.1 Disruptions to cerebral white matter

In this thesis (see Chapter 2) we replicated findings of widespread disorganisation in white matter pathways underlying speech and motor cortex in stuttering [249] that occurred predominantly bilaterally in all four cerebral lobes. To test specific hypotheses about major speech and motor pathways in stuttering, we used fibre tracking and confirmed disruption to major speech/language pathways that coordinate integration of speech sequence plans with speech content. disorganisation of these pathways is consistently reported in microstructural research in stuttering [173]. Disruptions in the motor pathways, however, were left-lateralised and further, within the posterior limb of the internal capsule, appeared to be specific to the fibres that subserve mouth-motor function via delivery of motor plans to the musculature through corticobulbar tracts.

Our findings of disruption to left lateralised descending motor fibres supports the “disconnection syndrome” hypothesis [220], which suggests that a neuroanatomical disconnection delays or disrupts delivery of higher-order motor plans necessary for speech and the result is stuttering. In our work, however, those disruptions did not occur in isolation, and were part of a much more distributed disorganisation in stuttering. The majority of disruptions occurred primarily bilaterally and many symmetrically, which may reflect reorganisation rather than causal disruption [249]. Widespread, bilaterally occurring disruptions are somewhat consistent with the hypothesis that disrupted myelination occurs shortly after birth in stuttering [64] because typically injury and disease- associated lesions are unilaterally, randomly, or less symmetrically distributed. We therefore cautiously interpret our findings as reflecting developmental origins, however in order to determine cause and effect, longitudinal studies of stuttering populations are necessary.

7.1.2 Disruptions to cerebellar pathways

We manually masked the middle of all three pairs of major pathways leading in and out of the cerebellum and found that these, as well, showed relative disorganisation in our stuttering sample compared to controls. Further, microstructure of the inferior cerebellar peduncle becomes more organised with age in stuttering, but

is unchanged with age in fluent controls. The inferior cerebellar peduncles carry sensory information from the body into the cerebellum to be integrated into internal models and therefore have potential relevance to the theory of altered sensorimotor integration in stuttering [160]. The observation of age-related changes in cerebellar pathways that are specific to stuttering further supports a need for longitudinal studies of brain development in this population. Overall, these disruptions could theoretically impact a variety of cerebellar functions and future investigations should attempt to replicate these critical findings, examine them longitudinally, and determine their relationships with age and speech fluency in different subgroups of people who stutter.

The methods selected in Chapter 2 were designed to first replicate previous findings using a conservative, whole-brain approach, and then extend previous work by testing hypotheses through the isolation of specific white matter pathways using tractography or manual masking. Ours was the first paper to take this tract-specific approach in stuttering. Rather than using tractography, which is a widely accepted tool for diffusion imaging data, when assessing the microstructure of the cerebellar peduncles, we elected to use a manual approach to defining only the centre of these peduncles. This method has been used previously [139] but is somewhat limiting because it does not allow examination of the branching before or after the peduncles and does not facilitate a streamline count that can be informative with regards to pathway strength. Part of this decision was made because of the confounds of the large voxel size (2.5 mm^3) in our study, and therefore a limit to the validity of identifying appropriate waypoints for the cerebellar peduncles, which have been successfully tracked at better spatial resolution than our study in normal controls [238].

In addition, both the superior and inferior cerebellar peduncles are relatively slender, and spatial averaging across participants would presumably result in a loss of sensitivity and certainty in identifying these structures, specifically. We felt a manual approach was needed. The other major factor in this decision was the uncertainty of automated methods in the presence of disorganisation: because we assumed cerebellar pathways would be disrupted, given previous findings [249], we could not be confident those pathways would still be intact enough to propagate the necessary streamlines for tractography. Finally, isolation of the centre of cerebellar peduncles through manual masking allows us to distinguish between effects observed within the peduncles, as we have described, versus differences in tracts that could actually be localised on either side of the peduncle itself, in the major decussation of motor fibres, for example, of the basal pons, or in reference to spinothalamic tracts and other major pathways that are located near the inferior cerebellar peduncles. Our selected approach was conservative and limited but is nonetheless replicable in other samples with smaller voxel size.

7.1.3 Replications and extensions of functional disruptions

Our key functional magnetic resonance imaging (fMRI) findings support the view that people who stutter use the same networks, to a similar degree, during speech as do fluent controls [123]. We further replicated increased recruitment of typical speech motor regions in response to increased planning task demands, which was previously shown in a similar experimental design using positron emission tomography (PET) imaging [123]. Specifically, in our study adults who stutter showed relatively decreased activation in the left language cortices and cerebellum relative to controls during reading, but increased activation particularly in premotor cortices and basal ganglia relative to controls during a picture description task. The contrast of these two tasks resulted in an increased activation in the left inferior frontal gyrus extending into ventral premotor cortex, the left preSMA, and the left dorsal striatum including caudate nucleus and putamen for picture description in stuttering relative to fluent controls, whereas fluent controls showed increased activation relative to stuttering individuals only within the left lobule VI of the cerebellum. Overall, we were not able to replicate classic meta-analytic findings in stuttering that identified “neural signatures” thought to exist regardless of task demands or specific states of fluency [40]. We instead observed evidence that the networks utilised for speech in stuttering and fluent adults primarily overlap, and subtle differences between groups are tightly linked to task demands.

Further, when we isolated activation underlying fluent speech in both groups, we did not observe widespread reductions in activation in PDS [249], but instead observed subtle but widespread increases throughout the typical speech network, including left-lateralised inferior frontal cortex, extending into mouth motor cortex, the left preSMA, and the left putamen during picture description. Our work suggests that this increased motor activity is associated with fluent speech, which is counter to theory that overactivation of motor cortex in stuttering is a disruptive mechanism that excessively inhibits auditory cortex activation [40]. Instead, this increased recruitment of motor regions could indicate compensatory rewiring to local cortex as a result of focal disruptions underlying central opercular cortex [220] or increased recruitment of these regions to compensate for weaker signals overall, caused by widespread white matter disruptions [249]. During sentence reading we observed superior temporal increases in PDS and decreases in inferior frontal gyrus near Broca’s area occurring bilaterally. This finding suggests that future research examine task-related differences in activation patterns between people who stutter and fluent controls.

During scans taken at rest, we also observed alterations in cortical connectivity within several networks and regions that replicated previous reports. Specifically, lateral and medial premotor cortices [152][263], inferior medial frontal cortex [263], precuneus cortex[263], central opercular cortex [152], and right-lateralised auditory

cortex [210][55], all showed increased connectivity within respective networks in stuttering. Within a resting state network that encompassed the entire cerebellum, we also replicated findings of altered connectivity in stuttering [210][267][152], as well as both positive and negative correlations to stuttering severity within the cerebellum [267]. For the most part, many of these regions have been implicated at rest in stuttering previously, though ours is the first work to implicate all of these regions within a single population. Our choice of data-driven methods provides an alternative to the traditional selection of a specific “functional network” based on spatial distribution of correlated activity at rest. Instead we simply examined group differences within each of the networks that resulted from a model-free independent component analysis of the group data. This approach resulted in a consensus and replication of the major findings of several studies, which do not replicate one another [123][152][263][55][51][210][267]. An obvious limitation of our work is that we did not investigate the degree to which signal within each of these networks at rest changes in response to task demands, and this is an important area of future research.

7.1.4 Focal behavioural disruptions

In spite of the widespread disorganisation of pathways throughout the brain and altered activation during rest, we found no evidence of general motor deficits that one would expect to coincide with structural and functional anomalies in stuttering [172]. Furthermore, we did not replicate previous reports of specific impairments/deficits in non-speech behaviour, including general slowing of reaction time [215], difficulty with motor sequence planning [113], or variability in movement timing [269]. Our failure to replicate these effects during an extensive motor battery argues against core principles in several theories of stuttering. One theory in particular predicted that movement durations would be longer in stuttering due to a disruption in integration of sensory processing and motor planning [160]. Though we did not observe longer movement durations, we obtained direct evidence of altered sensorimotor integration in the form of slower error reduction during visuomotor adaptation, which is consistent with the central theme of this theory [160][247].

We selected the tasks for our behavioural chapter based on specific neural correlates associated with a long history of research using these classical paradigms. As one would in a registered report, we also therefore designed our models as replications of the way these data are analysed in the literature in order to maximise comparability to previous reports. As such, we did not necessarily ask the same questions that other stuttering researchers asked, nor were we specifically trying to fish for a motor deficit. We used the battery to address several theories at once. It is likely we missed “statistically significant” findings because our focus was so

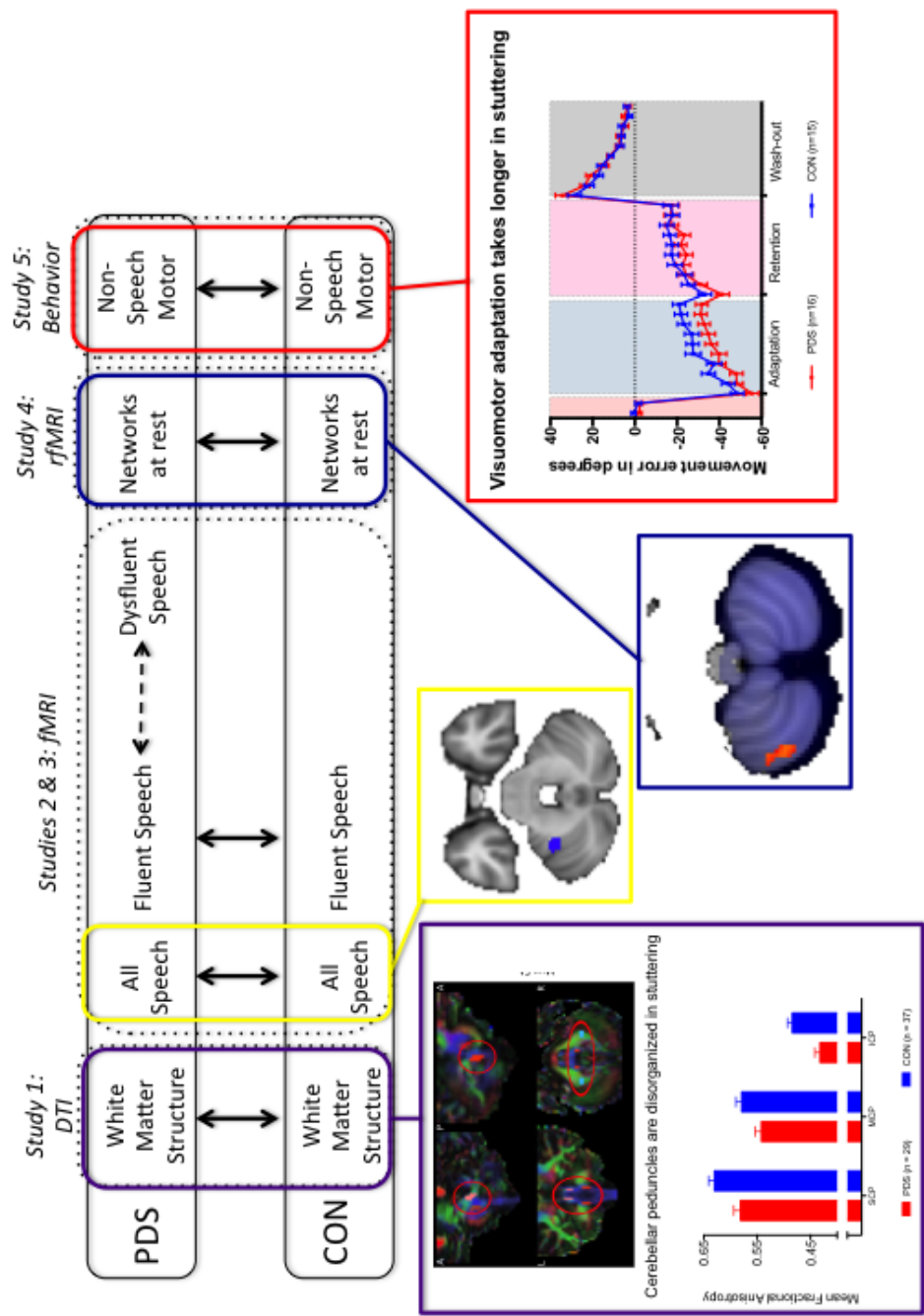
specific in each task. The dataset therefore provides an opportunity for further exploratory analyses to generate new hypotheses.

For example, we have a bulk of data reflecting the type of errors made by our participants in each study. Though we focused our analyses on correct responses, as do classic models of these tasks, these error data could be interesting for future research. We have not investigated, in depth, the types of errors made or whether they differed between groups. It is possible that, for example, during sequence learning, people who stuttered would make errors on the initial movement of a new sequence, even when ignorant of the sequence. It is also possible that people who stutter, who tended to show faster reaction times, also make more errors in this task. Further, they might make errors in a specific position, for example, following first repetition of the initial movement, in the form of restarting the sequence rather than continuing in the “middle”. Because stuttering symptoms typically involve production of the “correct” sound over and over, or no sound at all, we felt an examination of incorrect movements was of lower priority. It is certainly a future use of these data that would contribute to the literature on learning in stuttering. Overall, the only behavioural trait that distinguished between stuttering and fluent controls reflected altered integration of sensory information with internal motor plans, in the form of a delay in error reduction during visuomotor adaptation, which is thought to be subserved by the cerebellum [167][29].

7.1.5 Cerebellar disruption across studies

Across all of these studies a clear picture emerged: the cerebellum is disrupted in stuttering (Figure 7.1). We observed microstructural disorganisation in all of the major input and output pathways of the cerebellum. We observed decreased activation in the left motor region of the anterior lobe of the cerebellum (lobule VI) in stuttering relative to fluent controls during increased task demands of picture description relative to sentence reading. We also observed decreased activation of the cerebellar crus during each condition separately (left hemisphere during picture description and right hemisphere during sentence reading) in stuttering relative to fluent controls. We did not observe differences in cerebellar activation during fluent speech generally. However, we did observe differences in cerebellar recruitment within subgroups of PDS who differed in the frequency of dysfluent speech in the scanner. Specifically, during two different speaking conditions the more dysfluent subgroup showed decreased recruitment of left medial cerebellum (lobule VI) extending into the cerebellar vermis. We are unable to speculate whether this difference reflects compensatory overactivation in the more fluent subgroup or disruptive underactivation in the more dysfluent subgroup.

Figure 7.1: Summary of cerebellar trait effects observed in this thesis



The cerebellum shows disruptions in persistent developmental stuttering (PDS) relative to fluent controls in 1) Purple box: white matter microstructure of the peduncles which contain the input and output pathways of the cerebellum; 2) Green box: Reduced activation relative to controls in contrasts between spontaneous speech and reading tasks, whereas cortical areas showed increased recruitment in PDS; 3) Blue box: Within a cerebellar resting state network we observed increased coupling in left lateral posterior cerebellum in stuttering relative to fluent controls during eyes-open rest; and 4) Red box: Visuomotor adaptation was disrupted in stuttering, in that it took longer to reduce errors to the same amount as controls, however the stuttering group was eventually able to reduce errors to the same extent and showed similar learning overall. For all brain images, left is left.

We also observed increased connectivity of the left lateral cerebellum (crus II) in stuttering at rest within a network of cerebellar activation, as well as significant correlations with stuttering severity and brain activation at rest in the right cerebellar crus I and left cerebellar crus II that were positive and negative, respectively. Finally, we observed non-speech behavioural disruption that was specific to error reduction during visuomotor adaptation, which is dependent on the cerebellum [167][29]. Overall, we observed trait effects in the cerebellum using each of four different methods and found that speech-related activation of lobule VI was related to stuttering frequency in the scanner, whereas rest-related activation of the cerebellar crus (I & II) was related to stuttering severity outside of the scanner.

Though we observed disruptions in other brain regions in each of these studies in stuttering, the cerebellum was the only structure that was consistently implicated in each and every study. Altered cerebellar function has been previously demonstrated in stuttering [67], yet the convention is to interpret changes in this region as compensatory (e.g. [40][210]). Our findings are consistent with original theories of cerebellar contributions to stuttering [67] and provide evidence for a subcortical underpinning to the theory of altered sensory integration in persistent developmental stuttering that has instead primarily focused on cortical disruptions in stuttering [160]. The theory of altered sensory integration, as originally articulated, garnered support primarily from findings of altered primary sensory cortex activation in the form of decreased auditory cortex recruitment during speech in stuttering [160]. This theory was further supported by evidence that the mechanism of temporary fluency enhancement techniques which change sensory feedback lie in restoring synchrony between primary auditory and motor cortex activity in stuttering [246]. Further, this theory accounted for disruptions to cerebellar activation that had been described in stuttering and posited that the mechanism of increased recruitment was the need for excessive error detection [160]. However, direct tests of predictions made by this theory were inconclusive, as the relative strength of the suppression of auditory cortex activity during speech is no different in stuttering, but instead the timing of this effect is altered [19]. Finally, central mechanisms of the hypothesised altered sensory integration in stuttering would cause longer movement durations in general [160], a prediction which did not hold in our extensive battery of tasks and measurements of movement duration.

7.1.6 What broad cerebellar disruption adds to theories

We do not feel that the view of altered sensory integration should be disregarded, however. We believe the altered activation in primary sensory cortices could be a secondary effect of primary cerebellar disruption in stuttering. Our interpretation is largely based on a combination of the effects we observed across the thesis: 1) structural abnormalities were not observed within the cerebellar lobes but instead

was isolated to the cerebellar peduncles; 2) overt speech is associated with a decrease in cerebellar activation relative to controls; and 3) adults who stutter eventually do integrate information, but take longer to do so than do fluent controls. Combined with the knowledge that people who stutter are able to produce fluent speech in a number of different situations, it appears likely that the actual process of integrating information into internal representations is delayed rather than that “sensory processing” is deficient or the models themselves are disrupted.

Our conclusions are consistent with general clinical findings of the impact of injury in or around the cerebellum. In stuttering, concomitant behaviours are typically considered evidence for disruption to the dopaminergic system because of their similarities to dystonia or tics [4]. Lesions to the cerebellum or its input nuclei can produce a similar host of problems including intention tremor, lack of coordination or difficulty timing voluntary movements [72]. For example, concomitant movements observed during dysfluent speech states include tremor of the mouth or eyelids, which could be considered tremor associated with the intention to speak. Further, tremor of the mouth in particular is similar to what is described clinically as an essential tremor, which is thought to be caused by cerebellar damage [72] [150] and involves the inferior cerebellar peduncles, specifically [139]. We did not observe evidence of a lesion or drastic injury to the peduncles, but instead observed disorganisation that could have similar, but transient, effects in stuttering by temporarily delaying the transfer of signals. Because the frequency range of tremors due to subcortical injury overlap with one another and differ within individuals [183], an in-depth investigation of the frequencies of involuntary tremor in PDS could be a particularly interesting and fruitful area of future research.

The mechanism of disruption in stuttering is also consistent with evidence that the cerebellum coordinates with other regions including parietal cortex to determine the likelihood of movement success in dynamic environments [182]. Specifically, efferent copies of speech motor commands are thought to be processed by the cerebellum in order to predict the sensory consequences of the planned movements [257]. If this is the case, then part of the role of the cerebellum is to determine the likelihood of error-free movement associated with a specific plan. This is consistent with the original theory of altered sensory integration in stuttering which suggests that the cerebellum detects excessive errors in this process [160] and would therefore signal a mismatch to relevant premotor and sensory cortices, according to a leading theory of speech production [106]. However, in this case the problem is not caused by an increased inhibitory efferent copy originating in motor areas, as previously suggested [40], but instead by a delay in integration of information caused by disruptions to pathways leading in and out of the cerebellum. The superior cerebellar peduncles, which relay information to the cortex from the cerebellum have been previously described as a “bottle neck” [181] because of their

narrow proportions. Even subtle disruptions to the organisation of this “bottle neck” could cause significant signal delays similar to traffic jams observed at the entrances to highway tunnels.

An inefficiency in neural traffic flow entering or leaving the cerebellum could be further exacerbated by the presence of multiple copies of sensory expectations for each speech motor plan (dysfluent or fluent). Feedforward cerebellar models are thought to be stored specifically for acquisition context [198], which in the case of stuttering could be either one of two speech states (dysfluent and fluent). Thereby, increased cerebellar activity, caused by spikes in purkinje cell activity in response to unexpected sensory consequences of a movement [198], could theoretically be associated with either speech state. In other words, multiple acquisition contexts (speech states) means any sort of speech can result in unexpected sensory consequences, making altered cerebellar activation a consequence of the presence of multiple speech states in stuttering. The downstream delay in the updating of internal models that would result from having to process effectively twice as much information (one model for each speech state) could also work to maintain stuttering through, in effect, doubling the effort needed for complete integration of sensory and motor information [160]. This “bottle neck” effect supports a generalised disintegration syndrome view of stuttering that would have the consequences we observed throughout this thesis, and could provide the underlying mechanism behind theories of disruption to internal timing cues for portions of speech sequences [4], asynchronous internal timing cues [84], and unstable or insufficiently activated internal models [160] in stuttering.

7.2 Within subject effects in stuttering

In addition to the cerebellar disruptions that largely distinguished people who stutter from fluent controls, within stuttering we observed evidence that the underpinnings of specific states of speech may be linked to a different subcortical control system: the basal ganglia (Figure 7.2). Our examinations within stuttering groups in two studies in particular provided support for involvement of the dopaminergic system [4]. Specifically, we observed differences in basal ganglia activity in direct contrasts of stuttered speech to fluent speech, in which the specific nuclei and directions of these differences changed according to task demand. When contrasting subgroups of PDS based on the frequency of dysfluent speech in the scanner, we observed increased activity in the more fluent group in the basal ganglia occurring bilaterally. Finally, we observed behavioural evidence that supports a hypothesised imbalance in the dopaminergic system thought to differentiate subgroups of people who stutter [4]. For the relevant within PDS comparisons, our sample sizes are small ($n < 10$) in each subgroup, though they are similar to or even exceeded those

used in previous studies [216][70][260][121][262][204][176]. However, we believe the small sample size is a potential limitation, yet our findings are promising and should be considered guidance for replication in larger samples.

7.2.1 Methodological limitations to investigating states

Aside from the small sample sizes, another potential imitation to our work involves how we defined dysfluent states. Because our study design measured activity collectively for an entire speech utterance (typically a sentence), we defined any sentence containing any stuttering as a dysfluent speech state. We did not distinguish among specific types of symptoms occurring within states (blocks vs repetitions, for example), the amount or severity of symptoms within each dysfluent utterance, nor did we distinguish between more or less typical symptoms, which have shown separable neural activation patterns in other work [134]. The symptom-specific hyper and hypo activation within individuals [134] could account for previous reports of white matter structural disorganisation in stuttering that corresponded to abnormalities in BOLD activation differences in both directions [249]. We observed that our state effects were dependent on the task at hand, and if differences within tasks are related to symptoms, that would prove an interesting area for future research.

Our work was also vulnerable to a common limitation in stuttering research: stuttering frequency is variable. Across studies, dysfluent speech in the scanner is rare: most often observed in less than 10% of total trials [28]. Our decision to include longer utterances in an attempt to induce more stuttering was somewhat successful, still, in our sample the average frequency of dysfluent utterances was around 25%, with a vast range (1-79) of number of dysfluent utterances out of the total number of pictures described. Still, we did not model our contrasts according to the number of dysfluent utterances within a sentence, but instead pooled sentences with one stuttered word into the same analysis as those with several. The primary reason for this distinction was one of practicality and power: we had only nine participants who were reliably dysfluent more than ten times per scan session. Only eight of those were also reliably fluent more than ten times per scan session. Obvious future extensions of our work would include more in-depth examination within subjects to focus on multiple versus single stuttering events per utterance, symptom-specific neural activation, or even non-stuttered disfluencies within individuals such as interjections. While these would be interesting extensions, they were beyond the question we sought to answer, which was “what are the shared neurobiological underpinnings of specific states of stuttered speech.”

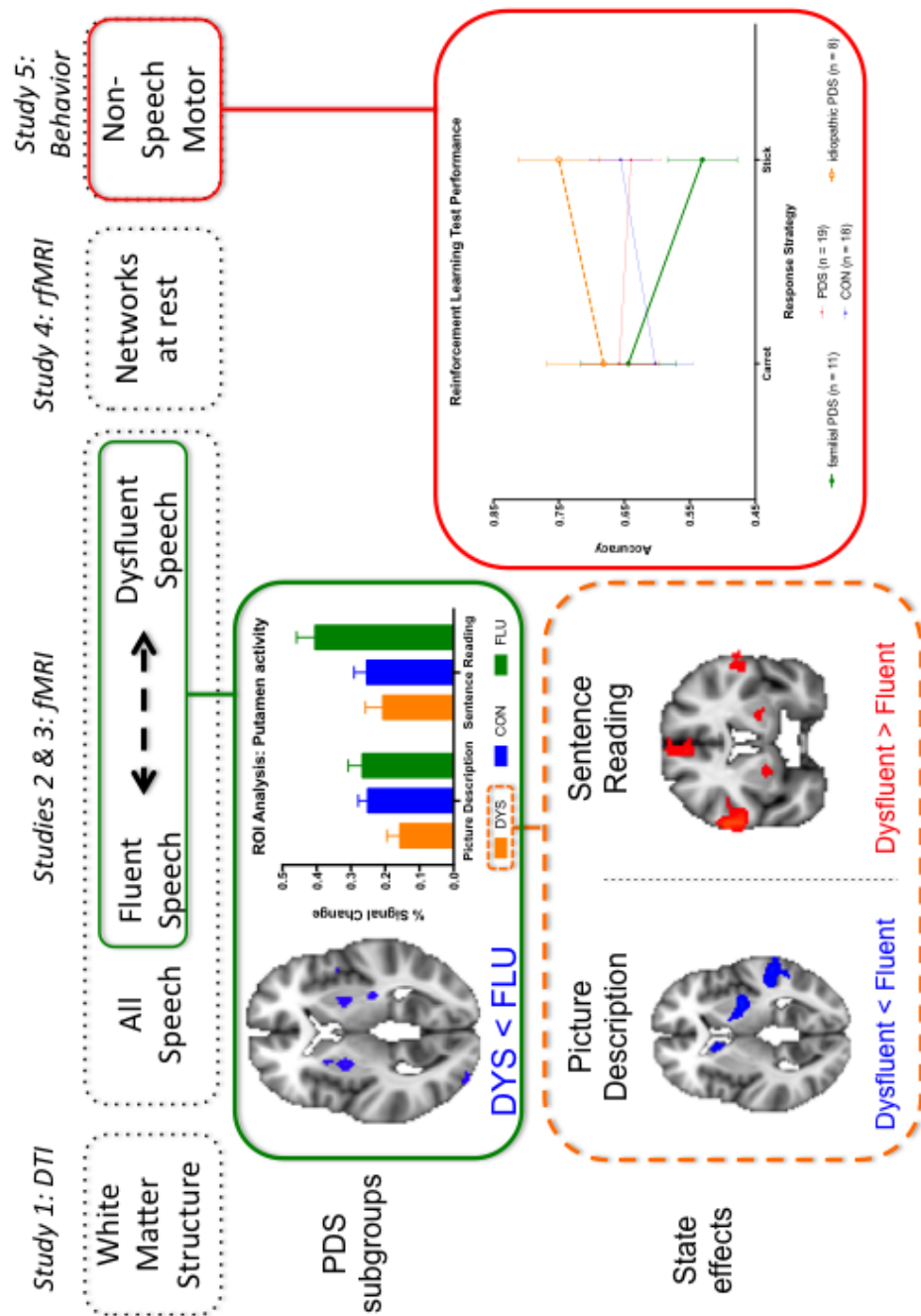
A final limitation to our work could be the temporal resolution of BOLD fMRI, which may not be sufficient to disentangle the bidirectional effects that are hypothesised in stuttering. There is some evidence that magnetoencephalography (MEG), which can measure on the order of a few hundred milliseconds, shows the hypothe-

sised hypo-hyper activation dichotomy within individuals who stutter. A “hyper activation” disruption in adults who stutter is consistent with excess preparatory activation, which extends throughout left frontoparietal cortex including mouth motor representations in PDS relative to fluent controls 100-200 ms after stimulus onset, but before speech production [206]. A “hypo activation” scenario, on the other hand, could easily result from delays or attenuated thresholds caused by white matter “disconnection” [220] to a variety of steps needed for fluent speech:

1. Premotor cortex activity occurs in coordinated bursts and subsides 500 ms prior to speech onset [89]. Corresponding MEG activation is stronger in fluent controls than in PDS [206].
2. Motor cortex activity is sustained prior to speech onset and continues throughout articulation [89]. During speech production, right frontoparietal MEG signal is stronger in fluent controls than PDS, and PDS further show a leftward asymmetry for mouth motor movements that is bilaterally distributed in controls [206].
3. Auditory cortex activity typically onsets suddenly with articulation [89] whereas absent or decreased activation of this region is a consistent trait of stuttering [40].

The interhemispheric imbalance observed using MEG in stuttering is not limited to speech production, but extends to auditory stimulation [207] and speech perception [208]. While in general the MEG findings support a theory of altered sensorimotor integration [160], the bidirectional patterns observed mean a dopaminergic imbalance cannot be ruled out [4], nor can it be addressed, given the limited resolution of MEG methods with reference to subcortical grey matter signal.

Figure 7.2: Summary of basal ganglia state effects observed in this thesis



The basal ganglia were implicated in two studies: Green box: In a comparison of speech-related activity between subgroups within people with persistent developmental stuttering (PDS) who differed according to the frequency of dysfluent speech in the scanner, we observed increased putamen activation for the more fluent subgroup in two speaking conditions. Axial image of brain shows Picture description condition, Left is left. Graph shows percent signal change in an ROI analysis of the putamen, with the control group (CON) for reference. ; 2) Orange box: Within the more dysfluent subgroup we directly compared activation during fluent and dysfluent utterances and observed that fluent utterances showed increased activation of the caudate nucleus in the left hemisphere and putamen/ globus pallidus in the right hemisphere relative to fluent. Left is Left; and 3) Red box: In a reinforcement learning task we observed a dissociation between subgroups of stuttering individuals who differed according to family history of stuttering. Different accuracies for the two subgroups were specific to the performance strategy that involved avoiding negative feedback (the stick) and is thought to be dependent specifically on tonic dopamine.

7.2.2 Theoretical potential of our investigation of states

Given methodological limitations and our small sample sizes, we do not wish to overspeculate. However, we did replicate evidence of basal ganglia disruption in two different methods and two different samples of people who stutter. We therefore are cautiously optimistic about the evidence we found in support of theories of dopaminergic imbalance in stuttering [4]. Critically, our findings do not support dysfunction specific to the putamen-SMA circuit that initiates and sustains sequential movements or a general dysfunction in production of timing cues, as was originally hypothesised [4]. Still, the mechanism that was central to these hypothesised disruptions, an imbalance in levels of different dopaminergic receptors [4], can account for our findings and is directly supported by behavioural evidence of dissociable reinforcement learning in subgroups of people who stutter.

7.2.2.1 State-related differences in basal ganglia activation

Within a small subgroup of individuals ($n=8$) we observed evidence of reductions in speech-related activity for dysfluent relative to fluent picture description within the left hemisphere caudate nucleus and the right hemisphere putamen. The left hemisphere cluster extended into the putamen and overlapped with a cluster in which fluent speech-related activity within the larger stuttering group was also increased relative to controls during picture description. The caudate nucleus, like its neighbouring putamen, provides an inhibitory function within the dopaminergic circuit on the globus pallidus, specifically, and receives projections from the higher order association areas in the cortex. The connectivity of the caudate nucleus within this loop, includes input from dorsolateral prefrontal cortex and output to the same cortex via the thalamus. Our findings suggest that increased recruitment of the caudate nucleus is associated with fluent states within individuals, and differentiates fluent speech from that of controls within speaking conditions that require generation of speech content. This effect could mirror the cognitive control function theorized by findings in bilingualism that the caudate nucleus plays a role in “controlling and selecting” the necessary motor sequences for inhibition/switching commands between languages [61]. The task-specific role of the caudate nucleus in stuttering fluency is an area that should be investigated in future research.

While the caudate nucleus is thought to contribute to higher level cognitive functions that would include cognitive control, monitoring, information manipulation, and allocating attentional resources, the putamen is connected within the more traditional “motor” loop associated with basal ganglia nuclei. We also observed differences in activation of the putamen related to stuttering states, specifically in increased recruitment during dysfluent relative to fluent states in the putamen bilaterally along with the medial premotor cortex and the brainstem at the level of

the basal pons. This is somewhat consistent with the observation that putamen structure and function are related to articulatory “effort” and engaged in a language that is not effectively mastered in multilingual subjects [1]. These effects were specific to a sentence reading condition, in which speech content was provided.

A disruption to the putamen-premotor circuit in particular is core to the dopaminergic imbalance theory of stuttering [4]. Computational models of the circuit disruption in stuttering simulate delays in signals from the putamen, which is responsible for gating the likelihood of success of motor plans, to the SMA, which is critical for initiating actions in a sequence [58]. Previous findings that are consistent with this theory primarily report decreased connectivity between the putamen and SMA [55], however, our findings of increased activation specifically during dysfluent states could indicate neural inefficiency in this circuit. Given that our findings were limited to a small subgroup of PDS ($n=8$) and specific to sentence reading, we believe it would be overly speculative to suggest the putamen-SMA circuit disruption is the core problem in stuttering, particularly given we did not observe thalamic disruption that would complete this loop.

A more general finding implicates the putamen in stuttering states, however, in our contrast of state-related subgroups. The dysfluent subgroup under-recruited the putamen, bilaterally, relative to the more fluent subgroup in both speaking conditions. This finding is consistent with other reports of basal ganglia activity responding to treatment gains [231], and distinguishing between recovered and persistent PDS [117][52]. Further, our study replicates previous findings of significant relationships between basal ganglia activity and the frequency stuttering specifically, (% stuttered syllables, [100][123]). The distinction between frequency of dysfluent states, which is often used as a “severity” metric in research and standard measures of stuttering severity (like the SSI [203] is critical for future work. Our subgroups differed according to frequency of dysfluent speech within the scanner, but not in stuttering severity. Though we do not have a candidate mechanism for the subgroup differences, we can speculate that subcortical involvement (the putamen and lobule VI of the cerebellum, specifically) could reflect the degree to which the scanning environment impacts fluency.

7.2.2.2 Behavioural evidence of dopaminergic imbalance

Finally, we observed direct evidence for hypothesised subgroups of stuttering that differ in dopaminergic balance [4]. Specifically, within small subgroups who performed an extensive motor learning battery, we observed a dissociation on decision accuracy based on negative reinforcement learning that mirrors the effects seen in Parkinson’s patients on and off medication. While original theories suggested these subgroups would differ on primary symptomology and response to medication [4], we instead created subgroups according to heritability. Specifically, we observed

evidence that an idiopathic subgroup of stuttering behaved similarly to Parkinson’s patients off of medication, and were high performers when avoiding punishment. The interpretation of this sort of result on the classic probabilistic selection task is that the idiopathic subgroup could have relatively more tonic dopamine (D2) and a dominant indirect pathway leading to increased inhibition of response [96]. According to the dopamine imbalance theory of stuttering, this would mean idiopathic stuttering are similar to the suggested “subtype II” and would improve with D2 blockers or worsen with stimulants [4]. Our familial subgroup, on the other hand, behaved similarly to Parkinson’s patients on medication, but only when avoiding punishment, which they were relatively poor at doing. This result suggests lower levels of tonic dopamine, which is consistent with “subtype I” who would worsen with dopamine blockers [4]. Given the small sample size we think this result merits future investigation but not further speculation.

Overall, we think it is clear that the basal ganglia and dopaminergic system are involved in specific stuttering states, as well as the relative likelihood of dysfluent speech states. One potential mechanism for this involvement is an imbalance specifically in the D2 system, which is consistent with a long-held theory of stuttering [4]. While the specific circuits implicated in the original theories and predictions of general motor timing deficits were not supported in this thesis, the underlying premise of dopaminergic imbalance is supported. Further, we speculate that one of the limitations contributing to lack of previous empirical support for this theory is a general failure to dissociate fluent from dysfluent speech or meaningful subgroups in stuttering research.

7.3 Variability within stuttering

The importance of identifying sources of variability within stuttering samples is a central theme of this thesis. Overall, we did not observe drastic effects of sex or handedness that would support the conventional approach to stuttering research, which examines only right-handed males. Though this subset makes up the largest “homogeneous” subgroup of stuttering, our work is consistent with reports that the inclusion of females and left handed individuals does not drastically impact crucial findings [122]. This was also the case in our behavioural study where neither handedness, nor familial heritability of sinistrality (left handedness) impacted motor performance across a variety of empirical and control tasks. The one exception to our general conclusion was observed using diffusion imaging, in that the left hemisphere corticospinal tract was disorganised relative to the right hemisphere corticospinal tract in right-handed individuals who stuttered only and this laterality was not observed in left-handed people who stutter. While including left handed individuals and females can introduce noise and variability into a study, the bottom

line is that excluding them limits one's ability to determine the extent to which sex and handedness are tied to the underpinnings of stuttering.

The role of gender in stuttering is part of a larger theory of dopaminergic imbalance in stuttering [4], which is of particular interest to us because we observed behavioural evidence that aetiology of stuttering may differ within subgroups of people who stutter. If so, this would have implications for a long-held theory of dopamine imbalance in stuttering [4]. Coincidentally, familial relative to idiopathic stuttering subgroups tend to have drastically different gender ratios: the number of males to females is up to "3.5 times higher" in idiopathic relative to familial stuttering [7]. We did not have sufficient sample size to address potential gender differences within subgroups. Averaging across these subgroups could obscure potentially meaningful gender effects as well as the theorised bidirectional findings in dopaminergic circuits. We recommend that future research take heritability and gender into account in much larger samples as a way to identify potential interactions between these factors and dopamine balance in stuttering. Further, it is likely that idiopathic stuttering is caused by spontaneous mutation rather than inherited mutations, which could mean this subgroup in particular (and not the more common familial subgroup) holds the key to understanding the genetic influence on stuttering.

We used a more data-driven approach to assessing variability in an fMRI study through first isolating activation related to fluent speech, then examining dysfluent speech states only within subjects who had been fluent at least ten times per scan session. Across our stuttering sample we observed individuals who ranged from having zero disfluencies within a condition of interest to others who spoke dysfluently within every single utterance in that same condition. This variability in individual stuttering frequencies and corresponding activation patterns has led to more focused within subject approaches [262][70][134] and calls into question attempts to average across neuroimaging studies in stuttering using meta-analysis ([40][42][28]).

Across our studies we used the same conventional tool for assessing stuttering severity [203]. This thesis includes data from individuals with very mild to very severe stuttering. Still, overall, relationships to stuttering severity varied from study to study. We did not observe any consistent effects suggesting stuttering severity is related to either trait or state effects observed. There were no significant relationships between stuttering severity and average signal in the putamen, though this region was related specifically to frequency of dysfluent states in the scanner. We suggest that future research assess the factors which compose stuttering severity measures to determine what processes are actually being represented by this metric, and whether they represent generalised or rather reading-related severity, as has been suggested before [5].

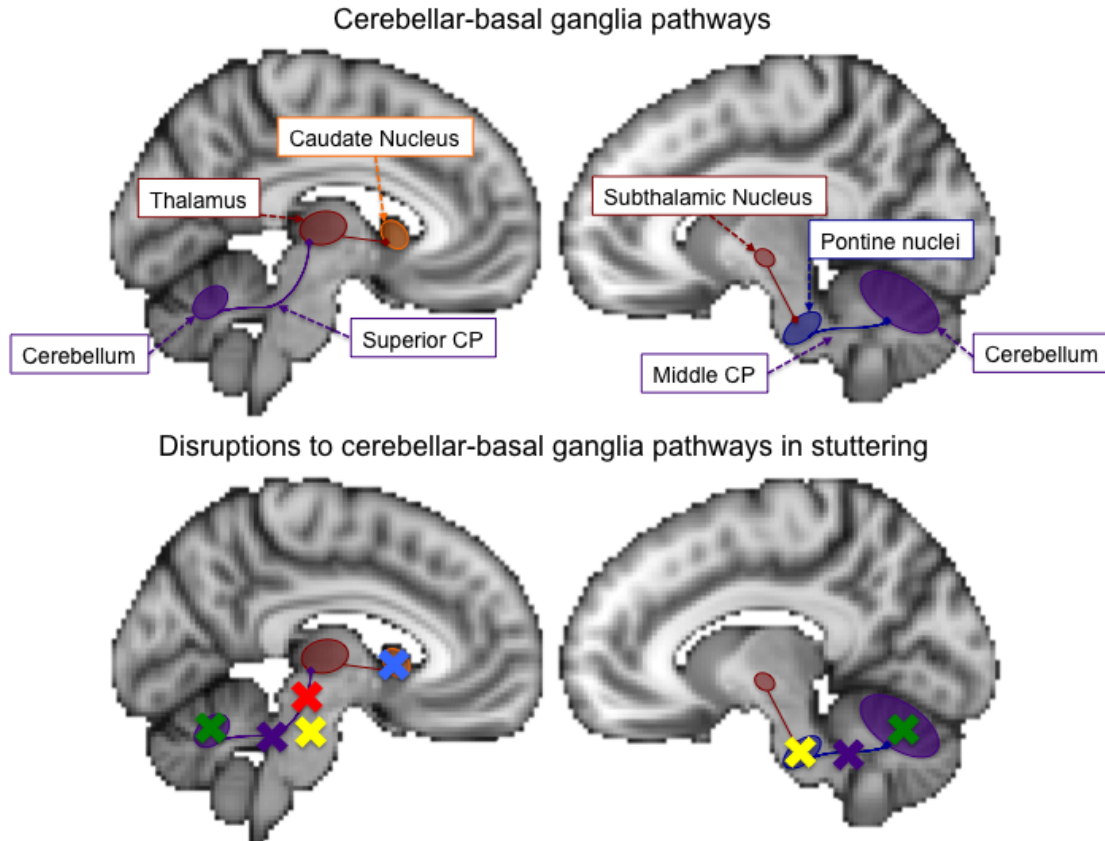
7.4 Integrating trait and state effects

Before we conclude, it may be prudent to resolve seemingly contradictory subcortical findings in stuttering: on the one hand, general traits reflect cerebellar disruption, on the other hand, basal ganglia disruption appears to drive the stuttering state and separate subgroups of people within stuttering. Classically, processes subserved by the cerebellum and basal ganglia have been theorised to occur in parallel loops, examples of which are frequently referred to as internal and external timing circuits [169]. The separation of these loops form the basis for two theories of stuttering [4][160]. In dominant models of speech production, these parallel loops are maintained: the cerebellum is involved in monitoring speech plans [106], whereas the basal ganglia are modelled in movement execution [106][58]. Given dopamine's rarity in the cerebellum, one might conclude its role is limited to mediation via influence on serotonergic and noradrennergic levels. More recently, however, a consensus has been reached regarding direct connections between the cerebellum and basal ganglia that would facilitate dopaminergic influence over cerebellar processes, and vice versa [45] (Figure 7.3, Top). In our neuroimaging work in particular we observed disruptions to several points along the shared cerebellar-basal ganglia pathways (Figure 7.3, Bottom) that also suggest the interaction between these subcortical systems underpins stuttering.

Specifically, tracer studies in non-human primates have confirmed connections from cerebellar cortex to the striatum via the dentate nucleus and thalamus, which provides pathways for the cerebellum to monitor basal ganglia activity similarly to how it is thought to monitor cortical activity [110]. Connections were observed in the opposite direction from the subthalamic nucleus via the pontine nuclei, into the cerebellum [37]. Diffusion tractography has confirmed similar, direct connections between the basal ganglia and cerebellum are present in humans [168]. The shared loop involving both the cerebellar and basal ganglia systems is theorised to subserve “model-based action selection and learning” (p 212 [45]). One example of shared function arising from the cerebellar to striatum loop is the dynamic modelling of action plans, which recruits both the striatum (caudate nucleus and putamen) and lobules VI and VIII of the cerebellum simultaneously [182]. Further, dopaminergic influence over cerebellar activity could arise from the subthalamic nucleus, which influences indirect pathway inhibition of motor function [133] and could theoretically exert influence regarding error avoidance, specifically, in the cerebellum. The potential of the subthalamic nucleus to induce abnormal cerebellar activity has also been recently theorized to play a role in symptoms of Parkinson's disease, including the tremor [45]. Overall, the two subcortical loops therefore provide multiple mechanisms by which trait and state effects could contribute to one another in stuttering.

The idea that multiple mechanisms could cause stuttering is not new, and is in

Figure 7.3: An integrated view of subcortical effects in stuttering



Top: An illustration of the two direct pathways thought to connect the cerebellum and basal ganglia based on tracer studies: 1) The cerebellum influences the striatum via the connections sent from the dentate nucleus (purple) through the superior cerebellar peduncle via the thalamus (red) to the striatum (caudate nucleus, orange) and 2) the output of the basal ganglia is also transferred to the cerebellum via connections with the subthalamic nucleus (red), via the pontine nuclei (blue) which connect to the middle cerebellar peduncles to input signal to cerebellar cortex (purple). CP = Cerebellar Peduncle. Bottom: An illustration of disruptions observed in our neuroimaging studies that impact the two direct pathways thought to connect the cerebellum and basal ganglia: 1) Left image: We observed microstructural disorganisation of the superior cerebellar peduncles (purple x) and cerebral peduncles (red x) in addition to state-related disruptions to the pons (yellow x) and the basal ganglia (blue x) and abnormal function of the cerebellum in several studies (green x). 2) Right Image: We observed microstructural disorganisation of the middle cerebellar peduncles (purple x), in addition to state-related disruptions to the pons (yellow x), and disruptions related to frequency of stuttering in the scanner as well as speech-related differences between controls in the cerebellar grey matter (green x).

fact central to a computational model of speech production, based on the DIVA model, which simulates stuttering [58]. This model accounts for stuttering-like speech interruptions through the disruption of a single circuit, the putamen-

premotor circuit, the specific cause of which could be either or both due to underlying neurochemical (dopamine imbalance) or neuroanatomical (white matter disconnection) anomalies. The neurochemical mechanism is theorised to result in a delay in activating the direct pathway motor program for the first syllable of speech, whereas the neuroanatomical mechanism results in weaker or delayed signals for indirect pathway processes important for sequencing [58]. While this simple model is somewhat consistent with findings in the literature, across our thesis we were not able to confirm its predictions with respect to behavioural disruptions, or altered speech-related activation, in particular a disruption arising from the putamen. We did, however, observe evidence of dopaminergic imbalance with regard to specific states and subgroups within stuttering in addition to physical evidence and plausible behavioural consequences of a neuroanatomical disruption to the cerebellum which would have similar impacts on speech. We thereby suggest expanding current theoretical models to include our findings regarding potential neurochemical and neuroanatomical underpinnings of persistent developmental stuttering.

7.4.1 A neurochemical account of stuttering

The original dopamine hypothesis of stuttering arose from evidence of “dopamine excess” in developmental stuttering in a small sample of males ($n=3$) who showed increased dopaminergic uptake in the medial frontal cortex and caudate nucleus relative to fluent controls [261]. The dramatic differences observed in that original study have not been replicated, presumably in part due to methodological concerns that could have exaggerated group differences. Also consistent with this theory was the impact of dopamine blockers in reducing some symptoms of stuttering in some people (*e.g.* [156]). More substantial experimental evidence for excessive dopamine has been relatively rare in developmental stuttering research. More modern theories such as an imbalance of dopamine [4] can better account for contradictory findings that include reports that dopamine blockers also induce stuttering [38]. Further support for this imbalance theory (rather than excess or depletion) comes from reports that stuttering severity is both positively correlated to activity in the caudate nucleus and negatively correlated to activity in the substantial nigra in the same subjects [100].

Indirect evidence of a disruption in the dopaminergic system function comes from observed abnormalities in the basal ganglia to SMA circuit that is theorised to be the core impairment caused by dopamine imbalance [4]. The functional connectivity between these regions is disrupted near the age of onset in stuttering [55] and persists into adulthood [263]. Of over thirty neuroimaging studies reviewed for this thesis, twenty-two implicate core structures of the dopaminergic system in stuttering, fifteen of which specifically mention the putamen. Likewise, acquired

stuttering that results from injuries to the basal ganglia, especially the putamen, produce symptoms similar to developmental stuttering [228]. In our work we observed evidence that the putamen is not disrupted relative to fluent controls during fluent speech, but it does show decreased recruitment within PDS in a subgroup who is more prone to dysfluent speech states in the scanner relative to a mostly fluent PDS subgroup. Dysfluent speech states during sentence reading also show increased recruitment of the putamen, bilaterally, relative to fluent states within this same relatively more dysfluent subgroup of people. This seemingly bidirectional finding within the same subjects could reflect the dopaminergic imbalance, but cannot corroborate that the “core impairment” is in the basal ganglia-SMA loop.

The behavioural evidence we observed during reinforcement learning suggested a neurochemical imbalance would likely impact the indirect dopamine pathway, specifically. In one subgroup of individuals, the impact would amount to an excess of dopamine in this pathway, which would lead to excessive sensitivity to negative feedback, and potentially increased inhibitory processes during speech sequence step selection and initiation. In the other subgroup of individuals, we observed evidence of a depletion in the indirect pathway, which would lead to weakened inhibitory processes necessary for speech sequence step selection and initiation. In this way, in contrast to computational theories that neurochemical imbalance would impact only the direct pathway [58], our findings suggest the opposite: that hypo and hyper dopaminergic subgroups exist within stuttering, and the impact of this imbalance would be most evident in the indirect pathway. In this way we are in agreement with both the dopaminergic imbalance theory of stuttering [4] and the conclusion that dysfluent speech states occur following failures to activate the necessary motor programs in a timely manner [58].

We further conjecture that an imbalance in the indirect pathway could also disrupt cerebellar function via connections between the output nucleus of that pathway (subthalamic nucleus) and the cerebellum. Specifically, if the striatal influence on the subthalamic nucleus is increased, an excess of “no-go” signals would be conveyed to the cerebellum, which could disrupt the processing of model-specific predictions subserved by these two circuits. In the same vein, if the striatal influence on the subthalamic nucleus is decreased, an absence or weakened “no-go” signal would be conveyed to the cerebellum, and corresponding model-based processes would be disrupted. We therefore theorise that an imbalance specific to the indirect pathway could lead to conflict between information coming from the subthalamic nucleus, the cortical-cerebellar loops, and sensory feedback which could result in a delay in updating internal representations and require an excess of cerebellar activity to resolve.

7.4.2 A neuroanatomical account of stuttering

An original neuroanatomical theory of stuttering suggested a coordination disorder results from competition for resources between multiple networks, but rather than implicating the dopaminergic system this theory suggested the blame lie within shared white matter pathways [242]. Our work indicates that a specific set of shared white matter pathways, the cerebellar peduncles, could reflect this sort of “bottle neck” [181] in the process necessary for updating internal sensorimotor representations. In contrast to abnormalities in cortical microstructure, which underlie reductions in neural activation, disorganisation in small pathways, such as the cerebral peduncles or caudal midbrain, are associated with hyper activation during speech in stuttering [249]. This hyper activation could reflect inefficient processing, or rather a “backlog” of signals indicating competition for resources.

Such a structural disruption could have downstream effects on dopaminergic levels in a variety of ways. First, the delays in cerebellar signals could impede the timely distribution of neurotransmitters, which would account for the dopaminergic imbalance in stuttering. The major bidirectional pathways of neurotransmitter distribution align in the midbrain, pons, and medulla near the openings into the cerebellar peduncles. Disruption to signal in nearby cerebellum could contribute to a fluctuation in neurotransmitter signals, which is similar to a theory of disruption in the “reticular formation” account of stuttering [30]. Second, the cerebellum is connected to the major input nuclei of the basal ganglia via the dentate nucleus and thalamus, which could facilitate monitoring of predictive actions in the striatum. If signals leaving the cerebellum are delayed or disrupted, then the monitoring process will also be delayed or disrupted, which could trigger hesitation by the striatum before gating the appropriate part of an action plan.

The final way in which a cerebellar disruption could impact the dopaminergic system is through changing the gating thresholds required for actions [58] through massive and ongoing cortical reorganisation attempts at compensating for the cerebellar bottleneck. If signals intended for speech-motor pathways are disrupted due to the aforementioned white matter disconnections, the brain could have an automatic plastic response that would reroute to nearby cortices as well as contralateral homologues. Such a compensatory response accounts for overactivity in the frontal cortex as well as nearby primary motor regions, as we observed during fluent speech in stuttering individuals relative to fluent controls. The detouring of vocalisation representation to nearby hand cortex could explain findings of altered motor excitability in stuttering [6][43], as well as reading related MEG activation that extends into hand areas in PDS, but not in fluent controls [206]. In order to produce fluent speech, an excitatory “speech” signal would need to be accompanied by simultaneous inhibitory “hand” signals, which would further disrupt and congest pathways. Though these explanations are speculative, it is critical that the joint

role of the basal ganglia and cerebellum in speech production and stuttering are assessed in more detail in future investigations.

7.5 Conclusion

Across this thesis we identified neurobiological underpinnings of specific speech states and general traits of stuttering that implicate some of the mammalian brain's oldest structures, the basal ganglia and cerebellum. Surrogates of cerebellar function were altered in stuttering: from organisation of white matter pathways to task-related activity, to activity at rest, this "little brain" functions differently in stuttering. Our observations of disrupted early motor learning suggest the critical sensory-motor integration function of the cerebellum is specifically disrupted in stuttering [160][249]. Whether this is a consequence or cause of the stuttering disorder is unknown, but interpreting cerebellar differences as compensatory, as is the overwhelming consensus in the literature, is not supported. During specific states of dysfluent speech the basal ganglia nuclei are differentially recruited, which provides support for theories of altered basal ganglia function in stuttering [4]. We did not observe general behavioural trends reflecting inefficient movement timing [84] but rather observed differences between subgroups of people who stutter that suggest the presence of both hypo- and hyper- tonic (D2) dopamine levels in stuttering that would impact feedback-based learning, specifically.

Critically, throughout this work our samples were small. The take home message is really a need for future research to:

1. Examine cerebellar function in stuttering without the a-priori assumption that abnormalities are compensatory.
2. Distinguish between effects related to general traits of stuttering and those underlying specific states of dysfluent speech.
3. Account for variability within stuttering groups through the use of meaningful aetiological subgroups.

The identification of subgroups is especially relevant for clinical work: some treatments may be more efficacious for certain individuals than for others. Finally, though we observed some cortical disruptions, the subcortical anomalies were more consistent across studies and support theoretical accounts of the disorder. The broader impact of this thesis is the acknowledgement that disruptions within subcortical control structures can influence higher-level processes such as speech. These subcortical control regions are critical, not only for motor initiation and coordination, but perhaps moreso for the initial learning stages that build the sequenced speech repertoire and facilitate smooth speech production.

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