Imaging structural and functional brain changes associated with long-term learning

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A thesis submitted for the degree of

Doctor of Philosophy

February 2013

Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

I declare that no part of this work has been submitted for a degree or any other qualification at this or any other university.

Cassandra Sampaio Baptista, February 2013

To my mother for being an amazon and to Pablo

Acknowledgements

The work presented in this thesis is highly interdisciplinary, involving different departments, specifically the FMRIB centre, the MRC Anatomical Neuropharmacology Unit, the Gray Institute for Radiation Oncology and Biology, Experimental Psychology and Neuropathology. I wish to thank all that have participated, helped and have made this work possible, in particular to the participants (human and rodents).

I would like to thank Professor Heidi Johansen-Berg for giving me the life changing opportunity to carry out my research project in her laboratory. In particular for her constant availability, for answering all my questions through many useful discussions, for being the best role model since Dana Scully and for making me believe that I could do it. I feel very fortunate to have done the Dphil with Heidi and her support during this bumpy trip has meant more to me than I can express with words.

I would also like to thank Professor David Bannerman for providing guidance in the capacity of co-supervisor with special input on the projects' behavioural aspect, and for his very useful comments on this thesis.

I wish to thank the following collaborators: Dr. Jeff Kleim, who trained me on the reaching task in his laboratory at the University of Florida; Dr. Paul Bolam for his advice on histology methods; Dr Pablo Henny for teaching me EM techniques in Dr. Bolam's lab; Dr. Steven Chance for advising about semi-quantitative measurements of optical density in immunostained brain tissue; Dr Gabriele De Luca

for training me and supporting me in the immunohistochemistry; Carolyn Sloan for cutting the brain tissue; Professor Niki Sibson for allowing me to use the MRI facilities at ROB; Dr. Alexandr Khrapichev, Dr. Sean Foxley and Dr. Karla Miller for support with rat scanning and sequence development; and Saad Jbabdi for his help with DTI analysis.

I thank FCT for providing my personal funding.

I would like to thank Dr. Nicola Filippini for being present in most of the juggling study data acquisition phases and for his help with RS data analysis, to Dr. Charlie Stagg for her input on MRS data, Dr. Jan Scholz for teaching me data acquisition and analysis and adapting TBSS analysis to the rat brain, Dr. Tamar Makin for useful discussions and much needed pub visits, Steve Knight for teaching me how to use the scanner, the FMRIB's analysis and IT guys for their help dealing with analysis problems and to my fellow lab colleagues for their support and science discussions.

A special mention goes to Theresa Schlagheck for performing part of the behavioural experiments on Chapter 6.

To my friends far away for their support and unwavering friendship, this includes but it is not limited to Angela Rodrigues, Liliana Amorim, Rita Flores, Andreia Luisa Vieites, Paula Alexandra, Andre Smith, Samuel Ginja, Igor Spinola, Nikolay Kukushkin and Katy Patzel.

I would like to thank my grandmother Delfina for helping to raise me, to my brother Edgar, sister Ines and my family for their support.

I thank my mother, Deolinda, for giving me the opportunity to do this through much personal sacrifice, for being a superhuman and the most extraordinary person I have ever met.

Finally, I thank Pablo for putting up with me, for his support and love, for making Oxford my home and for helping me see the light at the end of the tunnel in really dark times. I love you too.

Abstract

Learning induces functional and structural plasticity. This thesis used a range of neuroimaging approaches in both humans and rodents to address three main questions: (1) Can we predict learning performance using baseline imaging measures? (2) To what extent do performance outcomes or training amount determine experience-dependent plastic changes? (3) What biological mechanisms underlie white matter plasticity detected using MRI?

Effects of performance and amount of practice on brain structure were studied by varying the amount of juggling practice. Brain structure was found to predict performance on a complex juggling task before learning acquisition.

Both performance and practice were found to affect brain structure after learning. Overall, participants that achieved higher performances had higher grey matter (GM) and WM matter change. Also, participants that trained juggling for longer had higher positive brain changes than participants that practiced less.

The effects of juggling performance and practice in functional connectivity and GABA levels as measured by MR spectroscopy (MRS) were also investigated. High intensity training was found to decrease the motor resting-state network strength while lower intensity increased the network strength. The increase in strength was associated with a decrease in GABA concentration. A correlation was also found between motor resting-state strength change and GABA concentration change after learning.

Finally, since WM plasticity has not been thoroughly investigated and to understand which cellular events underlie WM change, an animal

model of motor learning was combined with diffusion tensor imaging (DTI) and immunohistochemistry. Learning a novel motor task increased WM fractional anisotropy, an indirect measure of WM microstructure, in the contralateral hemisphere to the used paw. Immunohistochemistry staining with myelin basic protein (MBP) antibody of this region revealed higher myelin stain intensity for the learning group that correlated with performance in the task.

Aims

Plasticity is an intrinsic property of the brain that allows the organism to process and adapt to external and internal stimuli. Experience-dependent brain plasticity that underlies behaviour can occur by functional modulation of already existing connections. Furthermore, when the existing connections cannot support some form of behaviour, changes in the anatomy of the brain occur. These structural changes can consist of modification of number of synapses, restructuring of dendritic and axonal branches, gliogenesis, including myelinogenesis, as well as neurogenesis. While most studies in this field have been carried out in animal models, due to the invasiveness of the methods necessary to study experience-dependent brain changes, more recently, studies have been performed in humans with non-invasive techniques such as magnetic resonance imaging (MRI). MRI also has other advantages over invasive techniques, such as acquisition of multiple time-points in the same subject and measurement of the whole brain, making it ideal for longitudinal studies of network-level brain plasticity.

However, MRI at the moment lacks the necessary resolution to address the cellular and molecular processes that underlie plasticity. Furthermore, MRI measures are often ambiguous and cannot discriminate between different underlying cellular processes, thus animal models are advantageous. Animal models of learning allow the acquisition of MRI and histology in the same specimen and can help to develop specific MRI markers for plasticity that can be applied to human studies. Moreover, histology has the potential to help clarify the origin of the signal change in neuroimaging techniques such as diffusion MRI. Consequently, the main aim of this thesis is to research structural and functional brain plasticity in response to learning in healthy brains in adult humans and rodents. More specifically, in the first study, I investigated (Chapter 3, 4 and 5) structural and

functional changes with learning in the human adult brain. In particular, Chapter 3 is dedicated to the behavioural effects of long-term learning and investigates how individual differences in brain structure at baseline can be used to predict responses to learning. In Chapter 4 I have studied whether structural brain changes are driven by amount of training and/or training outcome. In Chapter 5 I have explored the effects of long-term learning on functional connectivity across distributed networks and on brain chemistry as measured by MR spectroscopy. Finally, rodent research provides comparisons between diffusion imaging and histology (Chapter 6) in order to clarify which structural characteristics underlie MRI measures in long-term learning-dependent plasticity.

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Chapter 1

Introduction

1.1 Definition Of Neural Plasticity

One of the most striking characteristics of the brain is its ability to change and adapt in response to external and internal stimuli. Neural plasticity is a general term that refers to the functional and structural brain changes that allow the organism to adapt to the environment and to learn new information. Plasticity therefore determines the nervous system's capacity to adjust during development, interaction with the environment, aging and response to trauma. Plasticity is thus an intrinsic characteristic of the brain that is continuously occurring with every sensory input, every motor output and every thought. The distinction between functional and structural plasticity can be, on occasion, artificial since these mechanisms are interconnected, but for the purposes of this work I will distinguish them using the following definitions: Functional plasticity is the mechanism that changes the efficacy of an existing connection or synapse, by insertion or removal of pre or postsynaptic receptors or a change in the level of

presynaptic release of transmitters, thus including long-term potentiation (LTP) and long-term depression (LTD)-like mechanisms. At a systems-level, functional plasticity can be detected by changes in the strength of functional interactions between brain regions, for example using BOLD-signal based techniques like task-related functional MRI (fMRI) and resting-state fMRI (see Chapter 2 for details). Structural plasticity is any change in the anatomy of the brain, such as dendritic spine growth, that can be detected using microscopy. At a systems-level, such changes have the potential to affect measures from structural MRI like T1, T2, DTI among others (see Chapter 2 for details).

1.2 Historical Perspective On Neural Plasticity

The term plasticity was first used by William James in his seminal work "The Principles of Psychology" in 1890 while referring to the behavioural susceptibility to change (42). He defined plasticity as " ... the possession of a structure weak enough to yield to an influence, but strong enough not to yield all at once" considering that "Organic matter, especially nervous tissue, seems endowed with a very extraordinary degree of plasticity of this sort; so that we may without hesitation lay down as our first proposition the following, that the phenomena of habit in living beings are due to the plasticity of the organic materials of which their bodies are composed" (vol. I, p. 105). James not only introduces a new concept that would become extremely important decades later in the neuroscience field, he also makes the remarkable observation that brain changes lead to behavioural modification and vice-versa. Notably, at the time the brain was considered stable, with the exception of the decline that occurs with aging, and it was thought that

no new neurons were generated. Contradicting the neuroscience society views of the brain at the time, Ramón y Cajal (1894) suggested that memories and acquisition of new skills might be formed by increasing strength between neuronal connections. However, the most influential theory regarding synaptic plasticity was published by Hebb in 1949 in his book the "Organization of Behaviour" (39). Hebb stated that "when an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (39). With this appealing phrasing Hebb proposed a mechanism of memory storage through the synchronous firing of cell A and B. Interestingly, Hebb implicitly distinguishes between two types of plasticity: structural plasticity ("some growth process") to refer to anatomical changes in the brain; and functional plasticity ("metabolic change") to allude to changes that affect the excitability of the cell or strength of the connection such as removal or insertion of receptors. Furthermore, Hebb showed that rats housed in a complex environment improved their performance in a number of cognitive tasks and, thus hypothesized that complex environments could shape the brain through learning and experience (39). Since then a great number of studies, predominantly in rats, support Hebb's research by showing that spending time in an enriched environment induces structural brain reorganization, both in development and in the adult (14; 35; 55), through mechanisms such as dendritic growth and synaptic formation (35). The existence of some forms of functional and structural plasticity became increasingly more accepted in the field, but there was still one last dogma that remained: no new neurons are born in the adult mammalian brain. It was in 1967 that neurogenesis was first described in the dentate gyrus

of the hippocampus of the adult mammalian brain (2). However, it remained largely controversial and was forgotten by the scientific community until it was rediscovered in the 90's, putting an end to a long lasting dogma (25; 33; 64).

1.3 Mechanisms Of Structural Plasticity

Structural plasticity in response to learning encompasses many different types of molecular and cellular responses, as shown by animal studies, manifested through dendritic and synaptic restructuring (55), synaptogenesis (16; 36; 63), neurogenesis (25; 32; 33; 77), altered cortico-cortical wiring (40), gliogenesis and angiogenesis (3), and myelination processes (20). Structural plasticity also allows the central nervous system (CNS) to respond to insults such as stroke or traumatic injury by establishing new connections (18) and it is thought to be associated with "spontaneous" processes that underlie recovery from brain damage like in stroke (11; 80). Although the most widely reported changes with experience occur in grey matter (GM), there is evidence showing that experience-driven structural changes also take place in white matter (WM). Rearing in complex environments has been reported to increase the density and volume of oligodendrocytes in the visual cortex (78; 82), increase the number of myelinated neurons in the splenium of the corpus callosum (45) and increase the size of the corpus callosum in rhesus monkeys (71). On the other hand, it is still unknown if oligodendrocytes are sensitive to experience in adult brains, although it is widely known that myelination in the human brain continues into adulthood (31; 88). Myelination of unmyelinated axons, increase in myelination thickness of previously myelinated fibers or axonal sprouting could enhance the speed of the communication or alter

the synchronicity between connections in the adult brain and therefore could be an important mechanism for learning. However, evidence about how experience shapes WM is scarce, with few studies having investigated this. One of these studies found that adult rats housed in a complex environment for 2 months did not develop more myelinated axons, but had a higher number of unmyelinated axons and glial cells in the corpus callosum (56). This study suggests that myelination may be less sensitive to experience in adulthood but that non-myelinated axons do appear to respond to enrichement. However,another study recently reported an increase in volume of myelinated fibers and myelin sheaths in the corpus callosum with environmental enrichment in middle-aged rats (91). Furthermore, there is some evidence that myelin might be sensitive to learning. A higher expression of myelin basic protein (MBP) was found after spatial learning in rats (12). Taken together, these studies suggest learning and experience might trigger cellular events in WM. By combining neuroimaging and histological techniques in animal models we might be able to advance our understanding of WM plasticity in the in-vivo human brain.

1.4 Motor Skill Learning

Motor skills like riding a bicycle, writing, playing musical instruments or sports are acquired by repetitive practice. In recent years our knowledge about the brain areas involved in acquisition, consolidation and long-term retention of a motor skill has increased greatly due to technological advances in non-invasive neuroimaging and non-invasive brain stimulation. Since most skills are complex and consequently hard to study, sequence learning, where movements are learned

in a specific order, is often used in experimental studies as a model of motor learning though this requires a combination of skill learning and sequence learning (61; 69; 70). More recently a number of studies have focused on more naturalistic models like juggling (23; 73), golf (10; 43), typing (15), piano playing (7; 38) and basketball (59). While these studies have some advantages, like using real-life skill learning models and thus possessing higher ecological validity, there are associated disadvantages due to the complexity of the learning which makes it more challenging to assess performance objectively, control for external variables (like fatigue, training duration and schedule, learning strategies, etc.) and to provide an adequate control task and/or group.

1.5 Imaging Structural Plasticity With Learning In Humans

The first suggestion that MRI was a suitable technique to detect how the human brain structure was sensitive to experience and learning came from a cross-sectional study where it was shown that taxi drivers had a larger posterior hippocampus compared to controls and a smaller anterior hippocampus (54). The posterior hipocampus was positively correlated with the number of months spent as a taxi driver and the anterior hippocampus was negatively correlated (54). Although these differences could be due to genetics rather than to environmental influences, it opened the possibility of using non-invasive techniques to study how experience shapes the human brain. Since then several studies have used cross-sectional designs to look at the relationship between brain structure and

performance in several tasks. More recently, researchers have tried to show a causal relationship between brain structure and learning by using longitudinal designs.

1.6 Cross-sectional Studies Of Structural Brain Changes With Learning

While cross-sectional designs cannot show a causal relationship between learning and brain structure plasticity, these studies can provide valuable information about the types of behaviour and skills that correlate with individual structural differences, and could potentially offer outcome predictors in skill learning and even rehabilitation.

Cross-sectional studies have focused on GM density or volume, and more recently on WM parameters, with comparisons made between people with different levels of skill proficiency or using an expert population. For example, professional musicians were found to have increased GM density compared to non-musicians in the auditory, sensorimotor, premotor cortices and cerebellum (8; 9; 29; 30; 38). Practice intensity correlated with structural changes in these areas. Furthermore, WM changes were also found in the internal capsule, genu of the corpus callosum and corona radiata, with amount of practice again correlating with fractional anisotropy (FA), a measure of WM integrity, in the internal capsule (7; 38; 72). These studies suggest that the brain alterations associated with musical proficiency are related to the skills necessary to play instruments such as fine motor control, the ability to distinguish between different sounds and to memorise com-

plex musical pieces. Although we cannot directly conclude that musical training caused the detected structural changes, the correlations between practice intensity and brain changes suggest that this is the case.

Variations in brain structure are also associated with expertise in other sensorimotor domains. In professional typists, GM volume in prefrontal cortex (PFC), supplementary motor area (SMA) and cerebellum correlates with typing experience (15). Higher expertise in golf correlates with larger GM volumes in parietal and premotor cortices, and with lower FA in the external and internal capsule, and parietal operculum (43). Basketball players also have higher GM volume in the cerebellum (59).

There is also evidence that cognitive activity reorganizes brain structure. For instance, academic mathematicians were found to have larger GM density in the inferior parietal cortex, an area associated with arithmetic and mathematical reasoning, which correlated with time spent in academia (4). GM density correlates positively with second language proficiency and negatively with acquisition age in the left inferior parietal cortex (57). Vocabulary fluency also correlates with a similar region in monolingual adolescents, showing this area is generally concerned with vocabulary knowledge (50).

Differences in brain structure also correlate with task performance in non-expert normal populations (46). For instance, individual differences in bimanual co-ordination task performance correlate with WM FA in the body of the corpus callosum (44) and pre-SMA GM variability correlated with performance in an action selection task in the presence of conflict (87).

1.7 Longitudinal Studies Of Structural Brain Changes With Learning

The above-mentioned studies cannot clarify if the brain structure and behaviour correlations are due to genetic predisposition or due to practice effects. For example, a person can be drawn to music because he or she was born with brain structures that support this skill, thus improving their performance through training. However, it might be the case that practice itself drives the structural differences. To distinguish between these two factors, researchers have used longitudinal designs. The first study that showed that the adult human brain structure can change as a result of learning was conducted by Dranganski and colleagues (22). In this study, naïve participants were asked to learn to juggle for 3 months until they reached a determined amount of proficiency. They were scanned before and after the training period. GM density increased with juggling learning in a mid-temporal area (hMT/V5), which is involved in visual-motion information processing (74). After a subsequent three month period of no juggling, GM in this region decreased slightly. These results were partially replicated in a study with a higher temporal resolution in young adults (24). GM increases were found in the same area as before, but also in frontal lobe and cingulate gyrus. The authors reported a trend as early as 7 days in the right hMT/V5. Another study using elderly adults found additional increases in the hippocampus and precentral gyrus (13). Furthermore, Scholz and colleagues (2009), using a slightly different experimental design, showed an increase in GM density in occipital and parietal regions co-localised with FA increases in WM matter in participants that learned to juggle for 6 weeks (73). Curiously, over a subsequent 4 week period when par-

ticipants did not juggle, GM continued to increase while FA decreased. None of the above-mentioned studies showed a correlation between juggling performance and structural changes. In another study, involving a balancing task, increases in GM and decreases in FA were found after only two sessions of practice in frontal and parietal areas (85). A positive correlation was found between GM change in the oribitofrontal cortex and performance, and a negative correlation between FA and performance in prefrontal and parietal areas. Middle-aged golf novices asked to practice golf for 40 hours, with practicing schedules being under the golfers' control, showed an increased GM density in sensorimotor brain regions (10). Interestingly, a correlation was found between GM density in the occipital-parietal junction and practice intensity, defined as time needed to complete 40 hours of training (10). Structural changes have also been reported with cognitive tasks. Medical students showed enlargement of the hippocampus and parietal cortex after 3 months of intense study for an exam (23). Hippocampus GM continued to grow three months after the exam while the parietal cortex did not. No association between performance in the exam and brain structure was found. Taken together these studies do not show a clear association between performance, amount of training and structural changes. While some studies suggest that performance is important and might drive the structural brain changes, other studies do not show such relationships. This could be due to the sensitivity of behavioural measures that fail to capture important parameters of complex learning. As an alternative hypothesis, plasticity might be driven by the amount of practice rather than the outcome of that practice on performance. It is also possible that structural changes might result from a combination of the two. To complicate the matter even further, it is possible that different brain areas

and tissues (GM and WM) might be differently affected by performance and/or amount of practice.

1.8 Candidate Mechanisms For MRI Detected Structural Plasticity

The underlying cellular changes of structural plasticity are difficult to pinpoint as MRI measures are sensitive to a number of tissue properties (90). For example, an increase in GM density might reflect dendritic arborisation, increased number of spines, as well as non-neuronal morphological changes such as gliogenesis and angiogenesis. Similarly, markers of white matter structure can be modulated by different cellular components and mechanisms. Previous studies demonstrated that several WM features like membrane integrity, axon diameter, myelin thickness, and axon density modulate FA. Although Beaulieu and Allen (5; 6) showed that anisotropy in myelinated and nonmyelinated fibers is very similar, further studies proved that, although myelin may not be necessary for anisotropy, FA measures significantly change when myelin is absent or damaged. Specifically, Gulani and colleagues (2001) (37) showed in rats that the absence of myelin modulates FA values by approximately 20%. Finally, increases in axon packing density and decreases in axon diameter might increase FA in organized WM by decreasing perpendicular diffusivity due to the higher membrane density perpendicular to the axon (83).

To help interpret which cellular mechanisms could modulate GM and WM changes, a few studies have combined animal models of learning, MRI and his-

tology. Using a deformation-based technique, increases in mouse hippocampus or striatum were found depending on which version of the Morris water maze task was used (52). These increases correlated with growth associated protein 43 (GAP-43), a marker for neuronal process remodeling, but not with markers of neurogenesis, neuron or astrocyte number or size (52). Another study, using the same task, used DTI to investigate GM and WM changes (12). The authors found changes in several DTI parameters along with increases in synaptophysin, a synaptic marker, GFAP, an astrocyte marker and MBP, a myelin marker (12). More recently, a study showing a decrease in mean diffusivity (MD) in the human hippocampus after only 2 hours of playing a video game, directly compared its finding with rats trained for 2 hours in the water maze (67). The same decrease in MD was found in the rats' hippocampus along with glial fibrillary acidic protein (GFAP), synaptophysin and brain derived neurotrophic factor (BDNF), a neuroplasticity marker (67). This study highlights how fast neuroplasticity can occur and how sensitive MRI measures can be in detecting these changes.

Neurogenesis occurs mainly in the dentate gyrus and subventricular zone (SVZ) of the mammalian brain, including in humans (25; 32; 33; 77), and reports on neurogenesis in neocortical areas have been limited (34) and controversial (62). Therefore, neurogenesis is unlikely to account for structural changes that might be detected with MRI in brain regions other than the hippocampus. On the other hand, because synaptogenesis, dendritic, gliogenesis and angiogenesis have been widely reported to occur in neocortical areas with learning, these are strong candidate mechanisms to underlie structural variations detected with MRI in cortical GM, while axonal reorganization, including axonal sprouting and axonal diameter changes, and myelination, may contribute to MRI changes in

WM.

1.9 Imaging Functional Plasticity With Learning In Humans

While the motor models used may be different, most researchers agree that skill learning undergoes an early phase, where performance improves fast, and a slow phase, where improvements are slow and span multiple training sessions. The duration of each phase depends on the skill itself. The early phase in simple sequence learning may last one training session, but the same phase may last months when learning a complex musical piece on the piano. Furthermore, a consolidation phase is thought to occur between the fast and slow phases when learning becomes resistant to interference by other acquisitions (58). Consolidation can also refer to the off-line processes that occur after the practice session which improve performance, more specifically to the performance gains that occur between practice sessions, where sleep is thought to play an important role (26; 27). Finally, after acquisition and consolidation the learned skill may be retained for a long period of time and become automatic. Most of us have experienced that even in the absence of practice for years, we can still ride a bicycle, swim or drive a car. In the following sections I will focus on functional studies that show which brain areas are associated with each learning phase.

1.10 Early Fast Learning Phase

The early phase of motor sequence learning engages the primary motor cortex (M1), the presupplementary motor area (preSMA) and supplementary motor area (SMA), with these two last areas being concerned with the order of the movements (47; 76). As learning progresses, the premotor cortex, parietal areas, striatum and cerebellum become recruited, showing increased activation with learning (19; 28; 41; 69). The dorsolateral prefrontal cortex (DLPFC) is also involved in the early stages, primarily when learning is explicit and attentional demands are high (41; 69). Doyon and Ungerleider (2002) proposed a model that summarises the contributions of cortico-cerebellar and cortico-striatal loops to each learning phase (21). In the early phase both these loops are recruited and work in parallel to support learning; whereas the striatum contributes significantly to the consolidation phase, retention is supported by striatum, motor and parietal cortices (Fig. 1.1) (21). Hikosaka (2002) has proposed a different model that characterises motor learning based on the task components (Fig. 1.1) (41). In this model, motor learning is separated into a spatial component and a motor component, each supported by different neural circuits. Spatial learning activates frontal-parietal areas, cerebellum and basal ganglia. The motor component is controlled by a different circuit that includes M1, sensorimotor regions, cerebellum and striatum. The pre-SMA, SMA and premotor cortex act as mediators between these two parallel circuits. Spatial learning is considered to be faster and under explicit motor control, thus relying more on attentional and executive functions, whereas the motor component of learning occurs more slowly and is more implicit. Both these models acknowledge the contribution and collaboration between distinct

cortical and subcortical brain areas.

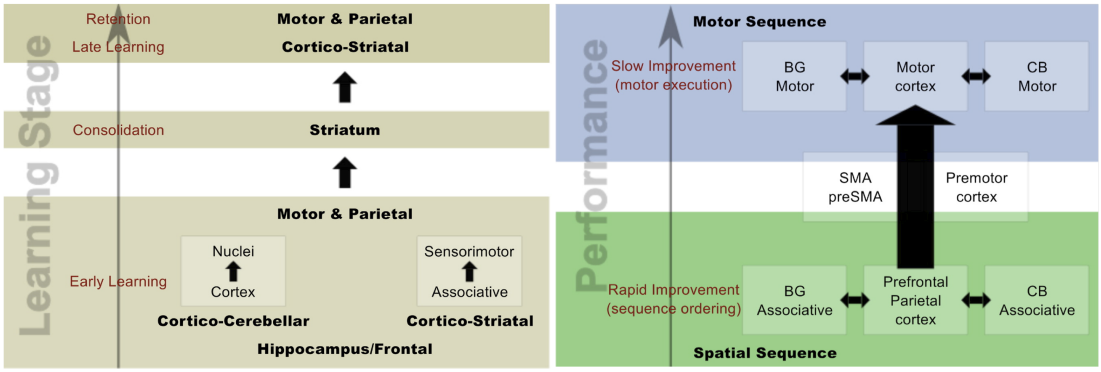

Figure 1.1: Motor Learning Models. Adapted from (61). The picture on the left summarises the Doyon and Ungerleider (2002) model (21) and the picture on the right summarises the Hikosaka model (41)

While both these models highlight the importance of brain areas working together as a network in motor learning, fMRI studies are mostly concerned with activation in isolated brain areas and not with how brain areas interact with each other to produce learning. However, there has been recent interest in researching interactions between different brain regions by means of functional connectivity analysis. Functional connectivity refers to the statistical covariance between brain regions' signal timecourses and it can be used to study how distant brain areas interact with each other. Recent studies using this technique have shown that M1, premotor cortex and SMA have higher covariance in the early stages of learning compared to later stages, within one session (81). Using a data driven method to study functional connectivity, Tamàs Kincses and colleagues (84) found a task related activation decreases in a fronto-parietal-cerebellum network and a sequence learning related activation in the posterior parietal and pre-motor cortex. Furthermore, the authors reported task-related deactivation in the previously identified default mode network (DMN) that correlated with

performance (84). Other studies have looked at the effects of motor learning on subsequent functional connectivity while the subject lies at rest in the scanner. The resting brain exhibits spontaneous activity that has been noted to form distinct temporally correlated patterns of functionally related brain regions. These patterns have been described as resting-state networks (RSNs) and it has been found that these resemble spatially and temporally task-related brain activation (79). Such RSNs have been shown to change in strength with long-term and short-term learning. Increases in strength in the fronto-parietal network and the cerebellum network after 11 minutes of learning were reported in one study (1). An increase in right postcentral gyrus (PCG) and in the supramarginal gyrus areas of the resting state motor network were reported in the first 2 weeks of a 4 week period of sequence learning, followed by a decrease in strength in the same areas in the last 2 weeks (53).

The underlying cellular mechanisms of motor learning in the early phase have been researched in both humans and animals. Studies in rodents show that LTP in M1 gets occluded after learning (65), suggesting LTP underlies motor learning. Similarly, studies with transcranial magnetic stimulation (TMS) in humans suggest that LTP-like mechanisms are present in M1 during the fast learning phase, while later stages might be supported by formation of new synapses (66).

1.11 Late Slow Learning Phase

The late phase of learning is usually researched by using several weeks of training in a specific task, often a complex sequence of movements. This phase is associated with increases of M1 activation, primary somatosensory cortex and

putamen (28). The motor cortex is a key structure not only during the early phase of learning but also in the late phase. Research shows that M1 BOLD signal progressively increases with learning (47; 48; 86) and the size of the motor maps increase as measured by TMS (60). This is supported by evidence from a rat study that showed motor map reorganization and synaptogenesis in the late phase (49).

The parietal cortex also plays a role in late stages of learning. A study by Sakai and colleagues (69) showed distinct time-course activations between DLPFC, pre-SMA, precuneus and the intraparietal sulcus (IPS). While the frontal areas were more activated in the early stages of learning, the parietal areas were more activated in the later stages of learning (68). Another study supports this shift from prefrontal regions to parietal cortex, cerebellum and premotor cortex during motor memory consolidation (75).

The striatum has been implicated in late learning stages and in automatic behaviour by several animal studies. In-vivo neuronal recordings show that the associative dorsomedial striatum is more involved in the initial phase, while the somatosensory dorsolateral striatum is engaged in the later phase (89). Neurons also refine their firing patterns in the slow stage of learning compared to the fast phase (17). These studies demonstrate that different stages of learning involve different striatal circuits and that different plastic processes occur in order to shape the circuits that sustain behaviour in later stages (17; 89). Similarly, a human fMRI study found progressive activation decreases throughout a month of learning a sequence of finger movements in the associative striatum and a progressive increase in the somatosensory striatum (51). Furthermore, performance accuracy was correlated with signal change in the areas activated during

the early learning phase, while performance speed was negatively correlated with signal change in areas activated in the late phase (51).

In summary, the late phase of learning is associated with functional reorganization and sculpting of the existent connections, as well as a shift from pre-frontal brain areas engaged in attentional processes to posterior brain areas involved in motor memory consolidation. Furthermore, this phase is associated with structural brain changes, both in GM and WM (22; 23; 73), as described previously.

1.12 Conclusions

The work described in this thesis provides a framework to understand and predict functional, structural and behavioural outcomes of motor learning in the healthy adult brain. Additionally, I have explored WM structural changes in an animal model of motor learning, in an effort to relate changes detected using MRI measurements to underlying cellular changes. Specifically, Chapter 2 provides an overview of the MRI methods and analysis used. Chapter 3 investigates how inter-individual differences in brain structure can be used to predict motor performance. Chapter 4 investigates whether structural brain changes are driven by amount of training and/or performance outcome. Chapter 5 explores the effects of long-term motor learning on functional connectivity and on brain chemistry, and the relationship between the two. In Chapter 6 I studied long-term motor learning-dependent plasticity effects on WM in rats using diffusion imaging and histology.

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Chapter 2

MRI Techniques And Derived Measures

Magnetic resonance imaging (MRI) techniques are non-invasive and do not use ionizing radiation (like computed tomography (CT) and X-rays), thus allowing repeated measures in the same subject. Furthermore, they have greater anatomical sensitivity compared to other imaging techniques. In the following sections I will explain the basic mechanisms of MRI and will go on to describe different imaging protocols, and which brain properties, functional and structural, can be investigated using these methods.

2.1 Introduction To MRI

MRI relies on nuclear magnetic resonance (NMR). Nuclei that contain an odd number of protons and/or neutrons spin around their own axis (Fig. 2.1a). The most commonly used nucleus in MRI is hydrogen (H), which is present in water

molecules and is widely available in body tissue. In a free environment the spins will be randomly oriented (Fig. 2.1b), however when placed in a magnetic field, the spins will align "slightly" with the field and precess around it (Fig. 2.1c). In MRI this field is generated by the scanner and is measured in tesla units. The frequency at which the nuclei precess depends on the type of nuclei and is directly proportional to the strength of the magnetic field, known as the resonant frequency.

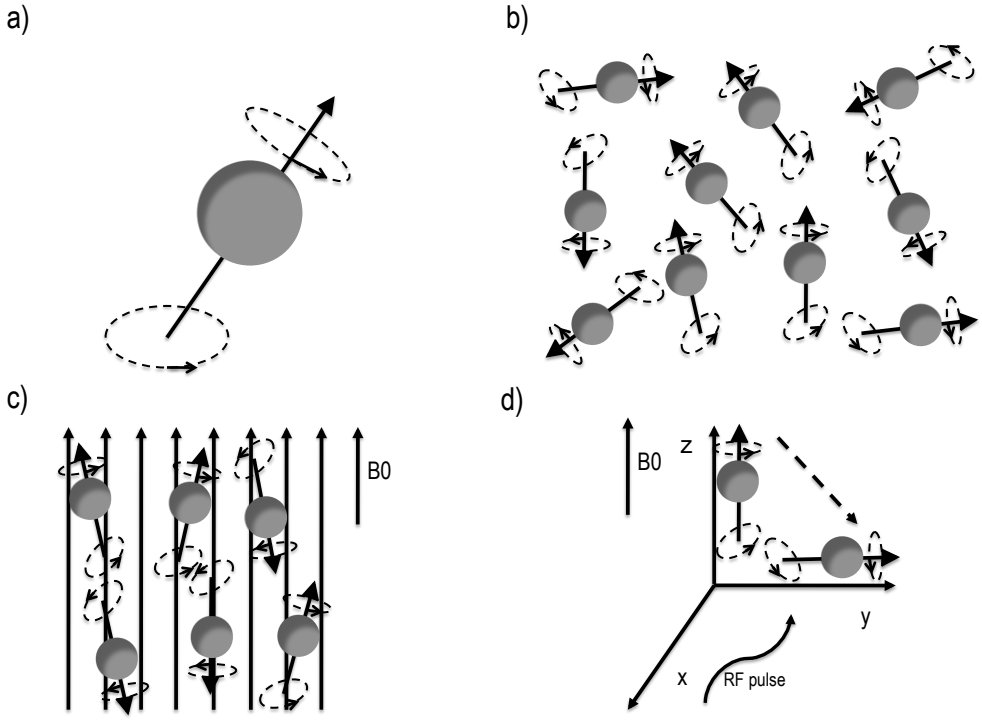

Figure 2.1: NMR Principles a) proton spinning around its own axis. b) Randomly oriented protons in free environment c) Proton aligning and precessing around an external magnetic field (b0) d) RF pulse flips proton spin and causes it to precess in the x,y axis.

In MRI, the alignment to the main magnetic field (named b0 and conventionally represented as the z axis) is perturbed by a perpendicular magnetic field

that emits a brief radio frequency (RF) pulse, tuned to the same frequency as the resonant frequency (Fig. 2.1d). This causes the protons to flip their spin and precess in the x,y axis, and can be detected as a current by a receiving coil (Fig. 2.1d). After the RF pulse is turned off, the protons' spin vector will become re-aligned with the main magnetic field (along the z axis) and precess around it.

The time between the RF pulse and signal acquisition, the echo time (TE), and the time between RF pulses, repetition time (TR), will have an impact on the image contrast. If the signal is acquired immediately after the RF pulse, most protons will be maximally aligned with the x,y axis and the resulting image will reflect proton density. On the other hand, if the signal acquisition is delayed, proton spins will start to re-align with the main magnetic field, resulting in signal decay in the measurable x,y axis. The recovery time in the longitudinal (z) axis is known as T1 and varies between different brain tissues which can be a source of tissue contrast. T2 reflects the decaying time in the transverse plane (x,y). This decay occurs faster than T1 due to the small magnetic fields around each proton that affect the spin of the neighbouring nuclei, causing them to dephase and decrease the signal. A second decaying time, T2* (T2 star), reflects inhomogeneities in the field. MRI sequences can be tuned in order to maximize or minimize the influence of each type of recovery or decaying time by manipulating the TR and TE in order to obtain images with high contrast between tissues. T1-weighted images, with short to medium TR and a short TE, allow for a high contrast between white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF), making it ideal for tissue segmentation.

To be able to image the location of the protons, it is necessary to use an additional set of small magnetic field gradients distributed across the x,y,z axis.

These gradients are rapidly turned on and off, after the RF pulse, to create small linear changes in the main magnetic field along the three orthogonal axes. These changes in the main magnetic field change the proton precession depending on their spatial position, which can be used to estimate the position of the nuclei. Fourier transforms are then used to reconstruct MR images from frequency space, conventionally known as k-space.

2.2 Diffusion Tensor Imaging (DTI)

Diffusion weighted-imaging (DWI) and diffusion tensor imaging (DTI) are two types of diffusion MRI methods that differ from conventional MRI in the sense that it quantifies the diffusion of local water molecules. This can be used to measure indirectly the microstructure of the brain in vivo, particularly the WM. Water molecules constantly move randomly across space. However, when constrained, diffusion of water molecules is directionally dependent. Biological tissues, cell membranes, fibres or macromolecules limit the motion of water molecules, which can be modeled in order to probe the tissue microstructure.

DTI is the most commonly used mathematical model to describe water diffusion in diffusion MRI by fitting an ellipsoid also known as a tensor (1). The tensor model permits us to quantify, not only the magnitude of global diffusivity or mean diffusivity (MD), but also the orientation and the directionality (fractional anisotropy), reflecting indirectly the orientation of the pathways (8).

The shape of the ellipsoid, whose principal axes correspond to the tensor eigenvectors, models the direction of the diffusion signal and the size reflects how freely the water molecules can move. The length of the eigenvectors axis represents the

tensor eigenvalues, with the first eigenvalue being the largest, representing the principal diffusion direction and often reflecting the main fiber orientation in WM. In the brain, a representative tensor of water diffusion in the ventricles is large and spherical in shape since there are few barriers, like cell membranes, and the diffusion is roughly the same in every direction (Fig.2.2).

In a fiber bundle there are many barriers such as the axonal membrane and myelin sheath so the diffusion is greater along the fiber than across it (1). For this reason the characteristic tensor in WM is elongated, reflecting the direction of the fiber (Fig. 2.2). In GM the tensor tends to be spherical but smaller compared with the ventricles' tensor, reflecting the fact that the water molecules are more constricted by barriers but there is not a preferential direction (at least not at the spatial resolution typically sampled in DTI) (Fig. 2.2).

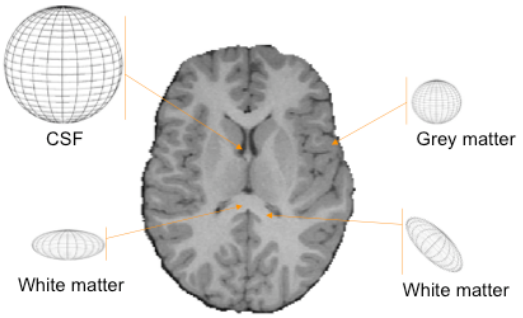

Figure 2.2: Vectors shape and size changes with tissue type. Image courtesy Saad Jbabdi.

Fractional anisotropy (FA) is the most commonly used parameter to quantify the direction of water diffusion in tissue, which ranges from zero (fully isotropic) to one (fully anisotropic). For example, the tensor in the ventricles could be described as isotropic with a value near zero because the diffusion is similar in all directions. However, in WM fibres the diffusion is not equal in all directions because there are barriers such as myelin or cell membranes. For that reason FA will be higher than zero and closer to one.

2.2.1 DTI As An Indirect Measure Of Microstructure

Understanding how the underlying biological factors like membrane integrity, axon diameter, myelin thickness and packing density, modulate DTI measurements can clarify which cellular events contribute to the measured signal and shed light on WM microstructure in vivo, making it a powerful tool to investigate WM plasticity in the adult brain.

As myelin is a unique characteristic of axons, it can be a major influence in the measured diffusivity. Beaulieu and Allen (1994) showed that anisotropy in myelinated and nonmyelinated fibres is very similar (2). However, further studies proved that although myelin may not be necessary for anisotropy, FA measures change significantly when myelin is damaged or absent like in multiple sclerosis (20). Gulani and colleagues (2001) also showed in mutant rats that the absence of myelin modulates FA values by about 10% (7). More recent studies show that myelin can modulate FA between 10% and 25% (11; 16; 19).

Takahashi and colleagues (2002) demonstrated that diffusion in lamprey large axons was found to be isotropic when measured in a single axon (17). This finding suggests that the cell membrane is the primary source of diffusion anisotropy in fiber tracts and not the microtubules or neurofilaments (17). Furthermore, the same study reported an increase in anisotropy with an increase of axonal density and a decrease of axonal diameter (17). Overall these findings point to the conclusion that internal organelles, like microtubules and neurofilaments, may have a slight contribution to the water diffusion and that the cell membrane is the principal barrier that forces water to diffuse in one direction, with myelin modulating FA by about 20%. Additionally, high FA can also reflect high axon

density or highly organized structures like parallel fibres. On the other hand, crossing fibres and large diameter axons are reflected in low FA values.

Taken together, evidence strongly suggests that there is not a single factor responsible for the DTI signal and correspondent values, hence it is crucial to conduct animal studies in order to understand the cellular basis of WM structural plasticity in humans as measured with DTI (Fig. 2.3)

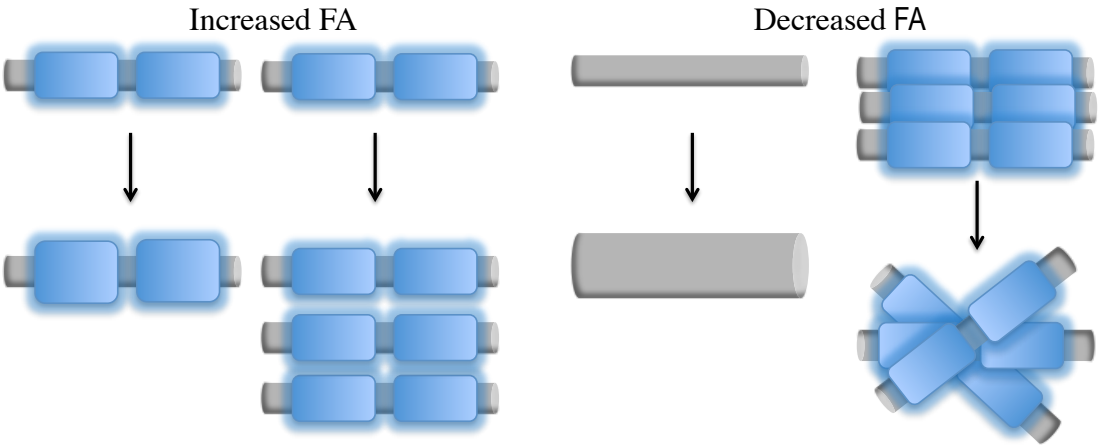

Figure 2.3: FA can be modulated by several WM feature,s which confound interpretation. FA increases with higher myelination and number of axons while it decreases with larger axon diameter and crossing fibres.

2.3 Functional Imaging

Functional MRI (fMRI) provides an indirect measure of brain activity by measuring changes in blood oxygenation, called blood-oxygenation-level dependent (BOLD) contrast. Deoxygenated haemoglobin is more paramagnetic than oxygenated haemoglobin, causing inhomogeneities in the field. It is assumed that neuronal activity increases the demands for energy and thus blood rushes to the active brain area. Although the blood flow increases, as well as the oxygen

consumption, only a fraction of the total oxygen is extracted, thus resulting in a decrease in magnetic susceptibility and an increase in MRI signal due to the presence of high levels of oxygenated haemoglobin. A recent study using optogenetics and fMRI showed that the BOLD signal is indeed related to neuronal activity (10).

This technique can be used for task-based fMRI, where stimuli are presented to participants, or resting-state fMRI (rs-fMRI), where no stimuli are presented and the BOLD signal is measured at rest. Experiments in this thesis tested the effects of learning to juggle on brain structure and function. Because participants are unable to juggle inside the scanner, I have used rs-fMRI to study the functional long-term effects of motor learning, amount of training and performance. At rest the brain exhibits spontaneous neuronal activity that modulates the BOLD signal and can be detected with rs-fMRI (4). It was noted that these low frequency BOLD fluctuations were functionally meaningful and not random noise. Biswal and colleagues (1995) reported that the fluctuation time-course in the right somatosensory cortex was correlated with resting BOLD signal in the left somatosensory cortex (4). This finding has been expanded by other groups showing that functionally related brain areas have correlated resting-state BOLD signal, now commonly known as resting-state networks (RSNs) (5; 13). These RSNs comprise brain areas that have similar fluctuation time-courses and reflect the brain's functional organisation and, in some cases, structure (9; 13).

2.4 Neuroimaging Analysis

All imaging analysis in this thesis was carried out using FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl) tools (15). A description of the analysis is provided below and specific details can be found in each chapter.

2.4.1 Structural Imaging Analysis

2.4.1.1 Voxel-Based Morphometry (VBM)

VBM is an unbiased, data driven analysis tool that allows for a whole brain investigation of brain tissue density. It can be used to investigate group differences in grey matter and white matter, in T1 images or any image that allows for sufficient contrast between tissues. This section will describe optimized VBM as it is implemented in FSL (6; 15).

First, the skull is removed with Brain Extraction Tool (BET) (12) and the brain is segmented into the different brain tissues (grey matter, white matter and cerebrospinal fluid) with FMRIB's Automated Segmentation Tool (FAST) (21), whilst also correcting for spatial intensity variations (also known as bias field or RF inhomogeneities). The segmentation generates images which quantify the estimation of the amount of each tissue type within each voxel, known as partial volume estimate (PVE). The PVE images of a representative number of subjects (different groups should be equally represented) are then individually aligned to a standard template with an affine transformation. The flipped versions of these images are also aligned to the template to avoid any lateral bias. All the aligned PVE images are then averaged to create a study specific template. The previous step is repeated with a non-linear transformation to the study spe-

cific template, and the resulting images are averaged. This resulting template is used as the target for all the subjects' PVEs. After non-linear alignment to the study-specific template, voxel-values of PVEs for each subject are modulated to correct for expansion and contraction effects that occurred during alignment (6). The modulated images are then smoothed and used as inputs for group comparisons with permutation-based non-parametric inference as implemented in the FSL tool, Randomise. While the same approach can be used for white matter analysis, it suffers from several problems like residual misalignments, which result in comparisons between different areas of the same tract making it difficult to interpret. For that reason Tract Based Spatial Statistics (TBSS) was used, rather than VBM, to analyse white matter (14).

2.4.1.2 Tract Based Spatial Statistics (TBSS)

TBSS has a very similar pipeline to VBM (14). First, FA images are created by fitting a tensor model to the raw diffusion data using FMRIB's Diffusion Toolbox (FDT), then the skull is stripped with BET and the edge voxels removed (12). The FA images are aligned into a common space using nonlinear registration and averaged to generate the study specific template. Next, the mean FA image is created and thinned to create a mean FA skeleton. The skeleton is thresholded to include only tracts that are common to all subjects. Each subject's aligned FA data are then projected onto this skeleton by searching a short distance along a line perpendicular to each skeleton voxel, finding the maximum FA value along that line and projecting that value onto the skeleton. The resulting aligned FA skeletons are fed into voxel-wise group-level statistics. Due to the lack of smoothing it is important to use permutation-based non-parametric inference,

since the data are not necessarily gaussian. Other DTI derived measures, such as mean diffusivity, can be projected onto the skeleton in the same way and fed into group-level statistics.

2.4.1.3 Avoiding Biased Registration In Longitudinal Data

For longitudinal data analysis a few more steps need to be added to avoid registration bias. When two or more images are collected from the same subject, aligning the second scan to the first scan will smooth the second scan but not the first. This affects the way tissues are segmented and can create false differences in tissue estimation. Thomas and colleagues (2009) demonstrated that biased registration can lead to false positives (18). It is possible to avoid this problem by registering all subjects' scans to midspace, a half-way space between scans, making smoothing uniform for all scans. All the T1 and DTI longitudinal data presented in this thesis were analysed with midspace tranformations. Details can be found in Chapter 4.

2.4.2 Functional Imaging Analysis: Resting-State fMRI

Analysis of resting FMRI data was carried out using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) (3). Independent Component Analysis (ICA) is a data driven method that decomposes the data into spatial components that are temporally correlated, while being independent from other spatial components. More specifically, ICA finds brain areas that have correlated BOLD fluctuations that are spatially independent from other brain areas with different fluctuation patterns. This technique can be used to identify not only the canonical resting-state networks but also artifacts like

head movements.

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Chapter 3

Inter-Individual Differences In Brain Structure Predict Juggling Learning Rate

In the past decade, MRI has been used to link inter-individual variability in behaviour and task performance to brain anatomy differences. In this chapter I have investigate if brain structure measured before learning can predict future performance.

3.1 Introduction

Inter-individual variability in brain structure can be linked to differences in task performance in expert populations of healthy individuals. For instance, professional musicians have increased grey matter density, compared to amateurs or controls (6; 7). There are several possible explanations for structure and

behaviour correlations. Individuals might possess innate brain structures that support a particular skill. Alternatively, an individual might, by experience-dependent plasticity, shape the underlying brain structure by engaging in particular tasks. Another possibility is that people that are genetically predisposed are more likely to engage and learn a specific activity, thus changing their brain structure and increasing their performance through learning. Although these studies hint that variation in brain structure can be related to differences in proficiency, the expert population is likely to be an extreme example.

There is evidence that meaningful behavioural variability is present as subtle differences in brain structure in non-expert populations that can be detected with MRI. For example, variation in performance in a bimanual coordination task was reflected in individual variability in white matter microstructure integrity in a corpus callosum area that connects to supplementary motor areas (SMA) (10). Another study found that high fractional anisotropy in the fornix was correlated with higher recollection, but not familiarity, in a memory task (13). Not only white matter but also grey matter variation has been linked to performance differences in non-experts. For example, grey matter density was correlated with action selection in the presence of conflict in pre-supplementary motor area (pre-SMA) (17).

These relationships between structure and behaviour offer insight about brain function and have the potential to help us understand behavioural disorders in the context of brain structure. More importantly, they provide the opportunity to make predictions about behaviour based on brain structure. This could be used in the context of talent identification like athlete, dancer and musician, and even in job recruitment. While repeated practice is a necessary condition

to become an elite athlete, it is often not sufficient and genetic predisposition is considered important to achieve high levels of performance (5; 12), although some authors disagree and put more emphasis on deliberate practice (4). Due to the complex interaction between nature and nurture it is quite difficult to identify who possesses the qualities to perform at a high level. As resources for extensive training are limited, and training itself takes several years, a lot of emphasis is put into identifying people that might benefit the most from training. Performance prediction based on brain structure variability can offer additional information when it comes to selection and recruitment of athletes or highly skilled populations such as surgeons.

While previous studies have shown correlations in an expert population after skill learning, or in a non-expert population with simple tasks or perceptual skills, there are no studies that show a relation between a complex skill and brain structure before skill acquisition. In this chapter I tested if different aspects of future performance in juggling were correlated with brain structure in naïve participants before learning. Furthermore, I tested if baseline brain structure could predict brain structure change after learning.

3.2 Methods

3.2.1 Participants

All subjects gave their informed consent to participate in the study in accordance with local ethics committee approval (REC B 07/Q1605/65).

Methodology was broadly based on a previous study (14) but with an ad-

ditional variable of practice time introduced to the design. This was done to separate effects of performance and practice in structural brain changes over time (see Chapter 4). In the current study, 66 naïve participants were recruited and randomly assigned to one of 3 groups: a high intensity training group that learned to juggle for 30 minutes per day, 5 days a week, for 6 weeks; a lower intensity group that practiced for 15 minutes per day, 5 days a week, for 6 weeks and a control group without any training. The training groups were scanned at baseline, after 6 weeks of training and 4 weeks after the previous scan. During the 4 week interval, participants were asked to stop juggling. The control group was scanned twice 6 weeks apart. From the initial 66 recruited participants only 60 completed the study (mean age 23.8, standard deviation 3.5; 31 female). Due to technical problems DTI was acquired only in 55 participants. Additionally, 20 control participants T1 scans (and 16 DTI scans) from a previous study (14) were used to increase the control group numbers. The final numbers per group can be found below (Fig. 3.1a). The current chapter only considers the baseline scan and behavioural results.

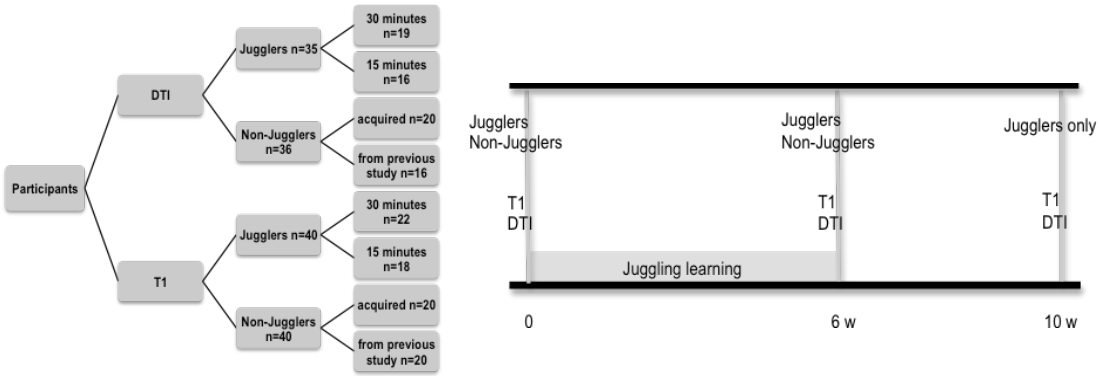

Figure 3.1: a) Final numbers per group per acquisition protocol: DTI and T1. b) The study lasts a total of 10 weeks for each participant. Subjects are scanned pre-training; after 6 weeks of juggling practice and 4 weeks after the post-training scan. The control group is scanned only twice, 6 weeks apart.

3.2.2 Behavioural Assessment

Participants in the training groups had a group juggling lesson on the first training day, where the fundamentals of the three-ball cascade were taught. Subsequently, subjects practiced daily at home and filmed themselves everyday while juggling for 29 days. After that, participants stopped juggling for 4 weeks. After the last scan participants were asked to film themselves again for 5 minutes while juggling. Volunteers who mastered the three-ball cascade before the end of the training period were encouraged to practice more advanced juggling patterns. The videos were uploaded to the anonymous file upload FMRIB website (http://www.fmrib.ox.ac.uk/cgi-bin/upload.cgi). This ensures compliance on the one hand and provides us with objective information for later assessment. The volunteers also rated themselves by reporting the best performance achieved on each day (0: 2-ball pattern; 1: 1 cycle of 3-ball cascade; 2: 2 cycles; 3: 3 cycles; 4: >3 cycles 5: 60 seconds of sustained 3-ball cascade). Final daily scores were derived from the volunteers' self-rating and the experimenter rating of each of the 29 training videos per participant. Compared to the self-report scale (0-5), the experimenter rating used a slightly more refined scale (0-10) of the performance (0: 2 balls; 1: 1 cycle of 3-ball cascade; 2: 2 cycles; 3: 3 cycles; 4: 5-10 seconds of sustained 3-ball cascade; 5: 10-20 seconds; 6: 20-30 seconds; 7: >30 seconds; 8: >60 seconds; 9: >60 seconds and at least one other pattern for <60 seconds; 10: >60 seconds and at least one other pattern for >60 seconds). From these scores the average over 29 days was calculated and the best performance for each participant that occurred was noted.

A learning curve was plotted for each participant based on the score for each day. A logarithm curve was then fitted to each participant's learning curve and the slope of the curve (learning rate) was calculated (Fig. 3.2). Repeated Measures ANOVA (RM - ANOVA) was used to test whether daily practice throughout the 30 days improved juggling performance. When Mauchly's test of sphericity is statistically significant, Greenhouse-Geisser F-test is used and the respective degrees of freedom are reported.

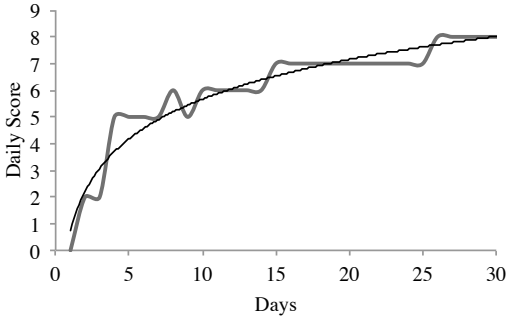

Figure 3.2: Typical participant learning curve (in black) and fitted logarithm curve (in grey) from which the slope was used to estimate learning rate.

3.2.3 MRI Acquisition

Data were acquired on a 3 Tesla Trio scanner (Siemens, Erlangen, Germany) with a 12-channel head coil. The same protocol was used for each volunteer and each scanning session. One axial T1-weighted anatomical image was acquired using a FLASH sequence (TR = 20.4 ms; TE = 4.7 ms; flip angle = 8; voxel size = 1 x 1 x 1 mm^3. Two sets of whole brain diffusion weighted volumes (60 directions; b = 1000 s/ mm^2; 65 slices; voxel size 2 x 2 x 2 mm^3; repetition time (TR) = 9.3 s; echo time (TE) = 94 ms) plus six volumes without diffusion weighting (b = 0 s-mm^2) were also acquired.

3.2.4 MRI Analysis: Preprocessing

Analyses were carried out with the FSL package, version 4.1 (for details see www.fmrib.ox.ac.uk/fsl). Analysed T1-weighted anatomical images were analysed using FSL's implementation (16) of voxel-based morphometry (VBM) (1; 8) (see section 2.4.1.1 for details). DTI data were analysed with FMRIB's Diffusion Toolbox (FDT). First, all data were corrected for eddy current distortions and head movements. Fractional anisotropy (FA) was estimated from the original DTI data with dtifit, and then analysed using the Tract Based Spatial Statistics (TBSS) approach (15) (see section 2.4.1.2 for details).

3.2.5 MRI Statistical Analysis

For statistical whole brain analyses, a voxel-wise generalised linear model (GLM) was applied using permutation-based non-parametric testing. Clusters were formed at $t > 2$ and tested for significance at $p < 0.05$, corrected for multiple comparisons across space. I tested whether baseline structural characteristics predict subsequent learning. I addressed this question using FA or GM density data from scan 1, and analysed this using a design matrix that included a regressor representing learning rate (slope) and average performance as measurements of performance.

3.3 Results

3.3.1 Behavioural Results

Participants were asked to practice juggling, while filming themselves, 5 days a week for 6 weeks, resulting in a total of 30 days training and 29 videos (excludes

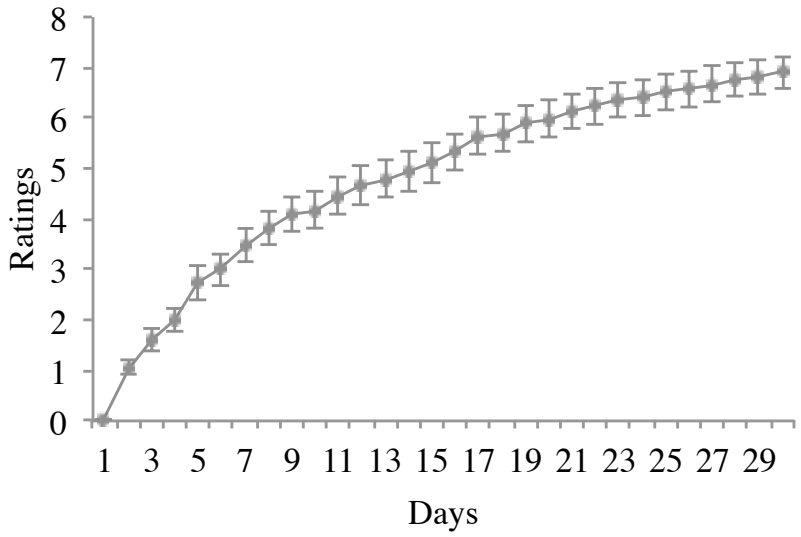

Figure 3.3: Average performance score per day (0: 2 balls; 1: 1 cycle of 3-ball cascade; 2: 2 cycles; 3: 3 cycles; 4: 5-10 seconds of sustained 3-ball cascade; 5: 10-20 seconds; 6: 20-30 seconds; 7: >30 seconds; 8: >60 seconds; 9: >60 seconds and at least one other pattern for <60 seconds; 10: >60 seconds and at least one other pattern for >60 seconds). There is a significant effect of day. Bars indicate standard error.

the juggling class).

Compliance was good: Subjects submitted on average 27.9 videos (SD = 2.2) and reported juggling on average for 28.6 days (SD = 0.9). To confirm performance improvement throughout the 30 days I used a RM - ANOVA (Fig. 3.3). The test revealed an effect of day on performance ($F(3.650,138.690) = 148.476$, $p < 0.001$). In summary, daily practice significantly improved juggling performance.

3.3.2 GM Density At Baseline Predicts How Fast Participants Learned To Juggle

GM density at baseline was found to be a good predictor of subsequent learning rate but not of average performance. Participants with higher GM density in right visual cortex (V1, V2, V4), right precuneus and right posterior cingulate at baseline learned to juggle faster than jugglers that had lower GM density in these regions (cluster p <0.05, corrected) (Fig. 3.4a,b). Interestingly, some of these brain areas partially overlap with regions that show GM change with learning in the same participants (see Chapter 4 for details) and in a previous study (14), particularly in precuneus, V1 and V2 areas (Fig. 3.4c).

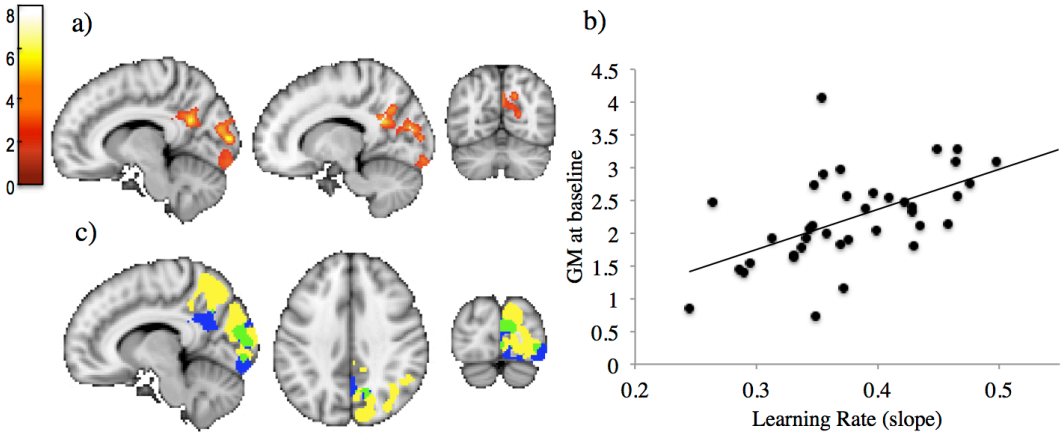

Figure 3.4: a) GM density in right visual and parietal cortex at baseline correlates with learning rate (p <0.05, corrected). Yellow-red voxels represent significant cluster superimposed on MNI template. Colour bar represents t-scores. b) Scatter-plot showing the correlation between brain areas in a) and learning rate. c) Yellow cluster corresponds to significant GM change after learning (from Chapter 4), blue cluster represents the GM correlation with learning rate at baseline (from Fig 4a) and green cluster shows the intersection between both clusters.

I further investigated if GM at baseline predicted the magnitude of GM change (as quantified in Chapter 4) in these overlapping regions, but no correlation was

found. I did not find any evidence that baseline FA was related to later performance even when the threshold was lowered or correction for multiple comparisons were not applied.

3.4 Discussion

Inter-individual differences in GM in occipital-parietal areas before learning were found to predict subsequent learning rate in a complex visuo-motor task. The identified brain areas have previously been implicated in the present task as part of a large GM cluster that was found to increase in density after juggling learning (14). The same areas overlapped with a significant increase in GM density after learning in the present group of participants (see Chapter 4).

These areas have been implicated in visual spatial processing and spatial attention processes that would be important for learning to juggle. More specifically, the posterior cingulate cortex (PCC) is involved in spatial attention shifting and in navigation tasks (9; 11). The precuneus is a major association area that connects to other parietal regions such as the intraparietal sulcus (IPS) as well as premotor regions like the supplementary motor areas (SMA) (2). Due to its anatomical connections this area has been considered part of a network that specializes in spatially guided behavior and in the spatial relationships of body movements, playing a role in the visual guidance of hand movements, reaching and grasping (for review see (3)). This region is also more active in complex bimanual tasks compared to a complex unilateral task (18). Taken together, all these areas are functionally relevant for juggling, which involves complex reaching and grasping movements. It is feasible that people who have greater eye-hand

coordination, that translates into higher GM density (either by previous learning or genetic predisposition) in brain areas that are related to this function, learn to juggle faster.

Most studies that have examined the relationship between behaviour and brain structure in non-expert populations have used simple tasks that can be performed with more or less difficulty by the participants (10; 13; 17). However, in this study the participants were scanned before they were able to juggle at all. The baseline correlation with subsequent learning rate in this complex task shows that the potential to learn complex skills is present and encoded in grey matter of functionally relevant areas. Even though some of these same areas increase in GM density after learning, the magnitude of subsequent GM change after learning could not be predicted based on GM density at baseline. This shows that GM density can increase independently of how much GM density was present to begin with. The ability to predict subsequent learning from baseline imaging measures is relevant to a number of real-life situations. For example, it could be relevant for talent identification in athletes, dancers and musicians. Furthermore, it can offer the opportunity to channel time-consuming training and limited resources by identifying people that might benefit the most from it, not only in sports but also in highly trained professional populations such as surgeons.

3.5 Conclusions

Individual differences in brain structure in areas that are functionally relevant to a specific skill predict how fast the skill can be acquired. On the other hand, in the next chapter it will be demonstrated that learning the skill itself drives

independent plastic processes that are specific and encode the skill acquisition.

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Chapter 4

Characterising Structural Change With Long-Term Learning In Healthy Adult Human Brain: Practice Versus Performance Outcome

In this study I have varied the amount of time subjects spent practicing in order to directly test whether structural changes are related to training time or training outcome.

4.1 Introduction

In everyday life we use motor skills that were previously acquired by repetitive practice, like tying our shoelaces. Animal studies show that motor learning is not only characterized by changes in functional responses, but also by structural remodelling (8; 23). More recently, MRI has been used to detect structural changes in humans in response to motor and cognitive learning (12; 13; 30; 34). However, it is still not known which aspects of motor learning drive the structural changes.

A number of previous human studies of structural change have used juggling as a training task. Those studies have predominantly used fixed training times, and have reported that changes in GM and WM are not correlated with how quickly subjects learn to juggle or how well they perform after training (6; 12; 14; 30). One possibility is that structural changes reflect the amount of time spent training rather than training outcome. The absence of a correlation between training outcome and structural changes in human neuroimaging studies is puzzling as functional plasticity and map reorganisation, as measured in animal studies, seem to be associated with learning outcome rather than with amount of practice (21; 27). It is not clear whether this apparent lack of a relationship between training outcome and structural brain change is real or reflects methodological factors. For example, the lack of an effect might be due to the assessment of the training outcome, i.e., the behavioural measures used might not be sensitive or might not represent the important aspects of learning that drive the structural changes.

In this study I have varied the amount of training time in order to directly test whether amount of practice or performance outcome drive structural brain

changes.

4.2 Methods

4.2.1 Participants

Participants are described in the previous chapter (section 3.2.1). Figure 4.1, which provides an overview of the participants and study design, is repeated below for information.

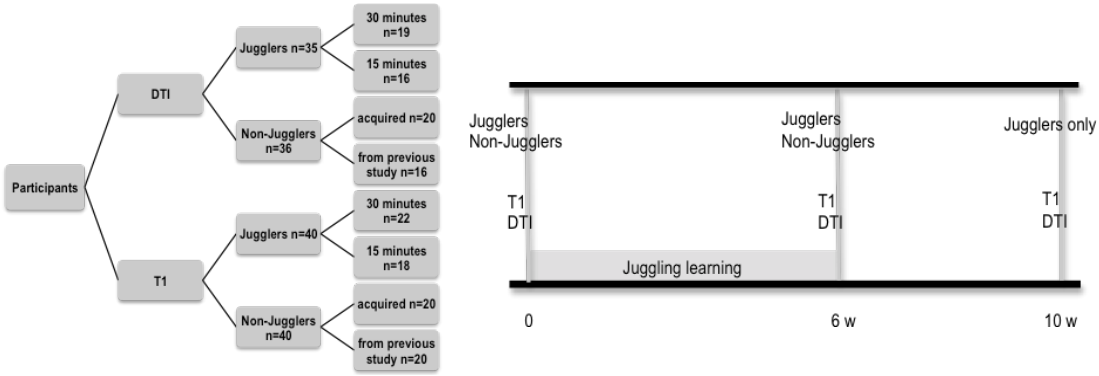


Figure 4.1: a) Final numbers per group per acquisition protocol: DTI and T1. b) The study lasted a total of 10 weeks for each participant. Subjects are scanned pre-training; after 6 weeks of juggling practice and 4 weeks after the post-training scan. The control group is scanned only twice, 6 weeks apart.

4.2.2 Behavioural Assessment

For details on behavioural assessment please refer to the previous chapter (chapter 3, section 3.2.2 Behavioural assessment). For each subject the following measures were calculated: daily performance score, best performance score over all training days, performance on the last day of practice, average performance over 29 days and learning rate.

Behavioural differences were explored over time and between groups with Repeated Measures ANOVA (RM - ANOVA) for the daily scores throughout the 30 days of training. When Mauchly's test of sphericity is statistically significant, Greenhouse-Geisser F-test is used and the respective degrees of freedom are reported.

Additionally, t-tests were used to investigate differences between groups' performance on the last day of practice, best performance, learning rate and the last performance measure acquired after the last scan (4 weeks after participants were asked to stop juggling). The difference between the last scan performance and average performance was calculated and a t-test was used to test differences between groups. Of the several performance parameters calculated, average performance over 29 days per participant (from now on referred to as average performance or just performance) (mean = 4.83, SD = 1.78) was used to test for effects of performance outcome on structural brain changes as this measure captured performance over the whole training period and showed a wide variation across subjects (Fig. 4.2).

4.2.3 MRI Acquisition

Details of MRI acquisition are provided in the previous chapter (Section 3.2.3).

4.2.4 MRI Analysis: Preprocessing

As for the previous chapter, image analyses were carried out with the FSL package, version 4.1 (www.fmrib.ox.ac.uk/fsl). However, a number of additional analysis steps were required due to the longitudinal nature of the data considered in

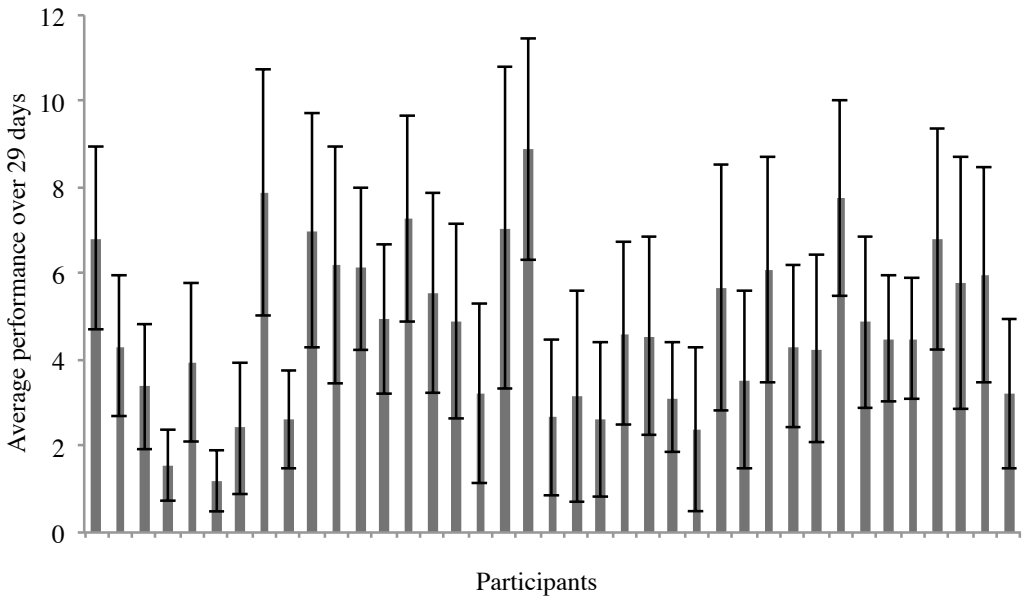


Figure 4.2: Average performance over 29 days per participant was used to test for structural brain changes in relation to performance outcome. Bars indicate standard deviation.

the current chapter.

T1-weighted anatomical images were analysed using FSL's implementation (33) of voxel-based morphometry (VBM) (4; 17). To avoid any registration bias, each scan was registered to the midspace between scans (11; 30). For each subject, the midspace between the 3 scans was calculated (or the 2 scans in the control group case) and aligned each scan with this mdispace using linear registration (FLIRT), after which all scans for a given subject were averaged in midspace (Fig. 4.3). For each subject, skull was stripped with BET (31) on the midspace averaged brain and back-projected the brain mask to each of the original (native space) scans. Then the brain-extracted native space images were segmented into GM, WM and CSF using FAST4 (38). The resulting GM partial volume images

were linearly transformed into that subjects' midspace, averaged and then aligned to MNI152 standard space using the nonlinear registration with FNIRT (2; 3). The resulting images were averaged across subjects to create a GM study-specific midspace template, to which the midspace GM images were then non-linearly re-registered. Then midway transformation were combined with the non-linear re-registration of the midspace images and applied it to the original GM images for each scan. The registered maps were modulated using the warp field of the non-linear transformation to correct for local geometric expansions or contractions. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 2 mm.

DTI data were analysed with FMRIB's Diffusion Toolbox (FDT). First, all data were corrected for eddy current distortions and head movements. Fractional anisotropy (FA) was estimated from the original DTI data with dtifit, and then analysed in a similar manner as described above using the Tract Based Spatial Statistics (TBSS) approach (32). Briefly, for each subject, FA maps were registered to a midspace between scans and averaged the registered maps. The averaged maps for each subject were non-linearly aligned to FSL's standard FA template and averaged to generate a study specific mean FA map. The white matter skeleton was then extracted from this mean FA image by thresholding it at an FA value of 0.2 to represent the center of the tracts common to all subjects. The tracts centres for each subject were projected onto the skeleton, which were then used for voxel-wise statistical comparisons.

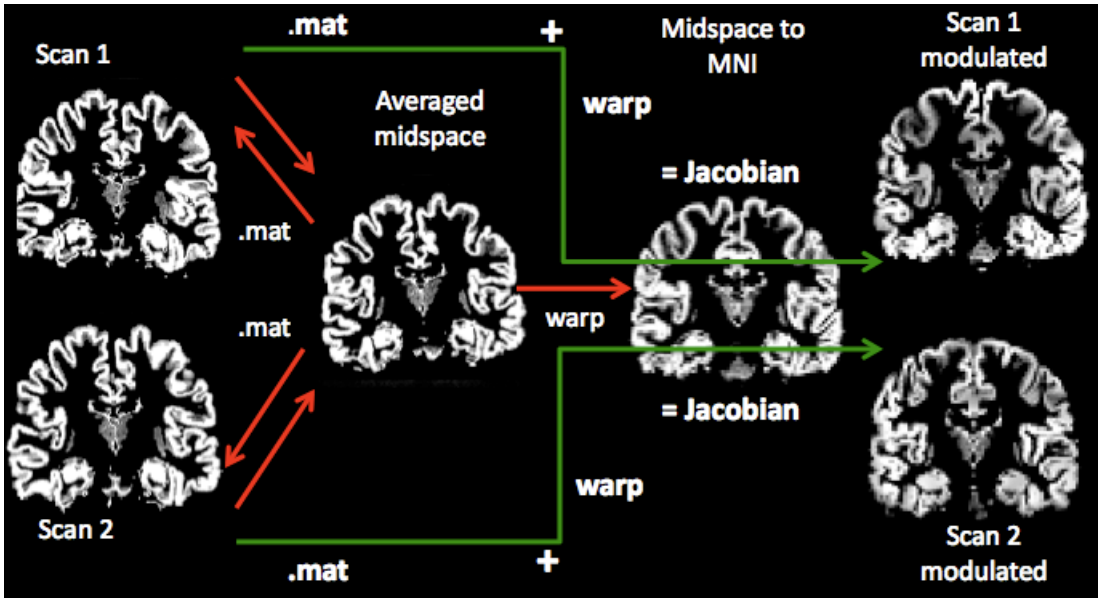


Figure 4.3: Schematic representation of midspace registration. Each brain scan is aligned to each other and the midspace transformation is extracted (forward red arrows with .mat legend). Brain extraction is done in midspace and then applied to each scan using the opposite midspace transformation (backwards red arrows with .mat legend). All images are then segmented and the GM midspace image is aligned to the GM template in order to extract the GM study specific template. This is then aligned to the MNI standard template (red arrow with warp legend). Finally the non-linear transformation to MNI is combined with the initial linear transformation to midspace and applied to the original scans (green arrows). The Jacobian modulation is then extracted for each brain scan and applied.

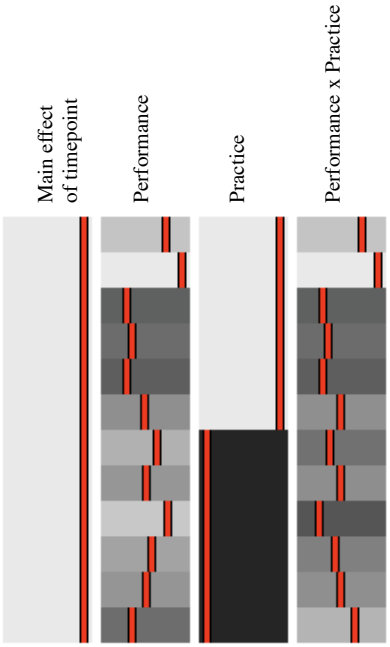
4.2.5 MRI Statistical Analysis

For statistical whole brain analysis voxelwise generalized linear models (GLM) were applied using permutation-based non-parametric testing, correcting for multiple comparisons across space. Clusters were formed at t >2 and tested for significance at p <0.05, corrected for multiple comparisons across space. For description purposes, trends between p <0.1 and p <0.05 (fully corrected for multiple comparisons) were also reported.

The primary research question concerned the effects of practice and perfor-

mance on structural brain change with training.

To address this question each pair of time points were considered separately (scan 1 vs 2; 2 vs 3; 1 vs 3), creating difference images between timepoints for both FA and GM density, which were then analysed separately with whole brain voxel-wise analysis. For each modality, and each pair of time points, a single design matrix was used to model both performance and amount of training, and the interaction between these variables (Fig. 4.4). This allowed to test for a main effect of time, as well as interactions between time and performance; time and practice; and time, practice and performance. The performance variable was demeaned across all subjects before entering into the design matrix. The "Performance x Practice" regressor was created by multiplying the performance regressor (column 2) with the practice regressor (column 3) (Fig. 4.4). For each contrast of interest both



Main effect of timepoint	1	0	0	0
	-1	0	0	0
Performance	0	1	0	0
	0	-1	0	0
Practice	0	0	1	0
	0	0	-1	0
Performance x Practice	0	0	0	1
	0	0	0	-1

Figure 4.4: Design matrix. Each variable (main effect of time, performance, practice and interaction between variables) is represented by a column. Each row represents a single subject. The first 6 rows are subjects from the 30 min group, the next 6 rows are subjects from the 15 min group (numbers are for illustrative purposes only). The bottom half of the figure represents the contrasts.

positive and negative contrasts were run.

The present results were also compared to those from a previous study that had considered a group undertaking 30 minutes of juggling training per day and a control group that received no training (30). For these analyses therefore the 30 minute group and control group only were considered. First, group by time interaction was tested using a whole brain voxel-wise analysis of FA and GM density maps before and after juggling, with RM - Anova. Second, the same analysis approach used in the previous publication was used, and the effects of time were tested separately within each group (30) . Finally, Scholz and colleagues (2009) GM and FA ROIs were used to test for differences between groups with RM - Anova(30) .

4.3 Results

4.3.1 Behavioural Results

Differences in performance scores between the 15 minutes group and the 30 minutes group (total n = 40) throughout the 30 days of juggling were explored with RM - ANOVA (Fig. 4.5). The test revealed an effect of day on performance ($F(4.127,152.704) = 157.5$, $p < 0.001$) but no interaction effect between day and group ($F(4.127,152.704) = 1.113$, $p = 0.353$) or main effect of group ($F(1, 38) = 0.064$, $p = 0.801$). To further explore differences between groups', less conservative tests were used. A t-test was used to investigate differences between the 15 minutes group (mean = 6.47, SD = 2.4) and the 30 minutes group (mean = 7.3, SD = 1.7) on the last day of practice; although final ratings were slightly

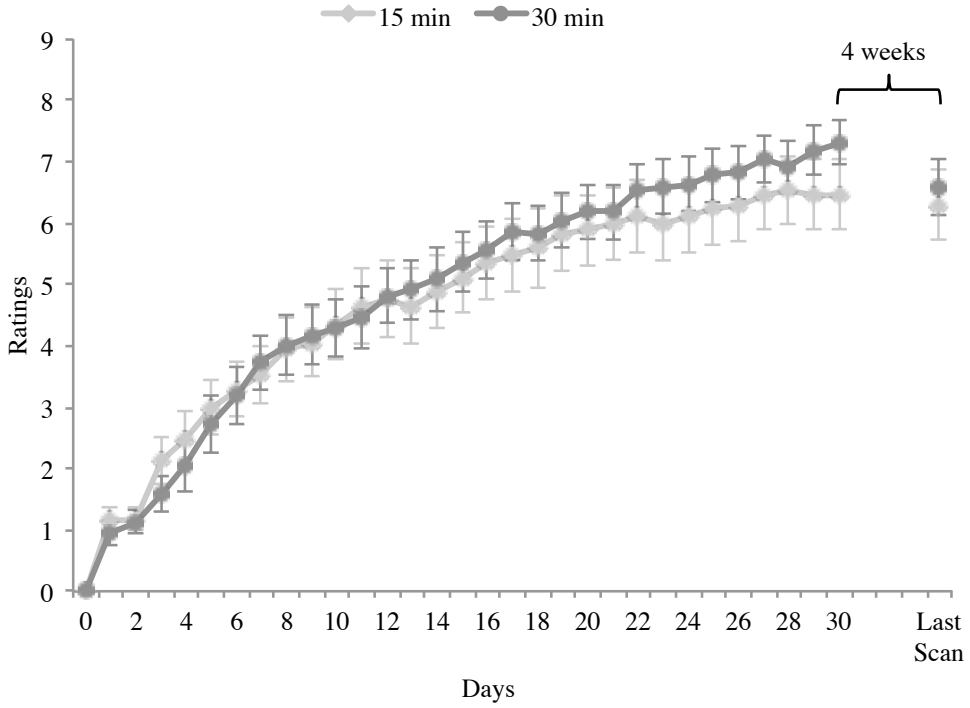


Figure 4.5: Average performance score for each group per day (15 minutes practice or 30 minutes practice) (0: 2 balls; 1: 1 cycle of 3-ball cascade; 2: 2 cycles; 3: 3 cycles; 4: 5-10 seconds of sustained 3-ball cascade; 5: 10-20 seconds; 6: 20-30 seconds; 7: >30 seconds; 8: >60 seconds; 9: >60 seconds and at least one other pattern for <60 seconds; 10: >60 seconds and at least one other pattern for >60 seconds). There is a significant effect of day but no significant interaction effect or significant differences between groups. Bars represent standard error.

higher for the 30 minutes group this difference was not significant ($t(38) = 1.29$, $p = 0.205$). There were no differences found between groups for learning rate (slope) ($t(1,38) = 1.47$, $p = 0.234$) or best performance ($t(1,38) = 1.439$, $p = 0.238$). I also tested for differences between groups in the last performance measure acquired after the last scan (4 weeks after participants were asked to stop juggling). There was no significant differences between groups at this last time point ($t(1,38) = 0.411$, $p = 0.683$). No differences between groups were

found when the difference between the last scan performance and average performance was calculated (t (1,38) = 0.504, p = 0.617). In summary, daily practice improved juggling performance but the amount of practice per day did not have any significant effect on performance outcome, even when less conservative tests were applied.

4.3.2 Neuroimaging Results: GM And WM Changes

4.3.2.1 What Drives The Structural Brain Changes? Effects Of Time, Amount Of Practice Or Performance.

For the trained groups, in order to distinguish the contribution of time, practice and performance outcome to structural brain plasticity a series of whole brain analyses were performed, in which the inputs were difference images (FA or GM) between each pair of time points (1 vs 2; 1 vs 3; 2 vs 3), and the regressors represented factors of practice (15 minutes vs 30 minutes groups) and performance outcome (average performance over 6 weeks of training) (Fig. 4.4). For each pair of timepoints, main effect of time was tested. Additionally, interactions were also tested between time and performance; time and practice; and time, practice and performance.

4.3.2.2 Training Period: Changes Between Scan 1 And Scan 2

No main effect of time was found, and no two-way interactions between time and performance or time and practice on GM between scan 1 and 2. However, significant interaction between time, performance and practice was found in the left motor and prefrontal cortex ($p < 0.05$, corrected) (Fig.4.6),

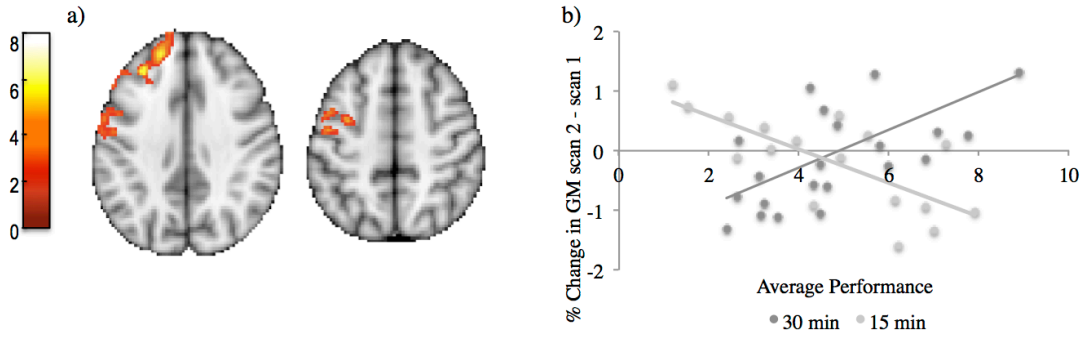


Figure 4.6: Interaction effect between practice group and average performance, between scan 1 and scan 2. a) yellow - red voxels correspond to the significant cluster, superimposed on MNI template. Colour bar represents t-scores. b) Correlation between the mean GM change and average performance for the 15 minute group (light grey symbols) and the 30 minute group (dark grey symbols) ($p < 0.05$, corrected).

demonstrating that the relationship between GM change over time and performance varied between practice groups.

The differences underlying this interaction can be seen in the scatter plot in Figure 4.6: For the 15 minute group, better average performance was associated with greater decreases in GM between scan 1 and 2 in left motor and prefrontal cortex, while for the 30 minute group, better average performance was associated with greater increases in GM between scan 1 and 2 in the same areas. No main effect of

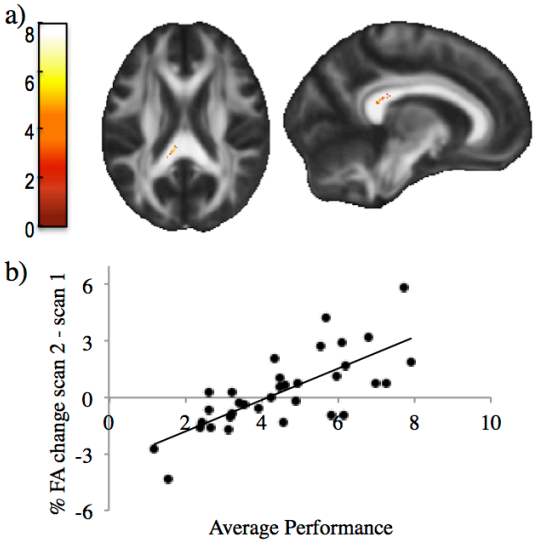


Figure 4.7: FA change between scan 1 and 2 correlated with average performance. a) yellow-red voxels correspond to significant cluster superimposed on FMRIB FA template ($p < 0.08$, corrected). Colour bar represents t-scores. b) Correlation with the clusters' mean FA change values and average performance.

time, and no interaction between time and practice group or three-way interaction between time, practice and performance was found for scan 1 to 2 for FA. A trend for an interaction between time and performance was found for FA change between scan 1 and 2 ($p = 0.08$, corrected) (Fig. 4.7). Jugglers that had higher FA increases in a posterior region of the left corpus callosum had higher average scores over the training period.

4.3.2.3 Longer Term Effects: Scan 1 To 3; Scan 2 To 3

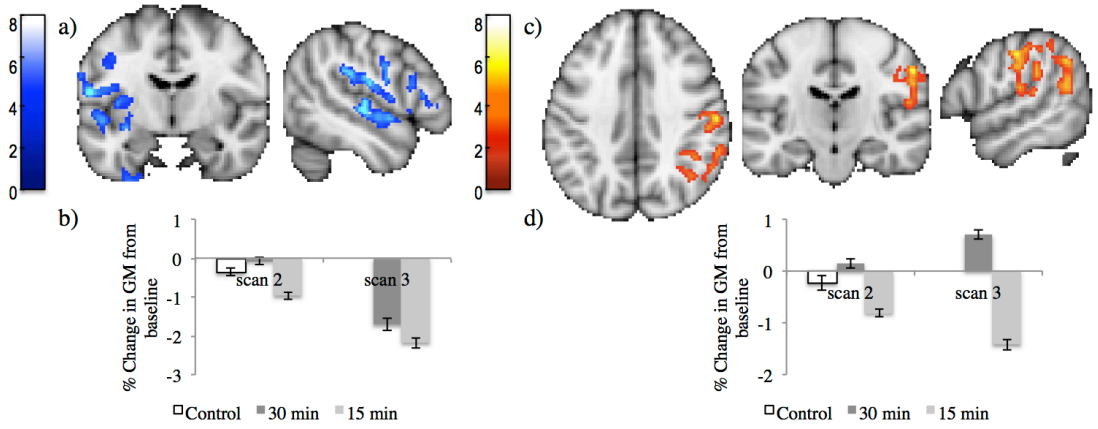


Figure 4.8: a) GM density decreases between scan 1 and 3 in the left temporal cortex, insula and operculum. Blue-dark blue voxels correspond to the significant cluster superimposed on the MNI template ($p < 0.05$, corrected). Colour bar represents negative t-scores. b) Mean GM values of the blue clusters throughout time relative to scan 1. c) Interaction effect of practice group and time, between scan 1 and scan 3. Yellow-red voxels correspond to the cluster showing a trend for an interaction superimposed on MNI template ($p < 0.08$, corrected). Colour bar represents t-scores. d) Mean GM values of the clusters throughout time relative to scan 1. Error bars represent standard error.

For GM, a main effect of time between scan 1 and 3 was found that corresponded to decreases in GM in the left operculum, insula and superior temporal gyrus ($p < 0.05$, corrected) (Fig. 4.8a). There was also a trend towards an interac-

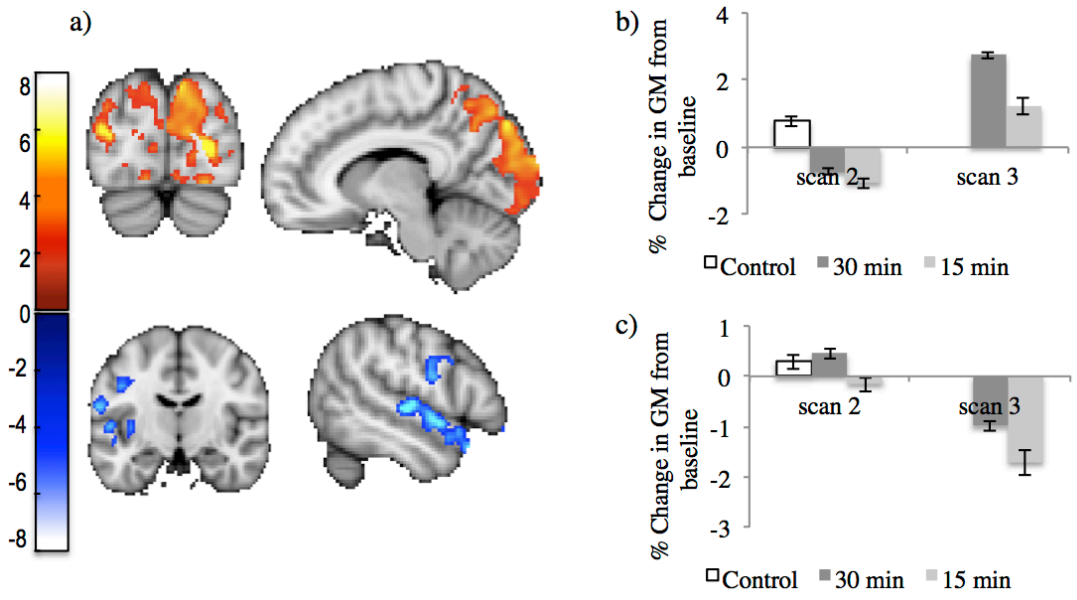


Figure 4.9: GM density increases between scan 2 and 3 in the visual and parietal cortex (Yellow-red voxels) and decreases between scan 2 and 3 in the superior temporal gyrus, insula and operculum (Blue-dark blue voxels). Clusters are superimposed on MNI template ($p < 0.05$, corrected). Colour bars represents t-scores. b) Mean GM values of the yellow-red clusters throughout time relative to scan 1. c) Mean GM values of the blue clusters throughout time relative to scan 1. Error bars represent standard error.

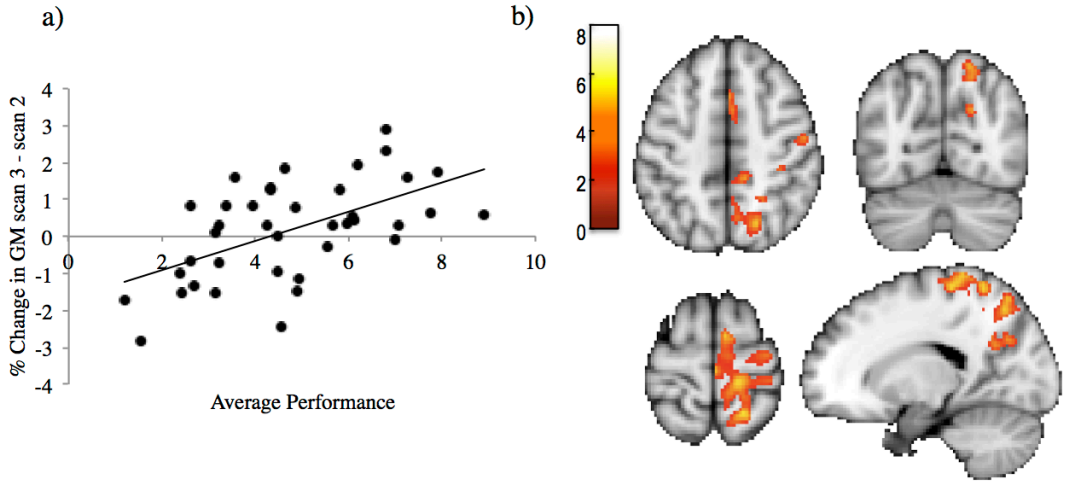


Figure 4.10: Correlation between the average performance and GM change after the learning period (between scan 2 and 3). Colour bar represents t-scores. b) Clusters' mean GM change values correlation with the average performance.

tion between time and practice group. The 30 minutes group showed an increase in GM density in the right parietal cortex and opercular areas while the 15 minutes group showed a decrease in the same areas ($p < 0.08$, corrected) (Fig. 4.8c). I did not find any interactions with performance for this pair of time-points.

During the follow up period (between scan 2 and 3) I found two main effects of time. Jugglers increase their GM density in occipital and parietal regions bilaterally ($p < 0.05$, corrected) (Fig. 4.9a,b), in similar areas to those reported by previous studies to change with juggling training (6; 12; 14; 30). Decreases in left operculum, insula and superior temporal gyrus were also found ($p < 0.05$, corrected) (Fig. 4.9a,c).

There was also an interaction between time and performance for GM change for this time period: jugglers that had higher GM increases in the right motor cortex, parietal cortex and pre-SMA also scored higher average performance during their learning period ($p < 0.05$, corrected) (Fig. 4.10). There were no interactions with practice group.

There were no significant main effects of time, or interactions of time with performance, or practice for FA. There were, however, trends for a main effect of time between scan 2 and scan 3 ($p < 0.09$, corrected) (Fig. 4.11a), and scan 1 and 3 ($p < 0.07$, corrected) (Fig. 4.11b).

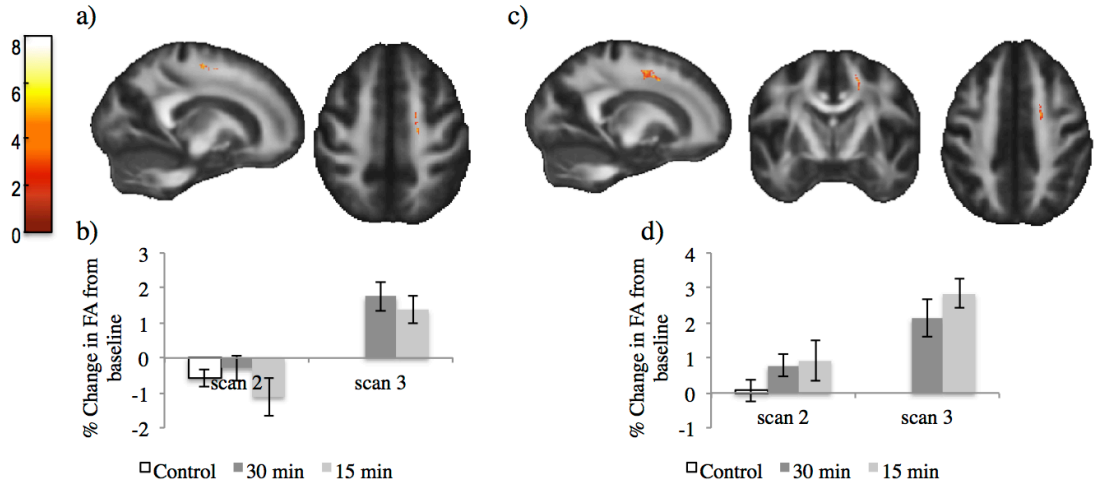


Figure 4.11: a) FA increases between scan 2 and 3. Yellow-red voxels correspond to trend cluster superimposed on FMRIB FA template (p <0.09, corrected). b) Mean FA values of the clusters throughout time relative to scan 1. c) FA increases between scan 1 and 3. Yellow-red voxels correspond to trend cluster superimposed on FMRIB FA template (p <0.07, corrected). d) Mean FA values of the clusters throughout time relative to scan 1. Colour bar represents t-scores. Error bars represent standard error.

4.3.2.4 GM And WM: Longitudinal Analysis Within Group

4.3.2.5 Juggling Training Effects On GM And FA Structure: Jugglers Vs Non-Jugglers

Group comparison between control participants and 30 minutes jugglers did not show any significant differences at baseline in GM or FA prior to any juggling training. The whole brain analysis RM-Anova did not reveal any significant differences between jugglers and non-jugglers for scan 2 compared to scan 1 in GM or FA.

A previous study used a very similar protocol to test for longitudinal brain change in jugglers who trained for 30 minutes per day, and compared this to controls (30). For comparison to that study, a longitudinal whole brain analysis

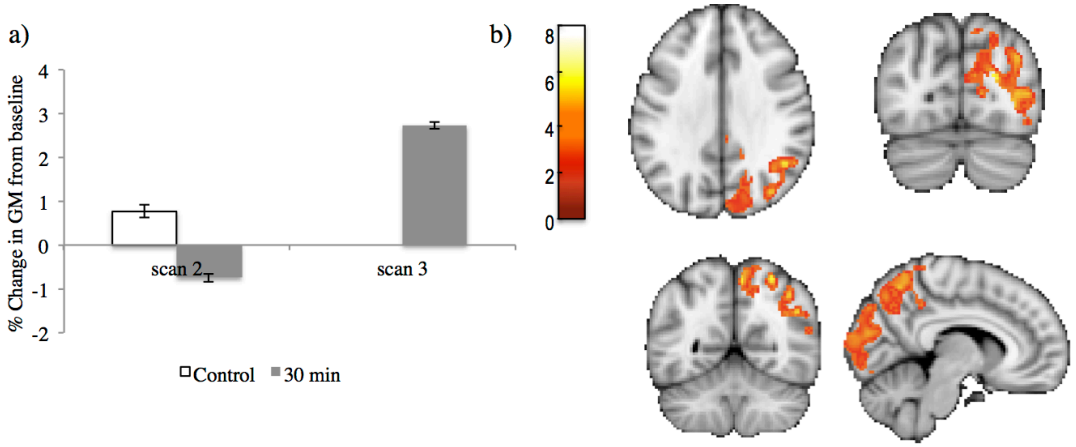


Figure 4.12: GM density increases between scan 2 and 3 in the right visual and parietal cortex. a) Yellow-red voxels correspond to significant cluster superimposed on MNI template (p <0.05, corrected). Colour bar represents t-scores. b) Mean GM values of the clusters throughout time relative to scan 1. Error bars represent standard error.

was performed within each equivalent group (30 minutes group and control group). No significant differences were detected between scan 1 and scan 2 for the jugglers or the control (both p <0.05, corrected) for GM or FA.

There was a significant GM density increase (p <0.05, corrected) between scan 2 and 3 in right occipital and parietal regions in the juggling group. These regions were anatomically similar to the previous study findings (Fig. 4.12).There was also a significant FA increase (p <0.05, corrected) between

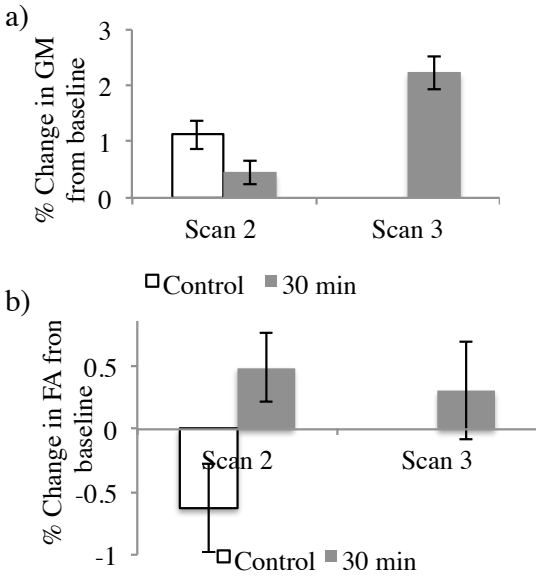


Figure 4.14: a) Mean GM values of (30) ROIs throughout time relative to scan 1. b) Mean FA values of (30) ROIs throughout time relative to scan 1. Error bars represent standard error.

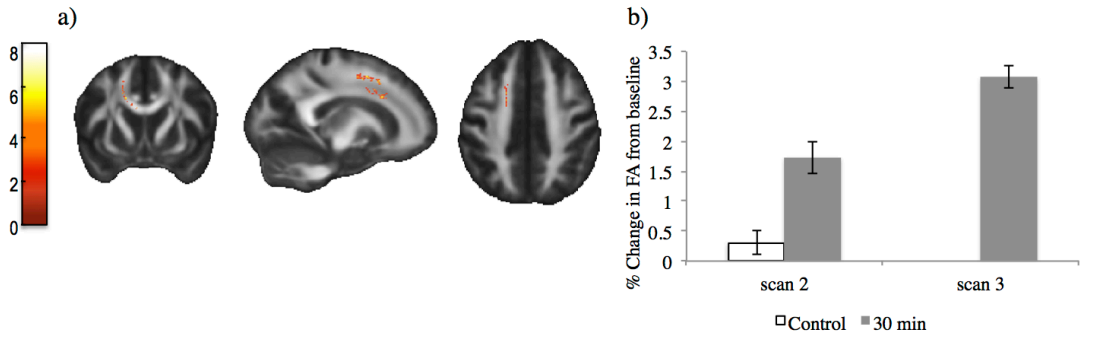


Figure 4.13: a) FA increases between scan 1 and 3. Yellow-red voxels correspond to significant cluster superimposed on FMRIB FA template ($p < 0.05$, corrected). b) Mean FA values of the clusters throughout time relative to scan 1. Colour bar represents t-scores. Error bars represent standard error.

scan 1 and 3 for the jugglers (Fig. 4.13).

Differences in GM and FA were also tested with Scholz and colleagues (2009) ROIs (30). I did not find any significant differences between the control group and the 30 minutes training group for GM density (Fig. 4.14). The RM-Anova revealed a trend for an interaction effect between scan and group ($F(1,53) = 3.654$, $p = 0.061$) but no main effects of scan ($F(1,53) = 0.078$, $p = 0.782$) or group ($F(1,53) = 0.030$, $p = 0.863$) for FA.

4.3.3 Summary Of Results

Results are summarised in the following table:

	Scan 1 to Scan 2		Scan 1 to Scan 3		Scan 2 to Scan 3	
	GM	FA	GM	FA	GM	FA
RM-ANOVA on 15 and 30 min juggling groups						
Main effect of Time			✓	✓ (trend)	✓	✓ (trend)
Time x Practice			✓ (trend)			
Time x Performance		✓ (trend)			✓	
Time x Practice x Performance	✓					
RM-ANOVA on 30 min group vs control group						
Group x Time						
RM-ANOVA on longitudinal analysis within 30 min group						
Main effect of Time				✓	✓	

Figure 4.15: Summary Of Results

4.4 Discussion

I set out to test directly for performance and practice effects on structural brain change. I found that performance outcome plays an important role in modulating positive structural brain change over certain timepoints in GM and WM (Fig.

4.10 and 4.7). I also found effects of practice, with higher amounts of practice resulting in higher GM density (Fig. 4.8c).

Participants with better performance had higher increases in GM density between scan 2 and 3 in dorsal parietal cortex and primary motor cortex (Fig. 4.10). These regions are relevant to the trained skill as they are involved in complex motor learning and eye-hand coordination (10). This finding contradicts previous reports where no correlations between GM structural change and performance were found (12; 14; 30). However, in the current study, the correlation related to structural brain changes was detected during the follow up period between scan 2 and 3, and might therefore be related to consolidation processes and long-term memory. As in the current study, previous studies have also reported that GM density continued to increase during a follow up period of weeks to months (16; 30), although those studies did not find any correlation between these late structural changes and performance.

An effect of amount of juggling training was also found in the right ventral parietal cortex, with GM decreases found in the 15 minutes group and increases in the 30 minutes group. Curiously, this result was mainly driven by unexpected decreases in the 15 minutes group (Fig. 4.8c). The right ventral parietal cortex has been implicated in multiple cognitive functions, particularly in visual-spatial attention orientation (9; 26), which could explain why this region may increase in the 30 minute group but does not help to understand why decreases are found here in the 15 minute group.

GM density also changed independently of performance and amount of training. Bilateral GM increases were found in widespread regions of visual and parietal cortex (Fig. 4.9), during the follow up period, in regions that coincide with

previous reports of GM change with juggling (12; 30). However, again, the increases detected in the current study were found at a later time-point compared to previous studies.

According to our results, GM change is driven by multiple aspects of training and performance, as well as, the training by itself. The change in the motor cortex and dorsal parietal cortex is modulated by performance outcome. Whereas some brain areas that overlap with regions reported in previous studies (12; 30), like the posterior parietal cortex and visual cortex, seem to increase their GM density in response to training regardless of training time or outcomes. On the other hand, GM increases in the right ventral parietal cortex appear to be modulated by the amount of practice itself.

Our findings were not limited to GM remodeling. During the training period, FA increases which correlated with performance were found in a posterior part of the corpus callosum, which connects both parietal and occipital cortical areas (Fig. 4.7). During the follow up period, FA progressively increased throughout time, independently of performance and amount of training in the corticospinal tract, motor and premotor areas of the corona radiata (Fig. 4.11).

The primary hypotheses concerned the effects of practice, time and performance outcomes on structural brain changes. However, as a control group was included in the design, I was also able to test for effects of juggling training per se, for comparison to previous studies of juggling training (6; 12; 14; 30). This is of particular interest as there has been recent skepticism over the validity of previous reports of structural brain changes with learning (35). In the main analysis I did find a main effect of time corresponding to increases in GM in brain areas that overlapped with those implicated in previous reports, but not for FA

(6; 12; 14; 30). However, the time-course was markedly different, with the increases in this data set being apparent during the follow up period only. When I tested for a group by time interaction, I did not find any significant effects. Furthermore, I was not able to reproduce previous results (30), even when the same statistical parameters were applied, or by using the previous study ROIs, although the data followed similar trends.

The differences between studies' results could be due to the employed training schedules and performance assessment. In the first study using the same behavioural manipulation, Draganski and colleagues (2004) asked participants to learn to juggle until 60 seconds of continuous juggling was achieved (12). The amount of daily practice was not fixed and participants that did not reach the criteria were not included in the study. Also, Draganski and colleagues (2004) did not find any evidence of increased GM 3 months later and participants were no longer able to juggle, while in the present work and in Scholz and colleagues (2009) study participants were still able to juggle 4 weeks later and GM continued to increase (12; 30). In an extension of Dranganski and colleagues (2004) study, participants were required to be able to juggle for at least 60 seconds at time point 2,and at least 120 seconds and 180 seconds at time point 3 and 4 (12; 14). The authors reported testing for correlations with behavior but did not find any.

The present study has used a similar methodology to Scholz and colleagues (2009) with a few differences (30). In the present work, I have direct evidence (through video recording) that participants practiced the designated amount of time. Although it is unlikely that participants in the previous study trained more than the designated amount, it is possible that they practiced less, which might have triggered distinct consolidation effects. Differences in participants'

performance between studies might account for differences in results. Approximately 50% of the participants in Scholz and colleagues' (2009) work were able to juggle for 60 seconds continuously, about the same amount as in the present study. However differences in other performance outcomes, such as learning rate which were not calculated in the previous study, cannot be excluded (30). The biggest difference between the two studies is the performance assessment. Due to video recording, I was also able to objectively assess daily performances and derive several measurements, such as an average value, that accounts for how much was learned. This more sensitive measure might account for the correlation between performance and GM and FA change not found in the previous studies. Also, by not imposing performance criteria there is a larger spread of performance, whereas participants in previous studies had very similar performance levels (with the exception of (30)).

4.4.1 Underlying Cellular Mechanisms

Because MRI does not allow us to differentiate the underlying cellular mechanisms, it is not straightforward to interpret the structural changes detected here. For example, the increases and decreases in GM density can be attributed to a variety of events, such as angiogenesis, synaptogenesis, spine formation or elimination, dendritic branching or pruning, gliogenesis and even neurogenesis. There is extensive evidence in the animal literature that all these events occur in response to experience and learning (1; 7; 15; 18; 19; 20; 28). There is less evidence from animal studies that WM undergoes neuroplastic changes with experience. On the other hand, FA is known to be modulated by a limited number of WM

features, such as myelin content, axon density and diameter, and fibre organization like crossing fibres. The few studies that examined WM plasticity in the adult brain in response to experience hint that any of these WM features can be modulated by experience. One study found that adult rats housed in a complex environment for 2 months had a higher number of unmyelinated axons and glial cells but not a higher number of myelinated axons in the corpus callosum (25). Another study, however, reported an increase in volume of myelinated fibers and myelin sheaths in the corpus callosum with environmental enrichment in middle-aged rats (39). There is also some evidence that myelin might be sensitive to learning. A higher expression of myelin basic protein (MBP) was found after spatial learning in rats in the corpus callosum (5).

Evidence from animal studies also raises some possible interpretations for some of the more counter-intuitive findings from the current study. For example, I found both decreases and increases in brain structural measures in task-relevant areas, some of which were modulated by practice or performance. Evidence from several animal and human studies suggests that the time-course of GM structural brain change might not be linear (22; 34). Therefore, different phases in learning might be associated with different cellular mechanisms that MRI is not able to distinguish. For instance, an initial learning phase might be associated with rapid and transient GM remodeling in functionally relevant brain areas, whereas more persistent GM changes might be associated with later learning phases, consolidation and long-term storage. Animal studies have provided evidence that this is indeed the case. Synaptogenesis and map reorganisation occur during the late phase in an animal model of motor learning, a reaching task, with synaptogenesis preceding map reorganisation, but not during the early learning phase (22).

Recent studies employing invasive in-vivo techniques have shown with the same model that new spines were formed within an hour of task acquisition and were formed in higher numbers during the early phase of learning compared to a later phase (36). The new spines formed during early acquisition were stabilized for a large period of time (120 days), while spines present before learning had a higher elimination rate compared to control animals (36). A different study, using a different model of motor learning, showed that performance correlated not only with new spine formation, but also with the elimination of existing spines (37). In summary these studies show that different cellular processes occur in different learning phases; that new spines that were relevant for task acquisition are stable and might be responsible for long-term memory; that learning destabilises older spines that are later eliminated; that not only spine formation but also spine elimination is important for coding learning and performance, thus circuit pruning is an essential aspect of neural plasticity. Due to the technique employed these studies are not able to track changes in the dendritic tree, however there is evidence from histological studies that dendritic trees can be remodeled by experience (for review see (24).

Although spine formation and elimination of old spines occur during early learning, with a small number of new spines persisting throughout life (37), it is not clear what happens at the structural level during later stages of learning. There is evidence that map reorganization, such as enlargement of the hand representation with motor skill learning (21), occurs in late stages preceded by synaptogenesis (22), but whether the map reorganization is sustained by synaptogenesis and functional changes alone, or by more complex structural reorganization is unknown. Also unclear is the time-course of non-neuronal events, like

angiogenesis or gliogenesis, which might underlie some of the MRI detected GM changes. Furthermore, late stages in most animal studies usually correspond to a few days or weeks. Learning stages will be intrinsically connected to the type and complexity of the task. A week can be enough to master a simple finger tapping sequence but it will take months or years to learn to play a complex piece of music on the piano. It seems likely that the more complex the skill, and the longer it takes to master, the greater the brain restructuring needs to be.

The recent animal studies highlight the fact that learning encompasses circuit refinement, thus including pruning as well as formation of new connections. This could explain why decreases in GM density were found in task relevant brain areas (Fig. 4.8a, b and Fig. 4.9a,c), and decreases and increases in GM in the parietal cortex were driven by differences in amounts of practice (Fig. 4.8c,d). Furthermore, I found an interaction between practice group, performance and time, where participants that perform better either increase or decrease their GM after learning in dorsolateral prefrontal cortex (DLPFC) depending on their practice group affiliation (Fig. 6). The DLPFC is involved in attention to action and in the integration of spatial and temporal information before specific motor actions are elicited, which is fundamental in a task like juggling (29). It is possible that, even though this is not expressed in performance differences, the two groups are at different stages of learning and thus the underlying cellular mechanisms do not overlap at that particular moment in time, or that distinct amounts of practice elicit different cellular mechanisms. It is conceivable that cellular mechanisms are dependent on practice levels, with lower amounts of practice eliciting more pruning mechanisms and relying mostly on previously established functional connections, and higher amounts of practice causing formation of new

connections.

4.4.2 Limitations

I did not find performance differences between naïve participants that learned to juggle for 15 minutes a day and participants that practiced twice as much, for the same period of time. It is possible that our measures are too crude to detect subtle differences between groups. I have only assessed the amount of sustained juggling each participant can perform on average in each training session as I have not quantified juggling speed or more importantly the quality of the movement. Even though our experimental assessment has limitations, I was still able to find a relationship between a derived performance outcome and brain structure change. This suggests that even though I was not able to differentiate between groups, our measurements still capture meaningful aspects of this task that correlate with brain structure change in areas known to be related to this skill.

Recently concerns have been raised about the current evidence from MRI studies supporting structural plasticity in the adult human brain (35). The authors mainly discuss flaws in the experimental design, statistical and analysis methods, reproducibility of the findings, and the relationship between the findings and behavioural performance (35). In this study I have addressed some of these concerns by using unbiased mid-space registration (as in the previous study by (30)), robust statistical analysis, and have found a correlation between GM and FA increases and behavioural performance. I was unable to completely reproduce previous results using an ROI based analysis, although data trends were similar. By using similar statistical approaches (within subject analysis) to pre-

vious studies, I found a GM increase in a later time point as previously described albeit in overlapping brain areas, and a progressive increase in FA in different WM tracts but still in areas that are related to motor control.

4.5 Conclusions

I found that both performance outcome and amount of practice play an important role in structural brain change in GM and WM. Previous MRI studies have painted a simplistic view of plasticity in relation to learning, the current study offers more complex findings that are consistent with animal reports on the topic. Although I can only speculate about the underlying cellular mechanisms, this work offers further evidence that MRI can be used to detect changes in structure that are related to specific learning variables.

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Chapter 5

Effects Of Long-Term Learning On Resting-State Networks And On GABA Concentration

The functional meaning of the resting networks is unknown although some have suggested a role in memory consolidation, rehearsal or future preparation (5). Recent evidence suggests that resting-state networks change as a result of motor learning (1; 14). Modulation of gamma-amino butyric acid (GABA), the major inhibitory neurotransmitter, by short-term learning has been previously characterized with MR spectroscopy. GABA levels are known to decrease with short-term motor learning (8). However, the effects of long-term learning on this neurotransmitter are unknown. This chapter is dedicated to the effects of long-term motor learning on resting-state networks strength and on neurotransmitter concentration.

5.1 Introduction

Motor skill learning, like riding a bicycle, is part of everyday life and it has been shown to induce structural and functional plasticity in the brain (e.g. (18; 19). While motor learning is known to involve several brain areas that work as a circuit (6; 11), such as M1, pre-SMA, SMA, striatum, cerebellum and parietal cortex, depending on the task and learning acquisition phase, most fMRI studies have focused on the effects of motor learning on discrete brain activation and not how brain areas interact with each other as a network.

One way of assessing network level changes in brain function is by investigating resting brain activity. The resting brain exhibits spontaneous activity that has been noted to form distinct temporally correlated patterns of functionally related brain regions. These patterns have been described as resting-state networks (RSN) and it has been found that these spatially resemble task-related activation (20). Studies have reported that functional connectivity is constrained by structural connectivity (12), suggesting that resting-state networks reflect brain areas that are functionally but also structurally interconnected. Variability of strength within a network is also modulated by disease, genetics and cognition (1; 7).

Changes in RSN strength with learning can be considered as an indirect marker for synaptic plasticity that has occurred during learning. Where synapses have been strengthened or increased in number one might expect increases in the strength of corresponding RSNs. Where there has been a net decrease in synaptic strength and number one might predict a decrease in RSN strength. Recently, RSNs have been studied in the context of motor-learning. One study found increases in strength in the fronto-parietal network and the cerebellum network

after 11 minutes of learning (1). An increase in right postcentral gyrus (PCG) and in the supramarginal gyrus areas of the resting state motor network were reported in the first 2 weeks of a 4 week period of sequence learning, followed by a decrease in strength in the same areas in the last 2 weeks (14). No correlations with performance were found in any of these studies.

In this chapter I have use resting-state fMRI to study how brain areas relate to each other and how experience affects the brain circuits involved. For a first approach I have used a data driven method and have focused on the motor resting state network and how the strength of the network is modulated by learning and different amounts of juggling practice and performance.

I have also investigated the effects of long-term motor learning on GABA levels, using MR spectroscopy (MRS), a non-invasive imaging technique used to measure the concentration of neurochemicals in a particular brain region. GABA is known to be important in modulating learning-related synaptic changes (9; 23). Previous studies have used MRS to show that GABA levels decrease with short-term motor learning, like sequence learning (8). However, there are no reports on modulation of GABA with long-term motor learning.

This chapter will also explore the relation between neurotransmitters and resting-state strength. It is not known if resting-state networks might reflect excitatory and/or inhibitory neurotransmitters levels, but previous work in our lab has provided evidence that motor RSN strength might reflect GABA levels, as individuals with higher motor RSN strength have lower GABA concentrations within M1 (22). In the context of the current study, I therefore hypothesised that GABA concentration change in response to learning is negatively correlated with the change in the strength of the motor resting-state network.

5.2 Methods

5.2.1 Participants

A full description of the participants, behavioural assessment and behavioural results is provided in chapter 3 (3.2.1 Participants; 3.2.2 Behavioural assessment; 3.3.1 Behavioural results). In summary, there were no significant differences for any of the behavioural measures between groups, although the 30 minute group performed slightly better than the 15 minute group on average.

5.2.2 MRI Acquisition

Whole-brain functional imaging was performed using a gradient echo EPI sequence while participants were at rest with eyes open (TR = 2,000 ms, TE = 28 ms, flip angle = 89, field of view = 224 mm, voxel dimension = 3 x 3 x 3.5 mm, acquisition time = 6 min 4 s). Due to technical problems resting state fMRI data were acquired for only 20 participants in the 30 minutes group, 16 in the 15 minutes group and 19 in the control group.

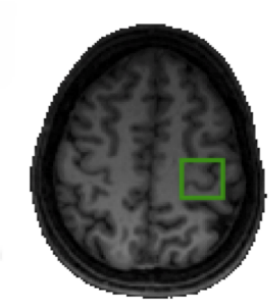

Figure 5.1: Placement of the MRS voxel over the hand motor representation area.

To assess metabolite concentration in the motor representation of the hand area, the Spin-Echo full intensity acquired localized (SPECIAL) spectroscopy technique was used (TR = 3000 ms; TE = 8.5 ms; flip angel = 90; voxel size = 20 x 20 x 20 mm3; total scan time = 9:48 minutes) (15). This sequence has the advantage of using only one refocusing pulse,

which shortens the minimum achievable TE and thus allows short TEs comparable to those attainable by STEAM (15). The sagittal and coronal T1-weighted scout images acquired and the axial T1-weighted anatomical image were used to place a 2 x 2 x 2 cm voxel manually over the left precentral knob, a landmark previously described to identify the hand motor representation in the human brain (24) (Fig. 5.1).

5.2.3 MRI Analysis

Data analysis was carried out using FSL tools (www.fmrib.ox.ac.uk/fsl). More specifically, fMRI analysis was carried out using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) (3). MELODIC is used to identify components containing brain areas with time-courses correlated with each other that are independent of other components.

Preprocessing included correction for head motion, brain extraction, spatial smoothing using a Gaussian kernel of full-width at half-maximum (FWHM) of 6 mm, and high-pass temporal filtering equivalent to 150 s (0.007 Hz). fMRI volumes were registered to the individual's structural scan using boundary-based registration (BBR) and then to standard space with FMRIB's Nonlinear Image Registration Tool (FNIRT) (2).

Preprocessed functional data containing 180 time points for each subject were temporally concatenated across subjects to create a single 4D data set. The concatenated files were used to identify previously described resting-state networks with Independent Component Analysis (ICA) (unconstrained) at a group level, for all time-points. The previously described canonical resting-state networks

were identified (motor, executive, auditory, default mode, left dorsal stream, right dorsal stream, medial visual and lateral visual) (4) (Fig. 5.2). A dual-regression approach was used to identify, within each subject's fMRI data set, subject-specific temporal dynamics and associated spatial maps for each resting-state network of interest (7):

1. Group-ICA maps were used as spatial regressors to find the time-courses for each subject associated with each group-level component;

2. The subjects' time-courses were used as regressors to identify subject specific spatial maps that correspond to the group analysis components;

3. Then the ICA output thresholded masks for each component of interest were used to calculate the mean value of the network for each participant's time-points. This mean value is interpreted as the mean strength of the network i.e. the higher the value the more correlated are the areas within the network.

GABA concentration was calculated automatically with LCModel (17). All metabolite concentrations are given as a ratio to creatine. Metabolite concentration with a %SD (Standard Deviation)>15%, a measure of reliability of the LCModel fit, were excluded from the analysis (2 in the control group, 2 in the low intensity group and 2 in the high intensity group).The GABA concentrations were corrected for the proportion of gray matter volume within the voxel [multiplied by [GM]/([GM] + [WM] + [CSF])] and creatine was corrected for the proportion of total brain tissue volume within the voxel [multiplied by ([GM] + [WM])/([GM] + [WM] + [CSF])] (21). FMRIB's Automated Segmentation Tool (FAST), part of the FMRIB software library (www.fmrib.ox.ac.uk/fsl), was used to calculate the relative quantities of grey matter and white matter within the voxel.

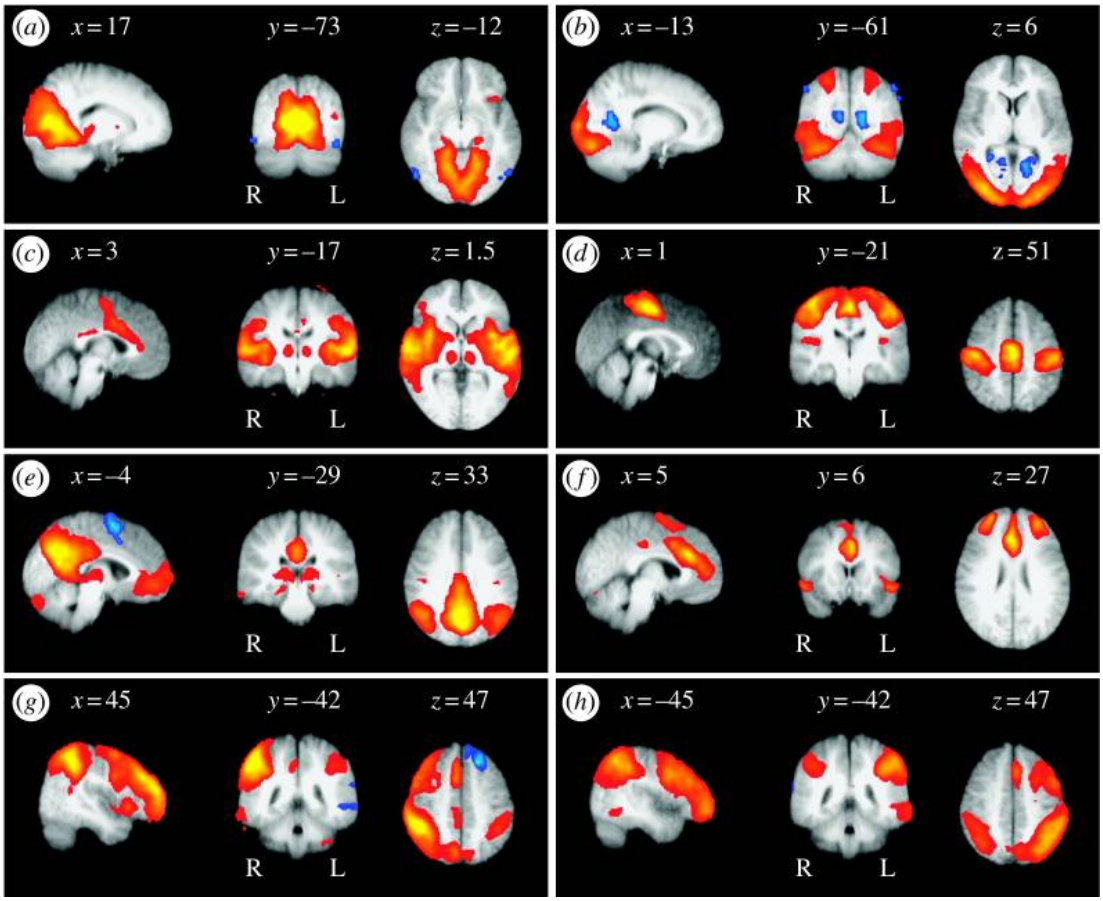

Figure 5.2: Resting State Networks (a) Medial visual cortical areas (b) Lateral visual cortical areas (c) Auditory system (d) Sensorymotorsystem (e) Visuo-spatial system (f) Executive control (g,h) Dorsal visual stream. Taken from (3)

5.2.4 Statistical Analysis

SPSS software was used to test for differences in resting-state network strength and neurotransmitter concentration throughout time and between the experimental groups.

Differences between groups (15 minute, 30 minute, control), time-points (1st scan and 2nd scan) and RSNs (motor, default mode) were explored with RM - Anova. The motor RSN was considered the network of interest and the default

mode RSN was used as a control. Change in RSN strength was quantified with learning as percent change from scan 1 to scan 2. Differences in percent change between groups was tested using a one-way Anova. Correlations between RSN strength change and performance change were also tested using Pearson's R. Normality was tested with Shapiro-Wilk and, when significant, Spearman tests were used. Differences in correlation strength were assessed using Fisher's r to z.

RM - Anova was used to test for differences between group and time-point for GABA concentration. Finally, it was tested if GABA change was negatively correlated with motor RSN strength change ($p < 0.05$, 1-tail as the direction of this relationship was predicted a priori).

5.3 Results

RM- Anova revealed a significant main effect of network ($F(1, 53) = 921.158$, $p < 0.001$), a trend for an interaction effect between time and group ($F(2, 53) = 3.101$, $p = 0.053$), and a group x time x network interaction ($F(2, 53) = 9.182$, $p < 0.001$). Long-term learning altered resting brain activity in a network specific manner. No effects or interactions were found for RM-Anova for the default mode RSN.

To further investigate this result a RM-Anova was run for each RSN. For the motor RSN, a significant interaction between time and group was found ($F(2, 53) = 9.176$, $p < 0.001$) but no significant main effect of time ($F(2, 53) = 0.105$, $p = 0.747$) or group ($F(2, 53) = 0.880$, $p < 0.421$) (Fig. 5.3a). I compared percent change in RSN strength from scan 1 to scan 2 between groups and found a significant effect of group ($F(2, 53) = 9.176$, $p < 0.001$) (Fig. 5.3c). The

post-hoc test revealed a significant difference between the 15 minutes and the 30 minutes group ($p < 0.001$, corrected for multiple comparisons with Tukey) and a trend for a significant difference between the 30 minutes and the control group ($p < 0.06$, corrected for multiple comparisons with Tukey) (Fig. 5.3c). The above results show that the motor RSN strength decreases with learning in the 30 minutes group and increases in the 15 minutes group (Fig. 5.3c).

There was a difference between groups for the motor network at baseline ($F(2, 53) = 4.160$, $p < 0.05$) but not for the default mode network ($F(2, 53) = 0.542$, $p = 0.585$). The post-hoc test revealed a signifcant difference at baseline between the 30 minute group and the 15 minute group ($p < 0.05$, corrected for multiple comparisons with Tukey). Differences within group between time-points were also tested using a one-way RM-Anova. An effect of time was found for the 30 minutes group ($F(2, 38) = 3.835$, $p < 0.05$). A paired t-test revealed a significant decrease in motor RSN strength between time point 1 and 2 ($t(19) = 2.787$, $p < 0.05$, 2-tail) (Fig. 5.3a). There was also an effect of time for the 15 minutes group ($F(2, 30) = 5.442$, $p < 0.01$). The paired t-test revealed significant increase in stregth between time point 1 and 2 ($t(15) = 3.283$, $p < 0.01$, 2-tail) and 1 and 3 ($t(15) = 2.347$, $p < 0.05$, 2-tail) (Fig. 5.3a). There was no differences between time point 1 and 2 for the control group ($t(19) = 0.243$, $p = 0.881$, 2-tail) (Fig. 5.3a). In summary, although there was differences at baseline between groups, the within group analysis also shows differences between scans for both experimental groups, while the control does not change with learning.

Additionally, the change in motor RSN with learning was found to be related to performance level. There was a significant negative correlation between the motor RSN decrease in the 30 minutes group and learning rate (Pearson r =

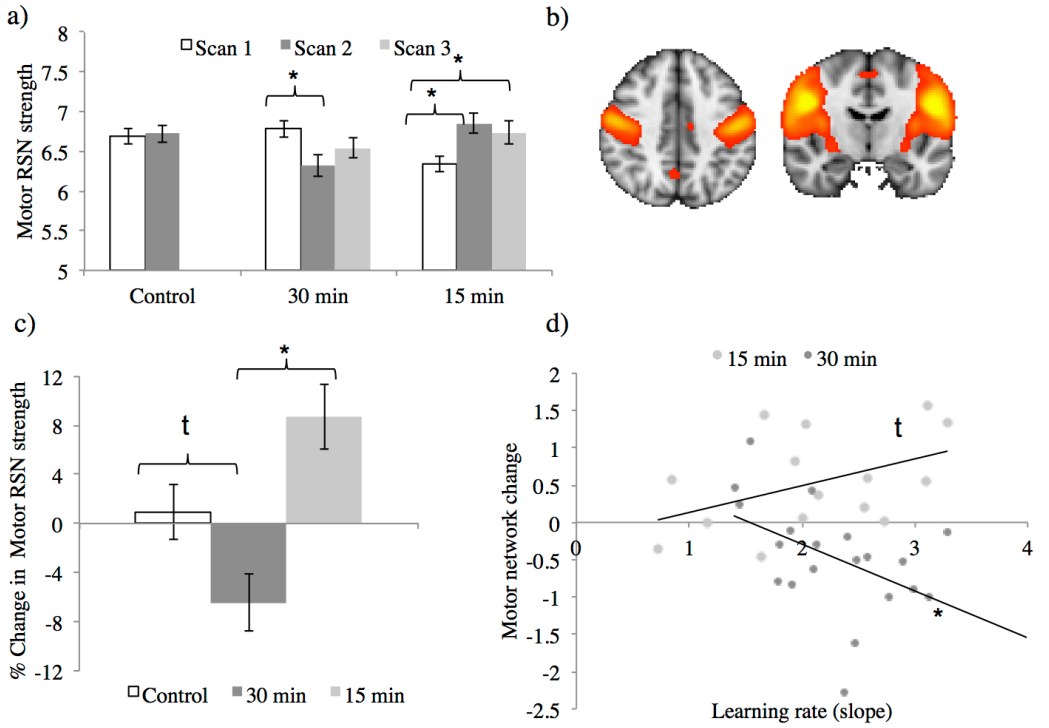

Figure 5.3: a) Motor resting-state network strength throughout the experiment. There is significant decrease between scan 1 and scan 2 for the 30 min group (* $t(19) = 2.787$, $p < 0.05$, 2-tail). The 15 min group shows significant increases between scan 1 and 2 (* $t(15) = 3.283$, $p < 0.01$, 2-tail) and 1 and 3 (* $t(15) = 2.347$, $p < 0.05$, 2-tail). b) Motor resting-state network defined by ICA analysis c) Significant interaction between group and time ($F(2, 53) = 9.176$, $p < 0.001$). 30 minutes motor RSN strength change is significantly different from the 15 minutes change after learning (* $p < 0.001$). There is a trend towards significance between the 30 minutes group change and the control group (t $p = 0.06$). d) Significant negative correlation between learning rate and motor RSN strength decrease in the 30 minutes group (* $p < 0.05$, 2-tail). There is a trend for the correlation between the 15 minutes group increase in motor RSN strength and learning rate (t $p = 0.097$, 2-tail). The two correlations are significantly different from each other ($z = -2.68$, $p < 0.01$).

- 0.471; p <0.05, 2-tail), and a trend towards a positive correlation in the 15 minutes group (Pearson r = 0.444, p = 0.097, 2-tail) (Fig. 5.3d). The difference between the two correlations was significant (z = -2.68, p <0.01) (Fig. 5.3d).

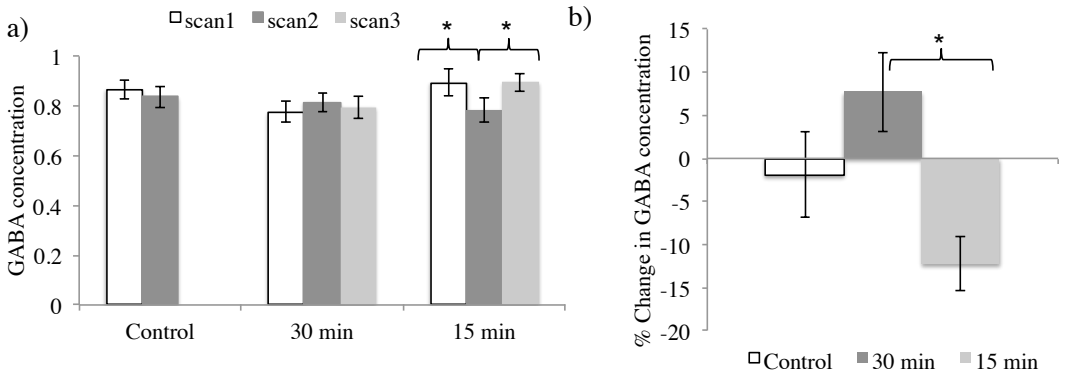

Figure 5.4: a) GABA concentration throughout time. There is a significant decrease between scan 1 and 2 (* t(13) = 3.899 , p <0.01, 2-tail) and a significant increase between scan 2 and 3 (* t(13) = 3.075 , p <0.01, 2-tail) for the 15 min group. b) Significant interaction between group and time (* F(2, 46) = 4.773 , p <0.05). The GABA concentration in the 15 minutes group decreases significantly after learning compared to the 30 minutes group (* p <0.01).

There was no difference between groups for GABA at baseline (F(2, 46) = 2.149, p = 0.128). The RM - Anova on GABA concentration values revealed an interaction effect between time and group (F(2, 46) = 4.655, p <0.05) but no main effect of time (F(2, 46) = 2.583, p = 0.115) or group (F(2, 46) = 0.597, p = 0.555) (Fig. 5.4a). Percent change in GABA was compared from scan 1 to scan 2 between groups and a significant effect of group was found (F(2, 46) = 4.773, p <0.05) (Fig. 5.4b).The post hoc test showed a significant difference between the 15 minutes group and the 30 minutes group (p <0.01, corrected for multiple comparisons with Tukey) (Fig. 5.4b). Differences within group between time-points were also tested using a one-way RM-Anova. There was no effect of time for the 30 minutes group (F(2, 34) = 1.067, p = 0.355) (Fig. 5.4a). There

was an effect of time for the 15 minutes group ($F(2, 26) = 7.446$, $p < 0.01$) (Fig. 5.4a). The paired t-test revealed significant differences between time point 1 and 2 ($t(13) = 3.899$, $p < 0.01$, 2-tail) and 2 and 3 ($t(13) = 3.075$, $p < 0.01$, 2-tail) (Fig. 5.4a). Differences between time point 1 and 2 for the control group were not found ($t(16) = 0.695$, $p = 0.497$, 2-tail) (Fig. 5.4a). In summary, GABA significantly decreases after learning and increases after learning is ceased.

Across all jugglers, GABA concentration change was negativelly correlated with motor RSN strength change (Shapiro-Wilk normality test was significant, Spearman r = - 0.326, $p < 0.05$, 1-tail). As GABA decreases motor RSN strength increases and vice-versa (Fig. 5.5).

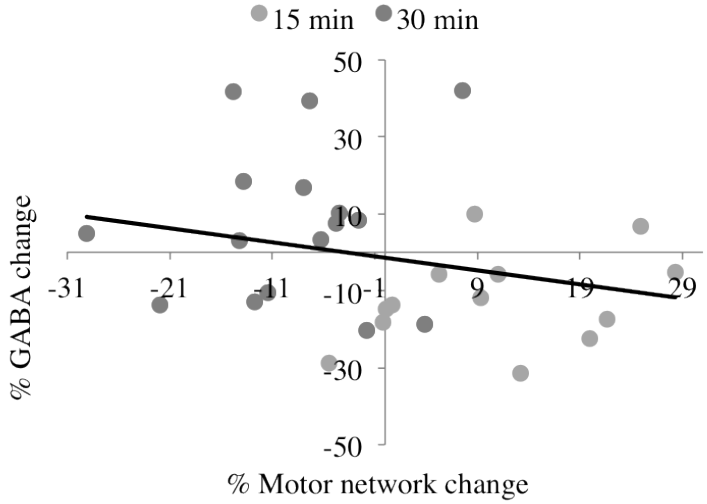

Figure 5.5: GABA concentration change is negatively correlated with motor RSN change after learning (* Spearman r = - 0.326, $p < 0.05$, 1-tail).

5.4 Discussion

Long-term motor learning altered resting brain activity specifically in the motor network. Furthermore, the amount of juggling training had distinct effects on the motor RSN strength with decreases found in the 30 minutes group and increases in the 15 minutes group. The amount of change in each group was related to performance. The differences found between groups in resting-state change with learning suggest different learning stages are present in each group.

The participants that practiced for 15 minutes have increased motor RSN strength, which is compatible with previous literature where it was reported that 11 minutes of visuo-motor learning increased component strength in RSNs related to the task (1). Another study of long-term finger sequence learning found an increase in correlation between M1 and S1 structures in the first 2 weeks of learning and a decrease in correlation between week 2 and week 4 (14). This suggests that the 15 minutes group might be at an earlier learning stage than the 30 minutes group, and might be relying (mostly) on increased correlated activity between brain areas that are part of cortical circuits that support early motor learning like the M1 and S1 (16). This is supported by the drop in GABA found only in the 15 minutes group which has been previously shown to be associated with early stages of learning within a single session of learning (8). The current study provides the first evidence that a GABA decrease is found in long-term motor learning in humans. It has been previously shown in animal studies that GABA is important for long-term plasticity mechanisms in the hippocampus and neocortex (10). GABA decreases by means of LTD-like plasticity have been reported in the neocortex including the sensorimotor cortex in response to learning

(9; 23).

On the other hand, the 30 minutes group could be relying more on subcortical circuits that include the striatum, known to be involved in later stages of learning and automatisation of the task. This might cause specific somatomotor cortical regions to become more correlated with subcortical areas and thus become less correlated with brain regions within the motor network, thus decreasing the motor network strength.

If the two groups are at different stages in learning then we might expect this to be reflected by differences in performance measures. However, no differences in performance were found between groups (see Chapter 4). Our measures might be too crude to assess automatisation or reveal behavioural differences between groups' as the quality of the movement or juggling speed was not assessed. Nevertheless, a correlation between performance and resting-state change was found in the 30 minutes group with participants exhibiting higher performance levels having the greater decreases in motor RSN strength. Likewise, a trend was found for a correlation in the opposite direction in the 15 minutes group where better performance was associated with greater increases in motor RSN strength. This shows that the two different processes that are occurring in each group are behaviourally meaningful. Previous resting-state studies have not reported behavioural correlations with resting-state changes in motor learning. However, a large number of studies using task-based fMRI have reported that functional activation is often correlated with performance in the task, and have found correlations with early stages and late stages of learning. For instance in one study performance accuracy was correlated with signal change in the areas activated during the early learning phase, while performance speed was negatively corre-

lated with signal change in areas activated in the late phase (13).

Finally a change in motor RSN strength was correlated with change in M1 GABA concentration. This offers insight into the cellular mechanisms that may underlie the change in resting-state networks with motor learning. According to our results, change in concentrations of GABA as a result of learning is reflected in a change in motor RSN strength, thus suggesting that GABA is not only important for motor learning, as previously demonstrated by animal studies, but is also linked to network level activity fluctuation change as measured by resting-state in response to motor learning. This offers further evidence that GABAergic networks are involved in within network functional connectivity as measured by resting-state fMRI (22).

5.5 Conclusions

Motor learning changes resting-state network in a specific way. In early stages motor learning seems to increase functional connectivity of brain areas involved in the task, while in later stages refinement of the circuit may occur, resulting in disruption of the network. GABA is downregulated for a longer period of time than previously thought. GABA concentration decreases with motor learning might be necessary in highly complex tasks at least during the early learning stages as suggested by our data.

Our results suggest that changes in resting-state networks might represent an indirect marker for synaptic plasticity, with increases in synaptic strength resulting in stronger RSNs and the opposite resulting in weaker networks. According to our results GABAergic neurons might underlie the strengthening and weakening

of the motor RSN after learning.

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Chapter 6

Animal Model Of White Matter Plasticity With Long-Term Motor Learning

In an attempt to understand mechanistically the structural changes seen in human studies I have chosen to study experience-dependent structural change in rats. For this purpose I have picked a motor learning paradigm that has been well described behaviourally (25) and for which structural plasticity in the grey matter has been extensively studied histologically (12). This study has two main aims: to investigate experience-dependent white matter (WM) changes with motor skill learning in the rat and to compare imaging and histology in order to identify the cellular mechanisms responsible for WM plasticity.

6.1 Introduction

Animal research has shown that the brain is shaped by experience and learning. This was first suggested by Hebb in 1949 and later experimentally confirmed by Rosenzweig who found increased brain weight in rats exposed to enriched environments (8; 22). Learning induces a variety of cellular events like dendritic sprouting, growth and elimination of spines, synaptogenesis, gliogenesis, angiogenesis and neurogenesis, among others (i.e. (2; 3; 5; 14; 26).

Although there is extensive evidence for grey matter (GM) structural plasticity with learning, evidence for white matter (WM) plasticity in the adult brain in response to experience is more limited. In human studies, learning to juggle is associated with increases in fractional anisotropy (FA), in an WM tract co-localised with a brain area known to be involved in eye-hand coordination (23). However, because FA is modulated by several aspects of WM structure, like myelination, axon diameter, axon density and fibre organisation, it is not possible to pinpoint what structural event underlies a change in FA. Animal studies provide a number of candidate events: for example, recently a few studies have reported that exposure to environmental enrichment during adulthood results in a higher number of unmyelinated and myelinated axons, and glial cells (18; 27). An attempt to clarify which cellular events underlie changes in MRI measurements with learning used an animal model and a spatial memory task and found increases in FA in the corpus callosum as well as increases in myelin expression as measured by immunohistochemistry in the rat (6), though direct correlations between the two were not reported. However, the hippocampus is one of the few brain structures that possess the capacity for adult neurogenesis in response to learning (7). Thus

WM changes here could be driven by new neurons establishing new efferent connections. Cortical neurogenesis has not been established and thus studying tasks that induce cortical plasticity allows for assessment of WM plasticity without the generation of new neurons.

I have elected to use a reaching task, a well-described paradigm of motor learning, known to induce GM structural and functional plasticity like synaptogenesis and map reorganization within the motor cortex (10; 11; 12). In rats, learning a novel skilled reaching task is associated with well-characterised functional reorganization of cortical motor maps, including expanded representation of the trained limbs (12; 13). This functional remapping is accompanied by a variety of structural changes, including synaptogenesis, increase in spine formation and glial changes (12; 26).

Furthermore, the skilled reaching behaviour shows very similar motor components in humans and in rats and thus can be considered a homologous behavior in rats and humans (9). The skilled reaching task is also sensitive to damage to the motor system and can be used to assess function recovery and compensation mechanisms (15). There are differences and similarities in the somatomotor organization between primates and rodents. For instance, the sensory and motor areas are anatomically distinct in primates but overlap in rodents. However, lesions of the primary motor of both primates and rodents produce consistent symptoms that are similar across species as diverse as rats and humans since the organization of cortical and basal-ganglia circuits that control movement are quite similar across mammalian species (16). Thus this rat reaching task can be considered behaviourally and anatomically homologous to human reaching tasks, being widely used for preclinical studies of human movement disorders and injury

models (1; 17; 20). Two conditions can be studied using this task: skilled and unskilled reaching, allowing for separation of effects due to learning and effects due to increased movement of the paw. Thus, this animal model of long-term motor learning has the advantage of well-documented microstructural changes and an appropriate control condition. Here, I investigated WM plasticity associated with learning of a novel motor skill in rats by combining MRI and immunohistochemistry. MRI has the advantage of whole brain coverage, thus offering insight as to where WM plasticity might be occurring and providing guidance to histology. On the other hand, histology offers the possibility to validate MRI measurements and to shed light on the cellular events that underlie the measures obtained in human neuroimaging studies of motor learning.

6.2 Methods

The study was approved by the U. K. Home Office and all procedures were performed according to the project and personal licenses held by the experimenters. 3 separate batches of 24 (total = 72), 4 - 5 months old, male Lister hooded rats (250 - 450 g) (Harlan, Bicester, UK) were housed in groups of 3 in standard laboratory conditions under a 12-h light/12-h dark cycle at 20 C temperature and 40% - 70% humidity. Each cage contained an animal corresponding to each experimental condition (n per group condition = 8 rats x 3 batches) (randomly assigned): skilled reach (SR), unskilled reach (UR) and untrained control condition (UC). The cages did not contain any environmental enrichment in addition to standard bedding and nesting material. All animals were given appropriate time to acclimatise after delivery and had ad libitum access to food and water.

After this period they were handled daily for several days before the start of the training sessions, while also being exposed to the sucrose pellets in their home cages in addition to the previous ad libitum conditions. All behavioural training and testing was performed during the light phase. All animals were then food-deprived to 85% - 90% of their free-feeding weight a week before the start of the behavioural training. The target weight was calculated as 85% - 90% of the average weight measured on 3 consecutive days. Weight was closely monitored throughout the experiment to ensure it did not fall below 85% of the original weight.

6.2.1 Behavioural Training

Overall, the behavioural paradigm followed previously published guidelines on a single-pellet reaching task, which has been widely characterised (25).

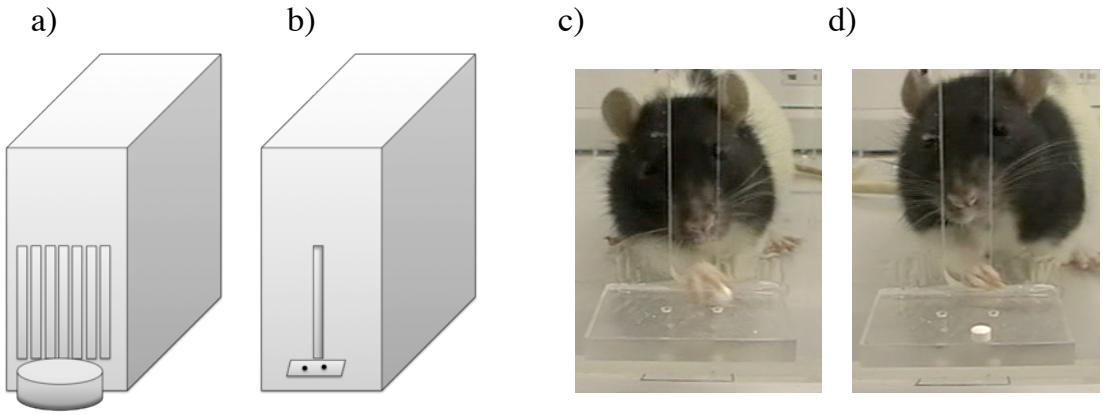

Figure 6.1: Experimental set up. a) Pre-training cage b) Training cage c) Skilled animal with pellet within reach d) Unskilled animal with pellet out of reach.

6.2.1.1 Pre-Training

Prior to behavioural training all animals underwent pre-training sessions of 30 min/day for 3 days. Animals were placed in a cage (17x38x40cm) with several 1 cm wide and 10 cm high openings on the front of the cage (Fig. 6.1a). A container filled with sucrose pellets (45 mg) (Bioserv, Frenchtown, NU, USA) was placed in front of the cage within easy reach. The rats were trained to reach into the container through the cage openings and retrieve pellets. The pre-training stopped when the animal had retrieved 10 pellets or 30 minutes had passed. The animals were permitted to use either limb. The preferred limb was noted for each animal and only this limb was reinforced in the next training phase (in both SR and UR groups).

6.2.1.2 Training

After three days of pre-training, only the SR and UR animals were further trained on the single-pellet-reaching task for 15 minutes daily for 11 consecutive days. The training sessions were conducted in a cage with just one opening and a tray with two small indentations centered on the edges of the opening, 1.3 cm away from the inside wall of the cage (Fig. 6.1b). The indentations indicate where the pellets should be put on the tray, contralateral to preferred limb. Animals were trained for 11 days, 15 minutes each day, to reach through the opening and retrieve a food pellet. On the first 3 days animals' behaviour was shaped in order for them to reach through the opening and retrieve the single pellet from its fixed location. First, the pellet was put on the tray beyond the opening within easy reach, i.e., animals only had to pick it up from the table. Subsequently the pellet

was put further away, with the purpose of inciting the animal to reach through the gap. The remaining training sessions were performed differently according to the experimental group the animals were in.

6.2.1.3 Skilled Reaching Condition

For this group the pellet was placed in the indentation within reach (Fig. 6.1c). The animal then had 5 attempts to successfully grasp the pellet, which together constitute one trial. Successful reaches were scored when an animal grasped the pellet (only when placed in the indentation) and guided it to its mouth without dropping it. When the rat made a successful reach in 1 out of 5 reaching attempts a pellet was dropped into the back of the cage. If after 5 consecutive reaches the animal failed to retrieve the pellet it was still prompted to go to the back of the cage but a pellet was not dropped. This was done to ensure that the rat left the opening after a successful reach or 5 consecutive failed attempts for the pellet to be replaced in the indentation.

6.2.1.4 Unskilled Reaching Condition

To control for increased motor activity of the forelimbs, UR animals were placed in the same conditions as the SR group but they were never allowed to grasp for the pellets and hence did not acquire the respective movement sequence of wrist and digits. To achieve this, after the animals started to reach through the opening in the cage, the pellet was placed out of reach, preventing the animals from developing reaching/grasping skills (Fig. 6.1d). To keep the animals motivated a pellet was dropped inside the cage periodically (every 5 attempts). On the last day of the experiment the UR group was tested for reaching accuracy by putting

the pellet within reach.

6.2.1.5 Untrained Control Condition

The control animals did not receive any training after completion of the 3 days of pre- training. However, they were handled daily and their weight monitored. In addition, they received a corresponding amount of sucrose pellets into their home cage in order to control for sucrose intake.

6.2.2 Behavioural Measures And Statistical Analysis

The accuracy score was calculated as: number of successful retrievals / total number of reaches. Number of total reaches corresponds to the sum of all reaches per day. Rate of learning was calculated as the slope of a fitted logarithmic model to the learning curve of each individual animal (Fig.6.2). A repeated measured Anova (RM - Anova) was used to investigate improvement in accuracy scores over time in the SR group. A separate RM Anova was run to investigate difference in total number of reaches between SR and UR. When Mauchly's test of sphericity is statistically significant, Greenhouse-Geisser F-test is used and the respective degrees of freedom are reported. To confirm the absence of skill learning in the UR group, accuracy scores on the final day of testing, when both groups were tested, were

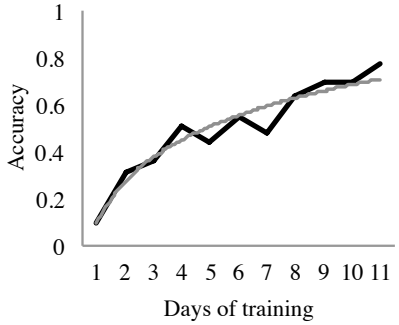

Figure 6.2: Typical animal learning curve (in black) and fitted logarithm curve (in grey) from which the slope was used to estimate learning rate.

compared with the SR group using a student's t-test. Accuracy and learning rate scores were correlated with histological and imaging measures as described below.

6.2.3 Perfusion And Brain Preparation

After training was completed, all animals were injected with 200 mg/kg sodium pentobarbital intraperitoneally and once pedal and corneal reflexes were negative, were perfused transcardially with saline solution (20 ml/minute) for 5 minutes and newly prepared PFA (4%). The second batch of animals was perfused with PFA (4%) with 0.1 M glutaraldehyde for 5 minutes (20ml/minute) for future electron microscopy (EM) analysis. Following perfusion, the brains were removed from the skull and stored in PFA until further processing. For DTI acquisition, in order to keep the brains in a fixed position, they were placed into falcon tubes (50ml) in pairs, one brain above the other, and embedded in 2% agarose gel (Sigma). The brains were aligned to each other along the posterior anterior axis. Air bubbles and hairs were removed before thickening of the agarose gel in order to prevent magnetic field distortions.

6.2.4 MRI Acquisition

Ex-vivo brains were scanned overnight using a multi-channel 7 Tesla MRI scanner (Varian Inc., Churchill Hospital, Oxford). DTI scanning parameters were as follows: 4 diffusion averages, 30 diffusion encoding directions per average, Spin-echo multi-slice (SEMS) diffusion sequence, 8 images with no diffusion weighting, 40 slices, slice thickness 0.5 mm, field of view 25 x 50 mm, matrix size 96 x 192, resolution 0.26 x 0.26 mm, b = 2000 s/mm^2.

6.2.5 MR Processing And Statistical Analysis

The data were pre-processed according to standard procedures. A modified version of Tract Based Spatial Statistics (TBSS) (24) was applied to the pre-processed data. FA images from animals that used their left paw for the motor task were flipped about the x-axis so that the hemisphere contralateral to the used limb was consistently aligned across animals. The FA images were first aligned with linear transformation (6 DOF) to an existing rat brain template. The aligned images were then averaged to create a study specific template. Next, the original FA images were aligned to the 6 DOF study specific templates with a linear transformation (12 DOF) and averaged. Again, original FA maps were aligned with a linear transformation to the 12 DOF study specific template and averaged to generate the final study template. All FA maps were aligned with linear and non-linear transformations to the final study template and averaged to generate the mean FA image, from which the white matter skeleton was extracted. The skeleton was thresholded at an FA value of 0.36 to contain only the major tracts. Finally the FA values of the tract centres (i.e., maximum FA values) were projected onto the skeleton for each rat brain and fed into statistical analysis. Permutation testing with a cluster threshold of t >2 and 5000 permutations was used to determine corrected p-values. We tested for differences between the SR group and the control groups (URT and CC conditions), and for whole brain correlations between FA and learning rate within the SR group.

6.2.6 Histology

Rat brain tissue was sectioned into blocks containing the regions of interest (ROIs), and embedded in paraffin blocks. Blocks were sectioned at 6 micrometers using a rotary microtome (Leica), then placed on a heated water bath to reduce creases. The sections were then mounted onto glass slides and dried. The tissue sections were dewaxed with 3 x 5 minutes immersion in histoclear and then rehydrated in decreasing ethanol concentrations (100% to 70%) and placed in distilled water. The sections were subsequently immersed in a solution of 10% Hydrogen Peroxide (25 ml Hydrogen Peroxide (H2O2) in 225 ml phosphate buffered saline (PBS), 0.1M, pH = 7.4) for 30 minutes in order to block any endogenous peroxidase activity. After washing the sections in tap and distilled water, the sections were placed in citrate buffer (pH = 6) for the duration of the antigen retrieval. For the antigen retrieval, the sections were heated in a microwave on full power for 5 minutes + 5 minutes cooling, repeated twice. After the treatment, the slides were mounted onto coverplates and into sequenzas (Shandon Sequenza, Thermo Electron Corporation) and washed twice with Tris-Buffered Saline Tween 20 (TBS/T, pH 7.6, DAKO REAL EnVision Kit detection system, K5007). The primary antibody was mixed with TBS/T according to the preferred dilution, proteolipid protein (PLP) 1/500 (Serotec), myelin basic protein (MBP) 1/500 (Millipore), pipetted onto the coverplates (120 l/ coverplate) and incubated for 1 hour at room temperature. After washing the sections twice with TBS/T, the secondary antibody (120 l of HRP Rabbit/Mouse serum (DAKO REAL EnVision Kit detection system, K5007) was added and allowed to incubate for 40 minutes at room temperature. After washing again twice with

TBS/T, 3,3'-diaminobenzidine (DAB) (mixed with the substrate buffer (volumes according to DAKO EnVision protocol)) was added for 5 minutes. In order to stop the reaction, the sections were again washed twice with TBS/T. Sections were not counterstained and were subsequently dehydrated in increasing concentrations of alcohol (70% to 100%), cleared with histoclear, and finally mounted with coverslips using DPX mountant. Counterstaining was only used during protocol optimisation to help visualize the cells structure (Fig. 6.3).

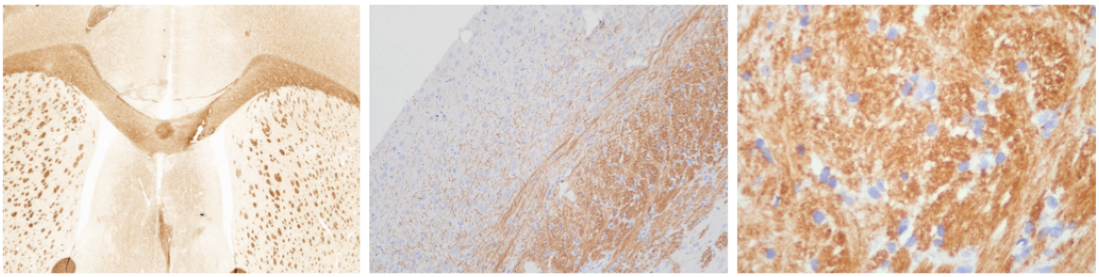

Figure 6.3: PLP expression in the WM. a) 10x magnification without counterstain b) 100x magnification with hematoxylin counterstain c) 400x magnification with hematoxylin counterstain.

6.2.7 Histology Measures And Statistical Analysis

Staining intensities were quantified by stereoinvestigator (MBF Bioscience) in one batch of animals (n=24; 8 animals per group). To correct for any light bias, background correction was performed by using an area without tissue within each slide. Images for all sections were acquired on the same day under the same light conditions for each antibody stain. ROIs were manually drawn (Fig. 6.4). Luminance measurements were extracted for each ROI. Luminance measures are between 0 and 254, with 0 corresponding to black and 254 to white. A low luminance measure reflects higher stain intensity.

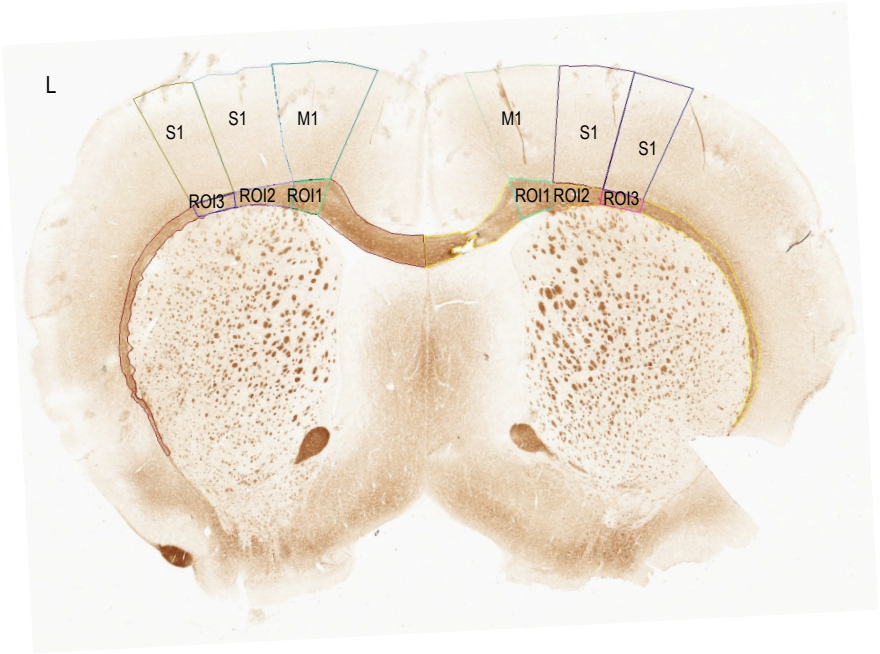

Figure 6.4: Anti-MBP stained section showing manually drawn ROIs. The section was automatically reconstructed from individual 10x magnification pictures. M1 corresponds to the primary motor cortex and S1 to the primary somatosensory cortex (21). L indicates the left hemisphere.

Differences in staining intensity between the SR and UR groups were tested using a student's t-test. Correlations between staining intensity and behavioural scores were tested using Pearson's correlation. Comparison of the strength of correlations were made using Fisher's r to z.

6.3 Results

6.3.1 Behavioural Results

For the SR group, a RM Anova on reaching accuracy scores over 11 days of testing showed a significant effect of day ($F(10,143.272) = 25.340$; $p < 0.001$), confirming that the rats learned the task (Fig. 6.5a). The UR group was tested on the skilled

version of the task on the 11th day. Their performance was significantly different from the SR group performance on the same day (t (46) = 3.379, p <0.001), the UR group has higher accuracy skills than the SR group performance on the 1st day (t (46) = 3.053, p <0.01), but UR accuracy is not significantly different from SR on the fourth day (t (46) = 0.481, p <0.633). This shows that UR group performance on the 11th day is comparable to the SR group accuracy on the 4th day (Fig. 6.5a).

For both groups, total number of reaches per day was recorded. RM - Anova on the total number of reaches per day in both UR and SR groups revealed a main effect of group (F(1,46) = 78.257; p = 0.001), a main effect of day (F(9,159.145) = 44.791; p <0.001), and an interaction between day and group (F(9,159.145) = 15.042; p <0.001) (Fig. 6.5b). I did not find any correlations between accuracy or number of reaches and body weight. In summary, SR animals learned the task and have significantly higher accuracy than UR rats. SR and UR animals reach progressively more across days, but UR animals make significantly more reaches than SR rats.

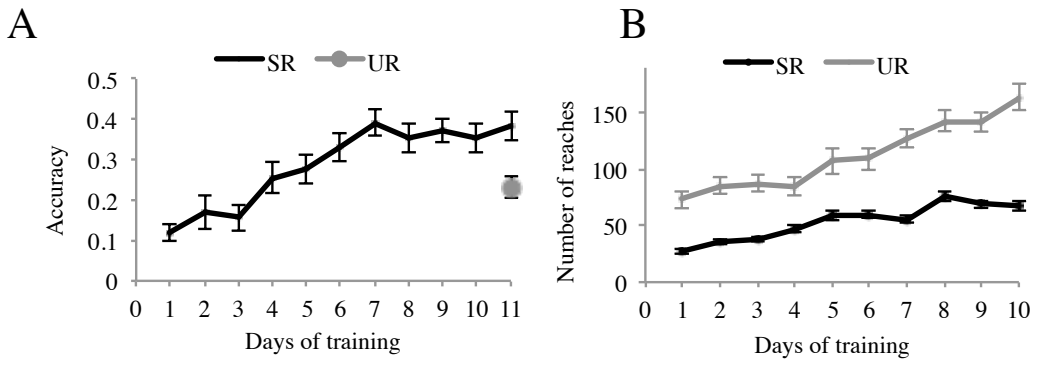

Figure 6.5: Behavioural results. a) average accuracy scores for SR animals for all training days (in black) and UR rats for 11th day test (in grey) (n = 24). b) Average number of reaches per day for SR (in black) and UR (in grey) (n = 24 animals per group). Error bars indicate standard error.

6.3.2 Neuroimaging Results

We found significant differences in FA between the SR animals and the control groups (URT and CC) ($p < 0.05$, corrected) ($n = 24$ animals per group) (Fig. 6.6). The cluster of significant FA difference was located in the external capsule, cingulum and corpus callosum, and co-localised with somatomotor GM areas in the contralateral hemisphere to the used paw (Fig. 6.6).

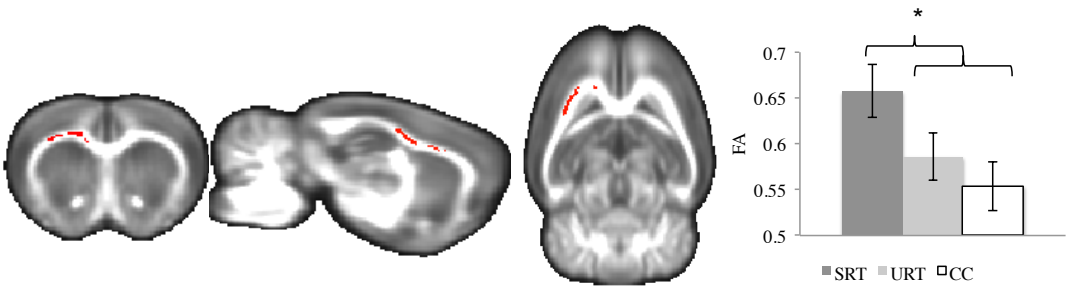

Figure 6.6: Significant cluster superimposed on rat template (*$p < 0.05$, corrected). SR animals have significantly higher FA than UR and CC animals in WM areas comprising the external capsule and cingulum co-localised with somatomotor GM areas in the contralateral hemisphere to the used paw.

Additionally, there was a positive correlation between FA and learning rate for the SR rats in the hemisphere contralateral to the used paw in widespread areas of WM ($p < 0.05$, corrected) (Fig. 6.7).

A subset of 24 brains were processed for histology (8 from each group) and stained with PLP and MBP. Regions of interest were defined according to the FA results. We mainly focused on the external capsule and cingulum WM co-localised with M1 and S1 (Fig. 6.8).

SR group was found to have significantly higher MBP staining intensity than UR and CC animals in the ROI1 (Fig. 6.8b) ($t(22) = 1.779$, $p < 0.05$). This area corresponds to the WM co-localised with M1, contralateral to the used paw and

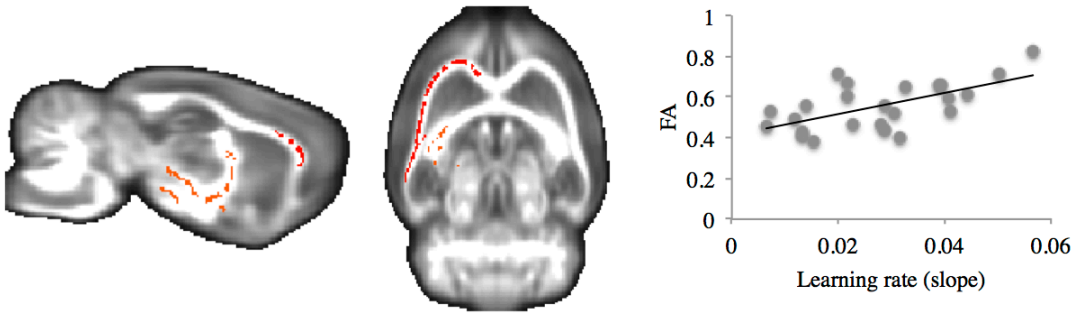

Figure 6.7: Significant clusters superimposed on rat template (p <0.05, corrected). Animals with higher performances have higher FA in subcortical WM areas and the external capsule co-localised with somatomotor cortical GM.

(Fig. 6.9a,b). No significant differences between groups for the ipsilateral side to the trained paw were found (t (22) =-0.203, p = 0.421). Staining intensity in the contralateral area correlated positively with learning rate for the SR rats (Pearson r = - 0.788; p <0.05; 2-tail) (Fig. 6.9c). The corresponding ROI in the hemisphere ipsilateral to the trained paw did not correlate with performance (Pearson r = 0.138; p = 0.744, 2-tail) (Fig. 6.9d). The correlation for the contralateral hemisphere was significantly stronger than those for the ipsilateral hemisphere (z = -1.7, p <0.05). No significant differences between groups were found for ROI2 (t (22) =0.801, p = 0.216) or ROI3 (t (22) =-0.122, p = 0.452). I did not find any correlation between MBP, PLP and FA. In summary, animals that learned the SR task have higher MBP expression in WM underneath M1 in the contralateral side to the used paw compared to a control group that learned the unskilled version of the task. MBP intensity is correlated with performance in the task: animals that perform better have higher MBP expression as measured by luminance (a low number in luminance represents higher stain intensity). We did not find any differences between groups in PLP expression.

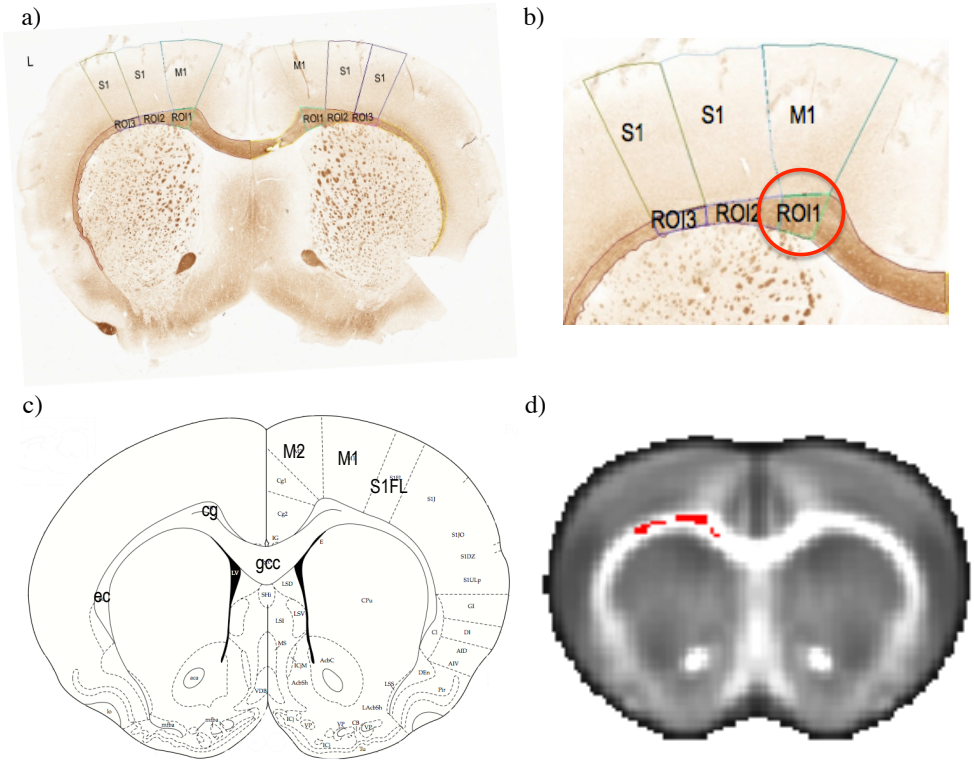

Figure 6.8: a) Anti-MBP stained section showing manually drawn ROIs. b) ROI with significant MBP differences between SR and UR group (t (14) = 1.905, p <0.05). c) Rat atlas section approximately corresponding to MBP and MRI slice (21). S1FL Forelimb S1; gcc genu of the corpus callosum; cg cingulum; ec external capsule d) MRI section showing significant FA cluster differences between SR and control groups.

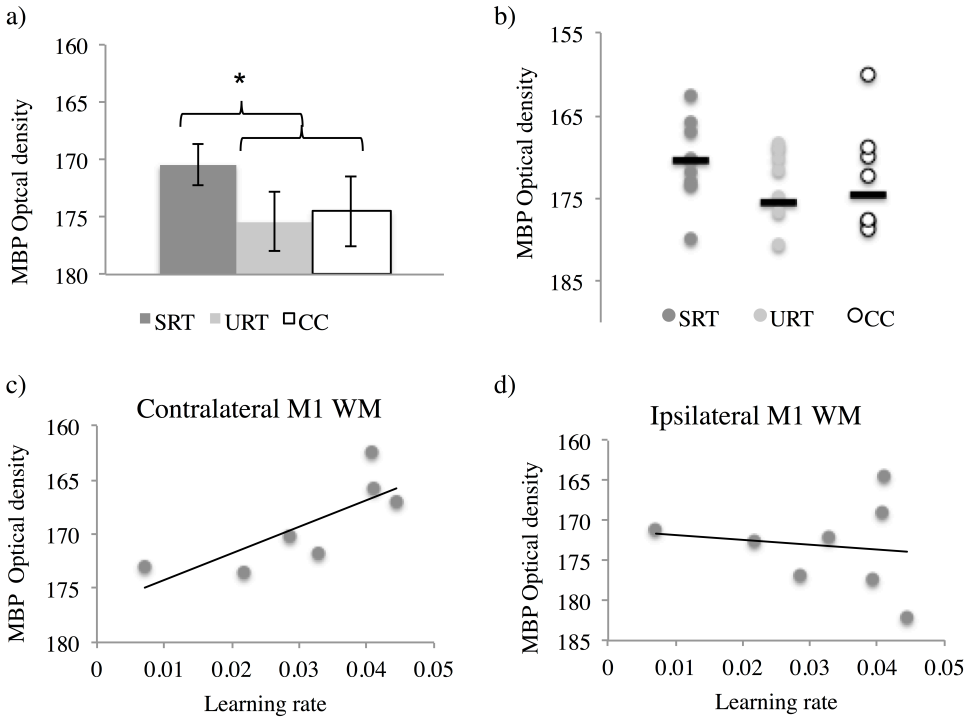

Figure 6.9: a) SR animals have significantly higher MBP stain intensity than UR and CC groups. b) Graph showing individual MBP values dispersion per group. c) Animals with higher MBP stain intensity in WM contralateral to the used paw perform better in the reaching task. d) No correlation with performance was found for the WM in the ipsilateral side. (n = 8 per group). Low luminance values reflect higher stain intensity. Vertical scales have been inverted for intuitive reading. Error bars indicate standard error.

6.4 Discussion

Learning a novel motor task results in increased FA in the contralateral external capsule, cingulum and corpus callosum, co-localised with the motor and somatosensory cortical representation of the forelimb of the rat. A higher MBP expression that correlated with performance was also found for the SR group. Previous research has shown that cortical synaptogenesis and motor map reorganisation occur in the last phases of learning in this paradigm (12), but no changes in the WM have been previously reported. While a previous study has WM changes following training on a hippocampal-dependent spatial navigation task ((6), the current study is the first to report experience-dependent WM plasticity after a cortical learning paradigm, during which neurogenesis is unlikely to occur. The current findings therefore suggest that WM structural plasticity, and growth of new myelin, can occur without the generation of new neurons.

The differences in FA found between SR and UR rats suggest that these effects are not due to increased movement of the paw but rather to the specific process involved in learning the precise reaching and grasping movement required to retrieve the single food pellet in the SR task. The behavioural data support this interpretation since the UR animals actually reach significantly more often than the SR group. No differences in FA were found between the UR group and the caged control group, suggesting that increased movement of the paw alone does not result in FA change. This is quite surprising as the UR animals learnt the gross movements to reach for the pellets and, on the 11th day test, achieve accuracy scores similar to the SR group 4th training day. This further suggests that the higher FA is not linked to the early stages of learning but to prolonged

task acquisition. This is in accordance to Kleim and colleagues (2004) reports that only late learning stages induce synaptogenesis and map reorganisation (12).

Furthermore, we found a positive correlation between learning rate and FA in the contralateral hemisphere to the used paw in WM areas that comprise the external capsule, cingulum, corpus callosum and internal capsule. Some of these WM regions overlap with the areas found to be significantly increased in the SR group.

A correlation with behavior might indicate that the apparent increase in FA after learning might be indeed related to the learning itself. However it is not possible to exclude the possibility that this correlation might be related to inter-individual differences pre-learning between animals in performance and brain structure. Only a longitudinal design would be able to clearly establish a relation between skill learning and FA change.

In accordance with the FA results, we found significantly higher expression of MBP in the region of interest identified by DTI. The increase in MBP expression in the hemisphere contralateral to the trained paw correlated with performance, while expression levels the ipsilateral hemisphere did not. Despite the consistency in the FA and histological results, we did not find any correlations between measures of MBP expression and FA. This might be due to the complexity of the FA signal. FA is modulated by several WM characteristics like axon diameter, axon packing and fibers organization (4). A previous study, that used a spatial learning task, also found an increase in MBP expression, along with increases in FA, in corpus callosum, but did not report any correlation between the two measures (6). It is possible that myelin is but one aspect of the WM that has undergone plasticity and FA is capturing several WM features, thus a correlation cannot be

established. Furthermore, the sample size used for histology in the current study is relatively small. It is possible that a larger sample size would be more sensitive to a correlation between histological measures and FA. Nevertheless, this work shows that myelin is involved in plastic processes after skill learning. In this study greater myelination is related to higher performance, which might be a result of increased synchronization between brain areas that are involved in the task. We did not find significant differences between groups with PLP, an alternative stain for myelin. There are differences in function between PLP and MBP that might account for this. While both MBP and PLP mediate myelin compaction, thickness is regulated by MBP. Thus this indicates that myelin thickness might be involved in WM plasticity in response to learning (19). To confirm this electron microscopy (EM) will need to be carried out. EM also has the potential to elucidate what other WM features might be modulating the FA measures.

6.5 Conclusions

We found higher FA and MBP expression, both of which correlated with performance, in a skilled learning group compared to the control groups. This was not caused by increased movement of the paw but specifically by the learning process involved in acquiring the novel unimanual reaching and grasp movement required to retrieve the food pellet in the SR task. This work has implications for the interpretation of human imaging studies of learning. Previous human neuroimaging studies have shown that FA changes in response to motor learning, however the underlying cellular events were unknown (23). Very few studies have investigated WM plasticity in the context of learning and experience thus this

work provides a valuable contribution to the field.

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Chapter 7

Conclusions and future directions

This thesis used a range of neuroimaging approaches in both humans and rodents to address three main questions: (1) Can we predict learning performance using baseline imaging measures? (2) To what extent do performance outcomes or training times determine experience-dependent plastic changes? (3) What biological mechanisms underlie white matter plasticity detected using MRI?

7.1 Predicting Performance

One question addressed in this thesis is whether baseline measures of brain structure of function can predict subsequent learning or brain plasticity. This is an important question as using brain structure to predict future performance could be a powerful tool for selecting and directing resources to the people most likely to benefit from it. For example, such an approach could be used in the context of elite sports and highly skilled professions, as well as in clinical domains such as predicting response to rehabilitation.

This question was addressed in Chapter 3, where I found that GM density in brain areas relevant to juggling learning predicted later performance in human volunteers. Some of these same brain areas were found to increase in GM density

after juggling learning in humans (Chapter 4), but the magnitude of these GM changes after learning could not be predicted based on GM density at baseline, showing that learning the skill itself drives independent plastic processes that are specific and encode the skill acquisition. This knowledge could be used to tailor training programs to people's needs, not only taking advantage of the natural inclinations but also maximising brain plasticity mechanisms through learning.

7.2 Separating Out Effects Of Performance Versus Amount Of Training On Functional And Structural Plasticity

Another aim of this thesis was to investigate the relative contribution of performance outcome versus amount of training in driving learning-dependent structural and functional plasticity. This was motivated by previous work that demonstrated, using a longitudinal design, an apparent increase in grey matter (GM) density in an area related to visual motion in subjects that learned to juggle (1). This study demonstrated that plastic changes occur in the human adult brain and these can be observed with MRI. However, no correlation with performance was found, in this or other follow up studies, thus giving rise to the question whether training intensity rather than performance were driving the structural brain changes. In Chapter 4, effects of performance and amount of practice on brain structure were studied by varying the amount of juggling practice. Some participants practiced juggling for 15 minutes a day while others practiced for 30 minutes. I found both effects of performance and practice on brain structure during the follow up period. Overall, participants that achieved higher performances had higher GM and WM matter change. Also, participants that trained juggling

for longer had higher positive brain changes than participants that practiced less.

Although I did not find structural brain changes during the learning period, I found changes in functional connectivity (Chapter 5). High intensity training decreased the motor resting-state network strength while lower intensity increased the network strength. The increase in motor strength was associated with a decrease in GABA concentration. A correlation was also found between motor resting-state strength change and GABA concentration change after learning. These results suggest that changes in functional connectivity could reflect changes in synaptic strength, mediated by GABA.

I speculated that the pattern of functional connectivity and GABA changes found reflect the 15 and 30 minute groups being at different stages of learning, if increases in network strength (and decreases in GABA) are found early in learning and the opposite patterns found once a skill is overlearnt. To test this possibility directly, further work would be necessary. For instance, a design with higher temporal resolution, where scans are acquired every week, could potentially clarify the time-course of the functional connectivity changes and GABA levels. It would be expected that the high intensity training group would have an increase in motor resting-state network, and a corresponding decrease in GABA concentration, in the first few weeks and a decrease in the latest weeks. Furthermore, it would be expected that the 15 minutes group would follow a similar pattern if training continued for a longer time period (superior to 6 weeks). This design could also potentially reveal when structural changes are evident and in which brain areas. It is possible that structural changes can be detected earlier in learning, when it is more likely that formation of new connections surpasses the elimination of old connections. Indeed, a previous study, that scanned participants 7 days after

the training started, found transient increases after the first scan in GM, when participants could juggle for 60 seconds, that disappeared by the second scan (2). In this study the amount of training was not controlled, but a performance criteria was defined for each scan, so it is not possible to dissociate the effects of training intensity from effects of performance.

7.3 Determining Underlying Biological Mechanisms For White Matter Plasticity

Very few studies have addressed the role of white matter (WM) in learning. Scholz and colleagues (3) were the first to use diffusion tensor imaging (DTI) to show that fractional anisotropy (FA), a measure of WM microstructure, is modulated in response to learning. However, the underlying mechanisms were not clear, since there is less evidence for WM plasticity in response to learning, compared to GM. Thus a combination of non-invasive MRI methods and immunohistochemistry were used to address this topic in an animal model of skill learning in Chapter 6. I found that learning a novel motor task increases WM fractional anisotropy, an indirect measure of WM microstructure, in the contralateral hemisphere to the used paw. Immunohistochemistry staining with myelin basic protein (MBP) antibody of this region revealed increased myelin stain intensity for the learning group that correlated with performance in the task. However, although both FA and MBP staining were increased in the learning group, I did not find a correlation between FA and MBP intensity. It is possible that FA is being modulated by more than one WM feature that has undergone structural plasticity. According to previous research, number of axons and axon diameter are possible candidates (4). I will carry out Electron Microscopy (EM) analysis as it allows to carry axon

counts, myelin thickness axon diameter measurements, and has the potential to clarify which WM features are not only modulating FA but undergo plasticity in response to skill learning.

7.4 Concluding Remarks

In summary, the work presented in this thesis shows that: (1) performance of complex skills can be predicted by baseline brain structure; (2) both performance outcome and training amount drive independent functional and structural plastic changes; (3) myelination is involved in experience-dependent plasticity in the adult brain, which can be indirectly detected with MRI.

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