Adaptive alterations in brain structure and function in young people with Tourette Syndrome

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Abstract

Tourette Syndrome (TS) is a developmental neurological disorder characterised by vocal and motor tics and is associated with cortical-striatal-thalamic-cortical circuit dysfunction and hyper-excitability within cortical motor areas. TS symptoms often become more controlled throughout adolescence until the individual is largely tic-free by early adulthood. It is likely that adaptive changes occur in the development of brain structure and function throughout the critical developmental period of adolescence in people with TS, which leads to tic remission in some individuals. To investigate this I used multiple brain-imaging approaches including diffusion tensor imaging to look at white matter microstructure, T1-weighted anatomical MR imaging to measure cortical grey matter thickness and MR-Spectroscopy (MRS) to measure neurotransmitters of interest (GABA and glutamate) in a group of young people with TS and a typically developing matched control group. Brain function (measures of excitation and inhibition in M1) was also considered by using transcranial magnetic stimulation. A significant positive relationship was found between white matter structural integrity (FA) measured from the body of the corpus callosum that contained projections to M1 or the SMA and motor tic severity. The TS group had increased levels of GABA in the SMA, as measured by MRS, compared to the control group. SMA- GABA levels had a significant positive relationship with FA from the SMA ROI but a negative relationship with TMS measures of cortical excitability during movement preparation. This suggests that those individuals with the least severe tic symptoms also have reduced callosal white matter from the SMA (an area implicated in the production and suppression of tics) in adolescents with TS, which relates to a reduction in task based cortical excitability and a reduction in SMA-GABA compared to those with more severe tics. The results from this thesis suggest that tic-suppression may occur through decreasing excitatory inputs to M1, either through increasing the inhibition (GABA levels) of the SMA, or by decreasing the number of excitatory interhemispheric inputs to sensorimotor regions.

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Chapter 1: Thesis Overview and Literature Review

1.1 Thesis overview

Within this thesis I used different cognitive neuroscience techniques to explore possible compensatory or adaptive brain alterations in young people with Tourette syndrome (TS). The background research leading up to the research thesis is presented in detail in the following sections of this chapter. Briefly, the majority of people diagnosed with TS in childhood become largely tic-free by early adulthood (Leckman et al., 1998), and the neurological mechanism by which tic remission may occur is explored in this thesis. The following two chapters in the thesis deal with adaptive alterations in brain structure in young people with TS (Chapters 2 and 3), following on to functional alterations (Chapter 4), and neurochemical alterations (Chapter 5). The final chapter combines structural and functional data with neuro-chemical results to draw conclusions on a possible mechanism to control tics based on local inhibition of motor and premotor cortical areas alongside developmental changes to white-matter pathways (Chapter 6).

In Chapter 2 I focus on the microstructure of white matter in the brain, firstly by reviewing the current literature on white matter alterations in people with TS, then by introducing Diffusion Tensor Imaging (DTI) as a technique to measure white matter microstructure in vivo. Following this, a novel DTI experiment is presented that tested a group of young people with TS and a typically developing matched control group. The data are analysed in three ways: a traditional tract-based-spatial statistics analysis, a modern machine-learning approach, and a region-of-interest probabilistic tractography method. The results of this experiment revealed that although traditional analysis showed very little

difference between the TS and the control group, the white matter microstructure in a large number of tracts are predictive of a diagnosis of TS. Furthermore, the structural integrity of the white matter that connects two key areas in tic production (the primary motor area and the supplementary motor area) across hemispheres through the corpus callosum, was found to be significantly related to the severity of tic symptoms. This lead to the conclusion that white matter in key areas of the corpus callosum may undergo adaptive changes in response to having TS, in line with past research (e.g. Jackson et al., 2011).

Chapter 3 follows on from Chapter 2, wherein I examined structural grey matter alterations in people with TS, focusing on the cortex rather than sub-cortical structures. The literature on structural changes in cortical grey matter in people with TS is reviewed, then a cortical thickness experiment on the same subjects as the DTI experiment in Chapter 2 is presented. Anatomical images were collected using a 3 Tesla MRI scanner and the thickness of cortical grey matter was measured using Freesurfer software. General linear modelling was used to examine the basic differences and age-related alterations between the control group and the TS group. Correlations between grey matter cortical thickness and tics were examined in the TS group. The results of these analyses showed widespread between-group differences in grey matter thickness, and many regions that correlate with tic symptom severity. In brief, the TS group tended to have thinner cortex that was increasing with age in areas where it was decreasing with age for the control group. Importantly, regions that were significantly thinner in TS than controls, and had a negative relationship with tic severity, included the sensorimotor regions identified in Chapter 2.

In Chapter 4 I addressed the functional or neurochemical alterations in TS, that may develop as a result of, or alongside, the structural changes reported in Chapters 2 and 3. Transcranial Magnetic Stimulation (TMS) was presented as a technique to measure physiological

changes in the primary motor cortex and motor pathways, and the literature on findings from TMS experiments with people with TS were presented. Following this, two separate TMS experiments using young people with TS and a typically developing matched control group were described. The first was a single pulse TMS experiment using a go/no go task in which subjects had to make a manual response (button push with right index finger) to visual stimuli. A single TMS pulse was delivered to primary motor cortex (M1) at the hotspot for the first dorsal interosseous muscle of the right hand in the interval between visual stimulus onset and button push response. The results of this experiment were that the TS group did not produce a sharp increase in the amplitude of the muscle response to TMS as they approached movement onset, whereas the control group did. Furthermore, those TS subjects with the most severe tics showed the least modulation of the muscle response preceding movement onset. This leads to the suggestion that people with TS reduce the excitability of M1 during a motor task to prevent interference from tics. Adaptive changes to reduce the excitability of M1 may lead to long-term reductions in tics. The second experiment used several paired-pulse TMS paradigms to examine inhibitory and excitatory functioning in M1 at rest. The results from this experiment were inconclusive due to the wide amount of variability in the data. However, there was a trend towards the previously reported effect of a reduced inhibitory response and an increased excitatory response in the TS group compared to the control group. Together, these two experiments suggest that there are alterations in the functioning of excitatory and inhibitory mechanisms in the motor cortex of young people with TS that may be adaptive.

Following on from the results of Chapter 4, in Chapter 5 I introduced Magnetic Resonance Spectroscopy (MRS) as a method for examining the key excitatory (glutamate) and inhibitory (GABA) neurotransmitters in regions of interest in the brain in vivo. An experiment was presented that measured neurotransmitters in M1 and the supplementary motor area (SMA), along with an occipital lobe control

site (V1), in a group of young people with TS and a matched control group. The main finding from this study was a significant increase in GABA for the young people with TS in the SMA region only. This result was explored in more detail in Chapter 6, in which I showed that the increase in GABA in the SMA is related to: a smaller muscle response to TMS in the last period before movement onset in TMS experiment 1, a smaller change in fMRI BOLD signal in the SMA to finger tapping vs. rest periods, and, finally, less white matter integrity in the area of the corpus callosum that joins the SMA across hemispheres. This leads to the final conclusion, presented in Chapter 7; that adaptive changes in white matter in TS may be developing to reduce excitability in the motor and premotor cortices, by reducing the amount of excitable cross-hemispheric inputs. If this reduction in inter-hemispheric white matter has not yet developed, the SMA compensates for excess excitatory input by increasing the inhibitory input, by means of increased GABA concentrations. This can be demonstrated in task-based physiological experiments (fMRI and TMS) and has the functional consequence of reducing the responsiveness of a sensorimotor cortical area, either seen by a reduced task-related change in fMRI BOLD signal or a reduced muscle response to TMS preceding movement onset. It is likely this mechanism has developed to avoid interference from tics to allow for successful voluntary movements.

1.2 What is Tourette Syndrome?

Tourette Syndrome (TS) is a movement disorder characterized by the presence of chronic motor and phonic tics. TS has been reported to affect between 0.042% - 3.8% of the population (Apter et al., 1993; Kurlan et al., 2001). An estimate of 1% of the population aged 5-18 years is considered reasonable (Robertson, 2008). In child populations of TS the ratio of males to females is estimated at around 5.2:1 but in adult samples this gender split is closer to equal with a ratio of 3:1 (Freeman, 2000).

According to the American Psychiatric Association Diagnostic and statistical manual of mental disorders (DSM-5), an individual has to have multiple motor and at least one phonic tic that persists for a minimum of one year with no more than three consecutive tic-free months, to receive a diagnosis of TS. Tics must also not be due to tic-provoking substance (e.g. stimulants). TS is usually diagnosed by clinical observation; assessment, including interview; and patient history.

Motor and phonic tics can be categorized as either "simple" or "complex". Simple tics are brief, meaningless, actions or utterances that usually involve only one muscle group (Singer, 2005). For example, a common simple motor tic is eye blinking, and a common simple phonic tic is throat clearing. Complex motor tics can be slower, and involve a sequence of coordinated movements or a cluster of simple tic-like movements (Singer, 2005). Complex phonic tics include words, phases, uttering obscenities (coprolalia), and repeating someone else's words (echolalia) (Singer, 2005). Although commonly portrayed in the media as a key symptom of TS, coprolalia is rare, only affecting about 10% of people diagnosed with TS (Goldenberg et al., 1994; Freeman et al., 2009). The kinds of tics performed are unique to each individual. Tics typically wax and wane in severity across the time, and become worse with stress (Robertson, 2000). At the height of severity tics can occur very frequently, with <0.5 seconds between successive tics (Peterson & Leckman, 1998). Simple motor tics usually appear first, between the ages of 3-8 years, with phonic tics typically appearing a few years after (Leckman, 2002). For most cases, tics reach their peak in severity in the early teen years of adolescence, and then show a decrease in severity until early adulthood when only a few mild tics remain. Indeed in a follow-up study, around 50% of children diagnosed with TS become completely tic-free by their early 20s (Leckman et al., 1998). However, Papert et al. (2003) reported that tics were still visible, although unnoticed, by 50% of subjects believed to be tic-free, suggesting that tics rarely completely disappear but become so mild as to not be noticed by the individual in the majority of cases.

Around 20% of individuals with a TS diagnosis continue to have debilitating tics into adulthood, with symptoms becoming more severe in some cases, resulting in considerable impairment in social functioning and quality of life (Cath et al., 2011). These individuals are often resistant to treatments for tics.

Some people with TS report feeling premonitory urges or sensations just before a tic (Bliss, 1980). Premonitory urges are uncomfortable bodily or cognitive sensations that require an action to diminish, for example the urge to sneeze or yawn (Banaschewski, Woerner & Rothenberger, 2003). People with TS can report feeling a tightness, itch, tingling, pressure or tension in a particular body part, or a general anxious or ill-at-ease feeling, that is relieved by performing a tic (Singer, 2005). The understanding of these premonitory sensations has lead to a new way of considering tics; tics can be thought of as a behavioural response to relieve uncomfortable bodily sensations (Capriotti et al., 2014). This would mean that tics have developed in people with TS through negative reinforcement; because they relieve uncomfortable sensations or urges (Capriotti et al., 2014)

However, not all cases report being aware of premonitory urges. Leckman et al. (2010) reported that children under 10 years of age who only present with simple tics typically do not report being aware of any premonitory sensations or urges. In a study of 254 children and adolescents with TS Banaschewski, Woerner & Rothenberger (2003) reported the percentage of young people who said they did experience premonitory sensations steadily rose with age, from 24% in 8-10 year olds to 57% in 15-19 year olds. This could demonstrate that tic-awareness increases with age, and that the ability to recognise and articulate premonitory sensations is a consequence of general cognitive development. Alternatively, premonitory urges may occur when people with TS supress a tic, and older children may have more experience of tic-suppression than younger children. For adult samples, Leckman, Walker

and Cohen (1993) reported 93% of patient's experienced premonitory sensations, and use this knowledge to suppress tics.

However, Banaschewski, Woerner and Rothenberger (2003) also note that awareness of premonitory sensations is not necessary for the successful suppression of tics, as 64% of their sample reported they were able to suppress their tics whereas only 37% reported premonitory sensations. This suggests that although premonitory urges could be helpful to suppress tics in adult samples (Leckman, Walker & Cohen, 1993), tic suppression, and tics, can occur without them for the majority of young people with TS. Indeed, Ganos et al., (2012) studied a sample of adults with TS, and although all reported experiencing premonitory urges, this was not related to tic inhibition measured by tic counting on a video of recording of subjects inhibiting tics using the Modified Rush Video Scale. Therefore, the evidence suggests that premonitory urges are experienced by the majority of adults with TS (Leckman, Walker and Cohen, 1993), but not for all tics (Ganos et al., 2012), and awareness of premonitory sensations develops throughout adolescence (Banaschewski, Woerner & Rothenberger, 2003) but is not necessary for tic inhibition (Ganos et al, 2012; Banaschewski, Woerner & Rothenberger, 2003).

TS is a complex disorder; most people with TS have a diagnosis of one of more co-morbid disorder (Robertson, 2000, Freeman et al., 2000). In a large sample of 3,500 subjects with TS, including both adults and children, from 65 sites in 22 countries, Freeman et al. reported only 12% of subjects had no co-morbidity, on average for a given site. The most common co-morbidity was attention-deficit hyperactivity disorder (ADHD); 60% of the sample had this additional diagnosis. Other common co-morbid disorders reported by Freeman et al. (2000) were: obsessive compulsive behaviors (OCB, 32%), obsessive compulsive disorder (OCD, 27%), specific learning difficulties (23%), mood disorder (20%), anxiety disorder (18%), oppositional defiant disorder (ODD) or conduct disorder (15% for ODD or CD) and self-injurious behaviors (SIB, 14%). Freeman et

al. (2000) also reported 20% of the sample had social skills problems, and TS is more prevalent in samples of young people with autistic spectrum disorder (ASD) than the general population: 6.5%-11% in the ASD sample compared to 1% of the general school-aged population (Baron-Cohen et al., 1999a; Canitano & Vivanti, 2007). ADHD, CD/ODD, specific learning disorders and SIB were all significantly more prevalent in male rather than female TS samples (Freeman et al., 2000).

1.3 Neurological Basis for Tourette Syndrome

It is generally agreed that the symptoms in TS stem from a disinhibition of the cortico-striato-thalamo-cortical circuitry (Albin & Mink, 2006). More specifically, TS is thought to be caused by a disinhibition of the striatum that leads to over-active sensorimotor areas and to the production of tics. This framework is presented below.

There are multiple, parallel but partially overlapping, cortico-striato-thalmo-cortical circuits including the limbic, associative and sensorimotor loops (Leckman et al., 2010). These circuits project information from the cerebral cortex to subcortical structures, which in turn send information back up to specific regions of the cortex. Efferent neurons from the cortex project to the medium spiny neurons in two distinct compartments of the striatum: striosomes and matrix (Leckman et al., 2010). The striosome neurons receive input from the limbic and pre-limbic areas, including the cingulate and the insula, whereas the matrix neurons receive input from primary motor and primary somatosensory cortex (Leckman, 2002). Although the focus in TS has mainly been on the sensorimotor circuit because of it's obvious relationship to motor tics, it is likely that alterations also affect the limbic and associative loops, and can account for more complex tics and co-morbidities (Ganos, Roessner & Münchau, 2013). The response of the medium spiny neurons in both compartments is dependant on the perceived salience of the stimuli, which could be aversive or rewarding (Canales & Graybiel 2000). Efferent

medium spiny neurons from both the striosomes and the matrix project to the pallidum and the subthalamic nucleus, whereas only striosomal efferent neurons project to the substantia nigra pars compacta (Ganos, Roessner & Münchau, 2013). All areas subsequently project back up to the cortex via the thalamus.

The output of striatal medium spinal neurons is modulated by tonically active cholinergic neurons and inhibitory GABA-ergic interneurons (Leckman et al., 2010). Post-mortem studies have shown that people with TS have a reduced number of GABA-ergic interneurons and tonically active cholinergic neurons in the sensorimotor areas of the striatum but similar numbers of medium spiny neurons (Kalanithi et al., 2005; Kataoka et al., 2010). Kalanithi et al. (2005) have also shown that people with TS have increased GABA-ergic interneurons in the internal segment of the globus pallidus, which lead them to suggest that TS results from an imbalance between excitatory and inhibitory mechanisms in the sensorimotor and limbic cortico-striato-thalamo-cortical loops, possibly due to interneuron migration errors. Indeed, Albin & Mink (2006) hypothesise that TS results from striatal medium spiny neurons becoming active inappropriately, which in turn disinhibit motor pattern generators in the cortex, resulting in an unwanted movement.

Leckman et al. (2010) describes tics as "routines that link sensory cues with specific motor actions" and in this way they are similar to habits. Tics, like habits, can also be thought of as learned responses to uncomfortable bodily sensations (pre-monitory urges) that are mediated by dopaminergic activity (Palminteri et al., 2009; Palminteri et al., 2011). Without pre-monitory urges, tics could develop through inappropriate reinforcement of behaviours by means of normal habit-formation mechanisms. The response of medium spiny neurons in the striatum can be dependant on salient perceptual cues (Blazquez et al., 2002). For example, tonically active neurons in the striatum respond to dopamine input from the substantial nigra and are involved in the judgement of the

salience of a stimulus. In TS, sensory stimuli could be inappropriately regarded as salient, through dysfunctional striatal activity, leading to an inappropriate response to that stimulus (tic) becoming a habit.

Furthermore, dopamine plays a key role in habit formation, and there is evidence for disrupted dopamine system in TS. Indeed, dopamine D2 receptor-blocking drugs are often administered as a pharmacological treatment for tics (Leckman, 2002). In a study of monozygotic twins with TS, Wolf et al. (1996) found that the severity of TS symptoms was related to the density of D2 receptors in the caudate nucleus. Yoon et al. (2007) reported increased D2 receptors in M1 and premotor cortex in a post-mortem study of 3 TS patients. Palminteri et al. (2009) reported that blocking dopamine transmission in young adults with TS increased punishment avoidance and impaired reward seeking in a reinforcement motor learning task. The TS subjects who were not medicated tended towards improved reward based reinforcement learning, demonstrating that dopamine levels in TS may be reinforcing tics (Palminteri et al., 2011).

Taken together, the evidence leads to a model where tics are caused by abnormal development of the striatum, leading to inappropriate actions, through normal habit formation mechanisms, which are reinforced by enhance dopamine activity. Abnormal development of the striatum is likely to have a knock on effect on the development of connected brain structures. Indeed, widespread structural and/or functional alterations in brain areas that are part of the cortico-striato-thalamo-cortical circuitry have been reported in people with TS, which I will discuss in the following sections.

1.4 Alterations in brain structure in Tourette Syndrome

Basal Ganglia

There is much evidence from volumetric MRI studies that people with TS have alterations in the structure of the basal ganglia. Alterations include reduced volume or loss of right-left asymmetry in the lenticular nuclei (Peterson et al., 1993; Singer et al., 1993; Swerdlow et al., 2001), reduced volume of the globus pallidus and putamen (Peterson et al., 2003), but also increased grey matter volume in the ventral putamen (Ludolph et al., 2006), and finally reduced volume of the caudate nucleus (Hyde et al., 1995; Peterson et al., 2003; Müller-Vahl et al., 2009). In a follow-up study Bloch et al. (2005) found an inverse relationship between caudate volume measured in childhood and tic-severity in early adulthood. There was no relationship between tic scores and caudate volume at the initial time of testing in childhood (Bloch et al., 2005). This result can be taken as evidence that caudate volume is involved in the pathogenesis of TS. Furthermore, in an early fMRI experiment Peterson et al. (1998) found activation in regions of the prefrontal cortex and caudate nucleus, and deactivation of the globus pallidus and caudate bilaterally during voluntary tic-suppression. This demonstrates the basal ganglia's role in tic production and suppression.

Thalamus

The thalamus may be important for tic production, and has been reported as increased in volume in unmedicated boys with TS (Lee et al., 2006). A more recent study found no differences in thalamic volume in adults with TS compared to a typically developed control group (Wang et al., 2007), so the development of the thalamus may not be abnormal in all cases of TS. However, neurosurgical treatment of TS involving lesions to the thalamus or deep brain stimulation have been successful in reducing tic symptom severity for people with very severe, life-limiting tics (Rauch

et al., 1995; Vandewalle et al., 1999; Rickards et al., 2008). This is likely to be because the thalamus is a key structure that relays information from the striatum to the cortex, and if some of the signals from the over-active striatum can be interrupted here less tics will be produced. Although the thalamus itself is unlikely to have developed abnormally in TS, it is an important structure in the relay of information that leads to the production of tics.

Cortex

There is now mounting evidence for structural abnormalities in the cortex of people with TS, as well as the sub-cortical structures. Alterations in cortical volume are often age-dependant. For example, Peterson et al., (2001) reported increased volume of dorsolateral prefrontal regions in children with TS compared to a matched typically developing group, but decreased cortical volume in the same area for adults with TS. This study also reported that increased volume of orbitofrontal and parietal lobe were associated with fewer tic symptoms (Peterson et al., 2001). Müller-Vahl et al. (2009) reported reduced cortical grey matter in the prefrontal, anterior cingulate, sensorimotor, the primary sensory cortices for adults with TS. There is also evidence for increased white matter in the right frontal lobe (Fredericksen et al., 2002; Hong et al., 2002), and decreased white matter in left frontal lobe (Kates et al., 2002) for children with TS.

There is evidence of cortical thinning in the sensorimotor cortices in children with TS (Sowell et al., 2008). Sowell et al. (2008) reported that those with the most severe TS symptoms also had the thinnest cortex in sensorimotor regions, suggesting that these areas play a key role in the production of tics. This finding has since been replicated, with the grey matter thickness in specific regions of the somatosensory and motor strips relating to not only symptom severity, but also the body part where the tics are most often located (Fahim et al., 2010; Worbe et al., 2010). This is evidence that the structure of the sensorimotor cortices can be linked to

the clinical symptoms that present. However, it is important to bare in mind the possibility that the cortex develops in this manner because of the tic, rather than the other way around.

Functional brain-imaging studies demonstrated significant increases in pre-frontal cortex activation during active tic suppression (Peterson et al., 1998, Stern et al., 2000). For tic-production Bohlhalter et al. (2006) reported increased activation in primary sensorimotor cortices, the thalamus and cerebellum at tic-onset; areas that are responsible for motor production. Importantly, activation of the insular, parietal and cingulate cortices and supplementary motor area were reported 2 seconds before tic onset. This suggests that these regions may be related to the pre-monitory sensations or urge to tic (Bolhalter et al., 2006).

White Matter connectivity

Makki et al. (2009) used a probabilistic tractography method to study anatomical connectivity of fronto-striato-thalamic circuits in children with TS. They reported a reduction in connectivity for the TS group between the caudate nucleus and anterior dorsolateral frontal cortex. This finding has also been replicated in adults with TS (Neuner et al., 2011). Thomalla et al. (2009) reported widespread increases in FA (Fractional Anisotropy: a measure of white matter structural integrity) in the white matter that made up part of the primary sensory and motor pathways in adults with TS. The average amount of FA taken from these regions was also found to have a negative correlation with tic severity (Thomalla et al., 2009). Together these findings reinforced the hypothesis that alterations in the cortico-striato-thamalic-cortical circuitry are the root cause of TS.

There is evidence for alterations in the corpus callosum volume (Peterson et al., 1994; Baumgardner et al., 1996; Moriarty et al., 1997) and area (Plessen et al., 2004) measured in the mid-sagittal plane. However,

this finding is inconsistent, with increased (Moriarty et al., 1997) and decreased (Peterson et al., 1994) corpus callosum volume reported in adults with TS. In children with TS, the corpus callosum volume has been reported as larger (Baumgardner et al., 1996) and the mid-sagittal area of the structure had a positive correlation with motor tic severity (Plessen et al., 2004). A more recent diffusion tensor imagining (DTI) study reported reduced fractional anisotropy (FA), a measure of structural integrity of white matter fibre bundles, in the corpus callosum of children with TS (Plessen et al., 2006). Corpus callosum alterations and some grey matter cortical alterations reported in TS have been hypothesised to develop as an adaption to, rather than cause of, the disorder (Jackson et al., 2011). The theory of a neurological compensatory mechanism is discussed in the following section.

1.5 Theories of a Compensatory Mechanism in Tourette Syndrome

Plessen, Bansal & Peterson (2009) argued that the reduction in tic symptoms that is observed in many people diagnosed with TS in childhood (Leckman, 2002) might be a consequence of normal developmental processes. In the typically developing brain, a large amount of synaptic pruning, cortical thinning and myelination of white matter occurs during adolescence, alongside improvements in impulse control (Huttenlocher, 1984; Giedd et al., 1999). It is likely that young people with TS, through the process of synaptic pruning and active tic-suppression, are able to gain some control over their tics. Indeed, the age-dependant alterations in frontal lobe volume of people with TS could be indicative that the continual need to suppress tics may alter the development of the frontal lobe in adolescence (Marsh et al., 2008).

Support for this theory comes from the observation that adolescents with TS perform better on tasks of cognitive control than an age and gender matched typically developing control group (Mueller et al., 2006; Jackson et al., 2007; Jackson et al., 2011; Jung et al., 2013). Even

when TS groups do not out-perform typically developing control groups, better task performance has been related to less severe tic scores, suggesting that control of tics is related to general improvement in cognitive control (Baym et al., 2008; Jackson et al. 2011; Jung et al., 2012). This enhanced cognitive control of action has been related to increases in a measure of structural integrity of white matter fibre bundles (fractional anisotropy) in the forceps minor, a white matter bundle that projects to the prefrontal cortex (Jackson et al., 2011). The amount of FA in the forceps minor positively correlated with motor tic scores, demonstrating that those subjects with the least FA in the forceps minor, i.e. those who were furthest away from the typically developing control group, had the least severe motor tics. This can be taken as evidence that the white matter development in the frontal lobe may be involved in developing enhanced cognitive control of action, which in turn, leads to control of tics.

Further support for the role of the pre-frontal cortex in inhibitory control comes from Marsh et al. (2007), who reported normal age-related improvement in performance of a Stroop task in people with TS, but the TS group did not see the same reduction in prefrontal cortex activity (as assessed via fMRI) with age as the control group. This suggests that young people with TS engage the prefrontal cortex to a greater extent to allow them to successfully complete the Stroop task. More recent studies have reported increased activation in pre-frontal cortex during cognitive control tasks for the TS group compared to typically developing control groups (Baym et al., 2008; Raz et al., 2009; Jackson et al. 2011; Jung et al., 2012). Furthermore, a positive relationship between motor tic severity and task-related BOLD signal has been reported in left inferior prefrontal regions (Raz et al., 2009), and bilateral premotor cortex, left superior frontal gyrus, right inferior frontal gyrus and bilateral middle frontal gyrus (Baym et al., 2008). This leads to the suggestion that the pre-frontal cortex has to be engaged to a greater extent in people with more severe tics, perhaps because it already exhibits top down control over dysfunctional cortico-striato-thalamo-cortical loops to control tics (Baym et al., 2008),

i.e. is it already engaged in tic-suppression, then works *even harder* (as demonstrated by increased blood flow to this region) to allow the subject to perform the task. Put another way, in order to successfully complete the cognitive control task, people with more severe tics have to put in more cognitive effort to avoid a tic interrupting their action.

In a recent review, Ganos, Roessner and Munchau (2013), posed the theory that erratic and dysfunctional striato-thalamic firing leads to tics in the idle brain, but that this erratic activity can be mediated by top-down inhibitory control from pre-frontal cortex to enable voluntary movements. Furthermore, cortico-striatal inhibition could be achieved by enhanced functional connectivity between not only pre-frontal and pre-motor areas, but also between pre-motor, SMA and primary motor cortices. Evidence for this comes from TMS studies that have shown a reduction in gain during voluntary movements (Heise et al., 2010; Jackson et al., 2012; Draper et al., 2014) and MEG studies that demonstrate stronger SMA-M1 coupling during voluntary movement in TS (Franzkowiak et al., 2012).

However, increased M1-SMA connectivity may not always be helpful. A study by Hampson et al. (2009) trained typically developing control subjects to mimic tic-like movements, and compared the fMRI BOLD signal during these minic-tics to genuine tics produced by adults with TS. Both groups showed a similar pattern of activity across the motor system during tics or mimic tics. However, the TS group showed significantly increased cross-correlation between M1 and the SMA during tics. This suggests that increased connectivity between M1 and SMA could be partly what is leading to tics. Furthermore, inhibitory rTMS delivered to SMA has been shown to reduce tics (Mantovani et al., 2007; Kwon et al., 2011). This demonstrates that over-activity in the SMA plays a key role in tic production, and reducing the excitability in this area, or reducing it's input to M1, may reduce tics (Ganos, Roessner & Munchau, 2013).

Jung et al. (2013) proposed a novel theory of how a compensatory mechanism may develop in TS, with the focus on local inhibitory mechanisms rather than top down control from the prefrontal cortex. This view is similar to that proposed by Ganos, Roessner and Munchau (2013) presented previously, but takes it one step further by offering a different perspective on the role of the prefrontal cortex. The alternative view, proposed by Jung et al. (2013), is that the prefrontal cortex does not exhibit inhibitory control over motor output to aid in tic suppression, but instead plays a facilitatory role to bias response competition in premotor and sensorimotor regions (Sumner et al., 2007; Munakata et al., 2011). This viewpoint takes the stance that the increased activity in prefrontal cortex might actually contribute to the occurrence of tics, by enhancing the activity of an already over-active sensorimotor cortex (Albin & Mink, 2006). A compensatory mechanism to control tics could therefore develop to isolate sensorimotor cortex from upstream influence of brain regions such as the prefrontal cortex (Jung et al., 2013). Evidence to support this hypothesis comes from the finding that young people with TS have decreased white matter in the corpus callosum, including projections from the prefrontal cortex (forceps minor), which is positively correlated with tic severity (Jackson et al., 2011; Plessen et al., 2004). This suggests that the less cortico-cortico connectivity a young person with TS has, the less severe their tics are.

Furthermore, Jung et al. (2013) suggest that tics could be controlled by localised suppression of cortical excitability in motor cortical areas, perhaps through the modulation of local GABA-ergic interneurons. Evidence for this theory comes from fMRI experiments of cognitive control tasks which show that the TS group have a reduced BOLD response in primary motor cortex compared to a typically developing control group, despite similar behavioural performance (Jackson et al., 2011; Jung et al., 2013). Further evidence comes from TMS studies that have shown motor cortical excitability is significantly reduced prior to movement onset in TS (Heise et al., 2010; Jackson et al., 2012), which suggests local inhibition of

this area takes place to aid in successful voluntary movements. Finally, reducing the excitability of the SMA through inhibitory rTMS has been reported to reduce tics (Mantovani et al., 2007; Kwon et al., 2011). Taken together, this evidence can support the view that reducing the hyper-exciatbility of premotor and sensorimotor cortices through the enhanced activity of local GABA-ergic interneurons may help in the execution of voluntary actions and reduce tics (Jung et al., 2013).

1.6 Research Aims

Keeping in mind recent theories of compensation via increased prefrontal-premotor or premotor-sensorimotor connectivity (Ganos, Roessner & Munchau, 2013) or via local inhibition of sensorimotor cortex and SMA (Jung et al, 2013), I used multiple techniques to examine possible adaptations in brain structure or neurochemistry in a cross sectional study of young people with TS. Importantly, there was a focus on how brain structure or chemistry related to measures of tic severity. Structural magnetic resonance imagining and diffusion tensor imaging was used to examine compensatory structural changes in white matter micro-structure, particularly the corpus callosum, and the grey matter of the cortex. Transcranial magnetic stimulation was used to measure the modulation of cortical excitability during volitional movement, and excitation and inhibition of the motor pathways at rest. Magnetic resonance spectroscopy was used to measure neurochemistry in the primary motor cortex and SMA. Finally, information from these techniques was combined for a deeper examination of the role of GABA in the supplementary motor area.

Chapter 2: Structural Connectivity in Tourette Syndrome assessed by Diffusion Tensor Imaging

2.1 Introduction

Tourette Syndrome (TS) is generally thought to arise through dysfunction in the basal ganglia-thalamic-cortical circuits (Albin & Mink, 2006), as discussed extensively in the previous chapter. The many areas implicated in TS are distinct and are structurally connected through white matter tracts. Considering structural connectivity is a key step in understanding the neurological basis of TS. The brain does not have a rigid, unchanging, structure; plastic changes occur throughout childhood, adolescence, and even adulthood, which could allow for compensatory adaptations in brain structure to take place. In this introduction I will present the current understanding of white matter maturation through adolescence and evidence for training-based plasticity in typically developing young people. Following this I will present evidence for white matter alterations in young people with TS, which may be related to the control of tics.

The typical development of white matter from childhood to young adulthood

Kochunov et al. (2012) conducted a DTI study to look at age-related changed in FA by recruiting a large cross-sectional sample of typically developing individuals aged 11-85 years. They examined the change in average FA with age in several of the main white matter fiber bundles: corpus callosum, cingulum, corona radiate, cortico-spinal, external capsule, internal capsule, fronto-occipital fasciculi, superior longitudinal fasciculus and sagittal striatum. They reported that FA stayed stable throughout adolescence and adulthood for the cortico-spinal tract, but FA steadily increased with age until it reached a peak in early adulthood for all other tracts. This increase in FA is thought to mainly reflect myelination of fiber bundles or increased axon density (Lebel et al., 2012). In the corpus

callosum FA did not reach it's peak until 29.9 years for the splenium, 31.8 for the body and 34.2 for the genu, suggesting that sensorimotor regions of the corpus callosum develop at a faster rate to the fibers from the genu that project to frontal regions. Furthermore, Lebel et al. (2012) reported a sharp increase in white matter volume in the whole brain, and FA in specific tracts including the corpus callosum, between the age of 5 and 25 in a cross sectional study of 403 healthy subjects. Lebel et al.'s (2012) results concurred with Kochunov et al.'s (2012) finding that the change in FA for the body of the corpus callosum reached its peak earlier than for the genu. Lebel et al. (2012) added that the age-related changes in FA in the body of the corpus callosum were smaller than those of the genu.

Peters et al. (2012) used DTI to assess white matter development in adolescents, using a large sample of 8-21 year olds. The aim of this cross-sectional study was to identify which white matter tracts showed the largest age-related changes in this key period of development. These tracts were identified as left superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, and anterior thalamic radiation. This finding was supported by Schmithorst et al. (2002) who reported a positive correlation between FA values in the internal capsule, corticospinal tract, left arcuate fasciculus, and right inferior longitudinal fasciculus with age in a cross-section of 5-18 year olds. This relationship was the most pronounced in the fibers that descend from M1 and SMA, and the authors suggest this is due to the development of fine motor skills (Schmithorst et al., 2002).

Lebel and Beaulieu (2011) collected longitudinal data on a sample of 103 individuals aged 5-29 years, scanned at a minimum of 2 time points. They showed that white matter volume increased and grey matter volume decreased over time for the majority of subjects tested, and with age when looking at the group as a whole. They also reported that FA increased with age in all major white matter tracts. Callosal tracts tended to have more of an increase in FA in subjects aged 5-15, with most older subjects showing

very little change, which supports the notion that the corpus callosum develops rapidly in childhood and early adolescence (Lebel, Caverhill-Godkewitsch & Beauliuem 2010).

These age-related changes in white matter occur alongside cognitive development and learning (Nagy, Westerberg & Klingberg, 2004; Paus, 2005; Zatorre, Fields & Johansen-Berg, 2012). Nagy, Westerberg & Klingberg reported that working memory capacity had a positive correlation with the maturation of white matter fibres (FA values) in the frontal lobe, and reading ability in with the white matter of the temporal lobe in a cross section of children aged 8-18 years. These findings were, unsurprisingly, also correlated with age, suggesting that the white matter maturation that occurs during adolescence and childhood is also closely related to age-related development of cognitive abilities. This research also demonstrates that white matter microstructure in specific regions of the brain relate to specific cognitive skills, in this case working memory in the frontal lobe, and reading ability in the language centers of the left temporal lobe. However, from this research it is difficult to determine whether white matter development leads to improved reading and memory skills, or whether the environmental pressure of practicing reading and memory skills lead to the development of white matter. The following section considers changes in white matter development that are likely to occur because of environmental pressures, i.e. training in a certain skill.

White matter plasticity in the typically developing population

Research has suggested that structural grey and white matter changes can result from training in a certain skill, even in adulthood. Perhaps the most famous example of this is the increased posterior hippocampal volume found in London taxi drivers, thought to develop through years of demanding spatial navigation (Maguire et al., 2000). In this study, years of experience had a positive correlation with hippocampal volume, suggesting the plastic change in volume was a consequence of the

work the drivers (Maguire et al., 2000). Another common example is that musicians have been found to have thicker grey matter in the auditory cortex, which is likely due to their musical training (Bermudez et al., 2009). Indeed, Bengtsson et al. (2005) examined the impact that piano playing during critical developmental periods (before 11 years old, 12-16 years old and adulthood) has on white matter microstructure in adulthood. They found that hours of piano practiced during childhood had a significant positive relationship with FA values in the corpus callosum and internal capsule. The authors conclude that training in playing the piano during critical periods of white matter development (childhood and adolescence) leads to plastic changes in white matter development that are apparent in adulthood. The same positive relationship was also observed in the corpus callosum when correlated with hours of practice during adolescence, however childhood practice and adolescent practice where highly correlated with one another, so it is difficult to determine which period of development is more critical for white matter plasticity (Bengtsson et al., 2005). However, this research demonstrates clearly that learning and practicing a complex skill, such as piano playing, during development has a long-term impact on white matter microstructure.

Changes in white matter microstructure can also occur during adulthood after only brief training in a new skill. Tang et al. (2012) examined the affect of mindfulness meditation on white matter microstructure. They reported increased FA in the corpus callosum and corona radiata after just 4 weeks of meditation training, compared to a control group given relaxation training. The authors suggest that meditation training either increased axon density or myelination, perhaps as a consequence of increased neuronal firing in specific white matter pathways. This research suggests that alterations in the white matter microstructure in the corpus callosum can occur due to training, even after the critical period of development (adolescence) and after only a short period of training. This study did not report any follow-up scans so it cannot be determined

whether these alterations in FA are long term or if they would revert back to their original state if the meditation were discontinued.

In summary, white matter microstructure, measured by FA values from DTI scans, has a positive relationship with age, that is most apparent from 5-25years, but which can peak in the early 30s. The development of white matter is highly correlated with the development of cognitive abilities in a regionally specific way. Furthermore, the development of white matter can be influenced by training in a particular skill during the critical period of development, such as playing piano. Some degree of plasticity remains in adulthood; increases in FA can occur after only short-term intensive training, but the longevity of these alterations is uncertain.

White matter plasticity in TS

The majority of cases of TS show a large reduction in their symptoms throughout adolescence, until they have very mild or no tics by the time they are in their early to mid 20s (Leckman et al., 1998). This may occur because of plastic changes in white matter connectivity that develops throughout adolescence as an adaptation to TS (Jackson et al., 2011). Being able to identify tracts that are related to a reduction in TS symptoms may be critical to understanding the mechanisms underlying recovery, and to aid in the successful treatment of tics.

As tics are undesirable in many situations, active tic-suppression, over time, may lead to the reduction in tics that occurs in 70% of TS cases. It has been suggested that active tic-suppression leads to an enhanced overall control over actions. This is because TS groups have been observed to out-perform groups of typically developing matched controls in tasks thought to measure cognitive control over action (Mueller et al., 2006; Jackson et al., 2007; Jackson et al., 2011; Jung et al., 2013). Furthermore, task performance has been related to tic severity such that those with the most severe tics had the slowest reaction times in a condition that was demanded a large degree

of cognitive control (Jackson et al., 2011). This suggests that those individuals who have less tics have better cognitive control. It is possible that some subjects have reduced their tics through the practise of active tic suppression, which has led them to have better cognitive control. This enhanced cognitive control could then be applied to other tasks, leading to the improved performance demonstrated by Jackson et al., (2011).

Interestingly, motor tic scores also correlated positively with FA measured from the forceps minor, so that the higher the FA, the worse the tic symptoms, although the TS group show an overall decrease in FA compared to a control group (Jackson et al., 2011). The forceps minor are white matter tracts that connects the pre-frontal cortex across the hemispheres through the genu of the corpus callosum. This means that adolescents with the least severe tics have white matter structure that is furthest from that of the typically developing age-matched control subject. This could mean that practising cognitive control (through active tic suppression) leads to an altered development of forceps minor, perhaps in a similar way that piano practise impacted white matter development in the typical population (Bengtsson et al., 2005). As such, this result has been interpreted as evidence for an adaptive mechanism that involves the pre-frontal cortex exhibiting influence over motor areas to control unwanted movements. The adaptive mechanism has been hypothesised to develop through plastic changes in white matter connectivity from the pre-frontal cortex that takes place over adolescence as a result of, or to aid in, tic suppression and remission (Plessen, Bansal & Peterson, 2009), as discussed in Chapter 1.

However, the same positive relationship between tic scores and FA has been observed in the body of the corpus callosum in young people with TS (Jackson et al., 2011). This suggests that adaptive changes in white matter microstructure may be more widespread than just connections to and from the pre-frontal cortex in TS. Furthermore, Thomella et al. (2009) report a negative relationship between FA in the left mesial sub-central

white matter tract and tic severity, and an overall increase in FA in white matter tracts projecting from sensorimotor and pre motor cortices in adults with TS compared to a control group. This finding could represent the difference between those individuals that show tic remission by early adulthood and those who do not. Adolescents with TS have reduced FA in sensorimotor white matter tracts (Jackson et al., 2011) whereas adults have increased FA (Thomella et al., 2009), compared to typically developing control groups. It could be that those adolescents that develop lower FA projecting from sensorimotor cortices through the body of the corpus callosum, are more likely to have tic remission and therefore will not be included in adult samples. This hypothesis is supported by the finding that those with the least severe tics have the lowest FA values in the corpus callosum and forceps minor (Jackson et al., 2011).

Although it is likely that tics and tic suppression may alter the development of white matter through childhood and adolescence, the theory that control of tics occurs through enhanced connections from prefrontal cortex to motor areas (Plessen, Bansal & Peterson, 2009) is not necessarily supported by the current literature. It could be the case that increased connectivity with the pre-frontal cortex is not beneficial to overcoming tics. Indeed, it is counter-intuitive that a *decrease* in FA, observed in the forceps minor in young people with TS (Jackson et al., 2011), should be indicative of *increased* influence (Jung et al., 2013). This is not the case in typically developing individuals. For example, Lee et al. (2010) found that increased FA in white matter of the frontal lobe is related to expertise of the game Baduk. Baduk is a Korean game similar to chess that requires attention, concentration and control of action; many of the skills required to successfully suppress a tic (Lee et al., 2010). The expert subjects in this particular study started playing the game in childhood, and were in late adolescence or early adulthood at the time of testing. The authors argue that plastic changes in white matter structure develop throughout adolescence as a consequence of Baduk training. This is similar to the previous example of increased FA in the corpus callosum with piano practice (Bengtsson et al.,

2005), but is specific to the frontal lobe. Furthermore, in typically developing children and adolescents, increased FA in the frontal lobe is related to increased IQ independent of age (Schmithorst et al., 2005). This suggests that increased cognitive abilities relates to increased axon density in frontal white matter tracts. It is therefore difficult to comprehend how a *reduction* in FA in the forceps minor is evidence of a compensatory top-down control mechanism in TS.

In the following DTI study I will consider how white matter microstructure differs in adolescents with TS compared to a typically developing age- and gender-matched control group, and how these differences are related to symptom severity. This will be examined in 3 different ways: a tract based spatial statistics approach to compare TS vs. controls using standard statistical tests, a support vector machine approach to look for more widespread areas that may be predictive of a diagnosis of TS, and, finally, a tractography based approach to look at differences in specific regions of interest defined a-priori. By employing a combination of techniques, I will be able to identify which white matter tracts are related to a diagnosis of TS and which are also altered outside the range of values measured from of a typically developing population. More importantly, I will examine how the values in particular tracts of interest are related to tic symptoms, with the hypothesis that those areas that are closely related to symptom severity may also be subject to plastic changes that occur through adolescence as part of a compensatory mechanism that develops to reduce tics.

2.2 Diffusion Tensor Imaging

Diffusion Tensor Imaging (DTI) was used to measure structural connectivity by mapping the white-matter tracts in the brains of TS subjects and healthy control subjects. DTI measures the diffusion of water molecules in the brain. When water molecules are constrained inside a cell they move slower than they would in a larger, unconstrained space, (e.g.

the ventricles). Water molecules will also diffuse much more rapidly parallel to the orientation of a fibre bundle rather than perpendicular to it. DTI recruits this property to provide information about the precise location, and microstructure, of white matter fibre bundles (Jellison et al., 2004). Basser et al. (1994) introduced the diffusion tensor to describe the behaviour of anisotrophic water diffusion in biological tissue. The diffusion tensor is a 3x3x3 matrix that describes the covariance of diffusion displacements in 3D space (Alexander et al., 2008). The diffusion tensor can be represented by 3 principle Eigen vectors and their corresponding Eigen values, which describe the diffusion of water along the axes (x,y,z) of principle diffusion. A common visualisation of the diffusion tensor is to imagine water molecules constrained to an ellipsoid, the axes of the ellipsoid would be represented by the Eigen vectors, and the radius of the ellipsoid would be represented by the Eigen values (Alexander et al., 2008). If the ellipsoid was close to spherical the Eigen values would be very similar and diffusion would be considered isotropic. If the radius of the ellipsoid was much greater in one direction compared to the others the diffusion would be considered anisotropic and one Eigen value would be greater than the others. This is the case in homogenous white matter tracts in the brain.

Fractional anisotropy (FA) is a measure of the main direction of the random diffusion of water molecules (Basser et al., 1994). It is effectively a ratio of the 3 principle Eigen values (λ_1, λ_2, λ_3) the first indicating the main direction (i.e. the direction in which most of the water molecules diffuse), and other two being the directions the next largest amount of molecules are travelling in a given voxel. FA is given by the following equation, and is expressed as a decimal between 0 and 1:

$$FA = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}}{\sqrt{(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$$

FA is thought to be a measure of "structural integrity" of white matter fibre bundles, and reflects a combination of axon size, diameter, density and degree of myelination, and also the amount of crossing fibres within a given voxel (Wahl et al., 2007). Higher FA values are found in white matter tracts with higher levels of myelination. This is because the axonal cell membrane and myelin restrict the diffusion of water molecules, leading it only able to diffuse along the length of the fibre (Pierpaoli and Basser, 1996). Low FA values are found in areas where water molecules are largely unconstrained, such as the cerebral spinal fluid in the ventricles. FA values can also be low if there is more than one fibre bundle crossing in different directions within a given voxel. This is because diffusion will be high in more than one of the principle Eigen values, which would lead to a lower estimation of directional diffusivity.

Mean Diffusivity (MD) is a measure of the overall diffusion, regardless of direction, in a given voxel and is the mean of the 3 principle Eigen vectors. MD is likely to be high in areas where FA is low, because water molecules can move more rapidly when they are not constrained by cell membranes. Radial Diffusivity (RD) is the mean of the second two principle eigenvalues, and is a useful measure when considering the influence of crossing fibres on the FA value. An FA value may be low because of a reduction in myelination or axon density, but if RD is high FA is likely to be low because of branching or crossing fibres in the same voxel, meaning that the diffusion tensor is modelled well in multiple directions. However, RD can also reflect the degree of myelination of a fibre bundle. This is because diffusion in the perpendicular direction to the main Eigen vector relates to the permeability of axon membranes (Kochunov et al., 2012).

2.3 Assessing clinical measures

Tourette Syndrome symptoms were measured in each TS subject on the day of testing using the Yale Global Tic Severity Scale (YGTSS). The YGTSS involves conducting a semi-structured interview by a person trained in giving clinical interviews to people with Tourette syndrome. During this interview the TS subject is asked questions about their tics and behaviour over the past 2 weeks. Tics are separated into simple motor (e.g. eye blinking), complex motor (e.g. making a sequence of movements), simple phonic (e.g. throat clearing), and complex phonic (e.g. specific words or phrases). Subjects are questioned on each of these areas in turn and encouraged to describe their tics, including the frequency in which tics occur and the intensity of tics. Motor and phonic tics are scored by the researcher for their number, frequency, intensity, and complexity, and how much they interfere with normal day-to-day functioning. Questions regarding the overall impact that TS currently has on the individual's life (e.g. problems with self esteem, socialising with peers, and school work) are also asked to produce an impairment rating. The scale produces 3 separate scores: motor tics, phonic tics and impairment level. These can be summed to give an overall score of symptom severity for the last 2 weeks.

For all experiments described in this thesis, subjects were videoed during the interview. This meant that tics could be re-counted afterwards to aid the scoring process. Interviews were conducted either by a trained research nurse or by myself after training. As the impairment score is subjective, and can vary not only because of individual differences in personal experience, but also because of the subject's willingness to speak about their personal life, this score was not used in this thesis when relating the data collected to tic symptoms. Furthermore, most experiments in this thesis assessed motor functioning or motor areas of the brain, so the motor tic score was assumed the most appropriate measure to relate to such data.

Intelligence was measured using the Wechsler's Abbreviated Scale of Intelligence (WASI, Wechsler, 1997). The shortened version of the WASI was administered including the verbal reasoning and matrix reasoning sections (2 sub-tests). The verbal reasoning sub-test involved asking subjects to define a list of words that got increasingly more rare and scoring their response based on pre-written definitions. The matrix reasoning sub-test involved a series of pictures with a missing piece, and 5 possible solutions to the problem. Subjects had to indicate which solution best fitted the picture and there was only one correct answer. IQ scores were calculated dependant on the age of the subject. The WASI was administered to each subject (including control subjects) only once, when the volunteer was first recruited to take part in the experiments presented in this thesis. Only subjects with an IQ within the normal range (>80) were used. For each experiment a between-subjects t-test was performed to make sure the typically developing group did not have significantly higher or lower IQ scores than the TS group.

2.4 Subjects

16 adolescents with Tourette syndrome (13 males, mean age= 15.1 ± 3.7 years) were recruited from a specialized Tourette clinic based at Queen's Medical Centre, Nottingham. Details of TS subjects can be seen in Table 2.1. A group of typically developing age and gender-matched adolescents (3 female, mean age= 15.3 ± 3.7 years) were used as a control group. Each control subject was the same gender as, and born within 6 months of, their matched TS subject. On the day of testing the Yale Global Tic Severity Scale (YGSS) was administered to each TS subject by a trained clinical research nurse or myself. The Wechler Abbreviated Scale of Intelligence (WASI) matrix reasoning and verbal reasoning sub-tests were used to obtain an approximate IQ for TS and controls. A between-groups t-test confirmed there was no significant difference in IQ between TS and controls (TS mean = 108, control mean= 115, $t(30)= 1.6$, $p>0.1$)

Table 2.1: Clinical characteristics of subjects with Tourette Syndrome included in the DTI experiment (ADD = Attention Deficit Disorder, OCD= Obsessive Compulsive Disorder, ADHD= Attention Deficit Hyperactivity Disorder)

ID	Age (year.month)	Gender	IQ (WASI)	YGSS	Motor tic scores	Phonic tic scores	Co-morbidity	Medication
TS006	19.8	M	84	51	11	20	-	Clonidine
TS018	17.2	M	120	34	17	7	ADD	Clonidine
TS028	17.3	F	96	71	15	16	OCD	-
TS004	18.3	F	95	12	7	0	-	Clonidine
TS014	16.2	M	135	No tics last 2 months			-	-
TS043	11.3	M	123	66	13	13	-	Clonidine
TS031	16.3	M	133	56	24	14	-	Clonidine, Aripiprazol
TS048	13.8	M	118	43	13	0	-	Clonidine
TS049	20.2	M	116	64	21	13	-	Kapra
TS007	19.3	M	95	31	15	6	-	Clonidine
TS030	15.0	M	103	61	16	15	-	-
TS034	13.6	M	118	20	7	8	-	-
TS054	12.4	M	92	23	6	7	ADHD	-
TS058	7.8	M	96	39	16	8	-	-
TS059	8.5	M	142	34	14	10	ADHD	-
TS026	15.0	F	113	49	5	4	OCD	Fluoxetine

2.5 Procedure

A 3 Tesla Phillips MRI scanner with a 32-channel SENSE head coil was used to collect DTI data. DTI images were obtained using an EPI sequence consisting of 48 slices of 2x2x2mm voxels with a repetition time (TR) of 7803ms, 32 directions and a diffusion weighting (b-value) of 1000. This b-value was selected because it is in the ideal range for healthy adult brains of 800-1200. Lower b-vales risk no diffusion being recorded, whereas higher a b-value would risk no signal being recorded. Therefore a b-value of 1000 is thought to be a good compromise between diffusion and signal. The last volume for each subject has no diffusion weighting (b=0). This is so there is one clear image of the structure of the brain to align the diffusion-weighted data to during analysis. The slices were positioned using an initial survey scan; centred on the midline and angled along the anterior commissure - posterior commissure (AC-PC) line identified on the mid-sagittal slice.

A high-resolution T1-weighted anatomical image was collected in the same scan-session. The MPRAGE (Magnetization Prepared RApid Gradient Echo) consisted of 160 slices with 1x1x1mm voxel size, and a field of view of 240x160x224mm centred along the mid-plane of the brain and angled to follow the AP-PC line. The TR was 8.26ms, with a total scan time of roughly 4minutes.

Subjects lay flat on their back wearing ear plugs and ear defenders to protect from scanner noise. Foam pads were placed either side of the subject's ears to restrict head movement. Subjects were asked to keep as still as possible for the duration of each scan.

2.6 Between-Group Analyses: Data Processing

DTI data was processed using the FDT toolkit in FSL (Behrens et al., 2003a). Firstly all scans were eddy-current corrected, then the brain was extracted from the skull using the BET brain extraction tool, with a fractional intensity threshold of 0.3 (Smith, 2002). Diffusion maps were computed, including FA, MD, the 3 principle Eigen values ($\lambda_1, \lambda_2, \lambda_3$) and the 3 principle Eigen vectors (V_1, V_2, V_3). Diffusion maps were then transformed using nonlinear registration into 1x1x1mm standard MNI (Montreal Neurological Institute) space via a standard FA map template. 5 TS subjects and 3 control subjects had to be removed from further analysis following this stage due to a high level of noise in the data. This was attributed to head movement during the scan.

Further analysis was performed using FSL's Tract-Based Spatial Statistic (TBSS) toolbox (Smith et al., 2006). TBSS overcomes the limitations of a whole brain voxel-based approach by constraining the data that is used to the centres of main white matter tracts. This ensures that the FA or MD values used in the final analysis are from white matter of all the subjects. As such, an average white matter skeleton was created of the whole group using an intensity threshold of 0.2. The skeleton was used to extract just voxels of the main white matter tracts from each individual's FA and MD maps. This extracted data was analysed in 2 ways: a between groups voxel-wise permutation test to look at areas of significant difference between the TS group and the Control group, and a Support Vector Machine (SVM) analysis to find which voxels are predictors of a diagnosis of Tourette Syndrome.

2.7 TBSS results & Discussion

The between group voxel-wise test was calculated using the FSL function Randomize with 5000 permutations for FA and MD maps separately. Results were thresholded at $p<0.01$, corrected for multiple comparisons (family wise error). A cluster of significantly greater FA for the TS group compared to the controls was found (see Figure 2.1). This region was identified as part of the Cingulum (MNI co-ordinates: x=5, y=-2, z=43) by use of a human white matter atlas (Catani & Thiebaut de Schotten, 2012). No significant between-group differences in MD or RD were found.

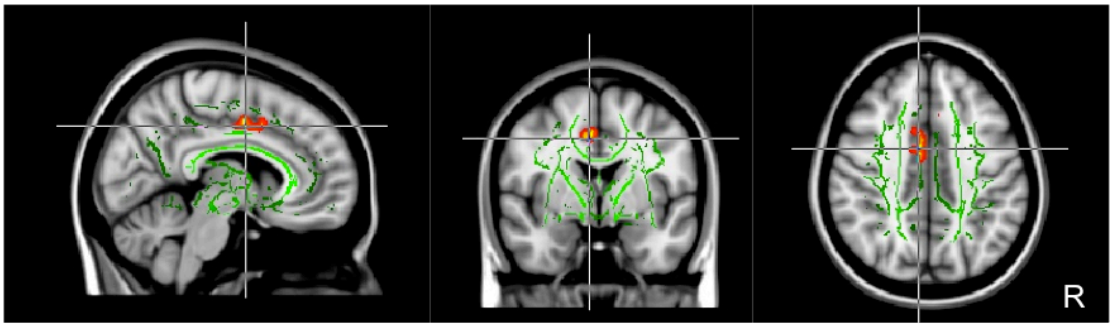

Figure 2.1: Region of increased FA in the TS group (yellow-red) overlayed on a standard MNI anatomical image. Green regions represent the average white matter skeleton. Cross-hairs positioned at MNI x,y,z co-ordinates: 5, -2, 43. This region has been identified as part of the Cingulum.

A region of interest of $5mm^3$ was centred at the peak difference of the identified cluster on each TS subject's standardized FA map. The average FA for this region of interest was calculated. The relationship between the average FA value for each subject with TS and their global tic score (YGSS), motor tic score, and phonic tic score, as measured by the Yale Global Tic Severity Scale on the day of testing, was then considered by a Pearson correlation analysis. As can be seen in Figure 2.2, there is a weak positive relationship between symptom severity and average FA in the cingulum, but this was not significant at a threshold of $p=0.05$ (Pearson's

R=0.47, p=0.068). No relationship with MD or RD with any measure of symptom severity was found.

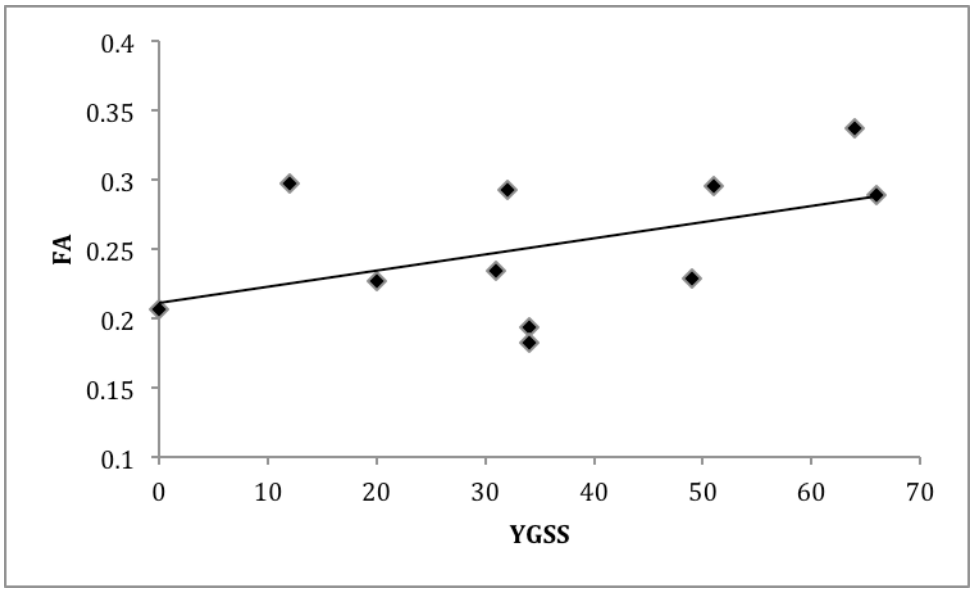

Figure 2.2: Relationship between FA value at region of interest and Yale Global Tic Severity Score (YGSS). Pearson's Correlation Co-efficient = 0.47, p=0.068.

The only area that was identified as significantly different between groups is a small section of the cingulum. The cingulum is a complex tract, made up of a range of fibres that project from and to many widespread areas, including: thalamus, anterior cingulate, pre-frontal cortex, insula, amygdala, the parahippocampal cortices, and it also contains multiple short association fibres (Jones et al., 2013). It is likely that this difference is a consequence of having TS, as it involves connections between areas in the basal ganglia-thalamic-cortical circuits previously implicated in TS (Albin & Mink, 2006). The white matter integrity measured from the Cingulum did not relate significantly to any measures of symptom severity, which suggests it does not play an important role in any compensatory mechanism to control tics, i.e. it is likely to be a result of having TS rather than an adaption to the disorder.

The TS group were not found to deviate significantly from that of a typically developing control group in the majority of white matter tracts. This does not concur with past research (e.g. Jackson et al., 2011; Thomalla et al., 2009), which highlighted different areas as significantly altered in TS, and found a relationship between white matter and tic symptoms. However, although white matter integrity is not outside the range of values in a typically developing population, there may be more subtle alterations that relate to the occurrence of or control of tics. The TBSS analysis is a univariate approach in which between group differences are assessed at each voxel independently, and then are subject to strict significance testing criteria in order to control for multiple comparisons. Although this strict threshold of significance provides strong evidence for between group differences in single voxels, a multivariate approach, such as machine learning algorithms, looks for patterns of voxel values that predict group membership. A multivariate approach may provide useful information about which tracts in the brain (or clusters of voxels) are related to a diagnosis of TS, without the need for strict significance testing. Following on from the initial TBSS analysis, a support vector machine learning approach was conducted to examine if there are more widespread white matter areas that are related to a diagnosis of TS.

2.8 Support Vector Machine Methods, Results & Discussion

Support Vector Machines (SVM) are learning machines that can classify sets of data into groups using pattern recognition and statistical modelling (Schölkopf et al., 1997). SVM achieves this by mapping the patterns non-linearly into a high dimensional space and searching for a hyperplane that best separates the data into specified classes (Schölkopf et al., 1997). Learning occurs by seeking out the most optimal hyperplane to classify the data. A very simple classification problem is depicted in Figure 2.3. In this example the SVM needs to find the most optimal function to separate the squares from the circles. You can think of the hyperplane as the boundary (2D line, in this example, but it would be a

plane in 3 dimensions) between the data points that would most successfully separate the 2 groups. However, often a straight line is not appropriate to separate the data based on its features. To get around this problem kernels are used with SVM. Kernels are similarity functions that allow the data to be transformed into higher (sometimes infinite) dimensions. The SVM can then more easily calculate the optimal separation boundary in this higher dimensional space.

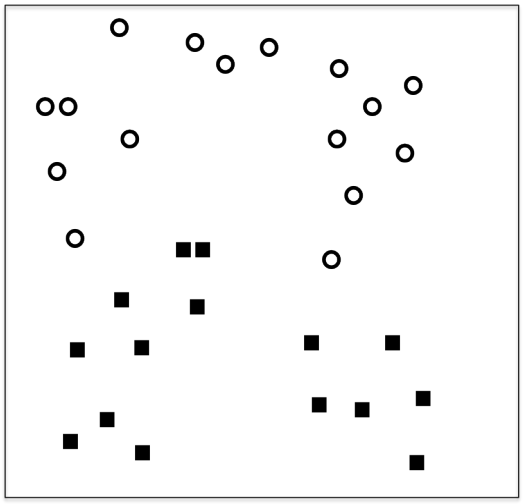

Figure 2.3: An example of a 2D classification problem: separate the squares from the circles.

In order to successfully create the optimal separation boundary to classify the data, the model needs a set of data to train on. This training data will encompass a set of attributes (i.e. features of the data, in Figure 2.3 these could be how round it is, or if it is filled or not) and each data point needs to be labelled with at least one target value (i.e. a classification, in the simple example in Figure 2.3 this would be squares vs. circles). If the SVM is successful it will produce a model that will correctly predict the classification of new (test) data, based on the new data's attributes, following training (Hsu, Chang & Lin, 2010). So if I were to enter a new square into the model, it should classify this data as square by placing it on the "square" side of the boundary.

Large sets of training data are required in order to create an optimal model to handle test data. However a "leave one out" method allows for the successful creation of an optimised model without the need for a large training set. The method involves classifying each data point based on training from the entire data set minus the current "test" data point. This way, the data set you are working with acts as both the training and the test sample. Because of the lack of a large training data set, this is the method I used for my research.

The data used were FA values extracted from each subject's normalised FA map using the average white matter skeleton as a mask. In this instance the magnitude of the FA value can be considered the attribute of the data, much like the roundness of a shape is in the example above. These values were entered into a 3D data matrix in Matlab (The Mathsworks). The data were represented in the matrix as such: each subject's FA values were entered into a 2D matrix so that the x and y axes represent the location of a particular white matter voxel. The z dimension represents the subject number. In essence, the matrix is composed of each subject's FA data in 2D space, aligned on top of one another, to produce a 3D matrix. A separate matrix labelled each grid of data as belonging to a TS or control subject. This "classifier" matrix meant the program could use the data to train on, as it labelled each data point with a target value of TS or control (much like the classifier of square or circle used in the example from Figure 2.3).

An in-house Matlab script using the libsvm toolbox (Chang & Lin, 2011) was used to implement the SVM analysis. A Radial Basis Function (RBF) kernel was used as follows:

$$K(x,y) = e^{-\gamma ||x - \gamma||2}$$

where (x,y) is the training data (inputs). The RBF kernel maps samples into higher dimensional space non-linearly. This means it can handle non-

linear relationships between attributes and classes, which may be appropriate particularly when the training dataset is small (Hsu, Chang & Lin, 2010). The RBF kernel has 2 parameters γ and C (the penalty parameter of the error term). A grid search was conducted to optimize parameters γ and C of the model. This meant that the model was trained over and over again using different combinations of γ and C, ranging from -20 to +20 in steps of 1. The values of γ and c that produced the model with the highest classification accuracy (i.e. that classified a data point as belonging to the correct group the most amount of times) were selected for the final model. As previously stated, a "leave one out" method was used. This meant that each voxel of interest for each subject was classified as belonging to a TS or control subject based on training from n-1 subjects. Once the same voxel had been classified for every subject the accuracy of the model to fit the data at that point was calculated. The data were then finally trained using the optimized model ($\gamma>14$, $C<-7$). This showed that a diagnosis of TS can be predicted from FA data with a maximum accuracy of 76.9% (see Figure 2.4). This means that at certain points in the data set (i.e. certain white matter voxels) a new test data point can be correctly classified as belonging to a control subject or an individual with TS about 77% of the time.

As can be seen in Figure 2.4, not all voxels were predictive (i.e. they were correctly classified as belonging to a TS or control brain less than 50% of the time). Therefore, this technique highlighted which white matter tracts are associated with TS to the extent that they are predictive of the disorder within the current dataset. By this I mean the FA values from specific tracts were correctly classified as belonging to the TS or CS groups on 77% of the time when tested. Identified tracts that demonstrated a high classification accuracy included: corpus callosum, anterior commissure, fornix, cortico-spinal tract, cingulum, inferior fronto-occipital fasciculus, and internal capsule (identified by use of a white-matter atlas: Catani & Thiebaut de Schotten (2012), see Figure 2.4).

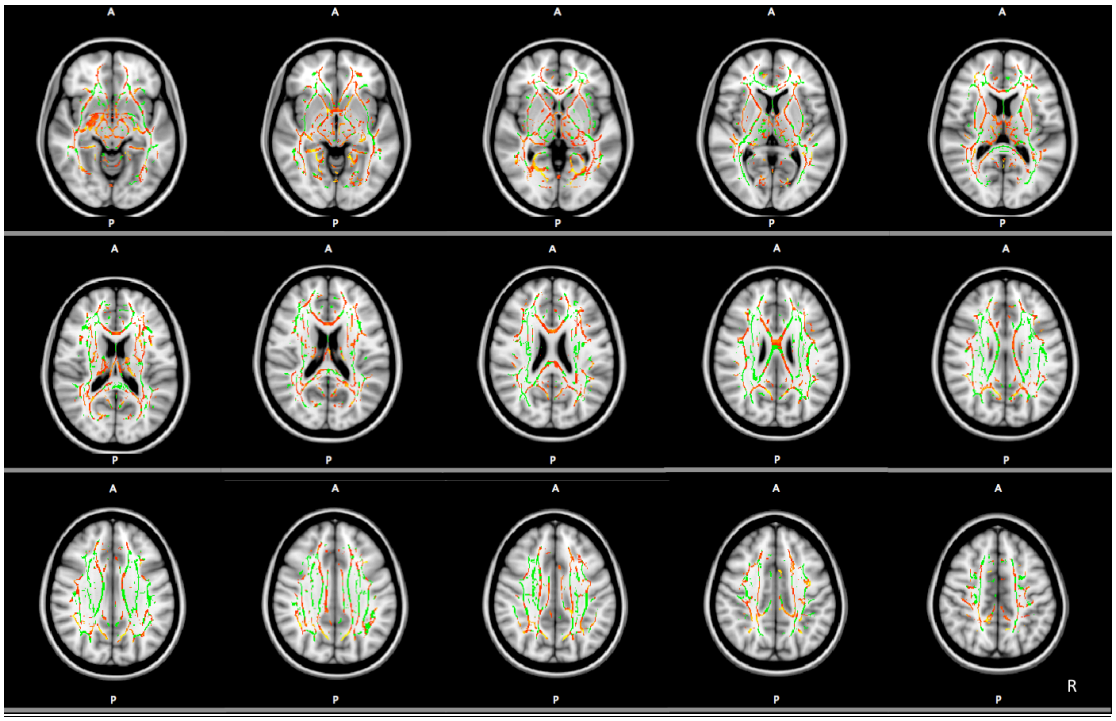

Figure 2.4: Regions of white matter that are predictive of a diagnosis of TS (yellow-red). Accuracy of prediction ranges from 57.7%-76.9%. Results are displayed in the axial plane in slices ranging from z=-9 (top left) to z=45 (bottom right) in MNI co-ordinates. Green regions represent the white matter voxels that were entered into the model (average skeleton).

The support vector machine learning approach has highlighted that although a univariate analysis of FA values indicates that FA does not differ significantly in TS from the FA values measured in a typically developing population, the multivariate analysis demonstrates that there are many tracts that may be strongly predictive of the disorder. Some of these are likely to be predictive because they are part of the cortico-striato-thalamo-cortical loops, thought to be dysfunctional in TS. The internal capsule and fornix are examples of this. The cortico-spinal tract may be reflective of the symptoms themselves; people with tics will make certain movements a lot more frequently than people without, therefore alternations in the direct pathways from cortex to muscles are likely to be affected. However, some tracts may also be predictive of TS because they are areas of compensatory changes that adapt in response to the disorder.

The corpus callosum may be an example of this, and was considered as such in a tractography-based approach.

2.9 Tractography of the Corpus Callosum in the TS group

Following on from the between-group analyses, it is clear that multiple white matter tracts are related to a diagnosis of TS. The corpus callosum is of particular interest because its role is to integrate information across the hemispheres. It achieves this by modulating excitability of homologous cortical areas, i.e. stimulation of a particular area of cortex in one hemisphere has an excitatory effect on the homologous area of the contra-lateral hemisphere (Bloom & Hyde, 2005). A relationship has previously been reported between motor cortical excitability, as measured by TMS motor threshold, and FA in the corpus callosum (Klöppel et al., 2008). Higher cortical excitability (i.e. lower motor thresholds) was related to higher FA values, which demonstrates further the role of corpus callosum in modulating cortical excitability. This is important because alterations in cortical excitability have been robustly demonstrated in TS. For example, there is evidence from Transcranial Magnetic Stimulation (TMS) studies of increased resting motor threshold and increased intra-cortical facilitation, suggesting increased excitability in primary motor cortex (M1) for adults with TS (Orth & Rothwell, 2009; Hiese et al. 2010). This increased excitability may be exacerbated by reduced intra-cortical inhibition that has also been observed in TS (Ziemann et al., 1997; Gilbert et al. 2004; Orth & Rothwell 2009).

There is also evidence for increased functional connectivity between M1 and the Supplementary Motor Area (SMA) in adults with TS during movement preparation and execution (Franzkowiak et al., 2012). The authors suggest that it is this increased input from the SMA that leads to the increased excitability in M1 that has been observed in subjects with TS. However, there is evidence from TMS studies of *decreased* M1 excitability during movement preparation in TS (Draper et al., 2013*;*

Jackson et al., 2013; Heise et al., 2010) and at rest (Orth et al., 2008). Furthermore, reduced fMRI BOLD signal change, and co-activation across hemispheres, in M1 and SMA has been observed during volitional movement in TS (Jackson et al., 2012; Thomalla et al., 2013). The decrease in excitability of M1 and SMA has a negative relationship with tic scores such that those with the lowest fMRI BOLD signal change have the least tics (Jackson et al., 2012). Furthermore, it has been demonstrated that tic symptoms can be dramatically reduced for up to 3 months following an inhibitory repetitive TMS paradigm, aimed at reducing cortical excitability, delivered to the SMA (Mantovani et al., 2007; Kwon et al., 2011). This suggests that a mechanism may be developing to reduce the excitability of M1 and SMA during volitional movement, which may be beneficial in controlling tic symptoms (Jung et al., 2013). For this reason, the regions in the body of the corpus callosum that connect M1 and SMA across hemisphere are of particular interest for the current study.

The corpus callosum has been the focus of attention in past research into structural abnormalities in TS. Although there is substantial evidence to suggest alterations in the corpus callosum in TS, the way in which it is altered, and the consequences of changes in connectivity, is unclear. In terms of the size of the structure, voxel-based morphometry studies have reported it to be increased in adults with TS (Peterson et al., 1994; Moriarty et al., 1997; Plessen et al., 2004) and both reduced (Plessen et al., 2004) and increased (Baumgardner et al., 1996) in children with TS. Diffusion Tensor imaging (DTI) studies have reported increased FA in the corpus callosum in adults with TS (Neuner et al., 2011), and reduced FA in children and adolescents with TS (Plessen et al., 2006; Jackson et al., 2011). The contradictory findings in corpus callosum size appear to be age-dependent and may indicate that those who continue to have tics into adulthood do not develop the same white matter microstructure as those who recover. Myelination of white matter of the corpus callosum continues to develop throughout puberty and into early adulthood (Keshava et al., 2002). Taken together the evidence suggests

that adaptive white matter changes in the corpus callosum may be developing over adolescence as individuals gain control over their tics, and may be reflected in altered cortical excitability in SMA and M1.

To investigate this, I considered if inter-hemispheric connectivity between M1 and SMA differs in adolescents with TS compared to a typically developing control group. By using a probabilistic tractography method I identified the regions in the body of the corpus callosum where the white matter projections from M1 and SMA cross the hemispheres. As previous research has highlighted the forceps minor as adaptively altered in TS (Jackson et al., 2011), the region in the genu of the corpus callosum that connects the prefrontal cortex (PFC) was also considered as a region of interest. Finally, the section in the splenium of the corpus callosum that connects to primary visual cortex was considered as a control site, as it is unlikely that adaptive alterations will occur in this pathway. A control site was used to examine if alterations in inter-hemispheric connectivity are region-specific, rather than apparent in the corpus callosum as a whole. I also considered how the white matter integrity in these regions is related to tic symptoms. If the connectivity of these regions is important for the development of control over tics, by altering levels of cortical excitability, is it likely that measures of white matter microstructure will relate to tic symptom severity.

2.10 Tractography: Data processing

Brain Voyager QX 2.3 software was used to process DTI data for probabilistic tractography. DTI data was co-registered to each subject's anatomical image (MP RAGE). Then FA and MD maps were created in native space, i.e. the data was not transformed into standard space. This tractography approach was performed entirely in native space because of white matter tracts being particularly susceptible to transformation errors when transforming into standard space. Specific tracts can be located and identified more accurately working in native space. The primary motor

cortex was identified on each subject's anatomical scan by finding the characteristic Ω shape in the axial plane of the motor hand area bilaterally. The central sulcus was then followed dorsally in the axial plane and the pre-central gyrus was marked as a ROI (Figure 2.5A). The fibres were then tracked from the primary motor cortex in both hemispheres. The area of the body of the corpus callosum where the fibres from each side crossed hemispheres was marked and a 6mm^3 ROI was centred on this position (Figure 2.5B). The fibres projecting from this ROI were then tracked back to the primary motor strip to check the ROI was correctly positioned (Figure 2.5C). The mean MD and FA values from just the central 6mm^3 ROI were then calculated.

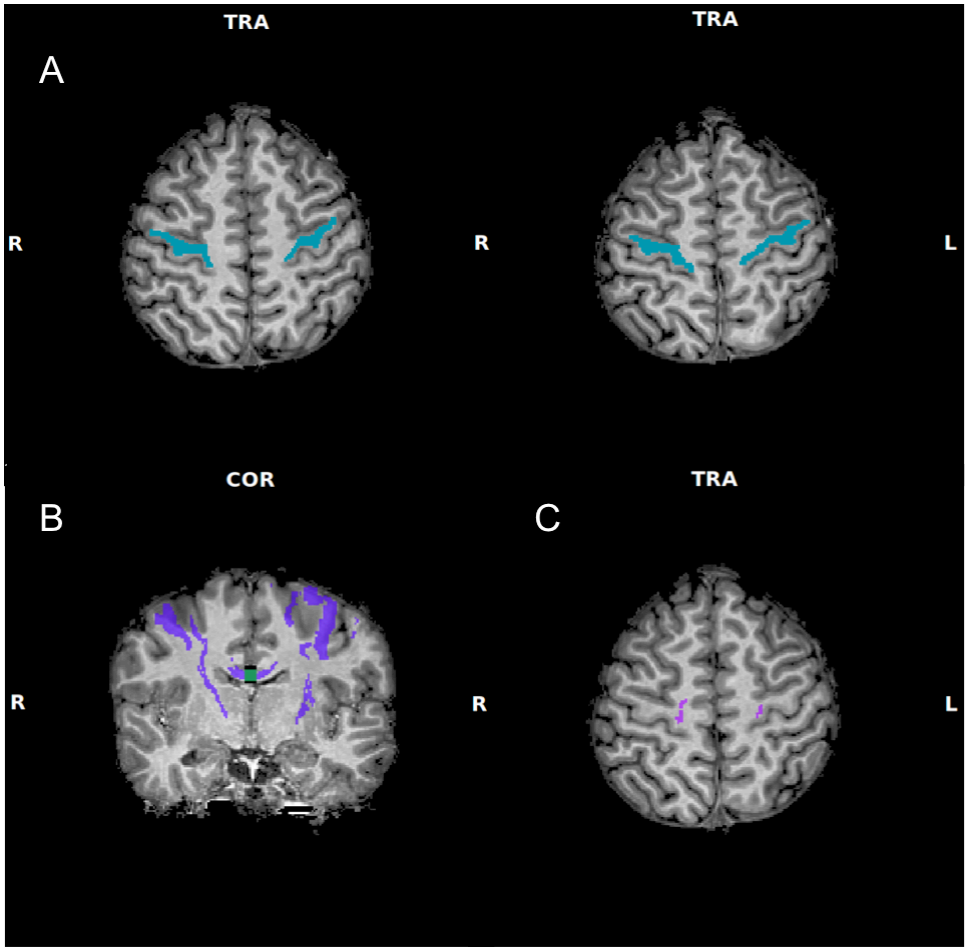

Figure 2.5: A*: Highlighted example of an ROI in the primary motor strip in 2 axial slices.* B*: Fibres tracked from the primary motor strip (purple) and the location of the 6mm^3 ROI in the body of the corpus callosum where the fibres cross hemispheres.* C*: Fibres tracked from the central 6mm^3 ROI terminate in the primary motor strip.*

The Supplementary Motor Area (SMA) was identified as the medial section of the gyrus anterior to the pre-central gyrus (the superior frontal gyrus). The SMA was marked as a ROI and the fibres tracked from this ROI (Figure 2.6A and B). A 6mm^3 ROI was placed in the body of the corpus callosum where the fibres crossed hemisphere (Figure 2.6B). The fibres from this central ROI were then tracked back up to the SMA (Figure 2.6C). The mean MD and FA values from the central 6mm^3 ROI were then extracted.

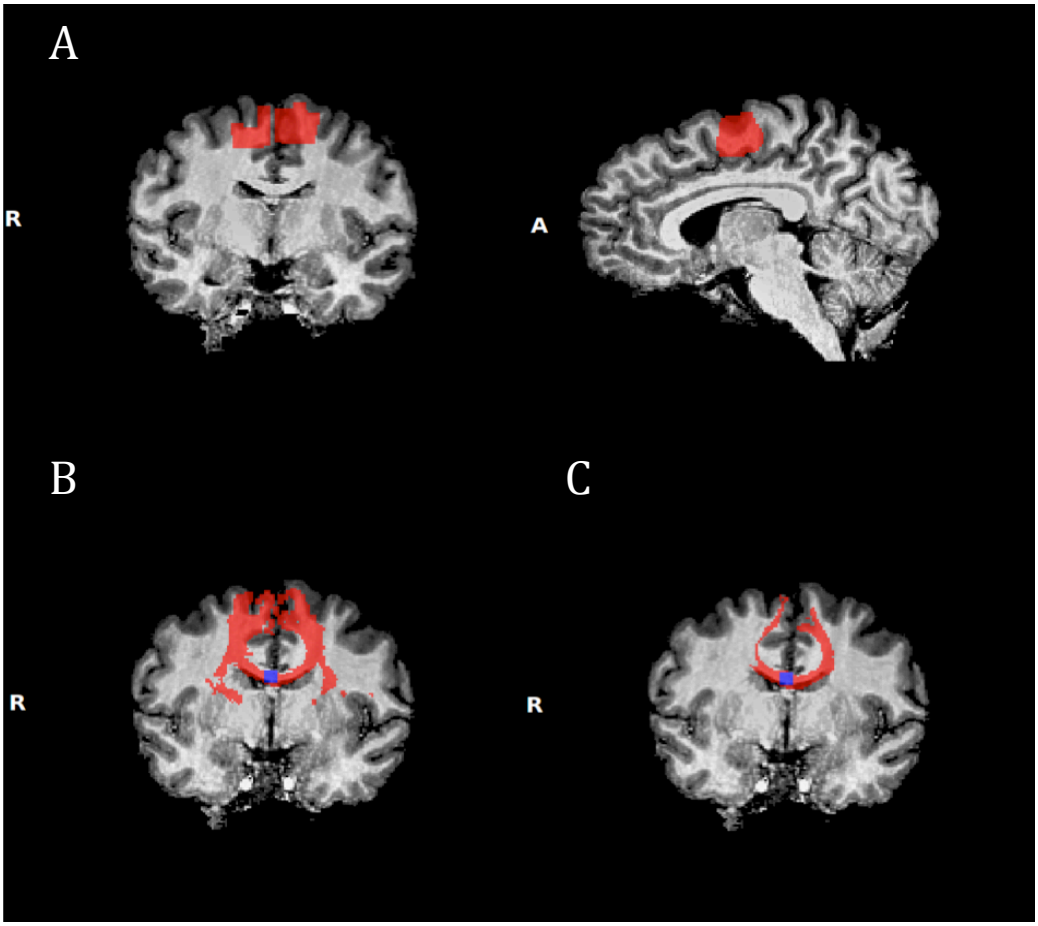

Figure 2.6: A*: Highlighted example of an ROI in the supplementary motor area in the axial and sagittal plane.* B*: Fibres tracked from the SMA (red) and the location of the 6mm^3 ROI in the body of the corpus callosum where the fibres cross hemispheres (blue).* C*: Fibres tracked from the central 6mm^3 ROI terminate in the SMA.*

The forceps minor were identified by highlighting the pre-frontal cortex and tracking the fibres from this region to the genu of the corpus callosum. A 6mm cubic ROI was placed in the genu where the fibres crossed hemisphere and FA and MD values from this region were extracted. Finally, the area surrounding the calcarine sulcus bilaterally was identified as the primary visual cortex (V1) to be considered as a control site. A 6mm ROI was placed in the splenium of the corpus callosum where the fibres tracked from V1 crossed hemispheres. The mean MD and FA values for this V1 ROI were extracted for each subject.

2.11 Tractography Results & Discussion

Firstly, a series of between-groups t-tests compared the control group and the TS group in measures of FA or MD for the PFC, V1, M1 and SMA ROIs, and found no significant between-group differences ($t(22)<1.4$, $p>0.1$ for all tests). Then, TS subjects' individual FA and MD score for each ROI were correlated with measures of tic symptoms from the YGTSS (motor tic score, phonic tic score and YGSS) and their age, using Pearson's R.

One subject (TS014) was removed from the correlation with tic scores, as they had no reported tics in the past 2 months, meaning the YGTSS could not be completed. A significant positive relationship between motor tic scores and FA in SMA (Pearson's $R=0.87$, $p<0.001$) and M1 (Pearson's $R=0.69$, $p<0.05$) was found (see Figure 2.7A and 2.7B). A negative relationship of the same strength was found between MD and motor tic scores in SMA (Pearson's $R=-0.85$, $p<0.005$) and M1 (Pearson's $R=-0.69$, $p<0.05$, see Figure 2.7C and 2.7D). There were no significant correlations between FA or MD and any other YGTSS measure, or subject age. There was no significant correlation between any YGTSS measure and FA or MD measured from the V1 voxel (max $R=0.35$, $p>0.2$), or the PFC voxel (max $R= 0.50$, $p>0.08$). See Figure 2.8 for V1 and PFC correlations.

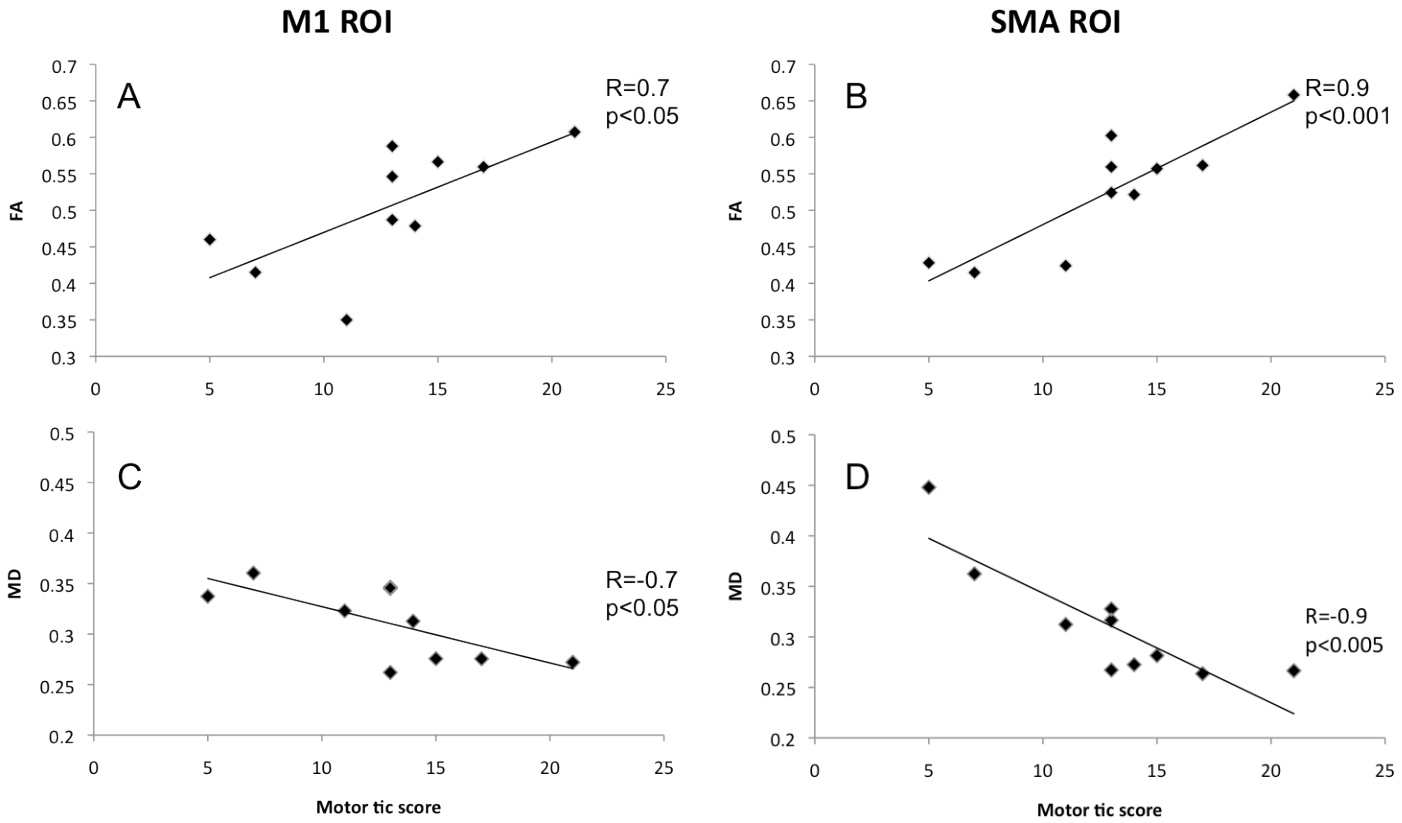

Figure 2.7: The relationship between motor tic scores and amount of FA (A and B) and MD (C and D) in a 6mm³ ROI centred in the region of the body of the corpus callosum that projects to M1 (A and C) or SMA (B and D).

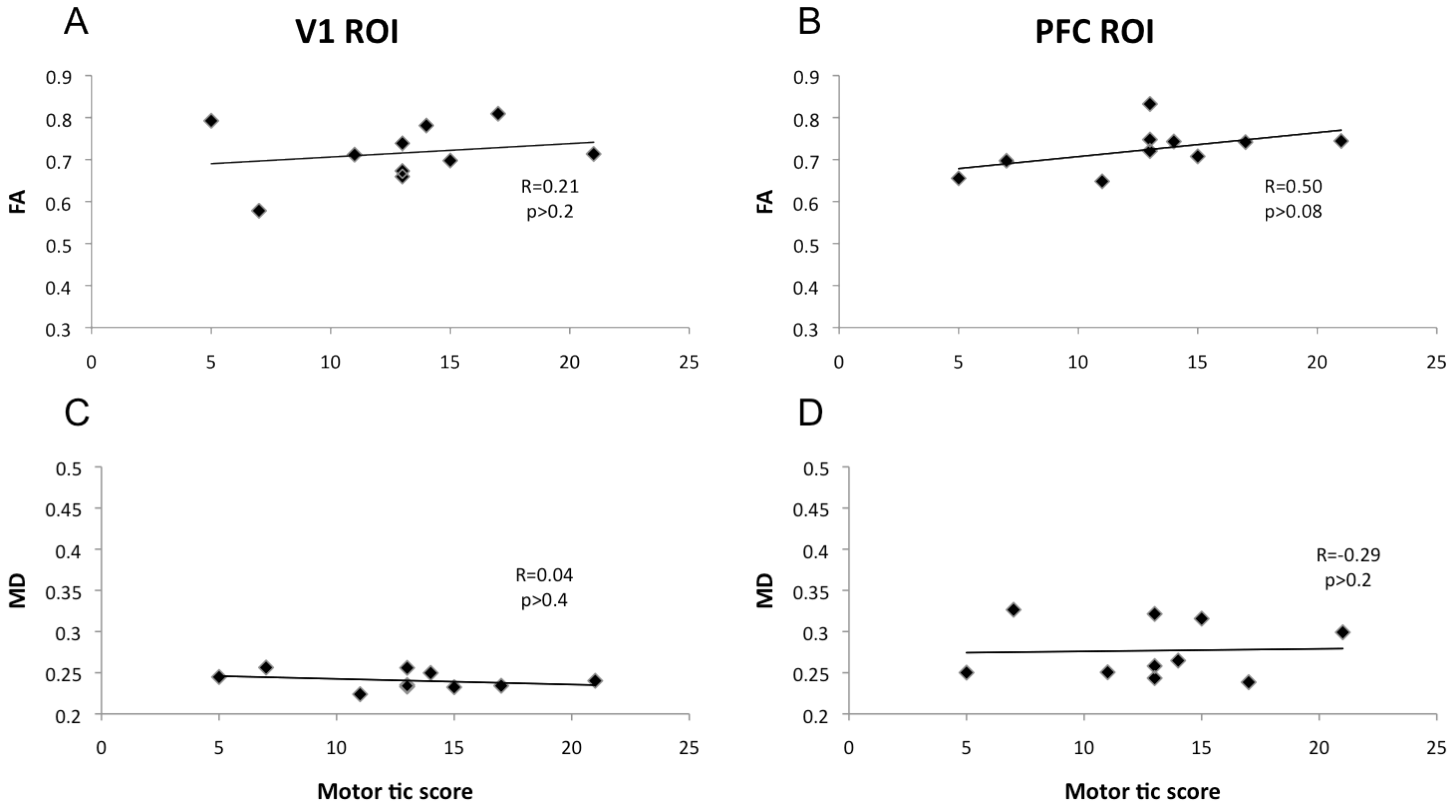

Figure 2.8: The relationship between motor tic scores and amount of FA (A and B) and MD (C and D) in a 6mm³ ROI centred in the region of the splenium of the corpus callosum that projects to V1 (A and C) or the region of the genu of the corpus callosum that projects to PFC (B and D).

DTI and probabilistic tractography were used to measure the integrity of inter-hemispheric connections, across the body of the corpus callosum, between M1 and SMA in a group of adolescents with TS. It was found that the stronger the integrity of the white matter fibre bundles within these areas (i.e. higher FA value), the more severe were the motor tic symptoms for that subject. The same relationship was not found in tracts that lead to V1 or PFC, and therefore cannot be accounted for by age of subject. There was also no significant difference between the TS group and an age and gender matched control group in FA or MD in the areas of the corpus callosum that were measured. This means that, although white matter integrity is not different to a typically developing matched population, it is nevertheless still predictive of tic symptoms. The lack of a relationship with V1 projections suggests that only distinct regions of the corpus callosum, that are involved the production and control of movement, are related to TS symptoms, rather than the structure as a whole. Although there is a slight trend between FA measured from the ROI from the forceps minor and motor tic score, the relationship does not reach statistical significance.

2.12 Discussion

Diffusion tensor imaging was implemented to measure white matter structure in a group of adolescents with Tourette syndrome and a matched group of typically developing controls. A standard tract-based spatial statistics (TBSS) approach revealed a significant increase in FA for the TS group compared to control group in a small region of the Cingulum. However, a support vector machine approach showed that a much wider set of white matter pathways are predictive of a clinical diagnosis of TS. This suggests that subtle alterations in white matter structure may be quite wide spread in TS, but that these differences do not deviate enough from the typically developing population to produce a significant result in the TBSS analysis. Finally, by using precise probabilistic tractography methods, in regions thought to be associated with tics, I have

demonstrated that the white matter of the body of the corpus callosum is highly correlated with symptom severity, suggesting that it might be important for the control of , or production of tics, even if the white matter is not significantly different to an aged-matched control group. This relationship is only apparent in the inter-hemispheric connections of bilateral SMA and M1, and is not reproduced in inter-hemispheric connections of pre frontal cortex or primary visual cortex.

One interpretation of the positive relationship between motor tic scores and white matter integrity is that those with lower tic scores, through the natural occurrence of white matter development that is on-going throughout adolescence, do not develop the same amount of white matter compared to those that continue to have more severe symptoms. This could be achieved either by a reduction in the amount of fibres (decreased axon density), or a reduction in myelination, as FA is a reflection of both of these in the body of the corpus callosum. As the corpus callosum exhibits an excitatory influence to integrate information across hemispheres, reducing this cross talk is one way to decrease the hyper-excitability in motor cortical areas found in subjects with TS (Orth & Rothwell, 2009; Hiese et al. 2010). Suppressing the activity of the SMA through inhibitory rTMS has been shown to reduce tic symptoms (Mantovani et al., 2007; Kwon et al., 2011). It is therefore possible that reducing the amount of excitatory connections across hemispheres to this area has the same effect. This is further supported by the observation that reduced cortical excitability (in this case measured as fMRI BOLD signal change prior to a volitional movement) in M1 and SMA is also related to a reduction in tics (Jackson et al., 2012; Thomalla et al., 2013). The same relationship is also reflected in reduced inter-hemispheric co-activation of the SMA during a motor task (Thomalla et al., 2013). This supports the proposal that a reduction in inter-hemispheric connectivity through the corpus callosum leads to a reduction in excitability in the SMA and M1, which may be beneficial to reducing tic symptoms. This concurs with the

theory proposed by Jung et al. (2013) that a reduction in tics may occur through local changes to the excitability of sensorimotor cortex.

However, the current method of research cannot determine whether white matter in the corpus callosum is adaptively altered as a reaction to over-stimulation from a dysfunctional basal ganglia, or if those who have milder tic symptoms just happen to be genetically predisposed to develop less white matter connections in these areas. Those who are genetically pre-disposed to develop a larger corpus callosum, and have less potential for white-matter plasticity, may struggle to gain control of their tics as a consequence. This highlights the need for a longitudinal study to map changes in connectivity, along with symptom severity, throughout adolescence.

It is also important to note that FA is not a straightforward measure; it is assumed to reflect a combination of axon size, density and degree of myelination, and can be affected by crossing fibres. However, the ROIs were selected from a region with a high density of uniform, not crossing, axon fibres. It is likely that FA measured from these regions mainly reflects fibre density (Wahl et al., 2007). FA measured from the body of the corpus callosum that projects to M1 has also been found to directly relate to measures of inter-hemispheric functional connectivity (Wahl et al., 2007). Therefore I can assume that the relationship found in the current study is as follows: a reduction in the density of axons connecting SMA and M1 across hemispheres relates to less severe tic symptoms, which is likely due to a reduction in excitatory co-activation (functional connectivity) across hemispheres.

The observation that adults with TS have larger corpus callosum volumes than children with TS could demonstrate that a reduction FA in this area is important for tic remission; those who do not develop in this way are those who do not show a remission in symptoms (Plessen et al., 2004). Active tic-suppression could lead to plastic alterations in white

matter development over adolescence in TS, leading to the reduction in corpus callosum volume. This potential mechanism has been previously demonstrated in typically developed individuals. For example, FA measured from the region of the body of the corpus callosum that projects to the SMA has been found to be related to bimanual motor skill proficiency in healthy adults (Johansen-Berg et al., 2007). The authors suggest this relationship may occur through environmental pressures during key stages of white matter development, e.g. learning a complex motor skill such as playing piano, may lead to plastic changes in white matter micro-structure, which in term, lead to better performance in other bimanual motor tasks (Johansen-Berg et al., 2007; Schlaug et al., 1995). Tics and tic suppression can be considered as environmental pressures that affect the development of the body of the corpus callosum. Indeed, the enhanced performance in motor control tasks that has been demonstrated in TS is a key example of motor task performance relating to white matter structure (Mueller et al., 2006; Jackson et al., 2007; Jackson et al., 2011).

Although the current research did not find any significant between-group differences in the corpus callosum in a TBSS analysis, this could be a consequence of the sample used. As it was quite small, and included a range of ages throughout late childhood and adolescence, it may have not had a large enough of a sample to observe the alterations that have previously been shown by traditional TBSS methods (e.g., Jackson et al., 2011). However, the machine learning approach clearly outlined predictive tracts, highlighting many tracts that have been previously related to TS, including most of the corpus callosum. This suggests that the sample is representative of the disorder in the age range tested. Machine learning approaches are valuable and should be incorporated in future research, perhaps alongside traditional statistical tests, to highlight areas that are related to a certain disorder that might be missed by the strict thresholds of standard univariate tests, particularly in clinical groups where sample sizes are usually small. There is also the potential of using these kinds of techniques to aid in diagnosis of disorders that are difficult

to diagnose from observing behaviour alone (Ecker et al., 2010). With longitudinal data models could be developed that can predict clinical outcome (e.g. who is likely to have a reduction in symptoms in early adulthood), and responsiveness to certain therapies, based on brain structure alone.

Previous research has also suggested that tic reduction is largely mediated through the prefrontal cortex exhibiting greater influence over motor areas through enhanced connectivity (Plessen, Bansal & Peterson, 2009). However, my current findings suggest that changes in local white matter microstructure of the SMA and M1 predict tic symptoms, and are likely to play a key role in tic reduction and modulating cortical excitability. These regions are structurally and functionally distinct from the pre-frontal cortex, and the connectivity of these areas has also been related to motor task performance (Jackson et al., 2011). Although the pre-frontal cortex undoubtedly plays a key role in control of behaviour, the current results highlight the importance of considering the role of development of local, inter-hemispheric connectivity in the control of tics.

To sum up, it was found that the amount of FA and MD, measured from the body of the corpus callosum where fibres project to the SMA and M1, are highly correlated to tic symptoms in a group of adolescents with TS. It is hypothesized that alterations in white matter development of these key areas contribute to the reduction of tics seen in the majority of TS cases. This is likely to occur via modulating the cortical excitability in these areas through reducing inter-hemispheric excitation. To confirm this, a longitudinal study would allow white matter development to be followed throughout adolescence and how it relates to tic symptoms in TS could be evaluated. Machine learning algorithms could be used to assess more widespread changes in white matter that are related to a disorder but do not deviate far enough from the normal range to be highlighted in traditional statistical tests. By using longitudinal data, potential models could be developed to predict clinical outcome.

Chapter 3: Grey Matter thickness in Tourette Syndrome

3.1 Introduction

The previous chapter used diffusion tensor imaging to identify widespread differences in white matter micro-structure for subjects with TS. It is therefore reasonable to expect that these white-matter alterations will also be reflected in the cortical grey matter. This issue will be examined in this chapter.

An early study of alterations to cortical structure in children and adults with TS reported widespread changes in dorsal pre-frontal, parieto-occipital and inferior occipital regions (Peterson et al., 2001). More specifically, Peterson et al. (2001) reported larger dorsal prefrontal volumes for the children with TS, which was associated with less severe tic symptoms. The adults with TS tended towards smaller dorsal prefrontal volumes, although this finding was not significantly different to the control group. The children also had smaller volumes in premotor regions, including the SMA, whereas the adults had increased premotor volumes when compared to control groups. Children and adults with TS had larger parieto-occipital volumes but only the child group had larger inferior occipital volumes and smaller subgenual volumes. From these findings the authors propose a hypothesis that the prefrontal, parieto-occipital and inferior occipital regions form an action-attention system that is engaged to successfully suppress tics. Conversely, premotor and subgenu regions contribute to the motivation, planning and execution of actions, and alterations in these areas are likely to contribute towards tic production, or be consequence of long-term tics. Peterson et al. (2001) also comment that the differences between the adult and the child sample are because those who continued to have tics into adulthood did not develop the same compensatory cortical alterations.

Following on from this, Sowell et al. (2008) looked at cortical thickness in children with TS and reported widespread thinning in the frontal and parietal lobes compared to a matched typically developing control group. This thinning was most apparent in the dorsal regions of sensorimotor areas that are involved in vocalisations and movements of the hands, arms, neck and face. These areas are involved in typical tic-expression and cortical thickness was negatively related to tic severity. This means that the subjects with more severe tic symptoms had the thinnest cortex in dorsal regions of sensorimotor areas. This led the authors to suggest that cortical thinning in specific regions of the sensorimotor cortices are evidence for the involvement of these regions in the pathogenesis of Tourette syndrome. A further interesting finding by Sowell et al. (2008) is that the younger children in the sample showed no significant difference in terms of cortical thickness in the sensorimotor regions compared to healthy controls. From this evidence they concluded that the cortex thickens with age in typically developing individuals and that this process is somehow impaired in TS.

However, without longitudinal data it is very difficult to support this hypothesis. Indeed, longitudinal data from typically developing children has been examined by Giorgio et al., (2010) who report widespread reductions in cortical thickness with age throughout adolescence, including the postcentral gyrus (Giorgio et al., 2010). Furthermore, Lebel et al. (2008) report that in typically developing populations total brain volume stays constant from around the age of 5 years, to at least 30 years. This is because as white matter volume increases with age (as discussed in Chapter 2), grey matter decreases with age over adolescence, which keeps total brain volume at a constant. Kochunov et al. (2011) also report grey matter decreases with age, but they refer specifically to cortical grey matter thickness, not just whole-brain grey matter volume. In concurrence with Lebel et al. (2008), Kochunov et al. (2011) report a steady decrease in grey matter thickness with age throughout the lifespan. In their large cross-sectional sample, this

decrease in grey matter thickness occurs alongside increases in white matter FA reported between the ages of 10 years and early thirties (Kochunov et al., 2011). This means that maximum grey matter thickness must occur before the age of 10 years, which sheds doubt on Sowell et al.'s (2008) conclusion that the TS group is developmentally delayed.

The association between the thickness of specific regions of sensorimotor cortices and specific tic symptoms has since been reported in adults (Worbe et al., 2010) and young people (Fahim et al., 2010) with TS. Fahim et al. (2010) reported cortical thinning in their TS group vs. a control group in the left primary motor and somatosensory cortices and right parietal and orbitofrontal regions. The thickness of the right orbitofrontal cortex and left somatosensory cortex had a negative correlation with tic severity. Furthermore, although both groups showed that cortical thickness reduces with age, the TS group had more cortical thinning with age compared to the typically developing control group in the right parietal cortex and right orbitofrontal cortex.

Worbe et al. (2010) split their adult sample into 3 sub-groups: just simple tics, simple and complex tics, and co-morbid OCD. For the entire sample, widespread cortical thinning was reported in the frontal lobe, including motor cortex, superior frontal gyrus, middle frontal gyrus and the orbito frontal gyrus. For the simple tics group, cortical thinning was most prominent in premotor cortex, and the section of primary motor cortex that contains representations for the upper face and upper and lower limbs. For the complex tics group cortical thinning was observed in the posterior middle and inferior frontal gyrus, the region of motor and somatosensory cortex that contain face representation, the anterior and inferior parietal cortex, and the lateral orbito frontal group. For both groups there was a negative relationship between tic scores and cortical thickness. Interestingly, the OCD sub group did not have any significant alterations in grey matter volume in the same regions as the uncomplicated TS groups, but a region of interest approached revealed

that they did have a significant reduction in hippocampal volume not present in the TS-only groups.

Together, these 4 studies present a somewhat confused picture. What is clear is that adults and children with TS have reduced cortical thickness in the primary sensorimotor cortices, and the thickness of these areas is negatively correlated with tic symptom severity (Worbe et al., 2010; Fahim et al., 2010; Sowell et al., 2008). This has lead to the hypothesis that reduced volume in the primary sensorimotor cortices is part of the pathogenesis of TS, as they form part of the cortico-striatal-thalamic-cortical circuitry that is dysfunctional, and because the subjects with less severe tics also had greater grey-matter volumes in these areas. The volume of the dorsolateral prefrontal cortex has been reported as increased in children and decreased in adults with TS and it has been reasoned to develop as part of an inhibitory mechanism to reduce tics (Peterson et al., 2001). However, this finding has not been replicated in more recent studies of cortical grey matter volume of children and young people with TS (Sowell et al., 2008; Fahim et al., 2010), but Worbe et al. (2010) have reported reduced pre-frontal volumes in adults with TS.

In this chapter I will present a cortical thickness analysis that was used to examine any differences in the grey matter structure in a group of young people with Tourette syndrome and an age and gender matched typically developing control group. Based past research on cortical thickness, it was predicted that there will be widespread alterations in the frontal, parietal and occipital lobe for the TS group compared to the control group. It was also expected that grey matter thickness in sensorimotor cortices would be negatively related to tic scores from the YGTSS taken on the day of testing.

3.2 Magnectic Resonance Imaging Procedure

High-resolution T1-weighted anatomical images were taken of the TS and matched control group. The MPRAGE (Magnetization Prepared RApid Gradient Echo) was created using a 3Teslsa Phillips MRI scanner. The MPRAGE consisted of 160 slices with 1x1x1mm voxel size, and a field of view of 240x160x224mm centered along the mid-plane of the brain and angled to follow the AP-PC line, using an initial survey scan. TR was 8.26ms. During this 4-minute scan subjects were instructed to remain as still as possible. This was part of the same scanning session as the DTI scans described in Chapter 2; subject details can be seen there (see Table 2.1, page 40)

3.3 Data processing

Images were transformed into nifti format and entered into FreeSurfer software (http://surfer.nmr.mgh.harvard.edu/) for cortical thickness analysis. This software pre-processes image data by correcting for motion, extracting brain tissue, transforming into standard MNI space, and performing intensity normalisation. The normalised images are segmented into anatomical structures (e.g. amygdala, hippocampus, caudate, ventricles, putamen). Figure 3.1 shows an example from one subject's segmented data. Then, surface deformation is performed by following intensity gradients to closely estimate the position of the grey matter-white matter and the grey matter-cerebrospinal fluid boundaries, which are defined by identifying the location of the greatest shift in intensity (Fischl, Sereno & Dale, 1999a; Dale, Fischl & Sereno, 1999). Grey-matter thickness was measured as the closest distance between these 2 boundaries (Fischl & Dale, 2000).

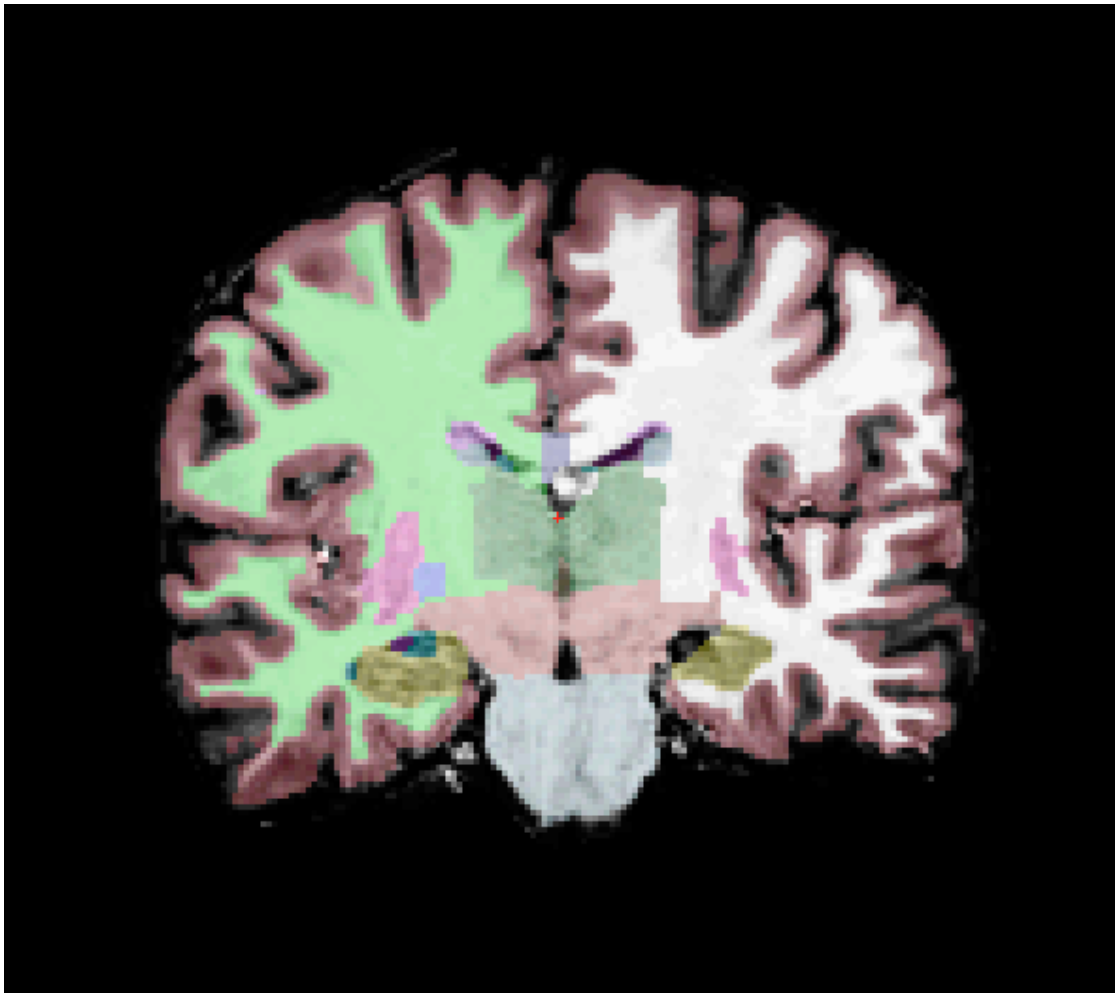

Figure 3.1: Segmented and normalized mid-coronal slice from one subject. Segmented structures include cerebral grey matter (red) and white matter (white or green), hippocampus (yellow), thalamus (dark green), brain stem (grey), putamen (pink), pallidum (purple). Grey matter thickness was calculated at the smallest distance between the grey matter and white matter boundaries.

3.4 Results: Between-group differences in grey-matter thickness

Once every subject has been pre-processed using FreeSurfer software a GLM (general linear model) analysis was used to assess between-group differences. Labels were assigned by entering the MNI co-ordinates of the centre of each significant cluster into FSL's Harvard-Oxford Cortical Structural Atlas (Desikan et al., 2006). Firstly, the control group was compared with the TS group directly to look for areas of

significant difference in grey matter volume. The groups were age and gender-matched so this analysis should show differences that are due to a diagnosis of TS. See Figure 3.2 and Table 3.1 and 3.2 for clusters of significant between-group differences in grey matter volume. Secondly, age was entered into the model as a covariate. This was because, in the typically developing brain, cortical thickness decreases throughout adolescence (Giorgio et al., 2010). If changes in cortical grey matter thickness in TS are a consequence of adaptation to tics, the relationship between age and grey matter thickness may be different to that of the typically developing brain. For example, increased volume of the pre-frontal cortex was reported previously in children with TS (Peterson et al., 2001), which may be due to a compensatory mechanism that allows children with TS to gain control over their tics (Marsh et al., 2008; Plessen, Bansal & Peterson, 2009). Therefore the negative correlation between age and grey matter thickness in the frontal lobe may be apparent in the control group but not the TS group. As can be seen in Table 3.4 and Figure 3.3 there were many clusters where the TS group exhibited a significant positive correlation between grey matter thickness and age, but where the control group had the typical negative relationship. Table 3.3 indicates there were also a few clusters where the TS group had a negative correlation between age and grey matter thickness, but where the control group has a positive correlation.

Table 3.1 Clusters where TS group has significantly thicker grey matter volume than the CS group (p<0.005).

Value (-log(10)p)	**Cluster size** (mm^2)	**MNI coordinates**			**Label**
		x	**y**	**z**	
3.7766	64.94	69.0	-43.3	-9.8	right middle temporal gyrus
3.0713	854.8	-16.7	-105.	-3.4	left occipital pole
2.8537	79.46	14.9	-92.3	19.6	right occipital pole
2.7821	48.32	50.3	-22.5	6.3	right planum temporale
2.6723	46.84	38.4	-9.5	6.8	right insula cortex

Table 3.2 Clusters where the TS group have significantly thinner grey matter volume than the CS group (p<0.005).

Value (-log(10)p)	**Cluster size** (mm^2)	**MNI coordinates**			**Label**
		x	**y**	**z**	
-2.9858	129.19	28.4	-23.8	-32.6	right posterior temporal fusiform gyrus
-2.9043	109.74	-22.7	-67.6	34.7	left superior lateral occipital cortex
-2.8839	159.53	-29.0	0.9	-39.3	left anterior temporal fusiform cortex
-2.835	135.97	-42.9	-81.8	-1.4	left lateral occipital gyrus
-2.8103	43.33	20.5	-37.6	54.5	right postcentral gyrus (S1)
-2.7519	58.44	-32.3	-4.6	47.7	left precentral gyrus (M1)
-2.7099	64.31	-16.4	47.6	34.4	left frontal pole
-2.6522	77.54	-46.5	-4.8	-42.1	left inferior temporal gyrus
-2.6265	33.11	-41.4	-71.2	22.1	left superior lateral occipital cortex
-2.5608	51.49	-7.2	-45.1	52.9	left precuneus cortex
-2.4802	39.23	-44.3	-52.7	22.7	left angular gyrus
-2.4201	33.99	-51.4	-65.2	9.6	left inferior lateral occipital cortex
-2.3918	24.95	21.7	40.1	42.7	right frontal pole
-2.3173	24.91	-49.1	-21.6	-37.7	left posterior interior temporal gyrus

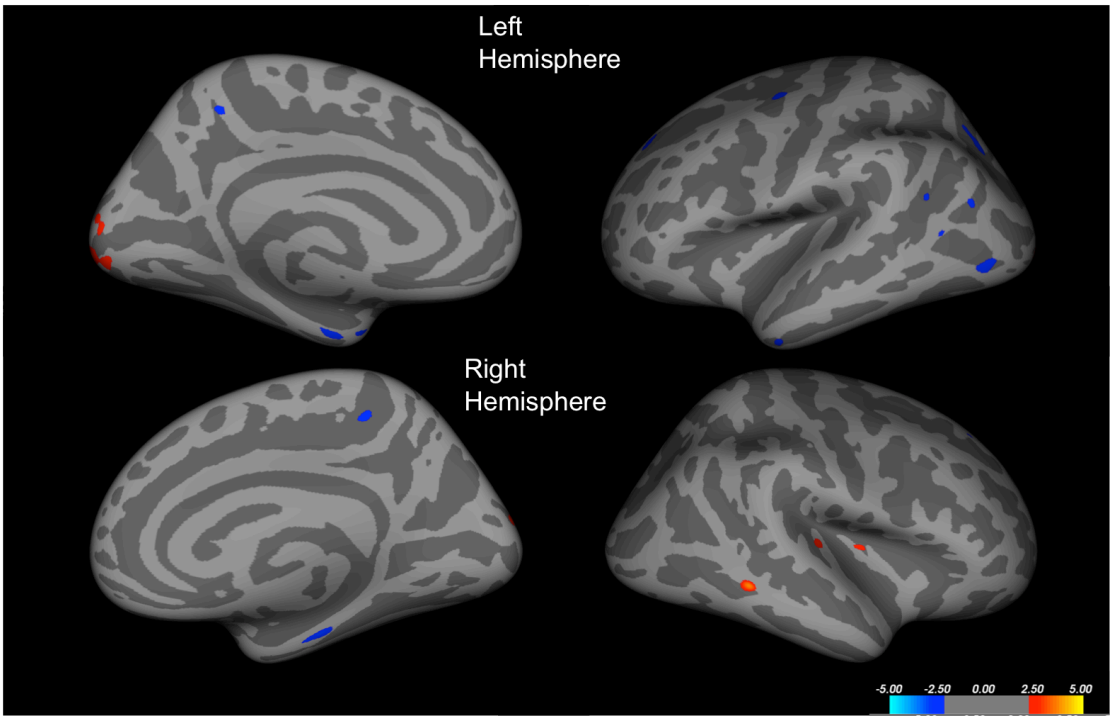

Figure 3.2 Clusters where grey matter thickness is significantly different in the TS group vs. control group (blue= TS>CS, orange = TS<CS).

Table 3.3 Clusters where grey matter decreases with age in the TS group and increases with age in the CS group. The between-group interaction effects between age and grey matter thickness were found to be significant at a threshold of p<0.005.

Value (-log(10)p)	**Cluster size** (mm^2)	**MNI coordinates**			**Label**
		x	**y**	**z**	
-3.6857	129.03	-27.4	27.0	-12.3	left frontal orbital cortex
-2.5388	30.55	44.0	17.4	17.8	right pars opercularis

Table 3.4 Clusters where grey matter increases with age in the TS group and decreases with age in the CS group. The between-group interaction effects between age and grey matter thickness were found to be significant at a threshold of p<0.005.

Value (-log(10)p)	**Cluster size** (mm^2)	**MNI coordinates**			**Label**
		x	**y**	**z**	
3.3572	67.95	-34.0	-42.9	42.4	left superior parietal lobule
2.911	73.56	53.2	-56.1	46.0	right superior lateral occiptial cortex
2.8943	67.57	-13.4	-69.2	42.5	left precuneus cortex
2.7852	57.51	43.0	23.0	37.2	right middle frontal gyrus
2.6336	88.07	-36.0	37.0	8.9	left frontal pole
2.6148	58.06	22.3	25.2	57.7	right superior frontal gyrus
2.5745	58.83	-15.2	-41.9	64.1	left postcentral gyrus (S1)
2.4828	32.25	50.7	39.0	21.1	right frontal pole
2.4747	59.46	40.4	-12.0	20.0	right central opercular cortex
2.4477	35.98	-5.7	-1.5	41.0	left anterior cingulate gyrus
2.3179	42.6	-11.5	-53.7	64.9	left precuneus cortex
2.3114	30.49	-43.7	-67.8	13.9	left superior lateral occipital cortex

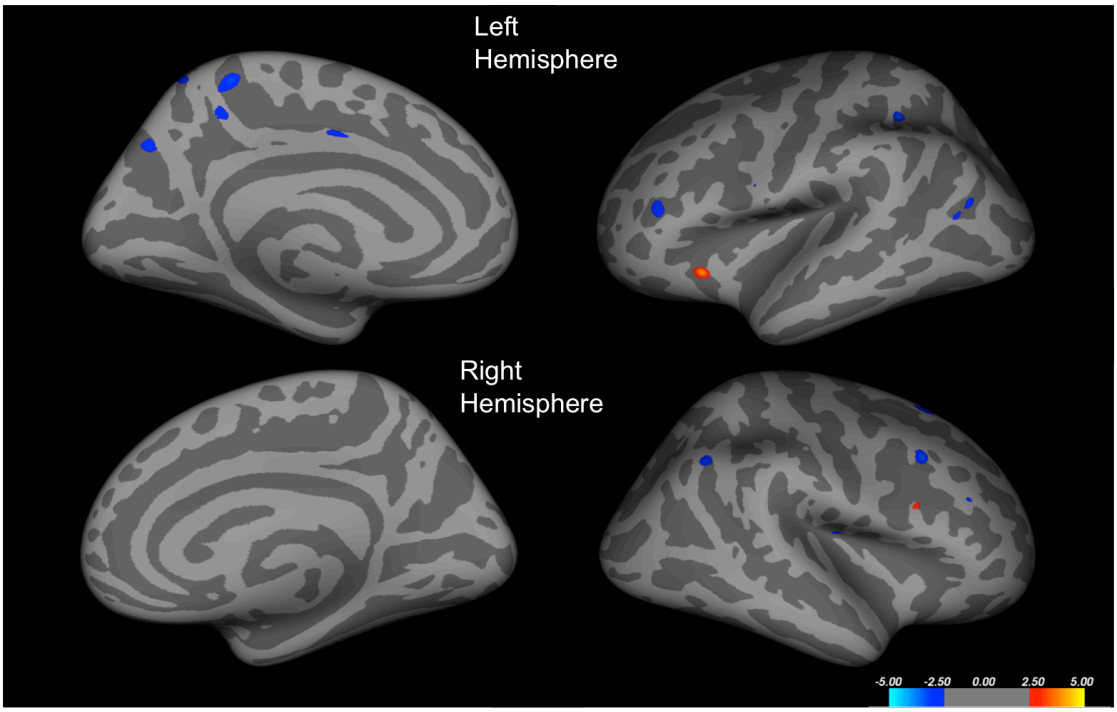

Figure 3.3: Clusters where the age vs. grey matter thickness correlation is significantly different between TS subjects and control subjects.

3.5 Results: Relationship between grey-matter thickness and tic scores

The relationship between TS symptom severity and grey matter thickness was then examined, by looking for clusters where there was a significant positive or negative correlation between thickness and motor and phonic tic scores. Phonic and motor tic scores were examined separately because some of the cortical areas used to produce these tics differ. For example, there may be a correlation between phonic tic scores and the thickness of grey matter in the language centres of the brain that may not be apparent when looking at just motor tic scores or a combined score of motor + phonic tics. As can be seen in Tables 3.5 -3.8 and Figures 3.4 and 3.5, there were widespread regions of grey matter thickness that correlated significantly with tic scores.

Table 3.5. Clusters where grey matter thickness has a significant negative correlation with motor tic scores (p<0.005).

Value (-log(10)p)	**Cluster size** (mm^2)	**MNI coordinates**			**Label**
		x	**y**	**z**	
-5.1644	333.32	-38.6	-31.6	52.8	left post central gyrus (S1)
-4.0196	230.6	24.0	0.9	58.5	right superior frontal gyrus
-3.843	383.79	39.3	15.0	-46.5	left temporal pole
-3.7053	70.1	-3.5	41.3	-31.7	left frontal medial cortex
-3.4841	287.17	-46.7	8.6	2.3	left central opercular cortex
-3.3922	323.07	-37.7	19.1	30.8	left middle frontal gyrus
-3.3268	243.07	42.2	-91.2	0.9	right occipital pole
-3.3014	239.26	-43.8	5.5	41.7	left precentral gyrus (M1)
-3.192	400.14	-5.4	37.5	6.7	left anterior cingulate gyrus
-3.1187	111.78	60.3	-59.1	-15.8	right inferior temporal gyrus
-3.1038	46.24	-8.5	-48.5	75.4	left post central gyrus (S1)
-3.0721	64.38	-41.2	-63.2	51.8	left superior lateral occipital cortex
-3.0569	65.39	-45.2	-66.0	13.7	left inferior lateral occipital cortex
-3.0255	311.11	47.8	-69.9	-5.7	right inferior lateral occipital cortex
-3.0129	58.29	-39.2	-9.5	45.1	left precentral gyrus (M1)
-2.978	101.78	23.1	-77.6	-6.5	right occipital fusiform gyrus
-2.9567	55.36	-6.4	-67.9	35.3	left precuneus cortex
-2.7639	48.84	22.7	-56.8	24.2	right precuneus cortex
-2.7413	75.46	39.7	17.6	-10.8	right insular cortex
-2.6797	43.88	43.1	-66.7	15.2	right inferior lateral occipital cortex
-2.6463	81.73	-29.7	20.6	-25.2	right frontal orbital cortex
-2.628	135.27	-21.4	28.7	45.6	left superior frontal gyrus
-2.6074	59.93	-43.3	-84.1	-11.4	left inferior lateral occipital cortex
-2.585	42.87	-48.1	-28.3	35.8	left post central gyrus (S1)
-2.5668	61.81	45.1	2.3	43.8	right precentral gyrus (M1)
-2.5176	48.61	-63.6	-31.5	32.0	left anterior supramarginal gyrus
-2.4573	38.38	-28.1	10.7	52.8	left middle frontal gyrus
-2.4301	35.79	58.1	11.7	26.0	right precentral gyrus (M1)
-2.4214	40.11	51.3	-48.9	10.6	right middle temporal gyrus
-2.392	21.82	35.6	-47.0	57.1	right superior parietal lobule
-2.3345	35.12	-30.0	1.7	-45.6	left fusiform gyrus
-2.3326	82.03	-24.6	-61.4	38.1	left superior lateral occipital cortex
-2.3251	33.87	-32.1	-75.2	24.5	left superior lateral occipital cortex

Table 3.6 Clusters where grey matter thickness has a significant positive correlation with motor tic scores (p<0.005).

Value (-log(10)p)	**Cluster size** (mm^2)	**MNI coordinates**			**Label**
		x	y	z	
2.4504	28.06	7.6	21.5	-25.4	left lingual gyrus
2.5537	71.22	-12.2	-67.0	3.1	right subcallosal cortex

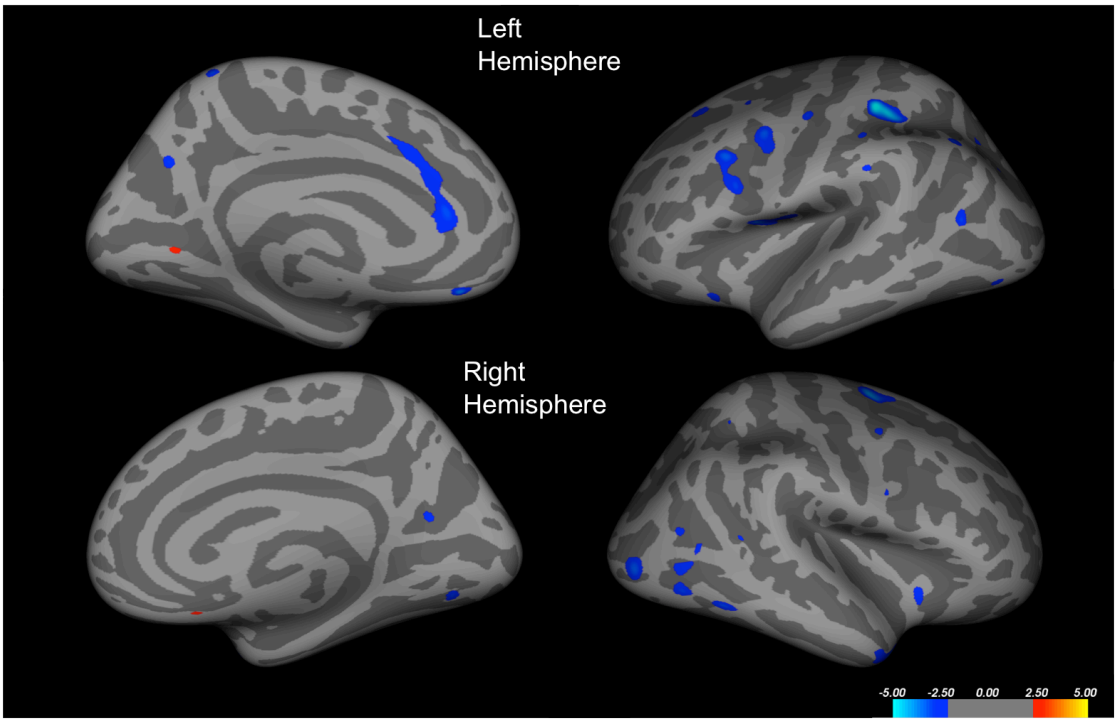

Figure 3.4: Clusters where grey matter thickness is significantly correlated with motor tic scores (blue=negative correlation, orange=positive correlation).

Table 3.7. Clusters where grey matter thickness has a significant negative correlation with phonic tic scores (p<0.005).

Value (-log(10)p)	**Cluster size** (mm2)	**MNI coordinates**			**Label**
		x	**y**	**z**	
-3.496	174.14	-20.5	-0.3	52.3	left superior frontal gyrus
-3.2289	101.22	-55.3	-35.2	30.3	left anterior supramarginal gyrus
-3.177	176.84	15.0	64.0	15.8	right frontal pole
-2.8233	5.17	-21.2	-20.2	-30.6	left anterior parahippocampal gyrus
-2.72	16.11	-32.3	-43.8	43.0	left superior parietal lobule
-2.5334	38.77	-24.2	41.2	17.7	left frontal pole
-2.517	62.14	-12.1	61.4	19.4	left frontal pole
-2.5038	52.67	-59.7	4.7	33.7	left precentral gyrus (M1)
-2.4943	50.1	44.4	46.4	-22.1	right frontal pole

Table 3.8. Clusters where grey matter thickness has a significant positive correlation with phonic tic scores (p<0.005).

Value (-log(10)p)	**Cluster size** (mm2)	**MNI coordinates**			**Label**
		x	**y**	**z**	
3.8273	254.58	23.0	-69.3	35.4	right superior lateral occipital cortex
3.6445	129.92	-20.4	-57.9	-1.7	left lingual gyrus
3.1657	198.92	-13.7	-93.7	-5.0	left occipital pole
2.92	53.85	15.1	-40.4	80.3	right postcentral gyrus (S1)
2.7837	53.18	-64.8	-45.9	-8.4	left middle temporal gyrus
2.7714	102.61	42.8	-53.9	-21.6	right temporal occipital fusiform cortex
2.7391	42.36	30.3	-39.2	60.2	right superior parietal lobule
2.6772	89.21	35.0	-28.5	72.4	right postcentral gyrus (S1)
2.5632	36.76	-24.7	-54.9	-5.1	left lingual gyrus
2.5042	20.39	-63.6	-6.1	10.8	left postcentral gyrus (S1)
2.4804	53.79	-22.3	-64.2	11.3	left intracalcarine cortex
2.4345	33.97	-5.4	28.3	-33.3	left frontal medial cortex
2.3786	25.37	37.9	-56.1	46.3	right angular gyrus
2.3196	105.81	-13.9	-76.8	-8.1	left lingual gyrus
2.3109	35.61	45.9	-21.7	64.2	right postcentral gyrus (S1)

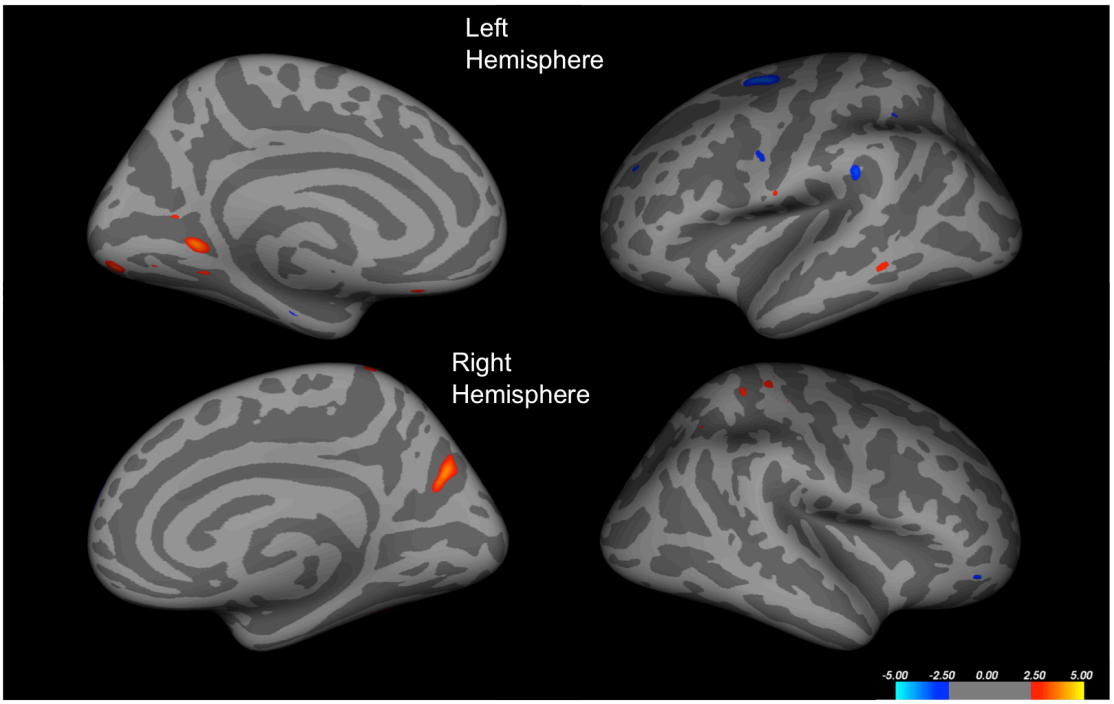

Figure 3.5: Clusters where grey matter thickness is significantly correlated with phonic tic scores (blue=negative correlation, orange=positive correlation).

3.6 Discussion

The results from this assessment of cortical grey matter thickness showed that there are widespread regions of alterations to the development of cortical grey matter associated with TS. For clarity, I will assess the results by splitting them into regions: temporal lobe, cingulate and insular lobe, occipital lobe, parietal lobe, sensorimotor cortices, and prefrontal cortex.

Temporal lobe

The TS group has significantly decreased grey matter thickness in the fusiform cortex and gyrus, and the inferior region of the temporal gyrus compared to the typically developing control group (Table 3.2). There is also a significant negative relationship between motor tic scores and grey matter thickness in the left fusiform gyrus and right inferior

temporal gyrus (Table 3.5). This means that those individuals furthest away from the control group, with lower grey matter thickness in the fusiform cortex and the inferior temporal gyrus have the most motor tic symptoms. This negative relationship is also reported in the left temporal pole, although the groups do not differ in grey matter thickness in this area.

The TS group has significantly increased grey matter thickness in the right middle temporal gyrus and planum temporale compared to the control group (Table 3.1). There is also a negative relationship between grey matter thickness in the right middle temporal gyrus and motor tic scores, which suggests that increased grey matter in this area is related to lower motor symptom severity. However, there is a positive relationship in grey matter thickness of the left middle temporal gyrus and phonic tic scores, suggesting that in the left hemisphere, increased grey matter of the middle temporal gyrus is related to more severe phonic tics. This may be partly related to the left hemisphere's language dominance (Vigneau et al., 2006).

Occipital lobe

The results of the cortical thickness analysis revealed many occipital lobe alterations in young people with TS, and some of these were related to tic symptom severity. These include reduced grey matter in the inferior and superior lateral occipital cortex, which increases with age in the TS group, but not the control group, and has a negative relationship with motor tic scores. However, it is difficult to determine in this small, cross-sectional sample whether those older individuals with TS had thicker occipital cortices to begin with, or if they have failed to decrease the grey matter thickness in such areas with development, leading to the positive age-thickness correlation.

The bilateral occipital poles are significantly increased in the TS group, and this increase in grey matter thickness has a negative relationship with motor tic scores on the right and a positive relationship with phonic tic scores on the left. However, other than abnormal oculomotor responses, which have been reported to be impaired (Mostofsky et al., 2001; Munoz, LeVasseur & Flanagan, 2002), or enhanced (Tajik-Parvinchi & Sandor, 2011; Jackson et al., 2007) in people with TS, there is very little evidence of visual attention deficits or enhanced visual processing in TS. Indeed, oculomotor responses have been related to motor areas that control eye movements, not to the visual processing areas of the occipital lobe. It is therefore unclear of how alterations of the grey matter structure in the occipital lobe relate to tic production or control. One possible theory, outlined by Peterson et al. (2001), is that regions in the lateral occipital lobe involved in attention form part of an inhibitory control network, along with prefrontal cortex and parietal regions, that is activated to suppress tics. Having a thicker lateral occipital cortex did relate to less severe motor tic symptoms, and this area tended to thicken with age for the TS group, which does suggest its development has some role in tic reduction. However, this theory would be better supported by increased grey matter in the lateral occipital cortex for the TS group, which is not what was observed in the current analysis.

Parietal lobe

Firstly, the TS group have significantly decreased grey matter thickness in the left precuneus cortex (Table 3.2), and this area increases with age for the TS group, and decreases with age for the control group (Table 3.4). There is also a negative relationship between grey matter thickness in bilateral precuneus cortex and motor tic scores (Table 3.5). Alterations in this area could be related to its connectivity to the thalamus, striatum and sensorimotor regions of the SMA, premotor cortex and S1 (Cavanna & Trimble, 2006).

The TS group also have decreased grey matter in the left angular gyrus (Table 3.2), although there is no relationship between this region when age is taken into account, nor is there a relationship with motor tic scores. There is, however, a positive relationship between the grey matter thickness of the right angular gyrus and phonic tic score (Table 3.8). Alterations in the angular gyrus may be because of this area's associated role with language processing, which could be why it is related to phonic but not motor tics (Vigneau et al., 2006). Furthermore, there is a negative relationship between both motor and phonic tics for another area of the inferior parietal lobule associated with language processing: the left anterior supramarginal gyrus, although this area is no different grey matter thickness to the control group. Both of these regions have multimodal input from sub-cortical structures and cortex, including the insula, caudate, prefrontal cortex, parahippocampal gyrus and precuneus. They are thus thought to integrate multisensory information to aid in problem solving, orient attention appropriately, and manipulate mental representations (Seghier, 2013).

The left superior parietal lobe gets thicker with age for the TS group, and gets thinner with age for the control group (Table 3.4). There is a negative relationship between phonic tic scores and grey matter thickness in this area (Table 3.7).

Insular & limbic lobe

Although there were no absolute differences between the TS group and control group in the insular lobe, there was age-related alterations in the right central opercular cortex (Table 3.4). Motor tic scores were also found to have a negative relationship with grey matter thickness in the left central opercular cortex and the right insula cortex. This could relate to the insula's role in the urge-for-action network, which could account for the premonitory sensations that can occur before a tic (Jackson et al., 2011b).

For the limbic lobe, the left anterior cingulate gyrus increases with age for the TS group, and decreases with age for the CS group (Table 3.7), and has a negative relationship with motor tic scores (Table 3.5). This suggests that this region increases (or fails to decrease) in size as young people with TS go through adolescence, and that those who develop the largest anterior cingulate gyrus also have the least amount of motor tics. This area has multiple outputs, including: lateral prefrontal cortex, anterior insula, premotor cortex, and the amygdala, and is thought to be involved in working with the dorsolateral prefrontal cortex to resolve conflict in tasks that require cognitive control (Carter & Van Veen, 2007). Therefore this area could be increasing in size with TS group with age because it may be engaged in active tic-suppression, and develops larger as a consequence of years of tic-supression. Indeed, those subjects with the least severe tics also had the thickest cortex in the left anterior cingulate. The cingulate also has been implicated in the urge-for action network, so alterations in this region may also relate to premonitory urges (Jackson et al., 2011b).

Primary motor and somatosensory cortices

The TS group have significantly reduced grey matter thickness compared to the control group in the left primary motor cortex (M1), and the right primary somatosensory cortex (S1). There is a negative relationship between grey matter thickness of bilateral M1 and motor tic score, and left M1 and phonic tic scores. This suggests that having reduced grey matter in M1 is related to more severe tics, in agreement with findings from Sowell et al., (2008), Fahim et al., (2010) and Worbe et al., (2010). The findings from S1 are not so easily interpreted. Left S1 has a positive relationship with age in the TS group, that is the opposite in the control group. Left S1 also has a negative relationship with motor tic scores, again in line with previous findings (Sowell et al., 2008; Fahim et al., 2010; Worbe et al., 2010). However, bilateral S1 has a positive

relationship with phonic tic scores, meaning that more severe phonic tics are associated with thicker primary somatosensory cortex. The core findings from the sensorimotor cortices are in line with past research and support the theory that cortical thinning in these areas relates to more severe motor tics (Sowell et al., 2008; Fahim et al., 2010; Worbe et al., 2010).

Prefrontal cortex

For the prefrontal cortex, not only is bilateral frontal pole significantly reduced compared to the control group, and this reduction is related to increased phonic tics, but the TS group also have significant increases in the grey matter volume of the frontal pole with age, where the control group have the opposite (Table 3.4). This result concurs with the hypothesis that prefrontal cortical volume increases, rather than decreases with age in TS to and is related to a reduction in tics (Marsh et al., 2008). However, this is not the full story. If young people with TS develop increased frontal lobe volumes because of attempted control of tics, you would expect for this increase to be apparent at the group level, where what is actually observed is a significant *reduction* in grey matter volume in bilateral frontal pole. This suggests that although grey matter thickness is increasing with age in the TS group, it is not reaching the volume of the average typically developing control. As past research has indicated grey matter thickness decreases with age in typically developing adolescents (Lebel et al., 2008; Giorgio et al., 2010; Kochunov et al., 2011), and this sample is cross-sectional, it could be that the older members of the TS sample have not reduced their grey matter thickness in this area, whereas the older members of the control group have. Therefore, it is more likely that this positive relationship between cortical thickness and age in the TS group represents a failure to decrease grey matter thickness, rather than an actual increase in grey matter with age. Longitudinal data could determine whether this is the case.

Furthermore, the region of the prefrontal cortex known as the frontal orbital cortex decreases with age for the TS group in the left hemisphere, but still has a negative relationship with motor tics in the right hemisphere. However, the TS group has significant increases with age in the grey matter thickness in sections of 2 other regions of prefrontal cortex; the superior frontal gyrus and the middle frontal gyrus (Table 3.4). Increased grey matter thickness in the superior frontal gyrus bilaterally and the left middle frontal gyrus is related to reduced motor and phonic tic scores, in line with the pre-frontal control hypothesis (Marsh et al., 2008; Plessen, Bansal & Peterson, 2009).

Conclusion

Typically developing adolescents go through a period of synaptic pruning, which results in thinner sensorimotor cortical grey matter (Huttenlocher, 1984; Giedd et al., 1999; Giorgio et al., 2010). Although it is unlikely that it is a reduction in synapses per se that reduces grey matter thickness (Bourgeois & Raki, 1993; Paus, Keshavan & Giedd, 2008). Instead, a reduction in synapses would lead to a reduction in metabolic demand, so the reduced grey matter thickness could be due to a reduction in glial cells, or vascular restrictions (Paus, Keshavan & Giedd, 2008). Furthermore, increased myelination of the axons of useful connections would lead to some voxels that were previously classified as grey matter in a T1 weighted MRI to be classified as white matter, which, in itself, would decrease measures of grey matter thickness (Paus, 2008). It is currently unknown what exactly grey cortical matter thinning relates to, it is likely to be a combination of reduced metabolic demand and increased myelin, which develop in adolescence to streamline the flow of information (Paus, 2008).

There are widespread alterations in grey matter thickness in young people with TS. For the purposes of this thesis the findings in the sensorimotor and prefrontal cortex are of most interest. The findings from

the pre-frontal cortex are inconclusive, with both increased and decrease volume relating to tic symptom severity in different sections. However, there is no doubt that there are structural changes in this region, but the role of these alterations is unclear. In the previous chapter I reported there were no between group differences in the callosal white matter from the pre-frontal cortex, and no relationship between the structural integrity of this tract and symptom severity. There was, however, a relationship between tic scores and the structural integrity of callosal white matter projecting from bilateral M1, so motor and pre-motor cortex will continue to be the main focus of this thesis.

In line with past research, the findings presented in this chapter show that adolescents with TS have reduced grey matter volumes in the sensorimotor cortices, and that the thinner these areas are, the more severe the tic symptoms are. Sowell et al. (2008) explained this finding as evidence for cortical thinning in sensorimotor regions to be part of the pathogenesis of TS. However, it could also be thought of as a developmental consequence of long-term tics. If the sensorimotor cortex were constantly receiving inappropriate stimulation from the striatum it would be in a state of heightened excitability and require less input to produce a response. It stands to reason that people with TS would prune earlier, and to a greater extent than typically developing adolescents, because of the excess input. They therefore may reduce the number of glial cells due to decreased metabolic demand, and myelinate these over-stimulated pathways earlier than their neuro-typical peers. Those individuals with more tics are likely to have more input to sensorimotor regions than those with less severe tics, and would hence might prune to a greater extent, which would explain the negative relationship between grey matter thickness and tic scores. In the following chapter I will consider the functional consequence of cortical thinning in the primary motor cortex, and explore cortical excitability of M1, using Transcranial Magnetic Stimulation.

Chapter 4: Cortical Excitability in Tourette Syndrome Measured by Transcranial Magnetic Stimulation

4.1 Introduction

The previous chapters have shown structural changes in M1 in adolescents with TS that relate to clinical symptoms. The DTI probalistic tractography showed that more inter-hemispheric connectivity through the corpus callosum of bilateral M1 is related to having more severe motor tics. The cortical thickness analysis showed that cortical thinning in M1 was related to more severe symptoms. Although these findings may at first glance, appear contradictory, they concur with normal brain development, i.e. increased FA occurs alongside decreased cortical grey matter during aging of typically developing young people (Kochunov et al., 2011). This means that the subjects with more severe tics would look more like an older population of typically developing individuals than like their age-matched peers. This suggests that individuals with severe TS might go through developmental changes in certain brain structures earlier, and perhaps to a greater extent, than typically developing individuals. As changes in white and grey matter occur alongside the development of cognitive abilities, discussed in detail in Chapter 2, it is likely that structural changes in people with TS may develop because of striving to control tics. Changes in brain structure are likely to lead to, or perhaps be the result of, functional changes in the affected area. In this chapter I will investigate functional changes in the excitability of M1 using transcranial magnetic stimulation.

4.2 Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation (TMS) is a technique that can be used to measure the excitability of a region of cortex. TMS involves placing an electro-magnet (coil) onto the scalp of a subject and delivering a very brief magnetic pulse. This magnetic pulse, if administered at a high enough

intensity, will induce a current in the brain tissue directly below the coil. If TMS is delivered to M1, this current induces axon potentials in cortical neurons, which, through synaptic transfer, excite pyramidal neurons that synapse with spinal alpha motoneurons and cause a response in the muscles of the body. Which muscles respond depends on which section of M1 was stimulated. The excitability of the motor cortex can be estimated by the size of the muscle response to the stimulation. This motor evoke potential (MEP) can be recorded using surface electrodes placed on the target muscle.

Single and paired-pulse Transcranial Magnet Stimulation (TMS) has been used on a single site, left hemisphere primary motor cortex, to measure intracortical excitability in Tourette Syndrome (Ziemann et al., 1997; Moll et al., 1999; Gilbert et al., 2004; Orth et al., 2005; Gilbert et al., 2005; Gilbert et al., 2007; Orth et al., 2008; Orth & Rothwell, 2009; Hiese et al., 2010). Resting and active motor thresholds are the most basic measure of cortical excitability. Resting motor threshold represents the intensity of a single pulse of TMS from a coil positioned on a motor "hotspot" (i.e. a position over M1 that produces the largest response from a particular target muscle, often the *first dorsal interosseous (FDI) hand muscle*) to produce a motor evoked potential (MEP) of a certain amplitude whilst the muscle is at rest (Rossini et al., 1999). Intuitively, active motor threshold represents the same thing but is measured whilst the muscle is in an active state (e.g. whilst applying constant pressure to a solid object held between thumb and index finger). It is thought that motor threshold represents the excitability of the corticospinal projections from M1 to the targeted muscle through the direct stimulation of corticospinal fibres by TMS and by the stimulation of cortico-cortical fibres that have glutamatergic synapses with corticospinal tracts (Ziemann, 2013). Evidence for this theory comes from the finding that ketamine, an NMDA receptor antagonist that increases glutamate neurotransmission indirectly, decreases the motor threshold, i.e. increasing glutamate release increases motor cortical excitability (Di Lazzaro et al., 2003). As such, the MEP amplitude is a

measure of the fraction of corticospinal neurons activated by TMS and increases with increased stimulus intensity (Rossini et al., 1999).

Neuronal excitability can also be estimated using a paired-pulse TMS paradigm known as intracortical facilitation (ICF). ICF is observed when a sub-threshold pulse is delivered 7-20ms before a supra-threshold pulse, and results in an increased in MEP amplitude to the second pulse compared to an unconditioned supra-threshold pulse of the same intensity. Although ICF is an excitatory paradigm, it is likely to be mediated by both glutamatergic and GABA-ergic neurotransmission, as it is a result of increased facilitation and decreased inhibition (Paulus et al., 2008). As such, the ICF effect can be reduced by administering Benzodiazepines, which are positive modulators of $GABA_A$ receptors (Ziemann et al., 1996b), and increased by positive modulators in the Norepinephrine system (Gilbert et al., 2006).

Short interval intracortical inhibition (SICI) and long interval intracortical inhibition (LICI) are paired-pulse TMS paradigms that provide an indirect estimate of $GABA_A$ and $GABA_B$ activity respectively. The standard procedure for SICI involves delivering a sub- motor threshold pulse to the motor hotspot 2-5ms before a supra-threshold pulse and measuring the MEP response to the second pulse. The target muscle is kept at rest through the procedure and the average MEP amplitude to conditioned trials is compared to the average MEP amplitude to a single (unconditioned) supra-threshold pulse of the same intensity. It is thought that the conditioning sub-threshold pulse activates a low threshold inhibitory circuit, probably by stimulating GABA-ergic interneurons, which inhibits the action potential of some corticospinal neurons, leading to a smaller response to the second supra-threshold pulse (Ziemann, 2013). Administering a $GABA_A$ receptor agonist can increase the SICI effect (Paulus et al., 2008). This is evidence that the SICI effect is largely a reflection of $GABA_A$ synaptic activity, and corticospinal responsiveness to TMS.

A LICI paradigm is similar to the SICI paradigm but both pulses are above threshold and delivered with an inter-stimulus interval (ISI) of 100-150ms. As a LICI effect is observed at such a large range of ISIs it is thought that it results from slower inhibitory processes mediated by the $GABA_B$ receptor activity, a mechanism distinct from that of SICI (Zieman, 2013). McDonnell et al. (2006) demonstrated that administering a $GABA_B$ receptor agonist to subjects could increase the LICI effect, which supports the claim that the LICI effect is a reflection of $GABA_B$ synaptic activity. GABA is an inhibitory neurotransmitter, so LICI paradigm typically causes a reduction in MEP size compared to a single (unconditioned) supra-threshold pulse of the same intensity. As with SICI, the LICI effect is usually quantified by calculating the percentage change in average MEP amplitude to a conditioned pulse of TMS compared to the average MEP amplitude to an unconditioned pulse of the same magnitude.

4.3 Altered Cortical Excitability in TS

TMS has been used to investigate cortical excitability and inhibition in both adults and children with TS. For example, Orth and Rothwell (2009) have reported increased active and resting motor thresholds in adults with TS compared to a neuro-typical control group. This suggests there may be subtle increases in cortical excitability as a consequence of TS. However, several other studies have reported no difference in motor threshold between TS subjects and neuro-typical controls (Ziemann et al., 1997; Moll et al., 1999; Moll et al., 2001; Orth et al., 2005). Resting and active motor thresholds may not be the most sensitive measure of changes in cortical excitability that may relate to TS. Paired-pulse TMS paradigms can provide more specific information about the balance between excitation and inhibition in cortex.

As such, some studies have reported that subjects with TS have reduced SICI effects compared to control groups (Orth & Rothwell, 2009; Orth et al., 2008; Orth et al., 2005a; Moll et al., 2001; Ziemann et al., 1997) and increased ICF effects (Orth & Rothwell, 2009). These findings have been used as evidence that people with TS have increased excitability in motor cortical areas because of increased, dysfunctional input from the striatum, which is exacerbated by a reduction in local inhibition, as evidenced by the reduced SICI effect. However, other studies have reported no differences between subjects with TS and control groups in SICI (Moll et al., 1999). Furthermore, the increased ICF result was only apparent in a sub group who had a diagnosis of co-morbid ADHD and may be related to ADHD rather than TS (Orth & Rothwell, 2009). The reduced SICI effect was also more strongly apparent in subjects with co-morbid ADHD, and related to ADHD symptom severity (Moll et al., 2001; Gilbert et al., 2004; Gilbert et al., 2005; Gilbert et al., 2007).

This highlights a problem in drawing meaningful conclusions about TS when using samples with co-morbid disorders. Although the reduced SICI effect in TS has been reproduced using different samples, suggesting that this effect is robust (even if it is also related to a diagnosis of ADHD), it is important to take co-morbid disorders into account when interpreting results. Furthermore, the inconclusive findings in the Tourette's TMS literature may also be due to differences in the age of the samples. Some studies include just children with TS (Moll et al., 1999; Moll et al., 2006; Hiese et al., 2008), some just adults (Baumer et al., 2010; Heise et al., 2010; Orth et al., 2005), and some a mixed sample of adults and children with TS (Gilbert et al., 2004; Orth et al., 2008). As around 80% of children with TS become largely tic-free by adulthood (Leckman et al., 1998) it is likely that the neurological profile of those who continue to have debilitating tics into adulthood is different to children with TS. Therefore it is useful to look at adult samples and child samples separately.

Inter-hemispheric influence has been examined in subjects with TS using dual-site paired-pulse TMS (Baumer et al. 2010; Jackson et al., 2012). This was measured by delivering a conditioning pulse of TMS to one hemisphere's hand area of M1 and a test pulse to the contra-lateral hemisphere's hand area of M1 and recording the MEP to the test pulse. The test-pulse MEP was then compared to MEPs from unconditioned test pulses and the resulting change in MEP amplitude was taken as a measure of inter-hemispheric influence. Baumer et al. (2010) reported that adults with TS have reduced right-to-left inter-hemispheric influence compared to controls, i.e. their MEP amplitudes did not increase to the same extent as the controls when a conditioning pulse was delivered to the right hemisphere ahead of a test pulse to the left hemisphere. They also used DTI to quantify the connectivity between both M1s through the corpus callosum. Baumer et al. report a relationship between callosal FA and inter-hemispheric influence in the control group, that is not present in the TS group. They suggest the TS group have altered inter-hemispheric connectivity of M1, which agrees with my DTI findings from Chapter 2. Jackson et al. (2012) supported this finding by showing the MEP amplitudes were modulated to a greater extent in the dual site condition compared to the single site condition for the control group. However, the difference between groups in the study did not reach statistical significance. It is important to note that Jackson et al. (2012) used a dual site paired-pulse paradigm during a manual task where Baumer et al. (2010) were measuring inter-hemispheric influence at rest. This may explain why Jackson et al.'s (2012) findings did not reach statistical significance.

TMS can also be used to measure the change in cortical excitability whilst the subject is engaged in an active task. Heise et al. (2010) investigated the modulation of excitability of the motor cortex during the preparation of a voluntary movement using TMS. Eleven adults with pure Tourette syndrome and eleven age-matched controls were asked to move their right index finger as quickly as possible when a 'GO' signal appeared.

The hand area of M1 was stimulated during 6 different time points between the 'GO' signal and the onset of movement. These time points were set to equal steps between 40% and 100% of each individual's reaction time. Single or paired pulse TMS was used. In the paired pulse condition a conditioning pulse was applied at 80% of motor threshold with an ISI of 3ms to evaluate short-interval intracortical inhibition (SICI), and 10ms to evaluate intracortical facilitation (ICF). EMG activity was recorded from the FDI muscle. In the unconditioned pulse condition subjects with TS had less of an increase in motor evoked potential (MEP) amplitude at the third, fourth and fifth time points compared to controls. In the SICI condition subjects with TS had less inhibition than controls at the first time point, but followed the same patterned for all other time points. In the intracortical facilitation condition subjects with TS and controls were comparable. However, during the late pre-movement phase modulation of excitability was correlated with reaction time for subjects with TS but not controls. The authors conclude that subjects with TS have a more disinhibited and excitable primary motor system.

However, this result could also be interpreted as the TS group reducing their levels of excitability in the pre-movement period in order to successfully complete a volitional movement. This is because the TS group does not have the same increase in MEP amplitude during the last time periods that the controls show in the single pulse condition, which suggests they are inhibiting M1 to such an extent that the usual modulation of (or gain in) excitability does not occur. Furthermore, in the SICI condition the TS group increase their $GABA_A$ levels as they get closer to movement onset, until they reach a similar level to the controls. This suggests that GABA-ergic inter neurons are more active in the period directly preceding movement onset, suggesting that people with TS increase their inhibitory response in M1 in order to successfully engage in a voluntary action. This may be an adaptation that allows for the suppression of tics in order to complete a desired movement. Jackson et al. (2012) also demonstrated reduced MEP amplitude in subjects with TS

compared to controls during the pre-movement period, this time in an inter-manual conflict task.

In this chapter I will give details of two TMS studies I conducted with subjects with TS and their matched control subjects. The first experiment used a GO / NO GO task and single pulse TMS to evaluate cortical excitability in the pre-movement period. The second experiment used 3 paired pulse TMS paradigms to measure cortical excitability at rest. The aim of these experiments was to investigate functional differences in M1 that are related to TS; i.e. if the circuits of excitation & inhibition are functioning the same as typically developing subjects of the same age, both at rest and in an active task. Heise et al. (2010) have demonstrated that there is a reduction in cortical excitability preceding a volitional movement in adults with TS, I investigated if these mechanisms of task-related increased local inhibition are already apparent in adolescents with TS, and importantly, how this relates to symptom severity.

4.4 Experiment 1: Subjects

10 adolescents (aged 11-20 years, mean age 15.3 years) with a diagnosis of Tourette syndrome took part in this study. 10 age and gender-matched (1 female, 9 male, mean age 15.3 years) typically developing adolescents acted as a control group. Controls were matched one-to-one to a subject with TS of the same gender and within 6 months of the same age. Tic symptoms were measured on the day of testing using the Yale Global Tic Severity Scale (Leckman et al., 1989). Two subjects had a diagnosis of co-morbid Obsessive Compulsive Disorder and one had a diagnosis of Attention Deficit Disorder. Details of TS subjects can be seen in Table 4.1.

Table 4.1*: Clinical characteristics of subjects with Tourette Syndrome that took part in TMS experiment 1* (note: YGSS = Yale Global Tic Severity Scale).

ID	Age	Gender	YGSS	Motor tic scores	Phonic tic scores	Co-morbitity	Medication
TS006	20	M	40	14	16	-	Clonidine
TS018	17	M	22	12	0	ADD	Clonidine
TS028	17	F	41	10	11	OCD	-
TS071	12	M	33	15	13	OCD	-
TS013	17	M	47	11	6	-	-
TS067	17	M	29	14	0	-	Clonidine
TS069	11	M	24	14	5	-	-
TS074	12	M	33	14	9	-	-
TS034	14	M	25	7	8	-	-
TS062	16	M	65	24	21	-	Citalopram

4.5 Experiment 1: Transcranical Magnetic Stimulation Procedure

A Magstim Rapid 200 TMS monophasic stimulator unit (Magstim Ltd., UK) with a 70mm figure of 8 coil was used to deliver a single pulse of TMS to the left motor cortex. The coil was positioned over the motor hotspot for the first dorsal interosseous (FDI) muscle of the right hand. The motor hotspot was found by a trial and error process that involved delivering a single TMS pulse in various likely locations until an optimal muscle twitch was observed consistently. The coil was held securely in place by a mechanical arm and the position continuously observed and corrected for any movement between trials by the experimenter. Resting motor threshold (RMT) was estimated using an adapted PEST procedure (T.M.S. Motor Threshold Assessment Tool, as described in Brockardt et al., 2006) and was defined as the lowest intensity to produce a visually observable twitch in the FDI muscle while the hand was at rest. The estimated RMT was checked and accepted if it produced a muscle twitch in 5 out of 10 consecutive trials. TMS pulse intensity was set to 110% of RMT throughout the experiment.

Single-use ECG electrodes (Covidien™, 2011) with a 5mm diameter were positioned in a belly-tendon configuration, with the active electrode positioned on the centre of the FDI muscle and the reference 2cm above it on the tendon of the right hand. A ground electrode was positioned on the right wrist.

4.6 Experiment 1: Experimental Procedure

Subjects were seated with their head resting comfortably in a chin rest positioned 50cm away from a 17inch monitor that displayed the stimuli.
They completed a standard GO/NO GO motor task. On GO trials subjects pushed a button with their right index finger in response to a green 4cm diameter circle on a grey background. A red circle indicated a NO GO trial

and subjects withheld a response. Trials were presented in a pseudo-random order, with one NO GO stimulus appearing in a random position in every block of 6 trials. Each trial was terminated either by a response or automatically after 2 seconds. During the inter-stimulus interval a grey background was displayed for 5 seconds. The experiment was coded and run using the Cogent Graphics toolbox in Matlab (Mathsworks).

Subjects completed an initial block of 36 trials to practice the task and to obtain an estimate of each individual's median reaction time. During the 120 experimental trials this median reaction time was re-calculated every 6 trials to control for any change in average response latency. A single TMS pulse was delivered at 25%, 50% or 75% of the median reaction time after stimulus onset. TMS was triggered automatically from a laptop running Matlab via a National Instruments Data Acquisition Device, which also relayed inputs from the button box back to the computer. Electromyography (EMG) data was recorded on each trial for 3 seconds following stimulus onset in Matlab via a G-Tech g.USB Biosignal Amplifier (g-tech, 2009) with a sampling frequency of 1200Hz.

4.7 Experiment 1 Results: Between-group differences in performance

Independent t-tests revealed no significant difference between the TS group and the control group for accuracy on NO GO trials (TS mean = 18% inaccurate, SD= 15, CS mean = 22% inaccurate, SD=14, $t(18)=1.2$, $p>0.1$) or reaction time on GO trails (TS mean = 0.49sec, SD= 0.18, CS mean = 0.46sec, SD= 0.11, $t(18)=1.04$, $p>0.1$). Resting motor thresholds also did not differ significantly between groups (TS mean = 67.2% of maximum stimulator output, SD=5.0, CS mean = 66.4% of maximum stimulator output, SD=8.0, $t(18)=0.87$, $p>0.1$). NO GO trials in which an erroneous response was made were identified and excluded from MEP amplitude analyses.

4.8 Experiment 1 Results: Change in MEP amplitude during the pre-movement period

EMG data was analysed using an in-house built script in Matlab. MEPs were identified as the signal occurring directly after the characteristic TMS artefact peak. The peak-to-peak amplitude of the MEP on each trial was measured. Trials in which the MEP was ambiguous were excluded. The exact position during the pre-movement period that the TMS pulse was delivered was calculated for each GO trial and expressed as a percentage from stimulus onset (0%) to reaction time (100%) for that particular trial. The data was then binned into 3 categories: TMS delivered at 0-60%, 61-80% and 81-100% of reaction time. Mean MEP amplitudes were calculated for each bin and for NO GO trials separately (see Figure 4.1).

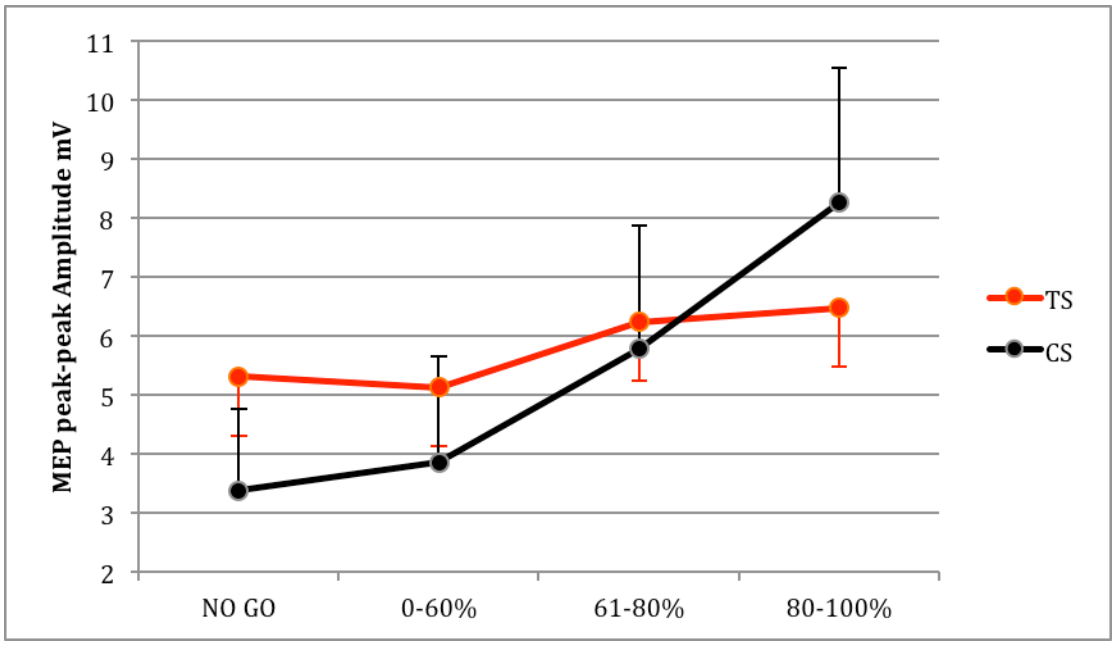

Figure 4.1: Mean MEP Amplitude for each binned condition. Error bars represent 1 standard deviation.

A 2-way mixed ANOVA (group (TS vs. CS) x period (NO GO, 0-60%, 61-80%, 80-100%)) revealed a significant main effect of period ($F(3, 18)=14.8$, $p<0.0001$) and a significant group x period interaction (F(3,

54)=4.353, p<0.01). Post-hoc within-group t-tests revealed a significant difference between the NO GO and the 61-80% period (t(9) = -4.501 p<0.05), and between the NO GO and 81-100% period (t(9) = -5.109 p <0.05) for the control group only. There were no significant differences between any of the conditions for the TS group. The mean amplitude for each condition and each group can be seen on Table 4.2.

Table 4.2 Mean and standard deviation of MEP peak-to-peak amplitude for the Control group (CS) and Tourette group (TS), for each condition.

	CS group MEP amp (mV)		TS group MEP amp (mV)	
	Mean	SD	Mean	SD
NO GO	3.3752	2.7877	5.3195	3.8818
0-60%	3.8570	3.6071	5.1389	3.7944
61-80%	5.7848	4.1793	6.2397	3.7933
80-100%	8.2639	4.5613	6.4866	3.6303

The mean MEP amplitude for NO GO trials was considered as a baseline measure for each subject. The ratio of baseline amplitude to the mean MEP amplitude for each period of the GO trials was calculated. This shows the change in MEP amplitude with time from baseline. As can be seen in Figure 4.2, the control group shows a greater increase in MEP size as they approach a movement. A 2-way mixed ANOVA (group (TS vs. CS) x period (0-60%, 61-80%, 81-100%)) revealed significant main effects of group (F(1, 18)= 5.5, p<0.05) and period (F(2, 18)=16.1, p<0.0001), and a group x period interaction that approached significance (F(2, 36) =2.9, p=0.068). Post-hoc between-group t-tests showed the change in MEP amplitude to be significantly greater for the control group than the TS group in the 61-80% period (t(18)=-2.77, p<0.05) and the 81-100% period (t(18)=-1.98, p<0.05). Within-group t-tests showed a significant difference between all periods: 0-60% vs. 61-80% (t(9) = -4.4, p<0.05), 0-60% vs. 81-100% (t(9) = -3.5, p <0.05) and 61-80% vs. 81-100% (t(9) = -2.8 p<0.05) for the control group, but only between 0-60% and 61-80% for the TS group (t(9) =-3.89, p<0.05).

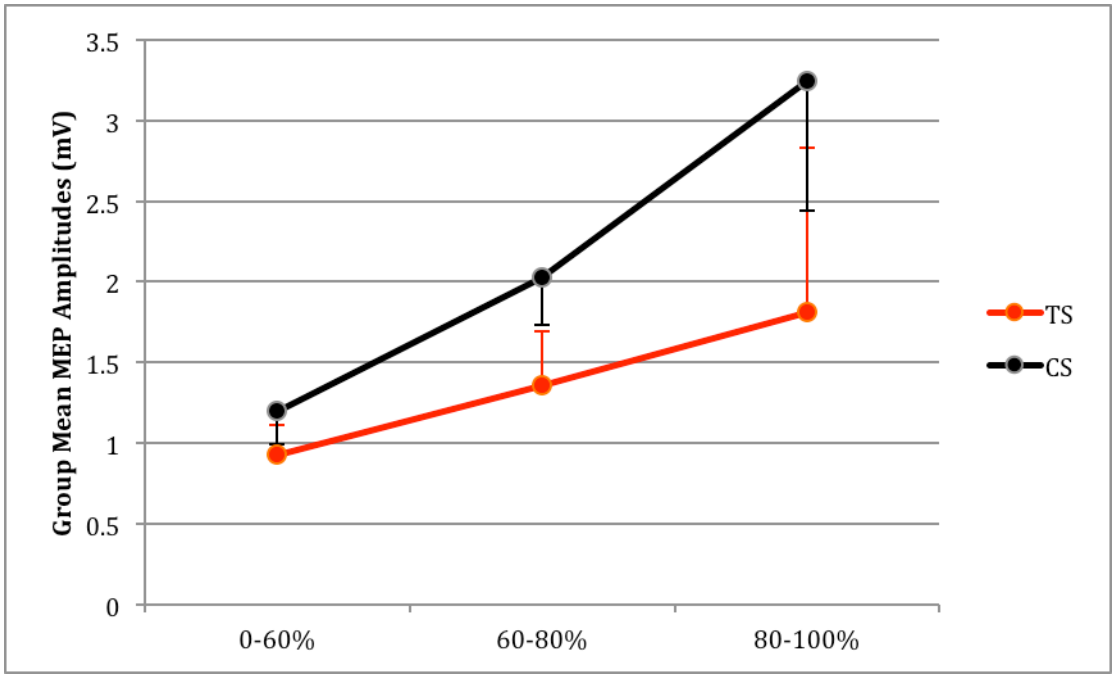

Figure 4.2: Change in MEP amplitude from baseline for each binned GO period. Error bars represent 1 standard deviation.

4.9 Experiment 1 Results: Change in Variability of MEP amplitude in the pre-movement period

The coefficient of variation was calculated as a measure of variability in MEP amplitude for each condition that can control for individual differences in mean MEP amplitude. This was calculated by taking the standard deviation from the mean MEP amplitude for each subject and each condition. As shown in Figure 4.3 there was a decrease in the variability of the MEP amplitude for the periods closest to movement onset for the control group only. This means that as MEP amplitude was increasing throughout the pre-movement period, the variability in MEP amplitude was decreasing for the control but not the TS group (see Figure 4.4).

A 2-way mixed ANOVA (group (TS vs. CS) x period (0-60%, 61-80%, 81-100%)) revealed a significant main effect of period ($F(2, 17)= 4.97$, $p<0.05$) and a significant group x period interaction ($F(2, 34)= 3.92$, $p<0.05$). Within-group t-tests showed a significant difference in variability

between all conditions: 0-60% vs. 61-80% ($t(9) = 2.806$ $p<0.05$), 0-60% vs. 81-100% ($t(9) = 3.241$ $p < 0.05$) and 61-80% vs. 81-100% ($t(9) = 2.352$ $p<0.05$) for the control group only. There were no significant between-group differences or any significant within- group difference between conditions for the TS group. Mean coefficients of variation for each condition and each group can be seen in Table 4.3.

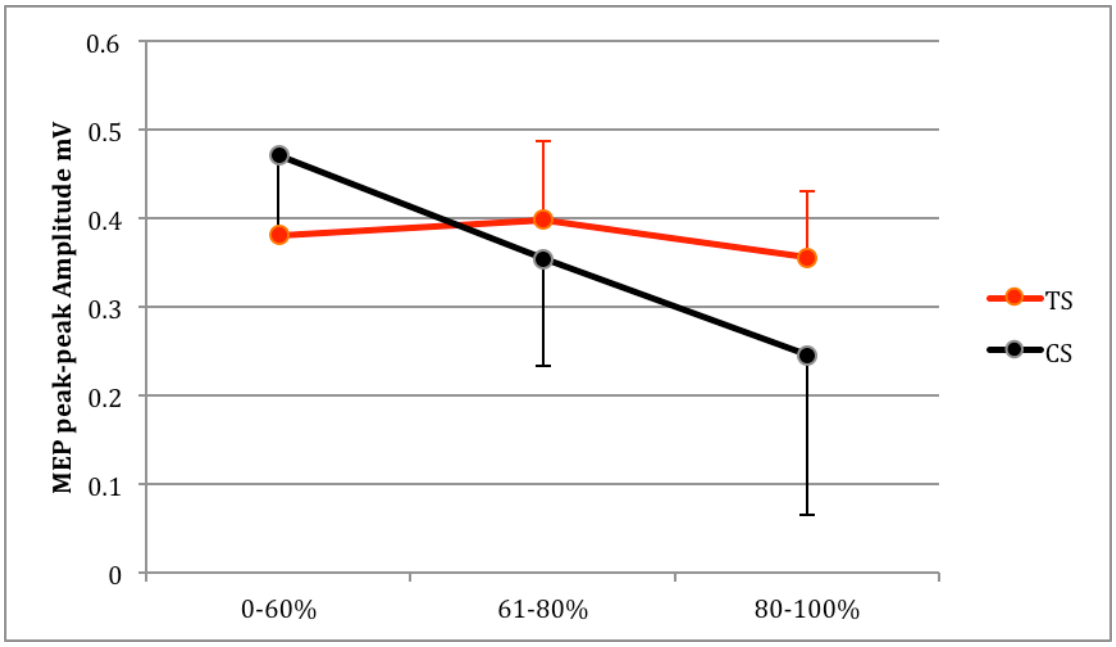

Figure 4.3: Change in the Coefficient of Variation for each period of GO trial. Error bars represent 1 standard deviation

Table 4.3 Mean and standard deviation of coefficients of variation for each group and each condition.

	CS group coefficient of variation		TS group coefficient of variation	
	Mean	SD	Mean	SD
0-60%	0.4709	0.1702	0.3814	0.1703
61-80%	0.3538	0.1775	0.3988	0.2418
80-100%	0.2456	0.1482	0.3560	0.3601

4.10 Experiment 1 Results: Relationship to tic scores

Finally, the relationship between clinical motor tic-scores, as measured by the YGTSS on the day of testing, and change in excitability

was investigated. A linear function was fitted to each TS subject's MEP amplitude against the position in the pre-movement period that the TMS pulse was delivered. The slope of this function was taken as a measure of cortical excitability leading up to movement onset. The steeper the slope, the larger the increase in MEP amplitude approaching movement. Slope was plotted against motor tic score. As can be seen in Figure 4.5, the steeper the slope the lower the motor tic score. This negative relationship was found to be significant (Pearson's R=-0.75, $p<0.01$).

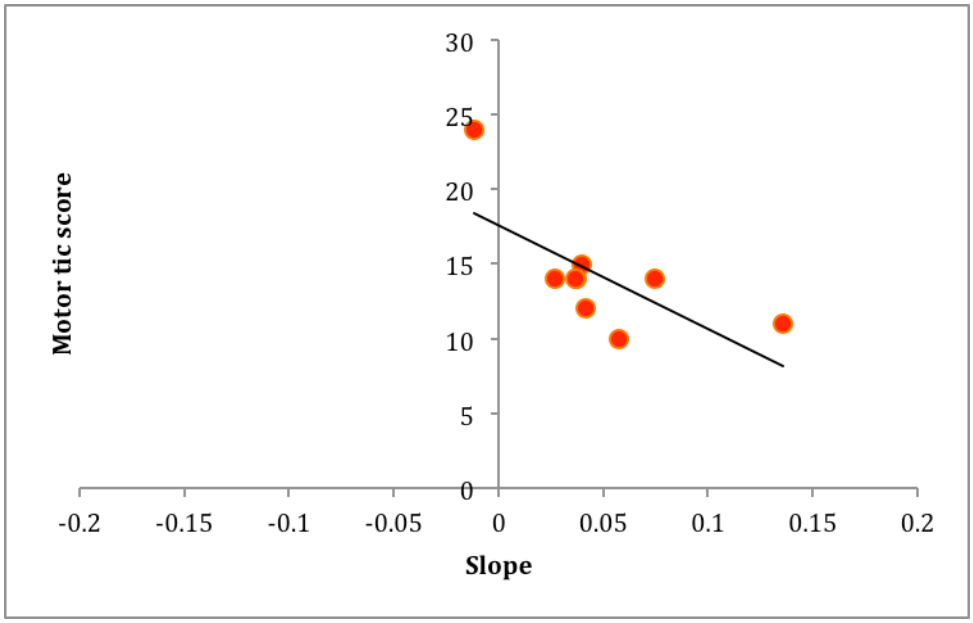

Figure 4.4: Relationship between motor tic scores from the YGTSS and slope of linear function of MEP amplitude vs. position in pre-movement period of TMS pulse delivery for TS Subjects.

4.11 Summary of Experiment 1

In Experiment 1, the modulation of cortical excitability over the pre-movement phase was examined in a group of young people with TS and a matched typically developing control group. This experiment showed that the TS group as compared to the control group, have significantly less of an increase in MEP size during the final stage of the pre-movement phase; the bin containing MEPs to a TMS pulse delivered at 81-100% of reaction time. They also do not show the same pattern of reduced variability in the MEP response as you approach movement onset that the control group show. Importantly, task performance and reaction times did not significantly differ between groups. This suggests that young people with TS do not increase their cortical excitability before making a voluntary movement in the same way as the typically developing population, in line with findings from Heise et al. (2010) and Jackson et al. (2012).

Klein-Flügge et al. (2013) investigated the change in the MEP response in the period preceding the onset of a voluntary action in a group of typical healthy adults. They report that, as you get closer to movement onset, the MEP amplitude increases and the coefficient of variability decreases. The results from the control group in Experiment 1 match this pattern. Klein-Flügge et al. suggest that the decrease in variability and increase in amplitude of the MEP during the later stages of movement preparation is unlikely to be solely due to changes at the spinal level. Instead it is likely to be influenced by inputs into M1 from premotor areas (Klein-Flügge et al., 2013). Research has shown that inputs to M1 from other cortical areas have a latency of between 2.4 and 7ms (Di Lazzaro, Ziemann & Lemon, 2008). Therefore these inputs would be largely affecting the period closest to movement onset, not the initial response.

If this is the case, the TS group in Experiment 1 may not be showing the same pattern of results as the control group due to less input from

premotor areas, such as pre-motor cortex and the SMA, to M1. This could be because the inhibition in these areas has been increased in order to interrupt dysfunctional signals from the striatum to control for tics during the motor task. This model can explain the current results in the following way: the TS group show the same pattern of results as the control group during the early stages of movement preparation, then the control group have inputs from other cortical areas, particularly premotor areas (Klein-Flügge et al., 2013), which leads them to show the typical increase in MEP amplitude and decrease in variability. The TS group, however, could be receiving much less input from other cortical areas, leading to their MEP amplitude to remain variable and increase less in the final stages of movement preparation.

The severity of motor tic symptoms was related to the modulation of cortical excitability in the pre-movement phase, so that those with the most severe motor tic symptoms showed the least modulation of their MEP amplitude. Put another way; those subjects who showed a similar pattern of cortical excitability as the control group in Experiment 1 tended to have less severe motor tics. This could be because those individuals with more severe tic symptoms receive more dysfunctional excitatory signals from the striatum. They then have to engage in local inhibition of premotor areas and M1 to a greater extent in order to keep their tics suppressed so that they can complete the actions that the task required. This concurs with the idea that increased local inhibition of the sensorimotor cortex in TS is a reaction to hyper excitability that leads to tics, which develops to enable voluntary movement (Jung et al., 2013). In Experiment 2 I investigated cortical excitability in young people with TS at rest.

4.12 Experiment 2: Subjects

1 female and 9 male subjects with pure TS aged 12-21 years (mean age 18.0 ± 3.1 years) took part in this experiment along with 10 age and gender matched typically developing subjects (mean age 17.8 ± 3.4 years). The YGSS was taken on the TS group on the day of testing. TS subjects with secondary diagnosis of any disorder, such as ADD, ADHD or OCD were excluded from this study, due to reported differences in SICI and ICF effects reported in subjects with such disorders (e.g. Gilbert et al., 2007; Orth & Rothwell, 2009). Details of TS subjects can be seen on Table 4.4.

Table 4.4 Details of TS subjects that took part in TMS experiment 2.

ID	**Age**	**Gender**	**YGTSS**	**Motor tic scores**	**Phonic tic scores**	**Medication**
TS006	21.1	M	45	12	23	-
TS030	16.5	M	24	12	12	-
TS013	17.7	M	33	16	7	Clonidine
TS069	12.0	M	14	9	5	-
TS002	21.4	M	8	8	0	-
TS008	21.5	M	19	11	8	-
TS048	15.4	M	24	12	7	-
TS062	17.5	M	42	21	21	Citalopram
TS085	21.2	F	52	18	19	Citalopram
TS075	15.3	M	56	18	18	Aripidrazole

4.13 Experiment 2: Transcranial Magnetic Stimulation Procedure

Paired-pulse TMS was delivered using a Magstim 200 BiStim TMS system (Magstim Ltd., UK) with a 70mm figure of 8 coil. The coil was positioned over the motor hotspot for the FDI muscle of the right hand, angled at 45° from the midline, as in Experiment 1. The coil location was maintained throughout the experiment via a Brain site neural navigation system (Rogue Research Inc., Montreal Quebec, Canada). This system involved using a headband with a tracker attached to the subject's head, and a second tracker on the TMS coil. The position of the motor hotspot in relation to these two trackers was constantly monitored and any movement of the coil from the optimum position was corrected online by the experimenter. A mechanical arm was used to hold the TMS coil in place, and the subjects sat comfortably with their head positioned in a chin rest. The subjects took regular breaks to stretch to minimise discomfort.

Single-use ECG electrodes (Covidien™, 2011) with a 5mm diameter were positioned in a belly-tendon configuration as in experiment 1. The signals were amplified and bandpass filtered (10 Hz- 2kHz, sampling rate 5kHz) and digitalized using Brainamp ExG (Brain Products GmbH, Gilching, Germany) controlled by Brain Vision Recorder (Brain Products GmbH, Gilching, Germany). Participants were encouraged to maintain their hand in a relaxed position on a table directly in front of them. Resting motor threshold (RMT) was determined as the lowest intensity needed to yield a MEP response of ≈50μV in the relaxed FDI muscle, in minimum of 5 of 10 trials. RMT was used to set the conditioning pulse. The test pulse was set to the lowest intensity needed to produce a MEP response of ≈1mV in 5 out of 10 trials.

Once the motor thresholds had been estimated there were 3 blocks of trials: a SICI, LICI and ICF paradigm. The order in which these paradigms were delivered was randomised for each subject to reduce

order effects. Each paradigm was controlled using an in-house built script and Matlab software. For SICI the conditioning pulse was set at 50, 60, 70 and 80% of RMT, with 15 trials for each intensity of conditioning pulse, plus 15 trials of unconditioned test pulses (baseline measure). The inter-stimulus interval (ISI) was 3ms, after which the test pulse set to the 1mV threshold was delivered. The paradigm was the same for ICF except the ISI was 15ms. For LICI there were 15 trials for each conditioning pulse intensity of 105, 110, 120 and 130% of RMT, with an ISI of 100ms, and 15 unconditioned test pulses. For every paradigm the 75 trials were delivered in a pseudo-random order. The 15 trials of each conditioning intensity were split into 3 blocks of 5 trials. The order of the blocks were randomised. There was a 5 second gap between each trial. If the subject requested a break the script was paused and then continued when they were ready.

4.14 Experiment 2: Results

Between-groups t-tests revealed no significant difference between the TS group and the control group for RMT (TS mean = 45.1 ± 6.5% of stimulator output, CS mean =42.6 ± 3.6% of stimulator output, $t(18)=1.6$, $p>0.1$) and 1mV threshold (TS mean = 54.5 ± 9.6% of stimulator output, CS mean= 51.6 ± 5.4% of stimulator output, $t(18)=1.4$, $p>0.1$).

The peak-to-peak MEP amplitude in response to the test pulse was measured using an in-house built script and Matlab. Each trial was visually inspected and any trial with evidence of muscle contraction was excluded from the analysis. On some LICI or SICI trials the MEP to the test pulse was not evident due to ceiling effects. These trials were also excluded from the analysis. For each subject the median MEP amplitude was calculated for each intensity of the conditioning pulse. The median MEP amplitude for unconditioned trials was also calculated and was considered the baseline. Each subject's data was then normalised into a percentage change from baseline for each conditioning pulse intensity. The mean change in MEP

amplitude for each conditioning pulse was then calculated for each group. The results of these can be seen in Figure 4.5 A-C.

As the MEP data was not normally distributed, log transformations were performed on each individual's data before entering it into a 2 (group) x 4 (intensity of conditioning pulse) mixed ANOVA for each paradigm. For the LICI paradigm, the ANOVA revealed a significant effect of conditioning intensity ($F(2, 17)=6.9$, $p<0.05$), but no significant conditioning intensity x group interactions ($F(2,17)=0.8$, $p>0.05$). Post-hoc pair-wise comparisons showed a significant difference between percentage change in MEP size to a 105% RMT conditioning pulse intensity compared to 130% RMT ($p>0.05$, Bonferroni corrected for multiple comparisons). This result shows that the LICI paradigm was successful in reducing MEP amplitude as the conditioning pulse intensity was increased from 105% to 130%, as can be seen in Figure 4.5A.

The ANOVA for SICI showed a significant effect of conditioning intensity ($F(2, 35)=8.5$, $p<0.05$) but no significant group x intensity interaction ($F(2, 35)=2.3$, $p>0.05$). Post-hoc pair-wise comparisons revealed a significant difference between the percentage change in MEP amplitude to a 50% RMT conditioning pulse compared to 60% and 70% ($p>0.05$, Bonferroni corrected for multiple comparisons). This shows that the SICI paradigm was successful in reducing MEP amplitude as the conditioning pulse intensity was increase from 50% RMT to 70% RMT, as can be seen in Figure 4.5B. By looking at Figure 4.5B, we can also see that the TS group have less inhibition than the control group at the higher intensities of conditioning pulse, although this difference does not reach statistical significance.

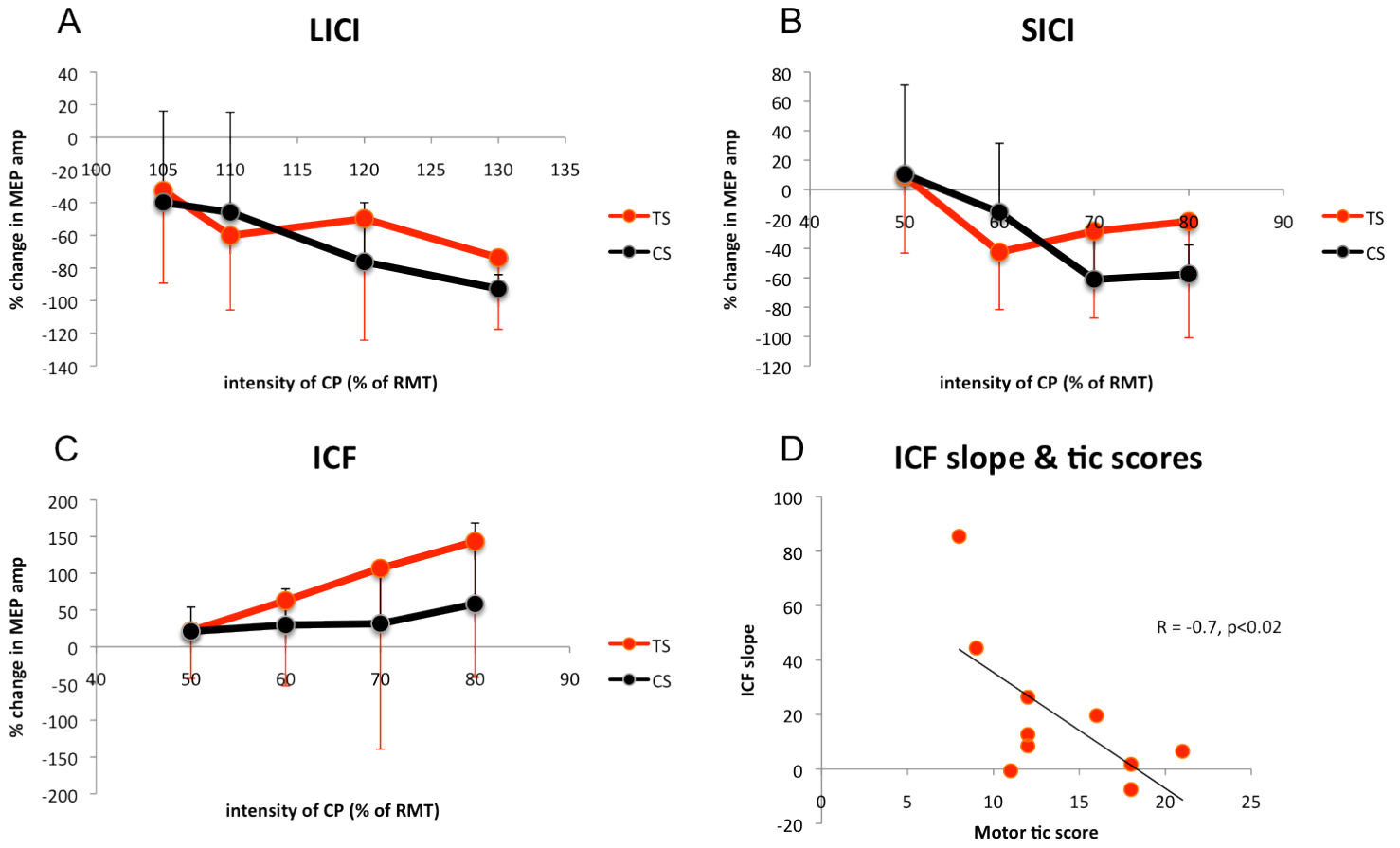

Figure 4.5. ***A-C:*** *Mean percentage change from baseline in MEP amplitude for different intensities of conditioning pulse when the inter-stimulus interval was:* ***A****: 100ms (LICI),* ***B****: 3ms (SICI) and* ***C****: 15ms (ICF). Error bars represent 1 standard deviation.* ***D:*** *Relationship between YALE motor tic scores and the slope of ICF function for TS group.*

The ANOVA for ICF showed no significant effect of conditioning pulse intensity ($F(2, 39)=0.99$, $p>0.05$) and no significant interaction effects ($F(2, 39)=1.3$, $p>0.05$). However, by looking at Figure 4.5C, we can see there is a trend towards the TS group having a larger ICF effect. However, the variability of the data is much too high, as the error bars show, the standard deviation was high for both groups, so finding significant between group effects is unlikely in this sample.

Finally, a linear regression of the relationship between conditioning pulse intensity and the percentage change in MEP size was plotted for each individual's SICI, LICI and ICF paradigm. The slopes of each function were compared between groups using 2-tailed between-group t-tests, and no significant difference in slope was observed (see Table 4.5). The relationship between slope for each paradigm and motor tic score was then examined for the TS group. Figure 4.5 D shows a significant negative correlation between ICF slope and motor tic score (Pearson's $R= -0.6$, $p<0.05$). No other relationship was significant. See Table 4.6 for all slope statistics.

Table 4.5 Between-group comparisons of conditioning pulse intensity vs. % change in MEP size slope for each paradigm. SD = 1 standard deviation.

Paradigm	**TS mean ± SD**	**CS mean ± SD**	**t-value**	**p-value**
LICI	-2.07 ± 2.12	-2.29 ± 2.21	0.23	0.82
SICI	-1.60 ±3.06	-3.48 ± 2.87	1.41	0.18
ICF	6.82 ± 12.98	2.84 ± 3.68	0.90	0.39

Table 4.6: Relationship between motor tic score and conditioning pulse intensity vs. % change in MEP size slope.

Paradigm	**Pearson's R**	**p-value**
LICI	-0.096	0.32
SICI	-0.36	0.15
ICF	-0.59	0.034

Finally, the percentage change in MEP amplitude from the conditioning pulse intensity that evoked the maximum median response for each individual was compared between groups for LICI, SICI and ICF. There is individual variability in the conditioning pulse intensity that provokes the strongest response; so looking just at the maximum change in MEP, at whichever conditioning intensity this may be, may pull out between-group differences that are not apparent when comparing binned data. Furthermore, the relationship between MEP amplitude and conditioning pulse in a SICI paradigm is typically U-shaped (Ziemann, 2013), which means the intensity which produced the largest decrease in MEP amplitude may not necessarily be the largest, or the same for each subject.

As can be seen in Figure 4.6, the TS group have a smaller maximum LICI effect and a larger ICF effect than the control group. A between-groups 2-tailed t-test revealed this difference is significant for LICI ($t(18)=2.2$, $p<0.05$) but not for ICF ($t(18)=1.7$, $p>0.05$). The maximum change in MEP amplitude for LICI, SICI and ICF was also correlated with motor tic score. As with the slope, there was a significant negative relationship between motor tic score and maximum percentage change in MEP amplitude for the ICF paradigm (Person's $R= - 0.73$, $p<0.01$, see Figure 4.7). No significant correlations were apparent in the SICI (Person's $R= - 0.32$, $p>0.1$) or LICI (Person's $R= - 0.43$, $p>0.1$) paradigms.

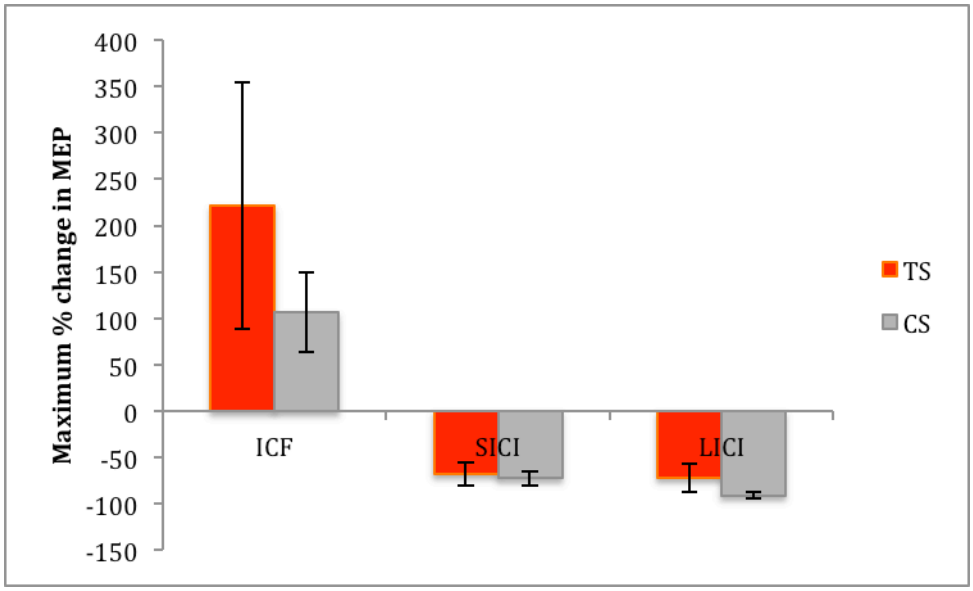

Figure 4.6: The difference between the TS group and the control group in the maximum change in MEP amplitude. Error bars represent 1 standard deviation of the mean.

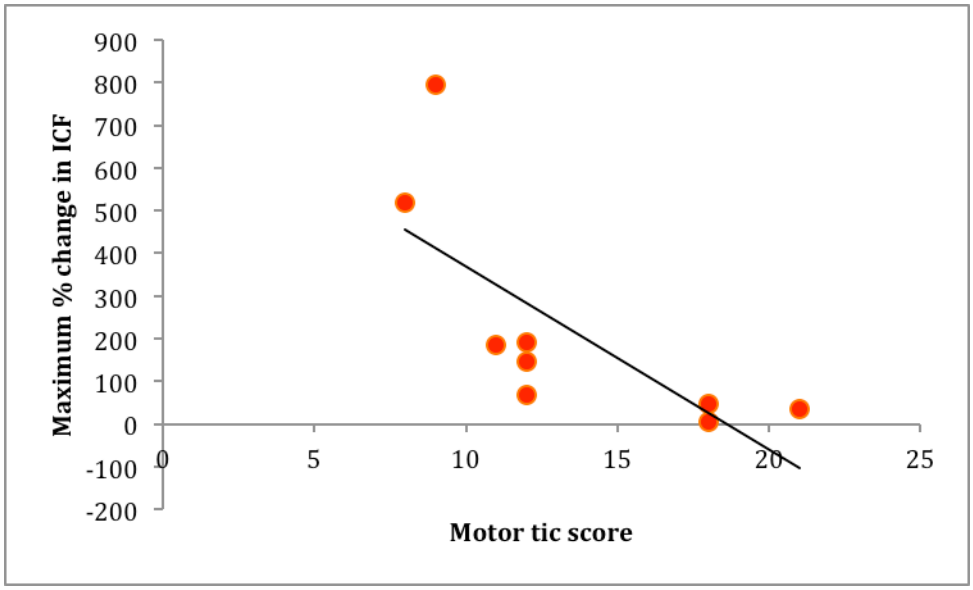

Figure 4.7: The relationship between motor tic score and the maximum % change in MEP amplitude for the ICF paradigm.

4.15 Discussion

By completing Experiment 2 I hoped to gain insight into cortical excitability at rest in young people with TS. Two paradigms that reflect inhibitory mechanisms (SICI and LICI), and one that reflects excitatory mechanisms (ICF) were conducted on a group of young people with "pure" TS and a typically developing matched control group. From the results of this experiment I cannot confidently conclude that the TS group have different inhibitory or excitatory responses to the typically developing population. However, the results do show a trend towards the TS group having a reduced SICI and LICI and increased ICF effect that has been previously reported in adults with TS (Orth & Rothwell, 2009).

Indeed, the extent to which the ICF paradigm had a facilitatory effect, as measured by slope or by maximum percentage change in MEP amplitude, was related to motor tic scores. This was a negative relationship in which those with the highest motor tic scores showed the smallest increase in MEP amplitude to a facilitatory conditioning pulse. This concurs with the finding from Experiment 1; that TS subjects with most severe symptoms also show less modulation, i.e. a shallower increase in MEP amplitude during the period preceding a volitional movement. As the size of the ICF effect is likely to reflect the balance of glutamate activity and GABA-ergic tone in the cortex, it is unclear from these results whether the negative relationship with tic scores is an indicator that those with the most severe tics have less glutamate activity or more GABA-ergic tone. I would argue that the latter is more likely, as tics are likely to be produced by increased glutamatergic input from the striatum via the thalamus (Albin & Mink, 2006), so those with more severe tics are likely to have increased glutamate activity. Increased glutamate could be driving up tonic levels of GABA, which could be influencing the ICF response. This idea will be explored in more depth using magnetic resonance spectroscopy as discussed in the following chapters.

The reason the between-groups results did not reach statistical significance in Experiment 2 in the ICF and SICI paradigms may be due to the large amount of variability in the data, which can be seen in the large standard deviations for both groups. This variability could have been reduced by increasing the number of trials, particularly in the baseline measure of MEP amplitude to an unconditioned pulse. A less variable baseline may have reduced the variability of the percentage change in amplitude to conditioned pulses. However, increasing the number of trials would have increased the amount of time the experiment took, which is unlikely to be well tolerated by all subjects, especially the younger members of the group. Furthermore, the ISIs that were chosen may not have been optimum for every subject to produce the largest effect. Testing a range of ISIs and conditioning pulse intensities would be ideal, however time constraints made this not reasonable.

Another issue was that there were ceiling effects for five TS and three control subjects for at least one conditioning pulse intensity in the LICI paradigm. This means that the LICI effect may not have been accurately represented in the binned data, as at the higher intensity conditioning pulses the MEP was inhibited to an extent that it was too small to measure. This was not the case for the SICI paradigm. Taking the percentage change in MEP amplitude from the conditioning pulse intensity that evoked the maximum median response (before ceiling) gets around this problem. Looking at the data in this way, the results show a significant decrease in the LICI effect for the TS group compared the control group. Together, these results imply a trend towards young people with TS having reduced $GABA_A$ and $GABA_B$ synaptic activity and increased glutamatergic activity compared to typically developing young people.

The large amount of individual variability in the data could be accounted for by the age of the subjects. Walther et al., (2009) reported that typically developing children and adolescents have higher motor thresholds and reduced SICI effects compared to their adult sample. LICI

was not tested in Walther et al.'s (2009) study, and no difference between children and adults were reported for the ICF paradigm. The authors suggest that their results reflect that the GABA system goes through a maturation process with age, and that children have not fully developed the structure for GABA-ergic intra-cortical inhibition. The glutamatergic excitatory system, however, is developed earlier, which is how they explained the intact ICF response. They go on to suggest that the maturation of synaptic plasticity is also mediated by the GABA-ergic system. A reduction in GABA-ergic signalling could relate to the enhanced synaptic plasticity needed for motor learning in childhood (Walther et al., 2009). They do not offer an explanation for the increased resting motor thresholds observed in children and adolescents.

The SICI and ICF results from Experiment 2 do not concur with these conclusions, as I found that a SICI effect was apparent to some extent in both groups, where the control group showed little evidence of an ICF effect (see Figure 4.5 B and C). However, Walther et al.'s (2009) SICI paradigm only tested one intensity of conditioning pulse, 80% of RMT, which may have not been optimum for all subjects. Furthermore, by looking at their reported results, I can see they had a larger degree of variability in MEP amplitude in their child and adolescent groups compared to their adult group. Considering just their 3ms SICI paradigm, the SEM was 0.15, with a range of 0.5 for adults, compared to a SEM of 1.27 and range of 3.45 for adolescents and SEM 0.47 of and range of 1.61 for children, with 10 subjects in each group (Walther et al., 2009). This is in agreement with the large amount of individual variability across my subjects, which suggests that SICI, LICI and ICF effects are less robust and MEP amplitudes more likely to be variable in adolescents compared to adults. It is likely that the optimum conditioning pulse intensity and ISI to produce a SICI, LICI or ICF effect differs from person to person, and is more variable during brain development in childhood and adolescence.

Taken together, these TMS experiments have shown that young people with TS do not show the same modulation of cortical excitability of M1 as a control group during an active task. This could be due to an increase in the inhibition of premotor areas to control for tics, leading to less excitatory inputs to M1 during the final period preceding movement onset. Measures of excitability and inhibition at rest suggest a reduced inhibitory response and increased facilitatory response in the TS group. However, the large amount of variability in the at-rest data makes this finding unreliable. A more direct measure of the main inhibitory & excitatory neuro-transmitters in cortex may present a clearer picture of local changes in cortical excitability in the TS group. Magnetic resonance spectroscopy (MRS) can be used to quantify certain metabolites in brain tissue, including GABA and glutamate. This method does not rely on the variable measure of MEP amplitudes, and can be used to measure the excitability of areas other than M1. This is useful as it is likely that the task-based alterations in cortical excitability could be a reflection of the inhibition of connected cortical areas which influence M1 during movement preparation (Klein-Flügge et al., 2013). A MRS experiment will be discussed in the following chapter.

Chapter 5: γ-amino-butyric acid and Glutamate concentrations in the Supplementary Motor Area and the Primary Motor Cortex in Tourette Syndrome, as measured by 7 Tesla Magnetic Resonance Spectroscopy

5.1 Introduction

The TMS experiments presented in the previous chapter showed that adolescents with TS have reduced gain in excitability of primary motor cortex in the period preceding movement onset compared to a typically developing control group. Furthermore, the ability to modulate cortical excitability, preceding a volitional movement or at rest, was found to be related to motor tic severity. These functional changes in cortical excitability may be reflective of the activity and balance between the main inhibitory neurotransmitter γ-Aminobutyric acid (GABA) and the main excitatory neurotransmitter Glutamate. Magnetic Resonance Spectroscopy (MRS) is a technique that can be used to quantify metabolites such as GABA and Glutamate in brain tissue in vivo. In this chapter, I will use MRS to quantify certain metabolites of interest in two cortical areas that have previously been related to TS: primary motor cortex and the supplementary motor area.

Several research studies have suggested that people with TS have alterations in GABA-ergic signalling or the number of GABA-ergic interneurons (Lerner et al., 2012; Kalanithi et al., 2005; Tian et al., 2011; Orth et al., 2009). Lerner et al. (2012) conducted a positron emission tomography (PET) procedure to investigate pathological changes in GABA-ergic receptor binding in a group of adults with TS. They found decreased binding of $GABA_A$ receptors in the striatum, thalamus & insula of their TS group compared to a matched control group. Post-mortem investigation has also revealed a reduction in GABA-ergic interneurons in the striatum of individuals with TS (Kalanithi et al., 2005). Furthermore, tic-severity

has been shown to correlate with an increase in expression of GABA-related genes in children with TS (Tian et al., 2011). Taken together, it is reasonable to suggest from this research that a GABA-ergic dysfunction in the striatum may play a role in the pathopysiology of TS.

Previous evidence from TMS studies has suggested alterations in GABA-ergic signalling in M1 in people with TS, as discussed in detail in Chapter 4 (Orth et al., 2009; Draper et al., 2013; Hiese et al., 2010). For example, Orth et al. (2009) reported a reduction in short interval cortical inhibition (SICI) effect in adults with TS. SICI is a paired-pulse trancranial magnetic stimulation paradgm thought to reflect $GABA_A$ synaptic activity in primary motor cortex (M1). This suggests that GABA-ergic dysfunction in TS is more widespread, affecting cortical as well as basal ganglia structures. Although I did not replicate this result in Experiment 2 within the previous chapter, MRS may reveal subtle alterations in GABA concentrations in young people with TS that were not observed at a level of statistical significance using TMS due to the variability of the MEP response.

Research on typically developing subjects has indicated that the GABA concentration in the primary visual cortex (Edden et al., 2009), the frontal eye fields (Sumner et al., 2010), the primary somatosensory cortex (Puts et al., 2011; Floyer-Lea et al., 2005), the supplementary motor area (Boy et al., 2010) and the primary motor cortex (Puts et al., 2011; Stagg et al., 2010) is predictive of performance on a specific task that is thought to recruit that area. These studies have demonstrated that a region-specific change in GABA concentration can be directly related to performance on a particular task. For example, GABA levels in the SMA were found to be predictive of performance on a subconscious motor control measure known as the negative compatibility effect (Boy et al., 2010). The negative compatibility effect is thought to measure automatic inhibitory mechanisms. This may be related to TS because local inhibitory mechanisms are likely to be involved during tic suppression and it stands

to reason that those with higher levels of GABA in the SMA would be more able to engage in successful tic-suppression (Jung et al., 2013).

Indeed, delivering inhibitory repetitive TMS to the supplementary motor area has been shown to reduce tics (Mantovani et al., 2007; Kwon et al., 2011). Therefore it is plausible that increased levels of GABA in this area may be beneficial in reducing tic symptoms. Furthermore, the results from the TMS Experiment 1 reported in the previous chapter suggest that the M1 is receiving less influence from connected cortical regions, such as the SMA, during movement preparation, perhaps to control for tics. For this reason the SMA was considered as a region of interest in the MRS experiment presented in this chapter. GABA concentration in the hand area of M1, after anodal transcrainial direct current stimulation (tDCS), has been found to be predictive of motor learning in typically developing individuals (Stagg et al., 2011). This suggests that the amount of GABA in the primary motor cortex is important for the control of action in a motor learning task. If the control of tics is facilitated through local inhibitory processes in motor cortical areas it is likely that GABA concentrations measured in M1 will be altered for people with TS, and may also be important for the control tics. Based on this evidence, M1 was also considered as a region of interest in this chapter.

The primary visual cortex (V1) was used as a control region. There are no recorded visual deficits in TS, therefore it was predicted that there will be no difference between TS subjects and control subjects in this area. This finding will imply that subjects with TS have region-specific differences in neurotransmitter concentrations rather than global changes. As levels of GABA and Glutamate change with age (Hare et al., 1982), and gender differences in the levels of these chemicals have been observed (Maffucci & Gore, 2009), the control subjects were age and gender matched to the TS group.

5.2 Subjects

15 adolescents (aged 11-20 years, mean age 15.8 ± 3.1 years) with a diagnosis of Tourette syndrome (TS) took part in this study. 14 age and gender-matched (1 female, 13 male, mean age 16.3 ± 3.3 years) typically developing adolescents acted as a control group. Tic symptoms were measured on the day of testing using the Yale Global Tic Severity Scale (Leckman et al., 1989). Three subjects had a diagnosis of co-morbid Obsessive Compulsive Disorder (OCD), one had an additional diagnosis of Attention Deficit Disorder (ADD) and one Attention Deficit Hyperactivity Disorder (ADHD). Details of TS subjects can be seen in Table 5.1. For both groups IQ was estimated by use of the Wechsler Abbreviated Scale of Intelligence (WASI) using just the two subtests of vocabulary and matrix reasoning. An independent t-test showed there was no significant difference in IQ between groups (TS mean=113, CS mean= 120, $t(29)=1.2$, $p>0.1$).

Table 5.1 Clinical characteristics of subjects with TS who took part in the MRS study (note: YGSS = Yale Global Tic Severity Scale, WASI= Wechsler Abbreviated Scale of Intelligence: vocabulary and matrix reasoning subtests).

ID	Age (year.month)	Gender	IQ (WASI)	YGSS	Motor tic scores	Phonic tic scores	Co-morbitity	Medication
TS006	20.0	M	84	50	14	16	-	Clonidine
TS018	18.2	M	120	41	13	5	ADD	Clonidine
TS028	17.8	F	96	41	10	15	OCD	-
TS071	12.8	M	118	83	22	11	OCD	-
TS013	17.2	M	135	47	11	6	OCD	-
TS043	11.5	M	123	67	17	10	-	Clonidine
TS069	11.7	M	133	20	10	0	-	-
TS034	13.8	M	118	No tics last 2 months			-	-
TS062	17.0	M	131	65	24	21	-	Citalopram
TS048	14.8	M	118	28	12	6	-	Clonidine
TS049	20.2	M	116	64	21	13	-	Kapra
TS007	19.7	M	95	25	5	0	-	Clonidine
TS030	15.0	M	103	61	16	15	-	-
TS076	14.2	M	85	40	13	8	ADHD	Methylphenidate, Melatonin
TS074	12.4	M	102	30	10	8	-	Risperidone

5.3 Magnetic Resonance Data Acquisition

Magnetic resonance data were acquired on a Philips Achieva 7 Tesla magnetic resonance imaging scanner with a 32-channel SENSE radio-frequency head coil. The subject's head was placed at the iso-center of the scanner with two foam pads either side to minimize head movements. Following an initial survey image, a Magnetisation Prepared RApid Gradient Echo (MP RAGE) anatomical image of the whole brain (120 slices, 1x1x1mm^3 voxel size, TR of 7.3ms) was taken to aid the placement of ROI's for spectroscopy, and for segmentation to allow measurement of voxel tissue content.

After this, subjects were asked to perform a brief fMRI task (EPI sequence with a repetition time (TR) of 2s, echo time (TE) of 25ms, 2x2x3mm^2 voxel size and a field of view of 192 x 60 x 192mm). The 20 slices were positioned to capture motor cortex, SMA and visual cortex, but not the subcortical structures in the brain. Subjects wore prism glasses in the scanner, which allowed them to see the screen where stimulus was projected. This stimulus consisted of the word TAP written in red capital letters for 24 seconds, followed by a white fixation dash (-) on a black background for 36 seconds. Whilst the word TAP was displayed subjects completed a bimanual finger-to-thumb opposition task in which they tapped, for each hand, their thumb to each finger sequentially, coordinating their movements so that both hands tapped simultaneously. This sequence was controlled using Presentation software and was repeated twice. The task was used in order to aid precisely locating the hand area of the primary motor cortex (M1) and the Supplementary Motor Ares (SMA). Significant regions of blood oxygenated level dependant (BOLD) signal activation for tap vs. rest were calculated in real-time using IViewBOLD software built in to the Philips MRI system.

MR Spectroscopy data were then sequentially collected from three different brain regions; primary visual cortex (V1), left hand motor cortex

(M1) and the supplementary motor area (SMA), using a cubic 2cm x 2cm x 2cm voxel for each area. The V1 voxel was positioned in the primary visual cortex, in the posterior region of the occipital lobe, centred on the mid-sagittal plane to cover both hemispheres, and by locating the calcarine sulcus from the MPRAGE as a landmark (see Figure 5.1A). The left hemisphere M1 voxel was positioned by identifying the hand area's characteristic Ω shape of the central sulcus on the MPRAGE as an anatomical landmark (figure 5.1B). This region was confirmed by consulting the statistical colour map from the fMRI and identifying the peak BOLD activation in this region. The SMA ROI was positioned on the mid-sagittal plane anterior to the central sulcus, using the peak BOLD activation in this region as a guide (Figure 5.1C). See Figure 5.1 for an example of voxel positions.

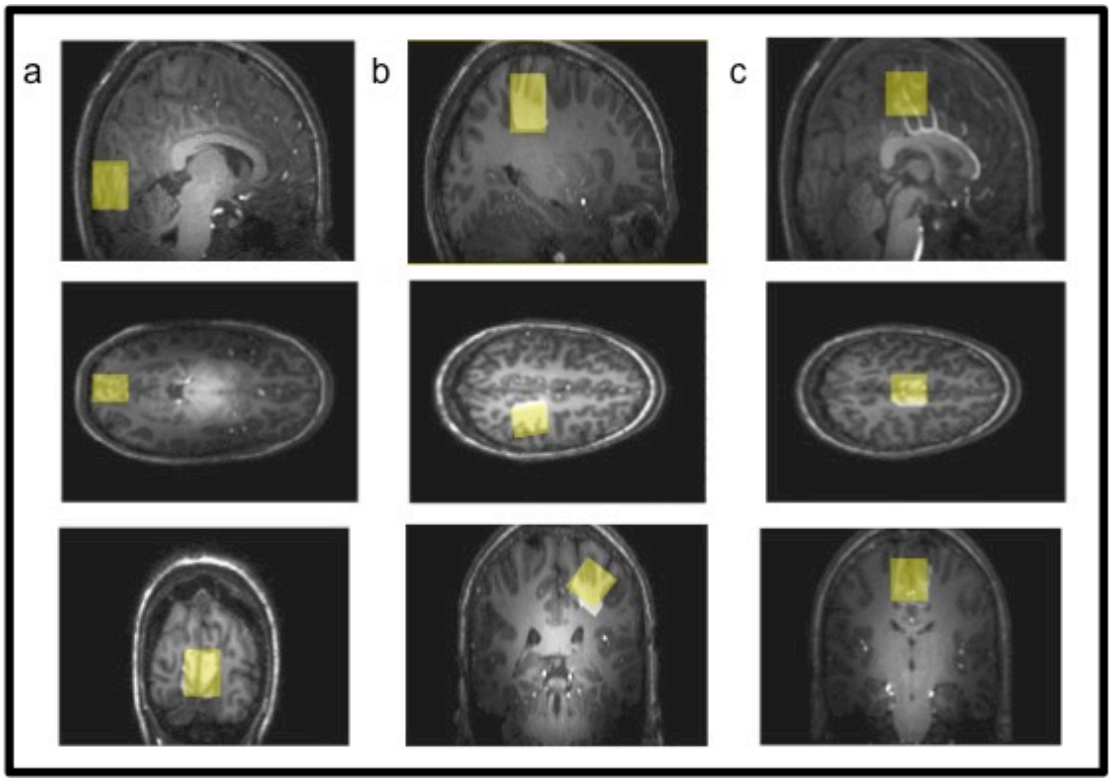

Figure 5.1: Example of ROI positions in sagittal, axial and coronal views from one control subject. ***A****: Primary Visual Cortex,* ***B****: Right hand area of the Primary Motor Cortex, and* ***C****: Supplementary Motor Area.*

MRS data were acquired using a Stimulated Echo Acquisition Mode (STEAM) sequence with echo time TE/TM/TR= 16/17/2000ms. The width of the acquired spectrum was 4,000Hz with 4096 time points. A B_0 field map and parcellated shimming approach was used to increase the B_0 homogeneity (Poole and Bowtell, 2008). 288 spectra were individually collected with Multiply Optimized Insensitive Suppression Train (MOIST) water suppression (Tarasow et al, 2003) and two spectra were acquired without water suppression for correction to absolute concentrations using water referencing. Each MRS voxel took approximately 10 minutes to acquire. During this time subjects were asked to remain as still as possible. This means that neurotransmitter concentrations were measured at rest.

5.4 MRS Data processing

For all individual MR spectra, data from each of the 32 coil elements were collected separately, and were realigned, phase corrected and averaged before being combined across coils using the theoretically optimized S/N^2 weighting, as described by Hall et al. (Hall et al., 2013) using an in-house Matlab script (Mathworks inc. Natick, USA).

Spectra were then analysed using LCModel (version 2.2-4, Provencher, 1993), using the unsuppressed water signal as an internal reference for metabolite quantification and for eddy current correction to gain concentrations of metabolites of interest (for an example of a spectra see Figure 5.2). The simulated spectra of 20 metabolites are in the LCModel basis dataset (Mekle et al., 2009), including: Gamma-aminobutyric acid (GABA), Glutamine (Gln), Glutamate (Glu), and N-acetylaspartate (NAA). Previously published chemical shifts and coupling constants with TE/TM values identical to those used for data acquisition were used to stimulate spectra for a STEAM sequence (Govindaraju et al. 2000). Any outputs with a standard deviation (S.D.) >30 were excluded from the analysis.

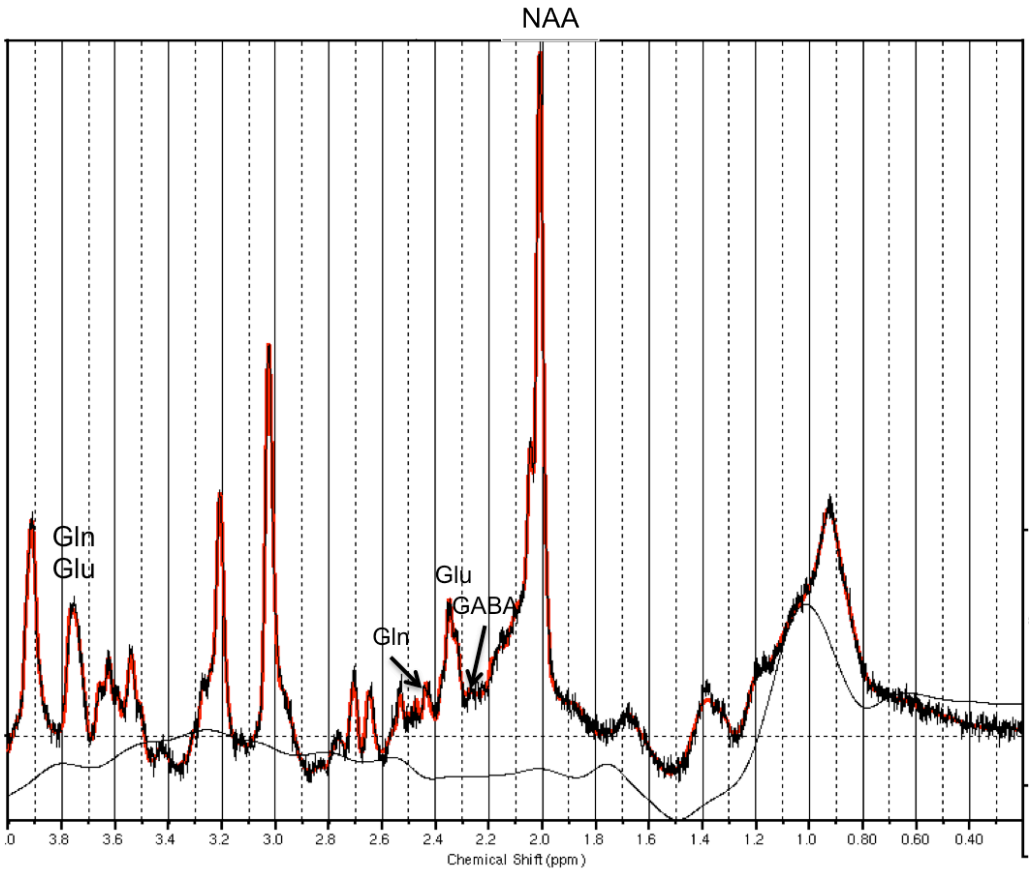

Figure 5.2: An example of spectra output from LC Model. This spectra was taken from one subject's SMA voxel and the main peaks of interest (Gln, Glu, GABA & NAA) have each been labelled.

SPM 8 Segment tool was used to create segmented binary maps of the volumes of white matter, grey matter and cerebral spinal fluid (CSF) respectively from each individual's MPRAGE image. The position of each VOI was then located and the proportions of grey matter, white matter and CSF within each VOI were calculated for each individual using and an in-house Matlab script. These were expressed as fractions.

5.5 Results

For one TS subject's M1 voxel and a different TS subject's SMA voxel the standard deviation of the measured concentrations of metabolites of interest were outside the normal range (Lin et al., 2012). Therefore the final analysis contained data from 14 TS subjects and 14 control subjects for M1 and SMA voxels and 15 TS subjects and 14 control subjects for the V1 voxel. To correct for tissue content, absolute concentrations of each metabolite were divided by white matter plus grey matter tissue fractions. This was done because individual differences in tissue content of the VOIs might affect the results. A between-groups t-test on tissue fractions was also completed for each VOI to confirm that there was no significant difference between tissue content for the groups (SMA: $t(25)=0.8$, $p=0.4$, M1: $t(25)= 0.8$, $p=0.4$, V1: $t(25)=0.7$, $p=0.5$).

Corrected concentrations of the main inhibitory neurotransmitter GABA were considered in each voxel. The TS group were found to have significantly more GABA in the SMA voxel compared to the control group in a between-group 2-tailed t-test (TS mean= 1.16 μmol/g, SD=0.47, control mean= 0.84 μmol/g, SD=0.30, $t(27)=2.1$, $p<0.05$). This difference was still apparent when GABA was taken as a ratio to NAA (TS mean= 0.14 μmol/g, SD=0.04, control mean = 0.11μmol/g, SD=0.04, $t(27)=2.0$, $p<0.05$). GABA:NAA ratio has been reported previously as standard to take into account individual differences in tissue content (for example Stagg et al., 2009). GABA and GABA:NAA concentrations did not significantly differ between groups in either the M1 or V1 voxel (see Figure 5.3A).

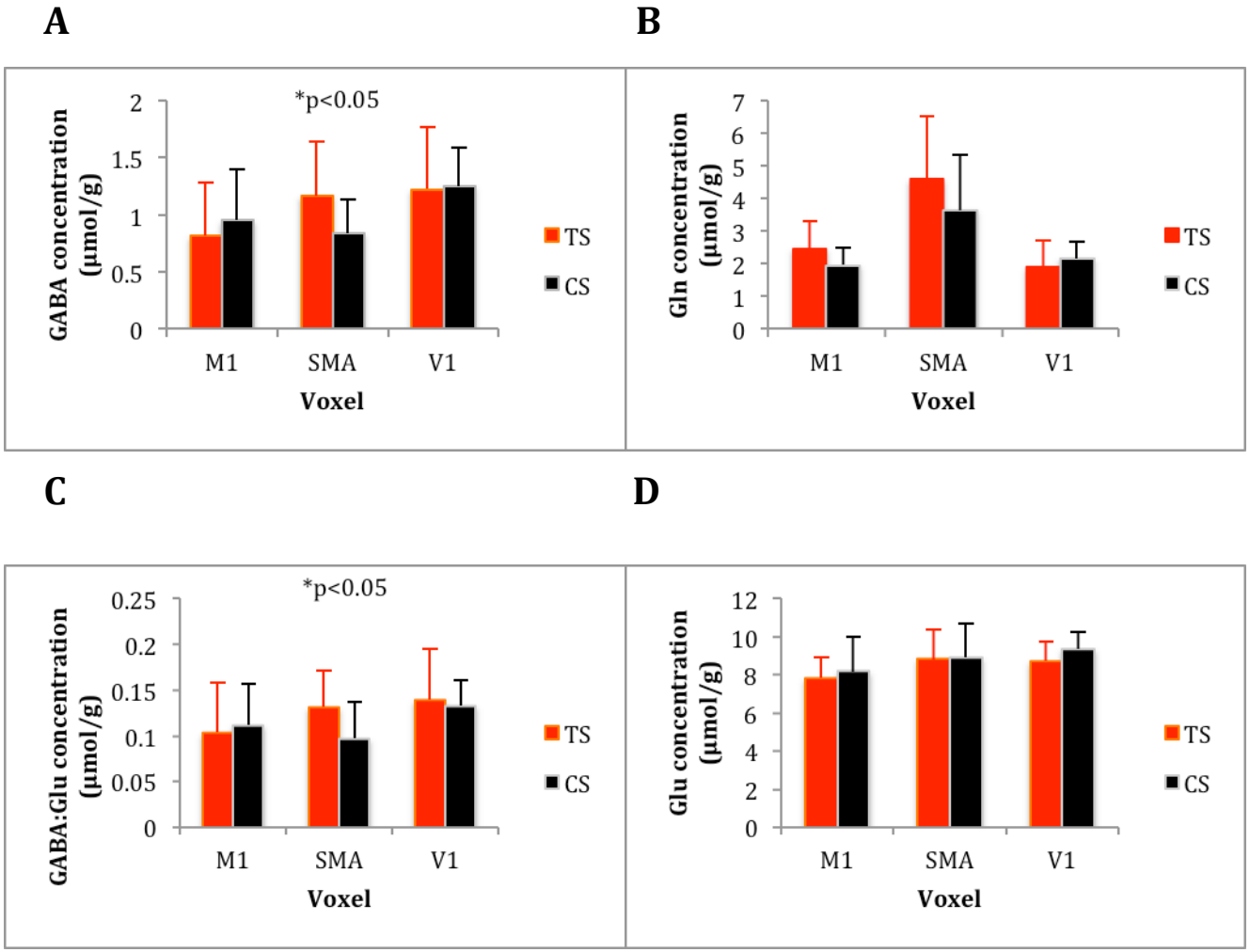

*Figure 5.3: Between-group differences in metabolite concentrations for each ROI: **A**: GABA, **B**: Glutamine, **C**: GABA:Glutamate ratio and **D**: Glutamate. Error bars represent 1 standard deviation*

Excitatory neurotransmitter metabolites glutamate (Glu) and glutamine (Gln) were also considered. Although there were no between-group differences in glutamate (see Figure 5.3D), the TS group were found to have slightly increased levels of glutamine in the M1 and SMA voxels (see Figure 5.3B), but this did not reach statistical significance in a 2-tailed between group t-test. The Glu:Gln ratio was also considered as a reflection of glutamatergic metabolic activity. As in the Glu results, there were no between-group differences in the Glu:Gln ratio in any of the voxels. As a measure of overall excitability in each voxel, the ratio of GABA:Glu was then calculated for each subject. A significant increase in the GABA:Glu ratio was observed for the TS group, again in the SMA voxel only (TS mean= 0.13 µmol/g, SD=0.04, control mean= 0.097 µmol/g, SD=0.04, $t(27)=2.2$, $p<0.05$, see Figure 5.3C).

A final analysis looked at the relationship between all metabolites of interest or ratios previously presented and tic scores by completing several correlations. The only significant relationship observed was a positive correlation between Glu:Gln ratio and motor tic scores in the SMA (Pearson's R= 0.47, $p<0.05$). See Figure 5.4.

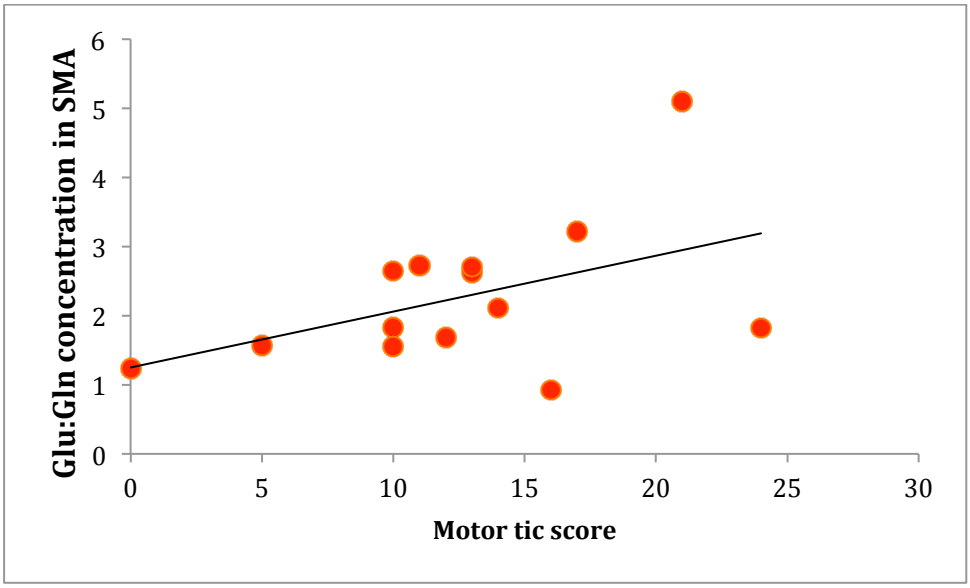

Figure 5.4: Correlation between Glu:Gln ratio from the SMA and motor tic scores measured by the YGTSS on the day of testing.

5.6 Discussion

The main finding from this MRS experiment was that the TS group has significantly increased GABA concentration in their SMA than a typically developing matched control group. This result is also reflected in the GABA:NAA and GABA:Glu ratios. This finding will be explored in more depth in the following chapter.

The TS group showed a trend towards increased in glutamine in M1 and SMA. The concentration of glutamine in brain tissue is thought to reflect the metabolic activity of glutamate. Too much glutamate is neuro-toxic to brain tissue and can cause seizures; so once it is released it is very quickly re-absorbed into the cell body or broken down. This is why Glutamate levels are fairly stable in both groups, as can be seen in Figure 5.3 D; Glutamate levels, on average, are very similar between groups, and the standard deviation is small. Glutamate is converted to glutamine by glial cells after it has been released by a glutamatergic neuron. It is then taken back into the neuron where it is converted back into glutamate (Ongur et al., 2011). Therefore elevated glutamine levels are thought to reflect increased glutamatergic neurotransmission (Xu. et al., 2005). Increased amounts of glutamine in the TS group suggests increased glumatergic activity in M1 and SMA, which concurs with the finding that adults with TS have increased cortical excitably in M1 at rest (Orth et al., 2009). Excessive excitatory inputs from the dysfunctional striatum to the SMA and M1 could be driving the increase in glutamatergic activity seen in the TS group. Previous research has shown that reducing the excitability in the SMA reduces tics symptoms (e.g. through inhibitory rTMS: Mantovani et al., 2007; Kwon et al., 2011), which suggests that reducing the excitability of the SMA, either through decreasing glutamate activity or increasing GABA-ergic release (which would, in effect, reduce Glutamate synaptic release) could reduce tic symptoms.

Furthermore, the Glutamate:Glutamine concentration ratio measured from the SMA had a weak positive relationship with motor tic scores. A high Glu:Gln ratio means a combination of high levels of Glutamate and low levels of Glutamine. If Glutamate activity was high you would expect high Glutamate and high Glutamine concentrations, which would result in a low Glu:Gln ratio. As already stated, Glutamate levels are kept stable, if they were not they cause neurotoxic effects, such as seizures. Therefore the Glu:Gln ratio is largely driven by Glutamine levels, and a high Glu:Gln would mean low levels of Glutamine, and thus low Glutamate activity. This means that those subjects with the more severe motor tic symptoms had the lowest glutamatergic metabolic activity. This could be a consequence of tic suppression that would occur in the scanner to remain still, and would require more effort for those subjects with more severe tics. However, if this were the case I would expect to see the same result replicated in the M1 voxel, which is not apparent. It is important to consider that the strength of the correlation, although significant, is weak (a correlation coefficient (Pearson's R) of only 0.47), $p<0.05$). Increased number of subjects and a stronger relationship would give more weight to this finding.

What is clear from this MRS experiment is that young people with TS have increased glutamatergic activity in the M1 and SMA, and increased tonic GABA levels in the SMA. I can speculate that these findings are likely to be confined to specific areas of cortex, particularly those related to the planning, control and execution of movements, because there are no significant, or trends towards, differences between groups in the occipital lobe (V1) cortical control site. However, it is unclear how increased GABA and increased Glutamatergic activity relate to symptom severity, and whether these findings are evidence of a adaptation to dysfunctional brain areas in TS, or evidence of the dysfunction itself. It is likely to be a reflection of both, i.e. increased Glutamatergic activity is a consequence of increased excitatory input from the dysfunctional striatum, and increased

GABA levels are an adaption to this, to reduce cortical excitability to engage in tic suppression and complete successful volitional movements.

It is important to point out here that the pool of GABA measured by MRS is largely thought to represent extra-synaptic, tonic levels of GABA, rather than GABA phasic synaptic activity (Stagg, Bachtiar & Johnasen-Berg, 2011). Evidence for this comes from research by Stagg et al. (2011) who reported no relationship between GABA measured by MRS and GABA measured by paired-pulse TMS (SICI and LICI paradigms). Stagg et al. (2011) did, however, report a significant relationship between MRS measured Glutamate and a TMS measure of cortical excitability (input-output curve). Therefore MRS measured Glutamate may represent Glutamatergic activity, whereas MRS measured GABA is unlikely to be directly related to GABA synaptic activity, but will instead provide important information about levels of extra-synaptic tonic GABA pools. The following chapter will explore the relationship between increased SMA tonic GABA levels and functional and structural alterations reported in previous chapters.

Chapter 6: Multi-modal exploration of GABA concentrations in SMA

6.1 Introduction

The main result from Chapter 5 was that the TS group has significantly increased concentrations of GABA compared to the typically developing control group in the supplementary motor area only. This finding of *increased* GABA in a premotor cortical area appears contrary to some previous research into GABA-ergic functioning in TS. For example, there is post-mortem evidence of a *reduction* of GABA-ergic interneurons in the striatum of individuals with TS (Kalanithi et al., 2005). Furthermore, Orth et al. (2008) found reduced SICI effects in adults with TS, which suggests reduced $GABA_A$ signalling in M1. In my paired-pulse TMS SICI paradigm described in Chapter 4 I found a trend towards supporting this finding in adolescents with TS, although it did not reach statistical significance. However, it has recently been demonstrated that GABA measured by MRS may not reflect GABA-ergic phasic signalling, which is indirectly measured in SICI and LICI paired-pulse TMS paradigms (Stagg et al., 2011; Stagg, Bachtiar & Johansen-Berg, 2011). Stagg et al. (2011) found that the level of intra-cortical inhibition, measured by SICI and LICI paradigms, were not related to MRS measures of GABA. They suggest that MRS measured GABA does not reflect GABA-ergic phasic synaptic activity, but instead is a measure of tonic levels of GABA in the extra-synaptic pool. This means that the finding from the previous chapter of increased GABA in the SMA for the TS group need not necessarily contradict previous findings of reduced SICI effects (Orth et al., 2008).

In this chapter I will explore how increased tonic levels of GABA in the SMA are related to cortical excitability and structural connectivity in a sub-set of subjects with TS. I will use evidence from the experiments outlined in Chapter 2 (DTI) and Chapter 4, Experiment 1 (TMS), plus new

evidence from a simple finger-tapping fMRI task completed for localisation purposes as part of the MRS protocol, described in Chapter 5. The purpose of this exploration is to better understand the functional consequences of increased tonic levels in TS in terms of excitability and the control of tics, and to examine the relationship between white matter structure and increased GABA. A further purpose of this exploration is to better understand if increased tonic GABA levels are a helpful adaptation to a dysfunctional motor system to help control tics.

6.2 Subjects

All 14 TS subjects that took part in the MRS experiment described in Chapter 5 also took part in at least 1 other relevant experiment within 6 months of the MRS experiment. Details of these subjects and which experiment they took part in can be seen in Table 6.1 below. 10 subjects took part in both the DTI and the TMS experiment, an extra 2 subjects apiece took part in the DTI and TMS experiments independently. All scores presented in Table 6.1 are taken from the day of the MRS experiment.

Table 6.1 Details of TS subjects. All subjects took part in MRS and fMRI task, YGTSS scores and age are taken from the day of MRS experiment. Colours indicate which other experiment the subject took part in: Red= TMS Experiment 1, Blue= DTI, Dark Purple = both TMS & DTI.

ID	Age (year.month)	Gender	IQ (WASI)	YGSS	Motor tic scores	Phonic tic scores	Co-morbitity	Medication
TS006	20.0	M	84	50	14	16	-	Clonidine
TS018	18.2	M	120	41	13	5	ADD	Clonidine
TS028	17.8	F	96	41	10	15	OCD	-
TS071	12.8	M	118	83	22	11	OCD	-
TS013	17.2	M	135	47	11	6	OCD	-
TS043	11.5	M	123	67	17	10	-	Clonidine
TS069	11.7	M	133	20	10	0	-	-
TS034	13.8	M	118	No tics last 2 months			-	-
TS062	17.0	M	131	65	24	21	-	Citalopram
TS048	14.8	M	118	28	12	6	-	Clonidine
TS049	20.2	M	116	64	21	13	-	Kapra
TS007	19.7	M	95	25	5	0	-	Clonidine
TS030	15.0	M	103	61	16	15	-	-
TS074	12.4	M	102	30	10	8	-	Risperidone

6.3 Cortical excitability preceding a volitional movement is related to GABA concentration in SMA.

In Experiment 1 in Chapter 4 the MEP amplitude to a single pulse of TMS delivered in the period preceding movement onset in a simple go/no go button push task was measured. It was found that the TS group did not show the same increase in MEP amplitude and reduction in MEP variability in the period closest to movement onset (81-100% of reaction time) that the control group showed. It was also found that the slope of the relationship between the onset of TMS and MEP amplitude was related to motor tic scores on the day of testing. This was such that those subjects with the most severe motor tics also had the least steep slopes; i.e. they showed the least modulation of their cortical excitability in the period preceding movement onset. As the SMA influences the response of neurons within M1, the increased tonic levels of (inhibitory) GABA in this area reported in Chapter 5 may be, in part, the cause of reduced task-based modulation of excitability observed particularly in those individuals with the most severe tics.

To investigate the relationship between cortical excitability before a volitional movement and MRS measured levels of GABA in the SMA, the median MEP amplitude in the period closest to movement onset was calculated for each TS subject. This period was chosen because it is the period where the TS subjects have significantly smaller MEP amplitudes compared to the control subjects, at the earlier periods the groups are similar. This is also the period where the MEP amplitude is thought to be influenced by the input of premotor cortical areas to M1 (Klien-Flügge et al., 2013). This single measure was then correlated with GABA and Glutamine concentrations measured from the SMA and M1. As can be seen in Table 6.2, there was no significant relationship between MEP amplitude in the period preceding movement onset and M1 GABA nor SMA Glutamine. There was, however, a significant negative relationship

between SMA GABA and MEP amplitude, and a significant positive relationship between M1 Glutamine and MEP amplitude (see Figure 6.1).

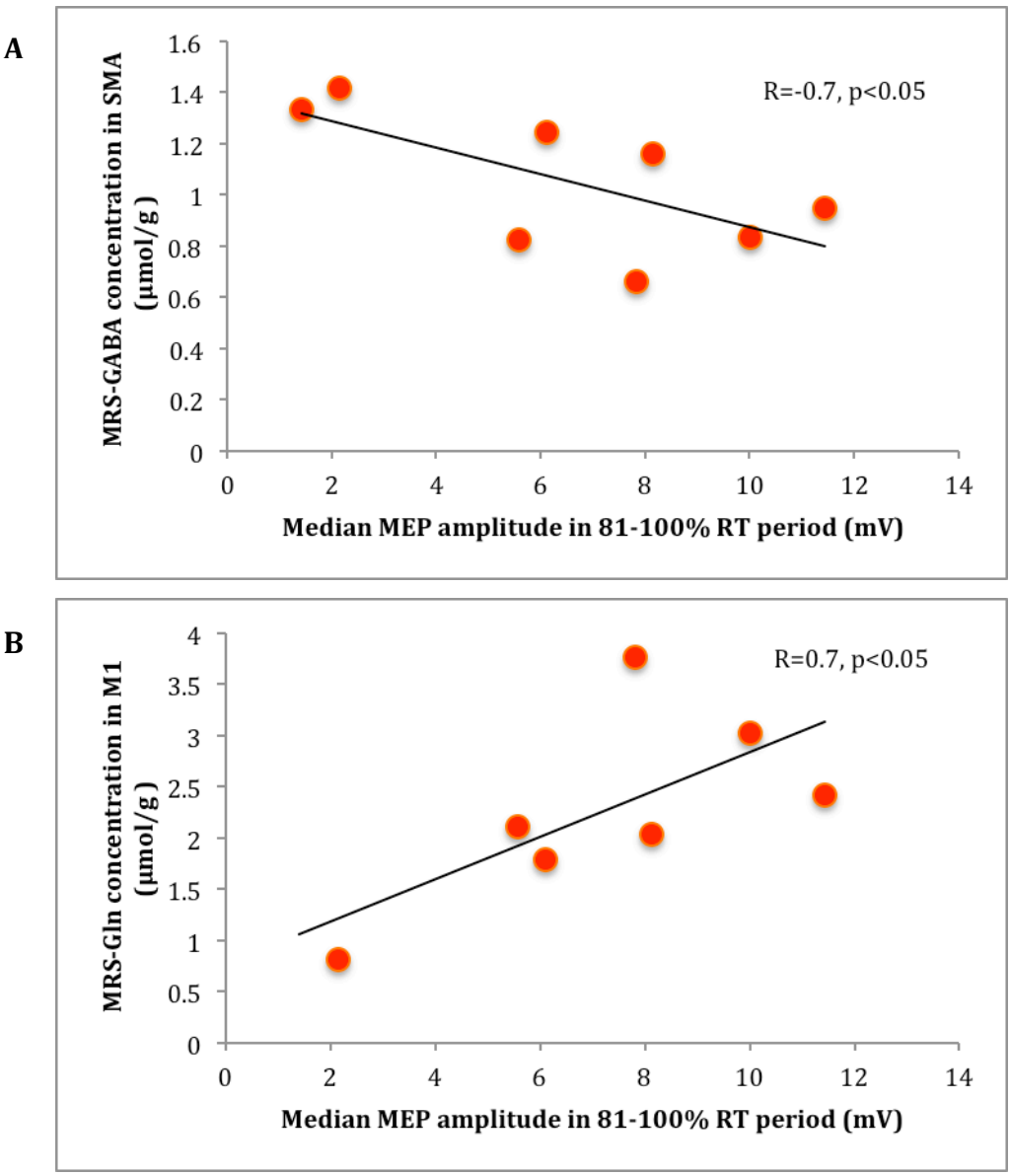

Figure 6.1: Relationship between MEP amplitude to a TMS pulse delivered at 81-100% of reaction time and: ***A:*** *MRS measured GABA concentrations in the SMA, and* ***B:*** *MRS measured Glutamine in M1.*

Table 6.2 Correlations between the median MEP amplitude to a TMS pulse delivered at 81-100% of reaction time and the concentration of GABA or Glutamine measured by MRS in M1 or SMA.

MEP amp correlated with:	**Pearson's R**	**p-value**
SMA GABA	-0.668	0.03
M1 GABA	0.039	0.46
SMA Glutamine	0.079	0.42
M1 Glutamine	0.676	0.03

By combining observations from a TMS experiment and a MRS experiment I can speculate that the reduced cortical excitability preceding movement onset could be related to increased GABA concentrations in SMA and reduced Glutamine activity in M1. As can be seen in Figure 6.1, those subjects with the lowest MEP amplitudes, i.e. the least modulation of cortical excitability, have the highest levels of SMA GABA and lowest levels of M1 Glutamine. This suggests that increased tonic levels of GABA in SMA that has been observed in the TS group may have the functional consequence of reducing cortical excitability of M1 when making voluntary movements. This is likely to be because if the SMA is inhibited by elevated GABA levels, it gives less excitatory input into M1, therefore the MEP amplitude will be smaller in the final period preceding movement onset compared to subjects with less SMA GABA (Klein-Flügge et al., 2013).

Evidence from Chapter 4 showed that those with the most severe motor tics had the least amount of modulation of cortical excitability preceding movement onset. These individuals are represented by the lowest MEP amplitudes in the 81-100% period; those who have the lowest levels of glutamine in M1 and highest levels of GABA in the SMA (see Figure 6.1). This finding concurs with the result from Chapter 5, that those with the lowest glutamatergic activity have the most severe motor tics (see Figure 5.4, Chapter 5), although this result referred to glutamatergic activity in the SMA, not M1. It is likely that those individuals with the most

severe tics have increased GABA levels, which in turn, influence M1 to reduce excitatory glutamate activity (represented by glutamine levels). This reduction in excitatory glutatmate activity is then reflected in the reduced modulation of cortical excitability preceding a volitional movement apparent in the TS group. This mechanism could develop to aid in successful tic-suppression, both to complete a motor task (as in TMS Experiment 1), and to remain still in the scanner.

However, the TMS experiment does not measure the excitability of SMA directly, which is the area of increased GABA levels for TS, and is a key area in tic production and suppression. I can only speculate that increased tonic GABA levels in the SMA may influence the cortical excitability of M1. Furthermore, the TMS experiment did not take place on the same day as the MRS study, so conclusions about the relationship between the two measures must be taken with caution. Therefore, a measure of task-related activity that is close in time to the MRS measurements would be beneficial. In the following section I will discuss the relationship between task-based activity (measured by fMRI BOLD) in the SMA and GABA concentrations. fMRI measurements were taken in the same scanning session as MRS.

6.4 The relationship between % BOLD signal change in a finger tapping task and GABA concentrations in the SMA.

As discussed in the previous chapter, an fMRI finger-tapping task was used as part of the MRS scanning protocol to aid in the localisation of MRS regions of interest. The data collected during this task was used to assess the level of activation of M1 and SMA whilst the subjects were making voluntary finger movements; this time bimanual sequential finger tapping (refer to Chapter 5 for fMRI protocol details). Functional MRI data was processed using SPM8 (The Welcome Trust Centre for Neuroimaging, London, UK). Data was realigned and re-sliced and kept in native space. A t-contrast map of tap vs. rest with a family-wise error corrected threshold

of p<0.05 was applied to each subject. See Figure 6.2 for an example of BOLD signal for tap vs. rest. The area of peak blood oxygenated level dependant (BOLD) signal in left hemisphere M1 and the SMA was identified. Two region of interest (ROI) 10mm^3 boxes were centred on each of these peak-activation co-ordinates using the Marsbar toolbox (version 0.43, Brett et al., 2002). Then the estimated average percentage of BOLD signal change for the tap vs. rest conditions was extracted from each ROI for each subject.

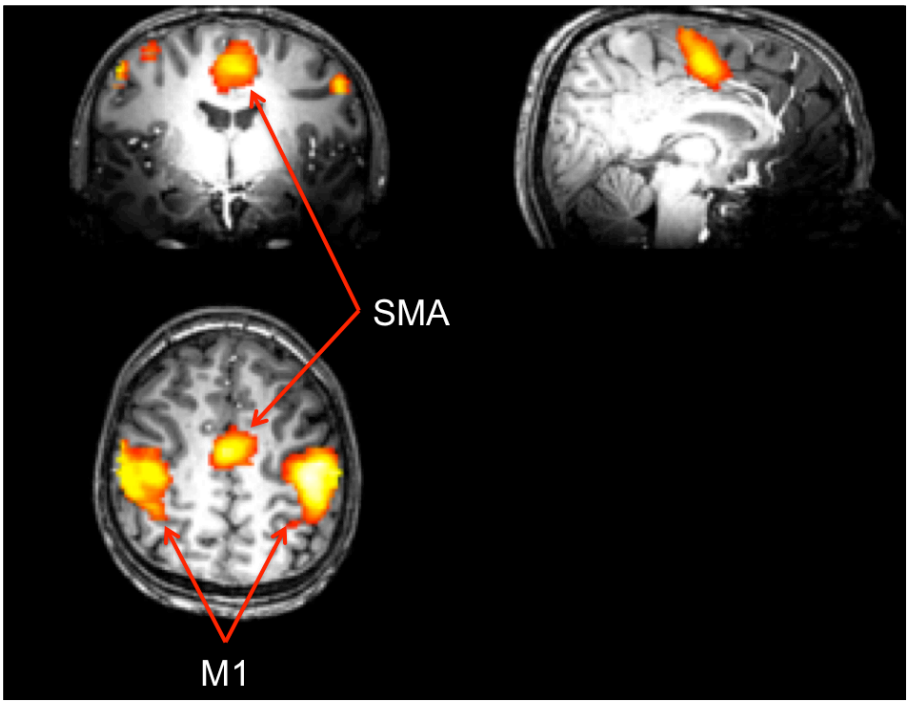

Figure 6.2: Example of BOLD activation for tap vs. rest condition for single TS subject. Arrows indicate SMA and M1.

Firstly, the difference between groups in the percentage of BOLD signal change was compared using a between-groups 2-tailed t-test. No significant between-group difference in BOLD signal change was found in M1 or SMA (largest t(24)= 1.8, p>0.08). The relationship between the amount of BOLD signal change for finger tapping vs. rest in M1 and SMA and metabolites of interest (GABA and Glutamine) was then considered. In the SMA a significant negative relationship between GABA

concentration and BOLD signal change was observed for the TS group (Pearson's R=-0.6, p<0.02, see Figure 6.3). No other significant correlations were observed.

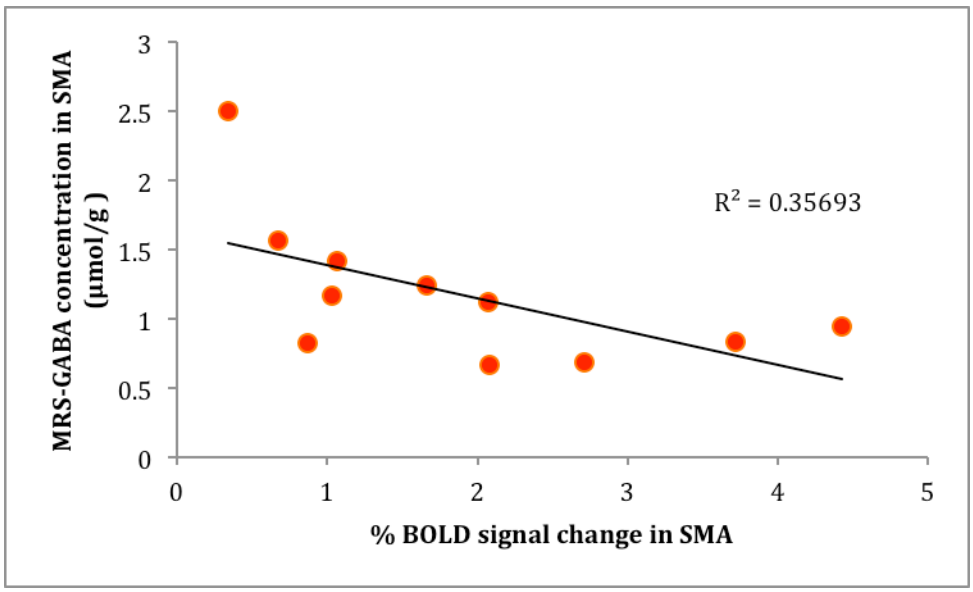

Figure 6.3: Negative correlation between % BOLD signal change in the SMA to finger tapping vs. rest and GABA concentration measured from SMA at rest in the TS group only.

In agreement with the cortical excitability results of the previous section, Figure 6.3 shows that percentage of task-related BOLD signal change has a negative relationship with MRS measured GABA in the SMA. This suggests that having higher levels of tonic GABA in the SMA may reduce SMA activity in a motor task. The positive relationship between M1 activity and glutamine seen in the previous section was not replicated. I can now speculate that a functional consequence of increased extra-cellular levels of GABA in young people with TS is a reduction in cortical activity during a task. As the TS group did not differ significantly from the control group for BOLD signal change, it is likely that the percentage of BOLD signal change in SMA and M1 ROIs mostly reflects the activity involved tapping, rather than activity during the rest period (e.g. if the TS group were using more effort to remain still during the rest period this

could produce between group differences in the percentage of BOLD signal change). In the next section I will examine the structural inter-hemispheric connectivity of the SMA and it's relationship to tonic GABA levels.

6.5 The relationship between GABA concentrations and the structural connectivity of the SMA.

In Chapter 2 I explored differences between the TS group and typically developing control group in white matter microstructure using diffusion tensor imaging (DTI). One of the most intriguing findings was that the inter-hemispheric connectivity of M1 and SMA was highly correlated with motor tic scores (see Chapter 2, Figure 2.7). TS subjects with the most severe motor tic symptoms also had the highest FA in a section of the corpus callosum that joined the SMA across hemispheres. The role of the corpus callosum is to integrate information across hemispheres. It does this by modulating excitability of homologous cortical areas (Bloom & Hyde, 2005). Indeed, FA measured from the area of the corpus callosum that projects to M1 has been found to relate to TMS motor threshold: a measure of cortical excitability (Klöppel et al., 2008). As findings from the previous two sections have implied that increased GABA levels in the SMA have the functional consequence of reducing cortical excitability, it is reasonable to explore how corpus callosum inputs relate to GABA levels. Mean FA values were extracted from an ROI in the body of the corpus that projects to SMA bilaterally (refer to Chapter 1 for methodological details of this process). These were then correlated with the metabolites of interest, GABA and glutamine.

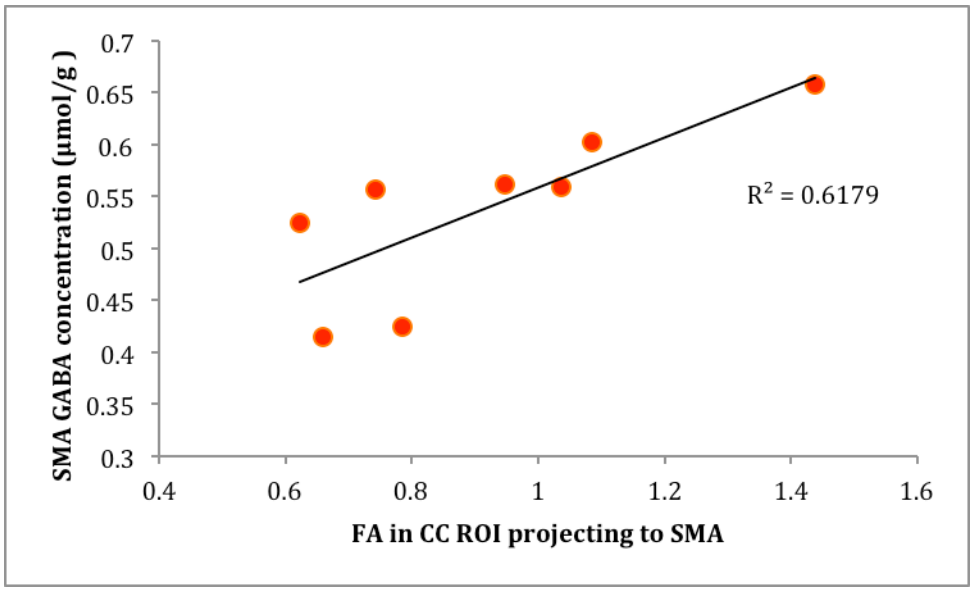

Figure 6.4: Relationship between GABA concentrations in SMA and FA measured from a ROI in the body of the corpus callosum where fibres project to SMA bilaterally.

As can be seen in Figure 6.4, there was a significant positive relationship between FA measured from the area in the body of the corpus callosum that projects to SMA and MRS-GABA concentration in SMA (Pearson's R=0.78, $p<0.01$). No other explored correlation was significant. In the body of the corpus callosum the fibres are all very uniform, so there is not a problem of crossing fibres. FA measured from such a uniform area is thought to mostly reflex axon density (Wahl et al., 2007). Therefore I can interpret Figure 6.4 as follows: TS subjects with increased inter-hemispheric connectivity between SMA bilaterally (i.e. higher FA), have the highest GABA concentrations in SMA, and also the highest motor tic scores (see Chapter 2, Figure 2.7B for the positive correlation between motor tic scores and callosal FA). This finding leads to the suggestion that increased tonic levels of GABA may be, in part, driven by inter-hemispheric inputs into that area.

6.7 Discussion

The multi-modal exploration of elevated GABA concentrations in the SMA in young people with TS has shown that increased SMA-GABA is related to: smaller MEP amplitude in the period preceding volitional movement, a reduced fMRI BOLD signal change in a finger-tapping task, and increased inter-hemispheric structural connectivity of the SMA. Firstly, I will consider the relationship between functional measures (cortical excitability and BOLD signal change) and increased GABA concentration in the SMA.

As GABA is an inhibitory neurotransmitter it is not surprising that elevated levels of MRS-GABA are related to reduced excitability, both in M1 and SMA. The interesting aspect of this result is that it allows me to speculate on the mechanism underlying the decrease in gain function reported in TMS studies discussed in Chapter 4 (Heise et al., 2010; Jackson et al., 2012; Draper et al., 2013). These studies show that people with TS do not show the same increase in cortical excitability as they approach a volitional movement that typically developing control groups show, possibly because of an inhibitory mechanism that develops to reduce hyper-excitability and enable voluntary actions without interference from tics (Jung et al., 2013). The findings from this chapter suggest that the extent to which inhibition of M1 occurs in the pre-movement period is related to tonic GABA concentrations measured from the SMA. This agrees with the interpretation of the modulation of the MEP response given by Klein-Flügge et al. (2013), that the increase in MEP amplitude and decrease in MEP variability in the period closest to movement onset seen in neurotypical populations is largely influenced by input from premotor cortical areas. My results suggest that people with TS do not show this pattern of modulation because of decreased input from the SMA to M1 due to increases in the inhibition of the SMA.

Indeed, previous research has shown increased functional coupling of the SMA and M1 during tics (Hampson et al., 2009), so perhaps reducing the excitatory input from the SMA to M1, via increased tonic inhibition of the SMA, may be beneficial when performing voluntary movements without interference from tics. Furthermore, it was found that performing inhibitory rTMS to the SMA reduced tic symptoms (Mantovani et al., 2007; Kwon et al., 2011) whereas inhibitory rTMS to premotor cortex or M1 did not reduce tic symptoms (Munchau et al., 2002; Orth et al., 2005b). This could be because the SMA receives a lot of input from the striatum, which is dysfunctional in TS, so increasing the inhibition of the SMA may reduce the amount of excitatory input sent on to M1. Indeed, Jung et al. (2013) reported that a reduction in BOLD signal change in both M1 and SMA was observed for subjects with TS during a cognitive control motor task. The reduction in BOLD was related to task performance such that those with the fastest response also had the least BOLD signal change. This suggests that reducing the excitability of these areas facilitates successful motor response. In my MRS study I found that elevated levels of GABA are related to reduced BOLD signal change in a motor task, which suggests that the task-related BOLD findings reported by Jung et al. (2013) may be partly driven by increased tonic GABA.

Although it has been demonstrated that people with TS have alterations in their cortical excitability, and my current data suggests that local increases in tonic GABA in the SMA may contribute to reducing hyper-excitability to enable voluntary movement, it is unclear whether this mechanism is responsible for the ultimate remission in tics that occurs in late adolescence for the majority of people diagnosed in childhood (Leckman, 2002). I found no significant relationship between tic scores and MRS-GABA concentration in M1 or SMA, or with BOLD signal change in the bimanual finger-tapping task.

There was, however, a significant relationship between motor tic scores and the slope of the relationship between MEP amplitude and TMS

onset, such that those with the steepest slope have the least amount of tics. This suggests that having severe motor tics could be influencing the modulation of cortical excitability preceding a movement. As tics are likely to be caused by a hyper-excitability of motor and pre-motor cortical areas, it is likely that those people with the most severe tics also have the higher levels of excitatory inputs to the SMA from the basal ganglia than those with less severe tics. Therefore, an elevated concentration of tonic GABA is likely to be a functional consequence of, or reaction to, hyper-excitability of the SMA, which produces tics. This means that although reducing the activity of M1 and SMA may aid in successful motor responses in subjects with TS (e.g. successful completion of a button push in a go/no go task), it may not be a mechanism that leads to a long-term reduction in tics. Rather, it is a reaction to an over-excitable system.

Further evidence that elevated levels of tonic GABA may be a reaction to hyper-excitability comes from the relationship between inter-hemispheric structural connectivity of the SMA, GABA levels, and motor tic scores. In this chapter I reported that increased FA from the body of the corpus callosum that contains fibres projecting to bilateral SMA relate to increased MRS-GABA in the SMA. In Chapter 2 I also reported that FA from this region has a positive relationship with motor tic scores, such that those with the most severe motor tics also have the most FA. FA, in such a uniform area as the body of the corpus callosum, is thought to mostly reflect axon density because there are a low number of crossing fibres (Wahl et al., 2007). Inter-hemispheric connectivity through the corpus callosum is largely excitatory (Bloom & Hyde, 2005). This leads to the interpretation that elevated tonic GABA levels in the SMA may be driven by increased excitatory input through the corpus callosum. Put another way, reducing the inter-hemispheric excitatory input to the SMA may lead to a reduction in tics by reducing the excitability of this area. If this occurs, there is less excitatory input driving up the glutamatergic activity of the SMA, and less need for reactive increases in tonic GABA to compensate for the over-activity.

Using this framework, a compensatory mechanism may develop throughout adolescence in people with TS that involves plastic changes to brain structure to reduce the number of excitatory inputs to the SMA, premotor cortex, and M1. Those individuals who continue to have a typical amount of excitatory input to M1 and SMA from other cortical areas have reactive inhibitory processes that enable them to control this excess activity to produce volitional actions, as demonstrated by reduced MEP amplitude and reduced BOLD signal change during motor tasks (Heise et al., 2010; Jung et al., 2013). Indeed, in my TMS study I found that those subjects with TS that showed a modulation of cortical excitability similar to the control group in the pre-movement period had the least severe tics. This framework can also explain the finding reported by Jackson et al. (2011) that increased FA in the prefrontal cortex is related to increased motor tics. This is because those individuals with lower FA (i.e. less connectivity) in the tracts leading from prefrontal cortex would be receiving less excitatory input to premotor and sensorimotor cortex from the prefrontal cortex, leading to fewer tics.

This does not mean that increased tonic levels of GABA in the SMA are not helpful in reducing tic symptoms. Indeed, inhibitory rTMS to the SMA has been shown to reduce tics, but this result can also be applied to the same framework. By increasing the inhibition of the SMA, SMA neurons are less likely to send signals to connected regions, i.e. to M1. Therefore, increased the inhibition of this region has the same functional effect as reducing the structural connectivity between M1 and SMA. Thus, it seems that isolating the sensorimotor cortex from the influence of other cortical areas may be beneficial in reducing tics. As SMA receives many inputs from the striatum, which is dysfunctional in TS, it is in an ideal location to interrupt these dysfunctional excitatory signals before they can be transferred to M1 and become a movement. By increasing the inhibition in the SMA only the strongest signals would result in enough Glutamate released to produce an axon potential that sends the signal on.

This would mean voluntary actions would still be coded, whereas the probability that weaker signals that might result in a tic would be sent on could be reduced.

Furthermore, Shima & Tanji (1998) showed that injecting a GABA agonist directly into the SMA of monkeys impaired their ability to produce previously learnt motor sequence. Self-initiated, voluntary movements, however, were still intact. This suggests that increased GABA in the SMA may specifically reduce the likelihood of learnt motor responses being produced, where controlled, voluntary movements would still be successfully processed. As tics can be thought of as learnt, automatic, responses to internal and environmental cues (Leckman, 2002), increasing the levels of tonic GABA in the SMA is likely to specifically inhibit tics, leaving voluntary movements intact.

To sum up, there are increased tonic levels of GABA in the SMA in young people with TS compared to an age and gender matched typically developing control group. GABA measured from the SMA is related to task-based cortical excitability in M1 and the SMA such that a higher GABA concentration is related to a lower change in BOLD signal or MEP amplitude. This suggests that increased tonic GABA is having an inhibitory effect on M1 via decreased input from the SMA, and may be part of a mechanism to reduce the hyper-excitability of M1 to perform a voluntary action without interference from tics. Finally, the amount of GABA in the SMA is related to the structural inter-hemispheric connectivity of the SMA such that a higher FA value is related to higher GABA levels. This suggests that increased GABA might be, in part, driven by inter-hemispheric excitatory inputs to the SMA.

Chapter 7: Discussion

Within this thesis I have used multiple cognitive neuroscience techniques to explore structural and functional alterations in specific areas of cortex in a group of young people with Tourette Syndrome. The results of the experiments presented in this thesis have both supported existing research into TS and provided new evidence that supports the theory of tic suppression via increased local inhibition (Jung et al., 2013) or decreased functional connectivity of motor and premotor cortical areas. The core findings from each research chapter will be summarised in this discussion, and will be put in the context of previous relevant research into TS and theories of a compensatory mechanism. For clarity, I will first present findings from my study of brain structure, and then of brain function (excitation and inhibition), and how the two might relate to one another. Finally, I will present a theory of adaptive changes that might be developing in young people with TS to isolate M1 from excess input, which may lead to eventual tic remission.

7.1 Tic-related alterations in brain structure in Tourette Syndrome

The defining symptoms in TS are chronic, repetitive motor and phonic tics, which can be either simple (brief, meaningless utterances or movements, such as coughing or eye blinking), or complex (slower and more purposeful, such as repeating a phrase or sequentially tapping objects) (Singer, 2005). The underlying neurological mechanism that produces a motor tic is thought to be the same as typical habit-related actions (Palminteri et al., 2011). That is to say through the functioning of cortio-striatal-thalamic-cortico loops. Sensory information is sent from the cortex to the striatum, which depending on the salience of the sensory stimuli, will send signals back to sensorimotor areas of the cortex, via the thalamus, to result in an appropriate action. In TS tics are thought to result from striatal medium spiny neurons becoming active inappropriately, which in turn disinhibit motor pattern generators in the cortex, resulting

in an unwanted movement, which is reinforced by faulty dopamine activity to become a habit or tic (Albin and Mink, 2006). This theory is supported by a wealth of evidence of abnormal volume of basal-ganglia structures in both adults and children with TS (Peterson et al., 1993; Singer et al., 1993; Hyde et al., 1995; Swerdlow et al., 2001; Peterson et al., 2003; Müller-Vahl et al., 2009) which suggests that the basal ganglia develops abnormally, leading to dysfunction (or hyper-excitability) in connected brain regions (Albin & Mink, 2006).

The majority of people diagnosed with TS in childhood are largely tic-free by early adulthood (Leckman et al., 2010). This suggests that throughout adolescence the brain develops in such a way that it compensates for the dysfunctional, or inappropriate, signalling from the basal ganglia, resulting in the majority of tics being controlled or eliminated. Typically, the brain goes through drastic changes in white and grey matter volume between late childhood and early adulthood, although these changes continue throughout adulthood, the rate of change reduces past the mid twenties (Lebel, Caverhill-Godkewitsch & Beauliuem 2010; Kochunov et al., 2012; Lebel et al., 2012). There are large reductions in cortical grey matter volume, and large increases in white matter volume, which is also reflected in increases in FA values in the major white matter tracts (Lebel et al., 2008; Kochunov et al., 2011; Lebel et al., 2012). These typical developmental changes can be influenced by environmental factors such as training **(**Bengtsson et al., 2005). Indeed, training-induced changes in cortical brain structure can even occur in adulthood (Tang et al., 2012). As tics can cause distress, particularly in social situations, and can interfere with normal functioning, young people with TS have a high incentive to supress and control tics. This is something they can do for certain periods of time, depending on the severity of their symptoms (Banaschewski, Woerner & Rothenberger, 2003). Tic-suppression can be thought of as training that is specific to people with TS, and as such, it is likely to influence the development of cortical grey matter and white matter cortical projections that are changing throughout adolescence.

The findings from the DTI experiment I presented in Chapter 2 demonstrated that the white matter in the section of the corpus callosum that projected to M1 or the SMA was highly correlated with motor tic symptom severity. This result concurs with previous findings from Jackson et al. (2011) and Plessen et al. (2004), who both report a positive correlation between motor tics and corpus callosum FA and area respectively. The corpus callosum is a structure that goes through changes in volume and myelination throughout adolescence and early adulthood (Kochunov et al., 2012). The relationship between motor tics and FA from the corpus callosum reported in Chapter 2 suggests that the development of the corpus callosum is strongly linked to, or influenced by, the severity of motor tics. As we know that white matter development can be affected by training, and the corpus callosum is not a structure that is directly part of the cortio-striatal-thalamic-cortico pathway, I can speculate that the relationship between tics and FA is likely to be a reaction to the tics themselves. The finding that there was no relationship between tic severity and sections of the corpus callosum that project to the prefrontal cortex and occipital lobe, areas that are not related to motor tic production, further supports the hypothesis that the development of the corpus callosum in sensorimotor regions is largely influenced by tics in TS.

The specific relationship reported in Chapter 2 was that those individuals with the most severe motor tics had the highest FA value in the section of the body of the corpus callosum that projected to the SMA or M1 bilaterally. As the sample was a cross section of young people with TS, it is unclear from this study whether those with the least severe tics had developed mechanisms that compensated for striatal dysfunction, and the relationship between callosal FA and tics is evidence of this compensation, or if those people who happened to develop more interhemispheric connections end up with more severe tics, perhaps partly contributed to by this increased excitatory input. It is also important to note that the corpus callosum is unlikely to be the only area that changes with

development in young people with TS. Indeed, the machine learning (SVM) analysis reported in Chapter 2 revealed that many, widespread white matter tracts are predictive of a diagnosis of TS. Future research should focus on collecting longitudinal DTI data on young people with TS and mapping the development of white and grey matter and how it relates to tic severity and remission. Such a longitudinal study could help to determine how the development of white matter microstructure relates to tic remission.

Cortical grey matter goes through developmental changes in volume throughout adolescence alongside white matter. Unsurprisingly, there is evidence of training-induced changes in grey matter volume, as well as white matter volume, suggesting that the development of the grey matter of the cortex can be influenced by the environment (Maguire et al., 2000). Past research has indicated that people with TS have thinner grey matter in sensorimotor regions of the cortex, and that the thickness of the cortex has a negative relationship with tic severity (Sowell et al., 2008; Fahim et al., 2010; Worbe et al., 2010). I have replicated this finding in young people with TS in my cortical thickness analysis reported in Chapter 3.

It is important here to consider what grey matter thickness, measured from T1 weighted MRI actually reflects. A cortical thickness or VBM analysis typically classifies tissue as white matter or grey matter based on MRI intensity gradients. The signal that is classified as grey matter is made up of axons, dendrites, dendritic spines, glia, cell bodies and blood vessels. However, we cannot determine what proportion of different cell types make up the MR signal classified as grey matter. There is evidence from post-mortem studies that show a reduction in the number of dendritic spines in human adolescents, and a study that shows dramatic decrease in the number of synapses in the cortex during puberty in non-human primates (Huttenlocher, 1984; Bourgeois & Rakic, 1993). These studies form the basis for the theory that synaptic pruning occurs

during adolescence to reduce the number of redundant connections and streamline the flow of information.

Synaptic pruning has previously been given as the reason for cortical grey matter thinning during adolescence (Plessen, Bansal & Peterson, 2009). However, Bourgeois and Raki, (1993) reported that the density of synapses did not relate to cortical grey matter volume in their non-human primate study, which suggests that it is not the reduction in synapses *per se* that produces a decrease in measures of grey matter thickness or volume (Bourgeois & Raki, 1993; Paus, Keshavan & Giedd, 2008). However, it is likely that a reduction in synapses would lead to a reduction in metabolic demand, so the reduced grey matter thickness could be due to a reduction in glial cells, or vascular restrictions (Paus, Keshavan & Giedd, 2008).

Furthermore, white matter volume is increasing at the same rate as grey matter decreases in typically developing adolescents, so that total brain volume remains largely constant (Lebel et al., 2008). The increase in white matter volume is likely to be partly due to increased myelination of useful connections. This could lead to some tissue that was previously classified as grey matter in a T1-weighted anatomical MRI to be classified as white matter. Hence measured grey matter thickness could decrease because of increased axon myelination: voxels on the grey matter / white matter boundary that were previously classified as grey matter during an analysis now contain more myelin, and become classified as white matter, leading to reduction in grey matter thickness and increases in white matter volume (Paus, 2005). Although it is still widely debated what a decrease in grey matter thickness with age actually represents (Paus, 2005; Paus, Keshavan & Giedd, 2008), it is likely to be a combination of reduced glia and vascular restrictions due to synaptic pruning leading to lower metabolic demand, and increased myelination of axons.

Thus far the explanation for the finding that people with TS have thinner sensorimotor cortex is that less grey matter in these regions is part of the underlying cause of tics (Worbe et al., 2010), or that young people with TS are "developmentally delayed" in the development of cortical grey matter (Sowell et al., 2008). While there is no doubt that tic severity is related to grey matter thickness in the sensorimotor cortices, it could be another example of tics shaping the development of brain structure, rather than brain structure causing tics. As previous models suggest (e.g. Albin and Mink, 2006), the underlying deficit in TS is abnormal basal-ganglia function. Much like the white matter development, it is likely that the sensorimotor regions of the cortex develop to be thinner because of dysfunctional input they receive from the striatum, as part of the cortio-striatal-thalamic-cortio circuit.

Take M1 as an example. To produce a motor tic, M1 needs to be stimulated to such an extent that it sends information down the cortical spinal tract to simulate the muscles into response (Bolhalter et al., 2006). In TS the striatum is sending signals inappropriately, in some cases very often. If those signals get through to M1, M1 will be in a heightened state of excitability, which has been demonstrated in adults with TS using TMS (Orth et al., 2008). Throughout adolescent development connections are streamlined, so that unnecessary synapses are pruned and useful connections are reinforced through increased myelination (Lebel et al., 2008).

If M1 is being over-stimulated throughout this period is it likely that many of the synapses there will become unnecessary, because M1 is in a heightened state of excitability it would only need a small amount of input to produce a response. This reduction in synapses could lead to a reduction in metabolic demand, and a reduction in glial cells. It stands to reason that those people with the most severe tics would have the most over-stimulated M1, which would lead them to reduce the number of synapses in this region to a greater extent, leading to the negative

relationship between tics and M1 grey matter thickness reported in Chapter 3 and in past research (Sowell et al., 2008; Fahim et al., 2010; Worbe et al., 2010). As the sensorimotor cortices are part of the cortio-striatal-thalamic-cortio circuitary, it is likely that the thinning of these regions is a reaction to over-stimulation from the striatum. However, as the relationship between tic severity and grey matter thickness is negative, and has been reported in adults with TS (Worbe et al., 2010), it is unlikely that reducing grey matter thickness is helpful for tic remission.

The findings from the grey matter thickness are also likely to be linked to the relationship between white matter and tics. If reducing the number of inputs to M1 is helpful in reducing tics, those people with the less white matter to M1 will also have thicker grey matter thickness because these inputs will not be there to be myelinated. This is reflected in the data I have collected: the people with the least severe tics have less interhemispheric connectivity between bilaterally M1 (fewer inputs), and also have thicker grey matter in sensorimotor regions. However, as stated previously, it is impossible from the current data to establish whether thinner grey matter and more white matter interhemispheric connectivity is the cause of more severe tics, or the cortex developed in this way in response to severe tics.

7.2 Functional alterations in mechanisms of excitation and inhibition

M1 is pretty much the end of the line when it comes to signals from the striatum resulting in a motor response. If these signals can be interrupted before they reach M1, this is likely to reduce tics. The supplementary motor area (SMA) is an ideal structure to interrupt these signals and reduce tics. This is because the SMA contains many connections from the basal ganglia and has reciprocal connections with M1. Indeed, unlike other motor cortical areas, the SMA has 3-4 times more connections from the basal ganglia than it does from the cerebellum (Akkal, Dum & Strick, 2007). The SMA also has a hyper-direct connection

to the subthalamic nucleus, a property that is thought to allow this area to act as a brake on striatal-thalamic-cortico circuitary (Nachev, Kennard, & Husain, 2008). Evidence for this theory comes from fMRI experiments that have reported increased SMA activity on trials when participants had to make a change to their usual actions, either through an unexpected change of plan or unexpected stop (Nachev et al., 2005; Curtis et al., 2005; Li et al., 2006; Aron & Poldrack, 2006). Furthermore, another role of the SMA is to link environmental and internal cues to particular actions during motor learning (Nachev, Kennard, & Husain, 2008). Evidence for this role comes from an animal study where monkeys were injected with a GABA agonist directly into the SMA. These monkeys had previously been trained in movement sequences, and after the GABA agonist had been administered they were impaired in performing these movement sequences from memory, but could successful perform self-initiated or externally cued actions (Shima & Tanji, 1998). This suggests that if the SMA is inhibited through increased GABA learned motor patterns (or perhaps automatic motor habits) are not executed, whereas self-initiated actions are intact. If we think about tics as learned habits to environmental or internal (i.e. pre-monitory urges) cues, then increasing GABA in this area might well have the effect of reducing tics but keeping voluntary actions intact.

In a recent review Ganos, Roessner and Munchau (2013) suggest that, in the active brain, tics could be reduced by enhanced functional connectivity between pre-motor, SMA and primary motor cortices. However, it has been reported that inhibitory rTMS delivered to the SMA, but not pre-motor cortex or M1, reduces tic symptoms (Munchau et al., 2002; Orth et al., 2005b; Mantovani et al., 2007; Kwon et al., 2011). Furthermore, Hampson et al. (2009) reported increased SMA-M1 functional coupling during tics for adults with TS compared to mimic-tics performed by a control group. Taken together this evidence suggests that increasing the inhibition of the SMA reduces tics by inhibiting dysfunctional signals from the basal-ganglia and by decreasing the amount of excitatory signals sent on to M1. This suggests that reducing, rather

than enhancing, the connectivity between the SMA and M1 is likely to be beneficial in reducing tics, contrary to Ganos, Roessner and Munchau's (2013) view.

Indeed, in the MRS experiment reported in Chapter 5, I found elevated GABA levels for the TS group compared to the control group in the SMA only. Elevated SMA-GABA also had a negative relationship with task-related percentage change in fMRI BOLD signal, so that those with the highest concentration of SMA GABA had the lowest BOLD response in a motor task. This suggests that the functional consequence of elevated GABA in young people with TS is to reduce the excitability, or responsiveness, of the SMA. A possible explanation of why this might occur, in line with the reasoning of the previous paragraphs, is that people with TS are increasing the inhibition of the SMA to interrupt excessive signals from the striatum. This means that fewer signals will be passed on to M1, leaving the levels of GABA and Glutamate in M1 relatively normal.

Two, independent review papers (Ganos, Roessner and Munchau 2013, and Jung et al., 2013) called on evidence from TMS studies (e.g. Heise et al., 2010; Jackson et al., 2012) to suggest that in the active state, people with TS have an inhibitory mechanism that occurs to allow them to produce voluntary movements without interference from tics. In Chapter 4 I have replicated the previous finding that people with TS do not show the same dramatic increase in cortical excitability in the period preceding volitional movement that typically developing young people show. I also found that the extent to which cortical excitability of M1 was modulated preceding movement onset was negatively related to tic severity, so that those with the most severe tics had the least amount of modulation. This could be because those with the most severe tics have the most dysfunctional firing from the striatum, which needs to be reduced in order to successfully perform a voluntary movement without interruption from tics. Effectively, tic suppression has to occur at the same time as preparing to engage in a voluntary action.

Furthermore, in Chapter 6, I reported that the MEP amplitude to a TMS pulse delivered in the final period before movement onset was negatively correlated with GABA levels in the SMA. This suggests that those individuals with the smallest MEP amplitudes, and thus those who did not have an increased in the excitability of M1 preceding movement onset, also have the most elevated GABA levels in the SMA. This result suggests that the MEP response in the last period before movement onset is largely influenced by inputs from the SMA, and by increasing the inhibition of the SMA it has reduced influence on the excitability of M1. Task performance, however, is not impaired, as evidenced by the similar performance between groups on the go/no go task used in Chapter 4 Experiment one. This is further evidence that reducing the input M1 has from the SMA may be beneficial in controlling tics and producing successful voluntary actions in young people with severe tics.

7.3 A local inhibition mechanism to control tics

My research so far leads to a situation where the brain is adapting to dysfunctional striatal activity through reducing the number of excitatory inputs to M1. The isolation of M1 from excessive excitatory signals would allow only the strongest signals to be passed on to become motor responses. This system is likely to favour voluntary movements, rather than tics. Reducing the excitatory input to M1 could, in the long run, lead to the tic remission that is reported in the majority of young adults with TS. The research in this thesis has pointed towards two possible mechanisms by which the amount of excitatory signals to M1 could be reduced. The first is by reducing the number of inter-hemispheric connecting fibres, as demonstrated by the positive relationship between motor tics and FA measured from the section in the body of the corpus callosum that contains projections to M1 or the SMA. By reducing this inter-hemispheric excitatory signalling, the overall level of excitation of M1 or the SMA would be reduced. The second mechanism is by increasing

the level of inhibitory neurotransmitter GABA in the SMA, as a means of controlling excess signalling before it reaches M1.

In Chapter 5 I demonstrated that FA from the section of the corpus callosum that contains projections to the SMA is positively correlated with GABA concentration from the SMA ROI. This suggests that increased levels of GABA are a reaction to excitatory input; those individuals with the most inter-hemispheric connectivity (i.e. excitatory inter-hemispheric input) also have the highest levels of GABA. It is therefore likely that elevated tonic GABA levels, and reduced modulation of cortical excitability in M1, are immediate reactions to an over-excitable system, which could have developed to keep levels of excitation under control as part of a natural homeostatic mechanism that typically keeps the balance of excitation and inhibition in check. These reactive adaptations are more apparent in those people with the most severe tics, perhaps because these people have the highest levels of dysfunctional striatal signalling. As such, they would have the most excitatory signals to M1, which need to be inhibited or interrupted in order to produce successful voluntary movements.

The development of white matter and grey matter during the critical period of adolescence is likely to be greatly influenced both by tics themselves, and by the conscious suppression and control of tics that many young people with TS attempt. Much like the musicians training in piano playing (Bengtsson et al., 2005), individuals with TS spend many hours attempting to suppress tics. It is likely that the eventual remission in tic symptoms that happens to the majority of young people diagnosed with TS happens because of how the development of white and grey matter is affected by this tic suppression, which concurs with Plessen, Bansal & Peterson's (2009) view. However, Plessen, Bansal & Peterson (2009) suggested a model where increased input from the dorsolateral prefrontal cortex to motor and premotor cortical areas would lead to greater cognitive control over tics. In my thesis I have presented an alternative

suggestion, where the eventual control of tics occurs through local inhibitory processes to reduce the amount of excitatory signalling to M1.

Evidence for this theory comes from fMRI experiments of cognitive control tasks which show that subjects with TS have a reduced BOLD response in primary motor cortex compared to a typically developing control group, despite similar behavioural performance (Jackson et al., 2011; Jung et al., 2013). This research suggests that having a less active M1 leads to successful task performance in people with TS. Using my view, increased influence from the dorsolateral prefrontal cortex to M1 would actually be detrimental in controlling tics, as it would introduce more excitatory input. This could explain why a positive relationship between white matter projecting from the prefrontal cortex and the severity of motor tic symptoms has been previous reported (Jackson et al., 2011). Those with more inputs from the prefrontal cortex have worse tics because they have more excitatory inputs, much like the increased interhemispheric connections. However, I did not find any relationship between white matter projecting from the prefrontal cortex and tic severity, so I cannot support this line of reasoning using my current data.

7.4 Limitations and considerations for future research

It is important to consider that TS is a very complex disorder. Many people with TS have at least one co-morbid disorder, including OCD and ADHD, and the samples used in this thesis were representative of the adolescent TS population in this respect. The focus of this research has been mainly on the motor system, and as such, has only focused on motor tics. I understand that in the majority of people with TS motor tics are not their only symptom, and that developmental changes in brain structure are likely to relate to a whole range of behaviours. A more extensive study of the range of symptoms and co-morbidities associated with TS, and their impact on brain development was beyond the scope of this thesis. For this reason, a region of interest approach was adopted, with a focus on M1 and

the SMA because of these areas involvement in tic production and suppression. Future research would benefit from a broader scope to better account for individual differences in symptoms and brain development.

In Chapter 2 I demonstrated the usefulness of adopting a multivariate analysis technique (SVM). Such multivariate analyses could be a good tool in future research to combine information from different clinical, psychological and neuroscience measures to create models that may be able to predict not just group ownership, but with a large longitudinal dataset, clinical outcome. The complexity of disorders such as TS would benefit from such statistical modelling, as it is likely that some findings are better predicted by a combination of traits rather than a single measure (e.g. motor tic score). For example the severity of ADHD traits alongside the severity of tics may be a better predictor of white matter development than just tics or ADHD traits alone. Classifying a range of behavioural traits and using a range of complementary techniques with a large sample size would likely lead to better models of the progress of and neuronal adaptation to developmental disorders.

The research presented in this thesis was based on a cross-sectional sample of adolescents with TS. This means that the conclusions I have drawn are based on correlations between tic severity at the time of testing, and the data collected. This snapshot approach, although useful, means I cannot confidently determine whether the adaptive changes I have highlighted are indeed what lead to tic remission. A longitudinal data set that followed children from diagnosis to early adulthood, and included the classification of a range of behaviours including tics, would provide clearer answers to how the brain develops to compensate for tics. This approach should also take into account medication and psychological therapies that may be influencing brain development.

Without the use of a longitudinal data set, a smaller-scale study could look at brain structure and measures of excitation and inhibition in a

group of adults who were diagnosed with TS in childhood but now are largely tic-free. This group could be compared to a neuro-typical aged matched control group, and an aged-match group of adults who continue to have noticeable tics. This approach is likely to shed some light on the eventual white matter structure that needs to develop for successful tic remission.

If increases in tonic levels of GABA in the SMA are beneficial in reducing tics, at least in the short term, there is potential for the use of brain stimulation therapies to increase GABA release in the SMA. Transcranial direct current stimuluation (tDCS) has been shown to influence levels of GABA and glutamate in the cortex (Stagg et al., 2009, Kim et al., 2014). Cathodal tDCS could be used to increased GABA levels in the SMA of people with TS, which may be beneficial in reducing tic symptoms. However, the effects of tDCS are variable, and more research into the effects of tDCS on the SMA of non neuro-typical people is needed before a clinical trial of this kind should be attempted.

7.5 Conclusions

In this thesis I used complementary cognitive neuroscience research techniques to explore possible developmental adaptations in a group of young people with Tourette syndrome. This investigation revealed that young people with TS tend to have thinner grey matter in the sensorimotor areas of the cortex, reduced gain in cortical excitability of M1 leading up to a movement, and increased levels of tonic GABA in the supplementary motor area. Young people with TS also have a significant relationship between the severity of motor tics and the white matter in the body of the corpus callosum, the thickness of cortical grey matter in sensorimotor regions, and the modulation of cortical excitability of M1 preceding a volitional movement. Elevated levels of GABA in the SMA of young people with TS was reported as related to: FA values from the SMA region of the corpus callosum, the percentage of BOLD signal change in the

SMA in an fMRI finger-tapping task, and MEP amplitude in the period directly preceding movement onset. Taken together, in the context of the primary deficit in TS being dysfunctional striatal firing leading to hyper-excitability of the sensorimotor cortex, the results suggest that in TS the adolescent brain is developing in such a way to isolate M1 from excessive expiatory input. This could be achieved by alterations to the white matter structure to reduce excitatory interhemispheric input, as shown by the relationship between callosal FA and tics, or by functional alterations to increase SMA GABA levels to prevent some dysfunctional signals being passed on to M1. These effects may be short-term reactions to enable the successful completion of voluntary movements without interference from tics, or may be part of the developmental process that leads to tic remission. A longitudinal study is needed in order to substantiate this. It is likely, however, to be a combination of both; that altered balance between excitation and inhibition, along with active tic-suppression, over time, lead to permanent alterations in white matter and cortical grey matter structure.

References

Akkal, D., Dum, R. P. & Strick, P. L. (2007). Supplementary motor area and presupplementary motor area: targets of basal ganglia and cerebellar output. *Journal of Neuroscience, 27*, 10659–10673.

Albin, R.L. & Mink, J.W. (2006). Recent advances in Tourette syndrome research. *TRENDS in Neurosciences, 29* (3), 175-182.

Alexander, A.L., Lee, J.E., Lazar, M. & Field, A.S. (2008). Diffusion Tensor Imaging of the Brain. *Neurotherapeutics, 4*(3), 316–329.

Apter, A., Pauls, D.L., Bleich, A., Zohar, A.H., Kron, S., Ratzoni, G., et al. (1993). An Epidemiologic Study of Gilles de la Tourette's Syndrome in Israel. *Archives of General Psychiatry, 50*, 734-738.

Aron, A. R. & Poldrack, R. A. (2006). Cortical and subcortical contributions to Stop signal response inhibition: role of the subthalamic nucleus. *Journal of Neuroscience, 26*, 2424–2433.

Baron-Cohen, S., Mortimore, C., Moriarty, J., Izaguirre, J. & Robertson, M.M. (1999a) The prevalence of Gilles de la Tourette's syndrome in children and adolescents with autism. *Journal of Child Psychology and Psychiatry, 40*, 213–218.

Banaschewki, T., Woerner, W. & Rothenberger, A. (2003). Premonitory sensory phenomena and suppression of tics in Tourette syndrome: developmental aspects in children and adolescents. *Developmental Medicine and Child Neurology, 45*, 700–703.

Basser, P.J., Mattiello, J. & LeBihan, D. (1994). Estimation of the effective self-diffusion tensor from the NMR spin echo. *Journal of Magnetic Resonance, Series B, 103*, 247–254.

Baumer, T., Thomalla, G., Kroeger, J., Jonas, M., Gerloff, C., Hummel, F. C., et al. (2010). Interhemispheric Motor Networks are Abnormal in Patients with Gilles de la Tourette Syndrome. *Movement Disorders, 25*(16), 2828-2837.

Baumgardner, T.L., Singer, H.S., Denckla, M.B., Rubin, M.A., Abrams, M.T. & Colli, M.J. (1996). Corpus callosum morphology in children with Tourette syndrome and attention deficit hyperactivity disorder. *Neurology, 47*, 477–82.

Baym, C.L., Corbett, B.A., Wright, S.B., Bunge, S.A., (2008). Neural correlates of tic severity and cognitive control in children with Tourette syndrome. *Brain, 131*, 165–179.

Behrens, T.E.J., Woolrich, M.W., Jenkinson, M., Johansen-Berg, H., Nunes, R.G., Clare, S., et al. (2003). Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magnetic Resonance in Medicine*, *50*(5), 1077-1088.

Bengtsson, S.L., Nagy, Z., Skare, S., Forsman, L., Forssberg, H. & Ullén, F. (2005). Extensive piano practicing has regionally specific effects on white matter development. *Nature Neuroscience, 8*, 1148–1150.

Bermudez, P., Lerch, J.P., Evans, A.C. & Zatorre, R.J. (2009). Neuroanatomical correlates of musicianship as revealed by cortical thickness and voxel-based morphometry. *Cerebral Cortex, 19*, 1583–1596.

Blazquez, P.M., Fujii, N., Kojima, J., & Graybiel, A.M. (2002). A network representation of response probability in the striatum. *Neuron, 33*, 973–982.

Bliss, J. (1980). Sensory experiences of Gilles de la Tourette syndrome. *Archieves of General Psychiatry, 37,* 1343-1347.

Bloch, M., Michael, H., Leckman, J.F., James, F., Zhu, H., Peterson, B.S., et al. (2005). Caudate volumes in childhood predict symptom severity in adults with Tourette syndrome. *Neurology, 65*, 1253–1258.

Bloom, J.S. & Hynd, G.W. (2005). The Role of the Corpus Callosum in Interhemispheric Transfer of Information: Excitation or Inhibition? *Neuropsychology Review, 15* (2), 59-71.

Bohlhalter, S., Goldfine, A., Matteson, S., Garraux, G., Hanakawa, T., Kansaku, K., et al. (2006). Neural correlates of tic generation in Tourette syndrome: an event-related functional MRI study. *Brain, 129*, 2029–2037.

Bourgeois, J.P. & Rakic, P. (1993) Changes of synaptic density in the primary visual cortex of the macaque monkey from fetal to adult stage. *Journal of Neuroscience, 13*(7), 2801–2820.

Boy, F., Evans, C.J., Edden, R.A.E., Singh, K.D., Husain, M. & Sumner, P. (2010). Individual Differences in Subconscious Motor Control Predicted by GABA Concentration in SMA. *Current Biology, 20*, 1779–1785.

Canales, J.J. & Graybiel, A.M. (2000). A measure of striatal function predicts motor stereotypy. *Nature Neuroscience, 3*, 377–383.

Canitano, R. & Vivanti, G (2007). Tics and Tourette syndrome in autism spectrum disorders. *Autism, 11*, 19–28.

Capriotti, M.R., Brandt, B.C., Turkel, J.E., Lee, H.J. & Woods, D.W. (2014). Negative Reinforcement and Premonitory Urges in Youth With Tourette Syndrome: An Experimental Evaluation. *Behavior Modification, 38*(2), 276-296.

Carter, C.S. & Van Veen, V. (2007). Anterior cingulate cortex and conflict detection: An update of theory and data. *Cognitive, Affective, & Behavioral Neuroscience, 7*(4), 367-379.

Catani, M. & Thiebaut de Schotten, M. (2012). *Atlas of Human Brain Connections.* Oxford: Oxford University Press,

Cath, D.C., Hedderly, T., Ludolph, A.G., Stern, J.S., Murphy, T., Hartmann, A., et al. (2011). European clinical guidelines for Tourette Syndrome and other tic disorders. Part I: assessment. *European Child and Adolescent Psychiatry, 20*, 155–171

Cavann, A.E. & Trimble, M.R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain, 129*, 564–583.

Chang, C.-C. & Lin, C.-J. (2011). LIBSVM : a library for support vector machines. *ACM Transactions on Intelligent Systems and Technology, 2* (27), 1-27.

Church, J.A., Wenger, K.K., Dosenbach, N.U.F., Miezin, F.M., Petersen, S.E., & Schlaggar, B.L. (2009). Task control signals in pediatric Tourette syndrome show evidence of immature and anomalous functional activity. *Frontiers in Human Neuroscience, 3*(38), 1-14.

Conelea, C.A. & Woods, D.W. (2008). The influence of contextual factors on tic expression in Tourette's syndrome: A review. *Journal of Psychosomatic Research, 65,* 487–496.

Curtis, C. E., Cole, M. W., Rao, V. Y. & D'Esposito, M. (2005). Canceling planned action: an fMRI study of countermanding saccades. *Cerebral Cortex, 15,* 1281–1289.

Dale, A.M., Fischl, B. & Sereno, M.I. (1999). Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage, 9*, 179–194.

Debes, N.M., Hansen, A., Skov, L., & Larsson, H. (2011). A functional magnetic resonance imaging study of a large clinical cohort of children with Tourette syndrome. *Journal of Child Neurology, 26*, 560–569.

Desikan, R.S., Ségonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., et al. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage, 31*(3), 968-980.

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M.A., Pilato, F., Zito, G. et al. (2003). Ketamine increases motor cortex excitability to transcranial magnetic stimulation. *Journal of Physiology,* 547, 485–496.

Di Lazzaro, V., Ziemann, U. & Lemon, R.N. (2008). State of the art: physiology of transcranial motor cortex stimulation. *Brain Stimulation, 1*, 345–362.

Dosenbach, N.U.F., Nardos, B., Cohen, A.L. , Fair, D.A., Power, J.D., Church, J.A. et al. (2010). Prediction of Individual Brain Maturity Using fMRI. *Science, 329*, 1358-1361

Draper, A., Jude, L., Jackson, G. M. and Jackson, S. R. (2013), Motor excitability during movement preparation in Tourette syndrome. *Journal of Neuropsychology* doi: 10.1111/jnp.12033

Draganski, B., Martino, D., Cavanna, A.E., Hutton, C., Orth, M., Robertson, M.M., et al. (2010). Multispectral brain morphometry in Tourette syndrome persisting into adulthood. *Brain, 133*, 3661–3675.

Ecker, C., Rocha-Rego, V., Johnston, P., Mourao-Miranda, J., Marquand, A., Daly, E.M., et al. (2010). Investigating the predictive value of whole-brain structural MR scans in autism: A pattern classification approach. *NeuroImage, 49*, 44–56.

Edden, R.A.E., Muthukumaraswamy, S.D., Freeman, T.C.A. & Singh K.D. (2009). Orientation Discrimination Performance Is Predicted by GABA Concentration and Gamma Oscillation Frequency in Human Primary Visual Cortex. *The Journal of Neuroscience, 29*(50), 15721–15726.

Eickhoff, S., Stephan, K.E., Mohlberg, H., Grefkes, C., Fink, G.R., Amunts, K., et al. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *NeuroImage, 25*(4), 1325-1335.

Fahim, C., Yoon, U., Das, S., Lyttelton, O., Chen, J., Arnaoutelis, R., et al. (2010). Somatosensory-motor bodily representation cortical thinning in Tourette: effects of tic severity, age and gender. *Cortex, 46*, 750–760.

Fischl, B. & Dale, A.M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *PNAS USA, 97*, 11050–11055.

Fischl, B., Sereno, M.I. & Dale, A.M. (1999a). Cortical surface-based analysis. II: Inflation, flattening, and a surface based coordinate system. *Neuroimage, 9,* 195–207.

Freeman, R.D., Fast, D.K., Burd, L., Kerbashian, J., Robertson, M.M. & Sandor, P. (2000). An international perspective on Tourette syndrome: selected findings from 3500 individuals in 22 countries. *Developmental Medicine & Child Neurology, 42,* 436–447.

Freeman, R.D., Zinner, S.H., Müller-Vahl, K.R., Fast, D.K., Burd, L.J., Kano, Y., et al. (2009). Coprophenomena in Tourette syndrome. *Developmental Medicine & Child Neurology, 51*(3), 218-227.

Floyer-Lea, A., Wylezinska, M., Kincses, T., & Matthews, P.M. (2005). Rapid Modulation of GABA Concentration in Human Sensorimotor Cortex During Motor Learning. *Journal of Neurophysiology, 95*, 1639-1644.

Franzkowiak S., Pollok B., Biermann-Ruben K., Sudmeyer M., Paszek J., Jonas, M., et al. (2010). Altered pattern of motor cortical activation-inhibition during voluntary movements in Tourette syndrome. *Movement Disorders, 25*, 1960–1966.

Franzkowiak, S., Pollok, B., Biermann-Ruben, K., Sudmeyer, M., Paszek, J., Thomalla, G., et al. (2012). Motor-Cortical Interaction in Gilles de la Tourette Syndrome. *PLoS ONE, 7*(1), e27850- e27850. doi:10.1371/journal.pone.0027850

Fredericksen, K.A., Cutting, L.E., Kates, W.R., Mostofsky, S., Singer, H., Cooper, K., et al. (2002). Disproportionate increases of white matter in right frontal lobe in Tourette syndrome. *Neurology, 58*, 85–89.

Ganos, C., Kahl, U., Schunke, O., Kühn, S., Haggard, P., Gerloff, C., et al. (2012). Are premonitory urges a prerequisite of tic inhibition in Gilles de la Tourette syndrome? *Journal of Neurology Neurosurgery & Psychiatry, 83*, 975-978.

Ganos, C., Roessner, V. & Münchau, A. (2013). The functional anatomy of Gilles de la Tourette syndrome. *Neuroscience and Biobehavioral Reviews, 37*, 1050–1062.

Gilbert, D. L., Bansal, A. S., Sethuraman, G., Sallee, F. R., Zhang, J., Lipps, T., et al. (2004). Association of cortical disinhibition with tic, ADHD, and OCD severity in Tourette syndrome. *Movement Disorders, 19*(4), 416-425.

Gilbert, D. L., Sallee, F. R., Zhang, J., Lipps, T. D., & Wassermann, E. M. (2005). Transcranial magnetic stimulation-evoked cortical inhibition: A consistent marker of attention-deficit/hyperactivity disorder scores in Tourette syndrome. *Biological Psychiatry, 57*(12), 1597-1600.

Gilbert, D.L., Ridel, K.R., Sallee, F.R., Zhang, J., Lipps, T.D. & Wassermann, E.M. (2006). Comparison of the inhibitory and excitatory effects of ADHD medications methylphenidate and atomoxetine on motor cortex. *Neuropsychopharmacology, 31*(2), 442–449.

Gilbert, D. L., Zhang, J., Lipps, T. D., Natarajan, N., Brandyberry, J., Wang, Z. W., et al. (2007). Atomoxetine treatment of ADHD in Tourette Syndrome: Reduction in motor cortex inhibition correlates with clinical improvement. *Clinical Neurophysiology, 118*(8), 1835-1841.

Giedd, J.N., Blumenthal, J., Jeffries, N., Castellanos, F., Liu, H., Zijdenbos, A., et al. (1999). Brain development during childhood and adolescence: a longitudinal mri study. *Nature Neuroscience, 2,* 861–863.

Giorgio, A., Watkins, K.E., Douaud, G., James, A.C., James, S., De Stefano, N., et al. (2008). Changes in white matter microstructure during adolescence. *NeuroImage, 39,* 52–61

Giorgio, A., Watkins, K.E., Chadwick, M., James, S., Winmill, L., Douaud, G., et al. (2010). Longitudinal changes in grey and white matter during adolescence. *NeuroImage, 49*, 94–103.

Goldenberg, J.N., Brown, S.B. & Weiner, W.J. (1994). Coprolalia in younger patients with Gilles de la Tourette syndrome. *Movement Disorders, 9,* 622–625.

Gooijers, J., Caeyenberghs, K., Sisti, H.M., Geurts, M., Heitger, M.H., Leemans, A. et al. (2011). Diffusion Tensor Imaging Metrics of the Corpus Callosum in Relation to Bimanual Coordination: Effect of Task Complexity and Sensory Feedback. *Human Brain Mapping, 34*(1), 241-252.

Govindan, R.M., Makki, M.I., Wilson, B.J., Behen, M.E. & Chugani, H.T. (2010). Abnormal water diffusivity in corticostriatal projections in children with Tourette syndrome. Human Brain Mapping 31, 1665–1674.

Govindaraju V., Young K., & Maudsley A.A. (2000). Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed, 13*, 129–53.

Haggard, P., (2008). Human volition: towards a neuroscience of will. *Nature Reviews Neuroscience, 9*, 934–946.

Hall E.L., Stephenson M.C., Price D., & Morris P.G., (2013). Methodology for improved detection of low concentration metabolites in MRS: Optimised combination of signals from multi-element coil arrays. *Neuroimage, 86,* 35-42

Hampson, M., Tokoglu, F., King, R.A., Constable, R.T. & Leckman, J.F. (2009). Brain areas coactivating with motor cortex during chronic motor tics and intentional movements. *Biological Psychiatry, 65*, 594–599.

Hare, T.A., Wood, J.H., Manyam, B.V., Gerner, R.H., Ballenger, J.C. & Post, R.M. (1982). Central Nervous System γ -Aminobutyric Acid Activity in Man: Relationship to Age and Sex as Reflected in CSF. *Archives of Neurology, 39*(4), 247-249.

Heise, C. A., Wanschura, V., Albrecht, B., Uebel, H., Roessner, V., Himpel, S., et al. (2008). Voluntary motor drive: possible reduction in Tourette syndrome. *Journal of Neural Transmission, 115*(6), 857-861.

Heise, K. F., Steven, B., Liuzzi, G., Thomalla, G., Jonas, M., Muller-Vahl, K., et al. (2010). Altered modulation of intracortical excitability during movement preparation in Gilles de la Tourette syndrome. *Brain, 133*, 580-590.

Hong, K.E., Ock, S.M., Kang, M.H., Kim, C.E., Bae, J.N., Lim, M.K., et al. (2002). The segmented regional volumes of the cerebrum and cerebellum in boys with Tourette syndrome. *Journal of Korean Medical Science, 17*, 530–536.

Huttenlocher, P.R. (1984). Synapse elimination and plasticity in developing human cerebral cortex*. American journal of mental deficiency, 88*, 488–496.

Hsu, C.-W. Chang, C.-C. & Lin, C.-J. (2010) *A Practical Guide to Support Vector Classification.* (http://www.csie.ntu.edu.tw/~cjlin/libsvm/) Taipei: Department of Computer Science and Information Engineering, National Taiwan University.

Hyde, T., Stacey, M., Coppola, R., Handel, S., Rickler, K., & Weinberger, D. (1995). Cerebral morphometric abnormalities in Tourette's syndrome: a quantitative MRI study of monozygotic twins. *Neurology, 45*, 1176–1182.

Jackson, G.M., Mueller, S.C., Hambleton, K., & Hollis, C.P. (2007). Enhance cognitive control in Tourette Syndrome during task uncertainty. *Experimental Brain Research, 182*, 357–364.

Jackson, S.R., Parkinson, A., Jung, J., Ryan, S.E., Morgan, P.S., Hollis, C. et al. (2011). Compensatory Neural Reorganizaton in Tourette Syndrom. *Current Biology, 21*(7), 580-585.

Jackson, S.R., Parkinson, A., Kim, S.Y., Schuermann, M. & Eickhoff, S.B. (2011b). On the functional anatomy of the urge-for-action. *Cognitive Neuroscience, 2*(3-4), 252–257.

Jackson, S.R., Parkinson A., Manfredi V., Millon G., Hollis C.P., Jackson G.M. (2013). Motor excitability is reduced prior to voluntary movements in children and adolescents with Tourette syndrome. *Journal of Neuropsychology, 7*(1), 29-44.

Jellison, B.J., Field, A.S., Medow, J., Lazar, M., Salamat, M.S., & Alexander, A.L. (2004). Diffusion Tensor Imaging of Cerebral White Matter: A Pictorial Review of Physics, Fiber Tract Anatomy, and Tumor Imaging Patterns. *American Journal of Neuroradiology, 25,* 356–369

Johansen-Berg, H., Della-Maggiore, V., Behrens, T.E.J., Smith, S.M. & Paus, T. (2007) Integrity of white matter in the corpus callosum correlates with bimanual co-ordination skills. *NeuroImage, 36*, T16-T21.

Jones, D.K., Christiansen, K.F., Chapman, R.J. & Aggleton, J.P. (2013). Distinct subdivisions of the cingulum bundle revealed by diffusion MRI fibre tracking: Implications for neuropsychological investigations. *Neuropsychologia, 51,* 67–78.

Jung J.Y., Jackson S.R., Parkinson A., Jackson G.M. (2013). Cognitive control over motor output in Tourette syndrome. *Neuroscience & Biobehavioural Reviews, 37,* 1016–1025.

Kalanithi, P.S., Zheng, W., Kataoka, Y., DiFiglia, M., Grantz, H., Saper, C.B., et al. (2005). Altered parvalbumin-positive neuron distribution in basal ganglia of individuals with Gilles de la Tourette syndrome*. Proceedings of the National Academy of Sciences of the United States of America, 102*, 13307- 13311.

Kataoka, Y., Kalanithi, P.S., Grantz, H., Schwartz, M.L., Saper, C., Leckman, J.F., et al. (2010). Decreased number of parvalbumin and cholinergic interneurons in the striatum of individuals with Tourette syndrome. *The Journal of Comparative Neurology, 518*, 277–291.

Kates, W.R., Frederiksen, M., Mostofsky, S.H., et al. (2002). MRI parcellation of the frontal lobe in boys with attention deficit hyperactivity disorder or Tourette syndrome. *Psychiatry Research*, 116, 63–81.

Keshavan, M.S., Diwadkar, V.A., DeBellis, M., Dick, E., Kotwal, R., Rosenberg, D.R., et al. (2002). Development of the corpus callosum in childhood, adolescence and early adulthood. *Life Sciences*, *70* (16), 1909-1922.

Kim, S., Stephenson, M.C., Morris, P.G. & Jackson, S.R. (2014). tDCS-induced alterations in GABA concentration within primary motor cortex predict motor learning and motor memory: a 7 T magnetic resonance spectroscopy study. *Neuroimage. 99*, 237-243.

Klein-Flügge, M.C., Nobbs, D., Pitcher, J.B. & Bestmann, S. (2013). Variability of Human Corticospinal Excitability Tracks the State of Action Preparation. *The Journal of Neuroscience, 33*(13), 5564-5572.

Klöppel, S., Bäumer, T., Kroeger, J., Koch, M.A., Büchel, C., Münchau, A., et al. (2008). The cortical motor threshold reflects microstructural properties of cerebral white matter. *NeuroImage, 40*, 1782–1791.

Kochunov, P., Williamson, D.E., Lancaster, J., Fox, P., Cornell, J., Blangero, J. et al. (2012). Fractional anisotropy of water diffusion in cerebral white matter across the lifespan. *Neurobiology of Aging, 33*(1), 9-20.

Kurlan R., McDermott M.P., Deeley C., Como P.G., Brower C., & Eapen S. (2001). Prevalence of tics in schoolchildren and association with placement in special education. *Neurology, 57*, 1383–8.

Kwon, H. J., Lim, W. S., Lim, M. H., Lee, S. J., Hyun, J. K., Chae, J. H., et al. (2011). 1-Hz low frequency repetitive transcranial magnetic stimulation in children with Tourette's syndrome. *Neuroscience Letters, 492*(1), 1-4.

Lebel, C., Caverhill-Godkewitsch, S. & Beaulieu, C. (2010). Age-related regional variations of the corpus callosum identified by diffusion tensor tractography. Neuroimage, 52(1), 20-31.

Lebel, C. & Beaulieu, C. (2011). Longitudinal development of human brain wiring continues from childhood into adulthood. *Journal of Neuroscience, 31,* 10937–10947.

Lebel, C., Gee, M., Camicioli, R., Wieler, M., Martin, .W & Beaulieu C. (2012) Diffusion tensor imaging of white matter tract evolution over the lifespan. *Neuroimage, 60*(1), 340-52.

Leckman, J.F., Riddle, M.A., Hardin, M.T., Ort, S.I., Swartz, K.L., Stevenson, J., et al. (1989). The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *Journal of the American Academy of Child and Adolescent Psychiatry, 28,* 566-73.

Leckman, J.F., Walker, D.E. & Cohen, D.J. (1993). Premonitory urges in Tourette's syndrome. *American Journal Psychiatry, 150*, 98-102.

Leckman, J.F., Zhang, H., Vitale, A., Lahnin, F., Lynch, K., Bondi, C., et al. (1998). Course of tic severity in Tourette syndrome: the first two decades. *Pediatrics, 102,* 14-9.

Leckman, J.F. (2002). Tourette's syndrome. *Lancet, 360,* 1577–86.

Leckman, J.F., Bloch, M.H., Smith, M.E., Larabi, D. & Hampson, M. (2010). Neurobiological Substrates of Tourette's Disorder. *Journal of child and adolescent psychopharmacology, 20*(4), 237-247.

Lee, J.S., Yoo, S.S., Cho, S.Y., Ock, S.M., Lim, M.K. & Panych, L.P. (2006). Abnormal thalamic volume in treatment-*naive* boys with Tourette syndrome. *Acta Psychiatrica Scandinavica, 113*, 64–67.

Lee, B., Park, J., Jung, W.H., Kim, H.S., Oh, J.S., Choi, C., et al. (2010). White matter neuroplastic changes in long-term trained players of the game of "Baduk" (GO): A voxel-based diffusion-tensor imaging study. *NeuroImage, 52*(1), 9-19.

Lerner, A., Bagic, A., Simmons, J.M., Mari, Z., Bonne, O., Xu, B., et al. (2012). Widespread abnormality of the γ -aminobutyric acid-ergic system in Tourette syndrome. *Brain, 135,* 1926–1936.

Li, C. S., Huang, C., Constable, R. T. & Sinha, R. (2006). Imaging response inhibition in a stop-signal task: neural correlates independent of signal monitoring and post-response processing. *Journal of Neuroscience, 26*, 186–192.

Lin, Y., Stephenson, M.C., Xin, L., Napolitano, A., & Morris, P.G. (2012). Investigating the metabolic changes due to visual stimulation using functional proton magnetic resonance spectroscopy at 7 T. *Journal of Cerebral Blood Flow & Metabolism*, 1–12.

Ludolph, A.G., Juengling, F.D., Libal, G., Ludolph, A.C., Fegert, J.M. & Kassubek, J. (2006) Grey-matter abnormalities in boys with Tourette syndrome: magnetic resonance imaging study using optimised voxelbased morphometry. *British Journal of Psychiatry, 188*, 484–485.

Nagy, Z., Westerberg, H. & Klingberg, T. (2004). Maturation of White Matter is Associated with the Development of Cognitive Functions during Childhood. *Journal of Cognitive Neuroscience, 16*(7), 1227–1233.

Nachev, P., Rees, G., Parton, A., Kennard, C. & Husain, M. (2005). Volition and conflict in human medial frontal cortex. *Current Biology, 15,* 122–128.

Nachev, P., Kennard, C. & Husain, M. (2008). Functional role of the supplementary and pre-supplementary motor areas. *Nature Reviews Neuroscience, 9,* 856-869.

Neuner, I., Kupriyanova, Y., Stocker, T., Huang, R., Posnansky, O., Schneider, F., et al. (2010). White-matter abnormalities in Tourette syndrome extend beyond motor pathways. *Neuroimage, 51,* 1184–1193.

Neuner, I., Kupriyanova, I., Stöcker, T., Huang, R., Posnansky, O., Schneider, O., et al. (2011). Microstructure assessment of grey matter nuclei in adult tourette patients by diffusion tensor imaging. *Neuroscience Letters, 487,* 22–26.

Nitsche, M. A., Nitsche, M.S., Klein, C.C., Tergau, F., Rothwell, J.C. & Paulus, W. (2003). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clinical Neurophysiology, 114*(4), 600–604.

Maffucci, J.A., & Gore, A.C. (2009). Chapter 2 Hypothalamic Neural Systems Controlling the Female Reproductive Life Cycle: Gonadotropin‐Releasing Hormone, Glutamate, and GABA. *International Review of Cell and Molecular Biology, 274*, 69-127.

Maguire, E.A., Gadian, D.G., Johnsrude, I.S., Good, C.D., Ashburner, J., Frackowiak, R.S.J. et al. (2000). Navigation-related structural change in the hippocampi of taxi drivers. *PNAS, 97*(8), 4398–4403.

Makki, M.I., Behen, M., Bhatt, A., Wilson, B. & Chugani, H.T. (2008). Microstructural abnormalities of striatum and thalamus in children with Tourette syndrome. *Movement Disorders, 23*, 2349–2356.

Makki, M.I., Govindan, R.M., Wilson, B.J., Behen, M.E. & Chugani, H.T. (2009). Altered fronto-striato-thalamic connectivity in children with Tourette syndrome assessed with diffusion tensor mri and probabilistic fiber tracking. *Journal of Child Neurology, 24*, 669–678.

Mantovani, A., Leckman, J. F., Grantz, H., King, R. A., Sporn, A. L., & Lisanb, S. H. (2007). Repetitive Transcranial magnetic stimulation of the supplementary motor area in the treatment of Tourette syndrome: Report of two cases. Clinical *Neurophysiology, 118*(10), 2314-2315.

Marsh, R., Zhu, H., Wang, Z., Skudlarski, P. & Peterson, B.S. (2007). A developmental fMRI study of self-regulatory control in Tourette's syndrome. *The American Journal of Psychiatry, 164,* 955–966.

Marsh, R., Gerber, A.J. & Peterson, B.S. (2008). Neuroimaging studies of normal brain development and their relevance for understanding childhood neuropsychiatric disorders. *Journal of the American Academy of Child and Adolescent Psychiatry, 47*, 1233–1251.

McDonnell, M.N., Orekhov, Y., & Ziemann, U. (2006). The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Experimental Brain Research, 173*(1), 86-93.

Mekle R., Mlynarik V., Gambarota G., Hergt M., Krueger G., & Gruetter R. (2009). MR spectroscopy of the human brain with enhanced singla intensity at ultrashort echo times on a clinical platform at 3T and 7T. *Magnetic Resonance in Medicine, 61*, 1279–1285.

Moll, G. H., Wischer, S., Heinrich, H., Tergau, F., Paulus, W., & Rothenberger, A. (1999). Deficient motor control in children with tic disorder: evidence from transcranial magnetic stimulation*. Neuroscience Letters, 272*(1), 37-40.

Moll, G.H., Heinrich, H., Trott, G.E., Wirth, S., Bock, N., & Rothenberger, A. (2001). Children with comorbid attention- deficit hyperactivity disorder and tic disorder: evidence for additive inhibitory deficits within the motor system. *Annals of Neurology, 49*, 393–396.

Moll, G. H., Heinrich, H., Gevensleben, H., & Rothenberger, A. (2006). Tic distribution and inhibitory processes in the sensorimotor circuit during adolescence: A cross-sectional TMS study. *Neuroscience Letters, 403*(1-2), 96-99.

Moriarty, J., Varma, A.R., Stevens, J., Fish, M., Trimble, M.R. & Robertson, M.M. (1997). A volumetric MRI study of Gilles de la Tourette's syndrome. *Neurology, 49*, 410–5.

Mostofsky, S.H., Lasker, A.G., Singer, H.S., Denckla, M.B. & Zee, D.S. (2001). Oculomotor Abnormalities in Boys With Tourette Syndrome With and Without ADHD. *Journal of the American Academy of Child & Adolescent Psychiatry, 40*(12), 1464–1472.

Müller-Vahl, K.R., Kaufmann, J., Grosskreutz, J., Dengler, R., Emrich, H.M. & Peschel, T. (2009). Prefrontal and anterior cingulate cortex abnormalities in Tourette syndrome: evidence from voxel-based morphometry and magnetization transfer imaging. *BMC Neuroscience, 10*, 47.

Munakata, Y., Herd, S.A., Chatham, C.H., Depue, B.E., Banich, M.T. & O'Reilly, R.C. (2011). A unified framework for inhibitory control. *Trends in Cognition Science, 15*, 453–459.

Munchau, A., Bloem, B. R., Thilo, K. V., Trimble, M. R., Rothwell, J. C., & Robertson, M. M. (2002). Repetitive transcranial magnetic stimulation for Tourette syndrome. *Neurology, 59*(11), 1789-1791.

Munoz, D.P., LeVasseur, A.L. & Flanagan, J.R. (2002). Control of volitional and reflexive saccades in Tourette's syndrome. *Progress in Brain Research, 140*, 467–481.

Öngür, D., Haddad, S., Prescot, A.P., Jensen, J.E., Siburian, R., Cohen, B.M., et al. (2011). Relationship Between Genetic Variation in the Glutaminase Gene GLS1 and Brain Glutamine/Glutamate Ratio Measured In Vivo. *Biological Psychiatry, 70*, 169–174.

Orth, M., Amann, B., Robertson, M. M., & Rothwell, J. C. (2005). Excitability of motor cortex inhibitory circuits in Tourette syndrome before and after single dose nicotine*. Brain, 128*, 1292-1300.

Orth, M., Kirby, R., Richardson, M. P., Snijders, A. H., Rothwell, J. C., Trimble, M. R., et al. (2005b). Subthreshold rTMS over pre-motor cortex has no effect on tics in patients with Gilles de la Tourette syndrome*. Clinical Neurophysiology, 116*(4), 764-768.

Orth, M., Munchau, A., & Rothwell, J. C. (2008). Corticospinal system excitability at rest is associated with tic severity in Tourette syndrome. *Biological Psychiatry, 64*(3), 248-251.

Orth, M., & Rothwell, J. C. (2009). Motor cortex excitability and comorbidity in Gilles de la Tourette syndrome. *Journal of Neurology Neurosurgery and Psychiatry, 80*(1), 29-34.

Palminteri, S., Lebreton, M., Worbe, Y., Grabli, D., Hartmann, A. & Pessiglione, M. (2009). Pharmacological modulation of subliminal learning in Parkinson's and Tourette's syndromes. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 19179–19184.

Palminteri, S., Lebreton, M., Worbe, Y., Hartmann, A., Lehericy, S., Vidailhet, M., et al. (2011). Dopamine-dependent reinforcement of motor skill learning: evidence from Gilles de la Tourette syndrome. *Brain, 134*, 2287–2301.

Pappert, E.J., Goetz, C.J., Louis, E.D., Blasucci, L. & Leurgans, S. (2003). Objective assessments of longitudinal outcome in Gilles de la Tourette's syndrome. *Neurology, 61,* 936–940.

Paulus, W., Classen, J., Cohen, L.G., Large, C.H., Di Lazzaro, V., Nitsche, M., et al. (2008). State of the art: Pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation. *Brain Stimulation, 1*(3), 151-163.

Paus, T. (2005). Mapping brain maturation and cognitive development during adolescence. *TRENDS in Cognitive Sciences, 9*(2), 60-68.

Paus, T., Keshavan, M. & Giedd, J.N. (2008). Why do many psychiatric disorders emerge during adolescence? *Nature Reviews Neuroscience, 9* (12), 947–957.

Peterson, B.S., Riddle, M.A., Cohen, D.J., Katz, L.D., Smith, J.C., Hardin, M.T., et al. (1993). Reduced basal ganglia volumes in Tourette's syndrome using three-dimensional reconstruction techniques from magnetic resonance images. *Neurology, 43*, 941–949.

Peters, B.D., Szeszko, P.R., Radua, J., Ikuta, T., Gruner, P., DeRosse, P. et al., (2012). White Matter Development in Adolescence: Diffusion Tensor Imaging and Meta-Analytic Results. *Schizophrenia Bulletin, 38*(6), 1308–1317.

Peterson, B.S., Leckman, J.F., Duncan, J.S., Wetzles, R., Riddle, M.A., Hardin, M.T., et al. (1994). Corpus callosum morphology from magnetic resonance images in Tourette's syndrome. *Psychiatry Research, 55*, 85–99.

Peterson, B.S. & Leckman, J.F. (1998). Temporal characterization of tics in Gilles de la Tourette's syndrome. *Biological Psychiatry, 44*, 1337–48.

Peterson, B.S., Skudlarski, P., Anderson, A.W., Zhang, H., Gatenby, J.C., Lacadie, C.M., et al. (1998). A functional magnetic resonance imaging study of tic suppression in Tourette syndrome. *Archives of General Psychiatry, 55*, 326–333.

Peterson, B.S., Staib, L., Scahill, L., Zhang, H., Anderson, C., Leckman, J.F., et al. (2001). Regional brain and ventricular volumes in Tourette syndrome. *Archives of General Psychiatry, 5*, 427–440.

Peterson, B.S., Thomas, P., Kane, M.J., Scahill, L., Zhang, H., Bronen, R., et al. (2003). Basal Ganglia volumes in patients with Gilles de la Tourette syndrome. *Archives of General Psychiatry*, *60*, 415–424.

Plessen, J.K., Wentzel-Larsen, T., Hugdahl, K., Feineigle, P., Klein, J., Staib, L.H., et al. (2004). Altered Interhemispheric Connectivity in Individuals With Tourette's Disorder. *American Journal of Psychiatry, 161*, 2028–2037.

Plessen, J.K., Gruner, R., Lundervold, A., Hirsch, J.G., Xu, D., Bansal, R., et al. (2006). Reduced white matter connectivity in the corpus callosum of children with Tourette syndrome. *Journal of Child Psychology and Psychiatry, 47*, 1013–1022.

Plessen, K.J., Bansal, R. & Peterson, B.S. (2009). Imaging evidence for anatomical disturbances and neuroplastic compensation in persons with Tourette syndrome. *Journal of Psychosomatic Research, 67*, 559–573.

Pierpaoli, C., & Basser, P.J. (1996). Toward a quantitative assessment of diffusion anisotropy. *Magnetic Resonance in Medicine, 36*, 893–906.

Provencher S.W., (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine, 30*, 672–679.

Puts, N.A.J., Edden, R.A.E, Evans, J.C., McGlone, F. & McGonigle, D.J. (2011) Regionally Specific Human GABA Concentration Correlates with Tactile Discrimination Thresholds. *The Journal of Neuroscience, 31*(46), 16556 –16560.

Rauch, S.L., Baer, L., Cosgrove, G.R. & Jenike, M.A. (1995). Neurosurgical treatment of Tourette's syndrome: a critical review. *Comprehensive Psychiatry*, 36, 141–156.

Raz, A., Zhu, H., Yu, S., Bansal, R., Wang, Z., Alexander, G.M., et al. (2009). Neural substrates of self-regulatory control in children and adults with Tourette syndrome. *Canadian Journal of Psychiatry, 54*, 579–588.

Rickards, H., Wood, C. & Cavanna, A.E. (2008). Hassler and Dieckmann's seminal paper on stereotactic thalamotomy for Gilles de la Tourette syndrome: translation and critical reappraisal. *Movement Disorders, 23*, 1966–1972.

Robertson, M.M. (2008) The prevalence and epidemiology of Gilles de la Tourette syndrome. Part 1: The epidemiological and prevalence studies. *Journal of Psychosomatic Research, 65,* 461–472.

Robertson, M.M. (2000). Tourette syndrome, associated conditions and the complexities of treatment. *Brain, 123*, 425–62.

Roessner, V., Overlack, S., Schmidt-Samoa, C., Baudewig, J., Dechent, P., Rothenberger, A. et al. (2011). Increased putamen and callosal motor subregion in treatment-naive boys with Tourette syndrome indicates changes in the bihemispheric motor network. *Journal of Child Psychology and Psychiatry, 52*, 306–314.

Roessner, V., Wittfoth, M., Schmidt-Samoa, C., Rothenberger, A., Dechent, P. & Baudewig, J., (2012). Altered motor network recruitment during finger tapping in boys with Tourette syndrome. *Human Brain Mapping, 33*, 666–675.

Rossini, P.M., Berardelli, A., Deuschl, G., Hallett, M., Maertens de Noordhout, A.M., Paulus W. et al. (1999). Applications of magnetic cortical stimulation. *Electroencephalography and Clinical Neurophysiology Supplement, 52,* 171–185.

Schmithorst, V.J., Wilke, M., Dardzinski, B.J. & Holland, S.K. (2002). Correlation of White Matter Diffusivity and Anisotropy with Age during Childhood and Adolescence: A Crosssectional Diffusion-Tensor MR Imaging Study. *Radiology, 222*(1), 212-218.

Schmithorst, V.J., Wilke, M., Dardzinski, B.J. & Holland, S.K. (2005). Cognitive Functions Correlate With White Matter Architecture in a Normal Pediatric Population: A Diffusion Tensor MRI Study. *Human Brain Mapping, 26*, 139 –147.

Schölkopf, B., Sung, K.K., Burges, C.J.C., Gorosi, F., Niyogi, P., Poggio, T., et al. (1997). Comparing Support Vector Machines with Gaussian Kernels to Radial Basis Function Classifiers*. IEEE Transactions On Signal Processing, 45*(11), 2758-2765.

Seghier, M.L. (2013). The Angular Gyrus: Multiple Functions and Multiple Subdivisions. *The Neuroscientist, 19*(1), 43–61.

Shima, K. & Tanji, J. (1998). Both supplementary and presupplementary motor areas are crucial for the temporal organization of multiple movements. *Journal of Neurophysiology, 80*, 3247–3260.

Singer, H.S. (2005). Tourette's syndrome: from behaviour to biology. *Lancet Neurology, 4,* 149–159.

Singer, H.S., Reiss, A.L., Brown, J.E., Aylward, E., Shih, B., Chee, E., et al. (1993). Volumetric MRI changes in basal ganglia of children with Tourette's syndrome. *Neurology, 43,* 950–956.

Smith, S.M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T.E., Mackay, C.E., et al. (2006). Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *NeuroImage, 31*, 1487-1505.

Smith, S.M. (2002). Fast robust automated brain extraction. *Human Brain Mapping, 17*(3), 143-155.

Stagg, C.J., Best, J.G., Stephenson, M.C., O'Shea, J., Wylezinska, M., Kincses, Z.T., et al. (2009). Polarity-Sensitive Modulation of Cortical Neurotransmitters by Transcranial Stimulation. *The Journal of Neuroscience, 29*(16), 5202–5206.

Stagg, C.J., Bestmann, S., Constantinescu, A.O., Moreno, L., Allman, C., Mekle, R., et al. (2011). Relationship between physiological measures of excitability and levels of glutamate and GABA in the human motor cortex. *The Journal of Physiology, 589*(23), 5845-5855.

Stagg, C., Bachtiar, V., & Johansen-Berg, H. (2011). The Role of GABA in Human Motor Learning, *Current Biology, 21*, 480–484.

Stagg, C.J. & Nitsche, M.A. (2011). Physiological Basis of Transcranial Direct Current Stimulation. *The Neuroscientist, 17*(1), 37 –53

Stern, E., Silbersweig, D.A., Chee, K., Holmes, A., Robertson, M.M., Trimble, M., et al. (2000). A Functional Neuroanatomy of Tics in Tourette Syndrome, *Archives of General Psychiatry, 57,* 741-749.

Sowell, E.R., Kan, E., Yoshii, J., Thompson, P.M., Bansal, R., Xu, D., et al. (2008) Thinning of sensorimotor cortices in children with Tourette syndrome. *Nature Neuroscience, 11*(6) 637-639

Sumner, P., Edden, R.A.E, Bompas, A., Evans, C.J., & Singh, K.D. (2010). More GABA, less distraction: a neurochemical predictor of motor decision speed. *Nature Neuroscience, 13*(7), 825-827.

Suppa, A., Bologna, M., Belvisi, D., Marsili, L., Berardelli, I., Moretti, G., et al. (2009). Synaptic plasticity at brainstem and cortical level in Tourette syndrome. *Movement Disorders, 24*, S62-S63.

Suppa, A., Belvisi, D., Bologna, M., Marsili, L., Berardelli, I., Moretti, G., et al. (2011). Abnormal Cortical and Brain Stem Plasticity in Gilles de la Tourette Syndrome. *Movement Disorders, 26*(9), 1703-1710.

Swain, J.E., Scahill, L., Lombroso, P.J., King, R.A. & Leckman, J.F. (2007) Tourette Syndrome and Tic Disorders: A Decade of Progress *Journal of the American Academy of Child & Adolescent Psychiatry,* 46(8), 947-969

Swerdlow, N.R., & Young, A.B. (2001). Neuropathology in Tourette syndrome: an update. *Advanced Neurology, 85,* 151–161.

Tajik-Parvinchi, D. & Sandor, P. (2011). Smooth Pursuit and Fixation Ability in Children With Tourette Syndrome*. Cognitive & Behavioral Neurology, 24*(4), 174–186.

Tarasów E., Wiercinska-Drapaö A., Kubas B., Dzienis W., Orzechowska-Bobkiewicz A., Prokopowicz D., et al. (2003). Cerebral MR spectroscopy in neurologically asymptomatic HIV-infected patients. *Acta Radiologica, 44*, 206–212.

Tang, Y., Lu, Q., Fan, M., Yang, Y. & Posner, M.I., (2012). Mechanisms of white matter changes induced by meditation. *PNAS, 109*(26), 10570–10574.

Thomalla, G., Siebner, H.R., Jonas, M., Baumer, T., Biermann-Ruben, K., Hummel, F., et al. (2009). Structural changes in the somatosensory system correlate with tic severity in Gilles de la Tourette syndrome. *Brain, 132*(3), 765-777

Tian, Y., Gunther, J.R., Liao, I.H., Liu, D., Ander, B.P., Stamova, B.S., et al. (2011). GABA- and acetylcholine-related gene expression in blood correlate with tic severity and microarray evidence for alternative splicing in Tourette syndrome: A pilot study. *Brain Research, 1381*, 228-236.

Vandewalle, V., Van Der Linden, C., Groenewegen, H.J. & Caemaert, J. (1999). Stereotactic treatment of Gilles de la Tourette syndrome by high frequency stimulation of thalamus. *Lancet, 353*, 724.

Vigneau, M., Beaucousin, V., Hervé, P.Y., Duffau, H., Crivello, F., Houdé, O., et al. (2006). Meta-analyzing left hemisphere language areas: Phonology, semantics, and sentence processing. *NeuroImage, 30*(4), 1414-1432.

Wahl, M., Lauterbach-Soon, B., Hattingen, E., Jung, P., Singer, O., Volz, S., et al. (2007). Human Motor Corpus Callosum: Topography, Somatotopy, and Link between Microstructure and Function. The *Journal of Neuroscience, 27*(45), 12132–12138.

Walther, M., Berweck, S., Schessl, J., Linder-Lucht, M., Fietzek, U.M., Glocker, F.X., et al. (2009). Maturation of inhibitory and excitatory motor cortex pathways in children. *Brain & Development, 31*, 562–567.

Wang, L., Lee, D.Y., Bailey, E., Hartlein, J.M., Gado, M.H., Miller, M.I., et al. (2007). Validity of large-deformation high dimensional brain mapping of the basal ganglia in adults with Tourette syndrome*. Psychiatry Research, 154*, 181–190

Wang, Z., Maia, T.V., Marsh, R., Colibazzi, T., Gerber, A. & Peterson, B.S. (2011). The neural circuits that generate tics in Tourette's syndrome. *American Journal of Psychiatry, 168*, 1326–1337.

Wardak, C. (2011). The Role of the Supplementary Motor Area in Inhibitory Control in Monkeys and Humans. *The Journal of Neuroscience, 31*(14), 5181–5183.

Worbe, Y., Gerardin, E., Hartmann, A., Valabregue, R., Chupin, M., Tremblay, L., et al. (2010a). Distinct structural changes underpin clinical phenotypes in patients with Gilles de la Tourette syndrome. *Brain, 133*, 3649–3660.

Worbe, Y., Malherbe, C., Hartmann, A., Pelegrini-Issac, P., Messe, A., Vidailhet, M., et al. (2012). Functional immaturity of cortico-basal ganglia networks in Gilles de la Tourette syndrome. *Brain, 135*(6), 1937-1946.

Wolf, S.S., Jones, D.W., Knable, M.B., Gorey, J.G., Lee, K.S., Hyde, T.M., et al. (1996). Tourette syndrome: prediction of phenotypic variation in monozygotic twins by caudate nucleus D2 receptor binding. *Science, 273*, 1225–1227

Wu, S.W. & Gilbert, D.L. (2011) Altered neurophysiologic response to intermittent theta burst stimulation in Tourette syndrome. *Brain Stimulation, 5*(3), 315-319.

Xu, S., Yang, J., Li, C.Q., Zhu, W. & Shen, J. (2005). Metabolic alterations in focally activated primary somatosensory cortex of alpha-chloralose-anesthetized rats measured by 1H MRS at 11.7 T. *Neuroimage, 28,* 401–409.

Yoon, D.Y., Gause, C.D., Leckman, J.F. & Singer, H.S., (2007). Frontal dopaminergic abnormality in Tourette syndrome: a postmortem analysis. *Journal of the neurological sciences, 255,* 50–56.

Zatorre, R.J., Fields, R.D. & Johansen-Berg, H. (2012). Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nature Neuroscience, 15*(4), 528-536.

Ziemann, U., Leonnecker, S., Steinhoff, B.J. & Paulus, W. (1996b). The effect of lorazepam on the motor cortical excitability in man. *Experimental Brain Research, 109*(1), 127–135.

Ziemann, U., Paulus, W., & Rothenberger, A. (1997). Decreased motor inhibition in Tourette's disorder: Evidence from transcranial magnetic stimulation. *American Journal of Psychiatry, 154*(9), 1277-1284.

Ziemann, U. (2013). Pharmaco-transcranial magnetic stimulation studies of motor excitability. In Lozano, A.M. & Hallett, M. (Eds.), *Handbook of Clinical Neurology, Brain Stimulation*, *116* (3rd series). Elsevier.