TITLE

Investigating Structural Plasticity In Musicians' Brains Using Structural Magnetic Resonance And Diffusion Tensor Imaging Techniques

Supervisors

DR VANESSA SLUMING

PROFESSOR ANDREJ STANCAK

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IHSSAN ADEEB ABDUL-KAREEM

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DECLARATION

This thesis is the result of my own work. The material contained in this thesis has not been presented, nor is currently being presented, either wholly or in part, for any other degree or qualification.

(IHSSAN ADEEB ABDUL-KAREEM)

STUDENT

(DR. VANESSA SLUMING)

SUPERVISOR

DEDICATION

This work is dedicated

To the soul of my mother, GOD bless her

To my beloved wife

To my lovely sons, Ahmed and Yousif

ACKNOWLEDGMENTS

I would like to thank all those who helped me throughout the preparation of my thesis. Specifically, I would like to thank my supervisors Dr Vanessa Sluming, Dr Andrej Stancak and Dr Laura Parkes. Special thanks to Dr Vanessa who was very kind, considerate and knowledgeable supervisor.

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The time I spent here in the city of Liverpool was fascinating. The knowledge I got was splendid. It was a wonderful experience that I will remember throughout my entire life.

TABLE OF CONTENTS

INDEX OF TABLES

INDEX OF FIGURES

ABBREVIATIONS

AP	Absolute pitch
AC	Anterior commissure
ACPC	Anterior commissure-posterior commissure
ADC	Apparent diffusion coefficient
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CC	Corpus callosum
CST	Corticospinal tract
DF	Degree of freedom
DTI	Diffusion tensor imaging
DTT	Diffusion tensor tractography
DWI	Diffusion- weighted image
EEG	Electroencephalography
FA	Fractional anisotropy
fMRI	Functional magnetic resonance imaging
GM	Gray matter
ICV	Intracranial volume
ICC	Intraclass correlation coefficient
IFG	Inferior frontal gyrus
LTD	Long-term depression
LTP	Long-term potentiation
MARIARC	Magnetic Resonance And Image Analysis Research centre
MRI	Magnetic resonance imaging
MPRAGE	Magnetization-Prepared Rapid Gradient-Echo imaging
MEG	Magnetoencephalography
MIT	Melodic intonation therapy
MCP	Middle cerebellar peduncle
Mm	Millimetre
MANOVA	Multivariate analysis of variance
NMDA	N-methyl-D-aspartate
PET	Positron emission tomography
POP	Pars opercularis
PTR	Pars triangularis
PoCG	Postcentral gyrus
PC	Posterior commissure
PrCG	Precentral gyrus
ROI	Region of interest

RA	Relative anisotropy
SNR	Signal to noise ratio
SPGR	Spoiled gradient echo
SD	Standard deviation
SCP	Superior cerebellar peduncle
T	Tesla
3D	Three dimensional
VBM	Voxel based morphometry
WM	White matter

Investigating Structural Plasticity In Musicians' Brains Using Structural Magnetic Resonance And Diffusion Tensor Imaging Techniques

IHSSAN ADEEB ABDUL-KAREEM

ABSTRACT

Neuroplasticity is the ability of the brain to change its structure and/or function in response to environmental stimuli. It is implicated in many processes, such as learning, maturation, skill acquisition, and rehabilitation following brain injury. With the advent of neuroimaging techniques, the study of neuroplasticity and its mechanisms have fascinated researchers given the wide scope with which this process is involved. Musicians have long been considered an ideal model to study neuroplasticity in humans. It has been shown that musicians with their early, intensive, and multimodal skilful practice have structural plasticity in different brain regions. The objective of this work was to extend these structural studies through examining different cohorts of musicians, using a multitude of imaging and morphometric techniques, and performing novel examinations of brain regions essential for enabling high level musical performance such as Broca's area, corpus callosum (CC), and cerebellum.

Three age-, gender- and handedness-matched cohorts were examined. The first cohort included 26 orchestral musicians and 26 non-musicians. High resolution T1-weighted structural MR images were acquired to measure gray and white matter volumes and cortical surface area of Broca's area subparts: pars opercularis/BA44 and pars triangularis/BA45. The second cohort included 12/12/12 professional musicians/amateur musicians/non-musicians. High resolution T1-weighted MR images were acquired to measure cross-sectional areas of four regions of the midsagittal CC: CC1 (rostrum/ genu/anterior body), CC2 (anterior midbody), CC3 (posterior midbody), and CC4 (isthmus and splenium). In the third cohort, 12/12 musicians and non-musicians were examined. High resolution T1-weighted structural MR images were acquired to measure cross-sectional areas of CC1-CC4 regions; and diffusion tensor imaging-based tractography was used to measure average fractional anisotropy (FA), mean diffusivity (MD), tract volume, and number of streamlines of the same regions. In a subset (10/10) of this cohort, high resolution structural scans were used to measure gray and white matter volumes of cerebellar hemispheres; and diffusion tensor imaging-based tractography was used to measure average FA, tract volume, and number of streamlines of superior (SCP) and middle (MCP) cerebellar peduncles. Outcome measures were compared between groups.

Compared to controls, musicians possessed greater gray matter volume and cortical surface area of left pars opercularis/BA44 in the first cohort. The volume of left pars opercularis was positively correlated with years of musical performance. Professional musicians possessed greater cross-sectional area of CC1 and CC4 regions compared to amateurs and non-musicians in the second cohort. In the third cohort, musicians possessed greater cross-sectional area, average FA/tract volume/number of streamlines, and lower MD in CC4 region. There was a negative correlation between cross-sectional area of CC4 region and age of starting musical training. There was a positive correlation between average FA values and cross-sectional area of CC4 region in all subjects. In addition, musicians had increased white matter volume of the right cerebellar hemisphere, increased tract volume and number of streamlines of right SCP, and tract volume of right MCP. I hypothesize that these findings represent use-dependent structural plasticity imposed by musical performance. At the microscopic level, these macroanatomical changes may reflect increased synaptogenesis and dendritic growth, generation of new axon collaterals, and formation of new neurons, which would support enhanced functional demands on musicians' brains.

CHAPTER 1

INTRODUCTION-

OVERVIEW, RELEVANT ANATOMY & NEUROPLASTICITY

1.1 AIM

This chapter is divided into three main parts. The first part presents a general overview of the present work, structure of the thesis, and role of co-authors in published articles. The second part provides an overview of anatomy of brain structures examined in this work namely, Broca’s area, corpus callosum (CC), and cerebellum. The third part provides a detailed description of experience-dependent neuroplasticity including its principles, microscopic mechanisms, and macroanatomical evidence of neuroplasticity in the human brain as revealed by neuroimaging studies.

1.2 OVERVIEW OF THE PRESENT WORK

The term ‘neuroplasticity’ describes structural-functional adjustment or adaptation of the brain to environmental stimuli, such as maturation, learning, and skill acquisition, or a compensation of some cerebral structures for injured parts (Zilles, 1992, Rauschecker, 1995). This concept, being critically involved in health and disease, has fascinated scientists to explore its underlying mechanisms and events. Until 1970, the common belief was that the nervous tissue is static throughout adult life; however, experiments performed on animals showed that adult brain has the lifelong ability for structural and functional reorganization induced by learning and skill acquisition. Postmortem examination of specimens obtained from actively trained animals has shown clear evidence of microstructural plasticity in the form of dendritic growth and axonal sprouting (Pascual-Leone et al., 1994, Hallett, 1995, Pascual-Leone et al., 2005), which could not be demonstrated in inactive animals (Black et al., 1990, Anderson et al., 1996, Kleim et al., 1998a, Anderson et al., 2002, Swain et al., 2003, Kleim et al., 2004, Matsuzaka et al., 2007). Furthermore, there is convincing evidence that new neurons could be generated in response to increased functional demands (Gould et al., 1999b, Alvarez-Buylla and Garcia-Verdugo, 2002, Duchesne et al., 2002, Fowler et al., 2002, Rakic, 2002). With the emergence of neuroimaging modalities notably, magnetic resonance imaging (MRI), neuroscientists were fascinated to investigate training-induced plastic changes in the human brain. Experiments were conducted on subjects with various professions, such as taxi drivers (Maguire et al., 2000, Maguire et al., 2006b), basket ball players (Park et al., 2006), jugglers (Draganski, 2004), and mathematicians (Aydin et al., 2007). However, musicians emerged as an ideal model to study plasticity in the human brain. First, they commonly start their training at an early age when many brain structures are still vulnerable to plastic changes. Second, their practice involves the acquisition of multimodal skills, including sensory, motor, auditory, and visuospatial skills, which should be synchronized and refined to produce the finest outcome. Third, they continue to practice even outside their working hours unlike subjects from other professions. In this contest, tens of structural (Schlaug et al., 1995a, Schlaug et al., 1995b, Keenan et al., 2001, Schmithorst and Wilke, 2002, Sluming et al., 2002, Gaser and Schlaug, 2003, Hutchinson et al., 2003, Bengtsson et al., 2005, Imfeld et al., 2009) and functional (Sergent et al., 1992, Elbert et al., 1995, Koeneke et al., 2004, Vuust et al., 2005, Bangert et al., 2006, Limb et al., 2006, Magne et al., 2006, Baumann et al., 2007, Sluming et al., 2007, Watanabe et al., 2007) studies were conducted during the last two decades to investigate neuroplasticity in musicians. Results revealed structural-functional changes in musicians relative to controls in different brain regions, including the motor cortex, auditory cortex, prefrontal cortex, inferior frontal gyrus, superior parietal lobule, cerebellum, CC, and corticospinal tract. In addition, professional musicians exhibited evidence of cross-modal integration essential for enabling high level musical performance, such as auditory-somatosensory (Schulz et al., 2003), auditory-motor (Bangert et al., 2006, Baumann et al., 2007), and visual-motor integration (Sluming et al., 2007). The correlation of these findings with musical expertise, age of commencing musical tuition, duration of musical performance, and instrument specialty has led to the assumption that they represent use-dependent adaptations imposed by enriched environment in the form of musical practice. In addition to providing compelling evidence of neuroplasticity in the human brain, results of these studies may enhance our understanding of the mechanisms of music-brain interaction, which might have useful applications in health and disease. From the structural point of view, there is much work to be done to expand our knowledge about structural plasticity in musicians' brains particularly in Broca's area, cerebellum, and CC regions, which are crucial for musical performance. Thus, I set the goal to extend the literature through using structural MR and diffusion tensor imaging (DTI) techniques to perform novel investigations of structural plasticity in these regions. A shared characteristic of these regions is their considerable connections with several brain parts. Additionally, recent functional studies have assigned diverse musically-relevant functions to these regions justifying structural examinations performed in the current work. Four studies were conducted in the present work:

Study 1- Investigating structural plasticity of Broca's area subparts: pars opercularis (POP) and pars triangularis (PTR) in musicians. In this study, gray matter (GM) volumes, white matter (WM) volumes, and cortical surface areas of POP/BA44 and PTR/BA45 were compared between musicians and non-musicians. This study is presented in **chapter 4**.

Study 2- Investigating the effect of musical expertise on regional morphometry of the CC. In this study, cross-sectional area measurements of four callosal regions were compared between professional musicians, amateur musicians, and non-musicians. This study is presented in **chapter 5**.

Study 3- Investigating regional macro- and microstructural plasticity of the CC in musicians. The macrostructure (cross-sectional areas) and microstructure (average fractional anisotropy (FA), mean diffusivity (MD), tract volume, and number of streamlines) of four callosal regions were compared between musicians and non-musicians using structural MR and DTI-based tractography, respectively. This study is presented in **chapter 6**.

Study 4- Investigating structural plasticity of cerebellar WM in musicians. In this study, DTI-based tractography was used to compare average FA, tract volume, and number of streamlines of SCP and MCP between musicians and non-musicians. In addition, GM and WM volumes of cerebellar hemispheres were compared between groups. This study is presented in **chapter 7**.

To perform these studies, three cohorts of age-, gender-, and handedness-matched subjects were recruited; the first and third cohorts included musicians and non-musicians, while the second cohort included professional musicians, amateur musicians and non-musicians. Cohorts were examined using structural MR (study 1-4) and DTI techniques (study 3 and 4).

1.3 STRUCTURE OF THE THESIS

The thesis is divided into eight chapters. The first chapter (this chapter) provides an introduction to the thesis, including a general overview, a brief description of relevant anatomy, and an introduction to the principles and mechanisms of neuroplasticity. The second chapter discusses neuroplasticity in musicians' brains, reviews previous reports regarding anatomical-functional plasticity in musicians, and summarizes the different factors that govern this process in musicians. The second chapter concludes by presenting key objectives and research hypotheses of the present work. The third chapter presents a summary of recruited cohorts and image acquisitions, in addition to presenting general principles of methodologies used in the current work. Methods used in more than one study are mentioned in chapter three, and are referred to when presenting individual studies in subsequent chapters. The fourth, fifth, sixth, and seventh chapters present the four studies conducted in the present work, respectively. To comply with University of Liverpool guidelines, chapters 4-7 are presented in a 'ready to submit' style with an abstract, introduction, subjects and methods, results, discussion, and conclusion sections. A general discussion, conclusion, and recommendations for future studies are presented in chapter eight. All references are cited at the end of the thesis under the 'references' section using the referencing style of the 'Journal of Neuroscience'. The appendix section contains published peer-reviewed journal articles, conference papers, and abstracts.

1.4 PEER REVIEWED JOURNAL ARTICLES PUBLISHED DURING THE PHD STUDY

Four articles were published during the PhD study period. Below I present them in a chronological order:

- Abdul-Kareem IA & Sluming V 2009: Comparison of scanning at different field strengths and pulse sequences using voxel-based morphometry. Proceedings of Salford Postgraduate Annual Research Conference, SPARC. University of Salford, Manchester.

- Abdul-Kareem IA, Stancak A, Parkes LM, Sluming V 2009: Regional corpus callosum morphometry: effect of field strength and pulse sequence. J Magn Reson Imaging, 30 (5):1184-1190.
- Abdul-Kareem IA, Stancak A, Parkes LM, Sluming V 2011: Increased gray matter volume of left pars opercularis in male orchestral musicians correlate positively with years of musical performance. J Magn Reson Imaging, 33 (1):24-32.
- Abdul-Kareem IA, Stancak A, Parkes L, Al-Ameen M, AlGhamdi J, Al-Dhafeeri F, Embleton K, Morris D & Sluming V 2011: Plasticity of the superior and middle cerebellar peduncles in musicians revealed by quantitative analysis of volume and number of fibers based on diffusion tensor tractography. Cerebellum, 10: 611-623.

1.5 ROLE OF CO-AUTHORS IN PUBLISHED ARTICLES

- Vanessa Sluming, Andrej Stancak and Laura Parkes - as supervisors, they participated in reviewing the manuscripts and provided corrections and suggestions. Vanessa Sluming recruited subjects of the first study.
- May Al-Ameen - performed inter- and intra-rater studies.
- Jamaan AlGhamdi and Faten Aldhafeeri - provided technical help regarding the use of FreeSurfer software to measure ICV, GM and WM volumes of cerebellar hemispheres.
- David Morris and Karl Embleton - helped in optimizing the final diffusion-weighted pulse sequence that was used to perform DTI examination of the CC, superior cerebellar peduncle (SCP) and middle cerebellar peduncle (MCP).

1.6 RELEVANT ANATOMY

1.6.1 GROSS ANATOMY OF THE BRAIN

The human brain is conventionally divided into three major divisions: The forebrain (diencephalon and cerebrum), the mid brain, and the hind brain (medulla oblongata, pons, and cerebellum). The cerebrum (figure 1.1), is the largest part of the brain consisting of two cerebral hemispheres connected by the CC. The surface of each hemisphere (the cortex) is composed of GM, which contains neurons supported by neuroglia. The cerebral cortex is thrown into gyri and sulci, thereby increasing its surface area. Within the hemisphere is a central core of WM, which is composed of myelinated nerve fibers of different diameters supported by neuroglia (Williams et al., 1995, Snell, 2001, Kiernan, 2005).

1.6.2 MICROSCOPIC ANATOMY OF THE BRAIN

The basic functional unit of the brain is the neuron, which is specialized for the reception of stimuli and the conduction of nerve impulses. Each possesses a cell body and neuritis; the latter comprise dendrites and axons. Dendrites are responsible for receiving information and conducting it toward the cell body; they often branch profusely to increase surface area for the reception of axons from other neurons. The axon is a single long tubular neurite that conducts impulses away from the cell body. A synapse is the site where two neurons come in close proximity, and functional inter-neuronal communications occur. Most neurons make synaptic connections to 1000 or more other neurons and may receive up to 10,000 connections from other neurons. Communication at a synapse takes place in one direction only (Snell, 2001). Apart from neurons, the central nervous system also contains supporting cells, the neuroglia. Their number exceeds that of neurons about 10-fold and constitutes about half the nervous tissue mass. The neuroglial cell types in the central nervous system are astroglia, oligodendroglia, and microglia (Williams et al., 1995, Snell, 2001, Kiernan, 2005).

1.6.3 THE BROCA'S AREA

Broca's area is located in the POP, Brodmann area (BA) 44 and PTR, BA45 of the inferior frontal gyrus of the dominant hemisphere; it directs the motor area for the production of speech (figure 1.1B). The homologue of Broca's area in the right hemisphere is believed to be involved in emotional aspect of speech (Williams et al., 1995). Broca's area is connected to the sensory (receptive) speech area by a bundle of WM called the arcuate fasciculus. The name Broca was derived from the name of a French neurologist, Pierre Paul Broca, who reported impairments of language in two patients who lost the ability to speak after injury to the posterior inferior frontal gyrus of the brain (Dronkers et al., 2007). Since then, the approximate region he identified has become known as Broca's area, and the deficit in language production as Broca's aphasia. Although traditionally considered the most important language region in the brain, functions of Broca's area are not limited to language (to be discussed in details in chapter 4).

1.6.3.1 THE PARS OPERCULARIS

The POP is the posterior part of Broca's area. It is bounded posteriorly by the inferior precentral sulcus, which separates it from the precentral gyrus. Superiorly, it is separated from the middle frontal gyrus by the inferior frontal sulcus, and anteriorly from the PTR by the anterior ascending ramus of the Sylvian fissure (Foundas et al., 1998, Duvernoy, 1999, Tomaiuolo et al., 1999, Petrides and Pandya, 2004, Petrides, 2006).

1.6.3.2 THE PARS TRIANGULARIS

The PTR is the anterior part of Broca's area. It is demarcated posteriorly from the POP by the anterior ascending ramus of the Sylvian fissure, superiorly from the middle frontal gyrus by the inferior frontal sulcus, and anteroinferiorly by the anterior horizontal ramus of the Sylvian fissure (Foundas et al., 1998, Duvernoy, 1999, Tomaiuolo et al., 1999, Petrides and Pandya, 2004, Petrides, 2006).

1.6.3.3 MICROSCOPIC ANATOMY OF POP AND PTR

Cytoarchitectonically, BA 44 and 45 (Amunts et al., 1999) contain magnopyramidal cells in lower layer III, medium-large pyramidal cells in upper layer V, and decreased cell density in lower layer V. There is a size gradient in the cells of layer III, with the smallest cells at its border with layer II, and the largest near layer IV. The main cytoarchitectonic difference between areas 44 and 45 is the prominence of layer IV and the total thickness of the cortex. In particular, layer IV in area 44 is dysgranular, and appears to be invaded by cells from layers III and V. In area 45, layer IV is granular and more prominent and creates a clear barrier between layers III and V. The cortex is notably thinner in area 45 than in area 44 (Amunts et al., 1999).

1.6.3.4 CONNECTIONS OF POP AND PTR

It has been demonstrated that both BA 44 and 45 have distinct connections with other cortical regions. Using DTI, it has been shown that the right and left POP are connected with a large number of clusters in the frontal, parietal, temporal, and occipital lobes. The right and left PTR were found to have overlapping connectivity in both hemispheres. They are connected to the left POP, right POP, precentral gyrus, left posterior middle temporal gyrus, posterior inferior temporal gyrus, left insula, right supramarginal gyrus, and postcentral gyrus (Xiang et al., 2009). The dorsal pathway (to the parietal lobe and perisylvian regions in the temporal lobe) is more dominant in POP, whereas for PTR, tracts into the dorsomedial prefrontal cortex are more prominent.

1.6.4 THE CORPUS CALLOSUM

The CC is the largest WM structure in the brain consisting of approximately 180 million contralateral axonal projections (Blume, 1984) (figure 1.1C). It connects homologous regions within the two cerebral hemispheres. It is divided into rostrum, genu, body, isthmus, and splenium. The body is further divided into anterior and posterior parts (de Lacoste et al., 1985). The rostrum and genu connect mainly prefrontal regions. The body of the CC connects frontal and parietal regions with fibers originating in the premotor cortices traverse the CC through the anterior part of the body, whereas primary motor, somatosensory, and posterior parietal fiber bundles traverse the CC through the posterior part of the body. The isthmus and splenium connect temporal, parietal, and occipital cortical regions (de Lacoste et al., 1985, Hofer and Frahm, 2006). The fibers of the genu curve forward into the frontal lobes forming forceps minor, fibers of the body extend laterally forming radiation of the CC, while fibers in the splenium arch backward into the occipital lobe forming forceps major (Snell, 2001).The callosal fibers interdigitate with association and projection fibers.

There have been few studies that examined the microscopic structure of the human CC. One of the pioneer studies of the human CC is that of Aboitiz and colleagues (Aboitiz et al., 1992) who performed postmortem examination of callosa of 20 subjects (10 males and 10 females). They found that thin fibers are most dense in the genu and decrease in density posteriorly towards the posterior body, where they reach a minimum. The density of thin fibers increases again towards the splenium. Large-diameter fibers showed a pattern complementary to that of thin fibers, having a peak of density in the posterior body, and a local increase of density in the posterior pole of the CC. There was no gender difference in fiber distribution within the CC.

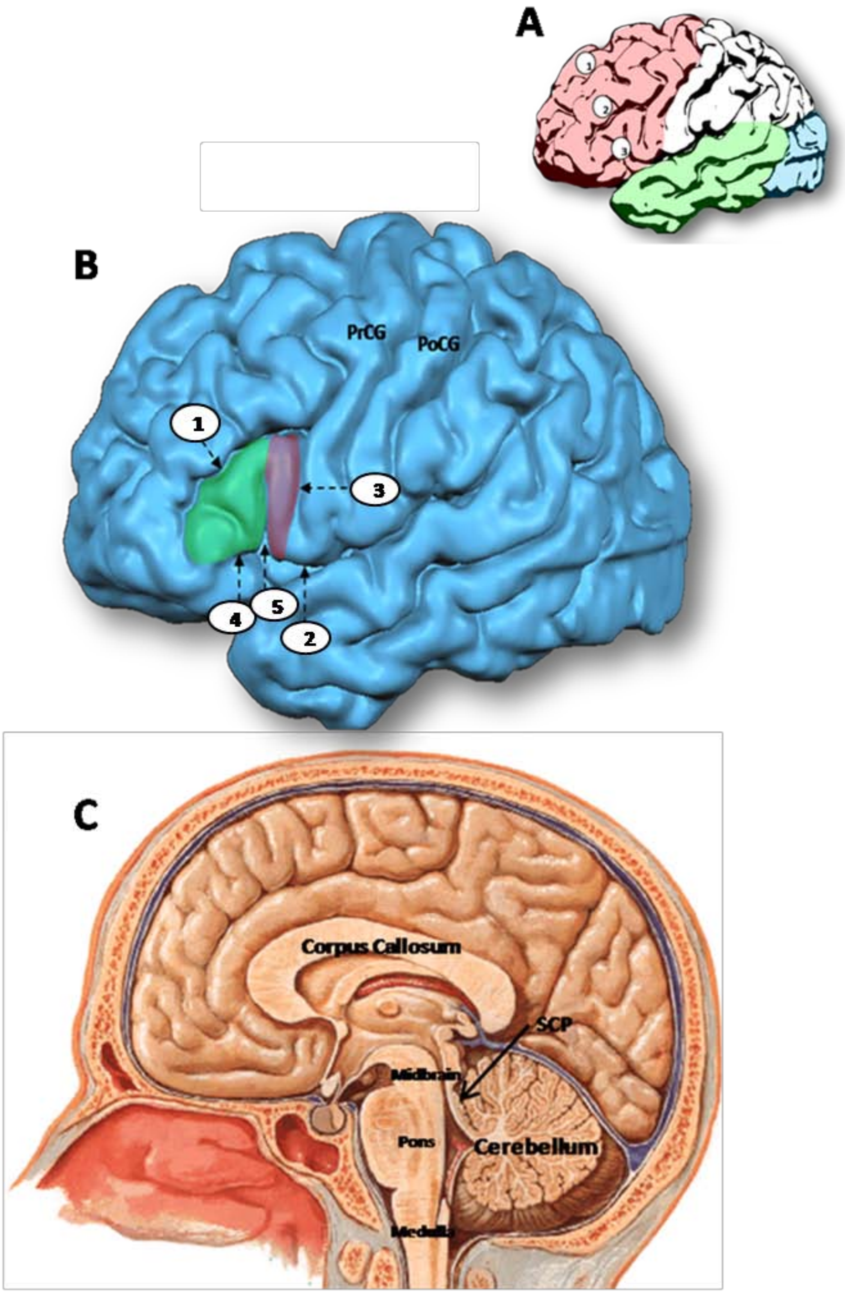


Fig.1.1. **A**, a three dimensional (3D) display of the left cerebral hemisphere demonstrating frontal (red), parietal (yellow), temporal (green), and occipital (blue) lobes. Numbers 1, 2, 3 represent superior, middle and inferior frontal gyri, respectively. **B**, a 3D display of the left hemisphere showing Broca's area and its subparts; POP (red) and PTR (green). 1, inferior frontal sulcus; 2, Sylvian fissure; 3, precentral sulcus; 4, anterior horizontal ramus; 5, anterior ascending ramus; PrCG, precentral gyrus; PoCG, postcentral gyrus. **C**, a sagittal view showing the medial surface of the cerebral hemisphere. SCP, superior cerebellar peduncle. Modified from (Netter, 2010).

1.6.5 THE CEREBELLUM

The cerebellum (Latin for little brain), is the largest part of the hind brain. It is located in the posterior cranial fossa. Anteriorly, the cerebellum is related to the pons and medulla oblongata. It is connected to the midbrain, pons, and medulla oblongata by the superior (SCP), middle (MCP), and inferior (ICP) cerebellar peduncles, respectively. The cerebellum is surfaced with a cortex of GM underneath which lies the WM, which is made up largely of myelinated nerve fibers running to and from the cortex. Embedded within the WM are four deep cerebellar nuclei situated near the roof of the fourth ventricle. The dentate nucleus is the largest of cerebellar nuclei forming a large crenated crescent opening toward the SCP (Snell, 2001, Sinnatamby, 2006).

1.6.5.1 CEREBELLAR PEDUNCLES

The SCP (figures 1.1C and 1.2B), also called the brachia conjunctiva, are two in number emerging from the upper and medial part of the white substance of the hemispheres, and are placed under cover of the upper part of the cerebellum. They are joined to each other across the midline by the anterior medullary velum. As they ascend they form the roof of the fourth ventricle. The fibers of the SCP are mainly derived from the cells of the dentate nucleus of the cerebellum. They continue upward where the two peduncles undergo a complete decussation ventral to the Sylvian aqueduct (Williams et al., 1995, Snell, 2001). The MCP (figure 1.2A) is the largest cerebellar peduncle, which is composed entirely of cortico-ponto-cerebellar fibers (Williams et al., 1995, Snell, 2001).

1.6.5.2 CEREBELLAR AFFERENTS

Afferent fibers to the cerebellum originate from the following regions:

- **Cerebral cortex** through the cortico-ponto-cerebellar fibers. These fibers arise from nerve cells in the frontal, parietal, temporal, and occipital lobes terminating in pontine nuclei. The pontine nuclei give rise to transverse fibers of the pons, which cross the midline and enter the opposite cerebellum as the MCP.

- **Spinal cord** through anterior and posterior spinocerebellar tracts, and cuneocerebellar tract. These tracts convey unconscious muscle joint sense. Axons of anterior spinocerebellar tract cross to the opposite side in the lateral column of the spinal cord. They then enter the cerebellum through SCP. Axons of the posterior spinocerebellar tract ascend in the lateral column of the spinal cord before entering the cerebellum through the ICP.

- **Vestibular nuclei**

1.6.5.3 CEREBELLAR EFFERENTS

- **Cerebral cortex**. The most extensive cerebellar efferent connections are those with the cerebral cortex. The cerebellum connects with the contralateral cerebral cortex by the dentothalamic tract. Fibers arising from dentate nucleus travel through the SCP, and cross the midline to the opposite side in the decussation of the SCP. The fibers end by synapsing with cells in the contralateral thalamus, and terminate in the primary motor and premotor areas of the cerebral cortex. By this pathway, the dentate nucleus can coordinate motor activity on the same side of the body as descending fibers from the primary motor cortex cross to the opposite side forming the corticospinal tract, which is the principal motor tract of the body (Snell, 2001).

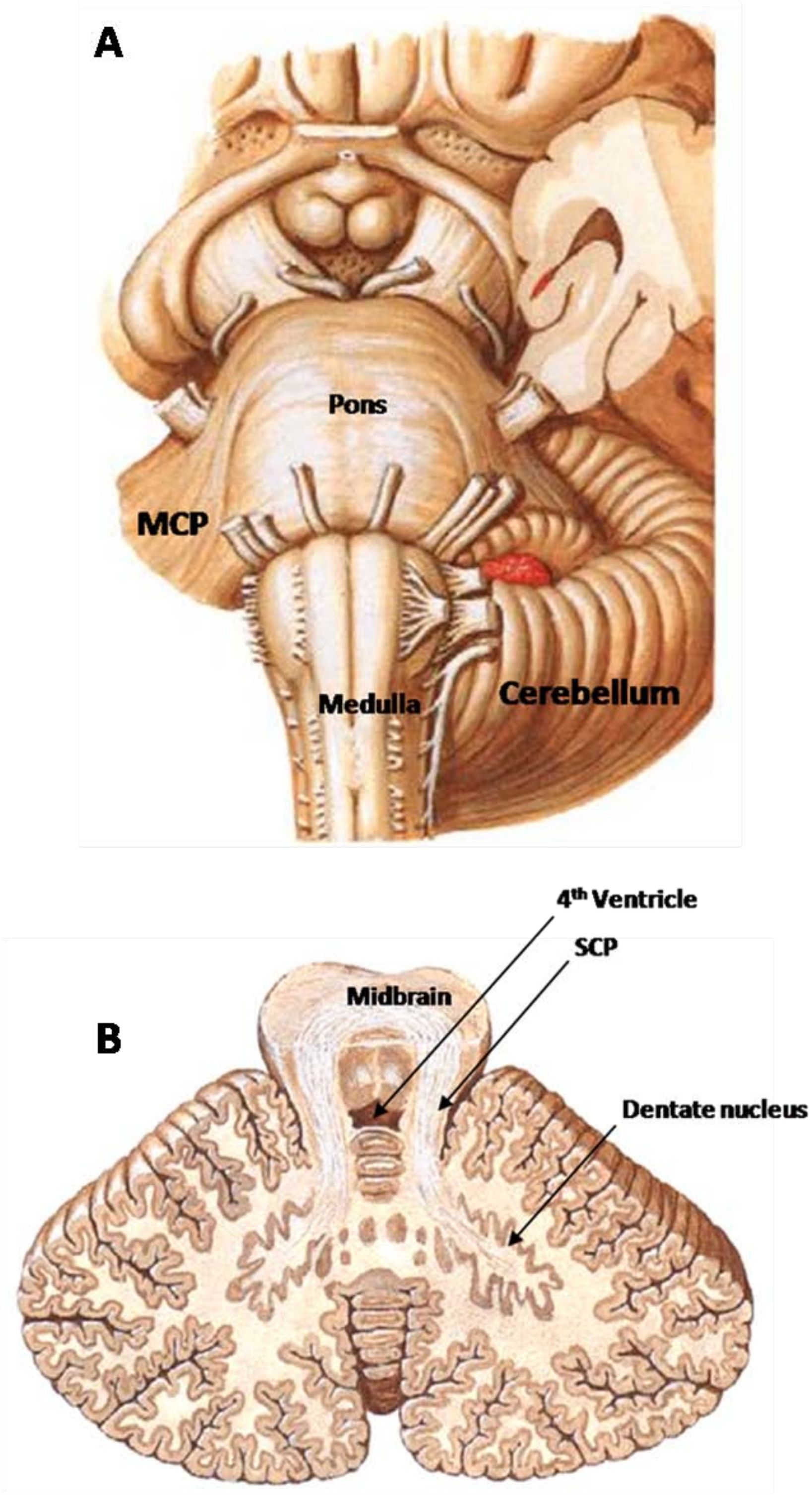


Fig.1.2. The superior and middle cerebellar peduncles. **A**, a ventral view of the brain showing the MCP. **B**, a coronal section through the cerebellum showing the SCP and dentate nucleus. SCP, superior cerebellar peduncle; MCP, middle cerebellar peduncle. Modified from (Netter, 2010).

1.7 NEUROPLASTICITY

1.7.1 OVERVIEW AND BACKGROUND

According to Cambridge dictionary, the word plasticity is defined as the quality of being soft enough to be changed into a new shape (Cambridge dictionaries online, http://dictionary.cambridge.org/). A defining characteristic of the brain is its remarkable capacity to undergo activity-dependent morphological and functional remodeling via mechanisms of plasticity that form the basis of our capacity to encode and retain memories. The process of neuroplasticity may occur during maturation, learning, skill acquisition, environmental challenges, or pathology (Zilles, 1992, Lledo et al., 2006). Through this process, neurons are able to change their function, chemical profile (such as the amount and type of neurotransmitters), and structure (Woolf and Salter, 2000), and can get beyond the limitations of their own genome (Pascual-Leone et al., 2005). A comprehensive understanding of the process of neuroplasticity and its mechanisms is therefore essential given the wide scope with which this process is implicated. The great challenge is to manipulate neuroplasticity for optimal outcome (Pascual-Leone et al., 2005). In rehabilitation medicine for example, the concept of neuroplasticity has been utilized to develop novel task-oriented therapies that could help in regaining functions following brain injury (Schneider et al., 2007, Schlaug et al., 2009, Lin et al., 2010a).

Until around the 1970s, an accepted idea across scientific literature was that the nervous tissue is essentially static throughout adulthood, both in terms of brain functions, as well as the idea that it is impossible for new neurons to develop after birth. However, over the past three decades, animal research has established two phenomena with a profound impact for understanding neuroplasticity. The first is significant microstructural plasticity in the adult brain, such as increased number of synapses, increased number of glial cells, and the formation of new blood capillaries, which account for learning and acquisition of new skills (Black et al., 1990, Anderson et al., 1996, Kleim et al., 1998a, Anderson et al., 2002, Swain et al., 2003, Kleim et al., 2004, Matsuzaka et al., 2007). The second is that brain re-mapping following injury could be stimulated by retraining animals to perform functions impaired by this injury (Nudo et al., 1996). These findings supported the assumption of a permanently plastic brain, and sparked an intense interest in studying neuroplasticity in humans.

1.7.2 PRINCIPLES OF EXPERIENCE-DEPENDENT NEUROPLASTICITY

1.7.2.1 ENRICHED ENVIRONMENT

Animal studies have indicated that the structure of the brain is, in part, a function of the environment in which an animal has been raised. Animals raised in a complex environment showed structural morphological changes compared to isolated rearing (Globus et al., 1973, Greenough and Volkmar, 1973). The concept of an enriched environment, first described by Hebb (Hebb, 1947), is the classical and most widely used experimental paradigm to study training-induced plasticity. Using this experimental paradigm, scientists could compare the behavior and morphological changes of animals living in an enriched environment with those living under the usual rather plain conditions of laboratory housing. It has been shown that extended training, which induces a specific brain function can lead to an enhancement of that function (Kleim and Jones, 2008), the concept of use it and improve it. In this context, several animal studies have reported training-induced plasticity in different brain regions (Anderson et al., 1996, Nudo et al., 1996, Kim et al., 2002, Cuppini et al., 2007, Ferreira et al., 2010, Real et al., 2010). For instance, monkeys trained to use a rake-shaped tool to retrieve a piece of food placed out of reach showed stronger and denser temporo-parietal junction connections in the intraparietal sulcus compared with naive monkeys (Hihara et al., 2006). Furthermore, rats trained to reach outside of their cage to retrieve food rewards were shown to have increased distal forepaw representations within the motor cortex (Kleim et al., 1998a, Kleim et al., 2002). Behavioral developments associated with environmental enrichment include enhanced skill learning and memory function, and reduced age-related memory decline (Pysh and Weiss, 1979, Anderson et al., 1996, Kleim et al., 1998b, Rampon et al., 2000, van Praag et al., 2000, Kim et al., 2002). At the microscopic level, environmental enrichment results in a range of morphological changes, such as cellular proliferation, angiogenesis, microglia activation (Gage, 2002), de novo synaptogenesis (Leuner et al., 2003), and conversion of silent to active synapses (Markham and Greenough, 2004, Butz et al., 2009). Results of training-induced plasticity in animals have sparked a considerable interest to study the effect of environmental enrichment in the human brain using *in vivo* neuroimaging techniques (Maguire et al., 1997, Maguire et al., 2000, Mechelli et al., 2004, Maguire et al., 2006a, Lee et al., 2007, Jancke et al., 2009, Park et al., 2009). Conversely, it has been shown that neurons not actively participating in neural activity for a long period of time start to degenerate, the concept of use it or lose it. This concept was first described by Hubel and Wiesel, 1965 who found that depriving a kitten's eye of light reduced the number of neurons in the visual cortex that responded to light (Hubel and Wiesel, 1965). Likewise, it has been shown that auditory deprivation of cats through cochlear ablation resulted in loss of auditory cortical sound representation (Reale et al., 1987), and reduction in synapse number (Perier et al., 1986). The above evidence indicates the presence of substantial environmental influence on the structure and function of the adult brain; however, genetic effects (Thompson et al., 2001) and interactions should also be taken into consideration to understand the true potency of neuroplasticity.

1.7.2.2 SPECIFICITY

The nature of the learnt skill dictates the pattern of the plasticity (Kleim and Jones, 2008). The literature indicates that the production of significant anatomical-functional changes in the brain requires learning or skill acquisition, rather than mere use. For example, it has been shown that rats allocated to an acrobatic training task have more parallel and climbing fiber synapses, and dendritic growth in the cerebellum than rats allocated for voluntary exercise condition or an inactive condition (Kleim et al., 1998b, Kim et al., 2002). Training-induced structural brain changes also show regional specificity. For example, rats learnt a spatial navigation task had increased dendritic length and branching in the visual cortex (Greenough et al., 1979, Kolb et al., 2008). Rats learnt unilateral motor reaching task demonstrated increased dendritic length and branching in the motor cortex contralateral to the trained limb (Greenough et al., 1985, Kolb et al., 2008), while those trained bilateral motor tasks showed increased dendritic growth in motor cortices in both cerebral hemispheres (Kolb et al., 2008). Additionally, classical conditioning in mice using whisker stimuli was shown to specifically expand cortical representation of trained whiskers (Siucinska and Kossut, 1996).

1.7.2.3 REPETITION OF STIMULATION

One of the key factors that drives plasticity is the presence of sufficient repetition of newly learned behavior (Kleim and Jones, 2008). Continuous rehearsal of the acquired skill is essential to induce lasting structural and functional brain changes resistant to decay when training ends (Monfils et al., 2005). For example, Trepel and colleagues (Trepel and Racine, 1998) investigated the effect of daily stimulation on cortical activation patterns in rats using electrodes implanted into the frontal and parietal regions of the brain. Lasting long-term potentiation (LTP) of synaptic responses could be induced by increasing the number of stimulation trains delivered in each session and by increasing the number of sessions; animals that received 60 trains per day demonstrated stronger response compared to those that received 5 and 10 trains per day. In humans, changes in cortical activation patterns induced by short- (20 minutes) and long-term (5 weeks) piano learning were investigated in musically naive subjects using auditory and motor tasks (Bangert and Altenmuller, 2003). Although activation patterns occurred after only 20 minutes, the effect did not reach a peak level until after 5 weeks of training.

1.7.2.4 INTENSITY OF STIMULATION

Apart from repetition, the intensity of training has a profound impact on driving plasticity. For example, in the above-mentioned experiment of Trepel and colleagues (Trepel and Racine, 1998), animals received stimulation trains with different pulse intensities of 16, 32,160, and 500 µA. Results showed that high-intensity stimulation trains of (500 µA) were sufficient to induce LTP, while animals received 16 and 32 µA trains showed no significant changes compared to controls. Additionally, microscopic evidence of structural plasticity in the form of increased dendritic arborization (Kleim et al., 2002) was demonstrated in animals trained to make 400 reaches per day to retrieve food pellets, but not in those trained to perform 60 reaches per day (Luke et al., 2004). In accord, the total size of the cerebellum in professional musicians was shown to be positively correlated with the intensity of musical performance (practice time per day) (Hutchinson et al., 2003).

1.7.2.5 EFFECT OF AGE

Early studies suggested that age-related cognitive decline arise primarily from reduction in the number of neurons; however, several recent studies have indicated that neuronal number is relatively preserved in the healthy aging brain (Peters et al., 1994, Peters and Sethares, 2002), and that aging is mainly associated with alterations in neuronal morphology in the form of regression of dendritic spines together with a reduction in the total number of synapses. Reduced synaptic density has been demonstrated in old rats (Chen et al., 1995, Long et al., 2009), monkeys (Peters et al., 2008, Hara et al., 2011) and humans (Masliah et al., 1993), and was shown to be associated with physiological degradation (Luebke et al., 2004). In accord, *in vivo* neuroimaging studies in humans have reported age-related alteration of global morphometric properties, including a decline in total brain volume (Blatter et al., 1995), GM volume (Jernigan et al., 1991), and cortical thickness (Salat et al., 2004). These age-related changes were hypothesized to be the inverse of development; the late maturing regions of the brain are especially susceptible to atrophic changes (McGinnis et al., 2011). It could thus be expected that plasticity responses are different in the aged brain (Nieto-Sampedro and Nieto-Diaz, 2005). Nevertheless, modern neuroscience adopts the assumption of a permanently plastic brain (Pascual-Leone et al., 2005). Activity-dependent plastic changes have been demonstrated in the aging brain though brain changes may be less prominent and/or slower to take place than those observed in younger brains (Green et al., 1983). For example, it has been demonstrated that exposure to an enriched environment is associated with enhanced neurogenesis and angiogenesis in aged animals (Kempermann et al., 2002, Ueda et al., 2005), which was associated with significant behavioral gain. Regarding humans, several studies have indicated that the human brain maintains the capacity to change its structure according to learning and cognitive exercise demands (Colcombe et al., 2006, Boyke et al., 2008, Engvig et al., 2010). For example, elderly subjects who learned to juggle showed increased GM in the visual cortex, left hippocampus, and in the nucleus accumbens bilaterally (Boyke et al., 2008). Furthermore, evidence of structural (Schlaug et al., 2009) and functional neuroplasticity (Schaechter et al., 2002) were reported in elderly stroke patients following the use of different rehabilitation regimes suggesting that age is not per se a limiting factor for neuroplasticity.

1.7.3 MECHANISMS OF NEUROPLASTICITY AT MICROSCOPIC LEVEL

A major goal of neuroscience has been to explore the cellular and synaptic mechanisms that underlie memory function, learning, aging, and lesions. With the advent of electron microscopy and modern electrophysiological, immunohistochemical, and *in vivo* microscopic imaging techniques, considerable progress has been made in describing cellular mechanisms for learning and plasticity (Denk et al., 1990, Raymond et al., 2002, Ferguson et al., 2004, Becker et al., 2008). The functional and anatomical malleability of the nervous system is, to a large extent, the plasticity of the synapses. At the microscopic level, cellular mechanisms underlying cortical plasticity include physiological mechanisms (functional reorganization of neurons and synapses) and structural mechanisms (rewiring of cortical circuits through the formation of new synapses, removal of old synapses, and morphological changes in the synapses) (Feldman, 2009). In general, it has been proposed that learning and skill acquisition induce rapid physiological plasticity through activation of N-methyl-D-aspartate (NMDA) receptor-dependent LTP and long term depression (LTD). These physiological mechanisms are followed by slower structural changes of nervous tissue to consolidate plasticity (Buonomano and Merzenich, 1998, Nieto-Sampedro and Nieto-Diaz, 2005). Here, I focus on several of the best-studied forms of physiological and structural mechanisms and their roles in neuroplasticity.

1.7.4 PHYSIOLOGICAL MECHANISMS OF NEUROPLASTICITY

1.7.4.1 SYNAPTIC STRENGTHENING: LONG-TERM POTENTIATION

Long-term potentiation is an enduring enhancement of synaptic transmission following high frequency electric stimulation. This process was originally suggested by Hebb (Hebb, 1947) who postulated that a synapse used repeatedly is made more efficient, i.e. reinforced (a hebbian synapse). Once reinforced, synaptic stimulation threshold becomes lower and the synapse can be activated by stimuli of lower intensity than originally required; or when activated by the same stimulus, it produces a response of greater amplitude (Nieto-Sampedro and Nieto-Diaz, 2005). Bliss and colleagues (Bliss and Gardner-Medwin, 1973, Bliss and Lomo, 1973) were the first to demonstrate LTP electrophysiologically in the neurons of the hippocampus. Later, LTP was shown to occur in many cortical regions (Tsumoto, 1992, Maren, 2005, Abidin et al., 2006, Bruel-Jungerman et al., 2007, Cui et al., 2011, Sheynikhovich et al., 2011, Meis et al., 2012). Repeated physiological activation of a synapse is translated into a set of morphological synaptic changes that ensure a record of long duration (from weeks to several months) (Bruel-Jungerman et al., 2007). Results of the past four decades of research have provided compelling support for the idea that LTP-induced synaptic changes play an essential role in adult learning and memory function (for review, see (Martin et al., 2000)). The primary mechanism for LTP induction is the activation of NMDA receptor which requires two simultaneous events: the binding of glutamate released from presynaptic boutons, and a sufficient level of postsynaptic depolarization mediated via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor activation (Nieto-Sampedro and Nieto-Diaz, 2005). When these conditions are fulfilled, calcium flows into the postsynaptic neuron through the NMDA channel, which is the triggering event in LTP (Bliss and Collingridge, 1993). The rise in intracellular calcium triggers a host of intracellular events, including activation of several kinases and transcription factors that ultimately result in modifications of the properties of synaptic receptors and induction of specific genomic programs (Huang et al., 1996, Kandel, 1997). Modifications of synaptic receptors include phosphorylation of their subunits and enrolment of extra-synaptic AMPA receptors (Bruel-Jungerman et al., 2007), while activation of genomic programs in neurons leads to de novo synthesis of proteins (Frey and Morris, 1997), and a number of morphological changes in synapses (see below). These changes result in lasting modifications of the synapse and long-term consolidation of memories.

1.7.4.2 SYNAPTIC ELIMINATION/WEAKENING: LONG-TERM DEPRESSION

Long term depression is an electrophysiological phenomenon equivalent but opposite to LTP (Ito, 1989), which can be induced by low frequency stimulation of a synapse. This process results in shrinkage or even disappearance of synaptic connections in neural networks (Zhou et al., 2004). The mechanism of LTD induction is essentially similar to that of LTP, involving NMDA receptor activation and elevation of intracellular calcium concentration; however, the increase in calcium concentration is much lower than that after LTP induction. Unlike LTP, which activates kinases, the mechanism of LTD involves activating calcineurine, a synaptic phosphatase with high affinity for calcium (Lisman, 1989). This would trigger several molecular mechanisms, including dephosphorylation of specific residues on glutamate-containing AMPA receptors and internalization of AMPA receptors (Collingridge et al., 2004). It was hypothesized that LTD could serve as a tool to weaken unused connections, thereby facilitating the emergence of reinforced connections in the network, which enhances the network's capacity for information storage (Bruel-Jungerman et al., 2007). Previous studies have indicated that LTD has a role in the formation of long-term memory and plasticity. For example, LTD mechanisms were shown to be the dominant form of plasticity process in the cerebellum (De Zeeuw and Yeo, 2005). In the hippocampus, it was demonstrated that LTD induction is essential for complex visuospatial learning in rats (Kemp and Manahan-Vaughan, 2007). Furthermore, it has been shown that induction of genetic mutations in mice, which impairs LTD in the hippocampus, could result in selective deficit in spatial memory (Etkin et al., 2006). Since most neural networks could support both LTP and LTD mechanisms, it was suggested that the main role of LTD in plasticity is to maintain a constant overall synaptic drive in a network, which would prevent chronic excitation of neurons and the saturation of neural circuits thereby maintaining the capacity for learning and memory storage (Moser et al., 1998).

1.7.5 STRUCTURAL MECHANISMS OF NEUROPLASTICITY

1.7.5.1 SYNAPTOGENESIS AND SYNAPSE REMODELING

Physiological patterns of plasticity may not be sufficient to stabilize neuronal activities. Structural synaptic changes may thus be required to hardwire functional changes. It has been shown that LTP and learning could induce remodeling of neural circuits through de novo synaptogenesis (the physiological process of increasing synapse number and dendritic complexity)(Chang et al., 1991, Toni et al., 1999) and synapse renewal (Markham and Greenough, 2004, Nieto-Sampedro and Nieto-Diaz, 2005). Renewal of synapses includes four main stages triggered by the release of neurotrophins (Trachtenberg et al., 2002, Feldman, 2009): retraction of dendritic spines and disconnection of old synapses, growth of new axons and axon sprouting, formation of new synaptic contacts, and maturation of the new synapses. Neural circuits with higher capacity for synapse renewal have a greater potential for plastic changes. In addition to synaptogenesis, environmental enrichment could also result in a host of morphological changes at existing synapses, such as increased growth, arborization, surface-to-volume ratio, and contact boutons of dendritic spines together with changes in the length of post synaptic density, number of synaptic vesicles, and number of vacant spines (Toni et al., 1999, Geinisman, 2000, Toni et al., 2001, Yuste and Bonhoeffer, 2004, De Paola et al., 2006, Holtmaat et al., 2006, Knott et al., 2006). Increased vacant synaptic elements, which are either newly formed or arise from the breakdown of existing synapses, can induce synaptic rewiring through stimulating the production of neurotrophic factors capable of inducing axonal outgrowth and sprouting (Holland and Brown, 1980), and re-routing of old axons to different postsynaptic targets (De Paola et al., 2006). Other morphological changes associated with LTP include changes in synapse curvature and the appearance of perforations in the synapses (Agnihotri et al., 1998, Weeks et al., 2001, Connor et al., 2006). It has been postulated that increased synaptic curvature brings the receptor closer to the synaptic cleft, which increases the possibility of activation of the postsynapse (Bruel-Jungerman et al., 2007). The appearance of perforated synapses was suggested to precede synapse splitting, giving rise to multiple morphologically distinct synapses (Luscher et al., 2000). The net effect of these structural synaptic changes is the increase in stability and strength of synapses, the emergence of new functional units, and the conversion of dormant to active synapses (Bruel-Jungerman et al., 2007). In contrast to the above mentioned morphological synaptic changes associated with LTP, it has been reported that induction of LTD causes retraction of axonal branches (Chang et al., 1995) and weakening of synapses and spines (Zhou et al., 2004). Weak synapses are liable to break again (Becker et al., 2008).

1.7.5.2 NEUROGENESIS

It was classically thought that neurogenesis, the generation of new neurons, is generally confined to a discrete developmental period; however, this phenomenon was clearly demonstrated in adult animals (Kornack and Rakic, 1999, Aberg et al., 2000) and humans (Eriksson et al., 1998, Eriksson, 2003, Knoth et al., 2010). The process of neurogenesis has been reported to take place in different brain regions, including the hippocampus and olfactory bulb (Alvarez-Buylla and Garcia-Verdugo, 2002, Rakic, 2002), neocortex (Gould et al., 1999b), striatum (Fowler et al., 2002), substantia nigra (Zhao et al., 2003), and amygdala (Duchesne et al., 2002). Adult neurogenesis involves proliferation of stem cells, morphological and physiological maturation of newborn cells, and synaptic incorporation into existing neural circuits (Bruel-Jungerman et al., 2007). Recent studies have indicated that adult neurogenesis can be stimulated by a variety of factors, including environmental enrichment, physical activity, and learning (Kempermann et al., 1997, van Praag et al., 1999a, van Praag et al., 1999b, Brown et al., 2003). For example, animals exposed to an enriched environment showed increased neurogenesis, which was correlated with enhanced performance in spatial memory (Nilsson et al., 1999, Dhanushkodi et al., 2007) and novel object recognition tasks (Bruel-Jungerman et al., 2005). Also, animals enrolled in running exercise demonstrated increased number of newborn cells compared with littermates enrolled in ordinary activities (van Praag et al., 1999b). Exercise does not only increase the number of new neurons but also influences the morphology of individual newly born cells, i.e., it has qualitative and quantitative effects (Zhao et al., 2006). Theories of training-induced neurogenesis include modulation of the function of neurotransmitters, increased expression of growth factors and neurotrophins, and enhanced angiogenesis. Excitatory neurotransmitters, such as glutaminergic system are up-regulated, whereas inhibitory neurotransmitters, such as gama-aminobutyric acid system are down-regulated (Molteni et al., 2002, Kleim et al., 2003a, Kleim et al., 2003b). The crucial role for adult neurogenesis in learning and memory was also derived from studies showing that ablation of the process of neurogenesis through irradiation, antimitotic agents, and genetic ablation, impairs spatial learning and performance in working memory tasks (Madsen et al., 2003, Winocur et al., 2006).

Although millions of new neurons are generated each day, a large number will not complete their maturation cycle and die within the first few weeks (Kempermann et al., 2003). It has been shown that the same factors implicated in neurogenesis, namely environmental enrichment, physical activity, and learning can rescue young neurons from death and increase their chance to survive (Gould et al., 1999a, Leuner et al., 2004, Wu et al., 2008, Epp et al., 2010). These young neurons have a lower threshold for LTP induction and produce stable LTP more readily than more mature neurons (Snyder et al., 2001, Saxe et al., 2006). It is thus expected that newly born neurons are more vulnerable to plastic effect and can increase the overall computational capacity of the neural circuit within which they are embedded.

1.7.6 NEUROPLASTICITY AT MACROSCOPIC LEVEL, EXPLORING EXPERIENCE-DEPENDENT PLASTICITY IN THE ADULT HUMAN BRAIN USING *IN VIVO* IMAGING

As described earlier, animal studies have indicated that the adult brain is not as hardwired as previously thought, and that it maintains the capacity for synaptic rewiring (Chklovskii et al., 2004). The search for anatomical markers of extraordinary skills has fascinated researchers for many years. Reports of experience-dependent microstructural plasticity in brains of adult animals (Black et al., 1990, Anderson et al., 1996, Kleim et al., 1998a, Anderson et al., 2002, Swain et al., 2003, Kleim et al., 2004, Matsuzaka et al., 2007) had excited neuroscientists to search for equivalent changes in humans. However, a crucial limitation of postmortem studies lies in their invasive nature, and the fact that they can identify only very localized morphological changes due to the nature of the techniques used. Imaging techniques offer the opportunity to investigate human brain organization *in vivo* noninvasively. In particular, MRI has become the preferred imaging technique for the detection of structural and functional evidence of neuroplasticity due to its non-invasiveness, high spatial resolution, and the ability to perform whole-brain examination of GM and WM regions. In this context, a vast number of cross-sectional imaging studies revealed structural (macroanatomical) and functional correlates of learning and experience in different cognitive domains in healthy brains shedding light on structural (Maguire et al., 1997, Maguire et al., 2000, Mechelli et al., 2004, Maguire et al., 2006a, Lee et al., 2007, Jancke et al., 2009, Park et al., 2009), and functional (Elbert et al., 1995, Sacco et al., 2009, Takeuchi et al., 2011) plasticity phenomena in adult humans. Examples of functional neuroplasticity include increased size of somatosensory cortical maps of the digits of the left hand in violinists (Elbert et al., 1995), increased auditory cortical representation of piano tones in keyboard players (Pantev et al., 1998), and increased cortical activations in inferior parietal cortex and occipito-temporal regions in healthy subjects who learned to decode Morse code (Schmidt-Wilcke et al., 2010). At the structural level, coarse anatomical plastic changes have been observed using *in vivo* MR morphometry, voxel-based morphometry (VBM), and DTI. A common finding across these studies is the enlargement of the representative area that underlies the particular skill. For example, Maguire and colleagues reported that the right hippocampus, which is crucial for spatial memory and navigation, is preferentially enlarged in London taxi drivers compared to control population (Maguire et al., 2000), and bus drivers (Maguire et al., 2006b). Other examples of training-induced GM changes include increased GM density in the inferior parietal lobe in academic mathematicians, which was significantly correlated with the time spent as an academician (Aydin et al., 2007); and increased GM volume in premotor and parietal regions in skilled golfers compared to less skilled golfers and control subjects (Jancke et al., 2009). Experience-dependent structural MR changes are not restricted to GM but were also reported in WM regions. Training of a complex visuomotor skill was shown to increase FA of the WM underlying the intraparietal sulcus (Scholz et al., 2009). Similarly, enhanced visual memory performance was shown to be correlated with anisotropy measures of WM regions (Tuch et al., 2005, Begre et al., 2007), prompting the suggestion that increased myelination plays an important role in driving training-induced plasticity. A recent study that investigated WM plasticity in long-term trained Baduk players, a game that requires higher order cognitive capacities, such as learning, spatial imagination, and abstract reasoning, has demonstrated increased FA measures in Baduk players in the frontal and cingulum regions, which support attentional control, working memory, and problem-solving ability compared to inexperienced control subjects (Lee et al., 2010). Other studies reported combined GM and WM changes induced by learning, such as increased GM density (Mechelli et al., 2004, Lee et al., 2007), and WM density (Golestani et al., 2002) in the parietal lobe of faster phonetic learners, and those with better vocabulary knowledge. Faster learners were also shown to have greater WM density in the left Heschl gyrus compared with slower learners (Golestani et al., 2007). Interestingly, morphometric measures of pre-defined brain regions were even used to predict learning capacities of novel speech sounds (Golestani et al., 2007, Golestani and Pallier, 2007). Taken together, the above evidence provides support for the assumption that enhanced performance is associated with greater structural measures of related brain regions. However, a considerable limitation of morphometric analyses using MR data acquired at a single time point is the inability to distinguish the independent effect of learning on brain structure from diverse environmental and/or genetic influences (Schlaug, 2001). Longitudinal studies are therefore needed to acquire imaging data at multiple time points to disclose temporal characteristics of experience-induced plasticity changes. In this context, an interesting longitudinal MR study has investigated plasticity in healthy adults trained to perform 3-ball cascade juggling, a complex visuomotor task (Draganski et al., 2004). It was shown that three months training on juggling established the ability to achieve the required minimum of one-minute 3-ball cascade juggling. Subject were scanned at three time points: before staring to learn how to juggle, three months later when their juggling ability was tested, and another three months later following a period of juggling cessation. Compared to control subjects, MR examination performed at the second time point revealed GM increase in the extrastriate motion specific area bilaterally and in the left inferior parietal sulcus in jugglers. At the third time point, these GM changes have disappeared almost to base line, which was associated with reduced juggling performance compared to the second time point.

In addition to the wealth of literature suggesting a clear correlation between skill acquisition and structural properties in the healthy brain, our knowledge of neuroplasticity in the adult human brain was further extended by results of clinical studies, which demonstrated morphological brain changes induced by nervous system injury. For example, patients with unilateral vestibulo-cochlear lesions exhibited increased GM density in the primary somatosensory cortex and motion-sensitive areas in the medial temporal gyrus, which was correlated with improvement in vestibular and hearing functions (Helmchen et al., 2011). Additionally, blindness-induced alteration in motor experience was shown to drive morphological adaptations in WM structure in the form of increased average FA of the corticospinal tract (Yu et al., 2007). MRI can also provide a biomarker of outcome after brain injury and following the application of rehabilitation regimes. For example, at the structural level, a DTI study reported that patients with chronic Broca’s aphasia who undergone intonation-based speech therapy showed increased number of fibers and volume of the arcuate fasciculus compared to pre-treatment assessments (Schlaug et al., 2009). At the functional level, hemiparetic stroke patients treated with constraint-induced movement therapy, a physical rehabilitation regime that has been shown to improve motor function, showed increased cortical activations in the contralesional hemisphere during movement of the affected and unaffected hands, which was associated with significant motor improvement (Lin et al., 2010b).

Unfortunately, the nature of the cellular events underlying *in vivo* macroscopic changes in brain structure cannot be explored with the current resolution of MR images. Results of animal studies (described above) could provide indirect evidence to the possible cellular events underlying MR-derived measures in humans, such as cellular proliferation (Gage, 2002), angiogenesis (Pereira et al., 2007), synaptogenesis (Kleim et al., 2002), increased dendritic growth and axonal fiber densities (McAllister et al., 1995, Markham and Greenough, 2004, Butz et al., 2009), increased synaptic connectivity patterns (Chklovskii et al., 2004), in addition to changes in gene expression and protein synthesis (McAllister et al., 1995, Kleim et al., 1996). Future advance in MR technology could make it possible to achieve higher anatomical resolution (Nakada et al., 2008, Duyn, 2010) to explore the microscopic structure of the human brain *in vivo* thereby expanding our knowledge about the process of neuroplasticity.

Over the past two decades, a considerable amount of research has been devoted to identifying evidence of training-induced plasticity in brains of musicians. Professional musicians were considered the ideal model to investigate experience-driven neuroplasticity in healthy adults as they have the advantage of being intensively exposed to a complex stimulus, music. A review of structural and functional studies conducted on musicians’ brains is provided in the next chapter (chapter 2).

CHAPTER 2

REVIEW OF LITERATURE

THE MUSICIAN'S BRAIN AS AN IDEAL MODEL TO STUDY PLASTICITY IN HUMAN

2.1 AIM

The aim of this chapter is to provide a literature review of anatomical and functional studies that investigated evidence of plasticity in musicians' brains. This is followed by presenting the rationale for conducting structural examinations in the present work, research aims, and research hypotheses.

2.2 THE MUSICIAN'S BRAIN AS AN IDEAL MODEL TO STUDY PLASTICITY IN HUMANS

"…the work of a pianist…is inaccessible for the untrained human, as the acquisition of new abilities requires many years of mental and physical practice. In order to fully understand this complicated phenomenon it is necessary to admit, in addition to the strengthening of pre-established organic pathways, the establishment of new ones, through ramification and progressive growth of dendritic arborizations and nervous terminals.…Such a development takes place in response to exercise, while it stops and may be reversed in brain spheres that are not cultivated".—Santiago Ramón y Cajal, Textura del Sistema Nervioso del Hombre y de los Vertebrado. Cited in (Pascual-Leone, 2001).

Music is one of the oldest and most basic sociocognitive domains of the human species. It was assumed that human musical abilities played a key phylogenetical role for the evolution of language, and that music making behavior covered important evolutionary functions, such as communication, group coordination, and social cohesion. Musical communication in early childhood (such as maternal music) was suggested to play a key role in cognitive, emotional, and social maturation of children (Trehub, 2003).

The musician's brain was considered an ideal model to study neuroplasticity in humans (for review see (Schlaug, 2001, Munte et al., 2002). Musicians differ from subjects with other professions in two major aspects. First, they begin their training at an earlier age when many brain regions have not approached optimum maturity, hence vulnerable to plastic changes imposed by multimodal stimuli (Watanabe et al., 2007). Second, to master their profession, musicians must practice for several hours per day even outside working hours. As playing music is an enjoying act, this would not result in physical fatigue offering them more time to practice (Watson, 2006). Taken together, the above evidence indicates that musicians have an early, long-term, and intensive skilful practice sufficient to impose plastic changes in their brain. Playing an instrument is a multisensory motor experience that requires several skills, including reading musical notes, which contain complex symbols followed by translating these notes into sequential motor activity. It also involves memorizing long musical passages and improvising within given musical parameters (Bengtsson et al., 2007). One can imagine the complexity of musical performance by the assumption that a pianist can coordinate the production of up to 1800 musical notes per minute (Munte et al., 2002). It is therefore not surprising that musicians have been the subject of tens of imaging studies set to search for evidence of plasticity in their brains.

Neuroimaging studies in musicians have provided compelling evidence that training and enhanced use of a body part cause plastic changes in brain architecture. Coarse anatomical plastic changes have been observed using in vivo MR morphometry, voxel-based morphometry (VBM), and DTI (Schlaug et al., 1995a, Schlaug et al., 1995b, Schmithorst and Wilke, 2002, Gaser and Schlaug, 2003a, Hutchinson et al., 2003, Bengtsson et al., 2005, Imfeld et al., 2009). In particular, those brain areas involved in controlling music specific behavior, such as motor control and auditory processing revealed anatomical differences compared to non-musicians. A common finding across these studies is the enlargement of the representative area that underlies that particular skill in musicians. On the other hand, evidence of functional plasticity in musicians was elucidated using techniques, such as functional MRI (fMRI), magnetoencephalography (MEG), electroencephalography (EEG), positron emission tomography (PET), and behavioral studies (Sergent et al., 1992, Elbert et al., 1995, Koeneke et al., 2004, Vuust et al., 2005, Bangert et al., 2006, Limb et al., 2006, Magne et al., 2006, Baumann et al., 2007, Sluming et al., 2007, Watanabe et al., 2007). Evidence of functional neuroplasticity includes cross-modal integration, increased size of cortical representation maps, enhanced performance in sensorimotor tasks, and increased functional activation.

2.3 STRUCTURAL EVIDENCE OF PLASTICITY IN THE MUSICIAN'S BRAIN

Studies that investigated structural differences between musicians and non-musicians could be divided into whole-brain studies, which used VBM and DTI techniques, and studies that targeted specific areas of the brain through manual delineation of regions of interest with a variety of metrics and definitions.

Two pioneer studies that performed whole-brain VBM investigation (Sluming et al., 2002, Gaser and Schlaug, 2003a) revealed different sets of results, although both examined male professional musicians. In the first study (Gaser and Schlaug, 2003a), professional musicians possessed greater GM density in areas known to be involved in auditory, motor, somatosensory, and visuospatial processing compared to both amateur musicians and non-musicians. These areas include the left Heschl gyrus, primary motor area, premotor area, left cerebellum, somatosensory area, inferior temporal gyrus, superior parietal lobe, and left inferior frontal gyrus. The second study (Sluming et al., 2002) detected greater GM density in Broca's area in male orchestral musicians, which was positively correlated with the duration of musical performance. Apart from variable scanning parameters, the differences in findings may be attributed to demographics of recruited musicians, such as age of starting musical training and instrument specialty. The age of commencing musical training was around 7 years in the first study compared to 10 years in the second study. This factor is important in view of several reports, which showed that early musical training is associated with more robust structural and functional plastic changes in different brain regions (Elbert et al., 1995, Schlaug et al., 1995a, Amunts et al., 1997a, Watanabe et al., 2007, Li et al., 2010). In addition, the study of (Gaser and Schlaug, 2003a) has exclusively recruited keyboard players thereby increasing the likelihood of detecting significant group differences in motor cortical areas, whereas the cohort of (Sluming et al., 2002) included multi-instrumentalists and did not demonstrate differences in motor areas. However, a shared finding of these studies was the lack of significant WM differences between musicians and non-musicians, which might in part be related to limitations in the VBM technique itself as it might be insensitive to WM differences caused by low-intensity contrast differences between groups (Ashburner and Friston, 2000, Della Nave et al., 2008), underlying the significance of using more sensitive methods for studying WM structure in musicians, such as DTI. This *in vivo* neuroimaging technique offers a complementary way of studying the brain by directing interest towards WM integrity and axonal connectivity (Basser and Jones, 2002). It provides a method of characterizing the diffusivity of water molecules in the human brain, and can provide important information about WM structure that is not available from other imaging techniques (Basser et al., 1994, Pierpaoli et al., 1996). One of the most widely used DTI-derived metric is FA, which has been used as a scalar indicator of the directionality and coherence of fiber tracts (Basser and Pierpaoli, 1996). It was proposed that FA could provide information about WM microstructure, such as intra-axonal organization (Kinoshita et al., 1999), density of fibers, degree of myelination (Song et al., 2005), neuroglial cell packing, individual fiber diameter, and immunohistochemical parameters (Mori and Zhang, 2006, Sun et al., 2006, Trivedi et al., 2009). Postmortem studies suggested that axon diameter and myelin sheath undergo conspicuous growth during the first two years of life, but may not be fully mature before late adolescence (Benes, 1989) or even late adulthood (Benes et al., 1994), underlying the significance of investigating WM plasticity in musicians. Unlike GM, the WM has not been studied as extensive in musicians. Whole brain DTI studies showed that, compared to non-musicians, professional musicians have increased FA value in the right posterior limb of the internal capsule (Bengtsson et al., 2005, Han et al., 2009), which contains fibers of the corticospinal tract, the main motor tract in the brain. Increased FA value was also reported in the central portion of the cerebellum (Schmithorst and Wilke, 2002), genu of the CC (Schmithorst and Wilke, 2002), and the left inferior frontal gyrus (Han et al., 2009). Studies that targeted specific brain regions revealed convincing evidence of structural plasticity in musicians. Most of these studies reported on structural plasticity in the auditory and motor brain regions.

2.3.1 THE AUDITORY SYSTEM

The fact that musicians are challenged by no other profession in their expertise in the auditory domain has fascinated neuroscientists to relate such exceptional auditory skill to changes in brain anatomy. Prior to the era of MRI, postmortem examination of the brains of two imminent musicians revealed that the superior temporal gyrus was larger than the normal size (Meyer, 1977). Conventional MR imaging revealed a similar tendency in structures that process auditory stimuli namely, the Heschl gyrus and planum temporale. The Heschl gyrus includes the primary auditory cortex, which is involved in initial processing of auditory stimuli (Penhune et al., 1996, Yoo et al., 2005, Wong et al., 2007), while the planum temporale contains the secondary auditory cortex responsible for perceptual analysis and integration of sound stimuli (Griffiths et al., 1998, Zatorre, 1998, Ohnishi et al., 2001). It has been shown that professional musicians posses greater GM volume of anteromedial portion of the Heschl gyrus compared to amateurs and non-musicians (Schneider et al., 2002). A recent study that combined VBM and cortical thickness measurement corroborated these findings through demonstrating increased GM density and cortical thickness of the right Heschl gyrus, although the difference was mainly localized in the posterolateral portion of the gyrus (Bermudez et al., 2009). Moreover, it has been shown that GM volume of the lateral portion of the Heschl gyrus is correlated with instrument specialty (Schneider et al., 2005). Musicians who used high-pitched instruments, such as piano, trumpet, drums , or guitar exhibited greater GM volume in the left lateral Heschl gyrus, while those who used low-pitched instruments, such as bassoon, saxophone, or French horn exhibited greater GM volume in the right lateral Heschl gyrus (Schneider et al., 2005).

The size of the planum temporale in musicians was shown to correlate with absolute pitch ability, defined as the ability to identify a tone in the absence of an external reference tone (Takeuchi and Hulse, 1993). Musicians who possess this ability demonstrated more pronounced left -sided asymmetry compared to non-possessors and non-musicians (Schlaug et al., 1995b, Zatorre et al., 1998, Keenan et al., 2001, Luders et al., 2004). In agreement with these findings, fiber tractography of the superior longitudinal fasciculus, a tract that connects the frontal and temporal regions of the brain (including the planum temporale), revealed left-sided asymmetry in FA values in musicians with absolute pitch ability compared to musicians without absolute pitch ability and non-musicians (Oechslin et al., 2009). Furthermore, an interesting study that used MR spectroscopy reported greater concentration of N-acetylaspartate (NAA) in the planum temporale in musicians, which was correlated with the duration of musical performance (Aydin et al., 2005). As increased concentration of this metabolite was known to correlate with neuronal density (Cheng et al., 2002), it was hypothesized that long-term musical practice has stimulated the generation of new synapses and neurons, which was reflected by increased concentration of NAA. This study was the first to provide a direct *in vivo* evidence of ultra-structural neuroplasticity in musicians.

2.3.2 THE MOTOR SYSTEM

The main components of the motor system include the primary motor cortex, located in the precentral gyrus, and responsible for initiating motor activity; and the premotor cortex, located in the posterior parts of the superior, middle, medial, and inferior frontal gyri, and responsible for programming of a strategy of voluntary movement that directs the primary motor area in its execution (Williams et al., 1995, Lundy-Ekman, 2007). Another integral component of the motor system is the cerebellum, which plays an important role in coordinating motor activity, and producing the finest hand/finger movements. Neuronal impulses generated in the motor cortex of the brain travel within the corticospinal tract, the descending fibers of this tract converge in the corona radiata, the posterior limb of the internal capsule, midbrain, and pons. In the medulla oblongata, most of these fibers decussate to the contralateral side and descend into the spinal cord to supply all skeletal muscles in the body. Each corticospinal tract would thus supply the opposite side of the body (Williams et al., 1995). Professional musicians, especially keyboard players, constitute a perfect population for studying long-term plasticity in the motor system as their daily practice requires complex bimanual hand/fingers movements that should be coordinated to produce the best outcome. Amunts and colleagues (Amunts et al., 1997a) conducted manual measurement of the size of the primary motor cortex by estimating the intrasulcal length of the precentral gyrus (ILPG), defined as the length of the posterior bank of the precentral gyrus bordering the central sulcus. As expected, right-handed non-musicians had pronounced left-to-right asymmetry in the size of the ILPG; however, musicians exhibited a relatively symmetrical ILPG due to greater size of the ILPG in the right, non dominant hemisphere, which was supported at the behavioral level by reduced hand skill asymmetry in musicians. In accord, it has been reported that transcallosal inter-hemispheric inhibition is reduced in musicians compared to non-musicians as measured by applying transmagnetic stimulation to the hand area of the motor cortex (Ridding et al., 2000). The relative symmetry of brain structure and function is highly important for professional musicians, especially keyboard players in whom bilateral coordination of motor function is invaluable for efficient outcome.

A more recent study has further investigated the relationship between central sulcus morphology and musicianship through measuring intrasulcal length, surface area, and mean surface variability of anterior and posterior walls of the central sulcus using a 3D surface reconstruction (Li et al., 2010). The anterior wall of the central sulcus represents the posterior wall of the precentral gyrus (primary motor cortex), and its posterior wall represents the anterior wall of the postcentral gyrus (primary somatosensory cortex). Although no significant group differences were reported in intrasulcal length and surface area, musicians showed greater local variability in the middle section (representing the hand area) of the central sulcus, which was negatively correlated with age of commencement of musical training. These findings highlight the significance of studying the regional shape difference of brain structures as a marker of structural neuroplasticity.

In an interesting study, the shape of the cortical hand region was so distinctive that it was possible to distinguish musicians from non-musicians, and to classify musicians according to their instrument specialty. Bangert and Schlaug (Bangert and Schlaug, 2006) have visually inspected the omega sign or the 'precentral knob' of the central sulcus, a gross anatomical landmark of the precentral gyrus that corresponds to the middle section of the central sulcus and is known to be a reliable landmark of hand movement representation (Yousry et al., 1997). Musicians had more pronounced omega sign compared to non- musicians. Also, string players had more pronounced omega sign expression on the right hemisphere unlike keyboard players who had more pronounced expression on the left hemisphere. These findings reflect different requirements for development of hand/finger motor skills among these musicians as string players need to develop motor skill in their left, while keyboard players require good skills in both hands or more in the right hand (Bangert and Schlaug, 2006). In agreement, it has been shown that somatosensory cortical representation of the digits of the left hand (the fingering hand) was larger in string players (Elbert et al., 1995), which tends to correlate with early age of musical training.

Conventional MR and DTI studies of the WM tracts have supported the findings of motor cortical plasticity as musicians exhibited greater size of the anterior half of the CC indicating enhanced interaction between the motor areas of the two hemispheres (Schlaug et al., 1995a, Lee et al., 2003). Also, probabilistic tractography of the corticospinal tract revealed greater mean diffusivity in musicians (Imfeld et al., 2009). High level musical performance requires precise timing and temporal integration of movement, which are mediated by the cerebellum. Although the total cerebellar size constitutes only one-tenth of the total brain size, the cerebellar cortex contains four times the number of cells in the cerebral cortex (Andersen et al., 1992). Evidence of structural cerebellar plasticity has been shown in several experimental paradigms in animals, which reported significant plasticity in relation to motor skill acquisition. For example, it has been shown that motor-skill learnt rats have more parallel and climbing fiber synapses (Anderson et al., 1996, Kim et al., 2002), and that the volume of the molecular layer of the cerebellar cortex is larger in trained rats and mice than in the inactive group (Pysh and Weiss, 1979, Kleim et al., 1998). In accord, professional musicians exhibited greater cerebellar GM density (Gaser and Schlaug, 2003b, a, Han et al., 2009), and total cerebellar size (both GM and WM) compared to non-musicians (Hutchinson et al., 2003). Structural plasticity of the cerebellum was correlated with musical expertise as professional musicians showed greater GM density compared to amateur musicians and non-musicians (Gaser and Schlaug, 2003b, a). Also, there was a positive correlation between the intensity of musical practice and total cerebellar size

(Hutchinson et al., 2003). Unfortunately, there were no reports on plasticity of the cerebellar WM and cerebellar peduncles in musicians.

2.3.3 THE CORPUS CALLOSUM

The CC has received considerable interest in neuroimaging studies being the largest WM tract in the brain (Blume, 1984, Witelson, 1985), the latest maturing neural network (Pujol et al., 1993, Hasan et al., 2008, Hasan et al., 2009), and the most easily identified structure on midsagittal MR images. The CC connects homologues cortical regions in each cerebral hemisphere thereby playing a crucial role in many forms of unified motor, sensory, and cognitive performances (Witelson, 1985). Manual cross-sectional area measurement of the midsagittal CC revealed that musicians had greater anterior (Schlaug et al., 1995a, Lee et al., 2003), and posterior (Ozturk et al., 2002) CC size compared to controls. These differences were related to gender as only male musicians have increased size of the anterior CC (Lee et al., 2003), and age of commencement of musical training as increased CC size was observed in a group of musicians who commenced training before age of 7 years. Possible factors contributing to increased callosal size include more fibers crossing through the CC, thicker fibers, higher proportion of myelinated fibers, or a combination of these changes (Cowell et al., 1992, Schlaug et al., 1995a). The increased CC size in musicians might support enhanced inter-hemispheric communication subserving bimanual motor control (anterior CC), and auditory processing (posterior CC). There were few DTI studies that examined the CC in musicians. Schmithorst and Wilke (Schmithorst and Wilke, 2002) performed whole-brain DTI examination in a cohort of five musicians and seven non-musicians and reported that musicians have greater FA value in the genu of the CC. Two other studies reported no significant differences between musicians and non-musicians in all CC regions (Bengtsson et al., 2005, Imfeld et al., 2009), although there was a significant correlation between FA value of the body and splenium of the CC and total hours of piano practicing in children aged less than 11 years (Bengtsson et al., 2005).

2.4 FUNCTIONAL EVIDENCE OF PLASTICITY IN THE MUSICIAN'S BRAIN

It should be noted that the objective of the present work was to investigate structural plasticity in musicians; however, I found it prudent to present functional evidence of neuroplasticity as it allows better understanding of the effect of music and musical training/practice on the brain.

2.4.1 AUDITORY AND SOMATOSENSORY SYSTEMS

Extensive musical training and practice were associated with enlarged cortical representations in somatosensory and auditory regions in professional musicians. In the somatosensory cortex, plastic changes were shown to be specific for the frequently used fingers. In string players for example, cortical representation of the thumb was the smallest compared to other fingers of the left hand and, in the case of the right hand, there were no differences between musicians and non-musicians (Elbert et al., 1995). In the auditory domain, neuronal representations were equivalent in both musicians and non-musicians for pure tones of equal fundamental frequency and loudness. However, when piano tones were presented, tonotopic cortical representation was 25% larger in professional musicians (Pantev et al., 1998, Shahin et al., 2008). Moreover, a comparison between violinists and trumpeters revealed that auditory cortical representations were most pronounced for timbres of the instrument of training (Pantev et al., 2001) suggesting that long-term musical performance has resulted in timbre specificity for auditory processing.

The ability to detect incongruent rhythm is of extreme importance in musical performance. Compared to non-musicians, professional musicians showed a mismatch negativity (MMN), a marker of the pre-attentive detection of changes in regular sequences of auditory stimuli (Picton et al., 2000), for subtle deviations of the rhythm (Koelsch et al., 1999, Russeler et al., 2001). Likewise, musician children outperformed non-musician children in the detection of incongruent pitch in both music and language (Magne et al., 2006). The ability to detect wrong notes in sequential stimuli acts as a feedback mechanism enabling musicians to adjust their performance for the best outcome.

Functional studies have elucidated that musicians and non-musicians have distinct strategies to lateralize musical stimuli (Evers et al., 1999, Ohnishi et al., 2001, Limb, 2006, Limb et al., 2006). When passively listening to music, both groups tend to have increased brain activity in different brain structures, including bilateral superior temporal areas, left inferior parietal lobule, and right POP. However, musicians exhibited greater left hemisphere lateralization in a network of neural structures, including the superior temporal gyrus, planum temporale, POP, and inferior parietal lobule (Ohnishi et al., 2001, Limb, 2006, Limb et al., 2006). Most of these regions are also involved in language processing, which has led to the assumption that musicians tend to utilize an analytic mode of music processing, which is much like that of language (Vuust et al., 2005, Limb et al., 2006).

2.4.2 AUDITORY-SOMATOSENSORY INTEGRATION

Musical performance is a multimodal task that requires interaction and integration of multiple sensory modalities. Multisensory integration was defined by (Meredith and Stein, 1983) as an increase in neural response to a stimulus consisting of a combination of modalities in comparison to the sum of neuronal responses to each stimulus separately. The integration of multiple sensory inputs is especially important for professional musicians as it contributes to new qualities of perception not available in each sensory modality. Multisensory integration was investigated in a cohort of trumpet players and non-musicians. Somatosensory (lips and digits) and auditory stimuli were presented alone or in combination and responses were recorded using MEG (Schulz et al., 2003). As expected, the unimodal auditory response was larger in musicians; however, the unimodal somatosensory responses for lips and digits did not differ between musicians and non-musicians. Combined auditory and somatosensory stimulation of the lips in professional trumpet players revealed a multimodal interaction (expressed as the difference between the multimodal response and the sum of unimodal responses) in the somatosensory cortex, which was not seen in the control group suggesting cross modal somatosensory-auditory plasticity in trumpet players that is essential to master their instrument.

2.4.3 THE MOTOR SYSTEM

In the motor domain, a shared finding across functional studies was a reduction in hemodynamic response in professional musicians relative to non-musicians when performing complex unimanual (Hund-Georgiadis and von Cramon, 1999, Krings et al., 2000, Meister et al., 2005), and bimanual (Jancke et al., 2000, Haslinger et al., 2004) motor tasks. Areas involved include the primary motor cortex, premotor cortex, and the cerebellum (Hund-Georgiadis and von Cramon, 1999, Jancke et al., 2000, Krings et al., 2000, Haslinger et al., 2004, Koeneke et al., 2004, Meister et al., 2005). Extensive hand skill training allowed professional musicians to perform given finger movements through recruiting smaller number of neurons compared to non-musicians. It was shown that learning a new motor paradigm is not only easier for professional musicians, but also accompanied by weaker hemodynamic responses in the prefrontal cortex (Haslinger et al., 2004), a region known to be essential for skill learning being involved in imitation of hand actions (Vogt et al., 2007), procedural sequence learning (Jenkins et al., 1994), attention (Lau et al., 2004), and working memory (Robertson et al., 2001). It appears that daily practicing of skilled movement has resulted in less attention to action and less requirements for selection of the correct movement (Deiber et al., 1991, Botvinick et al., 1999), and error detection (Cohen et al., 2000, Rowe et al., 2000). Moreover, behavioral studies have indicated that musicians who began their musical training at an earlier age (around seven years) exhibited better performance in motor tasks compared to musicians who commenced their training later (Hughes and Franz, 2007, Watanabe et al., 2007).

2.4.4 AUDITORY-MOTOR INTEGRATION

The unique auditory-motor expertise of professional musicians makes them an ideal model to study integration between auditory and motor modalities. Activity in motor areas was reported during sound listening, and auditory areas were active during movement performance in skilled musicians suggesting the existence of a strong auditory-motor link, so that co-activation of the other modality occurs even when the task involves only auditory or only motor processing (Haueisen and Knosche, 2001, Lotze et al., 2003, Bangert et al., 2006, Baumann et al., 2007). This auditory-motor integration is essential to provide fast feedback mechanism that enables musicians to continuously coordinate complex movement patterns according to auditory input (Bangert and Altenmuller, 2003).

2.4.5 VISUOSPATIAL COGNITION

Apart from efficient auditory and motor skills, dexterous musical performance requires competent visuospatial cognition. Musical sight reading is the ability to read musical notes and translate these notations into movement patterns (Sergent et al., 1992), which is of paramount importance for professional musicians as it influences a musician's ability to perform a repertoire of rehearsed music. Music reading can be considered as a visuospatial sensorimotor task that involves the visuospatial analysis of symbolic input and the consequent generation of coordinated motor output (Stewart et al., 2003). Interestingly, changing the systematic relationship between the vertical position of a musical note and its corresponding position on the keyboard adversely affected the performance of pianists but not musically naive subjects (Stewart et al., 2004). Regions involved in sight reading ability include the left premotor cortex, Broca's area (specifically the POP), and superior parietal lobule of both hemispheres (Sergent et al., 1992, Stewart et al., 2003), which were activated when subjects read, listen, and play piano. There is a good evidence that professional musicians perform significantly better than non-musicians in spatial ability tasks. Musicians exhibited better performance scores than non-musicians in a test of spatial ability, the Benton judgment of line orientation test, which was positively correlated with musicians' age (Sluming et al., 2002). Sluming and colleagues (Sluming et al., 2007) investigated musicians' performance on a 3D mental rotation task, which is a complex spatial ability test that involves stimulus identification, mental rotation, and decision making (Shepard and Metzler, 1971). Both musicians and non-musicians showed increased activations in premotor and superior parietal cortices; however, only musicians demonstrated increased activation in Broca's area. At the behavioral level, it has been shown that musicians, unlike non-musicians, exhibited shorter reaction times when asked to detect the position of a flashed target along the horizontal and vertical planes (Brochard et al., 2004, Patston et al., 2007). In support of the aforementioned functional evidence of enhanced visuospatial cognition in musicians, structural studies reported increased size of structures known to support visuospatial cognition, such as increased GM density in the superior parietal lobule (Gaser and Schlaug, 2003b, a) and Broca's area (Sluming et al., 2002) in professional musicians. Table 2.1 summarizes findings of previous neuroimaging studies in musicians.

Table 2.1. Summary of previous neuroimaging studies comparing musicians and non-musicians.

Motor system

Structural

- Increased GM density in Broca's area in the dominant hemisphere in male orchestral musicians (Sluming et al., 2002).
- Increased GM density in the left inferior frontal gyrus in male and female pianists (Han et al., 2009).
- Increased FA value in the right posterior limb of the internal capsule in musicians (Bengtsson et al., 2005, Han et al., 2009).
- Decreased FA value in the corona radiata and internal capsule bilaterally in musicians (Schmithorst and Wilke, 2002).
- Decreased FA value in the corticospinal tract in musicians, increased MD in the corticospinal tract in musicians who began training before age of 7 years compared to those who started later and non-musicians (Imfeld et al., 2009).
- Increased total cerebellar volume in male pianists (Schlaug et al., 1998, Hutchinson et al., 2003).
- Increased GM density in the right cerebellum in pianists (Han et al., 2009) .
- Musicians had more pronounced omega sign expression than controls. Keyboard-players had left hemisphere advantage, while string players had right hemisphere advantage (Bangert and Schlaug, 2006).
- Musicians showed greater local variability in the anterior wall of the middle section (somatotopic hand area) of the right central sulcus (Li et al., 2010).

- Increased intra-sulcal length of the precentral gyrus in the right hemisphere in musicians (Amunts et al., 1997b).
- Increased FA in the central aspect of the cerebellum in musicians (Schmithorst and Wilke, 2002).

Functional

- Reduced cerebellar recruitment in musicians during bimanual and unimanual motor tasks (Koeneke et al., 2004).
- Reduced recruitment of motor association areas during bimanual (Jancke et al., 2000, Haslinger et al., 2004), and unimanual (Hund-Georgiadis and von Cramon, 1999, Jancke et al., 2000, Krings et al., 2000, Meister et al., 2005) motor tasks in musicians.

Auditory system	Structural • Increased left-sided asymmetry of planum temporale in musicians with absolute pitch ability compared to those who don’t have this ability and non-musicians (Schlaug et al., 1995b, Zatorre et al., 1998, Keenan et al., 2001, Luders et al., 2004). • Increased volume of the anteromedial part of the Heschl gyrus in musicians (Schneider et al., 2002). • Fundamental pitch listeners had increased GM volume of the left lateral Heschl gyrus, whereas spectral pitch listeners had increased GM volume of the right lateral Heschl gyrus (Schneider et al., 2005). • Increased GM density and cortical thickness in the posterolateral aspect of the right Heschl gyrus in musicians (Bermudez et al., 2009). • Increased N-acetylaspartate concentrations in the planum temporale in musicians (Aydin et al., 2005). • Increased GM density in the right planum temporale in musicians (Bermudez and Zatorre, 2005). • Increased cortical thickness of the right and left planum temporale in musicians (Bermudez et al., 2009). Functional • Musicians had left hemisphere, while controls have right hemisphere lateralization of music perception as detected using trans-cranial doppler sonography (Evers et al., 1999) and fMRI (Ohnishi et al., 2001, Limb, 2006, Limb et al., 2006). • Increased auditory cortical representation of piano tones in pianists compared to control subjects (Pantev et al., 1998).

- Violinists and trumpeters showed evidence of timbre-specific enhancement of auditory cortical representation related to instrument specialty (Pantev et al., 2001).
- GM density and cortical thickness in the right Heschl sulcus and bilateral intra-parietal sulci predicted relative pitch task performance in musicians (Foster and Zatorre).
- Musicians had greater right posterior temporal and supramarginal gyrus activation during a pitch memory task (Gaab and Schlaug, 2003).
- Increased multimodal (auditory-somatosensory) integration in trumpet players compared to controls (Schulz et al., 2003).
- Evidence of shared auditory-sensorimotor activation in professional pianists while performing motor task or passive listening (Lotze et al., 2003, Bangert et al., 2006, Baumann et al., 2007).

Somatosensory System

<u>Structural</u>

- Musicians had increased GM density in the left primary sensorimotor cortex (Han et al., 2009).
- Musicians showed greater local variability in the anterior wall of the middle section (somatotopic hand area) of the right central sulcus (Li et al., 2010).

<u>Functional</u>

- Increased cortical representation of the digits (second to fifth) of the left hand in violinists (Elbert et al., 1995).
- Increased somatosensory cortical representation in skilled pianists (Pantev et al., 1998).

Visuospatial System	Structural • Increased GM density of the superior parietal lobule in professional musicians compared to amateurs and controls (Gaser and Schlaug, 2003a). • Increased GM density in Broca's area of the left inferior frontal gyrus in orchestral musicians (Sluming et al., 2002). Functional • Bilateral activation of the visual cortex during verbal memory retrieval task in musicians (Huang et al., 2010). • Increased activation in Broca's area when performing a 3D mental rotation (visuospatial) task in musicians (Sluming et al., 2007).
Higher cerebral functions/ Prefrontal cortex	Structural • No significant differences between musicians and non-musicians in total volumes of prefrontal cortical subfields (Sluming et al., 2002). • Increased cortical thickness of the dorsolateral frontal cortex in musicians (Bermudez et al., 2009). Functional • Reduced recruitment of the prefrontal cortex during bimanual motor task in musicians (Haslinger et al., 2004). • Increased activation of the prefrontal cortex during music improvisation in pianists (Bengtsson et al., 2007). • Involvement of the left dorsolateral subfield of the prefrontal cortex in music processing and perception (Zatorre et al., 1998, Ohnishi et al., 2001) in musicians with absolute ability but not in musicians

	without this ability (Zatorre et al., 1998), and non-musicians (Ohnishi et al., 2001). • Increased induced gamma-band response, a measure of attention, expectation, and memory retrieval in adult musicians and after one year of musical training in children musicians compared to non-musicians (Trainor et al., 2009b).
White matter **CC**	• Increased cross-sectional area of the anterior (Schlaug et al., 1995a, Ozturk et al., 2002, Lee et al., 2003), and posterior (Ozturk et al., 2002) halves of the midsagittal CC in musicians. • Evidence of gender versus musicianship interaction as only male musicians had increased size of the anterior CC (Lee et al., 2003). • Increased FA in the genu of the CC (Schmithorst and Wilke, 2002). • No significant differences between musicians and non-musicians in FA values in the anterior and posterior halves of the CC (Imfeld et al., 2009).
Superior Longitudinal Fasciculus	• Increased FA value of the superior longitudinal fasciculus in musicians with absolute pitch ability compared to musicians without this ability and non-musicians (Oechslin et al., 2009).

2.5 FACTORS GOVERNING USE-DEPENDENT NEUROPLASTICITY IN MUSICIANS

The literature suggests the presence of four factors that govern neuroplasticity in musicians' brains:

2.5.1 AGE OF STARTING MUSICAL TRAINING

Several studies reported a negative association between the observed structural-functional changes in musicians' brains and age of starting musical training. This means that the earlier the musicians started their training the more robust were the observed changes relative to non-musicians (Elbert et al., 1995, Schlaug et al., 1995a, Amunts et al., 1997b, Jancke et al., 1997, Pantev et al., 1998, Schlaug et al., 1998, Ohnishi et al., 2001, Schlaug, 2001, Lotze et al., 2003, Koeneke et al., 2004, Bengtsson et al., 2005, Imfeld et al., 2009, Li et al., 2010). It appears that a critical period exists in the human development when intensive brain stimulation would result in long lasting structural-functional changes. Considering that maturation cycle differs between GM and WM structures, and among different brain regions (Ostby et al., 2009, Giedd et al., 2010, Tiemeier et al., 2010), plastic changes in specific brain regions are expected to be most robust when musical training/practice takes place at a specific age that coincides with the peak of the maturation cycle of that region.

2.5.2 DURATION OF MUSICAL TRAINING AND PRACTICE INTENSITY

Some studies reported a positive correlation between long-term musical performance (years) and structural plasticity (Sluming et al., 2002, Hutchinson et al., 2003, Aydin et al., 2005, Abdul-Kareem et al., 2011). Although functional studies reported evidence of plasticity even after short-term musical training (minutes-days-weeks) in musically naive subjects (Pascual-Leone, 2001, Tremblay and Kraus, 2002, Bangert and Altenmuller, 2003, Lappe et al., 2008), a corresponding structural plasticity in such a short time may be difficult to demonstrate at the current resolution of MR scanners as most structural changes would be at the level of neuronal dendrites and synapses.

2.5.3 MUSICAL EXPERTISE

Few studies compared professional musicians with amateur musicians and non-musicians and revealed a significant effect of musical expertise, so that professionals had greater GM volume and functional activations compared to other groups (Schneider et al., 2002, Bangert and Altenmuller, 2003, Gaser and Schlaug, 2003b, Lotze et al., 2003).

2.5.4 TYPE OF INSTRUMENT

Playing a piano, a violin, or a trumpet might induce instrument-specific plastic changes, which means that instrument specialty should be taken into account when investigating neuroplasticity in musicians (Elbert et al., 1995, Pantev et al., 2001, Schneider et al., 2005, Bangert and Schlaug, 2006, Shahin et al., 2008). However, it is difficult to select a musician who is mono-instrumental as most musicians play more than one instrument. In addition, nearly all musicians play piano even when they specialize in different instrument. In the present work, multi-instrumental cohorts were recruited; as the regions examined (Broca's area, CC, and cerebellum) were known to subserve several musically relevant functions common to all musicians, it was predicted that structural changes could be seen in all musicians irrespective of their instrument specialty.

To conclude, the literature suggests that studying neuroplasticity offers better understanding of brain reorganization in health and disease, and that musicians represent an ideal model to explore this process in the human brain. There is convincing anatomical-functional evidence that musicians' brains differ from non-musicians. Possible microscopic events associated with reported macroanatomical changes in musicians' brains include strengthening of pre-existing synapses, generation of new synapses, increased dendritic arborization and axonal projections, and formation of new neurons. More work is required in the future to explore these microscopic changes *in vivo*. Although the influence of genetics on brain structure cannot be excluded (Tramo et al., 1998, Joshi et al., 2011), the observed anatomical-functional changes in brains of musicians were considered as evidence of use-dependent adaptation in response to environmental enrichment as they were correlated with factors, such as age of starting musical training, duration of musical performance, musical expertise, and instrument specialty.

2.6 IS IT POSSIBLE TO TRANSFER NEUROPLASTIC EFFECT OF MUSIC TO NON- MUSICIANS?

Although long-term musical practice has been linked to neuroplasticity (Sluming et al., 2002, Hutchinson et al., 2003, Aydin et al., 2005, Abdul-Kareem et al., 2011), evidence of brain plasticity was shown even following short-term musical training in musically naive subjects (Pascual-Leone, 2001, Tremblay and Kraus, 2002, Lappe et al., 2008). For instance, Bangert and Altenmuler (Bangert and Altenmuller, 2003) recorded cortical activation patterns after only 20 minutes of performing auditory and motor tasks. This effect was further enhanced after 5 weeks of practice. Similar effects were produced in musically naive subjects who mentally practiced motor tasks over five days as measured by mapping of the responses to transmagnetic stimulation (Pascual-Leone, 2001). Together, these findings indicate that music could induce instant plasticity in the brain within a short period of time. In line with these findings, several groups investigated the effect of music and musical training in learning and cognitive skill development. It has been shown that learning to play a musical instrument in childhood stimulates cognitive development, and leads to enhanced spatial, verbal, reading, and mathematical skills (Gardiner et al., 1996, Rauscher et al., 1997, Chan et al., 1998, Ho et al., 2003, Trainor et al., 2009a).

Apart from its pronounced effect on healthy subjects, music could have therapeutic effect. The wealth of literature suggesting music-induced plasticity in the human brain has sparked the emergence of a new field in rehabilitation medicine, ‘music therapy’ (for review, see (Hassler, 2000). This type of therapy uses music and musical instruments to restore, maintain, and improve neurological and psychological functions. For example, Schneider and colleagues (Schneider et al., 2007) designed a music-supported training program for motor skill rehabilitation in musically naive stroke patients. The program was based on results of previous studies, which suggested that playing music induce auditory-motor integration (Bangert et al., 2006, Baumann et al., 2007). It consisted of training the paralyzed extremity followed by training both the paralyzed and unparalyzed extremities using either a piano or electronic drum pads. After 3 weeks of training, patients showed significant improvement in speed, precision, and smoothness of movements, which was attributed to training-induced auditory-motor coupling that allowed patients to judge their execution of movements based on auditory feedback. This study set a good example of applying data derived from musicians' studies in the clinical field. Apart from stroke, music therapy was used for treating and rehabilitating a variety of disorders, such as schizophrenia (Ulrich et al., 2007), depression (Hsu and Lai, 2004), Parkinson's disease (Chuma, 2007), Alzheimer's disease (Irish et al., 2006, Svansdottir and Snaedal, 2006), chronic tinnitus (Argstatter et al., 2007), and several others. However, the exact way of how hearing or practicing music could produce therapeutic effects has not yet been resolved.

2.7 STATEMENT OF THE PROBLEM AND JUSTIFICATION OF THE WORK

After systematically reviewing structural studies conducted on musicians' brains, I found that more investigations are required to study structural plasticity in distinct brain regions, which have not been previously covered. The objective of this work was to investigate evidence of structural plasticity in three brain regions: Broca's area, CC, and cerebellum in musicians. A shared characteristic among these regions is their widely distributed cortical connections and involvement in diverse musically relevant functions. Four studies were conducted in the present work:

Study 1- Investigating structural plasticity of POP and PTR in musicians. This study is presented in **chapter 4**.

Study 2- Investigating the effect of musical expertise on regional morphometry of the CC. This study is presented in **chapter 5**.

Study 3- Investigating regional macro- and microstructural plasticity of the CC in musicians. This study is presented in **chapter 6**.

Study 4- Investigating structural plasticity of cerebellar WM in musicians. This study is presented in **chapter 7**.

Broca’s area is located within the inferior frontal gyrus and is composed of two subparts, POP and PTR (Snell, 2001). Although traditionally considered a language region, recent functional studies have assigned many functions to Broca’s area, which are of particular importance for musical performance (Sergent et al., 1992, Martinkauppi et al., 2000, Limb et al., 2006, Sluming et al., 2007). So far, previous studies have reported on GM density changes of Broca’s area using VBM (Sluming et al., 2002, Gaser and Schlaug, 2003b, a). No previous study has specifically examined the two parts of Broca’s area. A previous study performed at Magnetic Resonance and Image Analysis Research Centre (MARIARC) has used VBM to examine a cohort of 52 orchestral musicians and non-musicians (Sluming et al., 2002). Results showed that musicians had increased GM density in Broca’s area in the left dominant hemisphere. In the present work, I re-examined the same cohort using manual volumetry with the rationale of providing automatic-manual cross-validation of the VBM results and, most importantly, to perform separate morphometric analysis of Broca’s area subparts (POP and PTR) as it has been shown that they are anatomically (Amunts et al., 1999, Schenker et al., 2008), and functionally (Demb et al., 1995, Papathanassiou et al., 2000, Bookheimer, 2002, Chein et al., 2002, Heiser et al., 2003, Cone et al., 2008) segregated, and have distinct cortical connections (Xiang et al., 2009). In particular, recent studies have assigned a substantial role for the left POP in audiomotor integration (Lahav et al., 2007). Using stereology, I performed manual tracing of these subparts to measure GM and WM volumes of POP and PTR in each cerebral hemisphere. Furthermore, cortical surface areas of POP (BA44) and PTR (BA45) were measured to provide another perspective of plasticity in Broca’s area in musicians (study 1).

The structure of the CC was examined in two separate studies (study 2 and 3). These investigations were sought to address research questions not previously answered. First, the current evidence suggests that age of commencing musical tuition (Schlaug et al., 1995a) and gender (Lee et al., 2003) play a significant role in shaping structural plasticity of the CC in musicians. However, the literature suggests another important factor that influences plasticity in musicians’ brains namely, musical expertise. Compared to amateurs and non-musicians, a previous VBM study demonstrated that professional musicians had greater GM density in different brain regions, although it failed to find a significant effect on WM structures, including the CC, which was attributed to weakness of the VBM technique itself (Gaser and Schlaug, 2003a). Investigating structural differences between professionals, amateurs, and non-musicians might provide a good evidence to support the theory of training-induced structural plasticity. Therefore, I set the goal to examine the effect of musical expertise on CC structure through dividing the midsagittal CC into four callosal regions: CC1 (rostrum/genu/anterior body), CC2 (anterior midbody), CC3 (posterior midbody), and CC4 (isthmus/splenium) and comparing cross-sectional areas of these regions between professional musicians, amateur musicians, and non-musicians (study 2).

Second, most previous studies have used conventional MR to report on increased macrostructure (cross-sectional area) of the CC (Schlaug et al., 1995a, Ozturk et al., 2002, Lee et al., 2003). Postmortem studies in humans and animals have indicated that increased CC size might not actually reflect increased number of fibers crossing through the CC, but rather increased inter-fiber distance (Tomasch, 1954, Lamantia and Rakic, 1990, Aboitiz et al., 1992). In accord, recent DTI studies revealed great discrepancy between macro- and microstructure of the CC (Pfefferbaum and Sullivan, 2002, Westerhausen et al., 2006). Combining macrostructural measures with microstructural ones would thus be the best approach when studying the CC. To the best of my knowledge, no study has examined both regional macrostructure and microstructure of the CC in musicians. The latter may provide better evidence of structural plasticity of the CC in musicians. I therefore combined conventional MR morphometry and DTI-based tractography to compare regional macrostructure (cross-sectional area) and microstructure (average FA, MD, tract volume and number of streamlines) of four callosal regions (mentioned above) between musicians and non-musicians (study 3).

Apart from its role in movement coordination and motor learning (Mauk et al., 1998, Thach, 1998), the cerebellum is also involved in several other functions essential for enabling high level musical performance, such as memory retention, sensory acquisition, auditory discrimination, and cognition (Kim et al., 1994, Flament et al., 1996, Gao et al., 1996, Parsons et al., 1997, Belin et al., 1998, Hund-Georgiadis and von Cramon, 1999, Doyon et al., 2003, Parsons et al., 2009, Stoodley et al., 2010). To date, previous studies have reported on total volume and GM density of the cerebellum in musicians with no reports on cerebellar WM. With the exception of one study, which reported on total cerebellar volumes (GM and WM combined), results of structural studies that examined musicians' cerebella were always reported roughly as additional findings. In view of the diverse cerebellar connections and functions, investigating cerebellar WM plasticity in musicians is essential. The main WM tracts of the cerebellum are the SCP and MCP. The SCP is the major output pathway that links the cerebellum to the cerebrum, while the MCP carries fibers from the cerebrum to the cerebellum thus completing a circle that connects cerebral and cerebellar cortices. It is through this circle that the cerebellum exerts its control on cerebral cortical output thereby ensuring the most highly refined motor output (Snell, 2001). Another justification for studying cerebellar WM in musicians could be derived from developmental studies, which showed that while the cerebellar cortex (GM) reaches its maximum life time maturation by the age of two years, the WM of the cerebellum continues to develop into adolescence (Saksena et al., 2008, Ostby et al., 2009, Wu et al., 2011) making it more vulnerable to plastic changes. No previous study has specifically examined the WM of the cerebellum and, most importantly, there were no reports on SCP and MCP plasticity in musicians. To fill this gap, I used DTI-based tractography to examine the major cerebellar pathways, the SCP and MCP. Using this technique, quantitative measurements of average FA, tract volume, and number of streamlines of SCP and MCP were compared between musicians and non-musicians. In addition, high resolution anatomical images were automatically segmented to perform group-wise comparison of GM and WM volumes of cerebellar hemispheres (study 4).

2.8 SIGNIFICANCE OF THE PRESENT WORK

This work involved series of investigations set to explore structural plasticity in different brain regions using multiple cohorts and a multitude of morphometric techniques. Results of the present work were sought to extend the currently expanding literature suggesting the presence of use-dependent structural plasticity in musicians' brains. Findings would enhance our understanding of music-brain interaction, and may help to direct future structural and functional studies in musicians. In rehabilitation medicine, these findings may be used as baseline data when designing therapeutic models.

2.9 RESEARCH AIMS AND KEY OBJECTIVES

To summarize, the objective of the present work was to answer the following research questions:

- Are there differences between musicians and non-musicians in the GM /WM volumes of POP and PTR parts of Broca's area? Are there differences between musicians and non-musicians in cortical surface area measurements of BA44 and BA45? These research questions are addressed in **study 1, chapter 4.**

- Are there differences between professional musicians, amateur musicians, and non-musicians in regional cross-sectional area measurements of midsagittal CC? This research question is addressed in **study 2, chapter 5**.

- Are there differences between musicians and non-musicians in regional macrostructural (cross-sectional area) and microstructural (average FA, MD, tract volume, and number of streamlines) measures of the CC? Is there a correlation between macrostructure (cross-sectional area) and microstructure (average FA) of CC subregions? These research questions are addressed in **study 3, chapter 6**.

- Are there differences between musicians and non-musicians in microstructural measures (average FA, tract volume, and number of streamlines) of SCP and MCP? Are there differences between musicians and non-musicians in GM and WM volumes of cerebellar hemispheres? These research questions are addressed in **study 4, chapter 7**.

2.10 RESEARCH HYPOTHESES OF THE PRESENT WORK

2.10.1 STUDY 1-INVESTIGATING STRUCTURAL PLASTICITY OF POP AND PTR IN MUSICIANS

I hypothesized that orchestral musicians would show evidence of increased GM volume in the left POP region (BA44) of Broca's area of the inferior frontal gyrus of the left (dominant) cerebral hemisphere. This hypothesis was based on the following evidence: first, several musically relevant functions, such as musical syntax processing, detection of music-structural irregularities (Maess et al., 2001, Koelsch, 2006), audiospatial localization (Martinkauppi et al., 2000), and sight reading (Sergent et al., 1992) were shown to selectively activate the left POP/BA44 indicating a potential role of this region in subserving these functions. Second, musicians tend to use an analytic mode of music processing that is identical to that of language (Vuust et al., 2005, Limb et al., 2006) as evidenced by activation of the left POP and left planum temporale when passively listening to music (Ohnishi et al., 2001, Limb, 2006, Limb et al., 2006). This left hemisphere dominance in music processing in musicians contrasts with right hemisphere dominance in musically naive listeners (Johnson, 1977, Gordon, 1980). Therefore, I predicted that my right-handed orchestral musicians would show evidence of selective structural enlargement of the POP in the left (dominant) cerebral hemisphere. Third, in particular, the left POP/BA44 has been considered a central component of the human mirror neuron network (Iacoboni et al., 1999, Binkofski and Buccino, 2006) that is activated by the execution of manual action and by hearing of the same action performed by others (Tettamanti et al., 2005, Lahav et al., 2007) indicating its essential role in audiomotor integration. In this context, functional studies have indicated that, compared to non-musicians, professional musicians possess an auditory-motor network that includes the left POP (Haslinger et al., 2005, Bangert et al., 2006). This network insures co-activation of the other modality even when the task involves only auditory or only motor processing (Haueisen and Knosche, 2001, Lotze et al., 2003, Bangert et al., 2006, Baumann et al., 2007). In orchestral performance, the auditory-motor network has two main advantages: it allows fast feedback mechanism that enables musicians to continuously coordinate complex motor actions according to auditory input (Bangert and Altenmuller, 2003), and it maintains sequence-specific priming of action representations together with unconscious stimulations and predictions for the next coming action or sound (Lahav et al., 2007). The above-mentioned functions of the left POP/BA44 are essential for all musicians irrespective of their instrument specialty, hence of relevance to the multi-instrumental cohort examined in the present study. Furthermore, since previous studies have indicated that neuronal density is positively correlated with cortical surface area (Rockel et al., 1974, Henery and Mayhew, 1989, Sisodiya et al., 1996, Sisodiya and Free, 1997), I hypothesized that orchestral musicians would show increased cortical surface area in the left BA44.

2.10.2 STUDY 2-INVESTIGATING EFFECT OF MUSICAL EXPERTISE ON REGIONAL MORPHOMETRY OF THE CC

I hypothesized that professional musicians would show increased size of CC1 (rostrum, genu, and anterior body), CC3 (posterior midbody), and CC4 (isthmus and splenium) regions compared to amateur musicians and non-musicians. According to postmortem human studies, the size of the CC is positively correlated with the number of crossing fibers (Aboitiz et al., 1992). Consequently, structural enlargement of CC1, CC3, and CC4 regions is essential to support the need for stronger inter-hemispheric communication in musicians' brains.

The CC1 region contains fibers from the prefrontal cortex (Park et al., 2008), which is critically involved in several functions essential for enabling high level musical performance, such as skill acquisition (Vogt et al., 2007), attention (Lau et al., 2004), music processing and perception (Zatorre et al., 1998, Ohnishi et al., 2001), and spatial working memory (Robertson et al., 2001). A larger CC1 size is therefore essential to support these functions in professional musicians. Regarding CC3 region, several DTI reports have demonstrated that callosal motor fibers cross through this region (Hofer and Frahm, 2006, Park et al., 2008, Chao et al., 2009). In particular, CC3 contains fibers that connect hand areas of the primary motor cortex of the two cerebral hemispheres (Wahl et al., 2007). I hypothesized that, since previous studies reported reduced right-to-left asymmetry in the size of the primary motor cortex (Amunts et al., 1997a), and reduced transcallosal inter-hemispheric inhibition between hand motor cortical areas (Ridding et al., 2000) in professional musicians, which are reflected by increased hand skill symmetry and bimanual coordination skills compared to non-musicians (Amunts et al., 1997a, Jancke et al., 2000, Haslinger et al., 2004), the size of this region would be larger in professional musicians to maintain high-level bimanual motor skills. Similarly, since previous MR studies revealed evidence of structural enlargement of the parietal and temporal cortical regions in professional musicians, including the primary somatosensory cortex (Han et al., 2009), superior parietal lobule (Gaser and Schlaug, 2003a), Heschl gyrus (Schneider et al., 2002, Bermudez et al., 2009), and planum temporale (Bermudez et al., 2009), which was shown to be reflected at the functional level by enhanced somatosensory cortical representation (Elbert et al., 1995), better visuospatial performance (Sergent et al., 1992, Sluming et al., 2002, Stewart et al., 2003), and increased auditory cortical representation of music tones (Pantev et al., 1998), I hypothesized that professional musicians would show evidence of use-dependent structural adaptation in the form of increased size of CC4 region, which connects parietal (primary somatosensory cortex and superior parietal lobule), and auditory (Heschl gyrus and planum temporale) cortical regions.

2.10.3 STUDY 3- INVESTIGATING REGIONAL MACRO- AND MICROSTRUCTURAL PLASTICITY OF THE CC IN MUSICIANS

I hypothesized that my findings of increased macrostructure (cross-sectional areas) of CC1 and CC4 regions in professional musicians (reported in study 2) would be replicated in the present study. Furthermore, I proposed that increased size of CC1 and CC4 regions would be reflected, at the microstructural level, by increased fiber density, axonal projections, and sprouting (indicated by increased number of streamlines), thicker myelination and/or increased diameter of fibers (indicated by increased tract volume), increased coherence, directionality, and integrity of fiber bundles (indicated by increased average FA value), and more restrictions to diffusion by the surrounding cellular components (indicated by reduced MD value). Increased FA values in professional musicians would suggest that molecular displacement is stronger parallel to than perpendicular to the axon orientation indicating increased directionality and alignment of axonal fibers. Reduced MD values may suggest the presence of a larger number of axons (more membrane) and/or more myelin material (Beaulieu, 2002), which result in attenuation of molecular diffusion. I hypothesized that these microstructural changes are triggered by the need for stronger and more effective inter-hemispheric communications in professional musicians.

2.10.4 STUDY 4-INVESTIGATING PLASTICITY OF CEREBELLAR WHITE MATTER IN MUSICIANS

I hypothesized that professional musicians, being involved in complex sensorimotor and cognitive skill acquisition, would show evidence of structural plasticity in WM and GM regions of cerebellar hemispheres. In particular, I hypothesized that music performance would induce structural plastic changes in SCP and MCP tracts in the form of increased fiber density, axonal projections, and sprouting (indicated by increased number of streamlines), thicker myelination and/or increased diameter of fibers (indicated by increased tract volume), increased coherence, directionality and integrity of fiber bundles (indicated by increased average FA value). This hypothesis was based on findings of several studies that reported cerebellar involvement in a variety of musically relevant functions, such as motor skill acquisition and sequence learning (Anderson et al., 1996, Kim et al., 2002, Koeneke et al., 2004), auditory discrimination (Parsons et al., 2009), spatial (mental orientation) and working memory (Stoodley et al., 2010), cognition (Flament et al., 1996, Doyon et al., 2003), and early error detection and correction phase of motor and cognitive skill learning (Flament et al., 1996, Doyon et al., 2003). I proposed that skilful musical practice would enhance the neural activity thereby resulting in increased fiber density, diameter, myelination, and coherence of SCP/MCP tracts. In addition, the relatively longer maturation cycle of cerebellar WM compared to GM (Saksena et al., 2008, Ostby et al., 2009, Wu et al., 2011) may indicate that SCP and MCP are exposed to the effect of environmental enrichment, in the form of musical practice, for a long period of time sufficient to impose structural plastic changes in these tracts.

CHAPTER 3

GENERAL METHODS

3.1 AIM

The aim of this chapter is to present a general framework for methods used in the present work. First, a brief description of examined cohorts, measurements obtained in each cohort, and MR data acquisitions is presented, with detailed description provided in related chapters. Second, an introduction to general principles of methods used in the present work is provided namely, stereology, diffusion tensor imaging, and fiber tractography. Image analysis methods used in more than one study are presented in this chapter to avoid repetition in subsequent chapters. Other methods are presented in related chapters. Results and technical limitations of all methods are presented in 'results' and 'discussion' sections of related chapters, respectively.

3.2 COHORTS EXAMINED IN THE PRESENT WORK

As stated in chapter 2, four studies were conducted in the present work to answer the proposed research questions. To perform these studies, three cohorts of age, gender, and handedness-matched musicians and non-musicians were examined. Outcome measures were compared between groups. In addition, correlation analyses between outcome measures and demographic factors, such as gender, age of commencing musical tuition, and duration of musical performance were performed. Below is a summary of cohorts examined in the present work. A detailed description of these cohorts is presented in related chapters.

- **Cohort 1 (study 1) -** Retrospective data; these were collected by Vanessa Sluming at Magnetic Resonance and Image Analysis Research Centre (MARIARC), University of Liverpool. The cohort included 26 right-handed male orchestral musicians and 26 right-handed male non-musicians.
- **Cohort 2 (study 2) -** Retrospective data; these were collected by Peter Schneider at University of Heidelberg. The cohort included 36 right-handed male subjects: 12 professional musicians, 12 amateur musicians, and 12 non-musicians.

- **Cohort 3 (study 3 and 4) -** Data collected by the PhD student, Ihssan Adeeb Abdul-Kareem at MARIARC, University of Liverpool. The cohort included 13 musicians and 13 non-musicians. In each group, there were 12 right-handed subjects and 1 left-handed subject. Prior to performing DTI data analysis, fiber reconstruction of the CC, SCP and MCP was performed to verify the reliability of tracking method and its consistency with anatomical and DTI atlases. Reconstruction of the CC tracts was feasible in all subjects in a manner consistent with the description of known anatomical and DTI atlases. For reconstruction of the SCP and MCP tracts, this was only feasible in 20/26 subjects (10 musicians and 10 non-musicians; 9 right-handed /1 left-handed in each group). For subsequent studies, cohort 3 was divided into two subcohorts:
    - **Cohort 3A (study 3)**, for investigating regional macro- and microstructure of the CC in musicians, I examined 12 right-handed musicians (9 males and 3 females) and 12 right-handed non-musicians (9 males and 3 females). The two left-handed subjects (1 musician/1 non-musician) were excluded from this study (see chapter 6- subjects section 6.3.1, page 188 for details).
    - **Cohort 3B (study 4)**, for investigating SCP and MCP tractography and cerebellar morphometry in musicians, I examined 10 musicians (7 males and 3 females) and 10 non-musicians (7 males and 3 females). Each group has 9 right-handed subjects and 1 left-handed subject. The 6 subjects (3 musicians and 3 non-musicians) in whom tractography was not consistent with DTI atlases were excluded from this study (see chapter 7- results section 7.5.2.1, page 232 for details).

3.3 EXAMINATIONS PERFORMED IN EACH COHORT

Cohort one

- Comparison of GM and WM volumes of POP and PTR between orchestral musicians and non-musicians using a manual morphometric technique.
- Comparison of cortical surface area measurements of BA44 and BA45 between orchestral musicians and non-musicians using an automatic user- independent technique.

Cohort two

- Comparison of cross-sectional areas of four callosal regions: CC1 (rostrum/ genu/anterior body), CC2 (anterior midbody), CC3 (posterior midbody), and CC4 (isthmus and splenium) between professional musicians, amateur musicians and non-musicians. These measurements were obtained using a manual morphometric technique.

Cohort three

- **A**, comparison of cross-sectional areas of the CC1 (rostrum/genu/anterior body), CC2 (anterior midbody), CC3 (posterior midbody), and CC4 (isthmus and splenium) regions between musicians and non-musicians. These measurements were obtained using a manual morphometric technique. Also, comparison of average FA, MD, tract volume, and number of streamlines of corresponding callosal regions using DTI-based tractography.
- **B**, comparison of average FA, tract volume, and number of streamlines of SCP and MCP between musicians and non-musicians using DTI-based tractography; and comparison of GM and WM volumes of cerebellar hemispheres between musicians and non-musicians using an automatic morphometric technique.

3.4 MR DATA ACQUISITIONS OBTAINED IN THE PRESENT WORK

A number of imaging protocols were used in the present work. A summary of scanning parameters is provided in table 3.1. A detailed description of MR data acquisitions is provided in related chapters.

Table 3.1. Summary of image acquisitions obtained in the present work.

Scanning Parameters	**Study 1/Cohort 1**	**Study 2/Cohort2**	**Study 3 /Cohort 3A & Study 4/Cohort 3B**		
Field strength	1.5 Tesla	1.5 Tesla	3 Tesla	1.5 Tesla	3 Tesla
Pulse sequence	[1]SPGR	[2]MPRAGE	MPRAGE	MPRAGE	Diffusion-weighted sequence
Field of view/mm	200	256	256	256	267
TR/ms	34	2040	2040	1660	10100
TE/ms	9	3.93	5.57	3.04	106
No. Of slices	124	176	176	176	60
Slice Orientation	Coronal	Sagittal	Sagittal	Sagittal	Axial
Flip angle	30º	15º	8º	8º	8º
Acquisition time /min.	13.56	7.38	4.14	14.11	22.14
b value					1200

[1]SPGR, Spoiled Gradient Echo, [2]MPRAGE, Magnetization-Prepared Rapid Gradient-Echo imaging.

3.5 IMAGE ANALYSIS METHODS APPLIED IN THE PRESENT WORK

3.5.1 METHODS USED IN MORE THAN ONE STUDY

Methods used in more than one study are presented in this chapter to avoid repetition in subsequent chapters, these include:

- Manual measurement of intracranial volume (ICV) (**study 1 and 2; chapter 4 and 5, respectively**).
- Manual cross-sectional area measurement of CC regions (**study 2 and 3; chapter 5 and 6, respectively**).
- Pre-processing of DTI data and selection of tractography parameters (**study 3 and 4; chapter 6 and 7, respectively**).

Furthermore, an introduction to basic principles of stereology technique, DTI, and tractography is presented in this chapter.

3.5.2 METHODS PRESENTED IN SPECIFIC CHAPTERS

These methods are presented in related chapters, they include:

- Manual measurement of GM and WM volumes of POP and PTR regions (**study 1, chapter 4**).
- Automatic measurement of cortical surface area of BA44 and BA45 (**study 1, chapter 4**).
- Fiber tractography of CC regions (**study 3, chapter 6**).
- Automatic measurement of ICV (**study 3 and 4; chapter 6 and 7, respectively**).
- Fiber tractography of the SCP and MCP (**study 4, chapter 7**).
- Automatic measurement of GM and WM volumes of cerebellar hemispheres (**study 4, chapter 7**).

A summary of recruited cohorts, measurements obtained in each study, and image analysis softwares applied in the present work is presented in table 3.2. Results of all image analysis methods and technical limitations are presented in related chapters.

Table 3.2. Summary of cohorts, measurements, and image analysis softwares. M, musicians; N, non-musicians; P, professional musicians; A, amateur musicians.

	Cohorts	**Measurements**	**Manual/automatic technique**	**Software**
Study 1 **Chapter 4**	*Cohort 1* 26/26 M/N Males All right-handed	• ICV • GM and WM volumes of POP/PTR • Cortical surface areas of BA44/45	Manual Manual Automatic	EasyMeasure EasyMeasure BrainVoyager
Study 2 **Chapter 5**	*Cohort 2* 12/12/12 P/A/N Males All right-handed	• ICV • Cross-sectional areas of CC1-CC4 regions	Manual Manual	EasyMeasure ImageJ
Study 3 **Chapter 6**	*Cohort 3A* 12/12 M/N 9 males & 3 females All right-handed	• ICV • Cross-sectional areas of CC1-CC4 regions • Tractography metrics of CC1-CC4 tracts: FA, MD, tract volume, and number of streamlines	Automatic Manual Manual/Automatic	FreeSurfer ImageJ MedINRIA
Study 4 **Chapter 7**	*Cohort 3B* 10/10 M/N 7 males & 3 females 9 right-handed & 1 left-handed	• ICV • GM and WM volumes of cerebellar hemispheres • Tractography metrics of SCP and MCP tracts: FA, tract volume, and number of streamlines	Automatic Automatic Manual/Automatic	FreeSurfer FreeSurfer MedINRIA

3.6 PRINCIPLES OF STEREOLOGY

Stereology is a method used to manually estimate the volume of structures of arbitrary size and shape (Gundersen and Jensen, 1987). It enables accurate unbiased volume estimation to be obtained for only a reasonable workload (Roberts et al., 2000). Most stereology techniques use the Cavalieri method for volume estimation, which was named in honor of Bonaventura Cavalieri (1598-1647) who was the first to consider measurement of volume via the analysis of sections through three-dimensional (3D) solid objects.

3.7 THE CAVALIERI METHOD

The Cavalieri section method is an unbiased volume estimator technique of modern design stereology. Modern design based techniques use sampling strategies to maximize efficiency (Gundersen and Jenson, 1987, Roberts et al., 1994a). The Cavalieri sections method requires that, beginning from a uniform random starting position within the sectioning interval, a structure of interest is exhaustively sectioned with a series of parallel planes (or sections) at a constant distance apart. An estimate of volume V can be obtained without bias by multiplying the total area of all sections through the structure by the sectioning interval *T* (figure 3.1) as in Equation 3.1 (Roberts et al., 2000).

$$est_1 V \ = T\ (A_1 + A_2 + \cdots \ A_n) \qquad \textbf{\textit{Equation 3.1}}$$

Where A_1, A_2,... A_n denote the section areas, and *T* is the sectioning interval for the *n* consecutive sections.

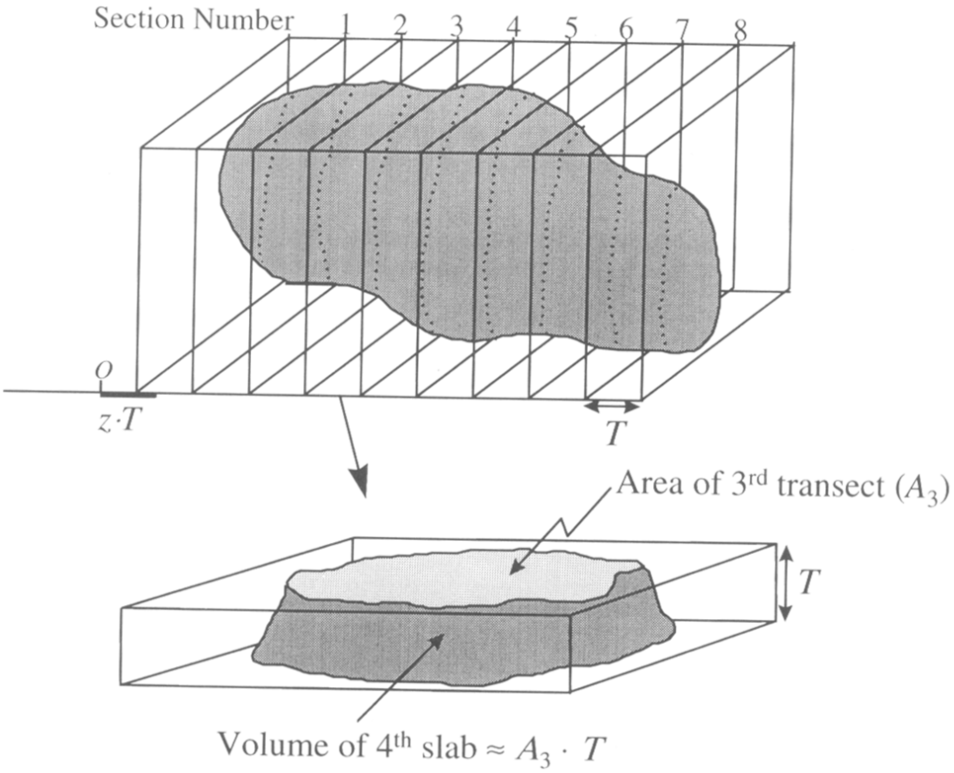


Fig.3.1. The basis of Cavalieri sections method of volume estimation. From (Howard and Reed, 1998).

3.7.1 VOLUME ESTIMATION IN MR IMAGES

Measurement of section areas is often difficult in 3D MR images. In this case, an unbiased estimate of volume can be obtained using an interactive point counting technique (Roberts et al., 1994b, Roberts et al., 2000, Garcia-Finana et al., 2003). It has been shown that this method of volume estimation is both more efficient and more precise than manual tracing (Gundersen and Osterby, 1981, Mathieu et al., 1981).

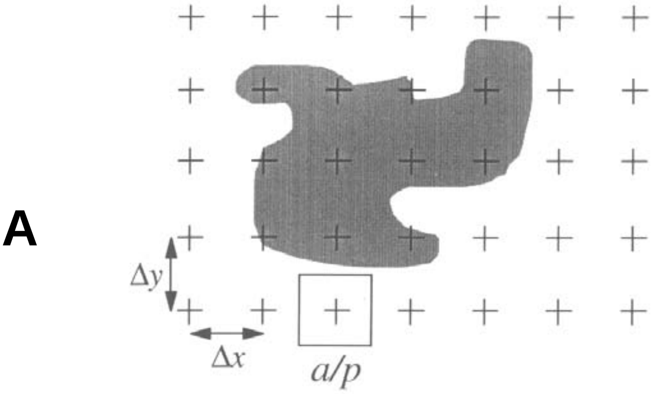
The point counting method involves overlying each MR image with a regular grid of test points (figure 3.2A). The test system should have new random position and orientation on each slice. After each superimposition, the number of test points hitting the structure of interest is counted on each section. An unbiased estimator of section area A_i becomes

$$estA_i = (a/p)\ P_i$$ **Equation 3.2**

Where P_i is the number of points counted and (a/p) represent the area associated with each point (figure 3.2A) corrected for any minification or magnification of the image as it is displayed on the computer screen. The volume estimator (in mm^3) using point count is thus

$$est_2V = T\ (a/p)\ (P_1 + P_2 + \cdots P_n)$$ **Equation 3.3**

Where P_1, P_2, ... P_n denote the point counts. The notation est_2V indicates that the volume estimator is based upon two sampling processes, sectioning the structure of interest into slices, and point counting on slices. In this work, the intersection of the upper right quadrant of the red cross was taken as the “point” (figure 3.2B).



$$\hat{A} = \frac{a}{p} \cdot P = \Delta x \cdot \Delta y \cdot P$$

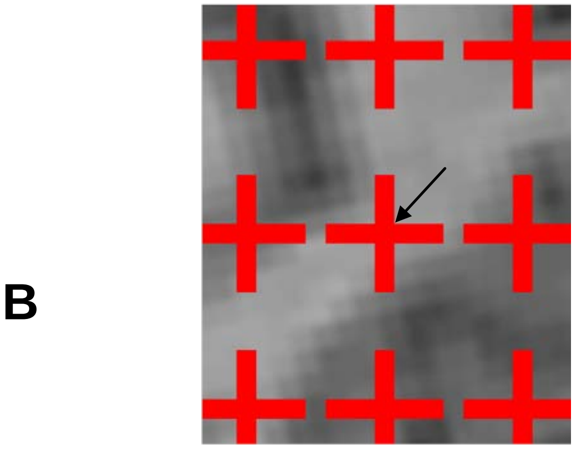


Fig.3.2. A**,** the grid system used for estimating slice volumes. The square around the cross on the bottom row indicates the area associated with that “point”. From (Howard and Reed, 1998). **B**, the intersection of the upper right quadrant of the red cross was taken as the “point”.

3.8 STEREOLOGICAL MEASUREMENTS PERFORMED IN THE PRESENT WORK

In the present work, stereology was used to measure ICVs and GM/WM volumes of POP and PTR regions. Method of ICV measurement is presented here, while method of measurement of GM/WM of POP and PTR is presented in chapter 4.

3.9 MEASUREMENT OF INTRACRANIAL VOLUME

Manual measurement of ICV using stereology was performed in study 1 and 2, which are presented in chapter 4 and 5, respectively.

3.9.1 IMAGE PRE-PROCESSING

This was performed using BrainVoyager software, version 1.9 (www.Brainvoyager.com, Brain Innovation, Maastricht, The Netherlands). The anatomical data of each subject were converted from (DICOM) format into BrainVoyager's internal (VMR) data format. Original coronal images were spatially transformed to the sagittal plane, and the acquired voxels of original size 0.781 X 0.781 X 1.6 mm were re-sampled into 1 X 1 X 1 mm isotropic voxels using cubic spline interpolation. Images were then re-aligned according to the bicommissural plane (anterior commissure-posterior commissure plane, ACPC plane). First, the anterior commissure (AC) was identified on a sagittal view closest to the midline and verified on transverse and coronal views. The sagittal image was then rotated such that the inferior part of the posterior commissure (PC) is viewable in transverse view. This was followed by rotating the coronal and axial images so that the brain is divided into symmetrical halves (figure 3.3). Images were then exported to (ANALYZE) format.

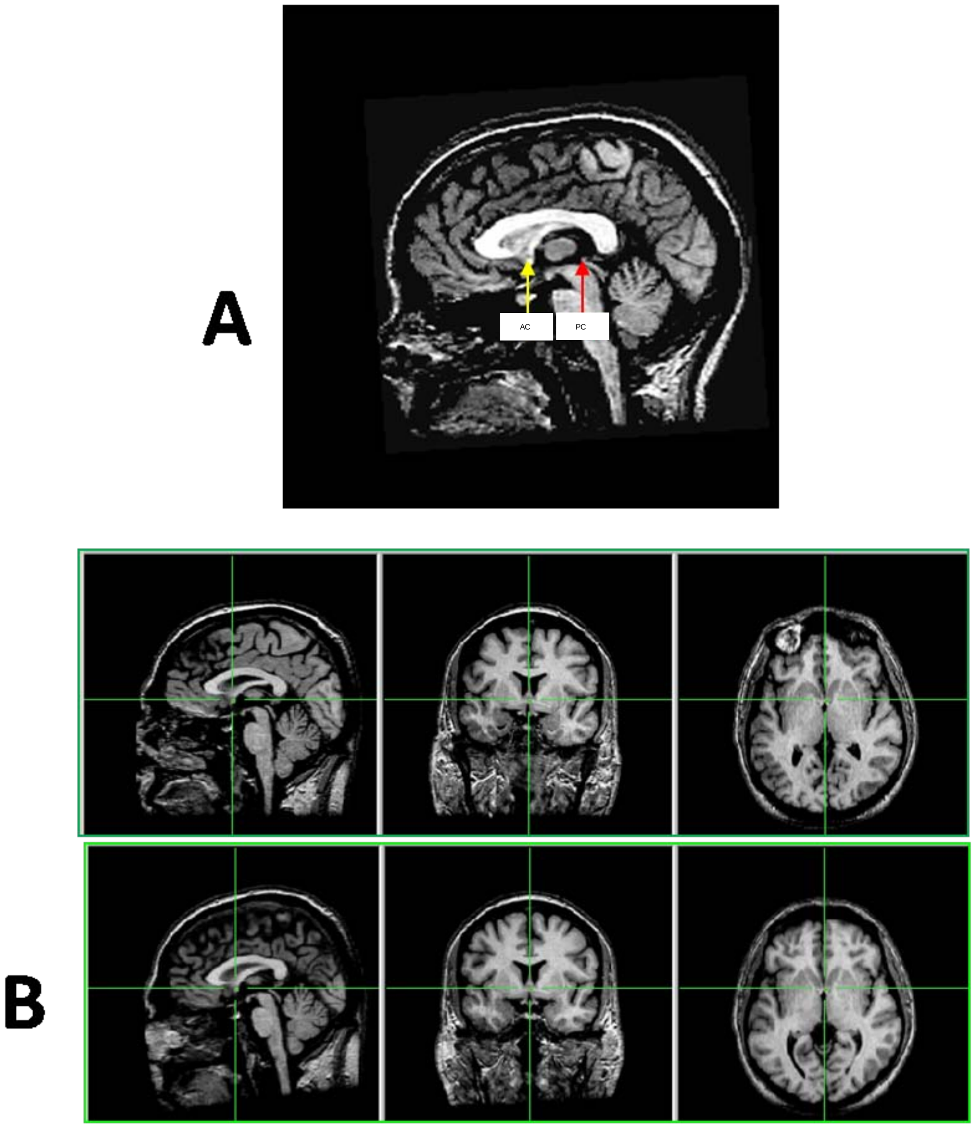


Fig. 3.3. A, the anterior (AC) and posterior commissures (PC) depicted on a sagittal T1-weighted MR image. **B**, method of re-alignment of brain images according to the ACPC plane. Top row, the AC was first demarcated on the sagittal view and verified on other views. Bottom row, the sagittal image was then rotated so that the brain is re-aligned according to ACPC plane. This was followed by rotating the coronal and axial images so that the green cross divides the brain into symmetrical halves.

3.9.2 DEFINITION OF INTRACRANIAL VOLUME

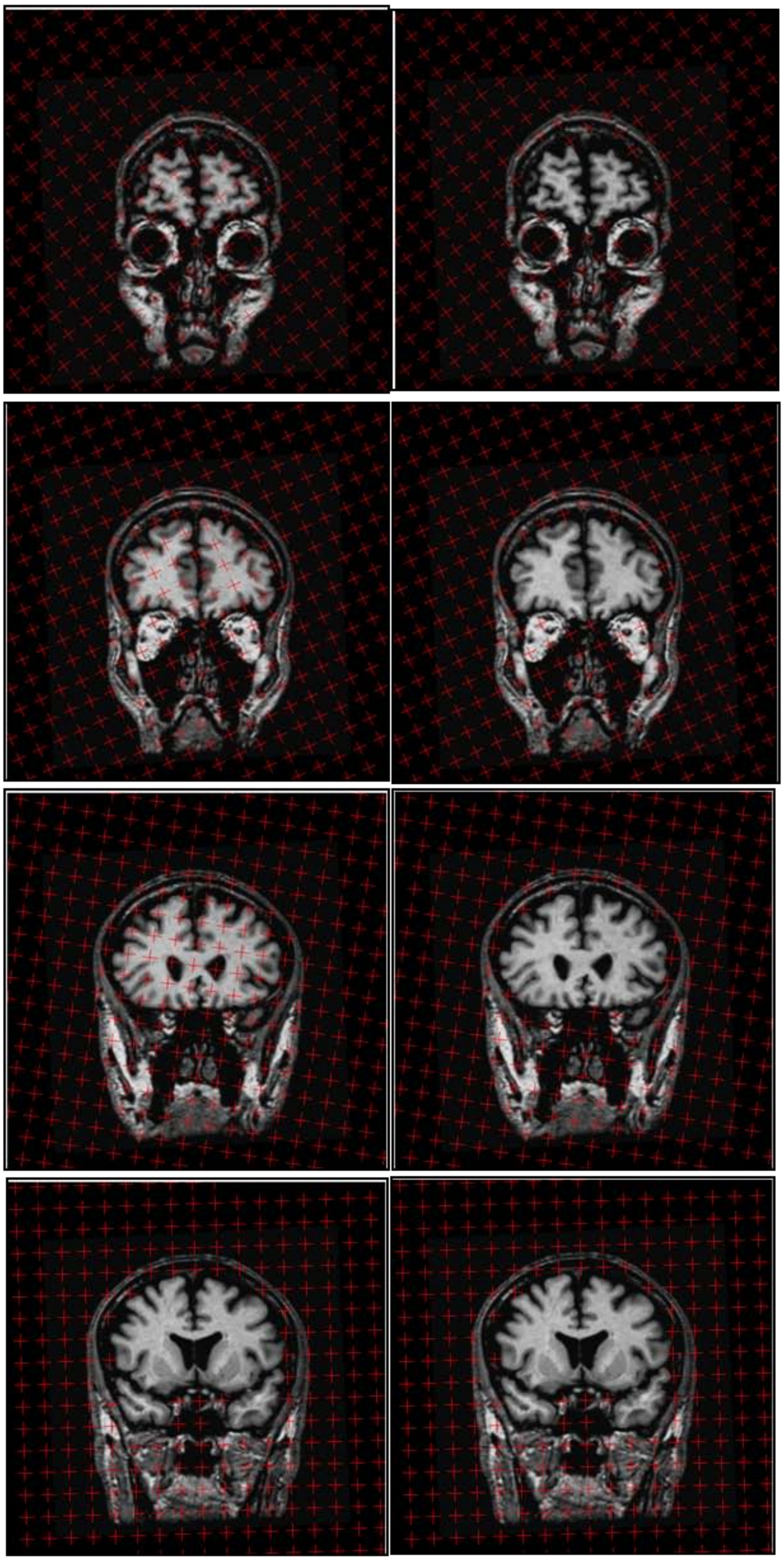
Intracranial volume was defined as the volume of the supra-tentorial space, including all cerebral tissue and cerebrospinal fluid spaces (figure 3.4). Posteriorly, the definition included the superior sagittal sinus and the confluence of sinuses. In slices where the cerebellum was not visible, the pontocrural sulcus provided the inferior boundary, as this is approximately the level where the tentorium cerebelli is attached to the anterior clinoid process. This level in the superior-inferior extent was maintained throughout the brainstem from posterior to anterior. If the pontocrural sulcus was not visible, then the narrowest part of the brainstem formed the inferior boundary.

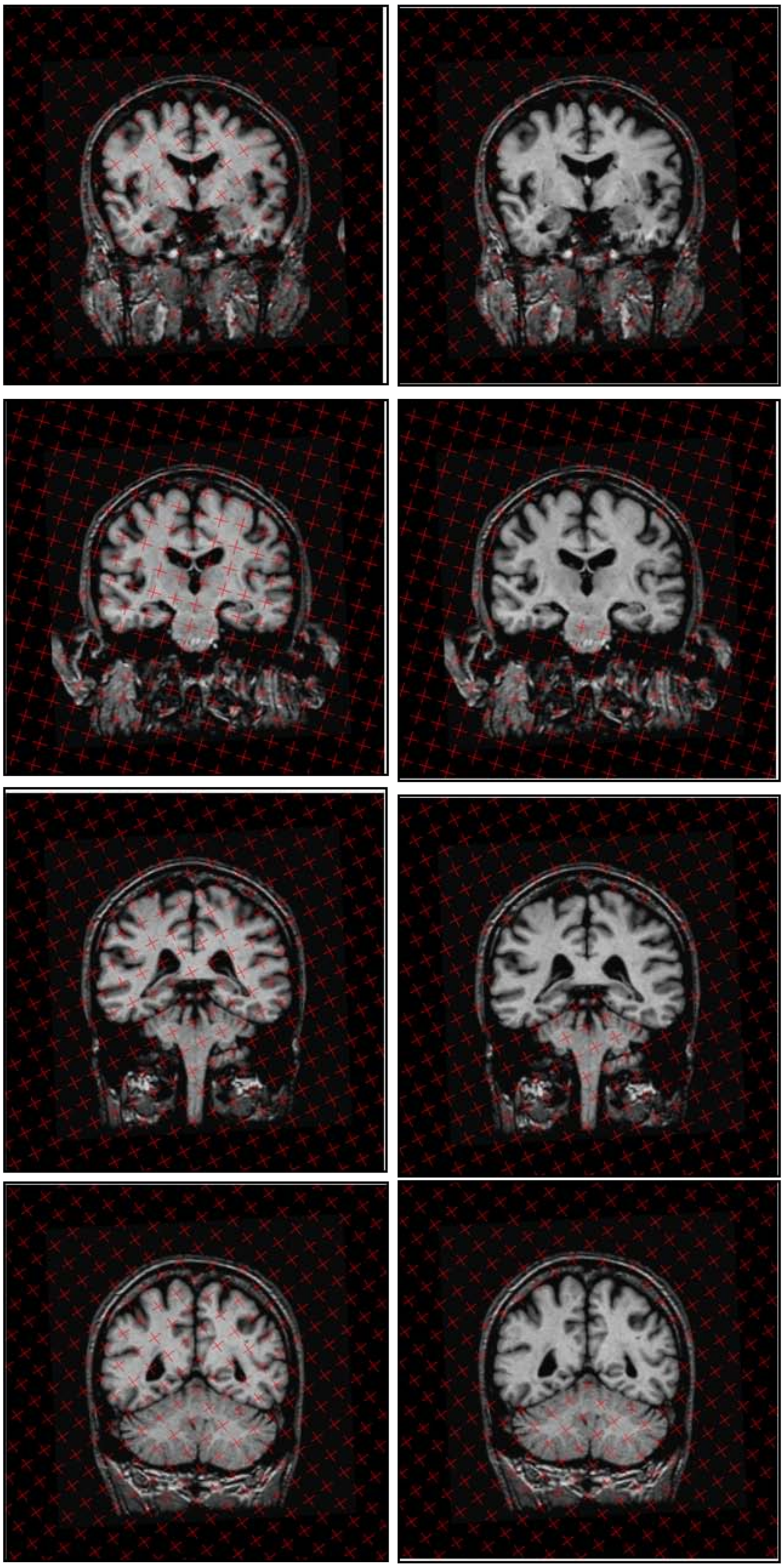
3.9.3 STEREOLOGICAL PARAMETERS FOR INTRACRANIAL VOLUME MEASUREMENT

Parameters selected for ICV measurements were a grid size (pixels) = 15 pixels, area associated with each test point, (a/p) = 225 mm^2 (Mackay et al., 1998), and section interval (T) was every 15 sections. Since final voxel size after iso-voxel transformation was 1 X 1 X 1 mm, then T = 15 X 1 mm = 15 mm.

3.9.4 STEREOLOGICAL MEASUREMENTS

Images were reoriented to the coronal plane using ImageJ software, version 1.38 available online at http://rsb.info.nih.gov/ij/, NIH, USA). Stereological measurements were performed using EasyMeasure software (Puddephat, 1999) on series of coronal MR sections. Images were always displayed using consistent image window and display levels on the same monitor with fixed screen contrast settings.





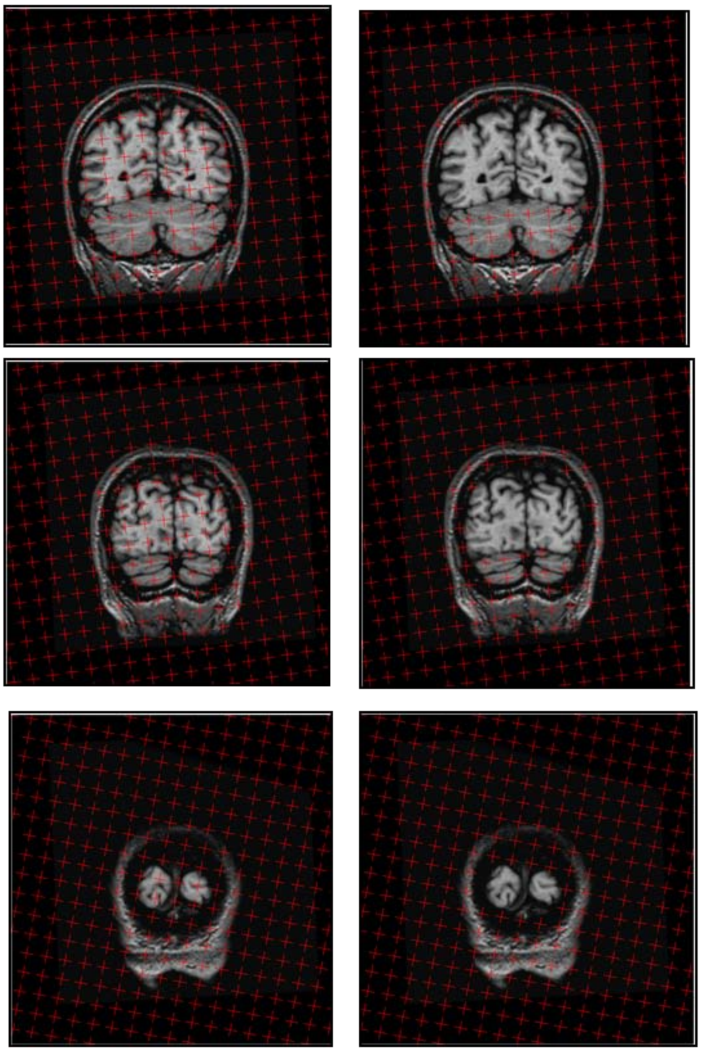


Fig 3.4. Illustration of Intracranial volume measurement using Cavalieri method with point counting, before (left) and after (right) point counting.

3.10 MEASUREMENT OF CROSS-SECTIONAL AREA OF CC REGIONS

Measurement of cross-sectional area of CC regions was performed in study 2 and 3, which are presented in chapter 5 and 6, respectively.

The fact that there are no macroscopic anatomical landmarks that clearly delimit distinct callosal areas in the midsagittal plane urged scientists to design partitioning schemes to divide the CC into regions (Clarke et al., 1989, Witelson, 1989, Denenberg et al., 1991, Weis et al., 1993, Rajapakse et al., 1996, Hampel et al., 1998) in order to facilitate structural-functional correlation. Most of previous studies used Witelson’s scheme for subdividing the CC into 7 regions. These callosal subdivisions were based mainly on studies of monkeys (Pandya et al., 1971, Barbas and Pandya, 1984, Cipolloni and Pandya, 1985), and postmortem studies in humans (de Lacoste et al., 1985). In the present work, a modification of Witelson’s scheme (Witelson, 1989) was used to divide the CC into 4 regions. The CC1 region corresponds to rostrum, genu, and anterior part of the body (areas 1-3 of Witelson’s scheme), CC2 region corresponds to anterior midbody (area 4 of Witelson’s scheme), CC3 region corresponds to posterior midbody (area 5 of Witelson’s scheme), and CC4 region corresponds to isthmus and splenium (areas 6-7 of Witelson’s scheme). The same division scheme was followed in previous studies (Jones and Basser, 2004, Shimony et al., 2006).

3.10.1 IMAGE PRE-PROCESSING

BrainVoyager was used to convert the anatomical data of each subject from (DICOM) format into BrainVoyager's internal (VMR) data format. Images were then exported to (ANALYZE) format. Using MRIcro (www.mricro.com, Chris Rorden, University of South Carolina, Columbia, SC, USA), images were first corrected for rotation through parallel alignment of the inter-hemispheric plane of the brain with the sagittal plane of images followed by extracting the midsagittal slice according to the following criteria (Ozturk et al., 2002) (figure 3.5):

- In the sagittal view, it is the slice in which the callosal sulcus, peak of 4th ventricle, and the cerebral aqueduct are distinct.

- In the transverse view, it is the slice that passes through the middle of 3rd ventricle, pineal gland, and cerebellar vermis.
- In the coronal view, it is the slice that passes through the septum pellucidum.

The midsagittal slice was then rotated, so that the line connecting the ventral most points of the anterior and posterior CC is horizontally oriented.

3.10.2 CROSS-SECTIONAL AREA MEASUREMENT

The maximal length of the CC was taken as the line joining the most anterior and posterior points of the CC on the midsagittal image. Three perpendicular lines were drawn at equal intervals along the widest antero-posterior line of the midsagittal CC, which resulted in dividing the CC into four regions (CC1-CC4) (figure 3.6). Manual cross-sectional area measurement of these regions was next performed using ImageJ software, version 1.38 available online at http://rsb.info.nih.gov/ij/, NIH, USA).

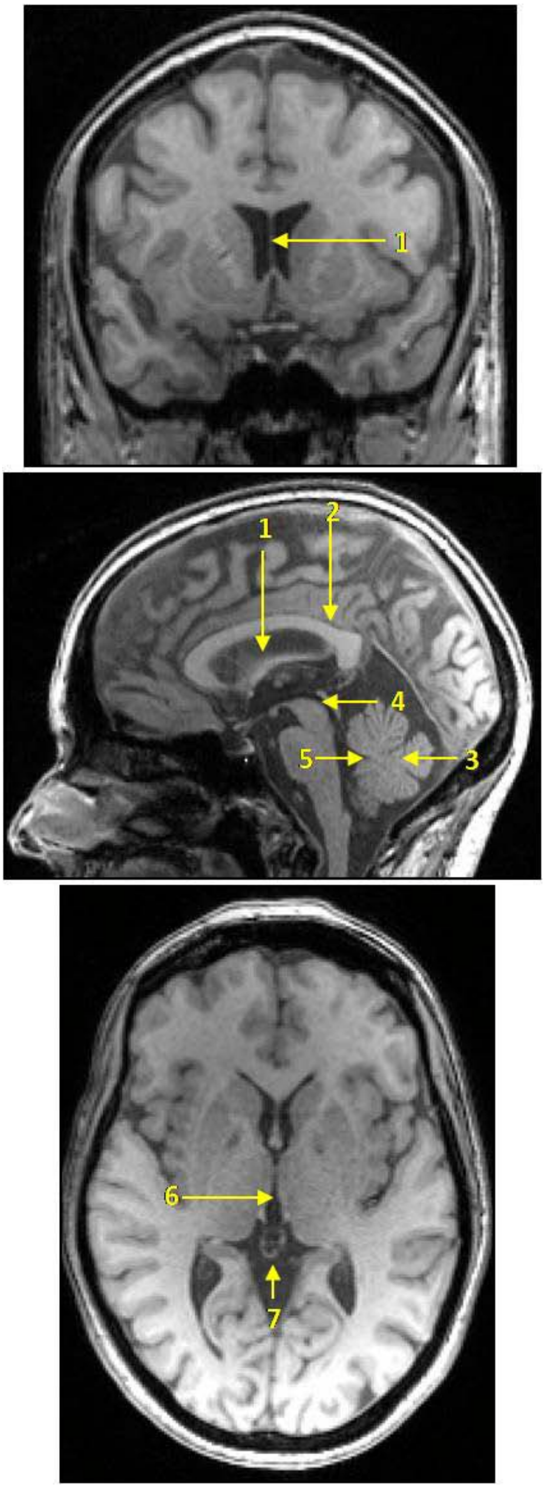


Fig. 3.5. Criteria for selecting the midsagittal slice. Top, coronal view; middle, sagittal view; bottom, transverse view. 1, septum pellucidum; 2, callosal sulcus; 3, cerebellar vermis; 4, cerebral aqueduct; 5, peak of 4th ventricle; 6, 3rd ventricle; 7, pineal gland.

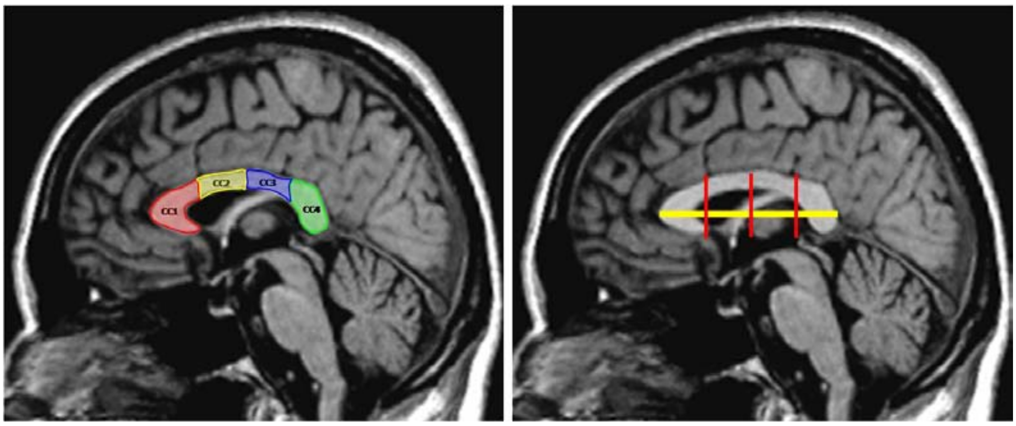


Fig. 3.6. Method of subdividing the CC on midsagittal T1-weighted MR image. Right, three perpendicular lines were drawn at equal intervals along the widest antero-posterior line of the CC. Left, the resulted four callosal regions (CC1-CC4) depicted in colours, red, yellow, blue and green, respectively.

3.11 INTRODUCTION TO PRINCIPLES OF DIFFUSION TENSOR IMAGING

3.11.1 OVERVIEW OF DIFFUSION TENSOR IMAGING

Diffusion tensor imaging (DTI) has revolutionalized the field of WM mapping. This MRI-based methodology, originally described by (Basser et al., 1994b, a), has emerged as an ideal tool for noninvasive assessment of WM structure in the human brain *in vivo* (Basser et al., 1994b, Pierpaoli et al., 1996). It is the method of choice for characterizing the diffusivity of water molecules in the human brain, and can provide unique biologically and clinically relevant information that is not available from other imaging modalities (Basser et al., 1994b, Pierpaoli et al., 1996). DTI-derived quantitative parameters could help to characterize tissue composition, the physical properties of tissue constituent, tissue microstructure, and its architectural organization. The approach also provides unique access to *in vivo* information about the topography of major fiber tracts.

3.12 DIFFUSION OF WATER AND DIFFUSION-WEIGHTED IMAGING

3.12.1 DIFFUSION OF WATER

The diffusion process refers to constant random movement of molecules driven by the ambient temperature of the substance of interest. This process is known as Brownian motion as Robert Brown first described it in 1827 to explain the motion of pollen grains suspended in water. In diffusion-weighted imaging, we are interested in the diffusion of water molecules driven by body temperature. The rate of diffusion at a fixed temperature can be described by the following Equation (Einstein, 1956):

$$D = < \mathit{\Delta} r^2 > / 2n\mathit{\Delta} t$$ ***Equation 3.4***

According to the Equation, the diffusion coefficient D (in mm^2/s) is proportional to the mean squared displacement $< \mathit{\Delta} r^2 >$ divided by the number of dimensions, n, and the diffusion time, $\mathit{\Delta} t$ (Alexander et al., 2007).

In biological tissues, millions of water molecules move inside, outside, around, and through cellular structures. Water diffusion is largely induced by random thermal fluctuations; however, it is also modulated by the interactions with various barriers and hindrances that interfere with *in vivo* diffusion, such as cell membranes, myelin sheath, protein filaments, and organelles (Thomsen et al., 1987, Alexander et al., 2007). Quantitation from diffusion-weighted imaging is therefore described in terms of an 'apparent diffusion coefficient' (ADC), rather than a 'diffusion coefficient' because we do not observe free diffusion of water (Parker, 2004, Roberts and Schwartz, 2007). For convenience, I will from now on mostly use the term 'apparent diffusion coefficient' and the symbol ADC instead of 'diffusion coefficient' and *D*. Changes in the degree of restriction to water diffusion (for example by changes in cellular density, degree of myelination, or average intercellular spacing) are reflected in changes in the diffusion-weighted signals. Therefore, the water molecules that influence the diffusion-weighted signal may be considered as probes that yield microstructural insights into the state of the tissue (Parker, 2004).

3.12.2 ISOTROPIC AND ANISOTROPIC DIFFUSION

The term isotropic diffusion describes equal rate of molecular motion in all directions (figure 3.7). In the human brain, isotropic diffusion may occur in the cerebrospinal fluid spaces (Pierpaoli et al., 1996), and may closely be approximated in GM regions where no dominant underlying structural orientation exists (Pierpaoli et al., 1996, Alexander et al., 2007). Conversely, in anisotropic diffusion, movement of water molecules is not equal for all directions as can be found in WM fiber tracts where axonal membranes and myelin sheaths present barriers to the diffusion of water. Water diffusion is highly restricted in directions perpendicular to the fiber orientation, while it is relatively unimpeded in the direction parallel to the fibers resulting in larger ADC values parallel to the WM tracts rather than orthogonal to them (Basser and Jones, 2002, Mukherjee et al., 2008a).

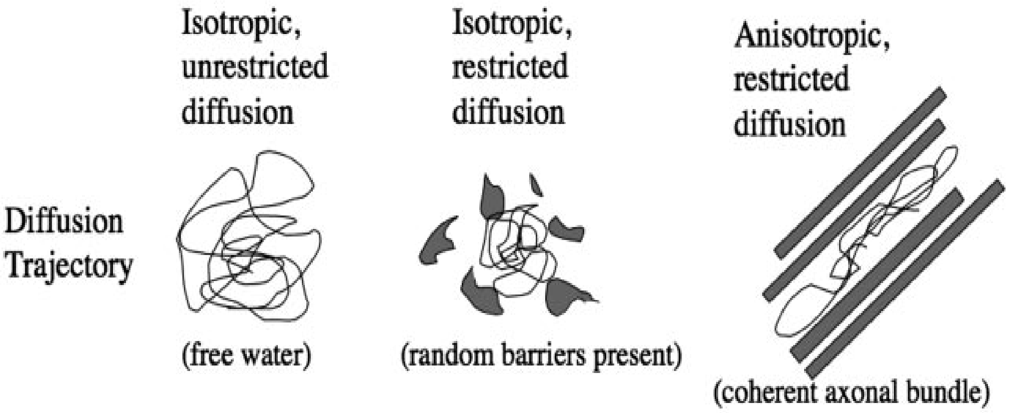


Fig. 3.7. Illustration of isotropic unrestricted diffusion, isotropic restricted diffusion, and anisotropic restricted diffusion. Modified from (Mukherjee et al., 2008a).

3.12.3 DIFFUSION-WEIGHTED IMAGE ACQUISITION

The most widely used diffusion-weighted imaging method is the pulsed-gradient spin echo pulse sequence with a single shot, echo-planar imaging readout (figure 3.8). A diffusion-weighted pulse sequence is created by the addition of a pair of gradient pulses (diffusion-sensitizing gradients) to a T2-weighted spin echo sequence. Gradient pulses of equal amplitude and duration are placed before and after the 180º refocusing pulse along the same directional axis (Stejskal-Tanner diffusion encoding (Stejskal and Tanner, 1965)). The first gradient pulse dephases, while the second pulse rephases the magnetization across the voxel. In the absence of molecular motion, rephasing will be exact and there will be no net loss of signal in the resultant spin echo. However, if there is translational motion (diffusion) of water molecules in the direction of the applied gradient, perfect refocusing will fail. Because the MR signal at each voxel represents the sum of signals from all the water molecules in that voxel, imperfect refocusing will lead to loss of signal in the resultant diffusion-weighted spin echo. In this way, the application of a pair of gradient pulses can sensitize the MR signal to the diffusion process of water, which is the basis of diffusion-weighting (Mori and Zhang, 2006, Alexander et al., 2007, Roberts and Schwartz, 2007, Mukherjee et al., 2008a). A characteristic feature of MR-based diffusion measurement is that it senses water movement only along the applied gradient axis. In the example presented in figure 3.9, only horizontal movement of water molecules results in signal loss as the gradient was applied along the horizontal orientation (X axis). In this particular example, the ADC is measured along the X axis. By combining the X, Y, and Z gradients, it is possible to measure ADC along any orientation (Mori and Zhang, 2006). Since ADC is equal for all directions in isotropic diffusion, the selection of direction for the applied diffusion-sensitizing gradient is not imperative. Conversely, more than one diffusion-encoding direction is needed to describe anisotropic diffusion of water (e.g. in WM regions). By obtaining diffusion-weighted images with diffusion sensitization in different directions, we can determine the magnitude and orientation of anisotropy (Alexander et al., 2007, Roberts and Schwartz, 2007, Mukherjee et al., 2008a).

The signal detected in the diffusion-weighted MR image is determined by the ADC and a measure of diffusion-weighting, *b*. The signal attenuation due to the application of the pulse gradients can be related to the amount of diffusion using the following Equation (Alexander et al., 2007, Mukherjee et al., 2008a):

$$S_i = S_0 \, e^{-bADC_i}$$ **Equation 3.5**

Where S_i is the diffusion-weighted signal intensity observed at a given voxel with the diffusion-sensitizing gradients applied along direction i, S_0 is the signal intensity at the same voxel measured without any diffusion-sensitizing gradients, and ADC_i is the ADC in the i direction. The diffusion-weighting factor, b is measured in s/mm^2 and calculated according to the following Equation (Stejskal and Tanner, 1965):

$$b = \gamma^2 \mathrm{G}^2 \delta^2 (\Delta - \delta/3)$$ **Equation 3.6**

Where γ is the gyromagnetic ratio, G is the amplitude of the applied diffusion-sensitizing gradient measured in milliteslas per meter, δ is the duration of each diffusion gradient in milliseconds and Δ is the time interval between these gradients, measured in milliseconds (Alexander et al., 2007, Mukherjee et al., 2008a). According to Equation 3.5, increasing the *b* value increases the degree of diffusion-weighting (i.e. signal attenuation S_i along direction i). From Equation 3.6, it can be seen that increasing the gradient amplitude and duration (G and δ, respectively) are the most efficient methods of raising the diffusion-weighting. Higher ADC values, which indicate greater amount of water motion, result in lower signal intensity in the diffusion-weighted MR image. On the other hand, reduced ADC values lead to higher signal intensity in the diffusion-weighted MR image (Mukherjee et al., 2008a).

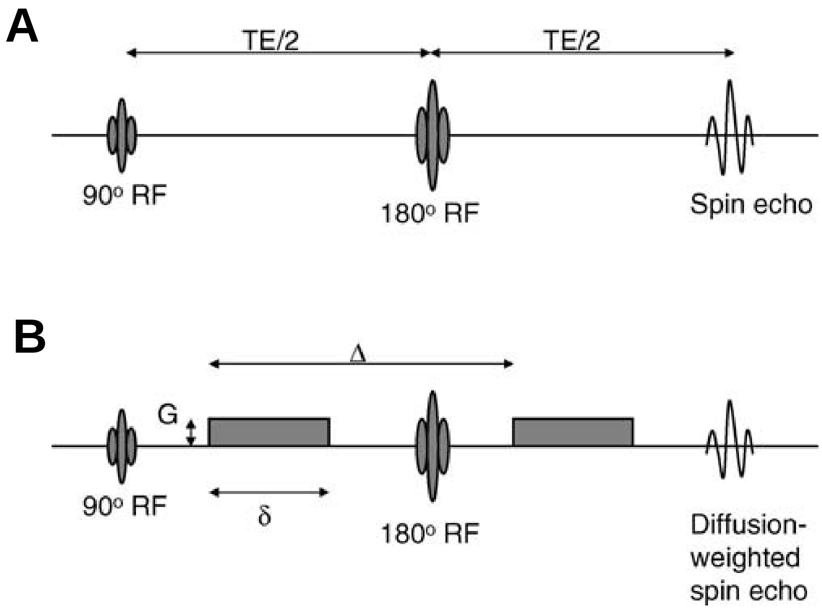


Fig. 3.8. A, Illustration of the spin echo sequence formed by applying 90° and 180° RF (radiofrequency) pulses. TE, echo time. **B**, adding two diffusion-sensitizing gradients (dark gray rectangles) transforms the spin echo sequence into a diffusion-weighted spin echo sequence (note the reduction in signal amplitude). The diffusion-weighting factor *b* depends on the amplitude of the diffusion gradient (G), the duration of the diffusion gradient (δ), and the separation between the diffusion gradients (Δ). Modified from (Roberts and Schwartz, 2007).

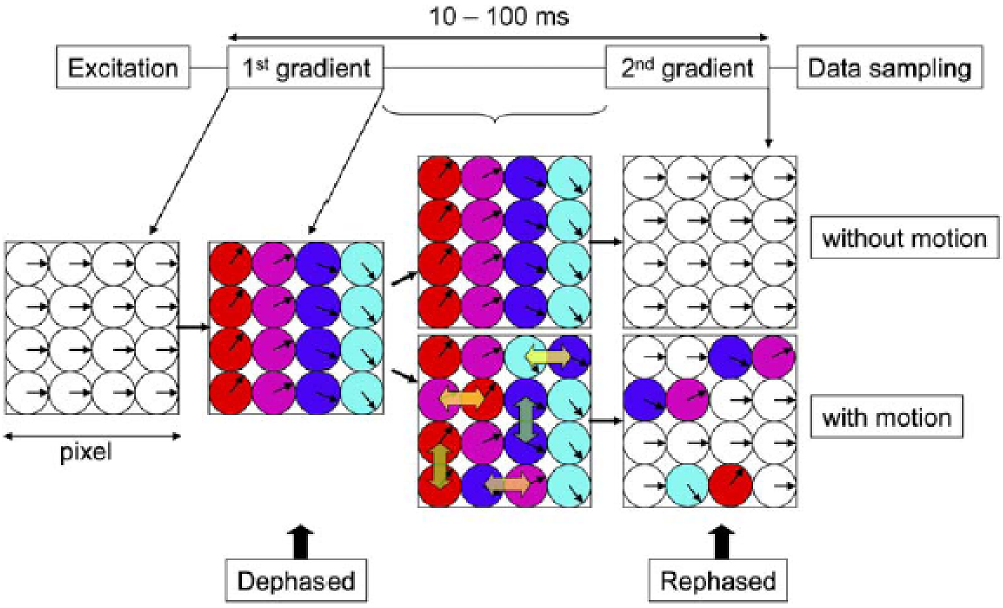


Fig. 3.9. A diagram demonstrating the relationship between diffusion of water molecules and gradient applications. Circles represent water molecules, with vectors (arrows) in the circles represent phases of the signal. The pulse gradient is applied along the horizontal orientation. Molecular motion between the two gradient applications causes imperfect refocusing of the phases by the second gradient resulting in signal loss. In this example, horizontal movement of water molecules (left-right yellow arrow) results in signal attenuation, while vertical movement (up-down green arrow) does not influence the signal intensity. Modified from (Mori and Zhang, 2006).

3.12.4 QUANTITATION OF ADC BY MRI

The signal intensity generated with diffusion-weighted imaging also depends on proton density, spin–lattice relaxation time (T1), and spin–spin relaxation time (T2), in addition to machine parameters, TR (repetition time) and TE (echo time) (Mori and Zhang, 2006). These additional weightings could result in erroneous analysis of a diffusion-weighted image when viewed in isolation. In particular, T2 prolongation resulting from pathology (e.g. in the presence of edema) may lead to diffusion-weighted signal hyperintensity, even when ADC values are not reduced, a phenomenon known as "T2 shine-through" (Rowley et al., 1999). On the other hand, reduction in T2 may mask a real reduction in ADC values, a phenomenon known as "T2 blackout" (Maldjian et al., 2001). This issue has received a considerable interest in clinical diagnosis.

To calculate pure ADC maps, where the ADC is the sole source of contrast, we need to acquire two sets of images with differing *b* values. In general, one set of images acquired at a low *b* value (≈ 0 s/mm^2) (non-diffusion-weighted), and a second set of diffusion-weighted images acquired at a high *b* value (e.g. 1000 s/mm^2), while other imaging parameters (TR and TE) remain the same. The pair of images with differing *b* values would eliminate the confounding weighting (from proton density and T2) (Mori and Zhang, 2006, Roberts and Schwartz, 2007). It is possible to quantify the ADC on a voxel-by-voxel basis by solving for ADC in Equation 3.5 (Stejskal and Tanner, 1965):

$$ADC_i = -\mathrm{In}\,\left(\frac{S_i}{S_0}\right)/b \qquad \textbf{Equation 3.7}$$

Where In is the natural logarithm. S_i is the signal intensity at a given voxel for the image acquired with higher *b* value. S_0 is the signal intensity at the same voxel of the image acquired with *b* = 0.

To calculate the diffusion tensor, **D**, at least six diffusion encoded image sets need to be acquired along noncollinear directions in addition to at least one *b* = 0 s/mm^2 (or low *b*) image set (Basser et al., 1994a) (see below).

3.12.5 ESTIMATION OF THE DIFFUSION TENSOR

The direction of maximum diffusivity has been shown to coincide with the WM fiber tract orientation (Moseley et al., 1990). In order to correctly estimate the orientation of the dominant direction of diffusion within a voxel (the orientation with the largest ADC), we need to measure diffusion along thousands of axes, which is impractical. The model of “diffusion tensor” was thus introduced by Basser and colleagues to accurately find the direction and degree of anisotropy (Basser et al., 1994b, a), hence the name ‘‘diffusion tensor imaging’’. The term DTI describes consecutive diffusion-weighted image acquisitions which have been obtained with encoding in different directions to estimate the diffusion tensor. The diffusion tensor, a 3 X 3 matrix representation, is a mathematical model of the 3D pattern of diffusion anisotropy of WM tracts:

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} \qquad \textbf{\textit{Equation 3.8}}$$

The tensor, **D**, is basically a matrix of numbers (diffusion coefficients) derived from diffusivity measurements in at least six noncollinear directions, which we can use to estimate the diffusivity in any arbitrary direction or determine the direction of maximum diffusivity. The elements of the tensor above the diagonal are always equal to those below the diagonal; the tensor matrix is therefore diagonally symmetric ($D_{ij} = D_{ji}$) with only six of the tensor’s matrix’s nine elements are independent necessitating a minimum of six diffusion-encoded measurements to correctly estimate the diffusion tensor. If we use more than six encoding directions, we can improve the accuracy of the tensor measurement for any arbitrary orientation (Jones et al., 1999a).There are three commonly used methods for estimating the tensor: nonlinear least squares, weighted linear least squares, and the most common is ordinary least squares (Basser et al., 1994a, Jones and Cercignani, 2010).

The tensor matrix is subjected to a linear algebraic procedure known as “matrix diagonalization”, which extracts the three eigenvectors of the diffusion tensor (the dominant direction of diffusion, V_1, in addition to orthogonal minor directional information, V_2 and V_3) together with the corresponding three eigenvalues (λ_1, λ_2, and λ_3) representing the ADCs along these axes.

The best way to understand the information provided by the diffusion tensor is to visualize it geometrically. The tensor efficiently fits the angular variation of the ADC values to the shape of a 3D ellipsoid (Mori and Zhang, 2006). The orientation of the ellipsoid is defined by the three eigenvectors of the tensor, and its shape is characterized by the three eigenvalues (Basser et al., 1994b). Three main classes of diffusion tensors may be described (Parker, 2004) (figure 3.10): class I represents the case where $\lambda 1 \approx \lambda 2 \approx \lambda 3$. The diffusion in this case is isotropic and the diffusion ellipsoid has the shape of a sphere as the ADC is equal for all directions. This is the form of tensor expected in cerebrospinal fluid spaces. Class II represents the case where $\lambda 1 > (\lambda 2 \approx \lambda 3)$ indicating a greater mean diffusion distance along the longest axis of the ellipsoid. The diffusion ellipsoid is cigar-shaped (prolate). Class III represents the case where $(\lambda 1 \approx \lambda 2) > \lambda 3$. The dominant direction of diffusion in this situation is less distinct indicating an ambiguous fiber orientation and the diffusion ellipsoid is disk-shaped (oblate). The vector of the longest axis (V_1), also named the principal eigenvector, with its associated eigenvalue λ_1 indicate the direction and magnitude of greatest water diffusion (maximum diffusivity), respectively (Mori and Zhang, 2006). The principal eigenvector is important for fiber tractography algorithms because it reflects the orientation of axonal fiber bundles.

3.12.6 COLOUR-CODED MAPS OF THE DIFFUSION TENSOR

Because it is very difficult to visualize 3D vectors on 2D images, the fiber orientation information inherent in the principal eigenvector can be visualized using a colour-coded orientation map (Pajevic and Pierpaoli, 1999). The most basic red–green–blue (RGB) colour-coded scheme indicates a colour for each orientation of the fibers X, Y and Z; the colour of the largest ADC is determined, and a colour is assigned for each pixel. Fibers running from left to right are represented in red because the ADC is greatest along the X-axis; likewise, fibers running antero-posteriorly are represented in green, and fibers running infero–superiorly are represented in blue (Mori and Zhang, 2006) (figure 3.11).

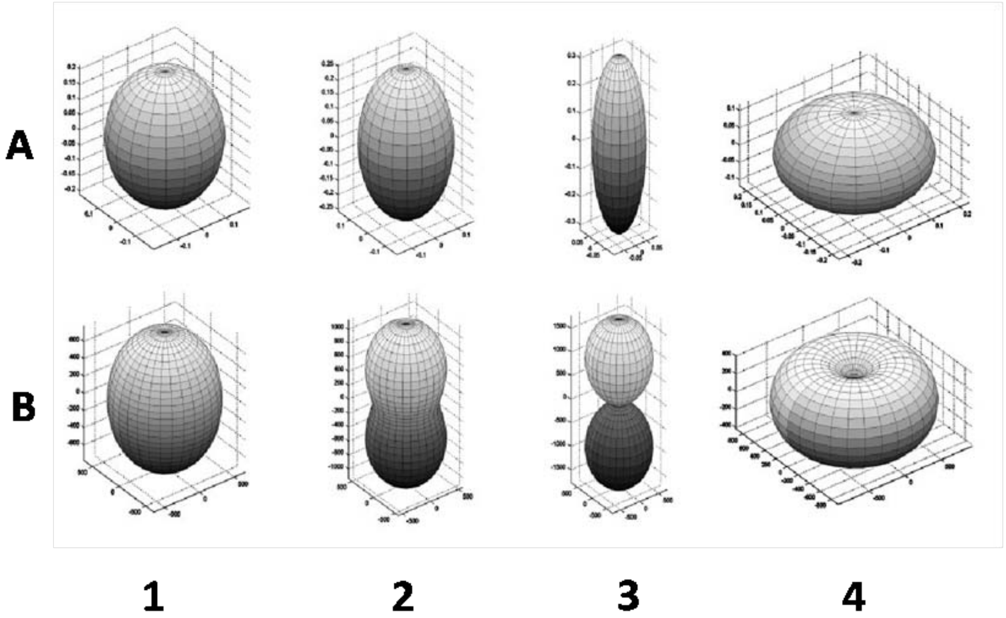
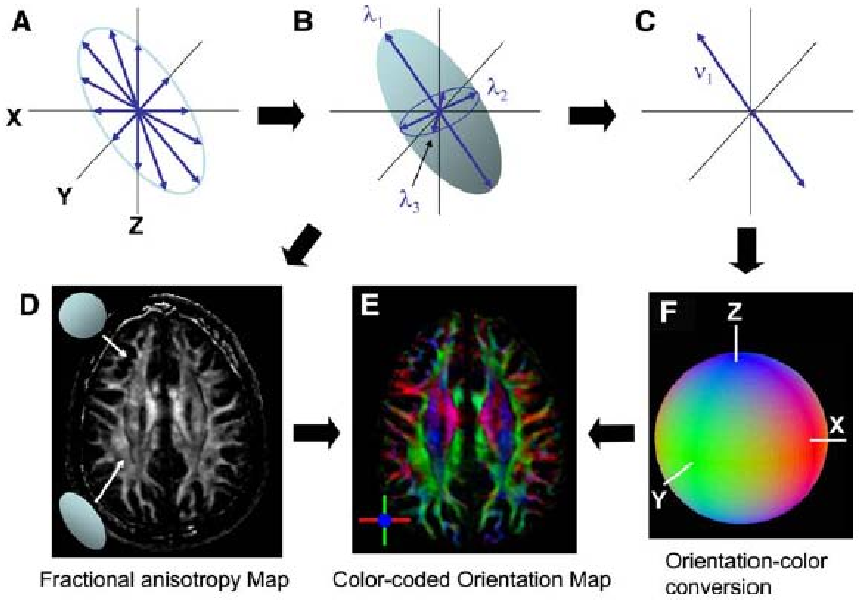


Fig. 3.10. Classes of diffusion tensor ellipsoids (**A**) and their ADC profiles (**B**). Eigenvalues (X 10^{-6} mm^2/s): 1, class **I**: 781, 670, 670; 2, class **II**: 1042, 529, 529; 3, class **II**: 1773, 164, 164; and 4, class **III**: 931, 931, 283. Trace = 2100 X 10^{-6} mm^2/s. ADC profile axes are represented in 10^{-6} mm^2/s. Modified from (Parker, 2004).



Fractional anisotropy Map

Color-coded Orientation Map

Orientation-color conversion

Fig. 3.11. The Principle of DTI. Diffusion measurements are obtained along multiple axes (**A**), which allow estimation of the shape and orientation of the 3D ellipsoid (**B**). A fractional anisotropy map (**D**) can be generated from the shape. Dark regions represent isotropic diffusion (spherical), and bright regions represent anisotropic diffusion (cigar-shaped). From the estimated 3D ellipsoid (**B**), the orientation of the longest axis can be identified (**C**), which reflects the orientation of axonal fiber bundles. This orientation information is converted to a colour (**F**) at each pixel. To visualize the fiber orientation information, a colour-coded map is generated (**E**). Modified from (Mori and Zhang, 2006).

3.13 QUANTITATIVE DTI-DERIVED METRICS

A number of diffusion metrics can be obtained from the three eigenvalues, which could then be used to probe the microstructure in a particular voxel.

3.13.1 TRACE OF THE TENSOR

The trace of the tensor (Tr) summarizes the total diffusivity, it is the sum of the three eigenvalues:

Trace (Tr) = $\lambda_1 + \lambda_2 + \lambda_3$ ***Equation 3.9***

3.13.2 MEAN DIFFUSIVITY

The mean diffusivity (MD) describes the directionally averaged diffusivity of water within a voxel. The MD is obtained from the following Equation:

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} = \mathrm{Tr}/3$$ ***Equation 3.10***

Diffusivity can be divided into an **axial (longitudinal) diffusivity** component ($\lambda \parallel$, diffusion along the axons), which corresponds to the principal eigenvalue of the diffusion tensor ($\lambda \parallel = \lambda 1$), and a **radial diffusivity** component ($\lambda \perp$, diffusion perpendicular to the axons), which is calculated by averaging the second and third eigenvalues: $\lambda \perp = (\lambda 2 + \lambda 3) / 2$ (Alexander et al., 2007).

3.13.3 FRACTIONAL ANISOTROPY AND RELATIVE ANISOTROPY

Fractional anisotropy (FA) and relative anisotropy (RA) characterize different ways in which the diffusion tensor field deviates from being isotropic. The FA and RA indices determine the degree of directionality of intra-voxel diffusivity (Mukherjee et al., 2008a). FA and RA can be defined by the following Equations (Basser et al., 1994b, Pierpaoli and Basser, 1996):

$$\mathrm{FA} = \sqrt{\frac{1}{2}}\,\frac{\sqrt{(\lambda_1-\lambda_2)^2+(\lambda_2-\lambda_3)^2+(\lambda_3-\lambda_1)^2}}{\sqrt{\lambda_1{}^2+\lambda_2{}^2+\lambda_3{}^2}}$$ ***Equation 3.11***

$$\mathrm{RA} = \frac{\sqrt{(\lambda_1-\lambda_2)^2+(\lambda_2-\lambda_3)^2+(\lambda_3-\lambda_1)^2}}{\lambda_1 + \lambda_2 + \lambda_3} \qquad \textbf{\textit{Equation 3.12}}$$

When λ_1 is much larger than the λ_2 and λ_3, anisotropy measures (FA and RA) will be high, representing a preferred direction of water diffusion (cigar-shaped diffusion). A cigar-shaped diffusion within a brain voxel usually represents a single coherent WM tract with axons coinciding with the principal eigenvector (Mukherjee et al., 2008a).

3.14 INTERPRETATION OF DTI-DERIVED METRICS

The literature indicates that DTI serves as a powerful tool for detecting microscopic changes in tissue properties. DTI-derived measures can provide information about the microstructural features of WM, which include intra-axonal organization, density of fibers, degree of myelination, and individual fiber diameter (Mori and Zhang, 2006, Sun et al., 2006). The FA has become the most widely used invariant measure of anisotropy in the DTI literature. In healthy WM, FA can range between values of 0.1 (isotropic; diffusivity is equal in all directions), as in cerebrospinal fluid and GM, to almost 1.0 (anisotropic, water diffusion is highly restricted in directions perpendicular to the fiber orientation, while it is relatively unimpeded in the direction parallel to the fibers) with a peak at 0.3 (Alexander et al., 2007). FA value has been used as a scalar indicator of the directionality and coherence of WM tracts and was considered a highly sensitive biomarker of WM integrity (Breier et al., 2011, Lin et al., 2012). The more coherent and organized are the fiber tracts, the higher their FA (Basser and Pierpaoli, 1996). FA and other DTI-derived measures, such as axial and radial diffusivity can identify age-related changes in WM structure (Barrick et al., 2010), and are sensitive to a broad spectrum of neuropathologic conditions (Bethune et al., 2011, Yu et al., 2011, Cirillo et al., 2012). Many pathologic conditions may alter FA because of changes in diffusivity and disorganization of the WM tracts leading to reduced anisotropy, such as in multiple sclerosis (Filippi et al., 2001). FA values may become abnormal even before the lesion is morphologically apparent on conventional MRIs and may therefore help in early detection and in defining the extent of these lesions (Zou et al., 2008). However, interpretation of DTI-derived measures is challenging and should be performed with great care. Many studies have focused mainly on the FA measure, which may not fully characterize changes in the microstructure. For example, reduced FA in neuropathologic conditions may be due to increased radial diffusivity or reduced axial diffusivity or both. Other DTI metrics, such as MD, axial, and radial diffusivities may thus be required to better understand changes in the diffusion tensor (Song et al., 2002, Alexander et al., 2007, Lochner et al., 2012, Zhuang et al., 2012). For instance, it has been shown that radial diffusivity, but not axial diffusivity, correlates with the demyelination process in WM tracts (Song et al., 2002, Song et al., 2005). Also, increased MD value was shown to reflect increased tissue water in edema (Kale et al., 2006), while reduced MD value could serve as a biomarker of cellular proliferation in tumors (Iacconi et al., 2010). Interpretation of DTI findings is also complicated by the sensitivity of anisotropy measures to several technical factors, such as noise, eddy current distortions, partial volume averaging, and issue of crossing fibers (Mukherjee et al., 2008b, Jones and Cercignani, 2010). Limitations of DTI and tractography are presented in chapter 6. These confounding factors may induce significant changes in anisotropy measures in the absence of any change in WM structure.

3.15 DTI-BASED TRACTOGRAPHY

DTI-based tractography provides unique access to *in vivo* information about the topography of major fiber tracts in the human brain. The objective of DTI-based tractography is to determine inter-voxel connectivity based on the anisotropic diffusion of water (Mori et al., 1999, Basser et al., 2000, Pfefferbaum et al., 2003). Since the orientation of the principal eigenvector is assumed to be parallel to the dominant direction of axonal tracts, tractography algorithms can use this information in each voxel to track a fiber tract in 3D from voxel to voxel (Berman et al., 2004). Compared to focal region of interest (ROI) analysis, quantitative fiber tracking has the advantages of providing visual depiction of WM tracts and the ability to examine the whole extent of a WM tract.

3.16 FIBER TRACKING METHODOLOGIES

Numerous DTI-based fiber tractography algorithms have been reported to date, such as line propagation (Mori and van Zijl, 2002), fast marching (Parker et al., 2002), regularization (Poupon et al., 2000), subvoxel interpolation of the diffusion tensor field (Basser et al., 2000), probabilistic (Behrens et al., 2003a), and stochastic labeling methods (Tench et al., 2002). In general, fiber tracking methodologies fall into two main categories (Parker, 2004, Mukherjee et al., 2008a): deterministic (Conturo et al., 1999, Jones et al., 1999b, Mori et al., 1999, Basser et al., 2000), and probabilistic methods (Behrens et al., 2003b, Lazar and Alexander, 2005, Behrens et al., 2007).

3.16.1 DETERMINISTIC FIBER TRACKING

Deterministic algorithms follow the major fiber directions as indicated by the diffusion model and create sequences of points that are considered as fibers. Several deterministic tractography algorithms were developed to date. The streamline algorithms were initially developed (Mori et al., 1999, Basser et al., 2000), followed by more complicated tensor deflection algorithms (Lazar et al., 2003), or more global approaches (Mangin et al., 2002). One of the most popular deterministic algorithms for tractography is known as fiber assignment by continuous tracking, FACT (Mori et al., 1999). Fiber trajectories (also called “streamlines”) are initiated from user-defined voxels (seed point). These streamlines propagate from voxel to voxel in 3D following the principal eigenvector in each voxel (Mori et al., 1999, Basser et al., 2000, Mori and Zhang, 2006). When the streamline approaches the edge of the voxel, its direction is changed to correspond to that of the principal eigenvector of the next voxel (figure 3.12). A modification of this simple linear approach can be used to generate a smooth (curved) trajectory, which can provide more accurate results when the curvature of a reconstructed streamline is steep with respect to imaging resolution (simple interpolation approach) (Mori and van Zijl, 2002).

3.16.1.1 TERMINATION CRITERIA

There are two important termination criteria for tractography; the first is the minimum FA threshold within a voxel, and the second is the maximum streamline turning angle between voxels. Typical minimum FA thresholds applied for tractography in adult human brain range from 0.1 to 0.3 (Mukherjee et al., 2008b). Fiber tracking should stop if the FA value of a voxel is less than a defined threshold as the voxel under examination probably contains GM or cerebrospinal fluid (Chung et al., 2011). It is thus expected that selecting low minimum FA thresholds will likely produce more and longer streamlines assuming that all other parameters are equal. However, a great care should be taken in such cases as selecting FA values, which are too low for the signal to noise ratio of the diffusion-weighted sequence is likely to produce false positive fiber tracking (Mukherjee et al., 2008b).

The angle change between pixels is another important criterion with typical maximum turning angle values range from 40° to 70°. Tracking errors are expected to occur if the angle transition is large. In certain circumstance, larger values may be used for proper definition of pathways with hairpin turns (e.g. the uncinate fasciculus), although such approach may considerably increase the number of spurious tracks and overall computational loads (Mukherjee et al., 2008b).

3.16.1.2 PLACEMENT OF THE SEED VOXELS

Placement of the seed voxels could influence tractography results. To identify WM tracts passing through certain ROIs, one can pre-assign seed voxels within a region of interest placed within the tract of interest (Mori and van Zijl, 2002). Another approach is to seed voxels within the entire brain region above a certain FA threshold, so that all possible WM streamlines within the range of computational considerations are identified (the brute force method) (Huang et al., 2004). Subsequently, streamlines crossing the pre-defined ROIs are then selected by filtering out those undesired ones. The brute force method has been considered technically superior as it may identify some tracks that are missed by ROI-based seeding and can substantially increase the validity of

DTI-based tractography (Huang et al., 2004); however, it adds huge loads to the computation process (Mukherjee et al., 2008b).

To improve localization of a specific WM tract, two or more seed regions may be placed and only the streamlines bisecting these regions are preserved (Jones and Basser, 2004). This multiple ROIs technique for isolating anatomically specific fiber pathways by using DTI-based tractography has been named “virtual dissection” (Catani et al., 2002). In this way, deterministic tractography algorithms were shown to be capable of generating anatomically plausible estimates of WM trajectories in the human brain, such as major projection pathways (e.g., corticospinal tract, corona radiata), commissural pathways (e.g., CC, anterior commissure), and association pathways (e.g., arcuate fasciculus, uncinate fasciculus, etc.) (Jellison et al., 2004, Hofer and Frahm, 2006, Yu et al., 2007, Matsumoto et al., 2008). A direct comparison of these DTI-based tract reconstructions with histology-based illustration revealed that tractography can achieve accurate reconstruction of core regions of major human neuroanatomical structures (Catani et al., 2002, Chung et al., 2011). However, Deterministic fiber tracking techniques are also sensitive to various sources of artifacts and technical limitations as discussed in chapters 6 and 7.

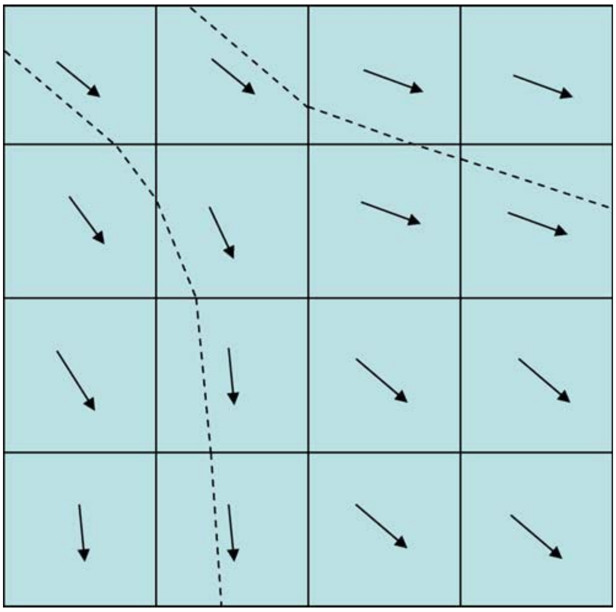


Fig. 3.12. A schematic illustration of the FACT algorithm. In this diagram, the principal eigenvector for each pixel is depicted as an arrow. Streamlines (dashed lines) are then determined by following the path of the principal eigenvector in each pixel and proceeding until a pixel is reached where anisotropy has fallen below a pre-defined threshold, at which point the tract terminates. Modified from (Roberts and Schwartz, 2007).

3.16.2 PROBABILISTIC FIBER TRACKING

Since probabilistic tractography was not performed in the present work, a brief review of the basic principles underlying this method is provided below; several more comprehensive reviews are provided elsewhere (Behrens et al., 2007, Berman et al., 2008, Jeurissen et al., 2011). Deterministic streamline fiber tracking is susceptible to noise, partial volume, subject motion, and distortion from other imaging artifacts, which can produce uncertainty in the orientation of the diffusion ellipsoid (Dietrich et al., 2001, Mori and van Zijl, 2002). Considering that errors due to the above-mentioned artifacts are random, it is predicted that they have low reproducibility if the same subject is repeatedly scanned and the results are superimposed. Probabilistic tractography provides information about the estimated probability distribution of the likelihood that two or more certain regions are interconnected (i.e., confidence mapping). For probabilistic fiber tracking, repeated image acquisitions are obtained from the same subject with the subsequent use of one tractography algorithm on these datasets to get several tractograms (Mori et al., 2002, Bammer et al., 2003). The probability of the presence of specific pathways in this subject is then determined by estimating the number of successful trackings, divided by the total number of datasets (Jones et al., 2002). Probabilistic tractography techniques tend to scatter fiber trajectories more than deterministic methods, and have the capacity to demarcate a larger portion of a WM tract (Mori et al., 2002, Mukherjee et al., 2008a).

3.17 VALIDATION OF DTI-BASED TRACTOGRAPHY

DTI-based tractography has been widely used in basic and clinical research being the only currently available technique to explore the anatomy of WM tracts in the human brain noninvasively. Unfortunately, results of DTI-based tractography in humans are difficult to validate *in vivo*. It is therefore essential to develop methods to verify whether DTI-derived tractography results match the real WM tracts. Several quality analysis methods were proposed to address this issue. First, it is important to compare fiber tracking results with those of published atlases (Wakana et al., 2004, Salamon et al., 2007, Catani and Thiebaut de Schotten, 2008, Lawes et al., 2008). This could be performed by assessing whether fiber tracking shows a tract that cannot be demonstrated with a gold-standard technique (false-positive results) or does not reveal a tract where the gold-standard demonstrates one (false-negative results) (Bammer et al., 2003). Despite the existence of several published DTI atlases, there is no unified concept about a proper gold-standard that can be used to evaluate the precision of DTI-based tractography in defining neural fiber direction. Second, comparison with physical or mathematic virtual fiber phantoms, such as curved 3D trajectories, merging/kissing fiber tracts, and parallel fiber bundles, is probably the best method that could be used to test the fidelity of tractography algorithms (Basser et al., 2000, Tournier et al., 2002, Cheng et al., 2006, Poupon et al., 2008). These phantoms may provide valuable tools to validate fiber tracking techniques with regard to varying SNR curvature and discontinuities (Bammer et al., 2003). Third, some animal studies compared 3D histological reconstruction of fiber tracts with DTI-based tractography to provide a standard reference for evaluating fiber tracking (Dauguet et al., 2006, 2007). However, this technique needs postmortem material and is subjected to several methodological problems notably, destruction of tissue material during preparation, which alters fiber orientations and causes a mismatch between specimens and DTI. Fourth, more direct information about axonal connectivity can be derived from the use of chemical tracers to track fibers with exact spatial registration. In this technique, chemical tracers are injected and their destinations confirmed histologically. Examples of such chemical tracers include divalent manganese ions (Mn^{+2}) (Lin et al., 2001) and biotinylated dextran amine (Dyrby et al., 2007). Tract-tracing techniques were considered one of the gold standard methods for connectivity studies. However, there are several difficulties in such a validation approach. First, although authors reported good correspondence between DTI-based tractography and $Mn^{+}2$-based enhancements in the optic tracts (Lin et al., 2001), the technique was shown to have limited ability to evaluate longer fiber tracts due to the slow spread of Mn^{+2} ions in the neural tracts and fast decay of enhancement. Second, a key advantage of the DTI-based tractography technique is its capacity to characterize the macroscopic WM structures in a short time. It is practically impossible to obtain similar datasets (there are millions of neurons inside the brain) using the single-cell level chemical tracer technique (Mori and van Zijl, 2002). Third, DTI examination of the human brain usually involves limited scanning time resulting in lower data quality compared to animal scans. It is thus expected that using the same technique in the human brain would show inferior performance.

3.18 ANALYSIS OF DTI DATA IN THE PRESENT WORK

DTI and fiber tractography were performed in study 3 and 4, which are presented in chapters 6 and 7, respectively.

3.18.1 DTI DATA PRE-PROCESSING

The following steps were performed to pre-process the DTI data:

- All diffusion–weighted images were visually inspected for apparent artifacts due to subject motion, magnetic susceptibility, and instrumental malfunction.
- Output files from the Siemens scanner (in DICOM format) were converted to (nii.gz) compressed data format using dcm2nii.exe program available with MRIcron version 12/2009 (Chris Rorden, University of South Carolina, Columbia, SC, USA; available online at http://www.mccauslandcenter.sc.edu/mricro/mricron/install.html).
- In order to remove the artifacts from subject motion and eddy current-induced distortion, I used FMRIB’s diffusion tool box (free software from Oxford Centre for Functional MRI of the brain, UK), available online

at http://www.fmrib.ox.ac.uk/fsl/ (Smith, 2002, Reese et al., 2003). Using the diffusion tensor analysis toolkit (FDT), spatial distortions produced by eddy currents were corrected using 12-parameter affine registration to the $b = 0$ image.

3.18.2 TRACTOGRAPHY PARAMETERS

After image pre-processing, tractography was applied to the DTI data to reconstruct WM tracts by successively following the path of preferred direction of water diffusion (Basser et al., 2000) using MedINRIA software v.1.9 for Linux (available online at http://www-sop.inria.fr/asclepios/software/MedINRIA/). It is important to note that differences in the algorithm and threshold used could induce biased results. I therefore used fixed parameters and applied ROIs according to fixed anatomical landmarks across all subjects to minimize bias. Fiber tracts were calculated by connecting adjacent voxels with similar principal eigenvectors, using the following parameters available in MedINRIA software:

- FA threshold = 0.2.
- Smoothing = medium.
- Smoothness factor (which corresponds to the straightness of each fiber) = 0.2.
- Minimum fiber length =10.
- Sampling =1.

Similar parameters were used by previous studies that applied continuous fiber tracking algorithm (Kim et al., 2005, Thomas et al., 2005, Schaechter et al., 2008).

3.18.3 TRACTOGRAPHY OF THE CORPUS CALLOSUM, SUPERIOR CEREBELLAR PEDUNCLE AND MIDDLE CEREBELLAR PEDUNCLE

A detailed description of methods used to reconstruct these tracts is provided in related chapters: reconstruction of the CC is described in chapter 6 and reconstruction of the SCP and MCP is described in chapter 7.

CHAPTER 4

INVESTIGATING STRUCTURAL PLASTICITY OF PARS OPERCULARIS & PARS TRIANGULARIS IN MUSICIANS

INCREASED GRAY MATTER VOLUME AND CORTICAL SURFACE AREA OF THE LEFT PARS OPERCULARIS/BA44 IN MALE ORCHESTRAL MUSICIANS

4.1 ABSTRACT

BACKGROUND AND PURPOSE To compare manual volumetry of gray matter (GM) and white matter (WM), and cortical surface area of Broca's area subparts: pars opercularis (POP)/BA44 and pars triangularis (PTR)/BA45 in both hemispheres between musicians and non- musicians, as it has been shown that these regions are crucial for musical abilities. A previous voxel-based morphometric (VBM) study conducted in our laboratory reported increased GM density in Broca's area of the left hemisphere in male orchestral musicians. Functional segregation of POP/PTR justified separate volumetric and surface area measurements of these parts.

SUBJECTS AND METHODS The same cohort of the VBM study was used. Manual morphometry (stereology) was used to compare volumes between 26/26 right-handed orchestral musicians/non-musicians. Cortical surface areas of BA 44/45 were also measured using an automatic method.

RESULTS Musicians showed significantly increased GM volume in the left POP ($p<0.001$). No significant results were detected in the right POP, left/right PTR GM volumes, and WM volumes for all regions. Results were positively correlated with years of orchestral performance ($r = 0.7$, $p = 0.0001$). In addition, musicians possessed significantly increased cortical surface area of the left BA44 ($p=0.01$) with no significant results in left BA45 and right BA44/45.

CONCLUSION The present findings corroborate the VBM study and are in line with the hypothesis of critical involvement of the POP in hearing-action integration being an integral component of the fronto-parieto-temporal mirror neuron network. I hypothesize that increased volume and cortical surface area of musicians' left POP/BA44 represent use-dependent structural adaptation triggered by intensive audiomotor skill acquisition.

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Abdul-Kareem IA, Stancak A, Parkes L & Sluming V (2011): Increased gray matter volume of left pars opercularis in male orchestral musicians correlate positively with years of musical performance. *JMRI*. 33(1):24-32.

4.2 INTRODUCTION

The posterior part of the inferior frontal gyrus in the left hemisphere is traditionally considered to constitute the classic Broca's area, which is critically involved in various aspects of expressive language processing (Petrides, 2006). This brain area includes two distinct regions: the POP, BA44 and the PTR, BA45.

A growing body of evidence suggests that language and music are intimately related. Like language, music involves organization of perceptually discrete elements into ordered sequences based on syntactic principles (Patel, 2003). The wealth of literature supports this assumption and assigns a substantial role for Broca's area in music, just as in language. Functional studies have reported involvement of Broca's area/left inferior frontal gyrus in music discrimination (Platel et al., 1997), visuospatial and audiospatial localization (Martinkauppi et al., 2000, Limb et al., 2006, Sluming et al., 2007), processing and organization of sequential sound stimuli (Platel et al., 1997), musical syntax processing (Maess et al., 2001, Koelsch, 2006), retrieval success for music memory (Watanabe et al., 2008), musical improvisation (Berkowitz and Ansari, 2008), sight reading (Sergent et al., 1992), and score reading while listening to the accuracy of musical performance (Parsons, 2001). Adding structural evidence to functional data, a previous VBM study reported increased GM density in Broca's area (left inferior frontal gyrus) in male orchestral musicians compared to non-musicians (Sluming et al., 2002). The latter study also compared manual volume measurements of prefrontal cortical subfields between musicians and non-musicians and reported no significant group differences. Other VBM studies also reported structural plasticity in the left inferior frontal gyrus in the form of increased GM density (Gaser and Schlaug, 2003b, a) and increased fractional anisotropy (Han et al., 2009).

In the present study, I re-examined Broca's area using the same cohort of the previous study (Sluming et al., 2002) with the rationale that this area includes two parts each behaves as one unit with its own connections and functions. Separate structural morphometry of these regions could thus give a clearer view about Broca's area plasticity in musicians. In addition, the previous study used an automatic technique

(VBM), which could be cross-validated through using manual volumetry, such as that used in the present study.

4.2.1 SEGREGATION OF PARS OPERCULARIS AND PARS TRIANGULARIS

Although both POP and PTR were collectively described as Broca's area, there are cytoarchitectonic, connectivity and functional data suggesting segregation of these parts. **Cytoarchitectonically**, POP (BA 44) contains a thin layer IV of small granular cells with pyramidal cells from deep layer IV (i.e. it is mostly dysgranular, motor), while BA 45 has densely packed granular cells in layer IV (i.e. it is mostly granular, sensory) (Amunts et al., 1999, Schenker et al., 2008). In particular, the POP/BA44 shares some cytoarchitectonic properties with the premotor cortex (BA6).

Connectivity data, structural characterization of a distinct cortical area just by its microstructure without taking into consideration its connections to other brain areas remains insufficient as cognitive functions depend on widespread networks, rather than isolated cortical areas. Both BA 44 and 45 have distinct cortical connections as revealed by diffusion tensor imaging (Xiang et al., 2009). Separation of BA44 and BA45 according to their connections was suggested to align with their macroanatomical boundaries (Anwander et al., 2007).

Functional, a corresponding functional division inside Broca's complex was suggested by functional neuroimaging studies employing language processing tasks (Demb et al., 1995, Bookheimer, 2002, Chein et al., 2002). Within the left inferior frontal gyrus, it was reported that BA 44 and parts of BA 6 have a role in syntactic processing and phonologically-based working memory, while BA 45 is mainly involved in semantic processing (Friederici et al., 2000), in addition to rhyming ability (Cone et al., 2008), phonological awareness (Demonet et al., 1992), and lexical decision making (Papathanassiou et al., 2000).

An important role for Broca's area has also been described for various aspects of motor processing, including motor sequence learning, action observation, motor imagery, and imitation (Iacoboni et al., 1999, Binkofski and Buccino, 2006). Importantly, functional MRI studies reported that POP is a key component of the human mirror neuron system activated during action observation and imitation, while the PTR is activated during action observation but not imitation (Molnar-Szakacs et al., 2005). Repetitive transmagnetic stimulation applied over the POP showed similar results through disrupting imitation of motor tasks (Heiser et al., 2003). Data from electrophysiological studies have pointed out that dysphasic speech arrest occurs most reliably from stimulation of the POP (Ojemann et al., 1989, Duffau et al., 2003), which, coupled with action observation and imitation data, suggest that the motor-related part of Broca's area is mainly localized within the POP.

The above evidence may suggest that morphometric analyses, such as volume and cortical surface area measurement of Broca's area as one entity remain incomplete without taking into consideration a separate analysis for POP and PTR. The primary goal of this study was therefore to measure volumes of POP and PTR separately through manual mapping according to previously established anatomical landmarks.

4.2.2 AUTOMATIC-MANUAL CROSS-VALIDATION OF BROCA'S AREA MORPHOMETRY

The VBM technique has been widely used in research as it provides unbiased, automatic, and objective morphometric analysis of GM and WM density. One advantage of VBM is its capacity to examine large cohorts in a short time. However, the VBM has been criticized (Crum et al., 2003), especially when performing morphometric analysis of WM (Gaser and Schlaug, 2003a, Thomann et al., 2006). In the normalization step, VBM registers all brain images with a reference template, which does not reflect the real structural anatomy of individual brains. This is especially true when considering morphometry of Broca's area as it is subjected to substantial inter-individual variations (Tomaiuolo et al., 1999, Keller et al., 2007). I therefore set the goal to cross-validate previous VBM results (Sluming et al., 2002) using manual volumetry as it allows more direct measurement of Broca's area with clear face validity. A similar automatic-manual cross-validation has been used in previous structural brain studies (Keller et al., 2002, Gong et al., 2005).

To summarize, the primary objective of the present study was to re-examine a previously published cohort (Sluming et al., 2002) of musicians and non-musicians to measure GM/WM volumes of Broca's area subparts (POP/PTR) in the left hemisphere and their homologue in the right hemisphere. I used manual morphometry (stereology), which allowed POP/PTR parcellation according to well-defined sulcal landmarks thereby enhancing the accuracy and validity of volume measurements. In addition to supporting previously published data of GM density differences in Broca's area, the estimation of volume may provide a better evidence (Rockel et al., 1980, Fischl and Dale, 2000) to support the theory of structural neuroplasticity acquired through skill acquisition (Schlaug et al., 1995a), and may also help to enhance our understanding of POP/PTR functional segregation. In addition to measuring volumes of POP and PTR, the second objective of the present study was to measure cortical surface areas of these regions. These measurements could provide another perspective to Broca's area plasticity in musicians. The present study was sought to extend previous structural reports in musicians (Schlaug et al., 1995a, Schlaug et al., 1995b, Ozturk et al., 2002, Schneider et al., 2002, Hutchinson et al., 2003, Lee et al., 2003, Schneider et al., 2005) through demonstrating structural plasticity in a region crucial for musical performance. Volume measurements of brain structures had been used extensively through applying the Cavalieri principle, but measurements of cortical surface area were much less frequently used possibly because it is easier to measure volume than surface area (Barta and Dazzan, 2003). The convoluted shape of the brain makes measuring cortical surface areas more difficult. In addition, application of stereological methods for cortical surface area measurements requires some degree of randomization when choosing a sectioning direction. Failure of performing this step could lead to bias (Haug, 1987, Steinmetz et al., 1989b). In the present study, I used an entirely automatic user-independent method for cortical surface area estimation to minimize bias.

4.2.3 HYPOTHESIS

I hypothesized that orchestral musicians would show increased GM volume and cortical surface area of the left POP/BA44. This hypothesis was based on the following evidence: first, the POP region (BA44) was shown to be involved in several musically relevant functions notably, musical syntax processing, detection of music-structural irregularities (Maess et al., 2001, Koelsch, 2006), audiospatial localization (Martinkauppi et al., 2000), sight reading (Sergent et al., 1992), and audiomotor integration (Tettamanti et al., 2005, Lahav et al., 2007), which are essential for orchestral performance by ensemble musicians irrespective of their instrument specialty. Second, several studies have shown that, unlike musically naive subjects, musicians tend to use the left hemisphere for music processing (Johnson, 1977, Gordon, 1980, Vuust et al., 2005, Limb et al., 2006), which was hypothesized to reflect the tendency to use an analytic mode of processing in musicians that is identical to that of language (Vuust et al., 2005, Limb et al., 2006). Therefore, I predicted that my right-handed orchestral musicians would show evidence of selective structural enlargement of the POP in the left (dominant) cerebral hemisphere. Third, in particular, the left POP/BA44 has been considered a central component of the human mirror neuron network (Iacoboni et al., 1999, Binkofski and Buccino, 2006) that is activated by the execution of manual action and by hearing of the same action performed by others (Tettamanti et al., 2005, Lahav et al., 2007) indicating its essential role in audiomotor integration. In orchestral performance, this auditory-motor network has two main advantages: it allows fast feedback mechanism that enables musicians to continuously coordinate complex motor actions according to auditory input (Bangert and Altenmuller, 2003), and it maintains sequence-specific priming of action representations together with unconscious stimulations and predictions for the next coming action or sound (Lahav et al., 2007). Furthermore, since previous studies have indicated that neuronal density is positively correlated with cortical surface area (Rockel et al., 1974, Henery and Mayhew, 1989, Sisodiya et al., 1996, Sisodiya and Free, 1997), I hypothesized that orchestral musicians would show increased cortical surface area in the left BA44.

4.3 SUBJECTS AND METHODS

4.3.1 SUBJECTS

The cohort included 52 age-, gender-, and handedness-matched subjects: 26 right-handed male musicians and 26 age-matched right-handed male non-musicians. All volunteers gave signed, informed written consent of their willingness to participate in this study and were screened for medical and neurological disorders. The study had Local Research Ethics Committee approval. Table 4.1 shows descriptive statistics for both groups. Handedness was assessed using the Edinburgh handedness inventory (Oldfield, 1971). Since the measured volumes and cortical surface areas are related to language, it was necessary to recruit subjects who were native English speakers to overcome any possible structural differences imposed by different languages. In addition, all subjects were assessed for intelligence using IQ test and selected IQ-matched subjects.

4.3.1.1 MUSICIANS' PROFILES

The musicians' group included 16 string players (9 violin, 2 viola, 2 cello, 3 double bass), 9 wind players (1 flute, 1 clarinet, 1 oboe, 1 bassoon, 4 French horn, 1 trombone), and 1 percussionist who were all members of a major British symphony orchestra. Descriptive statistics of age of starting musical training and duration of musical performance are provided in table 4.1. Three musicians commenced training at an age under 7 years, while seventeen musicians commenced training after the age of 10 years. Insufficient right-handed female musicians volunteered to warrant the inclusion of women in this study.

Table 4.1. Demographics of subjects recruited in this study.

	Groups	
	Musicians	Non-musicians
***Age (years) Mean / SD / range**	43.2 / 9.3 / 26-66	42.8 / 10.8 / 26-66
Duration of musical performance (years) Mean / SD / range	20.4 / 9.4 / 4-44	N/A
Age of starting musical training (years) Mean / SD/ range	9.6 / 2.4 / 4-13	N/A

*Student *t* test revealed no significant group difference regarding age (*p*=0.5).

4.3.2 MR DATA ACQUISITION

All volunteers were scanned using a protocol with identical sequence and parameters. High resolution T1-weighted images were obtained with a 1.5 Tesla Signa whole body MR imaging system (General Electric Medical Systems, Milwaukee, WI). A spoiled gradient echo (SPGR) pulse sequence was used with TE/TR = 9/34 ms, slice thickness =1.6 mm, field of view = 200 mm, which contained a 256 X 256-pixel matrix, voxel size = 1.6 X 0.781 X 0.781 mm, no. of slices =124 coronal slices, flip angle = 30°, and acquisition time = 13 min and 56 s.

4.4 METHODS

Image analysis methods used in this study include:

- Manual measurement of intracranial volume (ICV) using design-based stereology, which involved application of Cavalieri method combined with point counting (Roberts et al., 2000, Garcia-Finana et al., 2003).
- Manual measurement of GM/WM volumes of POP and PTR using design-based stereology, which involved application of Cavalieri method combined with point counting (Roberts et al., 2000, Garcia-Finana et al., 2003).
- Measurement of cortical surface areas of BA44 and BA45 using an automatic technique.

4.4.1 MEASUREMENT OF INTRACRANIAL VOLUME

To control for differences in head size, ICVs were measured using the method described in chapter 3, section 3.9, pages 74-79.

4.4.2 MEASUREMENT OF GM/WM VOLUMES OF POP AND PTR

4.4.2.1 IMAGE PRE-PROCESSING

This was performed using the method described in chapter 3, section 3.9.1, page 74.

4.4.2.2 DEFINITION OF POP AND PTR

The morphology of the sulcal contours defining and within the POP and PTR was assessed using a combination of multiple orthogonal MR sections and rendered surfaces of cerebral hemispheres using BrainVoyager software, version 1.9 (www.Brainvoyager.com, Brain Innovation, Maastricht, The Netherlands). Delineation of the POP and PTR followed anatomical definitions described by others (Foundas et al., 1998, Duvernoy, 1999, Tomaiuolo et al., 1999, Petrides and Pandya, 2004, Petrides, 2006). The POP was demarcated caudally from the precentral gyrus by the inferior precentral sulcus, dorsally from the middle frontal gyrus by the inferior frontal sulcus, and rostrally from the PTR by the anterior ascending ramus of the Sylvian fissure. The PTR was demarcated caudally from the POP by the anterior ascending ramus of the Sylvian fissure, dorsally from the middle frontal gyrus by the inferior frontal sulcus, and rostro-ventrally by the anterior horizontal ramus of the Sylvian fissure (figure 4.1).

4.4.2.3 STEREOLOGICAL PARAMETERS

Stereological parameters used for POP volume estimation:

- Grid size (pixels) = 3.
- Area associated with each test point (a/p) = 9 mm^2.
- The section interval (T) was every section. Since final voxel size after iso-voxel transformation was 1 X 1 X 1 mm, then T = 1 X 1 mm = 1 mm.

Stereological parameters used for PTR volume estimation:

- Grid size (pixels) = 3
- Area associated with each test point (a/p) = 9 mm^2
- The section interval (T) was every second section, so T = 2 X 1 mm = 2 mm.

The difference in the slice interval between structures was due to the fact that the PTR is usually longer (and thus larger) than the POP. Unbiased estimation of volume, V of structure of interest could be obtained using Equation 3.3 (chapter 3):

$$est_2V \ = \ T\ (a/p)\ (P_1 + P_2 + \cdots P_n)$$

Where P_1, P_2, ... P_n denote the point counts and (a/p) the area associated with each point.

Thus, estimation of GM and WM volumes of POP could be obtained using the following Equation:

$$V\text{ (POP)} = 1 \text{ X } 9 \text{ X } (P_1 + P_2 + \cdots P_n) \qquad \textbf{\textit{Equation 4.1}}$$

And for PTR volumes:

$$V\text{ (PTR)} = 2 \text{ X } 9 \text{ X } (P_1 + P_2 + \cdots P_n) \qquad \textbf{\textit{Equation 4.2}}$$

4.4.2.4 STEREOLOGICAL MEASUREMENT

Using BrainVoyager, markers were used to define sulcal landmarks of POP and PTR (see figure 4.1). For stereological measurements, images were reoriented to the coronal plane using ImageJ software, version 1.38 available online at http://rsb.info.nih.gov/ij/, NIH, USA). Estimation of GM and WM volumes of POP/PTR regions was performed using EasyMeasure software (Puddephat, 1999) as described by (Keller et al., 2007) (figures 4.2, 4.3, and 4.4). Images were always displayed using consistent image window and display levels on the same monitor with fixed screen contrast settings.

4.4.3 INTER- AND INTRA-RATER RELIABILITY

It is essential that volumetric measures are both repeatable and reproducible. Repeatability is the variability of repeated measures of a constant object by the same observer or rater (intra-rater), whereas reproducibility refers to the ability of several observers or raters (inter-rater) to obtain statistically similar results from measuring a constant object using the same methodology. Blind intra- and inter-rater studies were undertaken on all structures measured using the point counting technique namely, ICV and POP/PTR volumes.

The inter-rater study was conducted following the training period for measuring each structure. Ten randomly selected brain volume datasets were independently measured by two raters (MA and IA). MA was blinded to identity, grouping, and laterality of subjects. For intra-rater reliability, the structures of interest in the volume datasets of twenty randomly selected subjects were measured twice by IA, with a gap of three weeks between the first and second measuring sessions.

Reliability of a measure can be determined by making replicate measurements and using intra-class correlation coefficient (ICC) to test the null hypothesis that there is no association between the two sets of results. A coefficient greater than 0.80 was accepted as evidence of inter- and intra-rater reliability.

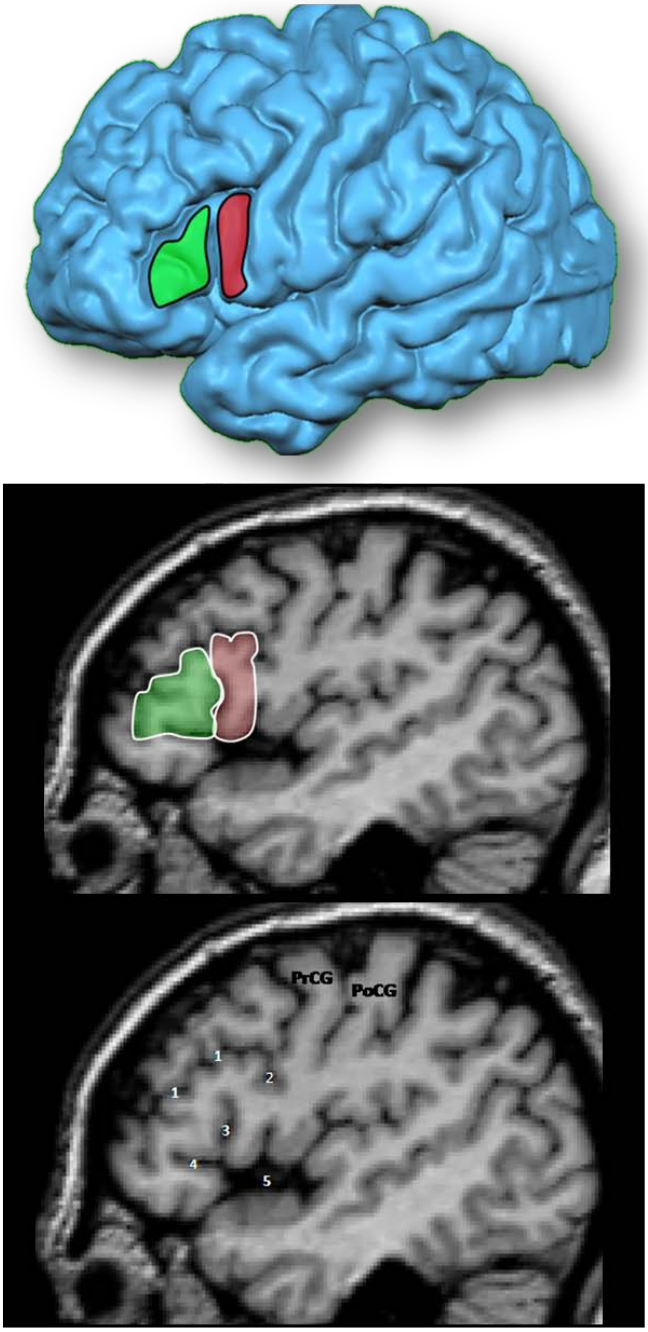


Fig.4.1. Depiction of POP (red) and PTR (green) on the left hemisphere of one subject. Top, outlines of POP and PTR on a 3D display. Middle, depiction of POP and PTR on a sagittal section. Bottom, anatomical boundaries of POP and PTR; 1, inferior frontal sulcus; 2, inferior precentral sulcus; 3, anterior ascending ramus of lateral fissure; 4, anterior horizontal ramus of lateral fissure; 5, lateral fissure; PrCG, precentral gyrus and PoCG, postcentral gyrus.

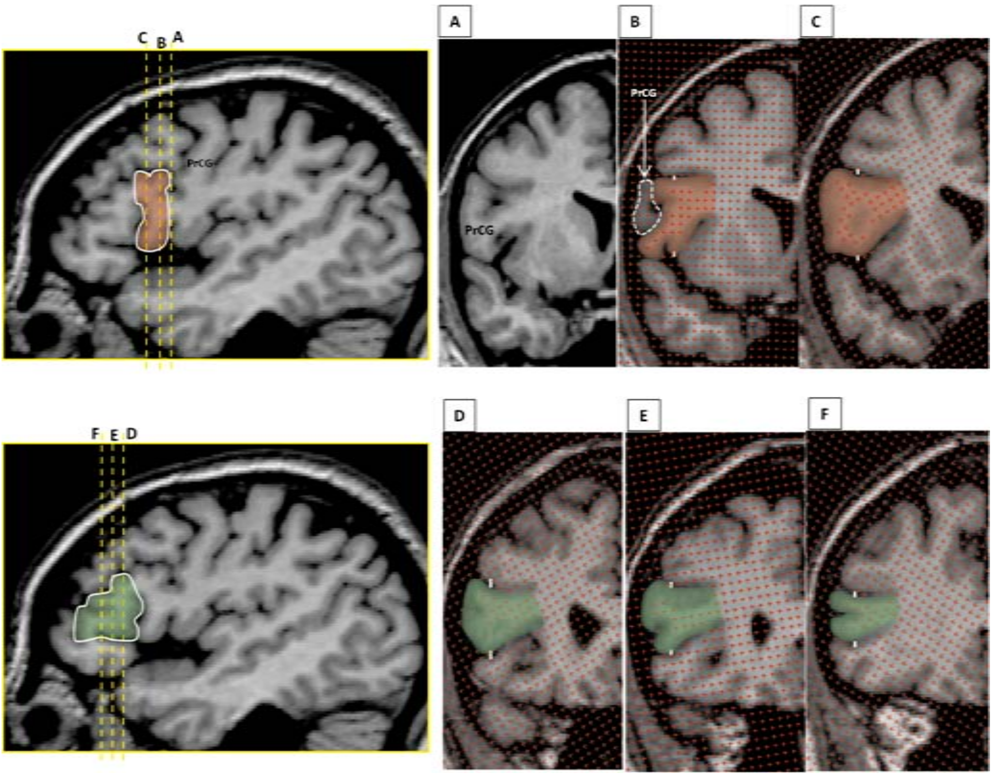
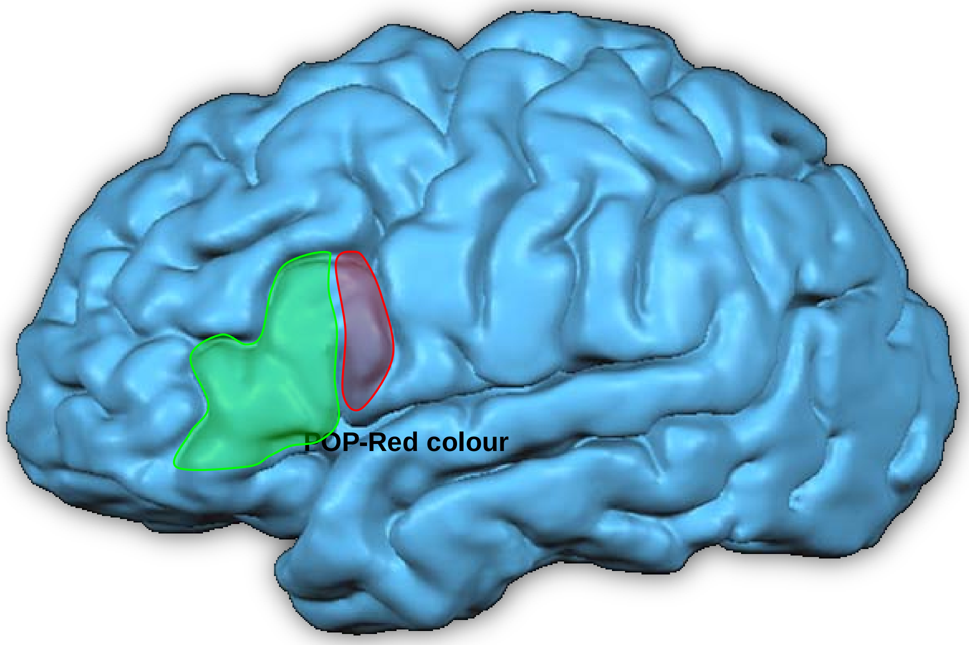
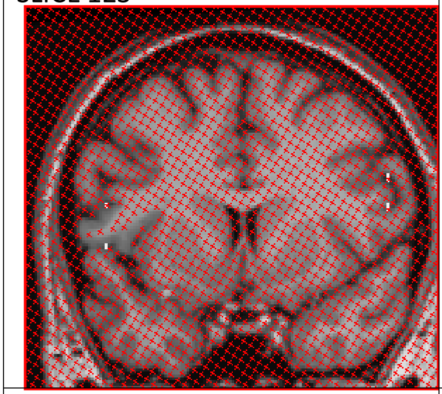
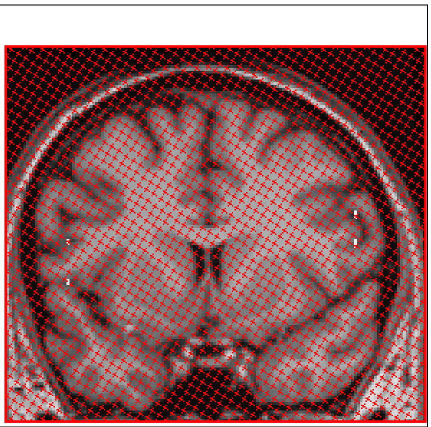
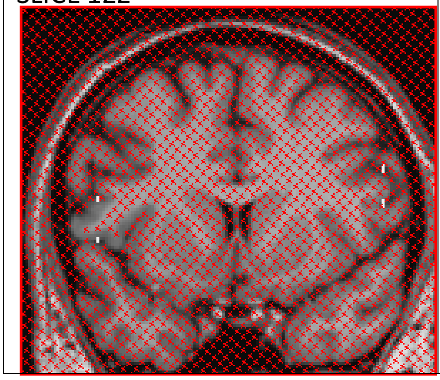
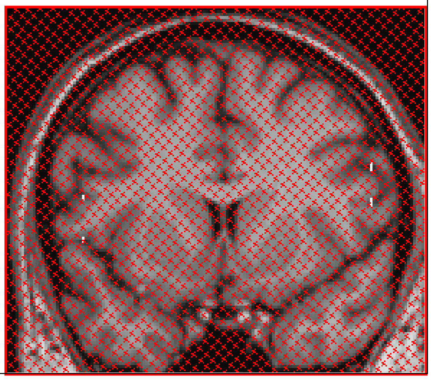
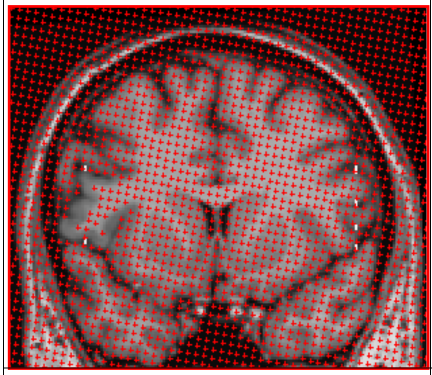
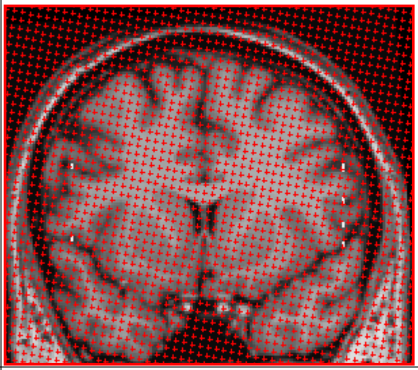
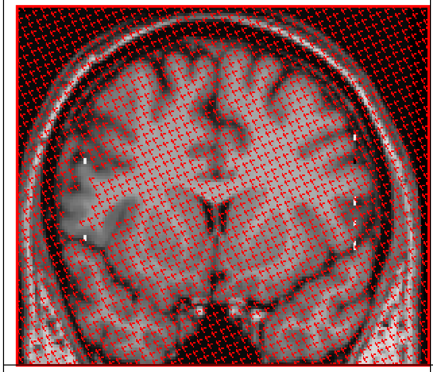
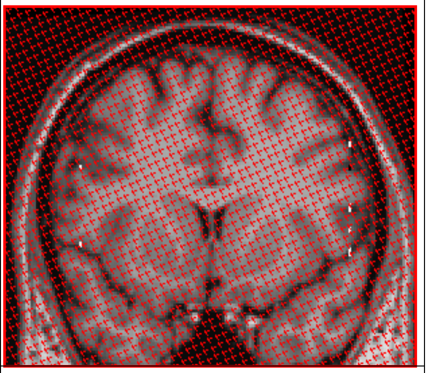
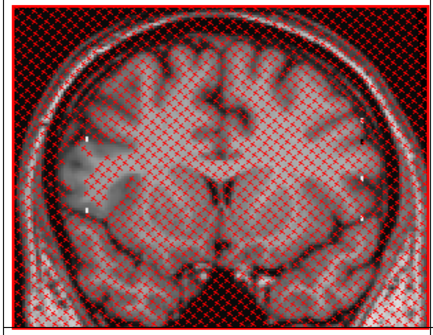
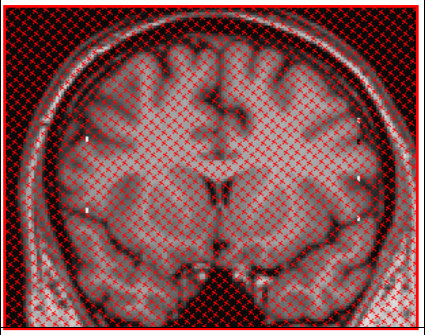
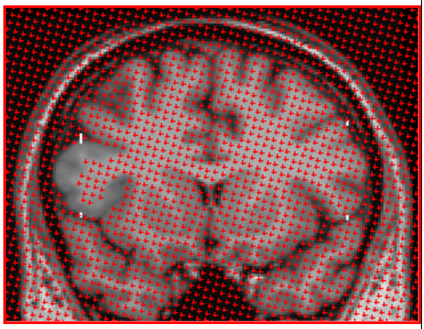
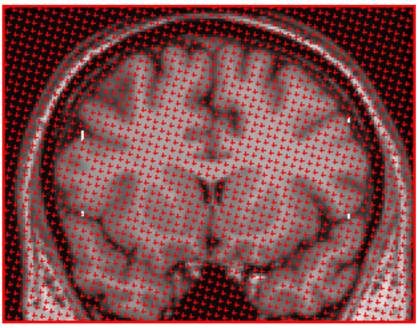


Fig.4.2. Demonstration of volume estimation of POP (red) and PTR (green) using stereology. GM volume estimation is demonstrated here. Sagittal sections used to show levels of coronal sections **A-F**. Section **A** represents the most anterior part of the precentral gyrus (PrCG) and the last coronal slice before the start of POP and was therefore not considered for point counting. A small part of PrCG that overlies POP was not considered for point counting (dotted line in section **B**). Sections **B** and **C** are taken through POP. Sections **D**, **E** and **F** are taken through PTR.



POP-Red colour

SLICE 123 	
SLICE 122 	

SLICE 121 	
SLICE 120 	
SLICE 119 	
SLICE 118 	

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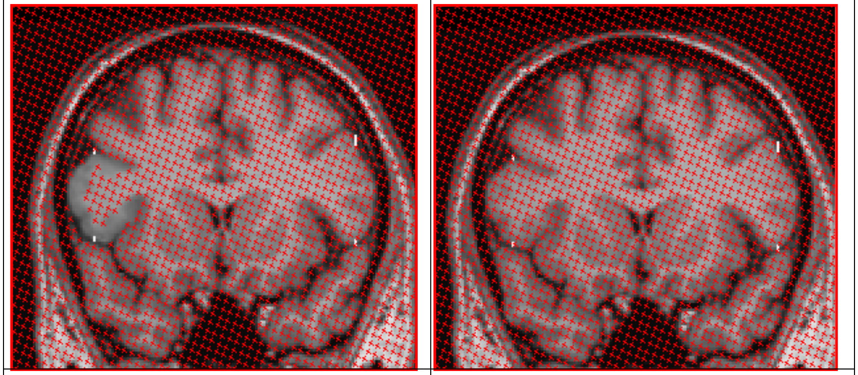

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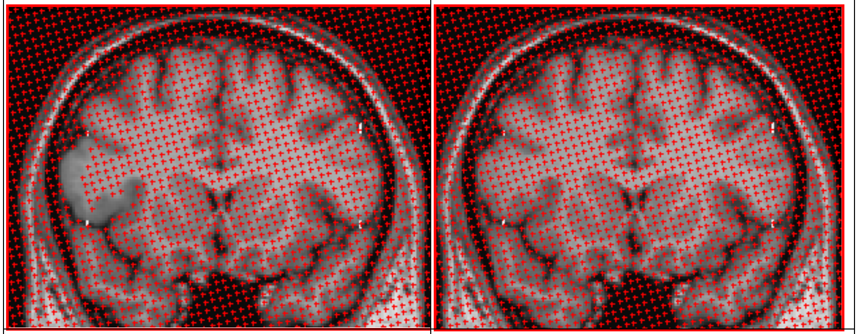

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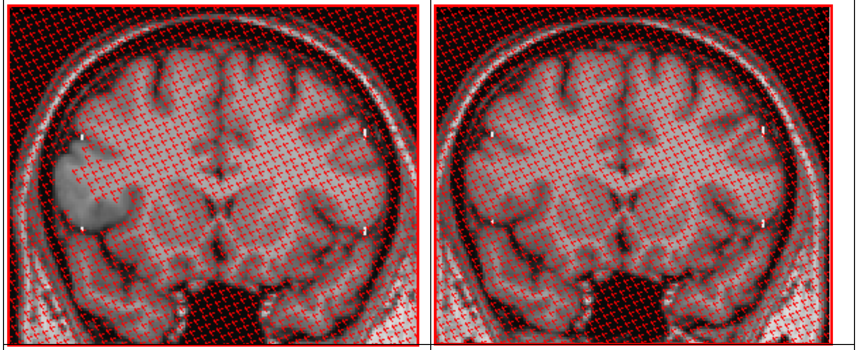

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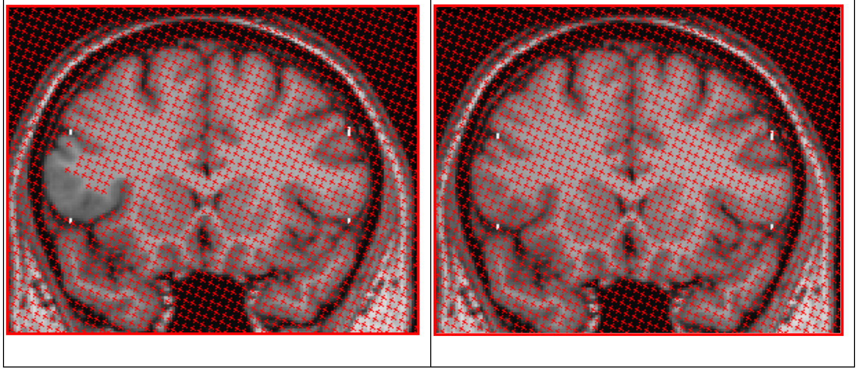


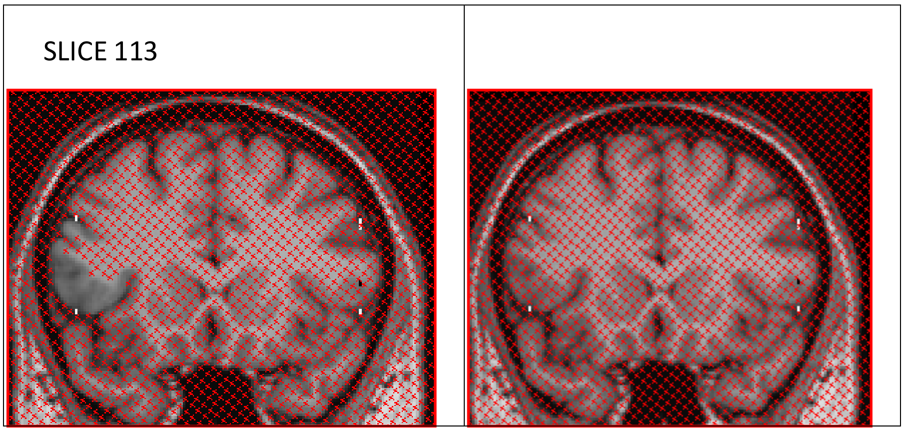
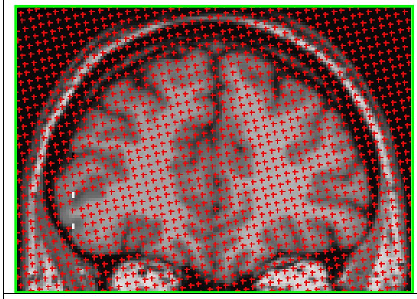
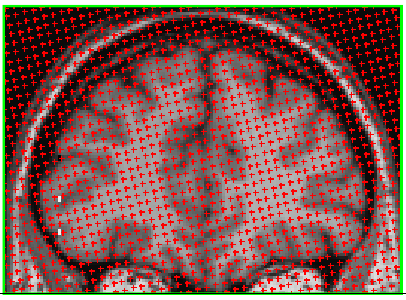
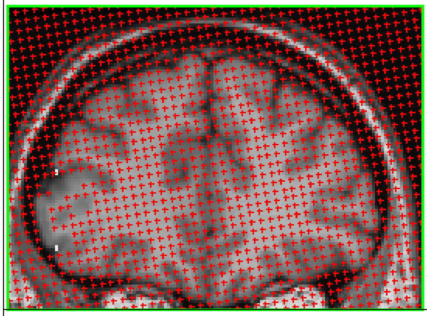
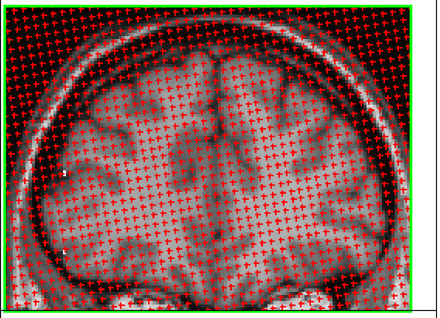
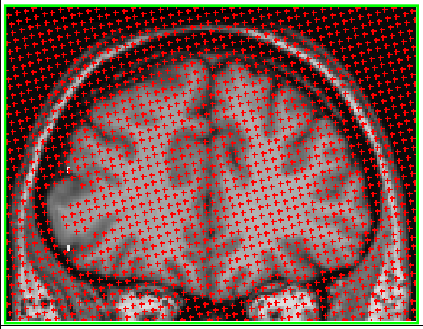
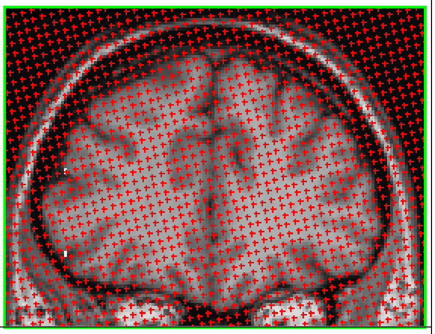
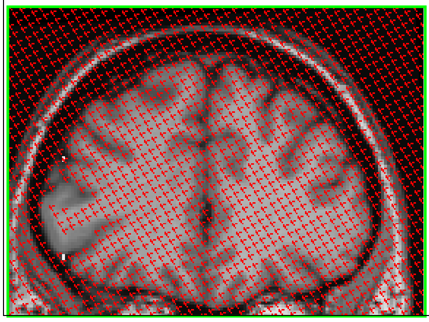
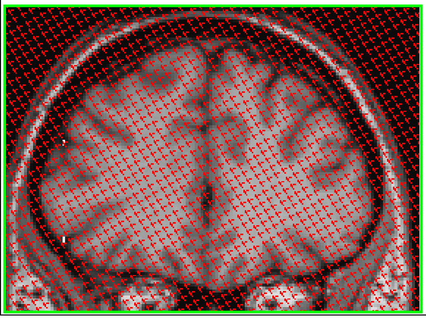


Fig.4.3. Demonstration of GM volume measurement of left POP using stereology. Right column (before) and left column (after) removal of crosses overlying region of interest. Upper and lower white dots represent superior and inferior boundaries, respectively.

PTR-Green colour

SLICE 47 	
SLICE 48 	
SLICE 49 	
SLICE 50 	

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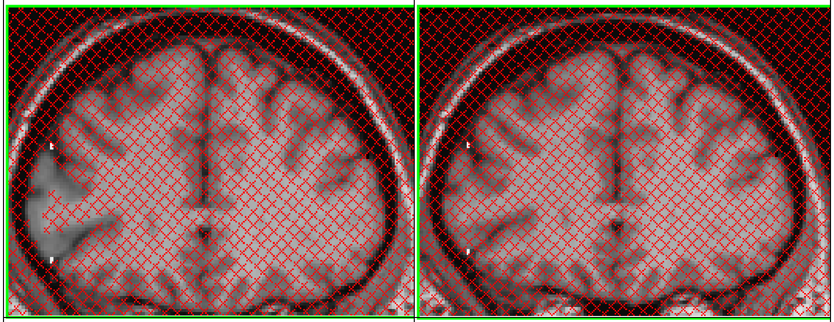

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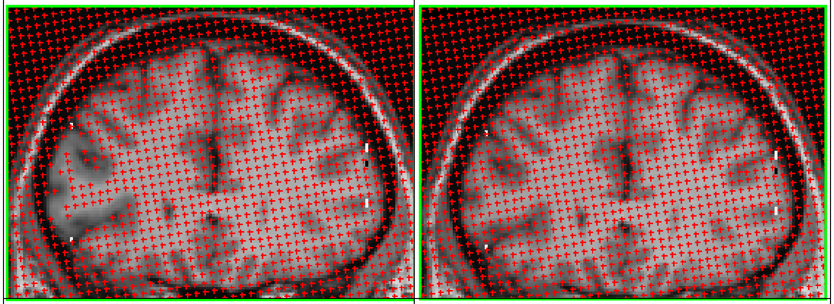

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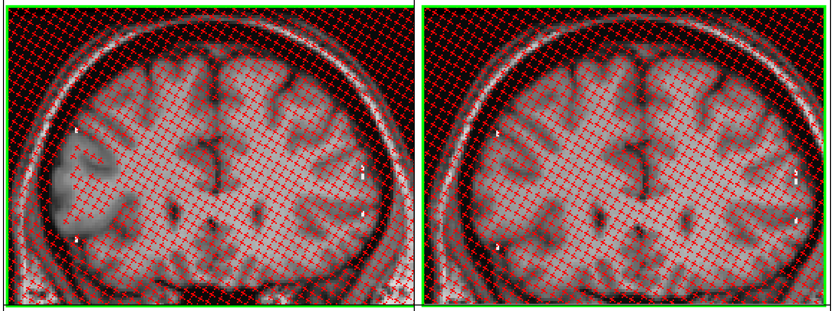

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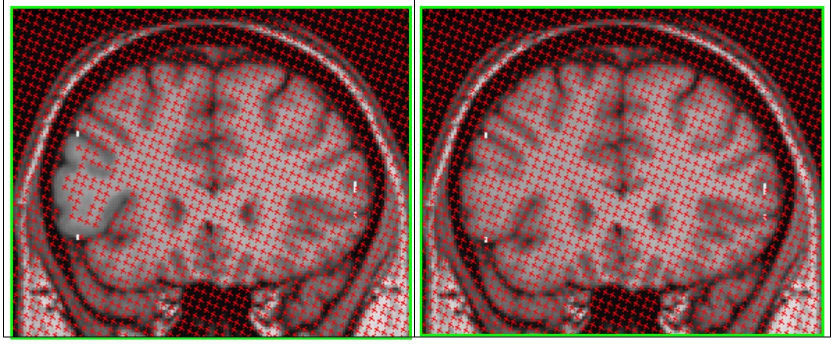
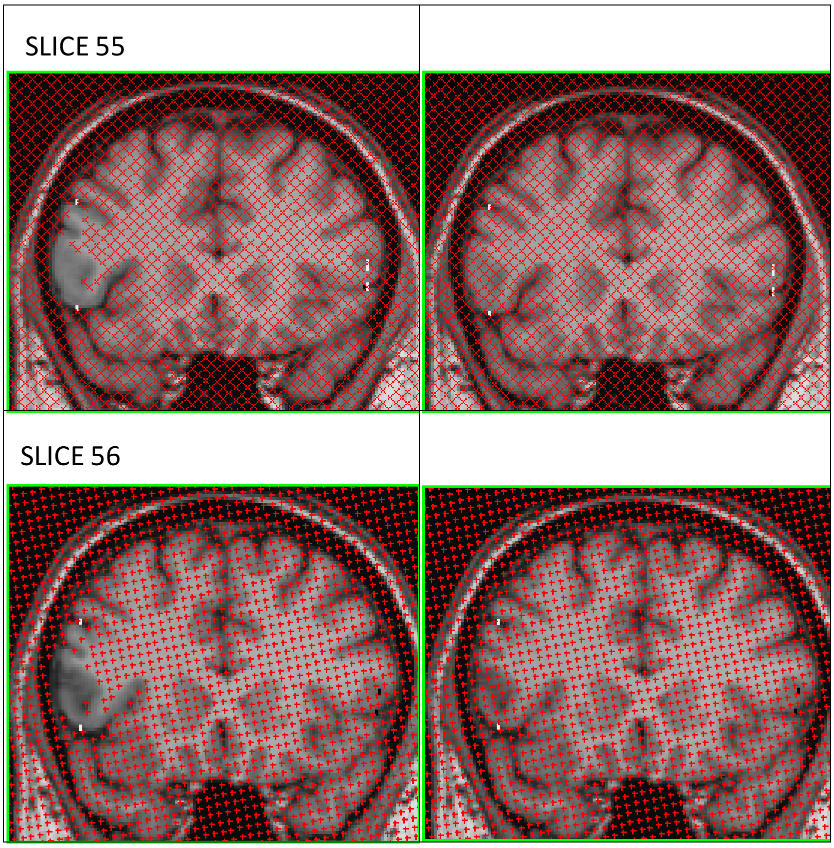




SLICE 55

SLICE 56

Fig.4.4. Demonstration of GM volume measurement of left PTR using stereology. Right column (before) and left column (after) removal of crosses overlying region of interest. Upper and lower white dots represent superior and inferior boundaries, respectively.

4.4.4 IDENTIFICATION OF THE DIAGONAL SULCUS IN PARS OPERCULARIS

The diagonal sulcus is a small sulcus located within the POP. It may lie within the POP without joining any of the sulci that surround the POP, or it may join the inferior frontal sulcus dorsally or the inferior precentral sulcus caudally (figure 4.5). The lower end of the diagonal sulcus may flow into the Sylvian fissure, or it may stay clearly separated from it by a bridge of cortex (Tomaiuolo et al., 1999). It has been suggested that right-left hemisphere differences in the number of diagonal sulci could result in right-to-left asymmetry in POP volume (Keller et al., 2007). I therefore estimated the number of diagonal sulci in the POP with the prediction that this may cause volume differences between groups.

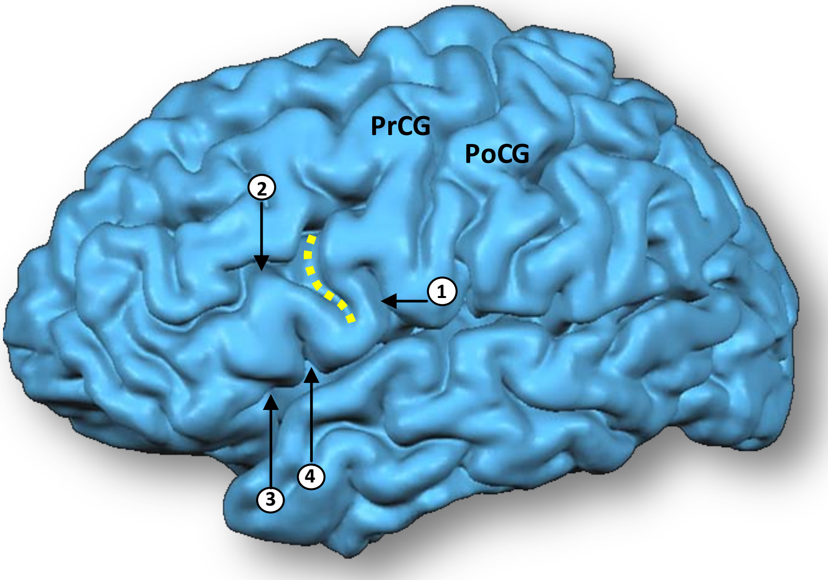


Fig.4.5. The diagonal sulcus. A three dimensional display of the left hemisphere showing the diagonal sulcus (yellow dotted line) arising from the inferior frontal sulcus. PrCG, precentral gyrus; PoCG, postcentral gyrus; 1, inferior precentral sulcus; 2, inferior frontal sulcus; 3, anterior horizontal ramus; 4, anterior ascending ramus.

4.4.5 MEASUREMENT OF CORTICAL SURFACE AREA

All steps for measuring cortical surface area were performed using BrainVoyager software, version 1.9 (www.Brainvoyager.com, Brain Innovation, Maastricht, The Netherlands). For cortical surface area measurement, images were first pre-processed, transformed into Talairach space, and subjected to cortex-based alignment.

4.4.5.1 IMAGE PRE-PROCESSING

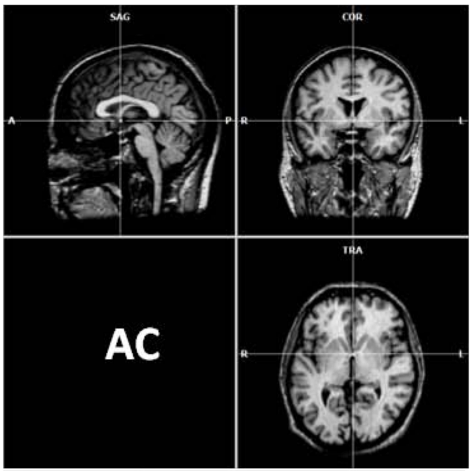
Image pre-processing was performed using the same steps described in chapter 3, section 3.9.1, page 74. This included spatial transformation of images to the sagittal plane, re-sampling of acquired voxels into 1 X 1 X 1 mm isotropic voxels using cubic spline interpolation, and re-alignment according to the ACPC plane. The resulted ACPC aligned MR images were then transformed into the Talairach coordinate system (Talairach and Tournoux, 1988) as described below.

4.4.5.2 TALAIRACH TRANSFORMATION

Talairach transformation was performed by specifying eight reference points within the ACPC transformed data set (figure 4.6): AC (anterior commissure), PC (posterior commissure), AP (the most anterior point of the cerebrum), PP (the most posterior point of the cerebrum), SP (the most superior point of the cerebrum), IP (the most inferior point of the cerebrum), RP (the most right point of the cerebrum), and LP (the most left point of the cerebrum). These points were then used to scale the 3D data set into the dimensions of the standard brain of the Talairach and Tournaux atlas using trilinear interpolation. The centre of the new Talairach data set is still the AC point and the brain is still aligned to the ACPC plane. The cerebrum’s size is; however, adjusted to fit into Talairach space. Table 4.2 shows orthogonal views used to localize landmark points.

Table 4.2. Orthogonal views used to localize landmark points for Talairach transformation.

Landmark Point	**View**
AC	Sagittal confirmed with transverse
PC	Sagittal confirmed with transverse
AP	Coronal
PP	Coronal
SP	Transverse
IP	Transverse
RP	Sagittal
LP	Sagittal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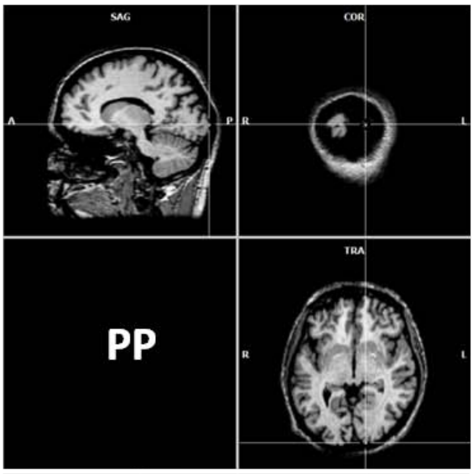
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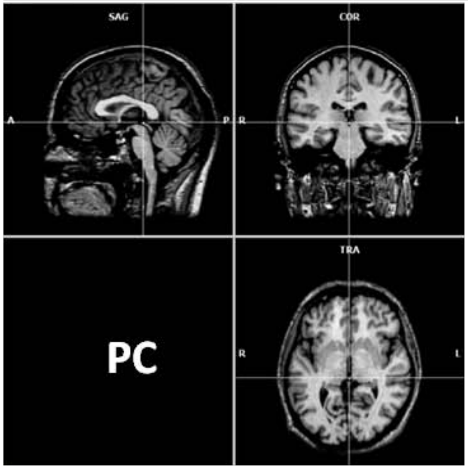
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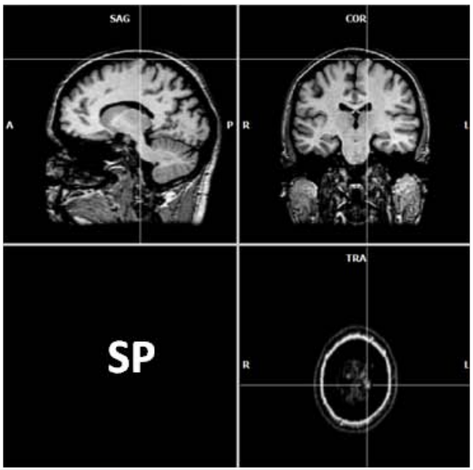
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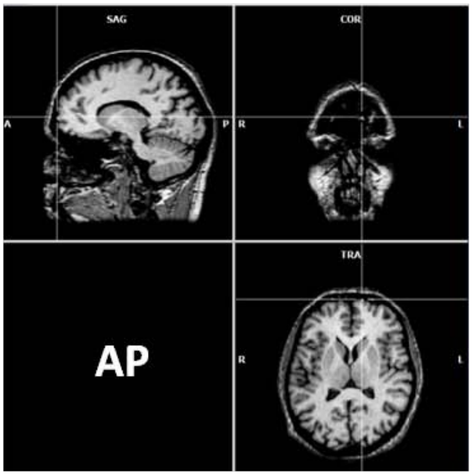
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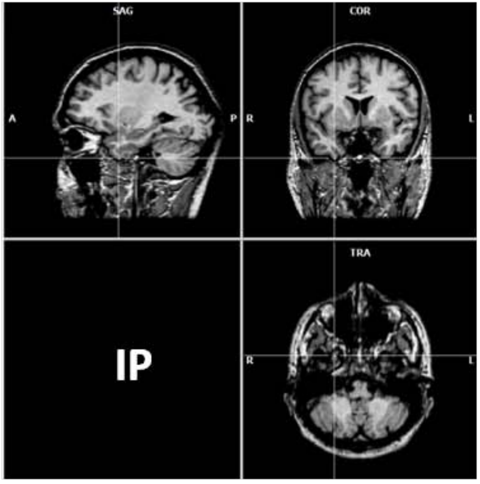
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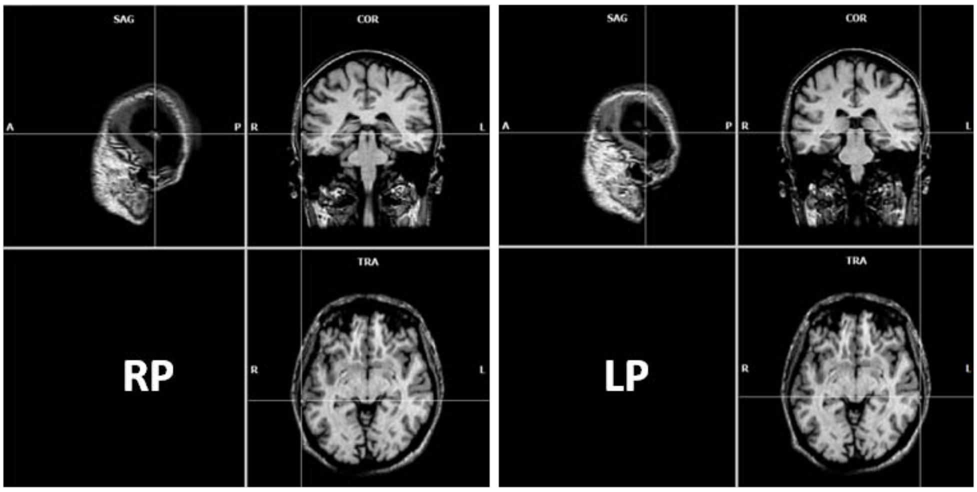

Fig.4.6. Talairach transformation. Identification of the 8 reference points: AC (anterior commissure), PC (posterior commissure), AP (the most anterior point of the cerebrum), PP (the most posterior point of the cerebrum), SP (the most superior point of the cerebrum), IP (the most inferior point of the cerebrum), RP (the most right point of the cerebrum), and LP (the most left point of the cerebrum).

4.4.5.3 CORTEX-BASED ALIGNMENT

A good match between corresponding brain regions is important for group-level statistical data analysis. It has been shown that a cortical matching approach substantially improves statistical group results by reducing anatomical variability (Fischl et al., 1999a, Fischl et al., 1999b). BrainVoyager offers an advanced cortical mapping approach to align brains using curvature information of the cortex (cortex-based alignment) (Goebel et al., 2006). Since the curvature of the cortex reflects the gyral/sulcal folding pattern of the brain, this brain matching approach essentially aligns gyri and sulci across brains. To apply the cortex-based matching approach for inter-subject alignment, the following steps were followed (Goebel et al., 2006):

1. Automatic cortical segmentation and reconstruction
2. Morphing reconstructed cortices to a sphere
3. Mapping a standard sphere to inflated sphere
4. Creating curvature maps for each re-sampled cortex mesh
5. Aligning source spheres to a moving target group average

1-Automatic cortical segmentation and reconstruction

Prior to this step, each brain was first segmented from surrounding head tissue using an automatic “brain peeling” tool (Goebel et al., 2006). This was followed by cortical segmentation and reconstruction of both cerebral hemispheres. The WM/GM boundary was segmented using an automatic segmentation algorithm (Kriegeskorte and Goebel, 2001). This process involves the following steps: edge-preserving smoothing (sigma filter), filling of ventricles, application of masks to label subcortical structures, creation and analysis of intensity histograms to detect WM and GM peaks, a region growing process, and several small morphological operations designed to smooth the borders of the segmented data and to separate the left from the right hemisphere. During segmentation, a window displaying a set of histograms was shown (figure 4.7A). The left peak in the histograms corresponds to GM and the right peak to WM. A dialog appeared to allow adjusting the WM/GM cut point, which was set in such a way that the white line is between the two peaks. Each segmented hemisphere was finally submitted to a “bridge removal” algorithm, which ensures the creation of topologically correct mesh representations (Kriegeskorte and Goebel, 2001)(figure 4.7B). The borders of the two resulting segmented subvolumes were tessellated to produce a surface reconstruction of the left and right hemispheres. The quality of the resulted segmentation was next verified in transverse, coronal, and sagittal views to check whether the mesh contour matches the WM/GM boundary (figure 4.7C).

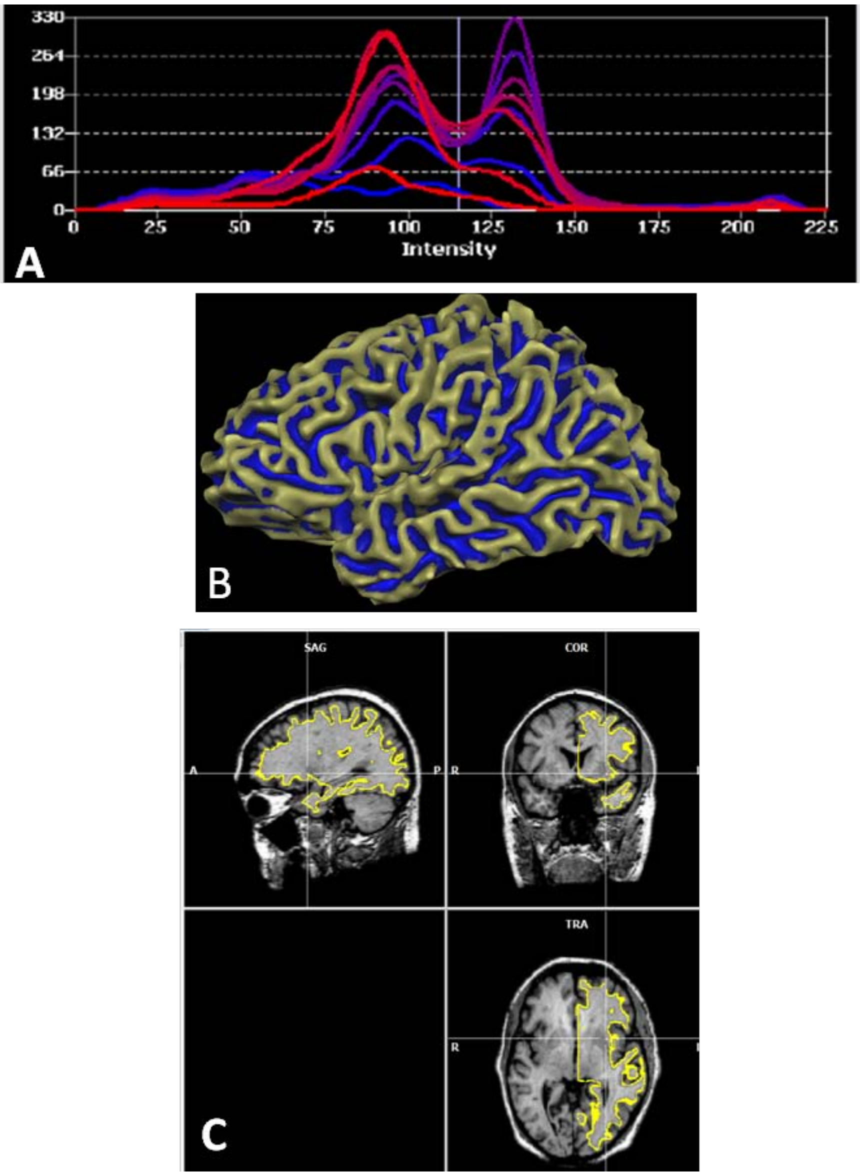

Fig.4.7. A, the smoothed intensity histogram displayed during automatic cortical segmentation process. **B**, visualization of the segmented cortex of the left hemisphere as a reconstructed mesh representation. Convex curvature (reflecting gyri) is colored in gold and concave curvature (reflecting sulci) is colored in blue. **C,** result of cortical segmentation visualized in orthographic slices of the 3D data; the yellow lines indicate the segmented WM/GM boundary.

2-Morphing reconstructed cortices to a sphere

This step involves two procedures: the first is morphing the folded cortex mesh of each hemisphere into a spherical representation, which provides a parameterizable surface ideal for across-subject non-rigid alignment (figure 4.8A). This step is essential since the cortex-based alignment algorithm works on superimposed spherical representations as a common space for all to-be-aligned cortex meshes. The morphing step is an iterative process, which uses several forces changed gradually over time. A smoothing force is used to remove the folding from the mesh, while a to-sphere force attempts to push the mesh vertices outward towards points on a sphere. The to-sphere morphing was performed separately for the cortex mesh of the left and right hemispheres of each subject. The second morphing procedure involves distortion correction of the resulted sphere (figure 4.8B). The default number of morphing steps was chosen for each procedure, 800 and 3000, respectively. Each vertex on the sphere (spherical coordinate system) corresponds to a vertex of the folded cortex (Cartesian coordinate system) and vice versa.

3-Mapping a standard sphere to inflated sphere

It is difficult to use spherical representation of cortex mesh directly for cortex-based alignment due to the presence of large number of vertices with different distances between adjacent vertices. To simplify and normalize the alignment process, the created spherical meshes are sampled from a standard sphere with 40962 vertices (figure 4.8C), which is the same for each subject. Sampling is performed by mapping each vertex of the standard sphere to the closest vertex of the morphed sphere. The final result is a mapping table stored in a sphere-to-sphere mapping file.

4-Creating curvature maps for each re-sampled cortex mesh

The created files in the previous step allow transferring information from the subject’s cortex sphere mesh to the standard sphere used during the subsequent alignment procedure. This step involves transferring the vertex position information resulting in folded mesh representation of the standard sphere. The curvature information is computed in the folded representation and preserved as a curvature map on the spherical representation. The generated curvature map file for the standard sphere provides an input for the alignment procedure (figure 4.8D). These files were created for all meshes of the same hemisphere.

5-Aligning source spheres to a moving target group average

All brains were aligned using the moving target group averaging approach in which the goal function is specified as a “moving target” computed repeatedly during the alignment process as the average curvature across all hemispheres at a given alignment stage. The procedure begins with the coarsest curvature maps then the next finer curvature maps are used and averaged with the acquired alignment result of the previous level (Goebel et al., 2006).

4.4.5.4 QUANTITATIVE MEASUREMENT OF CORTICAL SURFACE AREAS

BrainVoyager offers the option of using predefined regions of interest, such as BA44/45, in combination with cortex-based alignment for optimal group analysis. When not including the cortex-based alignment information into the region of interest labeling, any predefined area will not fit too well on a subject cortex. Regions of interest (in this case BA 44, BA45, frontal, parietal, temporal and occipital lobes) were automatically superimposed on aligned cortex mesh of hemispheres using the brain atlas available with BrainVoyager software package (figure 4.8E). Quantitative measurement of cortical surface area of a region of interest was calculated as follows: since the model of the cortical surface is a mesh of tessellated triangles (Fischl et al., 1999a, Goebel et al., 2006), the area of each triangle could be calculated using the following Equation:

Area of triangle =1/2 X triangle base X triangle height ***Equation 4.3***

Therefore, the total surface area of a region of interest is defined by the sum of areas of all triangles within that region (Feczko et al., 2009). The total cerebral cortical surface area, estimated by summating cortical surface areas of frontal, parietal, temporal, and occipital lobes of each brain, was measured to correct for differences in whole brain cortical surface area.

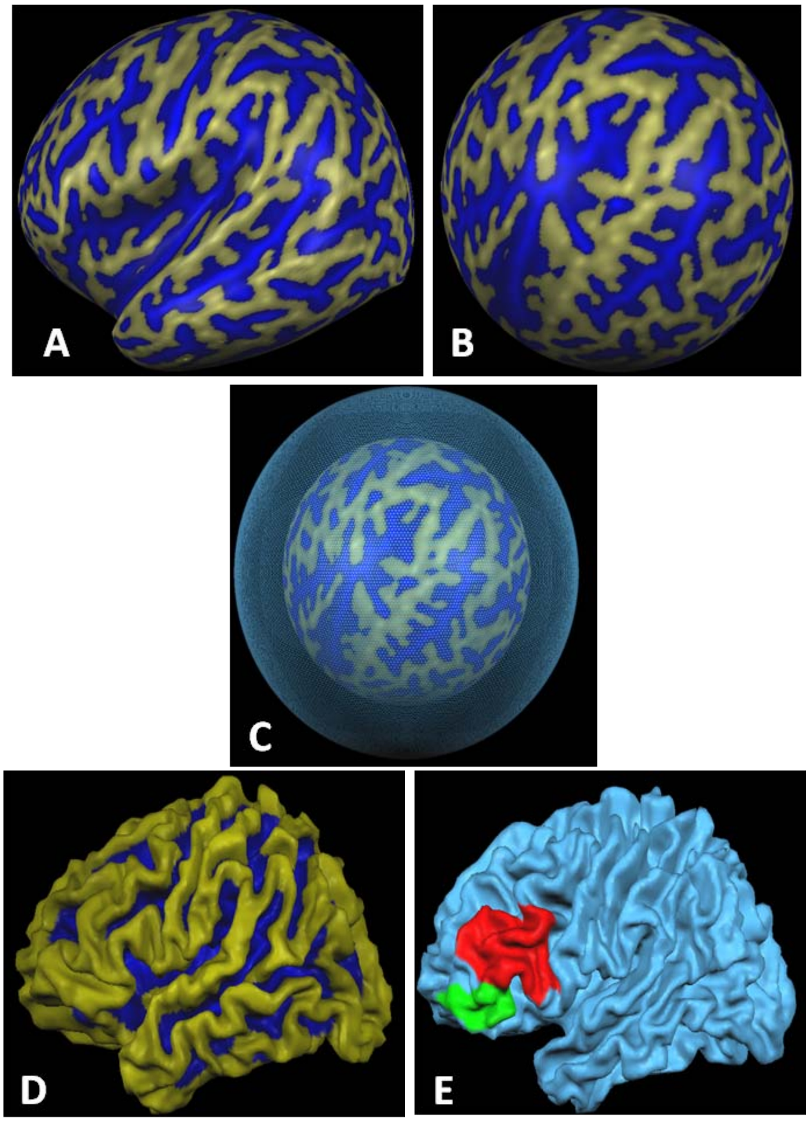

Fig.4.8. Steps of cortex-based alignment of the left hemisphere of one subject (**A-D**). **A**, visualization of an inflated representation of the cortex mesh. **B**, visualization of the final state after the distortion correction morphing step had been completed indicating that distortions had been reduced to a minimum. **C,** the standard sphere used for all subjects, it appears in wireframe mode (with vertices in blue colour) allowing to see that the inflated sphere is located inside the standard sphere. **D**, a folded sphere cortex mesh with overlaid curvature map. **E**, depiction of BA44 (red colour) and BA45 (green colour) on a 3D cortex mesh.

4.4.6 STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS v 16.0 and statistica program, v 6.0 (Statsoft Inc., USA). Shapiro-Wilk (S-W) was used to test normality of data distribution. Using SPSS, multivariate analysis of variance (MANOVA) was performed with GM and WM volumes of left POP/PTR and right POP/PTR corrected for ICV as dependent variables and musicianship (musicians versus non-musicians) as the independent variable with age as covariate; $p<0.05$ was considered significant after adjustment for multiple comparisons.

For cortical surface area analysis, a second MANOVA was performed with cortical surface area measurements of left BA 44/45 and right BA 44/45 corrected for total brain cortical surface area as dependent variables and musicianship as the independent variable with age as covariate; $p<0.05$ was considered significant after adjustment for multiple comparisons. Pearson’s correlation was used to perform correlation analyses. Student t test was used to compare ICV measurements between groups.

The most appropriate statistical test for assessing agreement in measurements obtained on two independent occasions, and particularly where the measurements are not totally objective, is the ICC. This takes into account the sum and difference of each pair of measurements and also their variance and will only yield a coefficient = 1 when all pairs of measurements fall on a straight line through the origin with slope unity (Armitage and Berry 1994) and is a good measure of reliability between raters. The ICC calculated by one-way random model was used to test intra- and inter-rater reliability. A coefficient greater than 0.80 was accepted as evidence of inter- and intra-rater reliability. Statistica program was used for creating figures 4.9, 4.10, and 4.13 and Microsoft Office Excel, 2007 for creating figure 4.11.

4.5 RESULTS

4.5.1 MEASUREMENT OF INTRACRANIAL VOLUME

There was no significant difference in ICV between groups (*p*=0.2).

4.5.2 VOLUME MEASUREMENTS OF POP AND PTR

4.5.2.1 EXCLUSION OF CASES FROM VOLUME MEASUREMENT

The morphology of the sulcal contours defining the POP and PTR described above prevented delineation of these structures for volumetric measurements in four of the 104 hemispheres for the following reasons: absent anterior ascending ramus (two left and one right hemispheres; no anterior/posterior boundary for the POP and PTR, respectively), and absent horizontal ramus (one left hemispheres; no anterior boundary for the PTR). Of these four hemispheres, one left hemisphere belongs to a musician and the rest belong to non-musicians. Therefore, analyses of POP and PTR volumes were performed in 26 right/25 left hemispheres in musicians and 25 right/24 left hemispheres in non-musicians.

4.5.2.2 COMPARISON OF GM/WM VOLUMES

MANOVA revealed that orchestral musicians possessed significantly larger GM volume of the left POP (F = 14.7, *p*<0.001) compared to non-musicians. No significant differences were detected for the left PTR, right POP/PTR GM volumes and for WM volumes in all regions. Means ± 95% confidence intervals of POP/PTR GM and WM volumes are presented in figures 4.9 and 4.10, respectively. A correlation analysis was next performed using Pearson's correlation between the significant region (left POP GM volume) and duration of musical performance/age of the commencement of musical training after controlling for subjects' age. There was a significant positive correlation (r = 0.7, *p*=0.0001) between GM volume of the left POP and years of musical performance (figure 4.11A). There was no significant correlation between the left POP GM volume and age of commencement of musical training. In musicians, there was a positive correlation between age and left POP GM volume (r =0.6, *p*=0.01), while there was a negative (but non-significant) correlation between age and left POP GM volume in non-musicians (r = -0.3, *p*=0.1). Figure 4.11B demonstrates a correlation between age and left POP GM volume in non-musicians. Figure 4.12 shows POP outlined on 3D views of left hemispheres of three musicians and three age-matched non-musicians. Table 4.3 shows the frequency distribution of diagonal sulci in the right and left POP in both groups.

4.5.3 INTRA- AND INTER-RATER STUDY

Results of intra-rater study for POP/PTR volumes and ICVs were good ranging between 0.91 to 0.96. Results of inter-rater study were also good ranging between 0.87 to 0.94.

4.5.4 CORTICAL SURFACE AREA MEASUREMENTS OF BA44 AND BA45

Musicians possessed greater cortical surface area of the left BA 44 (F = 6.1, *p*=0.010). No significant group differences were detected for the left BA45, right BA44, and right BA45 regions. A correlation analysis was next performed using Pearson's correlation between the significant region (left BA44 cortical surface area) and duration of musical performance/age of the commencement of musical training after controlling for subjects' age. There was no significant correlation between left BA44 cortical surface area and duration of musical performance/age of the commencement of musical training. Means ± 95% confidence intervals of BA44/BA45 cortical surface areas are presented in figure 4.13.

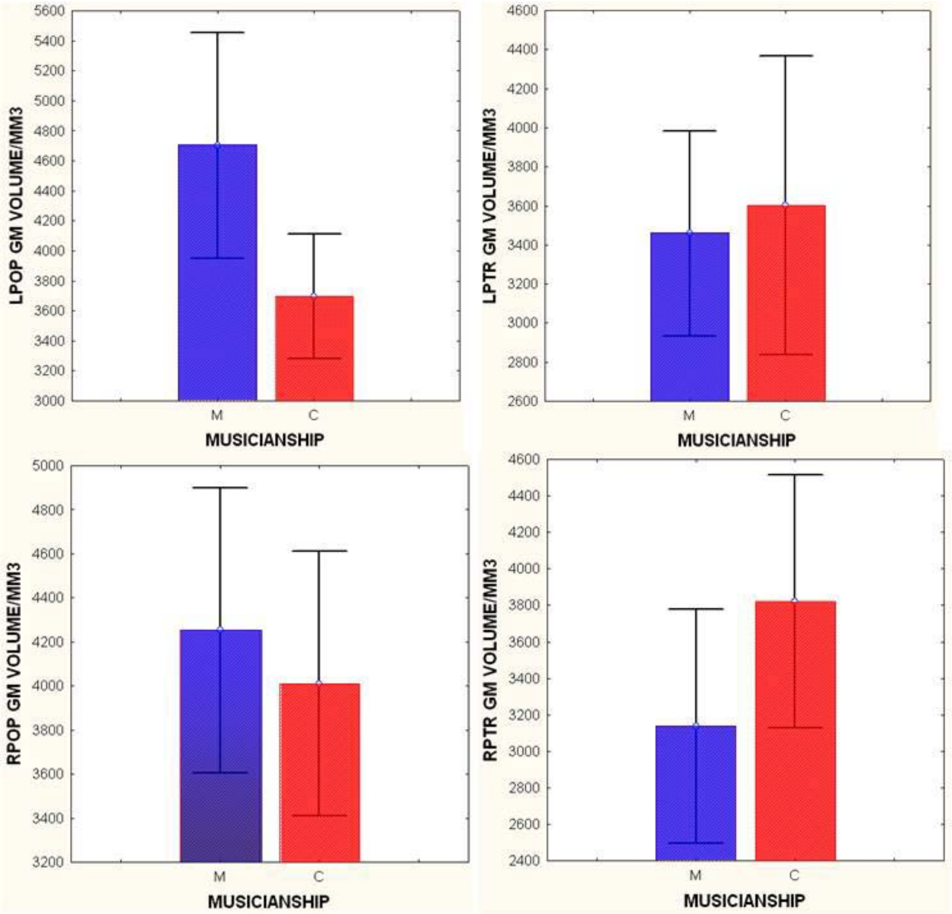

Fig.4.9. Mean ± 95% confidence intervals of LPOP/LPTR and RPOP/RPTR GM volume measurements (uncorrected for ICV) in musicians (blue, M) and non-musicians (red, C).

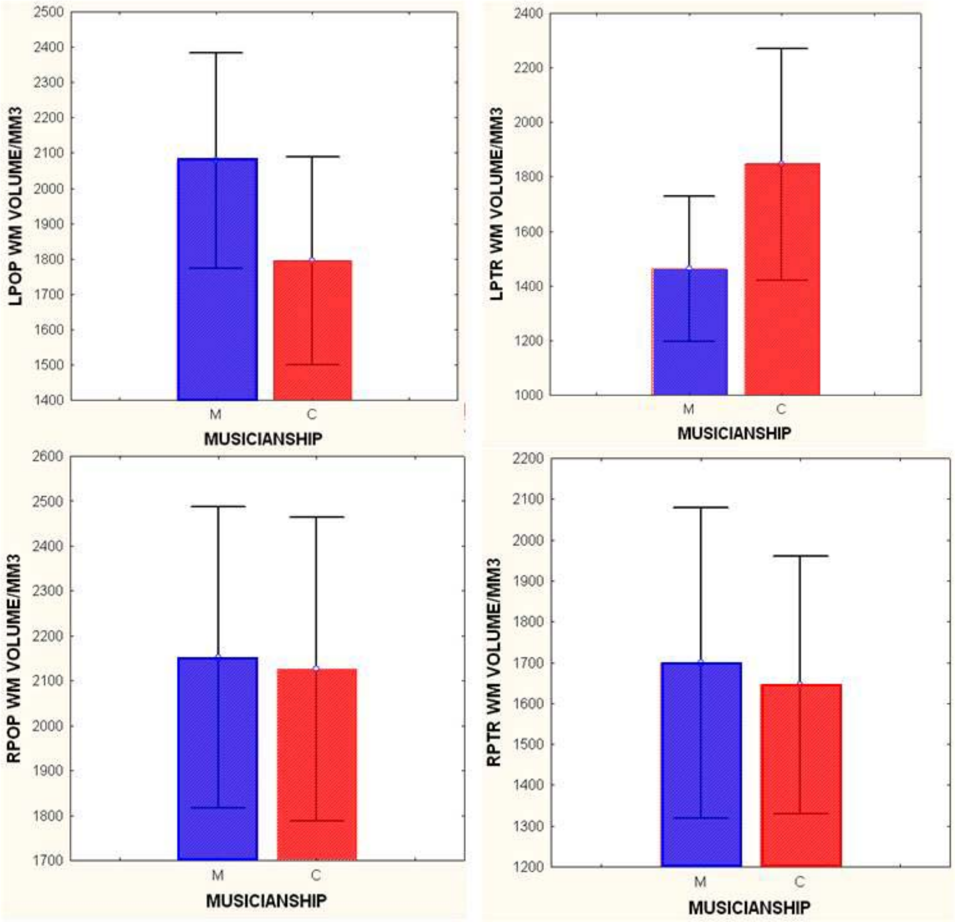

Fig.4.10. Mean ± 95% confidence intervals of LPOP/LPTR and RPOP/RPTR WM volume measurements (uncorrected for ICV) in musicians (blue colour, M) and non-musicians (red colour, C).

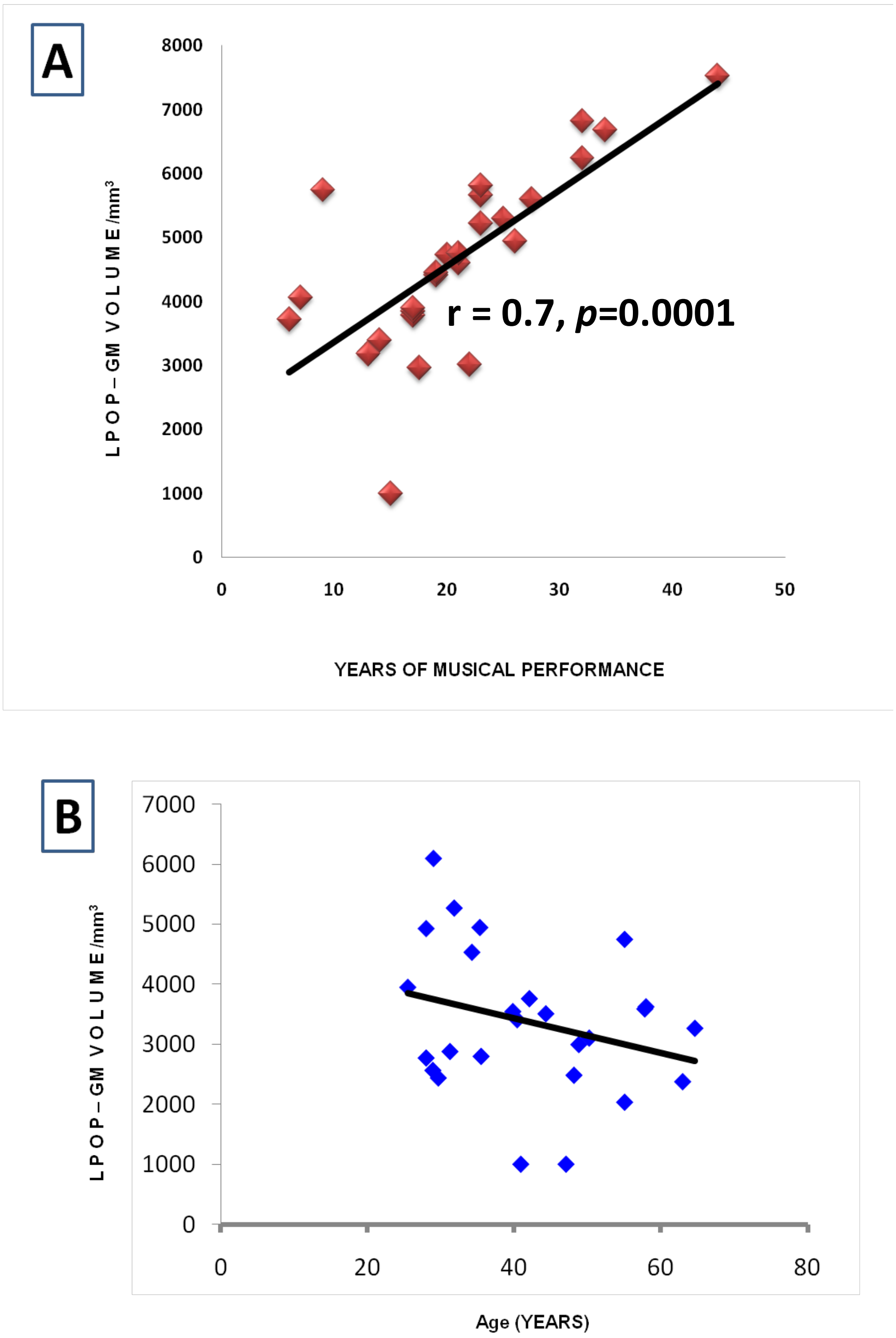

Fig.4.11. A, a graph showing the correlation between LPOP GM volume and years of musical performance in musicians. **B**, a graph showing the correlation between LPOP GM volume and age in non-musicians.

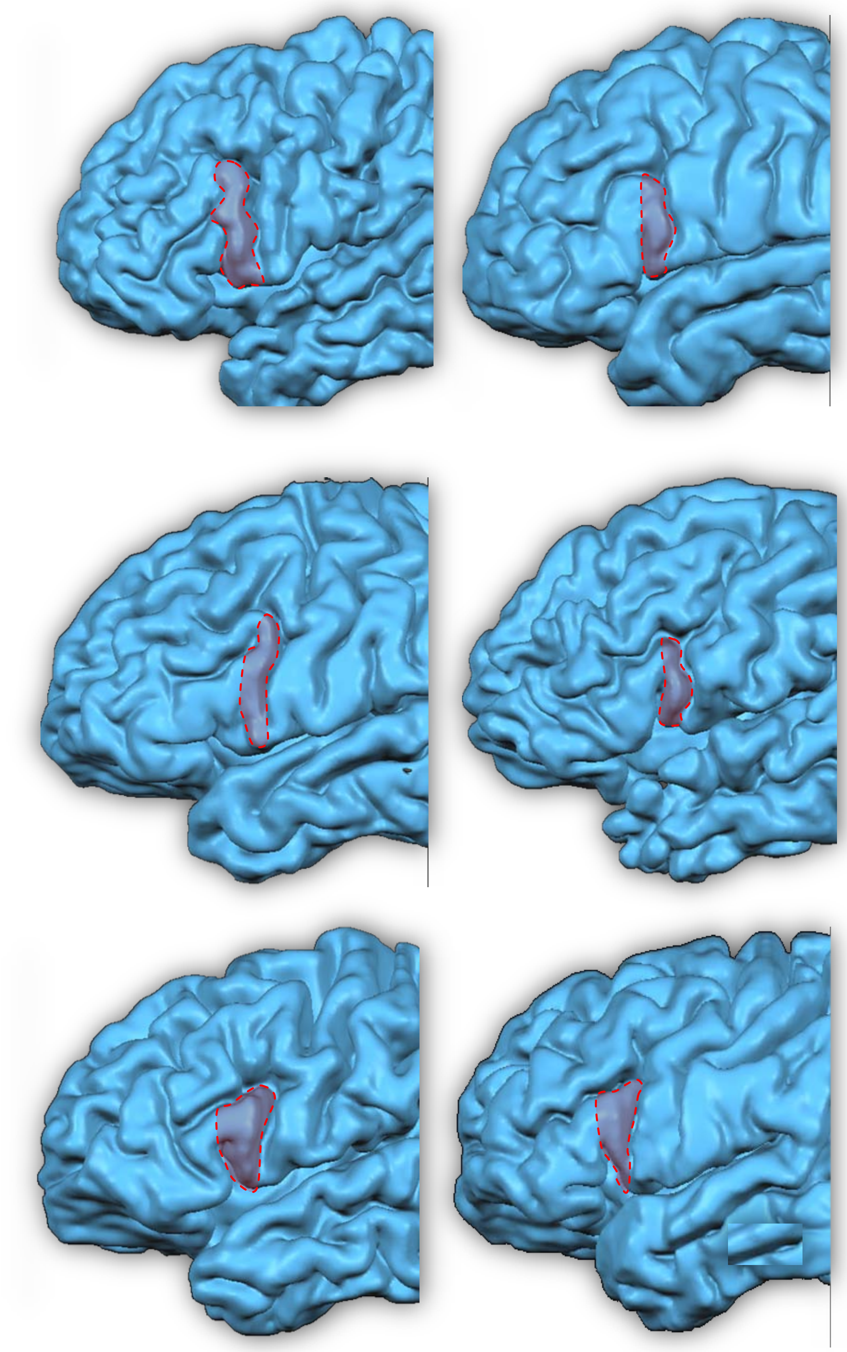

Fig.4.12. Depiction of POP (red) on 3D displays of the left hemispheres of age-matched musicians (left) and non-musicians (right).

Table 4.3. The frequency percentage of diagonal sulci in the right and left POP in both groups.

	Right	**Left**
Musicians	38.6%	40%
Non-musicians	53.8%	41%

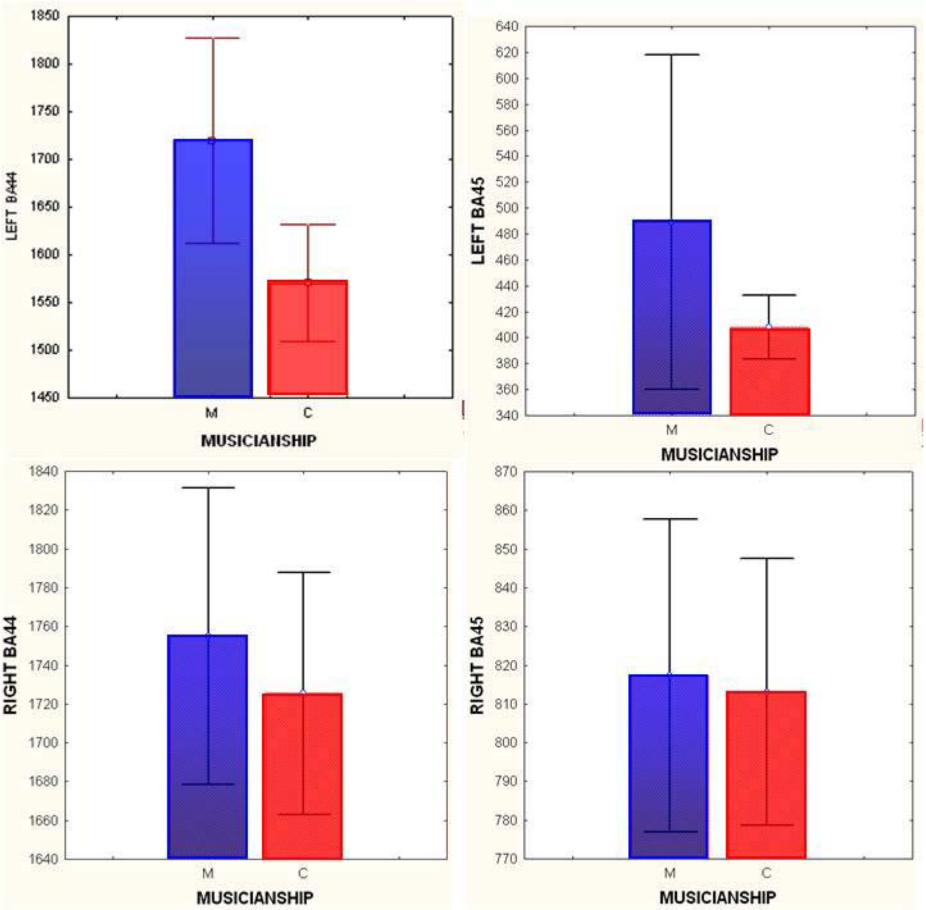

Fig.4.13. Mean ± 95% confidence intervals of Right BA44/45 and Left BA44/45 cortical surface area measurements (uncorrected for total cortical surface area of the brain) in musicians (blue, M) and non-musicians (red, C). Areas are mm^2.

4.6 DISCUSSION

The main finding of this study was increased GM volume of the left POP in orchestral musicians, which was positively correlated with the duration of musical practice. In addition to supporting previous finding of increased Broca's area GM density (Sluming et al., 2002) through automatic-manual cross validation, these findings may provide an additional support to the assumption of functional segregation of Broca's area as I found significant difference only in the POP region of Broca's area. I have also showed that musicians had increased cortical surface area of the left BA44, which may provide a new perspective to structural plasticity of Broca's area in musicians and support the volumetric findings.

Neuroplasticity refers to the brain's ability to change its structure and function during maturation, learning, environmental challenges, or pathology (Lledo et al., 2006). It enables the brain to get beyond the confinements of its own genome thus adapting to environmental pressures and experiences (Pascual-Leone et al., 2005). Microscopically, two steps of plasticity can be identified: unmasking existing connections, which may be followed by establishment of new ones through dendritic growth and arborization (Pascual-Leone et al., 2005). In addition, it has been postulated that the mammalian brain is capable of generating new neurons (Lledo et al., 2006). Over the past two decades, animal model research had documented two phenomena with a profound impact for understanding neuroplasticity. The first is significant plasticity in the adult brain that accounts for new learning (Black et al., 1990, Anderson et al., 1996, Kleim et al., 1998, Anderson et al., 2002, Swain et al., 2003, Kleim et al., 2004, Matsuzaka et al., 2007). The second is that brain re-mapping following injury could be stimulated by retraining animals to perform functions impaired by this injury (Nudo et al., 1996). Such findings have sparked an intense interest in the neuroplastic processes that underlie learning and skill acquisition, recovery of function, and rehabilitative change after brain damage, e.g. following stroke (Forrester et al., 2008). A comprehensive understanding of the processes and factors governing neuroplasticity is therefore essential given the wide scope with which this process is involved.

Musical practice is a multimodal task that requires the simultaneous integration of multimodal sensorimotor information with multimodal sensory feedback mechanisms to monitor performance. It is thus not surprising to find evidence of structural plasticity in different brain regions in musicians. In this context, there has been growing literature suggesting the presence of structural neuroplasticity in regions known to have particular relevance for enabling musical performance, such as motor regions, corpus callosum, auditory cortex, and cerebellum (Schlaug et al., 1995a, Schlaug et al., 1995b, Ozturk et al., 2002, Schneider et al., 2002, Hutchinson et al., 2003, Lee et al., 2003, Bermudez et al., 2009). The results of the present study extend this literature through revealing increased volume and cortical surface area of the left POP/BA44 in musicians. Although classically considered the most important language region in the brain, functions of Broca's area are not limited to language. Recent neuroimaging studies have documented that this region is essential for grasping, motor sequence learning, motor imagery, observation and preparation of action and imitation (Rizzolatti et al., 1996, Krams et al., 1998, Binkofski et al., 1999, Iacoboni et al., 1999, Koski et al., 2002, Mecklinger et al., 2002, Johnson-Frey et al., 2003), and hierarchical organization of behavior by implementing a specialized executive system to control the selection and nesting of action segments (Koechlin and Jubault, 2006). The proposal of a common neural substrate for music, language, and motor functions was also supported by evidence from clinical studies. For instance, it has been shown that children with dyslexia exhibit specific timing difficulties in the domain of music (Overy et al., 2003), motor control (Wolff and Lundberg, 2002), and language (Goswami et al., 2002); and that music lessons in children with dyslexia can lead to improvement in language skills (Overy et al., 2003). It has also been found that patients with severe non-fluent aphasia can benefit from music therapy, such as melodic intonation therapy (Albert et al., 1973, Sparks et al., 1974, Schlaug et al., 2009). I have reported that orchestral musicians possessed greater GM volumes of the left POP (BA 44), a region known to be critically important for language expression (Geschwind, 1970). The present results are in line with the assumption of overlap between music and language. In particular, the POP has been implicated in several musically relevant activities, such as musical syntax processing, calculating harmonic relations between a chord and a preceding harmonic sequence, and detecting music-structural irregularities (Maess et al., 2001, Koelsch, 2006). Furthermore, recent studies have assigned a new functional role for the POP, which is action understanding and imitation. Comparative neuroanatomy considers the POP as the human homolog of area F5 (ventral premotor cortex) in the monkey where multimodal mirror neurons have been located (Rizzolatti and Arbib, 1998). The POP is considered a central component ('hub') of the human mirror neuron network, which also includes the ventrolateral premotor cortex and anterior superior temporal gyrus (Iacoboni et al., 1999, Binkofski and Buccino, 2006). These regions are activated by the execution of manual action and by the observation of the same action performed by others (Binkofski and Buccino, 2006). Recently, it has been shown that this network is also involved in action-listening coupling. Functional studies have indicated that when musically naive subjects listen to action-related sentences (Tettamanti et al., 2005), and to previously practiced music (Lahav et al., 2007), there is differential activation of the POP in the dominant hemisphere, which underlies the importance of this region for audiomotor recognition. According to Baumgaertner et al, 2007 (Baumgaertner et al., 2007), the auditory and motor networks are strongly linked in the musician's brain, so that even when the task involved only auditory or only motor processing, co-activation phenomena within the receptive brain areas can be expected. The human mirror system may have different ways of coding actions in each hemisphere with actions coded through auditory, visual, and motor components in the left hemisphere, whereas in the right hemisphere action coding appears to occur only via the visual and motor channels (Aziz-Zadeh et al., 2004). This left lateralization of the human mirror neuron system in the auditory domain has been suggested to facilitate the emergence of language (Hauser et al., 2002). The findings of increased volume and surface area of the POP in the left (dominant) hemisphere observed in this study could thus be explained by its role in processing action-related sounds. This is in addition to its well-known role in language/music processing. Previous structural neuroimaging studies have clearly demonstrated structural plasticity in the frontal, parietal, and temporal lobes in musicians, such as increased GM density in the premotor cortex, inferior temporal gyrus, and superior parietal lobule (Gaser and Schlaug, 2003a), increased cortical thickness of the superior parietal lobe (Bermudez et al., 2009), increased cross-sectional area of the left planum temporale (Schlaug et al., 1995b), and increased size and cortical thickness of the Heschl gyrus (Schneider et al., 2002, Bermudez et al., 2009). If these results are combined with findings of the present study, it could be suggested that the functional fronto-parieto-temporal human mirror neuron network has a structural basis in musicians evidenced by increased size of its individual components.

The production of an internal duplicate of a heard action that is largely identical to an action performed by the self places the listening subject in the perspective of the acting one leading to the generation of a common link between them. It is therefore possible that, during action listening conditions, the left POP is involved in sequence-specific priming of action representations along with unconscious stimulations and predictions for the next coming action or sound (Lahav et al., 2007). Such neural equivalence between the sender and receiver of a message might be essential for communication (Liberman and Whalen, 2000, Fadiga et al., 2002), learning and skill acquisition (Haslinger et al., 2005), and therefore essential for enabling high level orchestral performance by ensemble musicians.

It has been suggested that right-to-left hemisphere differences in the number of diagonal sulci could result in right-left asymmetry in the volume of POP (Keller et al., 2007). I therefore estimated the number of diagonal sulci in right and left POP with the prediction that this may cause volume differences between musicians and non-musicians. In the left POP, the frequency percentage of diagonal sulci in musicians and non-musicians was nearly identical (40% and 41%, respectively), which may suggest that this factor was not related to the volume differences between the two groups in the present cohort.

There are topographical properties of the cerebral cortex, such as volume and surface area, which are of great importance in quantitative morphometry and cannot be estimated using the VBM technique (Rockel et al., 1980, Fischl and Dale, 2000). In particular, the surface area of the cerebral cortex is of considerable theoretical interest.

Recent studies suggested that increased cortical surface area is associated with enhanced cognitive performance (Koscik et al., 2009). Several reports postulated that the number of neurons in a particular cortical region is proportional to its surface area (Rockel et al., 1974, Henery and Mayhew, 1989, Sisodiya et al., 1996, Sisodiya and Free, 1997). I hypothesize that the finding of increased cortical surface area of the left BA44 in musicians might reflect increased number of cortical neurons in this region. More studies are required in the future to find out the exact correlation between MR-derived morphometric measures and histological properties of the nervous tissue.

4.6.1 TECHNICAL LIMITATIONS

4.6.1.1 STEREOLOGICAL MEASUREMENT OF PARS OPERCULARIS AND PARS TRIANGULARIS VOLUMES

Volume estimation of biologic structures from the analysis of sections of structure of interest is a problem that often arises in biomedical studies. Mathematic formulae and techniques have thus been developed to address this problem leading to the so-called stereological methods. These methods are well recognized for their easy implementation (compared, for example, to manual tracing methods), reliability, and high accuracy (Doherty et al., 2000). Their efficiency depends on generating non-biased encounters between randomly sampled sectional images and grids of systematically spaced test points superimposed upon them; these test points have new random position and orientation on each section to insure unbiasedness. In particular, the stereological Cavalieri method applied here has been designed to estimate the volume of any brain structure regardless of its shape, e.g. Broca's area (Keller et al., 2007), temporal lobe (Doherty et al., 2000), hippocampus (Keller et al., 2002), cerebellum (Karabekir et al., 2009), and brain tumor (Roberts et al., 2000). Despite the well-established advantages of the Cavalieri and point counting methods, they are not capable of correcting for the technical limitations of the MRI technique, which could compromise the precision and accuracy of the volume estimate. The use of Cavalieri method and point counting for volume estimation may be challenging when examining brain structures with irregular shapes and convolutions, such as the PTR, as a higher sampling attenuation is then required to estimate the volume with the same precision as when it is smooth (Garcia-Finana et al., 2003). Another source of error may be produced by inter-rater differences in measurement when identifying structure boundaries on MR images. Even when investigators have sufficient experience in neuroanatomy and image analysis methods, a consistent demarcation of features of interest in MR images may be challenging. In particular, the finite resolution of the images is recognized as an important problem of the MRI technique which may lead to errors related to partial volume effect. Partial volume effect is the loss of contrast or blurring between two adjacent tissues in an image caused by insufficient spatial resolution of MRI compared to the size of anatomical structures, so that more than one tissue type can occupy the same voxel. This problem is minimal with thin slice thickness and sufficiently high resolution, so that fat and water or other different structures are unlikely to occupy the same voxel (Acosta et al., 2008, Rueda et al., 2010). Although the MR images analyzed in the present study were obtained with relatively high resolution of 0.781 mm x 0.781 mm x 1.6 mm (which were re-sampled into 1 X 1 X 1 mm isotropic voxels), such resolution may unfortunately not be sufficient to solve the tissue border ambiguity. Such ambiguity was evident at the GM/CSF boundary, thereby influencing GM volume estimation, and at the GM/WM boundary, thereby influencing GM and WM volume estimation. Since the intersection of the upper right quadrant of the red cross was taken as the “point” in this study, it was sometimes difficult to indicate whether this small part of the red cross intersects GM, WM, or CSF pixel. Images were always displayed using consistent image window and display levels on the same monitor with fixed screen contrast settings; however, in cases of tissue border ambiguity, image window was enlarged to better visualize the ambiguous pixel. It should be noted that this problem involved only few red crosses in each slice and is not expected to change the results, especially with such high level of significance detected in GM volume of the left POP region (p<0.001). The latest generation of MR scanners allows the acquisition of higher resolution MR images, which can provide more accurate volume quantification. Also, methods for accurate classification of mixed voxels and correct estimation of the proportion of each pure tissue have been proposed (Acosta et al., 2008, Rueda et al., 2010), which may help to increase the precision of volume estimation in future studies.

Furthermore, another technical factor that may affect volume estimates of POP/PTR in the present study is inhomogeneities (e.g., shading) in signal intensity over the image field of view caused by the spatial variation in the transmission and reception sensitivities of the radiofrequency coil. Intensity inhomogeneity in MRI might alter image intensities that would otherwise be constant for the same tissue type regardless of its position in an image (Vovk et al., 2007). This has an impact on the demarcation of tissue boundaries, which may subtly differ in contrast from one region to another in the MR image. Sophisticated intensity inhomogeneity correction algorithms (for review see (Vovk et al., 2007)) have been proposed and could be incorporated in future studies. Other technical problems could arise from chemical shift and susceptibility artifacts as well as field of view and slice thickness calibration inaccuracies, which can compromise the accuracy of stereological measurements.

In order to enhance image analysis studies on the GM compartment, future data acquisition at MARIARC is considering the use of 3D T1- weighted Modified Driven Equilibrium Fourier Transform (MDEFT) sequence designed by (Deichmann et al., 2004) to improve contrast between GM and WM. Also, most studies are now transferred to a new 3 Tesla MR system, which offers increased signal to noise ratio thereby increasing accuracy of acquired stereological measures.

4.6.1.2 CORTICAL SURFACE AREA MEASUREMENT OF BA44/45

In the present study, an automatic method was used to estimate cortical surface area of BA44/45. The rationale was to avoid introducing bias when using other methods for surface area estimation, such as stereology (Haug, 1987, Steinmetz et al., 1989b). However, automatic methods are not free of limitations. First, brains were initially transformed to the Talairach space, which is a prerequisite step for achieving optimal results in BrainVoyager. This atlas is one of the most prevalent brain atlases of gross anatomy, the use of which is growing despite its well-known shortcomings (Steinmetz et al., 1989a, Nowinski, 2001, Nowinski and Thirunavuukarasuu, 2001, Maldjian et al., 2004, Nowinski, 2005). The coordinate system of Talairach space is based upon postmortem sections of a 60-year-old French female who had a smaller than average brain size (Talairach and Tournoux, 1988), which criticize the wide use of this coordinate system in MR studies. Also, previous studies have pointed out segmentation inaccuracies (Maldjian et al., 2004, Nowinski, 2005) and low spatial consistency (Nowinski and Thirunavuukarasuu, 2009) when using the Talairach coordinate system. It should be noted that the cortical parcellation scheme used in the present study was based not on cytoarchitecture, but on geometric features using outer anatomical landmarks determined by structural MRI. One could argue that BA44 and BA45 are strictly defined by cytoarchitecture, which cannot be directly observed in MRI scans. It has been assumed by many brain-mapping studies that the macroanatomical gyral and sulcal landmarks coincide with the borders of architectonic areas. That this hypothesis is not tenable in general has been shown previously (Rademacher et al., 1993, Amunts et al., 1999). For instance, Zilles and colleagues (Zilles et al., 1997) have noted that sulcal landmarks are not generally precise indicators of the borders of cytoarchitectonic areas. This concept is especially true in the case of Broca's area. Amunts and colleagues (Amunts et al., 1999) investigated the cytoarchitectural mapping of areas 44 and 45 in 10 human brains by means of an observer-independent technique. They reported that the cytoarchitectonic borders of these areas did not consistently coincide with sulcal contours and; consequently, they concluded that macroscopic features (sulcal landmarks) are not reliable landmarks of cytoarchitectonic borders. Furthermore, a great inter-subject variability in the microscopic (Amunts et al., 1999), and macroscopic (sulcal morphology) (Tomaiuolo et al., 1999, Keller et al., 2007) anatomy of BA44/45 was observed. The extremely variable extent and spatial layout of the BA44/45 imply that the use of an atlas with a single-brain–based template, like the Talairach and Tournoux, to locate these regions may have only an approximate relationship to the location of individual sulci and Brodmann's areas (Lancaster et al., 2000).

The main advantage of automatic methods for cortical surface-based analysis, such as that used in the present study, is the little requirement for user interaction and neuroanatomical expertise, thereby resulting in lower inter-rater variability. It is thus reasonable to use these methods when analyzing large cohorts. However, these methods neither guarantee accurate correspondences nor they directly make use of expert knowledge of the location and variability of specific sulcal features as in landmark-based methods (Pantazis et al., 2010). Even the most consistent gyri and sulci appearing in all normal subjects exhibit pronounced variability in size and configuration (Roland and Zilles, 1994). In accord, demarcation of sulcal landmarks of POP and PTR, such as the anterior ascending and diagonal sulci, was not always possible in the present study, thereby complicating the cortex-based alignment process. In this context, Pantazis and colleagues (Pantazis et al., 2010) compared automatic (using BrainVoyager and FreeSurfer image analysis softwares) and landmark-based methods for cortical surface registration and concluded that, in general, the landmark-based method is more reliable as it did not produce crude registration errors that are often present in automatic methods.

4.7 CONCLUSION

In conclusion, the present study has shown that male orchestral musicians had greater GM volume and cortical surface area of POP/BA44 in the left (dominant) hemisphere. These results corroborate those of the previous VBM study. I hypothesize that long-term skilful practice in the form of orchestral performance is an environmentally enriching activity resulting in structural reorganization through increased volume and cortical surface area of a brain region essential for such performance.

CHAPTER 5

INVESTIGATING THE EFFECT OF MUSICAL EXPERTISE ON REGIONAL MORPHOMETRY OF THE CORPUS CALLOSUM

EFFECT OF MUSICAL EXPERTISE ON REGIONAL CALLOSAL MORPHOMETRY

5.1 ABSTRACT

BACKGROUND AND PURPOSE The corpus callosum (CC) is the largest and latest maturing white matter (WM) tract in the brain that connects homologues cortical regions in both cerebral hemispheres thereby playing an integral role in relaying sensory, motor, and cognitive information. In musicians, previous studies have reported increased cross-sectional areas of the anterior and posterior CC. Gender and age of staring musical training were suggested to be crucial in dictating these differences. Here, I examined the effect of musical expertise on regional callosal morphometry.

SUBJECTS AND METHODS Thirty six right-handed subjects were categorized into three age- and gender-matched groups based on musical expertise: 12 professional musicians, 12 amateur musicians, and 12 non-musicians. T1-weighted 3D-MR images were obtained. Cross-sectional areas of four CC regions corresponding to rostrum/genu/anterior body (CC1), anterior midbody (CC2), posterior midbody (CC3), and isthmus/splenium (CC4) were measured and compared between groups.

RESULTS There were significant group differences in CC1 and CC4 regions. No significant differences were detected in other CC regions. Professional musicians had significantly larger CC1 region compared to amateurs (p=0.01) and non-musicians (p=0.01); and larger CC4 region compared to amateurs (p=0.01) and non-musicians (p=0.006). No significant differences were detected between amateurs and non-musicians in all CC regions.

CONCLUSION These findings may support the assumption that training-induced changes observed in musicians' brains represent use-dependent structural plasticity rather than genetic predisposition for being a musician. Results add another factor that could influence callosal plasticity in musicians namely, musical expertise.

5.2 INTRODUCTION

The CC is the largest commissural WM tract in the brain. It is a heterogeneous structure topographically organized into rostro-caudal sets of fibers each has distinct cortical connections (de Lacoste et al., 1985, Denenberg et al., 1991, Huang et al., 2005, Hofer and Frahm, 2006) and different microstructural properties, such as axon diameter and myelination (Aboitiz et al., 1992).

The heterogeneous structure of CC topography and the existence of specific cortical connections of each part underline the significance of studying regional morphometry of the CC. Cross-sectional area of the midsagittal CC has been examined by three previous studies in musicians. Schlaug and colleagues (Schlaug et al., 1995a) have examined CC morphometry in 30 professional musicians and 30 non-musicians; they reported increased cross-sectional area of the anterior CC half in musicians; interestingly, this difference was due to larger anterior CC in a subgroup who began their musical training before the age of 7 years. As an extension to the previous study, Lee et al. (Lee et al., 2003) recruited a larger cohort of 56 musicians and 56 non-musicians and replicated the finding of increased anterior CC size in musicians. In addition to testing the effect of musicianship on CC morphometry, the latter study investigated gender effect and reported significant gender by musicianship interaction with only male musicians having increased size of the anterior CC. Another study reported that musicians have increased size of both anterior and posterior halves of CC (Ozturk et al., 2002). It is commonly believed that the midsagittal callosal area is linked to the number of fibers crossing through (Aboitiz et al., 1992 , Highley et al., 1999, Bengtsson et al., 2005, Westerhausen et al., 2006). It was thus hypothesized that increased size of CC in musicians indicate structural plasticity during a maturation period essential for increased inter-hemispheric communication (Schlaug et al., 1995a). Such enhanced communication is crucial for subserving complex bimanual motor control and auditory perception.

According to the literature, factors that shape neuroplasticity in musicians include age of commencing musical tuition (Elbert et al., 1995, Schlaug et al., 1995a, Amunts et al., 1997, Jancke et al., 1997, Pantev et al., 1998, Schlaug et al., 1998, Ohnishi et al., 2001, Schlaug, 2001, Lotze et al., 2003, Koeneke et al., 2004, Bengtsson et al., 2005, Imfeld et al., 2009), duration of musical performance (Sluming et al., 2002, Hutchinson et al., 2003, Aydin et al., 2005, Abdul-Kareem et al., 2011), instrument specialty (Elbert et al., 1995, Pantev et al., 2001, Schneider et al., 2005, Bangert and Schlaug, 2006, Shahin et al., 2008), and musical expertise. The latter factor was examined in relatively few studies (Schneider et al., 2002, Gaser and Schlaug, 2003b, Lotze et al., 2003, Shahin et al., 2008). According to this factor, musicians were classified into 'professionals' and 'amateurs'. Schlaug and colleagues (Gaser and Schlaug, 2003a) defined 'professional musicians' as performing artists, full-time music teachers, or full-time conservatory students having an average daily practice time of at least one hour, while the term 'amateur musicians' indicates those who played a musical instrument regularly but whose profession was outside the field of music. Most previous studies reported that professional musicians had increased size of brain regions compared to amateurs and non-musicians. Using voxel-based morphometry (VBM), Gaser and Schlaug (Gaser and Schlaug, 2003a) examined 20 professional musicians, 20 amateur musicians, and 40 non-musicians. Results indicated a significant effect of musician's status on gray matter (GM) density in several brain regions. This means that GM density was highest in professional musicians, intermediate in amateur musicians, and lowest in non-musicians. Increased GM density in professionals was reported in motor areas, somatosensory areas, superior parietal gyrus, left Heschl gyrus, left cerebellum, left inferior frontal gyrus, and inferior temporal gyrus. However, in that same study, authors did not report significant findings in the CC, which was linked to the relative weakness of VBM in detecting WM density differences. The study of (Schneider et al., 2002) provided another example of the effect of musical expertise on brain morphometry through demonstrating increased volume of the Heschl gyrus (which contains the primary auditory cortex) in professional musicians compared to amateurs and non-musicians. In addition to the above mentioned structural markers of musical expertise, the literature suggests the existence of a corresponding correlation between musical expertise and brain function. Compared to amateurs, professional violinists were shown to generate higher electromyographic amplitudes during performance of Mozart's violin concerto and showed focused cerebral activations in the contralateral primary sensorimotor cortex, bilateral superior parietal lobes, and ipsilateral anterior cerebellar hemisphere (Lotze et al., 2003). Also, a study that recorded induced oscillatory gamma band activity (which correlates with attention, anticipation, and expectation) has demonstrated that professionals exhibited larger activity for music tones compared to pure tones indicating enhanced timbre specificity, while amateurs showed weak discrimination (Shahin et al., 2008). Likewise, professional musicians were reported to achieve more precise judgments of timbral dissimilarities of synthesized instrument sounds compared to amateurs and non-musicians (McAdams et al., 1995). The above evidence indicates that professional musicians, being involved in intensive highly skilled musical performance, have developed increased size of several brain regions and higher perceptual learning induced by auditory experience compared to amateurs and non-musicians. The aim of the present study was to extend the literature through exploring whether regional CC size differences (cross-sectional areas) exist between three matched groups of subjects (professional musicians, amateur musicians, and non-musicians) that differed in musical expertise.

5.2.1 HYPOTHESIS

I hypothesized that professional musicians, being involved in complex cognitive, sensorimotor, auditory, and visuospatial skill acquisition, would show evidence of increased size of CC1, CC3, and CC4 regions compared to amateur musicians and non-musicians. Fibers crossing these callosal regions connect cortical areas that were shown to be critically involved in enabling high level musical performance, including the prefrontal cortex (CC1), primary motor cortex (CC3), primary somatosensory cortex, superior parietal lobule, and auditory cortical areas (CC4). Since the size of the midsagittal CC was shown to be positively correlated with the number of crossing fibers (Aboitiz et al., 1992), structural enlargement of CC1, CC3, and CC4 regions is therefore essential to support the need for enhanced inter-hemispheric communication in professional musicians' brains.

5.3 SUBJECTS AND METHODS

5.3.1 SUBJECTS

The cohort included 36 right-handed male volunteers recruited after obtaining Local Research Ethics Committee approval. All subjects gave signed informed consent and were medically screened for neurological, cardiovascular, and endocrine disorders. Subjects were also questioned regarding their alcohol consumption. Handedness was assessed using Edinburgh Handedness Inventory (Oldfield, 1971). The cohort was categorized into three age-, gender-, and handedness matched groups: twelve professional musicians, twelve amateur musicians, and twelve non-musicians. Table 5.1 shows descriptive statistics for all groups. Data regarding the duration of musical practice were not taken so I did not include this factor in statistical analysis.

5.3.1.1 MUSICIANS' PROFILES

Classification of musicians into professionals and amateurs was validated by the measurement of music audition tonal test (Schneider et al., 2002). In this test, 30 pairs of short melodies were presented. These melodies have changes in pitch (10 pairs) or rhythm (10 pairs), or are unchanged (10 pairs). Subjects detected the modification in a three-way forced choice task. Only tonal raw test scores were calculated. Responses were calculated as 20 plus the number of correct responses, minus the number of false alarms. Control subjects scored less than 25 on a scale of 0−40 (range 17−24), professional musicians had a score of at least 26 (range 26−39), and amateur musicians scored in an intermediate range (18−33). Professional musicians were those who had undergone a professional music education ending with a diploma, and were actively performing at the time of examination, while amateur musicians were those who received special instruction in one or more musical instruments. Table 5.2 shows instrument specialty of professionals and amateurs.

Table 5.1. Demographics of subjects recruited in the present study.

Group	***Age (years) Mean / SD / range**	***Age of starting musical training (years) Mean / SD / range**
Professionals	38.3 / 9.5 / 29-55	5.2 / 1.1 / 3-7
Amateurs	41.5 / 11.8 / 24-62	6.1 / 0.9 / 5-7
Non-musicians	36.3 / 4.4 / 26-43	N/A

*One-way analysis of variance revealed no significant group difference in age (p=0.37). Independent student *t* test revealed no significant difference in age of starting musical training between professionals and amateurs (p=0.7).

Table 5.2. Instrument specialty of all musicians.

Professionals		**Amateurs**	
Instrument	No.	Instrument	No.
Piano	8	Piano	7
Violin	3	Violin	3
Viola	1	Viola	2

5.3.2 MR DATA ACQUISITION

All volunteers were scanned using a protocol with identical sequence and parameters. T1-weighted 3D-MRI images were obtained using Siemens symphony 1.5 T (Siemens, Magnetom, Germany). Magnetization-Prepared Rapid Gradient-Echo imaging (MPRAGE) sequence was used with TR/TE/TI= 2040/3.93/1100 ms, slice thickness = 1 mm, field of view = 256 mm, matrix size=256 X 224, voxel size = 1 X 1 X 1 mm, no. of slices =176 sagittal slices, flip angle = 15º, bandwidth = 130 Hz/Px, and acquisition time = 7 minutes and 38 seconds.

5.4 METHODS

Image analysis methods used in this study include:

- Measurement of intracranial volume (ICV) using design-based stereology, which involved application of Cavalieri method combined with point counting (Roberts et al., 2000, Garcia-Finana et al., 2003).
- Measurement of cross-sectional areas of CC regions.

5.4.1 MEASUREMENT OF INTRACRANIAL VOLUME

To control for head size, ICVs were measured using design-based stereology, which involved application of Cavalieri method combined with point counting as described in chapter 3, section 3.9, pages 74-79.

5.4.2 MEASUREMENT OF CROSS-SECTIONAL AREAS OF CC REGIONS

The method of measurement of cross-sectional areas of CC regions is described in chapter 3, section 3.10, pages 80-83.

5.4.3 INTRA- AND INTER-RATER RELIABILITY

Intra-rater reliability was analyzed by repeating ICV and CC measurements (cross-sectional areas of CC1-CC4) of all subjects by IA with at least 3 weeks between the two measurements. For inter-rater reliability, ICV and CC measurements were independently performed by two raters (MA and IA) for all subjects. An intra-class correlation coefficient greater than 0.80 was accepted as evidence of inter- and intra-rater reliability.

5.4.4 STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS v. 16.0 for Windows and statistica program, v. 6.0 (Statsoft Inc., USA). Shapiro-Wilk (S-W) was used to test normality of data distribution. Cross-sectional areas of CC1-CC4 regions were corrected for ICV as recommended by previous studies (Rauch and Jinkins, 1994, Steinmetz et al., 1995, Giedd et al., 1999, Smith, 2005, Phillips et al., 2007). To achieve this task, the square root of the cross-sectional area of each CC region was divided by the cube root of the ICV for each subject (Smith, 2005, Phillips et al., 2007).

Using SPSS, multivariate analysis of variance (MANOVA) was performed with ICV-corrected measures of CC1-CC4 regions as dependent variables and musicianship (professionals versus amateurs versus non-musicians) as the independent variable with age as covariate; $p<0.05$ was considered significant after adjustment for multiple comparisons. Pearson's correlation was used to perform correlation analyses. One-way analysis of variance was used to compare ICV measurements between groups. The intra-class correlation coefficient (ICC) calculated by one-way random model was used to test the intra- and inter-rater reliability. A coefficient greater than 0.80 was accepted as evidence of inter- and intra-rater reliability. Statistica program was used to produce figures 5.1 and 5.2.

5.5 RESULTS

5.5.1 MEASUREMENT OF INTRACRANIAL VOLUME

One-way analysis of variance revealed no significant group difference in ICV (*p*=0.32). Figure 5.1 illustrates Means ± 95% confidence intervals for ICV.

5.5.2 COMPARISON OF REGIONAL CROSS-SECTIONAL AREAS OF THE CC

Results revealed significant CC region by musicianship interaction in CC1 and CC4 regions (F = 5.85; *p*=0.003), and (F = 5.85; *p*=0.002), respectively. No significant difference was detected in other CC regions. Post hoc test (Bonferroni adjusted) revealed that professional musicians had significantly larger CC1 region size compared to amateurs (*p*=0.01) and non-musicians (*p*=0.01); and larger CC4 region size compared to amateurs (*p*=0.01) and non-musicians (*p*=0.006). No significant difference was detected between amateurs and non-musicians in all CC regions. Means ± 95% confidence intervals of cross-sectional areas of CC regions are illustrated in figure 5.2. Correlation analyses were next performed using Pearson’s correlation between age of starting musical training and cross-sectional areas of CC1/CC4 regions after controlling for subjects’ age. There was no significant correlation between cross-sectional area of CC1/CC4 regions and age of starting musical training (*p*>0.05) in professional musicians. Figure 5.3 shows midsagittal slices from professional, amateur, and control subjects.

5.5.3 INTER- AND INTRA-RATER STUDY

The result of inter-rater reliability was good ranging between 0.90-0.96. The result of intra-rater reliability was also good ranging between 0.93 and 0.98.

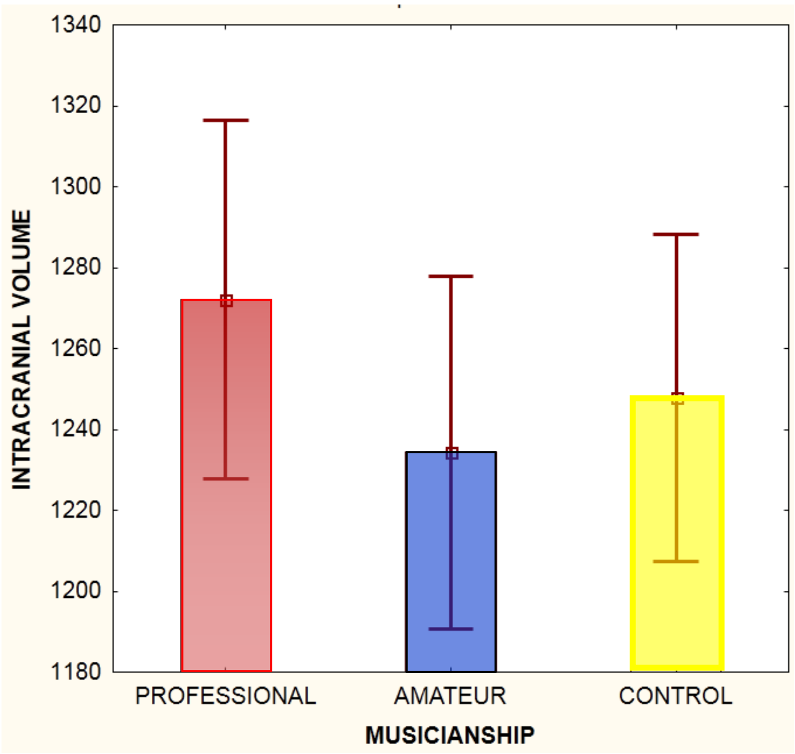

Fig.5.1. Means ± 95% confidence intervals of ICV in the three groups examined in the present study. Volumes are mm^3.

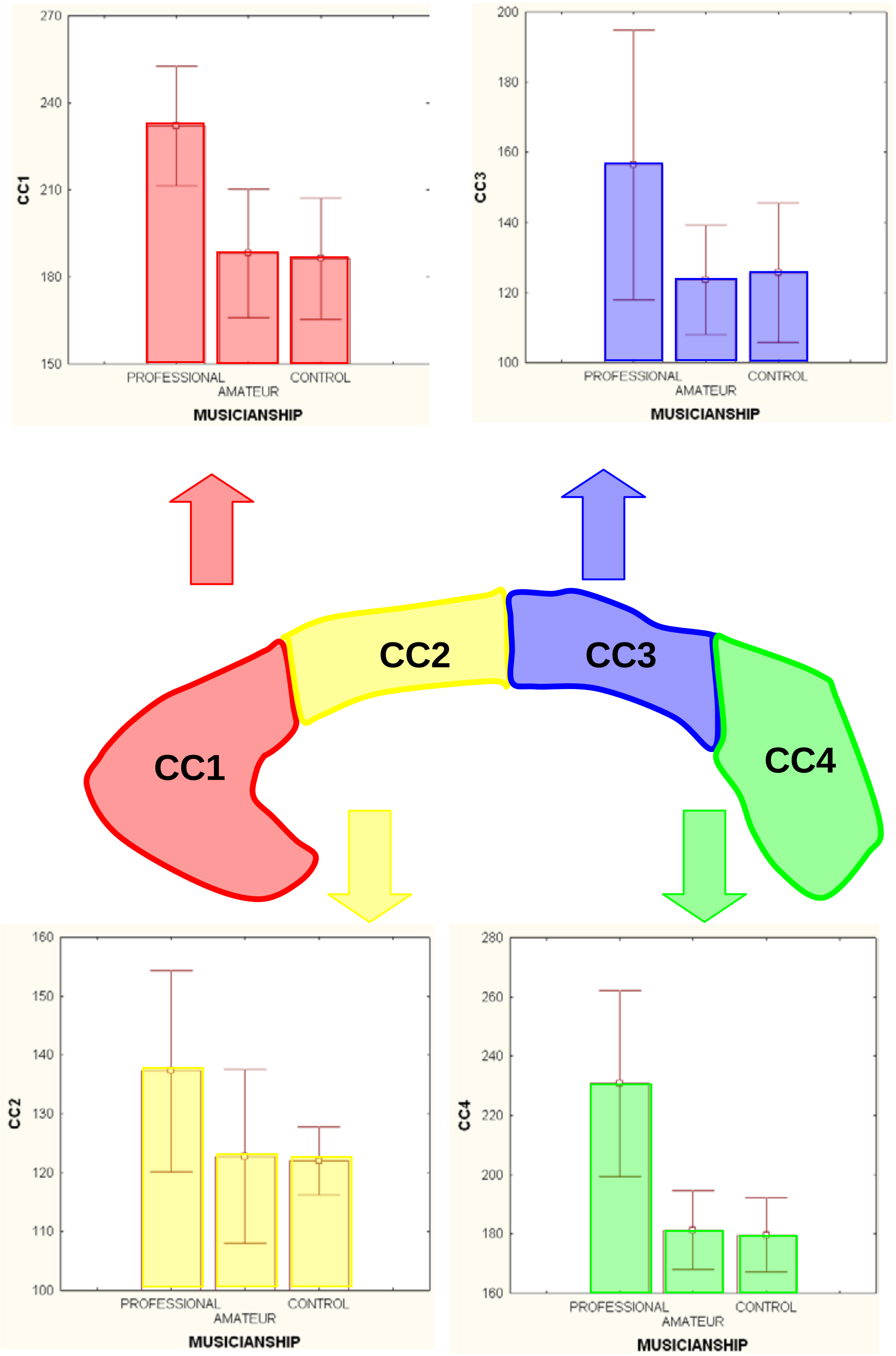

Fig.5.2. Mean ± 95% confidence intervals of cross-sectional areas of the four callosal regions (CC1-CC4) (uncorrected for ICV) in professional musicians, amateurs and non-musicians. Areas are mm^2.

Fig.5.3. Mid-sagittal MR sections showing the CC (yellow dashed box) in a professional musician (top), an amateur (middle), and a control subject (bottom) aligned along the maximum anteroposterior length of the CC.

5.6 DISCUSSION

Regional callosal morphometry based on MRI has been used extensively in previous studies owing to the ease with which the CC is identified on midsagittal MR sections, and the fact that the CC is extensively connected with the cerebral cortex making it an ideal tool to probe several behavioral, psychological, and pathological conditions. In accordance, regional morphometry of the CC has been used to investigate handedness (Cowell et al., 1993), sexual dimorphism (Bermudez and Zatorre, 2001), natural aging and development (Giedd et al., 1996, Keshavan et al., 2002), use-dependent structural changes in response to skill acquisition (Schlaug et al., 1995a, Ozturk et al., 2002, Lee et al., 2003), as well as in several neurological and psychological disorders, such as schizophrenia (Rotarska-Jagiela et al., 2008), depression (Lacerda et al., 2005), Alzheimer disease (Hallam et al., 2008, Li et al., 2008), dyslexia (Hynd et al., 1995, Rumsey et al., 1996), autism (Kilian et al., 2008), and several others. Here, I test the effect of musical expertise on CC structure. I have selected a cohort of male professionals and amateurs who commenced their musical training at or before age of 7 years to control for gender and age of commencement of musical training as previous studies suggested that these factors are important determinants of CC plasticity in musicians (Schlaug et al., 1995a, Lee et al., 2003).

5.6.1 WITELSON'S VERSUS DTI-BASED PARCELLATION OF CC TOPOGRAPHY

According to Witelson's scheme used in the present study (Witelson, 1989), the CC1 region (rostrum/genu/anterior part of the body) contains fibers from the prefrontal, premotor, and supplementary motor cortical regions; CC2 region (anterior midbody) contains fibers from the primary motor region; CC3 region (posterior midbody) contains fibers from the sensory and posterior parietal regions; and CC4 region (isthmus and splenium) contains most of the fibers from the parietal, temporal, and occipital regions. These callosal subdivisions were based mainly on studies of monkeys (Pandya et al., 1971, Barbas and Pandya, 1984, Cipolloni and Pandya, 1985) and postmortem studies in humans (de Lacoste et al., 1985). However, advance in DTI technique has allowed scientists to examine the callosal structure *in vivo*. Numerous studies based on diffusion tensor tractography have extended our knowledge regarding topological partitions of the CC through incorporating callosal parcellation with various region of interest selection methods, such as brief definition of cortical areas (Huang et al., 2005, Hofer and Frahm, 2006), determining geometric features of outer anatomical landmarks by structural MRI (Park et al., 2008), and clustering the CC subdivisions by parcellating neural pathways that connect the CC and distinct Brodmann's areas (Chao et al., 2009).These studies reported discrepancies between the DTI-based fiber topology of the human CC and Witelson's classification and showed that callosal fiber topology is subjected to considerable inter-individual variability along the antero-posterior axis. First, although DTI studies agreed with Witelson's scheme in the fact that prefrontal fibers cross through the CC1 region, they showed contradicting results regarding the full extent of these fibers across the antero-posterior dimension of the CC. Hofer and colleagues (Hofer and Frahm, 2006) demonstrated that prefrontal fibers occupy only the anterior 1/6th of the CC, while other studies showed that these fibers extend to anterior midbody (CC2) (Zarei et al., 2006, Chao et al., 2009), and may even reach the posterior midbody (CC3) (Park et al., 2008). Second, fibers from premotor and supplementary motor cortical regions do not only occupy CC1 region as Witelson's scheme suggested but also extend to anterior midbody (CC2) (Hofer and Frahm, 2006). Third, in contrast to Witelson's classification, DTI studies have indicated that callosal motor fiber bundles cross the CC in a much more posterior location than previously indicated. In other words, these fibers cross the posterior midbody (CC3) rather than the anterior midbody (CC2) (Hofer and Frahm, 2006, Wahl et al., 2007, Park et al., 2008, Chao et al., 2009). One possible explanation for the posterior shift of callosal motor fibers in humans relative to monkeys is the massive increase in prefrontal cortex volume, in particular the prefrontal WM, in humans (Schoenemann et al., 2005), which suggests connectional expansion (Wahl et al., 2007). A part from the above-mentioned discrepancies, both DTI and Witelson's schemes agreed that the isthmus and splenium (CC4) contain fibers from the primary somatosensory cortex (isthmus), and from parieto-temporo-occipital regions (splenium). DTI studies also showed that splenial fibers are arranged in a dorso-ventral dimension corresponding to the medio-lateral cortical axis with fibers from the parietal lobe occupying mainly the dorsal part of the splenium, while fibers from the temporal and occipital lobes occupying the ventral part of the splenium (Park et al., 2008). Despite these discrepancies, Witelson's scheme has been widely used in callosal studies as it is simple, reliable, and has less technical limitations known to DTI studies.

5.6.2 INCREASED CC1 AND CC4 SIZE IN PROFESSIONAL MUSICIANS

In the present study, I found increased size of CC1 and CC4 regions in professional musicians compared to both amateurs and non-musicians. In addition, amateurs did not show significant size differences compared to non-musicians. I have shown that regional CC macrostructure in musicians is affected by musician's expertise, which could be added to other previously reported factors, such as age of starting musical training (Schlaug et al., 1995a) and gender (Lee et al., 2003). Possible factors contributing to increased callosal size include more fibers crossing through the CC, thicker fibers, higher proportion of myelinated fibers, or a combination of these changes (Cowell et al., 1992, Schlaug et al., 1995a). In a postmortem study of the human CC, Aboitiz and colleagues (Aboitiz et al., 1992) reported that an increased callosal size may indicate increased total number of fibers crossing through. Furthermore, increased callosal size has been shown to correlate with increased fractional anisotropy (FA) (Hasan et al., 2008). The FA value has been related to intra-axonal organization (Kinoshita et al., 1999), density of fibers, and myelination (Mori and Zhang, 2006, Sun et al., 2006, Trivedi et al., 2009). It is thus possible that the increased size of CC1 and CC4 regions observed in professional musicians is related to increased fiber density and myelination, although one should use this interpretation with care when dealing with conventional MR methodologies. DTI studies are required in the future to provide additional information about structural plasticity of the CC in musicians.

Both DTI and postmortem studies (de Lacoste et al., 1985, Chao et al., 2009) agreed that the rostrum, genu, and anterior part of the callosal body (i.e. CC1) connect mainly prefrontal regions, while the isthmus and splenium (i.e. CC4) connect temporo-parieto-occipital regions. It has been shown that both the size (Narberhaus et al., 2008), and FA value (Salo et al., 2009) of the genu are correlated with cognitive functions, such as verbal fluency, attention, motor speed, executive functioning, and memory (Narberhaus et al., 2008). The prefrontal cortex is involved in several functions essential for musical performance, including skill acquisition, imitation learning of hand actions (Vogt et al., 2007), procedural sequence learning (Jenkins et al., 1994), attention (Lau et al., 2004), music processing and perception (Zatorre et al., 1998, Ohnishi et al., 2001), and spatial working memory (Robertson et al., 2001). In musicians, fMRI studies revealed that the prefrontal cortex plays a key role in the generation of musical structures during improvisation (Bengtsson et al., 2007). Furthermore, professional musicians showed weaker activations within the prefrontal cortex compared to non-musicians while performing a bimanual motor task (Haslinger et al., 2004). This might imply that professional musicians require less attention to action selection of the correct movement (Deiber et al., 1991, Botvinick et al., 1999). It is therefore possible that increased size of CC1 region reported here represent use-dependent structural adaptation essential for increasing inter-hemispheric communication between prefrontal cortices. The finding of increased CC1 size in professionals is in partial agreement with those of (Schlaug et al., 1995a, Ozturk et al., 2002, Lee et al., 2003) who reported increased size of the anterior half of CC. In the present study, the anterior CC half was further divided into rostrum/genu/anterior body and anterior midbody as these regions have distinct anatomical connections.

The isthmus part of the CC (the smallest part of CC4 region) contains fibers from the primary somatosensory cortex. Compared to non-musicians, musicians have greater sensory load imposed by their complex skilful performance. In accord, previous studies have shown evidence of use-dependent structural-functional adaptation in the primary somatosensory cortex in professional musicians, such as increased GM density in the left primary somatosensory cortex in right-handed pianists and increased cortical representation of the digits (second to fifth) of the left hand in string players. These findings are compatible with increased size of the isthmus reported here.

The splenium (the largest part of CC4 region) has a massive number of fibers crossing from parietal, temporal, and occipital regions. This part of the CC plays a key role in skill acquisition. Previous studies had indicated that it is crucial in relaying visuospatial cognitive information (Schmitt et al., 2001, Tomaiuolo et al., 2002, Pollmann et al., 2004, Fryer et al., 2008) through connecting visual ad parietal cortices. Musicians are well-known for their enhanced visuospatial performance (Sergent et al., 1992, Stewart et al., 2003). When practicing music, they need to read musical notations and translate them into movement patterns, a process known as sight reading (Sergent et al., 1992). Music reading can thus be considered as a visuospatial cognitive task in which vertically oriented musical notes are mapped onto the fingers, which strike horizontally oriented keyboard elements as in keyboard players (Stewart et al., 2003). In this context, professional musicians were reported to have increased GM density in the superior partial lobule (Gaser and Schlaug, 2003a), a region known to subserve visuomotor control (Jeannerod et al., 1994). The splenium also contains fibers from the superior temporal gyri in which the primary and secondary auditory cortices are located. Several studies reported structural-functional plasticity in auditory regions in musicians, such as increased volume and GM density of the Heschl gyrus (Schneider et al., 2002, Bermudez et al., 2009), increased size of the right and left planum temporale (Schlaug et al., 1995b, Bermudez and Zatorre, 2005), increased auditory cortical representation of piano tones (Pantev et al., 1998), increased multimodal auditory-somatosensory (Schulz et al., 2003), and auditory-motor integration (Lotze et al., 2003, Bangert et al., 2006, Baumann et al., 2007), and enhanced timbre specificity (McAdams et al., 1995, Shahin et al., 2008). Thus, I may also propose that structural enlargement of the splenium revealed in professional musicians represent use-dependent structural adaptation essential for increasing inter-hemispheric communication between visual, parietal, and auditory cortices. Results of the present study agree well with those of (Ozturk et al., 2002), which showed increased size of the posterior CC in musicians. The lack of significant differences in CC2 and CC3 regions contradicts with previous studies (Schlaug et al., 1995a, Ozturk et al., 2002, Lee et al., 2003). According to recently published DTI studies, CC2 region is mostly occupied by premotor and supplementary motor fibers, while CC3 region contains fibers from the primary motor cortex (Park et al., 2008, Chao et al., 2009). The lack of significant findings in these regions may indicate their additional involvement in a multitude of non-musical tasks that were practiced by amateurs and non-musicians. The curved shape of the CC and the parcellation scheme used in the present study have resulted in larger CC1 and CC4 regions compared to smaller CC2 and CC3 regions. It is possible for fibers from CC2 and CC3 to overlap with neighboring regions, as shown clearly from DTI studies, thereby averaging out subtle size differences between groups in these regions. Studies that have shown a positive correlation between musical expertise and neuroplasticity (Schneider et al., 2002, Gaser and Schlaug, 2003a, Lotze et al., 2003) might provide a good support to the assumption that plastic changes observed in musicians' brains represent use-dependent adaptations imposed by intensive skilful practice rather than an innate predisposition for being a musician. If the latter assumption is true, then we would expect similar changes in brains of professional and amateur musicians. Indeed in the present study I have not only observed increased callosal size in professionals relative to amateurs and non-musicians, but also no significant differences between amateurs and non-musicians. The fact that both groups of musicians were matched for age, gender, handedness, and age of starting musical training implies that playing musical instrument might not necessarily lead to induction of structural plastic changes in the candidate's brain; what is more important is the quantity of that training. Results of the present study suggest that this assumption is at least true when considering the callosal structure. In accord, it has been shown that there are no neural, cognitive, and motoric differences between children enrolled in music training program and those who are not planning to take music lessons (Norton et al., 2005).

5.6.3 METHODOLOGICAL CONSIDERATIONS AND LIMITATIONS OF THE STUDY

The present study suffers from some technical limitations common to MR studies, which may produce difficulties that can impact estimates of the callosal area. Partial volume and effects related to limited image resolution made it difficult to delineate the ambiguous boundaries of the CC (Clarke and Zaidel, 1994), such as, for example, the boundary between the dorsal border of the CC and the cingulate gyrus. In addition, it was challenging to disambiguate confusion due to non-callosal structures, such as that due to fornix and septum pellucidum visual encroachment onto the CC.

Comparison of current morphometric results with previous studies in musicians may be problematic when different criteria are used to measure the CC. First, despite the fact that previous studies used Witelson’s method to examine callosal morphology on midsagittal MR images, they often do not provide detailed information about the anatomic landmarks used to define the midsagittal plane. Without explicit measures to account for or to describe these features, all subsequent processing is prone to error in examining for inter-subject differences. In the present study, the midsagittal plane was carefully selected according to specific anatomical landmarks, which insured consistent segmentation results. In effect, intra- and inter-rater reliability measures were good ranging between 0.93-0.98 and 0.90-0.96, respectively. Second, the use of different methods to identify the CC plane along which vertical lines are applied can greatly impact the size of resulted CC subdivisions. For example, Schlaug and colleagues (Schlaug et al., 1995a) have set CC subdivisions according to the bicommissural plane, while in the present study, the CC was rotated so that the line connecting the ventral most points of the anterior and posterior CC is horizontally oriented before setting CC subdivisions along the maximum antero-posterior width of the CC. Third, although most previous studies followed Witelson’s scheme to segment the CC, they often interpret their findings based on different numbers of CC subdivisions, such as 2, 3, 5 and 9 subdivisions (Aboitiz et al., 1992 , Schlaug et al., 1995a, Highley et al., 1999, Westerhausen et al., 2004), which makes comparison of findings across studies extremely challenging.

Several geometric partitions have been proposed to subdivide the CC on midsagittal MR images in addition to Witelson's scheme used in the present study (Denenberg et al., 1991, Weis et al., 1993, Clarke and Zaidel, 1994, Rajapakse et al., 1996). It should be noted; however, that these partitioning methods are basically geometric and do not take into account neuronal fiber composition or fiber connections through the CC. As discussed previously, DTI studies have indicated that callosal fiber topology is complex and subjected to great inter-individual variations. Consequently, the use of geometric sectioning schemes may oversimplify callosal structure. Recently, several groups have used DTI to generate probabilistic topographic maps of the CC, which may provide more accurate CC segmentation scheme based on individual anatomical connectivity (Park et al., 2008, Chao et al., 2009) rather than crude geometric segmentation schemes.

5.7 CONCLUSION

In conclusion, it was shown that professional musicians had increased size of CC1 and CC4 regions compared to amateurs and non-musicians. These findings extend the currently expanding literature suggesting use-dependent structural adaptation in musicians' brains induced by skilful practice. I hypothesize that these structural changes are essential to support enhanced inter-hemispheric communication between cortical regions connected by fibers traversing the CC1 and CC4 regions. These results add another factor that could influence structural plasticity of the CC in musicians namely, musical expertise.

CHAPTER 6

INVESTIGATING REGIONAL MACRO- & MICROSTRUCTURAL PLASTICITY OF THE CORPUS CALLOSUM IN MUSICIANS

MACROSTRUCTURAL AND MICROSTRUCTURAL PLASTICITY OF THE ISTHMUS AND SPLENIUM IN MUSICIANS: EVIDENCE FROM CONVENTIONAL MAGNETIC RESONANCE AND DIFFUSION TENSOR TRACTOGRAPHY

6.1 ABSTRACT

BACKGROUND AND PURPOSE To investigate regional macrostructural and microstructural plasticity of the corpus callosum (CC) in musicians. Using conventional MR morphometry, previous studies reported increased CC macrostructure in musicians. Here, I add to the literature through reporting on regional macrostructure and microstructure of the CC using a combination of structural MR and diffusion tensor imaging (DTI) techniques, respectively.

SUBJECTS AND METHODS Twelve right handed musicians (9 males/3 females) and twelve age-, gender-, and handedness-matched controls were examined. Cross-sectional areas (macrostructure) of 4 callosal regions corresponding to rostrum, genu, and anterior body (CC1), anterior midbody (CC2), posterior midbody (CC3), and isthmus and splenium (CC4) were measured on midsagittal T1-weighted MR sections. Using DTI, fiber tractography of corresponding regions was performed to measure average fractional anisotropy (FA), mean diffusivity (MD), tract volume, and number of streamlines (microstructure) in each region. These measures were normalized for intracranial volumes and compared between groups.

RESULTS Statistical analysis revealed increased normalized cross-sectional area (p=0.001), average FA (p=0.001), normalized tract volume (p=0.003), normalized number of streamlines (p=0.002), and decreased MD (p=0.02) in CC4 region in musicians. No significant results were detected in other regions and no significant gender by musicianship interaction. Cross-sectional area of CC4 was negatively correlated with age of commencing musical tuition (r = -0.7, p=0.01). There was a significant positive correlation between cross-sectional area and average FA value in CC4 region in all subjects (r = 0.5, p=0.004).

CONCLUSION It could be hypothesized that increased macro- and microstructural measures in CC4 region represent structural plasticity crucial for supporting enhanced inter-hemispheric communication between parietal, temporal, and occipital cortical regions in musicians.

6.2 INTRODUCTION

The CC is the largest white matter (WM) commissure in the brain consisting of approximately 180 million contralateral axonal projections (Blume, 1984) that connect homologous cortical regions in both cerebral hemispheres (de Lacoste et al., 1985, Hofer and Frahm, 2006).

The technique of DTI has revolutionized the field of WM mapping. This MR-based methodology characterizes the diffusivity of water molecules in the human brain and can provide important information about WM microstructure that is not available from other imaging modalities (Basser et al., 1994, Pierpaoli et al., 1996). Diffusion-weighted imaging also offers the possibility of *in vivo* visualization of major fiber tracts, i.e. diffusion tensor tractography (DTT). This technique can provide quantitative data of reconstructed tracts, such as average FA, MD, tract volume, and number of streamlines, which may have different discriminative power (Correia et al., 2008, Tate et al., 2010). The FA and MD provide information about the microstructural features of WM, which include intra-axonal organization, density of fibers, degree of myelination, and individual fiber diameter (Mori and Zhang, 2006, Sun et al., 2006). In particular, tract volume and number of streamlines were suggested to provide useful tools for detecting subtle differences in WM integrity (Ciccarelli et al., 2003, Correia et al., 2008), and may provide results comparable to postmortem data (Ciccarelli et al., 2003). These DTI-derived metrics were used to quantify specific WM pathways, such as the corticospinal tract (Lindenberg et al., 2010), arcuate fasciculus (Matsumoto et al., 2008, Schlaug et al., 2009), CC, and cingulum (Thomas et al., 2005, Correia et al., 2008). Quantitative changes of these metrics have been linked to cognitive performance (Tate et al., 2010) and were suggested to provide structural markers of motor dysfunction (Hosomi et al., 2009) and plasticity (Ramu et al., 2008a, Schlaug et al., 2009). For example, Schlaug and colleagues (Schlaug et al., 2009) reported significant increase in the number of streamlines and tract volume of the arcuate fasciculus in patients with non-fluent aphasia who undergone intonation-based speech therapy. Another example is increased number of streamlines of internal capsule and cerebral peduncles six weeks following the induction of spinal cord injury in rats (Ramu et al., 2008b).

To date, most previous studies that examined the CC in musicians have used conventional MR to manually measure cross-sectional area of the CC on midsagittal images (Schlaug et al., 1995, Ozturk et al., 2002, Lee et al., 2003). Increased size of the anterior (Schlaug et al., 1995, Lee et al., 2003), and posterior (Ozturk et al., 2002) CC reported in these studies were hypothesized to indicate more fibers crossing through, hence a better capacity for inter-hemispheric transfer. However, it is possible that an increase in callosal size is associated with a decrease in fiber density resulting from an increased inter-fiber distance (Tomasch, 1954). In human, it has been reported that the correlation between callosal size and fiber density is partially determined by fiber size, with good correlation in fibers with a diameter around 0.4 - 1 µm but not for fibers larger than 3 µm in diameter (Aboitiz et al., 1992). Furthermore, a study performed in the macaque (Lamantia and Rakic, 1990) reported a significant negative correlation between fiber density and callosal size. This implies that increased callosal macrostructure (cross-sectional area) may not indicate a corresponding increase in microstructural parameters of the CC. Such macro-microstructural discrepancy has been reported in previous DTI studies. For instance, significant microstructural CC changes have been reported in the absence of macrostructural ones (Pfefferbaum and Sullivan, 2002, Westerhausen et al., 2006a). A combined use of structural MR and DTI techniques was postulated to provide better evidence when studying WM pathways (Pfefferbaum and Sullivan, 2002, Schulte et al., 2004, Westerhausen et al., 2004, 2006a, Westerhausen et al., 2006b, Hasan et al., 2008a). Expanding these studies, the present work represents the first report relating macro- and microstructural measures of the CC to musicianship. To date, DTI examinations of the CC in musicians have revealed controversial findings. Differences are more likely to be the result of the musical and experiential histories of participants studied, different scanning parameters, and diversity in the applied tracking algorithms. Schmithorst and Wilke (Schmithorst and Wilke, 2002) performed voxel-wise examination in a cohort of five musicians and seven non-musicians and reported increased FA value in the genu of the CC in musicians. Another VBM study (Bengtsson et al., 2005) investigated effects of piano practicing in childhood, adolescence, and adulthood on FA measures of the CC. Authors failed to report significant group differences in the CC, although there was a significant correlation between FA value of the body/splenium of the CC and total hours of piano practicing in children aged less than 11 years. To the best of my knowledge, only one study had used DTI-based tractography (probabilistic algorithm) to investigate the effect of musical training on CC structure. Authors reported no significant group differences in FA and MD of the anterior and posterior halves of the CC (Imfeld et al., 2009).

In this work, the aim was to examine regional CC macrostructure and microstructure, the latter of which may be more sensitive to the detection of subtle fiber differences than gross measurements of size. Accordingly, I used conventional MR to quantify regional cross-sectional area and DTI-based tractography to quantify average FA, MD, normalized tract volume, and normalized number of streamlines of four evenly divided callosal regions corresponding roughly to rostrum, genu, and anterior body (CC1), anterior midbody (CC2), posterior midbody (CC3), and isthmus and splenium (CC4).

6.2.1 HYPOTHESIS

I hypothesized that my previous findings of increased macrostructure (cross-sectional areas) of CC1 and CC4 regions in professional musicians (study 2) would be replicated in the present study. Furthermore, I proposed that increased size of these regions would be reflected, at the microstructural level, by increased fiber density, axonal projections, and sprouting (indicated by increased number of streamlines), thicker myelination and/or larger diameter of fibers (indicated by increased tract volume), increased coherence and integrity of fiber bundles (indicated by increased average FA value), and more restrictions to diffusion by the surrounding cellular components, which may suggest the presence of larger number of axons (more membrane) and/or more myelin material (indicated by reduced MD value). I hypothesized that these findings represent training-induced structural plastic changes triggered by the need for stronger and more effective inter-hemispheric communications in professional musicians.

6.3 SUBJECTS AND METHODS

6.3.1 SUBJECTS

As described in chapter 3, cohort 3 originally included 26 volunteers recruited after obtaining Local Research Ethics Committee approval. Two left-handed subjects (1 musician/1 control) were excluded from the present study since several reports have indicated a significant effect of handedness on callosal macrostructure (Witelson, 1989, Habib et al., 1991, Witelson and Goldsmith, 1991) and microstructure (Westerhausen et al., 2003, Westerhausen et al., 2004). The final cohort (cohort 3A) used for subsequent data analysis included 24 subjects: 12 right-handed musicians (9 males and 3 females), and 12 right-handed non-musicians (9 males and 3 females). Subjects were matched for age and gender. All subjects gave signed informed consent and were medically screened for neurological, cardiovascular, and endocrine disorders. Subjects were also questioned regarding their alcohol consumption. Handedness was assessed using the Edinburgh handedness inventory (Oldfield, 1971). During the first test session, musicians were questioned regarding their musical training history, in addition to instrumental and performance hours. Controls were also questioned regarding any musical training and also their interest in music. Descriptive statistics of volunteers' age and years of education are presented in table 6.1.

6.3.1.1 MUSICIANS' PROFILE

Professional musicians were recruited from a full-time playing major British symphony orchestra (9 subjects), and from the School of Music, University of Liverpool (3 subjects). There were 6 pianists and 6 string players (2 cello players, 3 violin players, and 1 viola player). Descriptive statistics of age of starting musical training and duration of musical performance are provided in table 6.1. Only two musicians started their musical training after the age of 7 years.

Table 6.1. Demographics of subjects recruited in this study.

	Groups	
	Musicians	Non-musicians
***Age (years) Mean / SD / range**	37 / 15.6 / 18-57	35.5 / 12.7 / 20-57
Duration of musical performance (years) Mean / SD / range	28.3 / 13.05 / 11-48	N/A
Age of starting musical training (years) Mean / SD / range	6.6 / 2.05 / 4-10	N/A
***Education (years) Mean / SD**	13.6 / 1.2	13.4 / 2.1

*Student *t* test revealed no significant group difference regarding age (*p*=0.5) and years of education (*p*=0.2).

6.3.2 MR DATA ACQUISITION

All volunteers were scanned using protocols with identical sequences and parameters.

6.3.2.1 HIGH RESOLUTION STRUCTURAL SCANS AT 3 TESLA

T1-weighted MR images were obtained using Trio 3 Tesla (Siemens, Magnetom, Germany) whole-body imaging system using a proprietary 8-channel head coil. These scans were used to measure cross-sectional areas of CC regions and ICVs. The Magnetization-Prepared Rapid Gradient-Echo (MPRAGE) imaging sequence was used with the following parameters: TR/TE = 2040/5.57 ms, flip angle = 8°, field of view = 256 mm, and acquisition time = 4 minutes, 14 seconds. A total of 176 sagittal slices with slice thickness of 1 mm, and in-plane resolution of 1 mm were obtained.

6.3.2.2 DIFFUSION-WEIGHTED MR DATA ACQUISITION

DTI data acquisition was acquired with Trio 3 Tesla whole-body MRI system (Siemens, Magnetom, Germany).The DTI scheme included the collection of 60 images with noncollinear diffusion gradients (b = 1200 s/mm^2), and five non-diffusion-weighted images (b = 0 s/mm^2), employing a single shot echo-planar imaging sequence. From each participant, 60 axial slices were collected. TR/TE = 10100/106 ms, field of view = 267 mm, the acquisition matrix was 112 X 128, and the slice thickness was 2 mm with no gap, which resulted in voxel dimensions of 2 X 2 X 2 mm. The total acquisition time was 22 minutes and 14 seconds. Prior to diffusion-weighted data acquisition, all subjects were given detailed explanation about the nature of the acquisition, and were asked to keep body motion to a minimum. Inside the scanner, motion was controlled with the use of head cushions provided by the scanner manufacturer.

6.4 METHODS

Image analysis methods used in this study include:

- Measurement of intracranial volume (ICV) using an automatic technique.
- Measurement of cross-sectional areas of CC regions using a manual morphometric technique.
- Fiber tractography of CC regions to measure average FA, MD, tract volume, and number of streamlines of tracts crossing through these regions.

6.4.1 MEASUREMENT OF INTRACRANIAL VOLUME

To control for differences in head size, ICVs were measured. Automatic measurement of ICV was performed using FreeSurfer software (version: stable v5.0 for Linux), available online at http://surfer.nmr.mgh.harvard.edu/fswiki/Download. High resolution T1 weighted MR images obtained using 1.5 Tesla MPRAGE protocol were initially planned to be used for ICV measurement (a detailed description of this imaging protocol is provided in chapter 7). The 1.5 Tesla MPRAGE protocol has longer acquisition time = 14 minutes compared to the 3 Tesla MPRAGE protocol and was optimized to obtain high resolution images ideal for GM/WM segmentation. I followed the recommendation of previous studies conducted at MARIARC, which revealed more satisfactory GM/WM segmentation results of the 1.5 T MPRAGE protocol when using FreeSurfer software. However, I compared ICVs from data obtained at 1.5 Tesla and 3 Tesla and found no significant results; therefore, statistical analyses were performed using only images obtained at the 3 Tesla scanner.

6.4.2 MEASUREMENT OF CROSS-SECTIONAL AREA OF CC REGIONS

The method of cross-sectional area measurement of CC regions is described in chapter 3, section 3.10, pages 80-83.

6.4.3 DTI DATA ANALYSIS

6.4.3.1 PRE-PROCESSING OF DTI DATA

Pre-processing of DTI data was performed as described in chapter 3, section 3.18.1, page 105.

6.4.3.2 TRACTOGRAPHY PARAMETERS

Tractography parameters are described in chapter 3, section 3.18.2, page 106.

6.4.3.3 FIBER TRACTOGRAPHY OF CC REGIONS

To constrain fiber tracts and to determine regional FA values, the midsagittal FA image was used. The CC was evenly divided into 4 quadrants along the antero-posterior axis. The same division scheme was followed in previous DTI studies (Shimony et al., 2006, Yu et al., 2007). The 4 quadrants were roughly corresponding to rostrum/genu/anterior body (CC1), anterior midbody (CC2), posterior midbody (CC3), and isthmus and splenium (CC4). Each region of the CC was traced by placing a seed region of interest (ROI) encompassing the corresponding quadrant (figure 6.1). After applying tractography, the identified fiber bundles were compared for four parameters: average FA, MD, normalized tract volume, and normalized number of streamlines. The FA values were calculated by averaging across all voxels in the tract. It should be emphasized that the reconstructed tracts represent streamlines based on tensor directions and not the real WM fibers (Matsumoto et al., 2008). Since we are at present not able to resolve fiber tracts at microscopic levels, DTI-based tractography is the only technique that allows identification of WM pathways in the living human brain. The calculated number of fibers represents the number of the reconstructed streamlines and the calculated tract volume represents the number of voxels that these streamlines traverse multiplied by the 2 mm^3 voxel size. Streamlines originating from different brain regions may converge along their pathway, so that twice the number of streamlines may not essentially represent twice the volume (number of voxels)(Reich et al., 2006).

6.4.4 INTRA- AND INTER-RATER RELIABILITY

6.4.4.1 MEASUREMENT OF CROSS-SECTIONAL AREAS OF CC REGIONS

Intra-rater reliability was analyzed by repeating CC measurements (cross-sectional areas of CC1-CC4) of all subjects by IA with at least 3 weeks between the two measurements. For inter-rater reliability, CC measurements were independently performed by two raters (IA and MA) for all subjects. An intra-class correlation coefficient (ICC) greater than 0.80 was accepted as evidence of inter- and intra-rater reliability.

6.4.4.2 FIBER TRACTOGRAPHY OF THE CC

All ROIs were placed by a single rater, IA who was blind to the identity, laterality, and grouping of subjects. Intra-rater study was performed by repeating DTI measurements by the same rater in all subjects at least three weeks after the first measurements. For inter-rater reliability, DTI measurements were independently performed by two raters (IA and MA) for all subjects. An ICC coefficient greater than 0.80 was accepted as evidence of inter- and intra-rater reliability.

6.4.5 STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS v. 16.0 and statistica program, v. 6.0 (Statsoft Inc., USA). Shapiro-Wilk (S-W) was used to test normality of data distribution. The values of the number of streamlines and tract volumes are likely to be affected by head size. Increased head size may result in increased number of streamlines and tract volume. I therefore followed the recommendations of previous studies (Ciccarelli et al., 2003, Correia et al., 2008, Tate et al., 2010) for normalization of tractography-derived metrics (tract volume and number of streamlines). I calculated the relative values of these metrics normalized by the ICV following the formula provided by (Correia et al., 2008). These metrics were divided by (V / V-), where V is the ICV of the subject and V- is the average ICV for the entire group (musicians and non-musicians). Cross-sectional area measurements of CC1-CC4 regions were also normalized by dividing the square root of each area by the cube root of the ICV (Smith, 2005, Phillips et al., 2007).

Using SPSS, multivariate analysis of variance (MANOVA) was performed with musicianship (musicians versus non-musicians) and gender (males versus females) as between subjects factors and CC1-CC4 measures: average FA, MD, normalized tract volume (NV), normalized number of streamlines (NS), and normalized cross-sectional areas (NA) as within subjects factors covarying for age; $p<0.05$ was considered significant after adjustment for multiple comparisons. Pearson's correlation was used to perform correlation analyses. Independent student *t* test was used to compare ICV measurements between groups. The ICC calculated by one-way random model was used to test the intra- and inter-rater reliability. A coefficient greater than 0.80 was accepted as evidence of inter- and intra-rater reliability. Microsoft Office Excel, 2007 software was used for creating figure 6.2.

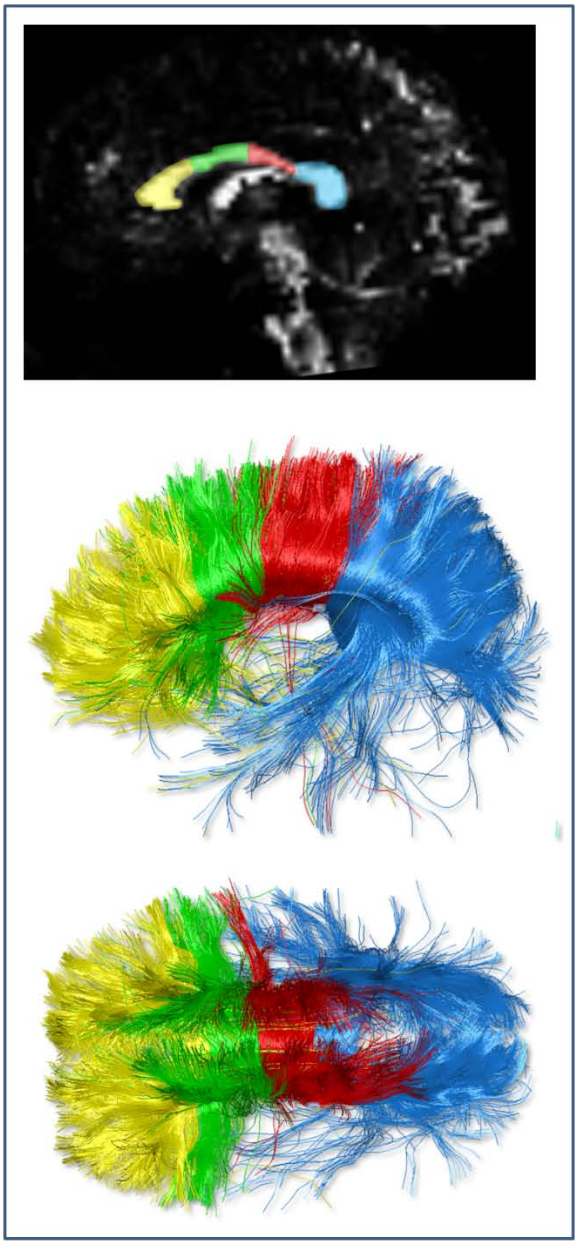

Fig.6.1. Regional tractography of the CC. Top, midsagittal FA map showing subdivisions of CC into regions; CC1 (yellow), CC2 (green), CC3 (red), and CC4 (blue). Middle, fiber tracking of CC1-CC4 regions depicted in colours (yellow, green, red and blue, respectively). Bottom, superior view of the reconstructed CC streamlines.

6.5 RESULTS

6.5.1 MEASUREMENT OF INTRACRANIAL VOLUME

Student *t* test revealed no significant difference in ICV between musicians and non-musicians (*p*=0.15).

6.5.2 FIBER TRACTOGRAPHY OF THE CC

Reconstruction of CC was feasible in all subjects (figures 6.3 and 6.4), in a manner consistent with the description of published DTI atlases (Jellison et al., 2004, Wakana et al., 2004, Salamon et al., 2007), which indicates the reliability of the applied tracking method.

6.5.3 COMPARISON OF REGIONAL MACRO- AND MICROSTRUCTURAL MEASURES OF THE CC

Compared to controls, professional musicians possessed greater average FA value (F=14.08, *p*=0.001), normalized tract volume (F=10.7, *p*=0.003), normalized number of streamlines (F= 12.5, *p*=0.002), and normalized cross-sectional area (F=12.7, *p*=0.001) in CC4 region. In addition, musicians showed lower MD values in CC4 region compared to controls (*p*=0.02). There was no significant group difference in any measure of CC1-CC3 regions, no significant effect of gender, and no significant gender by musicianship interaction. Means and standard deviations of macrostructural and microstructural CC measures are presented in table 6.2.

A correlation analysis was then performed between duration of musical performance/age of commencing musical training and CC4 metrics (cross-sectional area, average FA, MD, tract volume, and number of streamlines) after controlling for age. There was a significant negative correlation between age of commencing musical training and cross-sectional area of CC4 region (r = - 0.7, *p*=0.011) (figure 6.2). There was no significant correlation between age of commencing musical training and other CC4 measures (average FA, MD, tract volume, and number of streamlines). There was no significant correlation between duration of musical training and any of CC4 measures.

6.5.4 INTRA- AND INTER-RATER STUDY

A good agreement was revealed for cross-sectional area measurements with a range of 0.92-0.98 for intra-rater study and 0.90-0.96 for inter-rater study. For CC tractography, results showed good agreement with ICC ranging between 0.84-0.90 for intra-rater study and 0.82-0.88 for inter-rater study.

6.5.5 CORRELATION BETWEEN MACROSTRUCTURE (CROSS-SECTIONAL AREA) AND MICROSTRUCTURE (AVERAGE FA) ACROSS SUBJECTS

Correlation analyses were next performed between absolute measurements of cross-sectional areas of CC1-CC4 regions (uncorrected for ICV) and average FA values of CC1-CC4 tracts. There was a significant positive correlation between average FA and absolute cross-sectional area of CC4 region (r= 0.5, *p*=0.004) in all subjects (musicians and non-musicians). There were no significant correlations in CC1-CC3 regions.

Table 6.2. Means ± standard deviations of cross-sectional areas and tractography-derived metrics. Volumes are mm^3, areas are mm^2, and MD unit is mm^2 s^{-1} X 10^{-3}.

Measurement	**CC1**	**CC2**	**CC3**	**CC4**
Cross-sectional area				
Musicians	214±35	118±21	102±23	304±60
Controls	197±14.9	110±21	111±27	231±51
Normalized cross-sectional area				
Musicians	0.12±0.01	0.09±0.008	0.08±0.01	*0.15±0.01
Controls	0.12±0.008	0.09±0.01	0.08±0.01	0.12±0.02
Average FA				
Musicians	0.55±0.06	0.44±0.03	0.46±0.04	*0.60±0.06
Controls	0.59±0.06	0.50±0.04	0.40±0.05	0.52±0.07
MD				
Musicians	0.83±0.02	0.87±0.02	0.89±0.01	*0.73±0.01
Controls	0.84±0.02	0.88±0.02	0.86±0.02	0.80±0.02
Tract volume				
Musicians	111190±47322	70654±25656	155818±21842	197348±55000
Controls	94906±25526	65210±4906	54850±2166	141275±30350
Normalized tract volume				
Musicians	112130.8±50193	72058.1±29941	164027±23852	*912643±137827
Controls	96097±27707	65449±4869	55031±2062	772927±82888
Number of streamlines				
Musicians	2713.3±1336	1006.6±339	1073.5±249	3760.3±873
Controls	2059±538	1059±403	1064±256	2778±922
Normalized number of streamlines				
Musicians	2766±501	1021±368	1093±305	*3826±605
Controls	2087±402	1074±429	1070±256	2433±506

*Significant at $p<0.05$

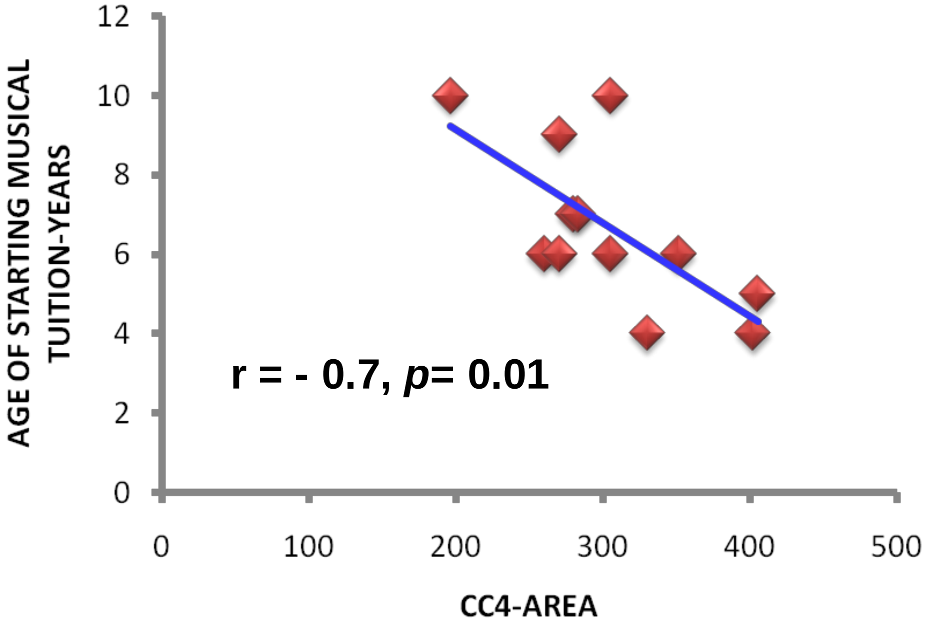

Fig.6.2. A graph showing correlation between age of starting musical training and cross-sectional area of CC4 region, areas are mm^2.

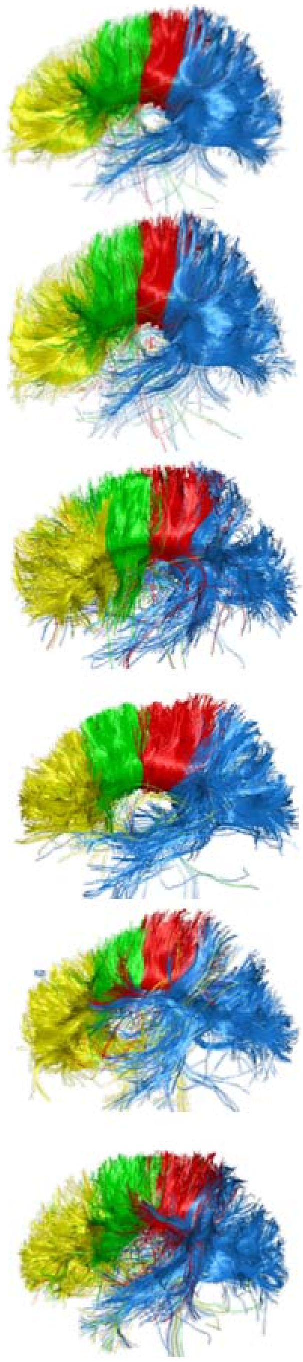

Fig.6.3. Illustration of regional CC tractography in 6 subjects.

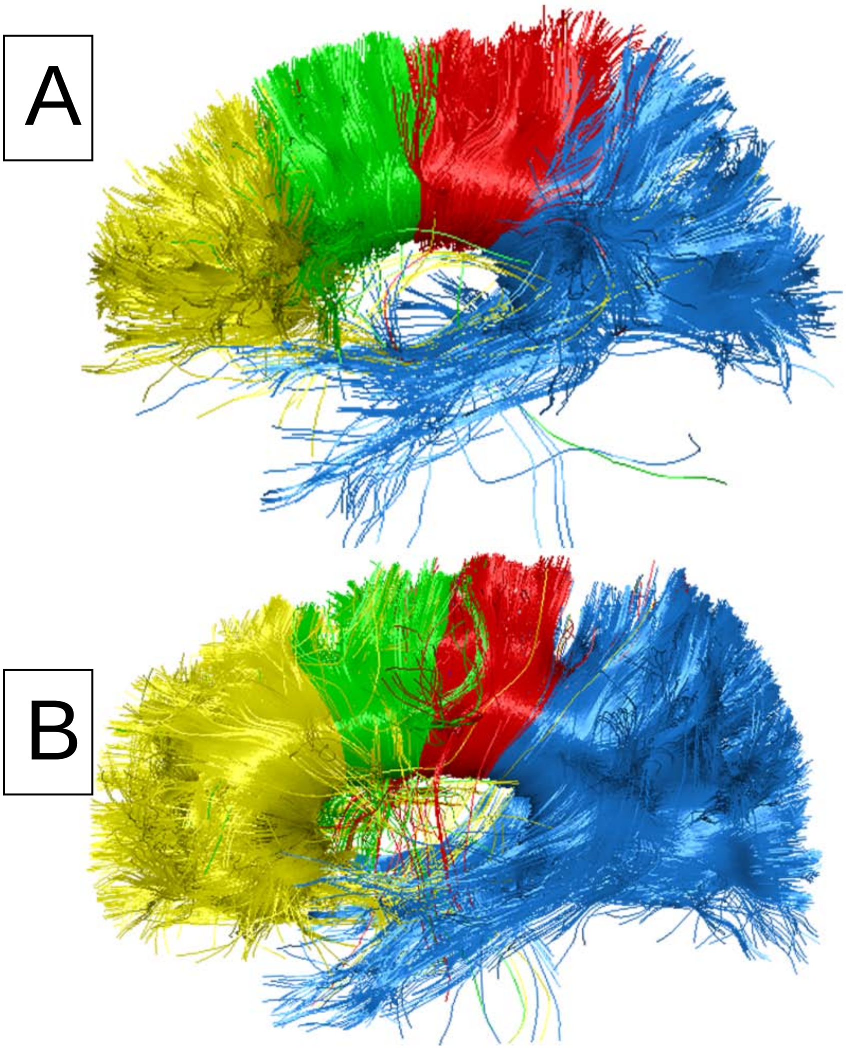

Fig.6.4. illustration of regional CC tractography in a non-musician, **A** and a musician, **B**.

6.6 DISCUSSION

6.6.1 MUSICIANS SHOWED EVIDENCE OF MACRO- AND MICROSTRUCTURAL PLASTICITY IN CC4 REGION

The present study was sought to extend previous CC studies in musicians through examining the macro- and microstructure of the CC. The combined use of structural MR and DTI techniques could provide a complementary approach to study the CC structure. Such approach has been used in several previous studies (Pfefferbaum and Sullivan, 2002, Schulte et al., 2004, Westerhausen et al., 2004, 2006a, Westerhausen et al., 2006b, Hasan et al., 2008a, Hasan et al., 2008b). Musicians revealed macrostructural changes (greater cross-sectional area) and microstructural changes (greater average FA, tract volume, number of streamlines, and lower MD) in CC4 region. There was a negative correlation between age of commencing musical tuition and cross-sectional area of CC4 region. This means that the earliest the musician commenced training the larger would be the size of CC4 region. These findings are in agreement with other reports (Schlaug et al., 1995, Bengtsson et al., 2005), which suggested greater malleability of CC structure in subjects who commenced musical training early in life. The increased cross-sectional area of CC4 region corroborates my previous findings in professional musicians in study 2 (chapter 5).

Regarding the CC microstructure, FA has become by far the most widely used measure of anisotropy in the DTI literature. It was proposed that FA could provide information about WM microstructure, such as intra-axonal organization (Kinoshita et al., 1999), fiber density, degree of myelination, neuroglial cell packing, individual fiber diameter, and immunohistochemical properties (Mori and Zhang, 2006, Sun et al., 2006, Trivedi et al., 2009), for review of literature see (Beaulieu, 2002). The FA is often considered an indicator of WM integrity (Alexander et al., 2007b), because of its increase during WM maturation (Eluvathingal et al., 2007), decrease during normal aging (Barrick et al., 2010), and in patients with neurodegenerative (Sundgren et al., 2004) and demyelinating (Filippi et al., 2001) disorders. Furthermore, the FA value reflects the directionality and coherence of fiber tracts (Basser and Pierpaoli, 1996). Since diffusion is mainly restricted by membranes and myelin sheaths that are oriented parallel to the long axis of the axons, the molecular displacement is stronger parallel to than perpendicular to the axon orientation. If the axons within a voxel are highly aligned, the diffusion in this voxel is directional (anisotropic). Thus, higher FA in CC4 region in musicians would reflect more parallel alignment of callosal axons in this region. However, measurement of FA may not be enough to characterize tissue changes. For example, an increase in FA values may result from decreased radial (perpendicular) diffusivity, increased axial (parallel) diffusivity, or both. Measurements of the MD may thus help to better understand how the diffusion tensor is changing. Results of the present study showed that musicians had lower MD values in CC4 region compared to non-musicians. A lower MD value reflects stronger attenuation of diffusion by the surrounding cellular components, particularly the axonal membranes and myelin sheaths (Beaulieu, 2002, Iacconi et al., 2010), which may thus indicate larger number of axons (more membrane) and/or thicker myelination (more myelin material) in musicians. The latter assumption may be supported by my findings of increased tract volume and number of streamlines in CC4 region. Taken together, higher FA and lower MD values in CC4 region in musicians could reflect increased WM coherence, fiber density, and thicker myelination in this region.

My results also showed that musicians had greater tract volume and number of streamlines in CC4 region. These data may provide complementary evidence to FA and MD measures. Before discussing the implications and interpretation of these findings, it is important to note that the reconstructed tracts do not represent actual callosal fibers, but rather streamlines based on mathematically derived tensor directions (Matsumoto et al., 2008). I investigated CC subregions by assessing tract volume and number of the reconstructed streamlines, which could quantify the microscopic architecture of the WM (Mori and Zhang, 2006). A similar approach has been used in previous studies (Nucifora et al., 2005, Thomas et al., 2005, Vernooij et al., 2007, Matsumoto et al., 2008, Schlaug et al., 2009, Liu et al., 2010a). These parameters might provide a different perspective for structural plasticity of CC in musicians, which was not provided by previous neuroimaging studies. A number of factors might contribute to the observed changes in tract volumes and number of streamlines in the present study, including increased myelination, increased axonal projections and collaterals, and increased fiber density due to new sprouting. It is possible that remodeling of trans-splenial fibers was triggered by the need for stronger and more effective inter-hemispheric communications. However, I could only provide hypotheses at this stage, and more studies are needed in the future to understand the exact correlation between tractography-derived quantitative metrics and microscopic anatomy of the nervous tissue.

The isthmus and splenium (CC4) contain fibers from parietal, temporal, and occipital regions (Hofer and Frahm, 2006, Park et al., 2008). Most of the fibers traversing these regions connect somatosensory, superior parietal, auditory, and visual cortices. The isthmus part of the CC (the smallest part of CC4 region) contains fibers from the primary somatosensory cortex. Compared to non-musicians, musicians have greater sensory load imposed by their complex skilful performance. In accord, previous studies have shown evidence of use-dependent structural-functional adaptation in the primary somatosensory cortex in professional musicians, such as increased GM density in the left primary somatosensory cortex in right-handed pianists and increased cortical representation of the digits (second to fifth) of the left hand in string players. These findings are compatible with increased size of the isthmus reported here. The splenium (the largest part of CC4 region) contains fibers from auditory and parietal cortical regions. Musicians have well established evidence of structural-functional plasticity in the auditory domain; examples include increased size and GM density of the Heschl gyrus (Schneider et al., 2002, Bermudez et al., 2009), increased cortical thickness of the right and left planum temporale (Bermudez et al., 2009), increased auditory cortical representation of piano tones (Pantev et al., 1998), increased multimodal auditory-somatosensory (Schulz et al., 2003) and auditory-motor integration (Bangert and Altenmuller, 2003, Lotze et al., 2003, Bangert et al., 2006, Baumann et al., 2007), and enhanced timbre specificity (McAdams et al., 1995, Shahin et al., 2008). Furthermore, previous callosal studies have assigned a substantial role for the splenium in relaying visuospatial cognitive information (Schmitt et al., 2001, Tomaiuolo et al., 2002, Pollmann et al., 2004, Fryer et al., 2008). Also, microstructural measures of the splenium, such as FA and relative anisotropy (RA) were found to be related to reading skills and phonological awareness (Frye et al., 2008). Musicians are well-known for their enhanced visuospatial performance (Sergent et al., 1992, Sluming et al., 2002, Sluming et al., 2007). When practicing music, they need to read musical notations and translate them into movement patterns, a process known as sight reading (Sergent et al., 1992). Music reading can thus be considered as a visuospatial cognitive task in which vertically oriented stimuli are mapped onto the fingers, which strike horizontally oriented keyboard elements as in pianists (Stewart et al., 2003). This visuospatial ability is essential to all musicians irrespective of instrument specialty, hence of relevance to the multi-instrumental cohort examined in the present study. Furthermore, FA value of the splenium was shown to be directly related to the speed of bimanual coordination (Muetzel et al., 2008) and enhanced attention to visual stimuli (Madden et al., 2004). It could thus be hypothesized that increased macrostructure and microstructure of the CC4 region in musicians represent structural adaptation imposed by intensive multimodal skill acquisition. I propose that these plastic changes would support functions crucial for musical performance, such as auditory processing, visuospatial cognition, and bimanual coordination. For the CC1-CC3 regions, no significant group differences were detected, which might indicate that these regions are also implicated in a number of non-musical tasks that were practiced by control subjects (Bengtsson et al., 2005).

In contrast to findings of the present study, Imfled and colleagues (Imfeld et al., 2009) investigated microstructural plasticity in the CC using probabilistic tractography and reported no significant group differences in FA and MD values of the anterior and posterior halves of the CC. The discrepancy in obtained results could be attributed to differences in musical and experiential histories of participants studied, different scanning parameters, and different tracking algorithms (deterministic versus probabilistic). In contrast to the study of Imfeld and colleagues (Imfeld et al., 2009) which divided the CC into 2 halves, tractography was performed in the present study after dividing the CC into 4 regions, which might provide a more sensitive way for detecting regional callosal differences between groups.

The DTI results reproduce findings from other studies (Pfefferbaum et al., 2000, Sullivan et al., 2001, Chepuri et al., 2002, Westerhausen et al., 2004, Hasan et al., 2008b, Barrick et al., 2010, Liu et al., 2010a), which reported that diffusion anisotropy is higher in the posterior than anterior CC regions. Possible explanations for these findings include higher proportion of myelin material per voxel (Lamantia and Rakic, 1990), thicker myelin sheaths (Miller et al., 1999), and greater coherence of posterior CC fibers (Pfefferbaum et al., 2000).

The fact that gross measures of the CC may not reflect fiber density (Lamantia and Rakic, 1990) highlights the significance of combining macro- and microstructural measures of the CC. In the present study, the increased size of CC4 region in musicians was associated with increased FA, tract volume, number of streamlines, and reduced MD in the same region. This macro-/microstructural correlation in CC4 region may add further support to the assumption that larger CC4 size reported in the present study, and in study 2, represent structural plasticity induced by musical practice and not the result of increased inter-fiber distance (Tomasch, 1954). Postmortem histological studies have reported that the relation between total fiber number and CC size is dependent on regional distribution of small axons (0.4-1 µm in diameter) (Aboitiz et al., 1992, Highley et al., 1999). These small axons are most abundant in the splenium (Aboitiz et al., 1992), which may support the finding of significant macro-/microstructural correlation within this callosal region. Such correlation between CC size and anisotropy has been shown in previous studies (Alexander et al., 2007a, Hasan et al., 2008a, Rotarska-Jagiela et al., 2008). However, other studies reported contradicting results. For instance, a negative correlation was reported between relative anisotropy and posterior CC size in right-handed subjects (Westerhausen et al., 2004) and significant microstructural findings were reported in the absence of macrostructural ones (Pfefferbaum and Sullivan, 2002, Westerhausen et al., 2006a). It was postulated that FA can be used to visualize and quantitatively characterize WM development even before the onset of myelination, which was attributed to several mechanisms, such as ion fluxes across the axolemma (Wimberger et al., 1995, Prayer et al., 2001) and proliferation of immature oligodendrocytes (Drobyshevsky et al., 2005). It is thus possible that microstructural changes may exist long before being apparent at the macroanatomical level thereby causing macro/microstructural discrepancy.

6.6.2 LACK OF SIGNIFICANT GENDER EFFECT ON MACRO- AND MICROSTRUCTURE OF THE CC

In the present study, there was no gender effect on CC structure and no gender by musicianship interaction. These results contrast those of (Lee et al., 2003) who reported significant gender by musicianship effect as only male musicians showed increased CC size. Failure to achieve similar results might in part be related to the small number of female subjects recruited. Gender might be one of the most extensively studied factors in relation to callosal morphology. Postmortem examination of the cross-sectional area of the CC in 161 brains revealed greater absolute CC size in males (Matano and Nakano, 1998); however, when corrected for midsagittal brain area, there was no gender difference. Other groups reported larger total CC size and larger splenium size in females (Holloway and de Lacoste, 1986). Also, comparison of fiber composition of 9 callosal regions between 10 males and 10 females revealed no significant gender difference (Aboitiz et al., 1992). Conventional MR studies revealed similar conflicting results. Pozzilli and colleagues (Pozzilli et al., 1994) examined 130 subjects and reported no gender effect on CC size, while others reported increased relative CC size in females (Mitchell et al., 2003). With the development of DTI, many studies have examined the effect of gender on CC microstructure. Some authors reported that males had greater FA values (Menzler et al., 2011), while others failed to observe gender differences (Sullivan et al., 2001). Different methods of examination (postmortem, conventional MRI, or DTI), diverse image acquisition and processing techniques could account for variable results across studies.

6.6.3 TECHNICAL LIMITATIONS OF THE PRESENT STUDY

DTI-based tractography offers promising perspective to examine WM tracts *in vivo*. Previous studies have indicated that this technique frequently generates results that are plausible and apparently accurate (Jellison et al., 2004, Wakana et al., 2004, Catani et al., 2005). On the other hand, this technique suffers from several types of limitations. First, DTI scans are usually acquired with lower resolution than conventional MR scans, which may lead to substantial partial volume effects, such as errors related to cerebrospinal fluid contamination and averaging of fibers with different orientations within a voxel (Jones and Cercignani, 2010). Second, there are limitations related to reduced ability to track through zones of low signal to noise ratio (SNR), low anisotropy (especially below the stopping threshold), and crossing fibers (Pierpaoli et al., 2001, Alexander et al., 2007b). Third, tracking limitations related to eddy current distortions, subject motion, and magnetic susceptibility (Jones and Cercignani, 2010). Fourth, there are errors related to inaccurate determination of the principal eigenvector orientation (Jones, 2003) and difficulty in following tract bifurcations (Mori and van Zijl, 2002). Fifth, histological studies that can determine the exact correlation between DTI-derived metrics and histological parameters of the human WM are still limited (Concha et al., 2010). Finally, techniques for validating tractography algorithms are still in their infancy and difficult to apply (Behrens et al., 2003). Thus, DTI-based tractography may be reasonably regarded as a technique with a finite rate of false-negative and false-positive outcomes. Below, methodological limitations relevant to the present study are addressed.

6.6.3.1 ISSUE OF CROSSING FIBERS

A primary limitation of the currently used tractography algorithm arises from partial volume averaging of complex fiber architecture (such as crossing fibers) into single voxels (Tuch et al., 2002). Since the voxel size in the present study was 2 X 2 X 2 mm, some WM voxels can easily contain multiple fiber populations with different orientations. The assumption that the largest diffusion axis corresponds to the fiber orientation is not true if there are two or more fiber populations (Mori and van Zijl, 2002). Thus, algorithms based upon the principal eigenvector, such as that used in the current study, are unable to resolve regions of crossing WM pathways. Such fiber crossing problem may result in failure to detect the primary diffusion direction and lead to increased uncertainty in fiber tracking. The presence of crossing fiber bundles can average out the diffusion anisotropy to such a degree that it may seem isotropic (Mori et al., 2002, Bammer et al., 2003, Mori and Zhang, 2006). The immediate consequence for fiber tracking in this case is either a false-positive directional selection for a continuation of the tracking algorithm or a complete termination of fiber tracking by reaching the lower anisotropy threshold. When performing tractography of the CC, tracking of fibers directed toward the lateral and inferior side of the brain has limited reliability because of such fiber crossing problem (Hofer and Frahm, 2006, Park et al., 2008). For example, in the lateral side of the brain, the CC is susceptible to the crossing fiber problem at the centrum semiovale (Okada et al., 2006), where a small number of callosal fibers intersect a large number of corona radiata and corticospinal fibers. In the present study, this was evident in the lack of lateral projections present in the reconstruction of the CC and in the sparseness of fibers in certain regions (e.g. the superior parietal region, see figure 6.1). Tractography results of the present study were therefore more reliable in the medial projections of the CC. Likewise, tracking of callosal fibers that connect temporal lobes (running inferolaterally) had limited reliability as these fibers cross various directional tracts, such as the internal capsule and inferior longitudinal fascicles (Park et al., 2008, Chao et al., 2009). Barrick and colleagues (Barrick and Clark, 2004) developed an automated procedure to detect points (singularities), such as that occur where there is fiber crossing, in the diffusion tensor field that can adversely affect the direction of maximum diffusion thereby leading to errors in fiber tracking. Authors demonstrated that such fiber crossing singularities could erroneously lead to termination of fibers running from the CC to the lateral frontal lobe. Several diffusion imaging methods have been developed to address the problem of crossing WM regions, such as diffusion spectrum imaging (Wedeen et al., 2005), q ball imaging (Tuch et al., 2003), high angular diffusion imaging (Frank, 2002), and combined hindered and restricted diffusion (Assaf and Basser, 2005). These methods call for higher diffusion-weighting (typically 3,000–15,000 s/mm^2) and take much more time to acquire (Alexander et al., 2007b). Furthermore, the use of tractography algorithms to deal with the problem of crossing fibers is not simple due to noise propagation into the diffusion profiles and the possibility of revealing false-positive crossings (Barrick et al., 2007). The relatively long acquisition time of these algorithms, as well as the increased complexity of data analysis may limit their extensive use in research. Despite the above-mentioned crossing fiber problem, DTI-based tractography algorithms were shown to accurately reconstruct the CC without using methodology to resolve fiber crossing (e.g. (Shimony et al., 2006, Yu et al., 2007)).

6.6.3.2 SENSITIVITY TO SUBJECT MOTION

Diffusion-weighted images are proposed to detect the microscopic diffusion of water, which is about 5–10 mm during the measurement time, but they are also susceptible to macroscopic movements, such as bulk subject motion, eye movements, cardiac pulsation, and pulsation of the cerebrospinal fluid (Basser and Jones, 2002). Subject motion during the DTI acquisition can induce additional dephasing of the magnetization thereby increasing diffusion-weighted signal attenuation. This leads to overestimation of the ADC and introduces bias to anisotropy measures (Mukherjee et al., 2008). The bias produced by subject motion fluctuates according to the orientation of fibers. The effect of subject motion will be less marked if the fiber orientation aligns with the axis of rotation. For instance, if a subject flexes his/her head (i.e. moves his/her head towards the chest), the axis of rotation will be along the left-right direction. In this case, horizontally oriented midline CC fibers will be least affected, while corticospinal fibers oriented perpendicular to the axis of rotation will be mostly biased (Jones and Cercignani, 2010).

Although the diffusion-weighted sequence used in the present study was relatively long (22 minutes), the datasets had qualitatively minimal motion artifacts. Prior to DTI examination, all subjects were given detailed explanation about the nature of the DTI scan and were asked to keep body motion to a minimum. Inside the scanner, motion was controlled with the use of head cushions provided by the scanner manufacturer. During the scan session, subjects received repeated reminders to remain motionless as much as they can. I found that these methods worked well for most cases. Acquired images were then corrected for motion artifacts using 12-parameter affine registration to the *b* = 0 image (Smith, 2002, Reese et al., 2003). Post-corrected images were visually inspected to verify the reliability of correction procedure and to screen for excessive motion artifacts. In two subjects, motion artifacts were sufficiently high, so subjects were asked to repeat the diffusion-weighted acquisition.

6.6.3.3 EFFECT OF NOISE AND LOW SIGNAL-TO-NOISE RATIO ON DTI

Unfortunately, diffusion-weighted imaging is particularly susceptible to the effect of noise and poor SNR values, which can adversely affect quantitation of DTI parameters. The effect of noise on DTI was originally described by (Pierpaoli and Basser, 1996). The presence of noise in the diffusion-weighted signals can produce significant bias in anisotropy measurements through making isotropic media appear anisotropic. The noisier the signals, the greater the inconsistency in eigenvalue measurements. However, noise effect is less distinct in regions with high anisotropy (Jones and Cercignani, 2010). It was suggested that the SNR of the non-diffusion-weighted images of a DTI acquisition should be at least 20 to obtain reliable anisotropy measures (Mukherjee et al., 2008). In the case of low SNR, weak diffusion-weighted signals that approach the background noise level tend to be overestimated by noise, which in turn results in the underestimation of diffusivity (Dietrich et al., 2001). In anisotropic structures, this effect is orientationally dependent. Since the diffusivity is greater along some orientations than others, the underestimation of diffusivity will appear along certain number of axes leading to a negative bias in anisotropy (Jones and Basser, 2004). Furthermore, at low SNR, coregistration of diffusion-weighted images to the *b* = 0 image to correct for subject motion and eddy current distortions is challenging (Mukherjee et al., 2008). The noise effect can be ameliorated by using techniques that average tensor information among adjacent voxels, although such approach results in the reduction of effective resolution (Mori et al., 2002).

In DTI-based tractography, noise can have a deleterious effect on the accuracy of fiber tracking as the calculated vector direction may diverge from the actual fiber orientation (Mori et al., 2002). In other words, the presence of noise can lead to uncertainty associated with every estimate of fiber orientation (Jones, 2003). These uncertainties tend to accumulate along the length of the trajectory path resulting in erroneous tracking results: trajectories might execute false paths leading to identification of spurious tracts with no actual anatomic existence (Yamada et al., 2008), and may even terminate erroneously (Liu et al., 2010b). The degree of these errors was shown to depend on the shape of the trajectory, resolution, and anisotropy (Basser et al., 2000). Several techniques were proposed to graphically depict the uncertainty in the orientation of the diffusion tensor field (Behrens et al., 2003, Jones, 2003, Jeong and Anderson, 2008). Although these tools are useful in checking the reliability of fiber tracking results, their complex nature is currently limiting their use.

A common consensus in DTI studies is that acquiring large number of diffusion-encoding directions would reduce the orientational dependence and increase the accuracy and precision of DTI measures (Mukherjee et al., 2008). At least 30 directions were shown to be necessary for accurate estimation of the principal eigenvector and MD (Jones, 2004). Since the diffusion-weighted acquisition used in this study involved acquiring 60 diffusion-encoding directions, a robust estimation of the diffusion tensor is expected. Taken together, the above evidence indicates that it is critical to acquire DTI data with adequate SNR. Although computation of SNR was not performed, the present study benefited from two factors known to improve SNR namely, long scan time and high field strength (3 Tesla) (Murphy et al., 2007, Tijssen et al., 2009). Tractography results were consistent with previously published DTI atlases (Jellison et al., 2004) and were faithful to the classical anatomical description (Williams et al., 1995, Snell, 2001, Kiernan, 2005).

6.6.3.4 ISSUE OF MAGNETIC SUSCEPTIBILITY EFFECTS

The presence of multiple tissue components (e.g. tissue, air, and bone), which have different magnetic susceptibility, can produce local alterations in the magnetic field B_0 leading to large discontinuities in bulk magnetic susceptibility at tissue-air interfaces. In the brain, magnetic susceptibility artifacts are particularly common in regions adjacent to the paranasal air sinuses, such as the temporal lobes (Basser and Jones, 2002). This discontinuity in susceptibility results in two adverse effects in diffusion-weighted imaging: first, the additional local gradients operate like diffusion gradients inducing spatial variations in the *b*-matrix (Basser and Jones, 2002); second, it produces geometric distortions in the acquired images (Jones and Cercignani, 2010). Theoretically, images of the present study were vulnerable to magnetic field inhomogeneities, which are known to be proportional to the magnetic field strength (3 Tesla). Therefore, all acquired images were visually inspected for magnetic susceptibility effects prior to inclusion in the group analysis. Future studies could benefit from the use of strategies proposed to correct magnetic susceptibility artifacts, such as measuring the B_0 field and implementing a retrospective correction scheme to the distorted data (Jezzard and Balaban, 1995, Reber et al., 1998).

6.6.3.5 ISSUE OF EDDY CURRENTS

Diffusion-weighted image acquisitions using single shot echo-planar imaging sequence are susceptible to distortions caused by eddy currents. Eddy currents are electric currents induced in the electrically conductive structures of the MRI scanner by rapid switching of the large diffusion sensitization gradients. These eddy currents generate unwanted, rapidly and slowly decaying local magnetic fields (Basser and Jones, 2002, Embleton et al., 2010). Eddy current-induced distortions vary with the strength and direction of the diffusion encoding gradients. Two adverse effects result from these currents: first, the field gradient at the sample diverges from the prescribed field gradient, which leads to discrepancy between the actual and prescribed *b*-matrix; second, the resulted diffusion-weighted images will suffer from geometric distortions. Uncorrected eddy current distortions can thus lead to erroneous fiber-tracking results (Basser and Jones, 2002). In the present study, eddy currents were anticipated owing to the long scan time and were thus corrected using 12-parameter affine registration to the $b = 0$ image (Smith, 2002, Reese et al., 2003). Post-corrected images were inspected to verify the reliability of correction procedure. In effect, tractography results obtained in this study were faithful to the classical descriptions of neuroanatomy that have previously been documented (e.g. (Hofer and Frahm, 2006)).

6.6.3.6 ANALYSIS OF DTI DATA

Several techniques were proposed to perform quantitative analysis of DTI data, including manual ROI-based analysis, voxel-based analysis, and tract-based spatial statistics. However, there is no consensus on the ideal way to perform DTI data analysis. The ROI-based analysis technique applied in the present study has been widely used in the DTI literature. However, this technique has intra-rater and inter-rater variability and is time consuming. A further limitation of this technique is the possibility of errors related to including other fiber tracts, or even GM and cerebrospinal fluid, along with WM structures of interest (Snook et al., 2007). In ROI-based analysis, the anatomical level at which seed points are applied could significantly influence tracking results (Hattingen et al., 2009). If the orientation of a section is slightly oblique, then seed points would be placed at a different anatomical level even if the ROI is chosen carefully. In this case, the seed points are probably placed in a subvoxel coordinate leading to imprecise tracking results.

Regarding CC tractography, unreliable results could be produced when ROIs are placed on a plane other than the midsagittal one. To avoid this bias in the present study, ROIs were placed on midsagittal slices, which were carefully identified according to specific anatomical landmarks (Ozturk et al., 2002), and consistently applied across all subjects. However, there was difficulty in outlining the boundaries of the CC due to limited resolution of the diffusion-weighted data, particularly in the body of the CC. In addition, as there was no line of separation between the ventral border of the CC and the fornix, it was not possible to separate these structures. In all these cases, the resulted callosal tracts were checked for stray streamlines and those related to other tracts. In such conditions, manual editing to trim away the portions of streamlines that stray from the expected route was performed using the cropping box tool of MedINRIA software. While I acknowledge that the above-mentioned limitations have affected the results, it should be noted that they do so equally in both musicians and non-musicians groups. Since the same fiber tracking parameters (such as placement of ROI, FA threshold, minimum fiber length, and smoothness factor) were used for all subjects, inter-group comparison was still possible and results of intra-and inter-rater reliability were acceptable.

Some authors used VBM technique to obtain quantitative DTI metrics, which has the advantage of being observer independent and can be applied to study the whole brain (Han et al., 2009). However, VBM also has some inherent shortcomings because of the requirements for spatial normalization and smoothing (Ashburner and Friston, 2000). Inter-individual differences in brain size and shape and in WM anatomy may limit adequate coregistration of DTI data across subjects. The tract-based spatial statistics (TBSS) technique is another automated method that can be used to detect group-wise changes in FA concurrently throughout the WM regions of the brain (Smith et al., 2006). This technique has been widely used due to its availability and ease of use, however, the TBSS method also requires accurate registration of subjects' data.

6.6.3.7 INTERPRETATION OF TRACTOGRAPHY RESULTS

A considerable care should be taken when interpreting quantitative DTI data. First, since microscopic axonal information of several compartments is averaged within a voxel, it should be emphasized that DTI cannot provide direct information on axonal connectivity at cellular level (Bammer et al., 2003). Second, the exact significance of DTI-derived metrics, such as FA, MD, tract volume, and number of streamlines measures has not yet been resolved. For instance, the calculated number of the reconstructed streamlines does not represent fiber counts (Reich et al., 2006). Histological studies are thus required to determine the exact correlation between DTI-derived metrics and histological parameters of the human WM. Such correlation has mostly been addressed in the postmortem state (Schmierer et al., 2007, Saksena et al., 2008), which has several shortcomings common to postmortem studies, including vulnerability of tissues to shrinkage, deformation, and damage during fixation (D'Arceuil et al., 2007). In addition, these studies were focused on specific tracts (Concha et al., 2010) and cannot be generalized. Third, there are certain parameters that should be taken into consideration when performing statistical data analysis in DTI studies. For example, the body mass index has been shown to correlate with diffusion anisotropy measures of the CC (Xu et al., 2011). Since calculating the body mass index requires measuring subjects' weight and height, which were not taken in the present study, it was not possible to determine the potential effect of the body mass index on anisotropy measures of the CC.

6.6.3.8 COMPARISON WITH PREVIOUS STUDIES

It was challenging to compare my tractography results with those derived from studies that used different tracking algorithms, such as probabilistic tractography (Imfeld et al., 2009), or studies that used automated voxel-based analysis method (Han et al., 2009). In addition, comparing data obtained at different field strengths is not straightforward. For instance, it was shown that working at 3 Tesla magnetic field results in enhanced visualization of a number of WM tracts (Okada et al., 2006) compared to 1.5 Tesla field. Another important factor that needs to be considered when comparing DTI results across studies is the type of image analysis software used for fiber tracking. Burgel and colleagues (Burgel et al., 2009) applied four different software algorithms (three of them were based on fiber assignment by continuous tracking) on a single data set to depict the corticospinal tract. The use of different software algorithms resulted in substantial inconsistency in 3D depiction of the CST. Interestingly, a recent study reported great discrepancies in tracking results when using different personal computers despite running the same tracking algorithm and selecting identical tractography parameters (Chung et al., 2011). Comparison of reported tractography results from studies with differences in methodology would therefore call for great attention.

6.6.3.9 VALIDATION OF DTI-BASED TRACTOGRAPHY

This issue is discussed in chapter 3, section 3.17, page 104.

6.7 CONCLUSION

In conclusion, the present study was the first to combine conventional MR and DTI to study macro- and microstructure of the CC in musicians. Results showed evidence of structural plasticity in the isthmus and splenium in musicians, which was negatively correlated with age of commencing musical tuition. The combined use of structural MRI and DTI techniques is the best approach when examining the CC in musicians. I hypothesize that the observed structural changes represent training-induced plasticity essential for enabling high level musical performance. It is possible that increased size, average FA, tract volume, and number of streamlines in the isthmus and splenium would support enhanced inter-hemispheric communication between parietal, temporal, and occipital lobes in musicians.

CHAPTER 7

INVESTIGATING STRUCTURAL PLASTICITY OF CEREBELLAR WHITE MATTER IN MUSICIANS

PLASTICITY OF THE SUPERIOR AND MIDDLE CEREBELLAR PEDUNCLES IN MUSICIANS REVEALED BY QUANTITATIVE ANALYSIS OF VOLUME AND NUMBER OF STREAMLINES BASED ON DIFFUSION TENSOR TRACTOGRAPHY

7.1 ABSTRACT

BACKGROUND AND PURPOSE To study plasticity of the superior (SCP) and middle (MCP) cerebellar peduncles in musicians. The cerebellum is well known to support several musically relevant motor, sensory, and cognitive functions. Previous studies reported increased cerebellar volume and gray matter (GM) density in musicians. Here, I report on plasticity of the white matter (WM) of the cerebellum.

SUBJECTS AND METHODS The cohort included 10/10 gender and handedness-matched musicians and non-musicians. Using diffusion tensor imaging (DTI), fiber tractography of the SCP and MCP was performed. The average fractional anisotropy (FA), tract volume, and number of streamlines of SCP/MCP were compared between groups. Automatic measurements of GM and WM volumes of right/left cerebellar hemispheres were also compared.

RESULTS Musicians had significantly increased right SCP tract volume (p=0.02) and number of streamlines (p=0.001), right MCP tract volume (p=0.004), and total WM volume of the right cerebellum (p=0.003). There were no significant differences in right MCP number of streamlines, left SCP/MCP tract volume and number of streamlines, SCP/MCP FA values, GM volume of the right cerebellum, and GM/WM volumes of the left cerebellum.

CONCLUSION I propose that increased tract volume and number of streamlines of the right cerebellar peduncles represent use-dependent structural adaptation essential to support enhanced sensorimotor and cognitive functional demands on the musician's cerebellum.

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7.2 INTRODUCTION

Musician's brain has excited the scientific community as it represents an ideal model to study neuroplasticity (for review see (Munte et al., 2002)). Musicians contrast with subjects with other professions in two major aspects. First, they begin their training at an earlier age when many brain regions have not gained optimum maturity, hence vulnerable to plastic changes imposed by a variety of sensorimotor stimuli (Watanabe et al., 2007). Second, to master their profession, they must practice for several hours per day even outside working hours. Taken together, this means that musicians have an intensive sensorimotor skill acquisition sufficient to impose structural plastic changes in different brain regions. In this context, there has been growing neuroimaging literature suggesting the presence of structural neuroplasticity in musicians' brains in structures known to have particular relevance for enabling musical performance (Sluming et al., 2002, Schneider et al., 2005, Abdul-Kareem et al., 2011).

Although the cerebellum comprises only one-tenth of the whole-brain volume, the number of cells in the human cerebellar cortex exceeds that in the cerebral cortex four-fold (Andersen et al., 1992). The MCP is the largest cerebellar peduncle and it is the major input pathway to the cerebellum. It is composed of afferent neural fibers that project from sensorimotor areas of the cerebral cortex to the pontine nuclei and subsequently cross on the pons to the contralateral cerebellum. Efferent fibers from the cerebellum arise primarily from cells in the dentate nucleus but also from emboliform and globose nuclei. These deeply seated cerebellar nuclei receive fibers from the cerebellar cortical purkinje cells. The dentate nucleus sends fibers through the SCP, which is the major output pathway from the cerebellum to the contralateral motor areas of the cerebral cortex. Two major pathways pass through this peduncle, the dentato-rubro-thalamo-cortical (dentate nucleus-red nucleus-thalamus-premotor cortex) and cerebello-thalamo-cortical (cerebellum-thalamus-premotor cortex). While there are some afferent fibers from the anterior spinocerebellar tract that are conveyed to the anterior cerebellar lobe via SCP, most of the fibers within this peduncle are efferents (Williams et al., 1995). Thus, it could be seen that MCP and SCP complete a circuit between the cerebral and cerebellar cortices. The main function of this circuit was suggested to coordinate motor output of the cerebral cortex (Snell, 2001).

Retrograde tracing of substances injected into the cerebral cortex of mammals had revealed wide connections of the cerebellum. In monkeys for example, about half of the cerebral cortex projects into the pontine nuclei (Glickstein et al., 1985). The densest areas of cells projecting from the cerebral cortex to the pontine nuclei are from the primary motor and premotor areas. There are also dense projections to the pons from other regions, such as the extrastriate visual areas essential for visuomotor integration (Glickstein and Doron, 2008), the frontal eye field, and the prefrontal cortex. Cerebellar efferents from the dentate nucleus were shown to project not only to motor areas but also to the prefrontal cortex (Middleton and Strick, 2001).

The cerebellum primarily supports coordination of motor movement (Snell, 2001). This function is obviously of extreme importance to musicians irrespective of their instrumental specialty. Owing to its wide connections with the cerebral cortex, it has been shown that the cerebellum also subserves other functions, which might be of relevance to musical performance, such as cognition, sensory discrimination, and memory retention (Kim et al., 1994, Gao et al., 1996, Paradiso et al., 1997, Parsons et al., 1997, Doyon et al., 2002, Matsumura et al., 2004, Stoodley et al., 2010). In this context, structural studies in musicians reported that male pianists had increased cerebellar volumes compared to controls (Schlaug et al., 1998, Hutchinson et al., 2003), which was positively correlated with the intensity of musical performance (practice time per day and across lifetime)(Schlaug et al., 1998, Hutchinson et al., 2003), and negatively correlated with age of starting instrument lessons (Schlaug et al., 1998). However, authors neither specified whether the observed difference is due to greater GM or WM volume nor did they examined plasticity in each cerebellar hemisphere. In a voxel-based morphometric (VBM) study (Gaser and Schlaug, 2003) that examined 20 right-handed professional musicians, 20 amateur musicians, and 40 non-musicians, it was reported that professional musicians had increased GM density in the left cerebellum in addition to several other brain regions compared to both amateurs and non-musicians. This study could not however elicit significant findings in the cerebellar

WM, which might in part be related to limitations in the VBM technique itself as it might be insensitive to WM differences caused by low-intensity contrast differences between groups (Ashburner and Friston, 2000, Della Nave et al., 2008), hence the rationale of using a relatively more sensitive method for examining the WM, such as DTI. A more recent study that combined VBM and DTI to examine a cohort of 18 right-handed pianists and 21 right-handed controls (Han et al., 2009) reported increased GM density in the right cerebellum with no significant results detected in cerebellar WM.

With the exception of the study of Hutchison et al., 2003 (Hutchinson et al., 2003), which specifically examined the cerebellum, structural changes in musicians' cerebella were always reported roughly as additional findings. While the focus of mainstream imaging-based neuropsychological research continues to be on function and anatomy of GM, the *in vivo* neuroimaging technique of DTI-based tractography offers a complementary way of studying the brain by directing interest towards WM and axonal connectivity. With the development of this technique, structural plasticity of WM has received considerable interest both in basic and clinical research (Rose et al., 2000, Roberts et al., 2005, Jones et al., 2006, Yu et al., 2007). Previous DTI studies in musicians had mainly focused on studying the corticospinal tract (Schmithorst and Wilke, 2002, Bengtsson et al., 2005, Imfeld et al., 2009) as it is the main motor tract in the body with only one DTI study reporting significant findings in the cerebellum through demonstrating increased FA in the central aspect of the cerebellum in musicians (Schmithorst and Wilke, 2002). To the best of my knowledge, no previous DTI study has specifically examined the WM tracts of the cerebellum, such as the SCP and MCP in musicians. The main aim of this study was therefore to perform DTI-based tractography to examine SCP and MCP in musicians through measuring average FA, number of streamlines, and volume of streamlines of these tracts. A second aim was to measure GM and WM volumes of the right and left cerebellar hemispheres.

7.2.1 HYPOTHESIS

I hypothesized that professional musicians, being involved in intensive sensorimotor and cognitive skill acquisition, would show evidence of structural plasticity in the WM of the cerebellum. This hypothesis was based on findings of several studies that reported cerebellar involvement in a variety of musically relevant functions, such as motor skill acquisition and sequence learning (Anderson et al., 1996, Kim et al., 2002, Koeneke et al., 2004), auditory discrimination (Parsons et al., 2009), spatial (mental orientation) and working memory (Stoodley et al., 2010), cognition (Flament et al., 1996, Doyon et al., 2003), and early error detection and correction phase of motor and cognitive skill learning (Flament et al., 1996, Doyon et al., 2003). I proposed that enhanced neural activity imposed by musical practice would result in increased WM volume of the cerebellum; and increased average FA, tract volume, and number of streamlines of SCP and MCP tracts in musicians. These microstructural changes in SCP/MCP tracts may reflect increased coherence, directionality, and integrity of fiber bundles (indicated by increased average FA value), thicker myelination and/or larger diameter of fibers (indicated by increased tract volume), and increased fiber density, axonal projections, and sprouting (indicated by increased number of streamlines). These structural changes are essential to support enhanced functional demands on the musician’s cerebellum.

7.3 SUBJECTS AND METHODS

7.3.1 SUBJECTS

Twenty six volunteers were recruited after obtaining Local Research Ethics Committee approval. All subjects gave signed informed consent and were medically screened for neurological, cardiovascular, and endocrine disorders. Subjects were also questioned regarding their alcohol consumption. Handedness was assessed using Edinburgh Handedness Inventory prior to undergoing MR examination of the brain (Oldfield, 1971). The cohort included 13 musicians/13 controls with 12 right-handed subjects and 1 left-handed subjects in each group. Final data analysis was applied on 20 subjects (see result section for details) including 10 musicians (7 males and 3 females) and 10 non-musicians (7 males and 3 females). From this point onward, I will present subjects' demographics and musicians' profile of only those participants who were included in the final analysis. In each group, 9 were right-handed and 1 was left-handed. Two left-handed subjects (1 musician/1 control) were included in the present study as previous manual and VBM studies revealed no significant effect of handedness on cerebellar structure, including total volume and GM/WM density (Good et al., 2001a, Szabo et al., 2003). In particular, a VBM study that examined a large cohort (465: 67 left-handed and 398 right-handed) (Good et al., 2001b) revealed no significant effect of handedness on cerebellar structure. Table 7.1 details descriptive statistics of participants included in the final analysis. During the first test session, musicians were questioned regarding their musical training history in addition to instrumental and performance hours. Controls were also questioned regarding any musical training, and also their interest in music.

<u>7.3.1.1 MUSICIANS' PROFILE</u>

Final data analysis was applied on 10 professional musicians who were recruited from a full-time playing major British symphony orchestra (8 subjects) and from the School of Music, University of Liverpool (2 subjects). There were 6 pianists and 4 string players. Descriptive statistics of age of starting musical training and duration of musical performance are provided in table 7.1. Only one musician started musical training after the age of 7 years.

Table 7.1. Demographics of subjects recruited in this study.

	Groups	
	Musicians	Controls
***Age (years) Mean / SD / range**	38 / 14.6 / 18-57	34.5 / 11.7 / 20-57
Duration of musical performance (years) Mean / SD / range	29.9 / 14.1 / 11-48	N/A
Age of starting musical training (years) Mean / SD / range	6.6 / 1.8 / 4-10	N/A
***Education (years) Mean / SD**	13.8 / 1.2	13.5 / 2.5

*Student *t* test revealed no significant group difference regarding age (*p*=0.5) and years of education (*p*=0.2).

7.3.2 MR DATA ACQUISITION

All volunteers were scanned using protocols with identical sequences and parameters.

7.3.2.1 HIGH RESOLUTION STRUCTURAL SCANS

High-resolution 3D T1-weighted MR images were acquired using a Symphony 1.5 Tesla whole-body MRI system (Siemens, Magnetom, Germany). These scans were used for automatic measurement of GM and WM volumes of the cerebellum and for measurement of intracranial volumes (ICVs). Images were obtained using an 8-channel receiver head coil and a whole-body transmit coil. The Magnetization-Prepared Rapid Gradient-Echo (MPRAGE) imaging sequence was used with the following scanning parameters: TR/TE/TI = 1660/3.04/1100 ms, flip angle = 8°, field of view = 256 mm, and acquisition time = 14 minutes, 11 seconds. A total of 176 sagittal slices with slice thickness of 1 mm, and in-plane resolution of 1 mm were obtained.

7.3.2.2 DIFFUSION-WEIGHTED MR DATA ACQUISITION

DTI data acquisition was acquired with Trio 3 Tesla whole-body MRI system (Siemens, Magnetom, Germany).The DTI scheme included the collection of 60 images with noncollinear diffusion gradients (b = 1200 s/mm^2), and five non-diffusion-weighted images (b = 0 s/mm^2), employing a single shot echo-planar imaging sequence. From each participant, 60 axial slices were collected. TR/TE = 10100/106 ms, field of view = 267 mm, the acquisition matrix was 112 X 128, and the slice thickness was 2 mm with no gap, which resulted in voxel dimensions of 2 X 2 X 2 mm. The total acquisition time was 22 minutes and 14 seconds. Prior to diffusion-weighted data acquisition, all subjects were given detailed explanation about the nature of the acquisition, and were asked to keep body motion to a minimum. Inside the scanner, motion was controlled with the use of head cushions provided by the scanner manufacturer.

7.4 METHODS

Image analysis methods used in this study include:

- Measurement of intracranial volume (ICV) using an automatic technique.
- Fiber tractography of SCP and MCP to measure average FA, tract volume, and number of streamlines of these tracts.
- Measurement of GM and WM volumes of cerebellar hemispheres using an automatic technique.

7.4.1 MEASUREMENT OF INTRACRANIAL VOLUME

To control for differences in head size, automatic measurement of ICV was performed using FreeSurfer software (version: stable v5.0 for Linux), available online at http://surfer.nmr.mgh.harvard.edu/fswiki/Download.

7.4.2 DTI DATA ANALYSIS

7.4.2.1 PRE-PROCESSING OF DTI DATA

Pre-processing of DTI data was performed as described in chapter 3, section 3.18.1, page 105.

7.4.2.2 TRACTOGRAPHY PARAMETERS

Tractography parameters are mentioned in chapter 3, section 3.18.2, page 106.

7.4.2.3 FIBER TRACTOGRAPHY OF THE SCP AND MCP

Reconstruction of SCP and MCP fibers was according to anatomical delineations provided by published DTI atlases (Jellison et al., 2004, Wakana et al., 2004, Salamon et al., 2007).

- **RECONSTRUCTION OF THE SCP**

The SCP could be easily identified on colour-coded maps with its superior-inferior oriented fibers coded with light blue colour (figure 7.1). It is located above the level of MCP. The SCP was demarcated through applying regions of interest (ROIs) on the coronal colour-coded map at the level of the middle portion of the splenium of the corpus callosum (CC).

- **RECONSTRUCTION OF THE MCP**

For reconstructing the MCP, ROIs were placed on the coronal colour-coded map where the MCP was greatest in volume (confirmed by viewing the orthogonal axial image), which corresponds to the level of the middle portion of the splenium of the CC. In the coronal colour-coded image, the MCP appears green because of its antero-posteriorly oriented fibers; it could be easily identified lateral to the pontine tegmentum. Two ROIs were placed at the left and right of the lateral pontine tegmentum (figure 7.2).

Once a fiber tract was reconstructed, the entire trajectory was verified on a slice-by-slice basis to ensure consistency with established anatomical landmarks (Williams et al., 1995, Snell, 2001, Kiernan, 2005), as well as the tractography-based atlases of fiber pathways of the human brain (Jellison et al., 2004, Wakana et al., 2004, Salamon et al., 2007). In situations where a particular tract showed some unexpected connectivity or if there was a deviation relative to the known landmarks, the tract was excluded from further analyses. After applying tractography, the identified fiber bundles were compared for three parameters: mean FA value, tract volume, and number of streamlines.

The FA values were calculated by averaging across all voxels in the tract. It should be emphasized that the reconstructed tracts represent streamlines based on tensor directions and not the real WM fibers (Matsumoto et al., 2008). Since we are at present not able to resolve fiber tracts at microscopic levels, DTI-based tractography is the only technique that allows identification of WM pathways in the living human brain. The calculated number of fibers represents the number of the reconstructed streamlines and the calculated tract volume represents the number of voxels that these streamlines traverse multiplied by the 2 mm voxel size. Streamlines originating from different brain regions may converge along their pathway, so that twice the number of streamlines may not essentially represent twice the volume (number of voxels)(Reich et al., 2006).

7.4.3 INTRA- AND INTER-RATER RELIABILITY

All ROIs were placed by a single rater, IA who was blind to the identity, laterality, and grouping of subjects. Intra-rater study was performed by repeating DTI measurements by the same rater in all subjects at least three weeks after the first measurement. For inter-rater reliability, DTI measurements were independently performed by two raters (MA and IA) for all subjects. An intra-class correlation coefficient greater than 0.80 was accepted as evidence of inter- and intra-rater reliability.

7.4.4 MEASUREMENT OF GM AND WM VOLUMES OF CEREBELLAR HEMISPHERES

Automatic measurement of GM and WM volumes of the right and left cerebellar hemispheres was performed using FreeSurfer software (version: stable v5.0 for Linux), available online at http://surfer.nmr.mgh.harvard.edu/fswiki/Download. The image files in DICOM format were transferred to a Linux workstation for morphometric analysis. The automated procedures for volumetric measurement of different brain structures were originally described by (Fischl et al., 2002). In contrast to previous automated techniques for brain tissue segmentation that label a small number of tissue classes, FreeSurfer can automatically segment, parcellate, and label up to 40 unique structures, including the cerebellum by assigning a neuroanatomical label to each voxel in an MRI volume based on probabilistic information automatically estimated from a manually labeled training set. An optimal linear transform is computed that maximizes the likelihood of the input image, given an atlas constructed from manually labeled images. A nonlinear transform is next initialized with the linear one, and the image is allowed to further deform to better match the atlas. Finally, a Bayesian segmentation procedure is carried out, and the maximum a posteriori estimate of the labeling is calculated (Fischl et al., 2002). The segmentation employs three pieces of information to disambiguate labels: the prior probability of a given tissue class occurring at a specific atlas location, the likelihood of the image intensity given that tissue class, and the probability of the local spatial configuration of labels given the tissue class. It has been shown that the technique used in this study could achieve results comparable in accuracy to manual tracings (Fischl et al., 2002, McDonald et al., 2008); however, segmentation results were visually inspected for accuracy prior to inclusion in the group analysis. No segmentation errors were detected, so all cases were included in the final analysis.

7.4.5 STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS v. 16.0 and statistica program, v. 6.0 (Statsoft Inc., USA). Shapiro-Wilk (S-W) was used to test normality of data distribution. Using SPSS, multivariate analysis of variance (MANOVA) was performed with average FA values, tract volumes, and number of streamlines of right/left SCP/MCP as dependent variables and musicianship (musicians versus non-musicians) and gender (males versus females) as independent variables covarying for age and ICV; $p<0.05$ was considered significant after adjustment for multiple comparisons. The values of number of streamlines and tract volumes are likely to be affected by head size. Increased head size may result in increased number of streamlines and tract volume. I therefore covaried for ICV to control for head size. In addition, I followed the recommendations of previous studies (Ciccarelli et al., 2003, Correia et al., 2008, Tate et al., 2010) for normalizing these metrics according to ICV. I calculated the relative values of the metrics normalized by the ICV following the formula provided by (Correia et al., 2008). These metrics were divided by (V / V-), where V is the ICV of the subject and V- is the average ICV for the entire group (musicians and non-musicians). I re-performed the above MANOVA using normalized values of tract volume and number of streamlines. A third MANOVA was performed to compare GM and WM volumes of the right and left cerebellar hemispheres with GM/WM volumes of right/left cerebellar hemispheres corrected for ICV as dependent variables and musicianship and gender as independent variables covarying for age. Independent student *t* test was used to compare ICV measurements between groups. Pearson’s correlation was used to perform correlation analyses. The intra-class correlation coefficient (ICC) calculated by one-way random model was used to test intra- and inter-rater reliability. A coefficient greater than 0.80 was accepted as evidence of inter- and intra-rater reliability. Statistica software was used to create figures 7.5, 7.6, and 7.7.

7.5 RESULTS

7.5.1 MEASUREMENT OF INTRACRANIAL VOLUME

There was no significant difference in ICV between groups (*p*=0.15).

7.5.2 RECONSTRUCTION OF THE SCP AND MCP

7.5.2.1 EXCLUSION OF CASES FOR TRACTOGRAPHY

As stated in chapter 3, reconstruction of SCP and MCP was not feasible in 6/26 subjects. In these subjects (3 musicians/ 3 non-musicians), tractography results were deviated from known landmarks and showed inconsistency compared to published atlases. There was inconsistent tracking of the left MCP in 3 subjects (2 musicians /1 non-musician) and of the right MCP in 3 subjects (1 musician /2 non-musicians). In these cases, most of trajectories of the MCPs were terminated at the level of the pons and did not pass to the cerebellar hemisphere. Consequently, these cases were excluded from this study.

In all remaining 20 subjects assigned for final data analysis, reconstruction of the SCP and MCP was consistent with the description of published DTI atlases (figures 7.1, 7.2, 7.3, and 7.4) (Jellison et al., 2004, Wakana et al., 2004, Salamon et al., 2007).

7.5.2.2 COMPARISON OF SCP AND MCP TRACTOGRAPHY MEASURES

MANOVA revealed significantly increased right MCP tract volume (F= 10.2, *p*=0.004), right SCP tract volume (F= 5.9, *p*=0.02), and right SCP number of streamlines (F=14.1, *p*=0.001) in musicians compared to non-musicians. There were no significant differences in left SCP tract volume, left MCP tract volume, left SCP number of streamlines, right MCP number of streamlines, right/left SCP average FA values, and right/left MCP average FA values. There was no significant gender by musicianship interaction. It should be noted that normalization of tractography metrics did not affect the final results. I next assessed the correlation between age of starting musical training/duration of musical performance and right SCP (tract volume and number of streamlines) and right MCP (tract volume) using Pearson’s correlation after controlling for subjects' age. There was no significant correlation between age of starting musical training/duration of musical performance and tractography measures of right SCP/MCP. Figures 7.5 and 7.6 show means ± 95% confidence intervals of SCP and MCP measures, respectively.

7.5.3 INTRA- AND INTER-RATER RELIABILITY

Results of both inter- and intra-rater comparisons showed good reproducibility between measurements with ICC range of 0.82-0.93 for inter-rater study and 0.87-0.96 for intra-rater study.

7.5.4 COMPARISON OF GM AND WM VOLUMES OF RIGHT AND LEFT CEREBELLAR HEMISPHERES

MANOVA revealed that musicians had significantly increased right cerebellar WM volumes (F= 11.02, *p*=0.003). There was no significant difference in right cerebellar GM volume and left cerebellar WM/GM volumes. There was no significant gender by musicianship interaction. Figure 7.7 shows means ± 95% confidence intervals of GM and WM volumes of the right and left cerebellar hemispheres.

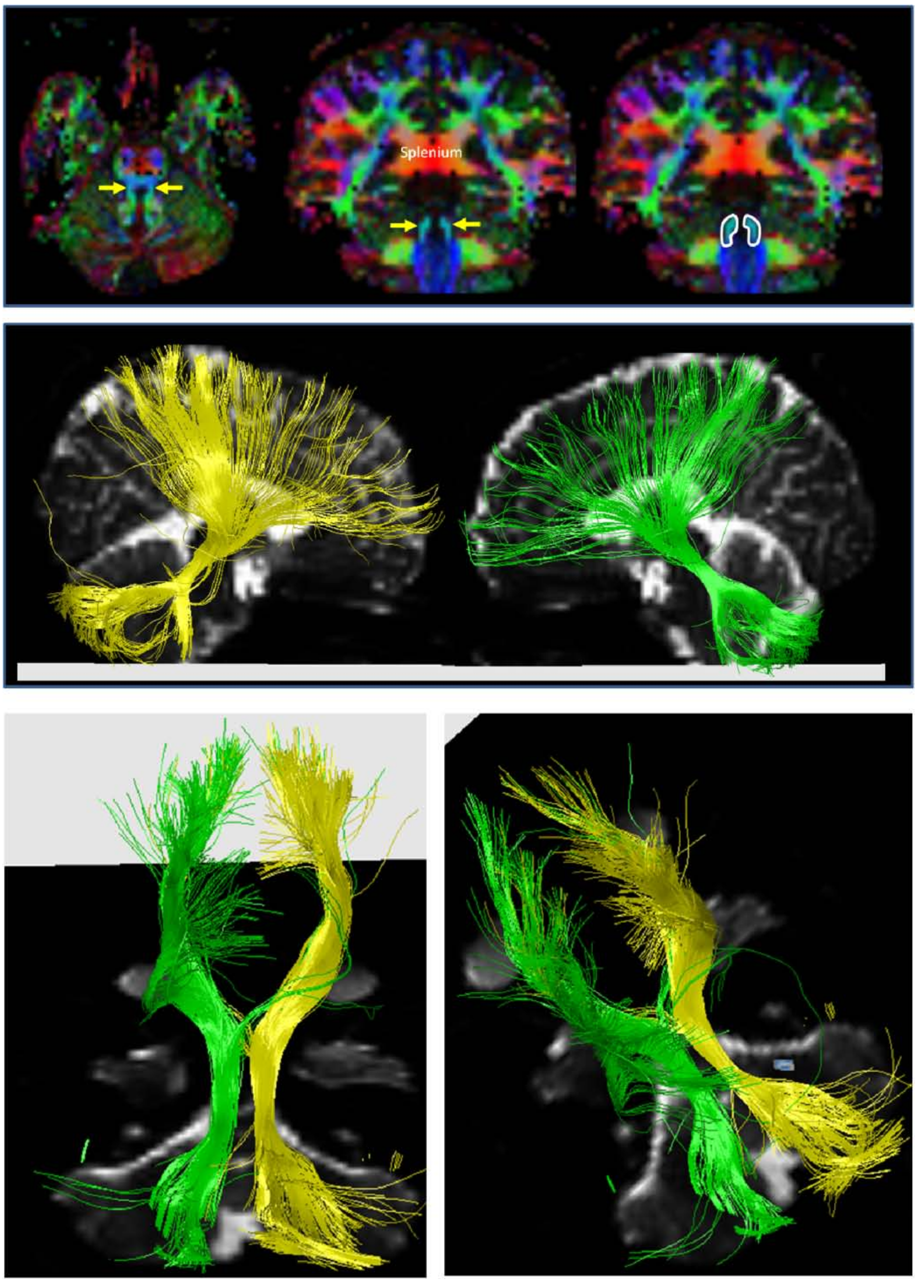

Fig.7.1. Fiber tracking of the SCP. Top row, axial and coronal colour-coded maps showing the SCP in blue colour (yellow arrows). Two ROIs were placed at the coronal image (outlined in white) to track SCP fibers. Middle row, depiction of left (green) and right (yellow) SCP fibers on sagittal T2-weighted images. Bottom row, right and left SCP fibers depicted on axial T2 weighted images.

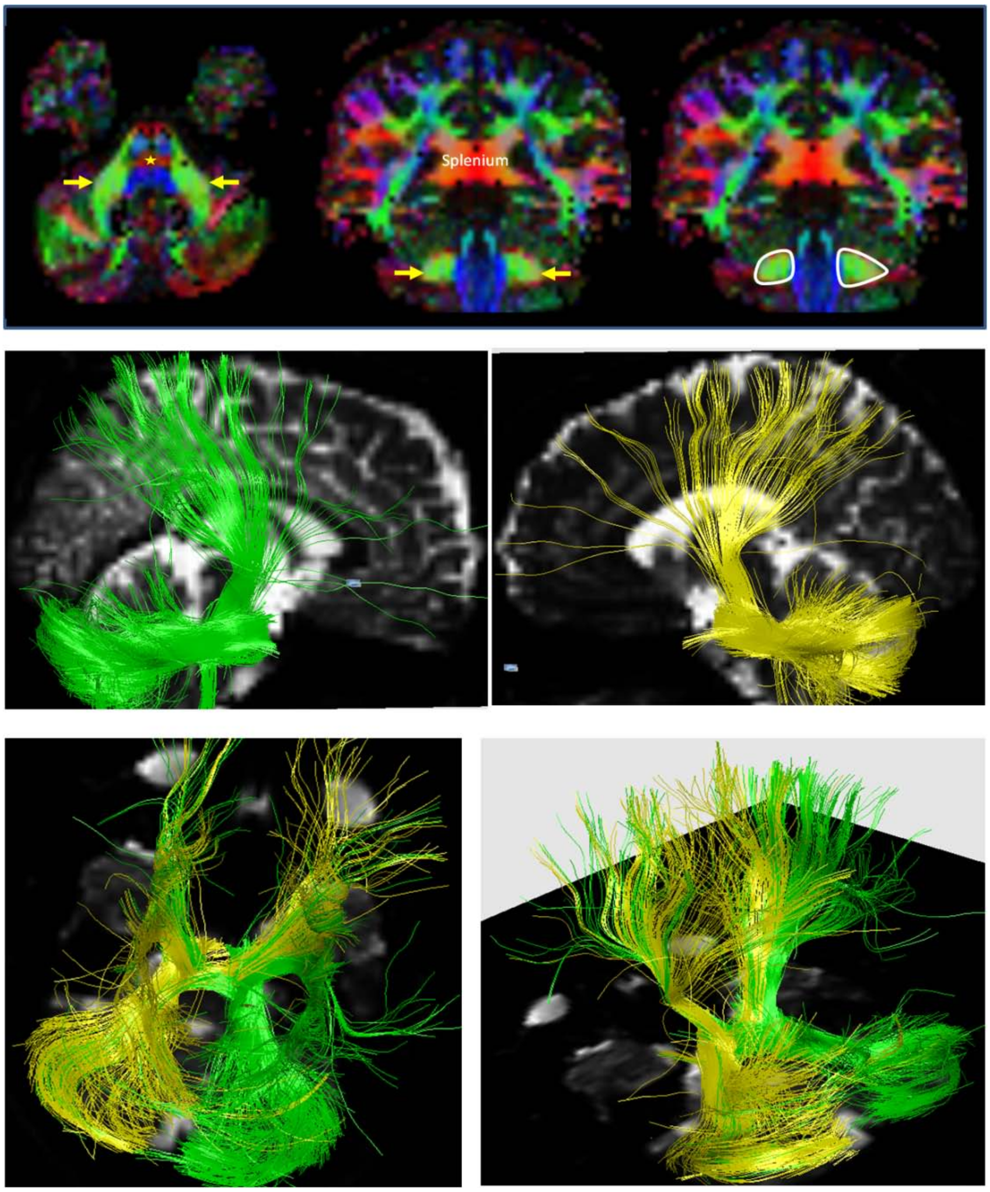

Fig. 7.2. Fiber tracking of the MCP. Top row, axial and coronal colour-coded maps showing the MCP in green colour (yellow arrows) and the pontine tegmentum (yellow star). Two ROIs were placed at the coronal image (outlined in white) to track MCP fibers. Middle row, depiction of left (yellow) and right (green) MCP fibers on sagittal T2-weighted images. Bottom row, right and left SCP fibers depicted on axial T2-weighted images.

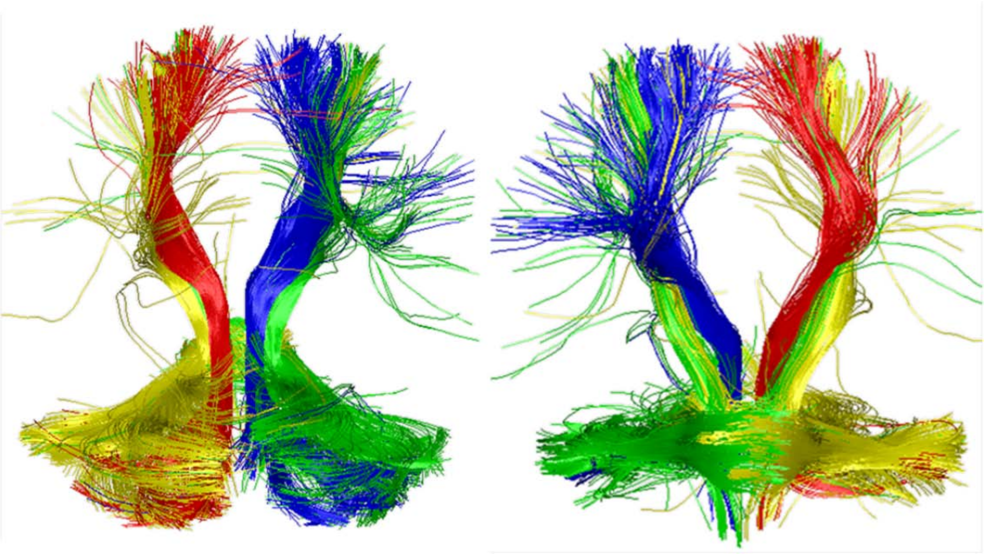

Fig. 7.3. Tractography of the SCP and MCP. Demonstration of right (blue) and left (red) SCP; right (green) and left (yellow) MCP. Left, posterior view and right, anterior view.

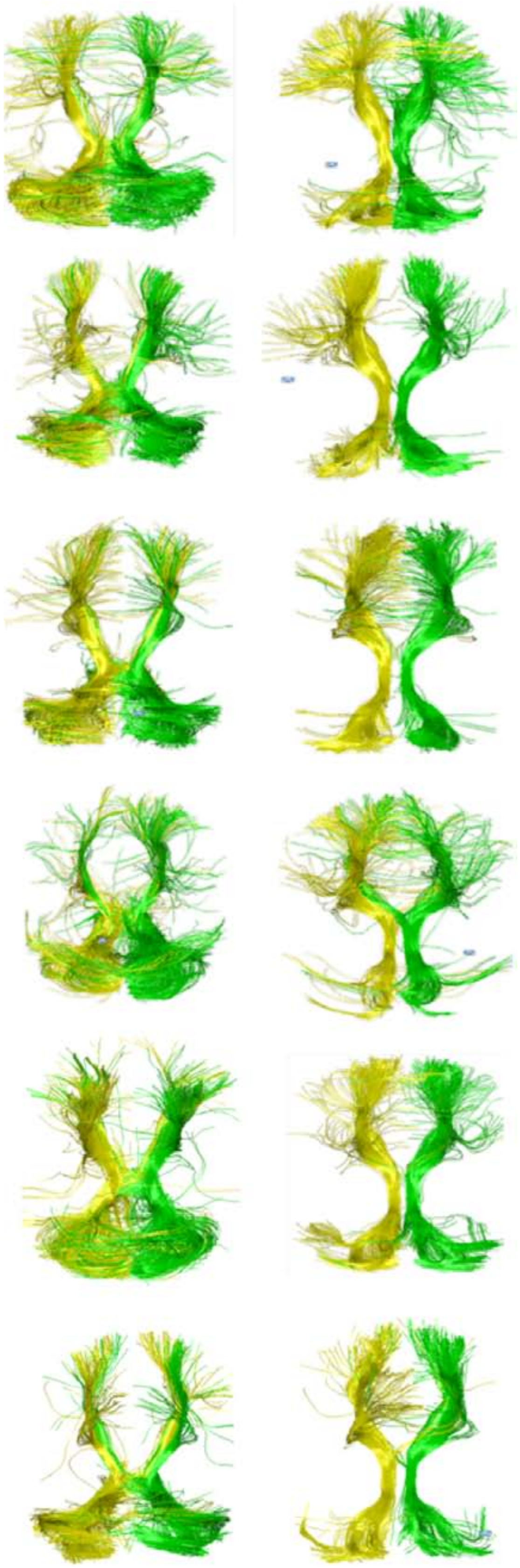

Fig. 7.4. Demonstration of MCP (left column) and SCP (right column) tracts in 6 subjects.

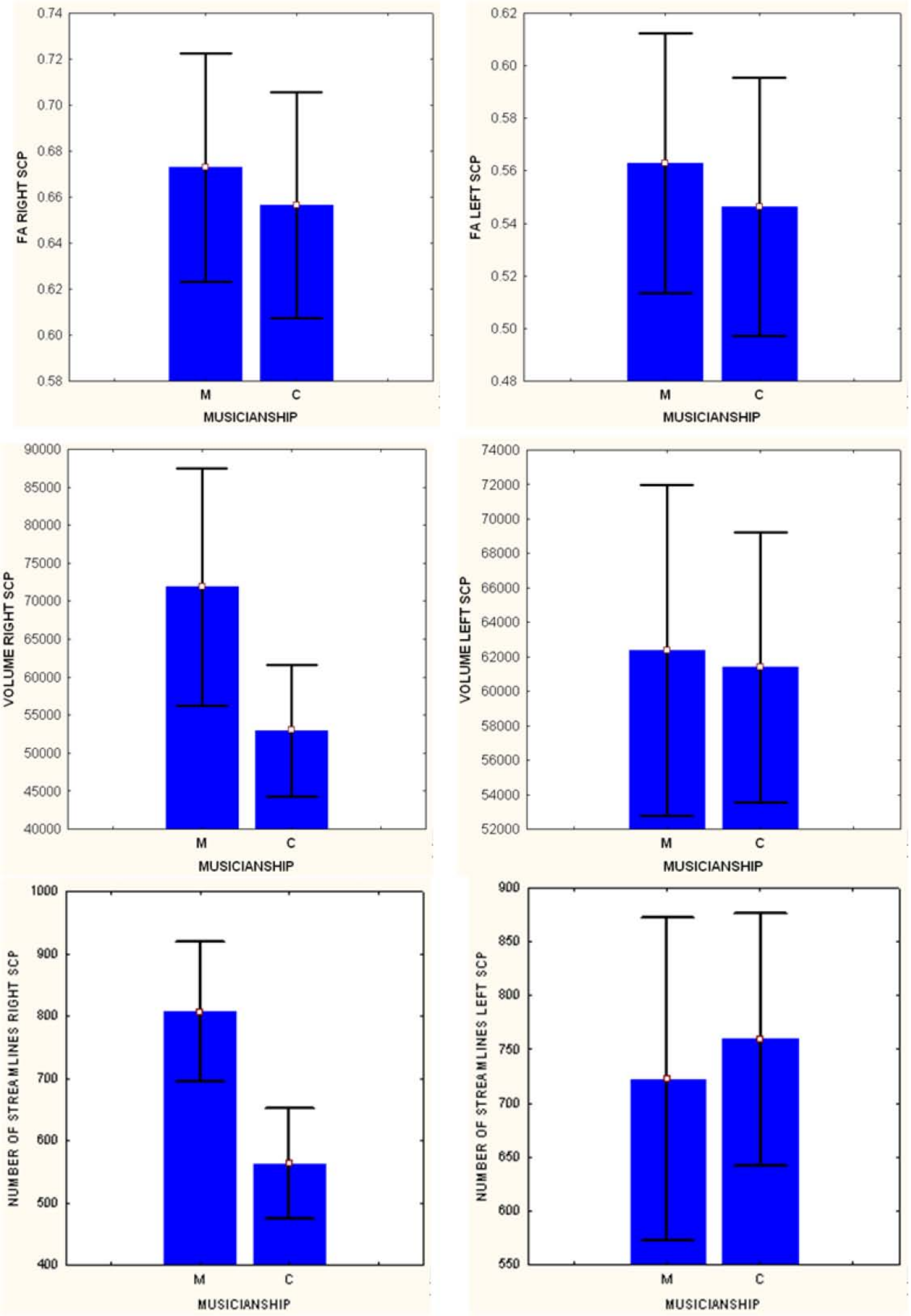

Fig. 7.5. Means ± 95% confidence intervals of average FA, tract volume, and number of streamlines of SCP (uncorrected for ICV).

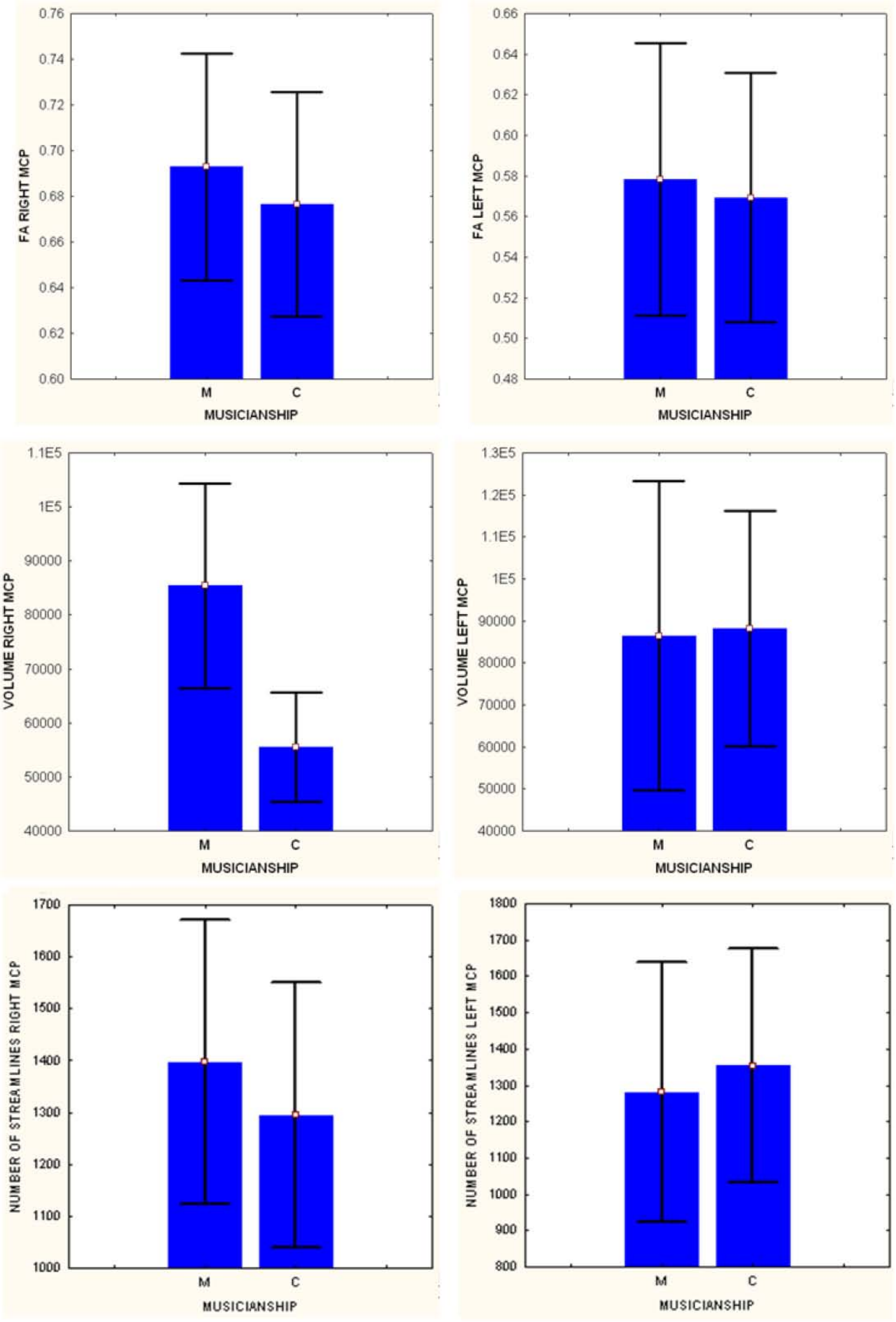

Fig. 7.6. Means ± 95% confidence intervals of average FA, tract volume and number of streamlines of MCP (uncorrected for ICV).

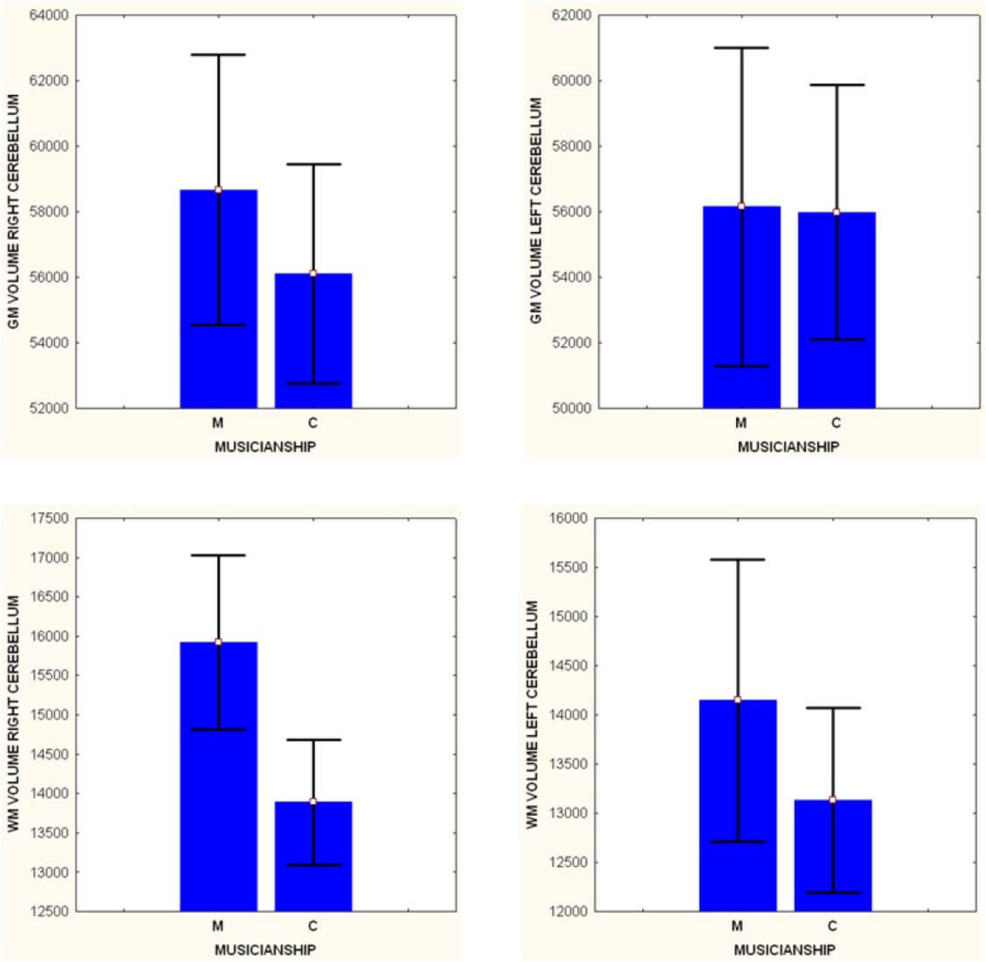

Fig. 7.7. Means ± 95% confidence intervals of GM and WM volumes of right and left cerebellar hemispheres (uncorrected for ICV). Volumes are mm^3.

7.6 DISCUSSION

Using fiber tractography, the present study revealed that musicians had significantly increased tract volume and number of streamlines of right SCP and volume of the right MCP. In addition, automatic measurement of cerebellar hemisphere volumes revealed increased WM volume of the right cerebellum in musicians. These findings were detected in a cohort of musicians and controls who showed no significant difference in ICV. I propose that these findings represent use-dependent structural plasticity imposed by early and intensive skilful performance. Each cerebellar hemisphere controls the ipsilateral side of the body, so the right cerebellum controls the right and left cerebellum controls the left side of the body (Snell, 2001). It is therefore expected to find evidence of plasticity in the right cerebellum as 18/20 of recruited subjects were right-handed; however, a VBM study that examined 465 right-handed normal subjects revealed no significant main effect of handedness on GM and WM density of the cerebellum (Good et al., 2001a). This might explain the discrepancy of findings of previous VBM studies in musicians as one study reported increased GM density in the right cerebellum (Han et al., 2009), yet another reported increased GM density in the left cerebellum (Gaser and Schlaug, 2003), although both investigated a cohort of right-handed keyboard players. Neuroplasticity refers to the brain’s ability to change its structure and function during maturation, learning, skill acquisition, environmental challenges, or pathology (Lledo et al., 2006). Through this process, neurons are able to change their function, chemical profile (such as the amount and types of neurotransmitters) or structure (Woolf and Salter, 2000). At the microscopic level, two steps of neuroplasticity can be identified: unmasking already established connections that may be followed by generation of new ones through dendritic growth, arborization, and axonal projections (Pascual-Leone et al., 2005). Recent studies showed that neuroplasticity can be modified by a variety of factors, including environmental enrichment and physical activity (van Praag et al., 1999). Evidence of structural plasticity in the cerebellum was reported in several experimental paradigms in animals. Many of these studies reported significant plasticity in relation to motor skill acquisition. For example, it has been shown that motor-skill learnt rats have more parallel and climbing fiber synapses (Anderson et al., 1996, Kim et al., 2002), and that the volume of the molecular layer of the cerebellar cortex is larger in trained rats and mice than in the inactive groups (Pysh and Weiss, 1979, Kleim et al., 1998). With the advent of neuroimaging techniques, it has been possible to study cerebellar plasticity in humans. Accordingly, evidence of structural cerebellar plasticity in animals has been paralleled by findings of structural plasticity in musicians in the form of increased absolute and relative cerebellar volumes (Hutchinson et al., 2003) and increased GM density of the left (Gaser and Schlaug, 2003) and right (Han et al., 2009) cerebellum. Results of the present study extend these findings through demonstrating evidence of WM plasticity in musicians' cerebellum. Interestingly, there are reports suggesting differential responses of neuroplasticity in animals according to exercise modules performed. For example, acrobatically trained rats have greater volume of the cerebellar molecular layer than rats that received forced or voluntary exercises, or were raised in inactive conditions (Black et al., 1990). This might in part explain why evidence of structural cerebellar plasticity has been reported in musicians but not in subjects with other professions like basket ball players (Park et al., 2006) as the two groups differ in the type of exercise they perform; musicians are involved in fine motor practice that recruits mainly small muscles of the hands, whereas basket ball players make coarser movements using mainly large muscles of the upper limb.

7.6.1 THE CEREBELLUM IS CRUCIAL FOR MUSICAL PERFORMANCE

Classically, it was thought that the function of the cerebellum is merely to coordinate motor activity; however, functional neuroimaging studies have shown that its function extends far beyond that. The cerebellum is involved in sensory acquisition and discrimination (Gao et al., 1996, Parsons et al., 1997), spatial (mental orientation) and working memory (Stoodley et al., 2010), language (Frings et al., 2006), and cognition (Kim et al., 1994). In the motor domain, fMRI studies in musicians revealed cerebellar activation while playing (Sergent et al., 1992) and during motor sequence learning (Hund-Georgiadis and von Cramon, 1999). In particular, the cerebellum appears to be important in the early error detection and correction phase of motor and cognitive skill learning (Flament et al., 1996, Doyon et al., 2003). This may suggest that subjects with fewer errors when performing motor tasks would show reduced recruitment of the cerebellum. In accordance, an fMRI study (Koeneke et al., 2004) reported reduced activations of the cerebellum in keyboard players when performing bimanual motor task compared to control subjects who showed increased activations. On the other hand, a sustained increase of activity with continued skilful practice was demonstrated in specific areas of the cerebellum, such as the dentate nucleus (Flament et al., 1996) and the posterior superior fissure (Imamizu et al., 2000), which might suggest that these cerebellar regions are part of the neuronal system engaged in the creation of a long-term representation of skilled movement necessary to execute those motor adaptation tasks proficiently.

In addition to the aforementioned functions, the cerebellum appears to have a crucial role in sensory perception (Parsons et al., 2009). It regulates the acquisition of incoming sensory data across all sensory modalities including those associated with motor as well as cognitive activities. Cerebellar involvement in sensory functions was suggested to be an indirect process through the initial acquisition of the sensory data on which motor and cognitive functions depend (Bower and Kassel, 1990, Gao et al., 1996, Parsons et al., 2009), and assuring that the best quality sensory data are gathered for use by the rest of the nervous system (Bower and Parsons, 2003). Furthermore, several neuroimaging studies have pointed out to a possible role of the cerebellum in auditory function, such as pitch (Parsons et al., 2009), intensity (Belin et al., 1998), and duration (Belin et al., 2002) discrimination of sound stimuli. Cerebellar activations were detected even when subjects passively listened to pure tones (Lockwood et al., 1999) and speech (Callan et al., 2006).

It could be hypothesized that, since spectral pitch discrimination and perception was shown to be lateralized to the right cerebellar hemisphere (Johnsrude et al., 2000, Zatorre and Belin, 2001, Schneider et al., 2005), the increased tract volumes and number of streamlines of right SCP/MCP reported here would support enhanced acquisition of auditory stimuli in musicians.

Quantitative MR studies reported decreased size of SCP and MCP in patients with neurodegenerative disorders, such as progressive supranuclear palsy (Paviour et al., 2006), multiple system atrophy (Paviour et al., 2006), Parkinson's disease (Gama et al., 2010), and Friedreich's ataxia. Atrophy of SCP and MCP on these patients was shown to be associated with variety of motor, sensory, and cognitive dysfunctions. These studies also suggested that measurement of volume/area/width of SCP and MCP is a useful strategy to improve the accuracy of diagnosis and can even help in differentiating between these disorders (Gama et al., 2010). In contrast to patients with these disorders, I proposed that musicians with their enhanced sensorimotor skill acquisition would show increased size of SCP and MCP, which was revealed in the present study. It has been suggested that quantitative tractography metrics, such as the number of streamlines and tract volume may provide a valid tool for detecting subtle group differences in structural integrity of WM tracts (Ciccarelli et al., 2003, Correia et al., 2008). Combined with average FA, these metrics may provide different discriminative power when addressing WM integrity (Correia et al., 2008). They have been used to quantify specific WM pathways, such as the corticospinal tract (Lindenberg et al., 2010), arcuate fasciculus (Matsumoto et al., 2008), CC, and cingulum (Ciccarelli et al., 2003, Correia et al., 2008). Quantitative changes of these metrics have been linked to cognitive performance (Tate et al., 2010) and were used as structural markers of motor dysfunction (Hosomi et al., 2009). Interestingly, the tract volume and number of streamlines have been used as markers of structural plasticity. For example, Schlaug and colleagues (Schlaug et al., 2009) reported significant increase in the number of streamlines and tract volume of the arcuate fasciculus in patients with non-fluent aphasia who undergone intonation-based speech therapy. Another example is increased number of streamlines of internal capsule and cerebral peduncles six weeks after the induction of spinal cord injury in rats (Ramu et al., 2008). Skilful musical practice may induce neural activity, which in turn may increase the number, diameter, myelination, and sprouting of SCP and MCP axons. These microstructural changes in musicians' cerebellum might be essential to support enhanced sensorimotor and cognitive demands on their brain. More studies are required in the future; however, to explain the exact relationship between DTI-derived measures and histological properties of the nervous system.

Results of the present study did not show significant differences in average FA between groups. Previous DTI studies in musicians reported inconsistent FA findings with some authors reported higher (Bengtsson et al., 2005, Han et al., 2009), while others reported lower (Schmithorst and Wilke, 2002, Imfeld et al., 2009) FA values compared to non-musicians; yet others revealed no significant differences in certain WM tracts, such as the CC (Imfeld et al., 2009). Although the FA values have been widely used in DTI literature as markers of WM integrity, the exact correlation between FA measures and microstructural tissue properties is still lacking. It is therefore reasonable to include other DTI parameters, such as volume and number of streamlines that could provide additional evidence of structural plasticity (Matsumoto et al., 2008).

7.6.2 LACK OF GM DIFFERENCES IN CEREBELLAR HEMISPHERES BETWEEN GROUPS

In the present study, I could not replicate findings of increased GM density in the cerebellum reported in previous VBM studies (Gaser and Schlaug, 2003, Han et al., 2009). This might be related to different sample characteristics (such as intensity of practice and instrument specialty), different scanning parameters, and different morphometric methods used. I have used a sequence specifically optimized to obtain high resolution scans that allowed optimum GM/WM segmentation by the FreeSurfer software. VBM was not originally designed for the analysis of subcortical structures and the complicated anatomy of the cerebellum and the surrounding cerebral tissue may impede automatic processing of VBM. The FreeSurfer (Fischl et al., 2002) software package used in the present study provides a completely automated parcellation of the cerebral and cerebellar cortices and subcortical structures. Several studies have validated the use of this method to quantify subcortical volume in aging (Walhovd et al., 2005), dementia (Pengas et al., 2009), and depression (Tae et al., 2008). Using FreeSurfer, I could still demonstrate significant WM differences in the right cerebellum in musicians, which support my findings of increased right SCP/MCP tractography measures. Results of the present study indicated that WM plasticity could still exist in the absence of significant GM plasticity. One possible explanation is that skilful musical practice has mainly induced WM changes in the form of increased axonal projections and collaterals with little effect on the cerebellar cortex. These findings underscore the importance of examining WM plasticity in musicians through using sensitive techniques like DTI.

7.6.3 CEREBELLAR ASYMMETRY

It is well known that the cerebellum does not have a symmetrical morphology. MR-based studies have demonstrated different patterns of asymmetry in the normal cerebellum. For instance, right-to-left asymmetry in total hemisphere volume (Rae et al., 2002, Szabo et al., 2003), and GM density (Barrick et al., 2005) of the cerebellum were reported using manual volumetry and VBM techniques, respectively. Several reports addressed cerebellar asymmetry after dividing each cerebellar hemisphere into anterior and posterior divisions. For instance, Snyder and colleagues (Snyder et al., 1995) reported right-to-left volume asymmetry in the anterior cerebellum and left-to-right volume asymmetry in the posterior cerebellum. This form of cerebellar asymmetry followed a pattern commonly found in the neocortex named as cerebral developmental torque wherein "the left occipital pole is often wider and usually protrudes more posteriorly than the right", and "if one frontal pole extends beyond the other it is usually the right" (LeMay, 1976). In contrast, a more recent VBM study detected left-to-right GM asymmetry in the anterior and superior parts of the cerebellum, while no asymmetry reported in the posterior cerebellum (Barrick et al., 2005). Other studies failed to reveal significant asymmetry in cerebellar volume (Luft et al., 1998).

The VBM technique has been widely used in research as it provides unbiased, automatic, and objective morphometric analysis of GM and WM density. One advantage of VBM is its capacity to examine large cohorts in a short time (Ashburner and Friston, 2000). Several studies have investigated cerebellar asymmetry using VBM (Good et al., 2001a, Good et al., 2001b, Luders et al., 2004, Park et al., 2004, Herve et al., 2006, Takao et al., 2011). A right-to-left asymmetry was observed in cerebellar GM density (Good et al., 2001b, Barrick et al., 2005, Herve et al., 2006, Takao et al., 2011), while the reverse (left-to-right asymmetry) was found in cerebellar WM density (Herve et al.,

2006) and FA of cerebellar hemispheres (Takao et al., 2011) and SCP (Park et al., 2004).

7.6.3.1 EFFECT OF HANDEDNESS ON CEREBELLAR ASYMMETRY

Cerebellar asymmetry has been linked to handedness patterns in adults (Snyder et al., 1995). In right-handed subjects, right-to-left cerebellar asymmetry was considered a reflection of left cerebral hemisphere dominance (Rae et al., 2002) as cerebellar dominance is contralateral to that of the cerebral cortex, i.e. ipsilateral to the hand (Leiner et al., 1991). Some reports have attributed right-to left cerebellar asymmetry in right-handed subjects to the role of the cerebellum in linguistic functions as evidenced by crossed cerebral and cerebellar language dominance, i.e. left cerebral and right cerebellar language dominance (Jansen et al., 2005). Studies that examined the effect of handedness on cohorts of right and left handers revealed contradicting results. As stated previously, Snyder and colleagues (Snyder et al., 1995) reported that dextral humans had greater cerebellar torque (a right anterior, left posterior bias of the cerebellum) than did nondextrals (left-handed and ambidextrous). A recent study (Powell et al., 2012) investigated the effect of handedness on WM anisotropy in a cohort of 42 right- and 40 left-handers. Authors reported greater leftward FA asymmetry in the posterior lobe of the cerebellum in left handers. In general, the effect of handedness seems to be inconsistent across studies. For example, a VBM study that examined a large cohort (465: 67 left-handed and 398 right-handed) (Good et al., 2001b) revealed no significant effect of handedness on cerebellar structure. These findings were replicated in another study that compared manual volumetric measurements of cerebellar hemispheres between 20 right-handed and 14 left-handed subjects (Szabo et al., 2003).

The discrepancies between the above mentioned MR studies could probably reflect multiple factors, such as differences in subjects' characteristics, sample size, image analysis methods (manual tracing on relatively few sections versus voxel-based analysis), and various anatomical definitions for cerebellar compartments.

7.6.3.2 STRUCTURAL ASYMMETRY IN MUSICIANS' BRAINS

Studies of structural brain asymmetry in professional musicians have primarily focused on the planum temporale and primary motor cortex. While most studies showed leftward structural asymmetry of the planum temporale in musicians with absolute pitch abilities (Schlaug et al., 1995, Zatorre et al., 1998, Keenan et al., 2001, Luders et al., 2004), there was a tendency for structural symmetry or reduced structural asymmetry in the primary motor cortex. For example, the size of the primary motor cortex was shown to be more symmetrical in the brains of right-handed keyboard players compared with that in right-handed non-musicians (Amunts et al., 1997), which was reflected at the behavioral level by reduced difference in the tapping rate between right and left hands in musicians (Jancke et al., 1997, Fujii and Oda, 2006). These findings of enhanced bimanual coordination skills in musicians may suggest that structural or hand skill asymmetry represent an obstacle for coordinated bimanual performance (Verheul and Geuze, 2004). It is thus expected that such structural-behavioral reduction in asymmetry in the motor domain in musicians would be reflected on the cerebellum being a key structure for motor coordination. However, a VBM study that examined 60 professional musicians demonstrated right-to-left GM asymmetry in most of the cerebellum (Luders et al., 2004), which was consistent with the general asymmetry pattern observed in non-musicians. Asymmetry of cerebellar WM and cerebellar peduncles has not yet been investigated in musicians. The cohort examined in the present study included a small number of musicians who were of mixed gender (7 males and 3 females) and specialized in different musical instruments (keyboard and string players). In particular, the type of musical instrument could have significant differential effect on the morphology of motor regions in musicians (Bangert and Schlaug, 2006). A larger cohort of uni-instrumental musicians is therefore required in the future to investigate asymmetry of cerebellar WM.

7.6.4 TECHNICAL LIMITATIONS OF THE PRESENT STUDY

In addition to general DTI limitations discussed in chapter 6 notably, effects of eddy currents, subject motion and SNR, there are other technical shortcomings specific to fiber reconstruction of the SCP and MCP that should be highlighted. First, the resolution of tractography images is several orders of magnitude lower than the nerve bundles under examination. For instance, the data in this study were acquired with a resolution of 2 x 2 x 2 mm, while the diameter of axons is typically 10 µm (Williams et al., 1995, Snell, 2001, Kiernan, 2005), which means that a voxel could contain a large number of axons. Estimation of tensor orientation in conventional tracking methods, such as that used in the present study, would thus represent an average of the orientations of all axons contained within a voxel. When axons are coherently organized in a parallel fashion, the orientation of the tensor will truly reflect the orientation of the underlying fibers. However, when axons are not oriented in a highly coherent fashion (e.g. when fibers cross within a voxel), then the voxel-averaged estimate of orientation cannot accurately summarize the orientation of the underlying fibers leading to tracking errors (Virta et al., 1999). In the present study, crossing fibers phenomenon was observed in the pons where afferent cerebellar fiber bundles carried by the MCP cross with bundles of the corticospinal tract thereby reducing reliability of the tracking algorithm.
Recognition of this potential limitation in fiber tracking has led to the development of techniques that permit more than one orientation to be determined in those voxels containing multi-orientational fiber populations (Frank, 2002, Tuch et al., 2003, Assaf and Basser, 2005, Wedeen et al., 2005). These techniques were proposed to provide more accurate tractography results and are the subject of considerable ongoing research. Ultimately, higher resolution diffusion-weighted imaging techniques are needed in the future to precisely delineate fiber pathways.

A further technical limitation that can affect the results unpredictably is the inability of the fiber tracking algorithm to distinguish “crossing” from “kissing” fiber bundles within a voxel (Basser et al., 2000). In the present study, the bulk of the reconstructed SCPs do not appear to decussate at the decussation of the SCP, as they are known anatomically to do, but the reconstructed trajectory remained in the same hemisphere (see figures

7.1, 7.3, and 7.4). This is due to the limitation of the fiber tracking algorithm that offers a “kissing” (><) solution when two fiber populations have a crossing (X) trajectory (Basser et al., 2000). Despite these sources of technical limitations, I expect that my approach identified voxels that make up the core of the SCP and MCP tracts. This was confirmed by visual inspection of the identified tracts, both within individual sections and in 3D reconstructions, which was performed for every individual studied. Reconstructed tracts were comparable to published DTI atlases (Wakana et al., 2004, Salamon et al., 2007, Lawes et al., 2008).

Another limitation is that DTI-based tractography cannot differentiate between afferent and efferent fibers (Mori and van Zijl, 2002, Nucifora et al., 2007). In DTI, we are observing the motion of water molecules, from which we cannot differentiate the directionality of axons. For example, at the level of the dentate nucleus, both afferent and efferent fibers intersect thereby reducing the likelihood of distinguishing afferent from efferent fibers within the MCP. The same problem was noticed when tracking the SCP; although the main bulk of the SCP is composed of efferent fibers of the dentothalamic tract, it also contains afferent axons of the anterior spinocerebellar tract. Therefore, one cannot be absolutely confident in naming these tracts as “afferent”, in the case of MCP and “efferent”, in the case of SCP since DTI does not allow separate visualization of the upward and downward orientation of fibers.

In the present study, ROI-based method was used for fiber tracking, which is known to be limited in reproducibility and susceptible to operator variability in the placement of ROIs. Also, no specific size was set for ROIs which might affect inter-rater reliability. However, to reduce bias, ROIs were placed on commonly identifiable anatomical landmarks based on previously proposed methods. The location of ROIs was further confirmed in each case by referring to sagittal and axial slices. As a result, reproducibility studies were generally good ranging between 0.87-0.96 for intra-rater study and 0.82-0.93 for inter-rater study.

The choice of cutoff thresholds for tract reconstruction can have a significant impact on the identification of fibers within a bundle (Stieltjes et al., 2001). The FA threshold (0.2) and minimum length criteria (10 mm) used in the present study were chosen to limit the number of spurious, anatomically implausible fibers while retaining a maximal number of anatomically plausible fibers. Lower FA threshold values are known to cause streamlines to intrude on regions that are obviously GM (Mori and van Zijl, 2002); however, it is also possible that adjusting these parameters could impact the magnitude of the obtained metrics. It was therefore prudent to repeat fiber tracking using different settings to test possible confounding effects on tractography measures (Correia et al., 2008), which was not performed in the present study.

In this study, cerebellar GM/WM volumes were obtained using an automated segmentation method provided by FreeSurfer software, which has some limitations inherent to fully automated segmentation softwares. Several technical factors could influence labeling of neuroanatomical structures by FreeSurfer software, such as section thickness, field strength, SNR, and anatomic boundary criteria. However, previous studies have indicated that volumetric data obtained with automated segmentation of the cerebellum approximate those obtained with manual tracings underscoring the utility of this method (Quattrone et al., 2008).

Manual-based volumetric measurements may be less susceptible to limitations associated with MR imaging such as image quality, tissue intensity that shift structure boundaries nonuniformly, or with motion artifacts, as the rater can apply neuroanatomical knowledge to overcome computational-based segmentation compared with completely automated segmentation (Oscar-Berman and Song, 2011). However, manual definition of ROI requires labor-intensive efforts, depends on rater experience, and prone to inter- and intra-rater variability. Automated volume measurement methods with high reproducibility and accuracy may thus be more efficient than manual volumetric methods.

Another methodological consideration is the small number of participants examined in the present work, which limited the power of the study and the inferences that could be made regarding the findings, especially when making so many statistical comparisons. It will be important to replicate these findings in larger future studies.

7.7 CONCLUSION

In conclusion, it was shown that musicians have WM plasticity in the cerebellum in the form of increased tract volume and number of streamlines of SCP and volume of MCP. These results extend the currently expanding musicians' literature suggesting structural plasticity in different brain regions. I hypothesize that these findings represent use-dependent structural adaptation resulting from enriched environmental stimuli in the form of skilful musical practice. These structural adaptations may be essential to support enhanced functional demands on the cerebellum in musicians. These results complement previously published GM findings in musicians' cerebella and highlight the importance of studying WM plasticity in musicians using DTI-based tractography.

CHAPTER 8

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

8.1 AIM

The aim of this chapter is to present a general discussion of results obtained in the present work. This include discussing differences/similarities in results of regional macrostructural measurements of the CC obtained in cohort 2 and 3A, and discussing possible roots of structural changes observed in musicians' brains notably, effect of age of starting musical training and developmental asynchronies. Furthermore, a discussion of whether the observed structural changes are training- or genetically-induced is provided. The chapter ends by providing general conclusion and recommendation for future studies.

8.2 GENERAL DISCUSSION

Findings of the present work showed that, compared to non-musicians, musicians had macroanatomical structural changes in Broca's area, CC, and cerebellum. Below is a summary of these findings:

8.2.1 STUDY 1- INVESTIGATING STRUCTURAL PLASTICITY OF POP AND PTR IN MUSICIANS

- Musicians possessed greater GM volume of the left POP, which was positively correlated with years of musical performance. There were no significant group differences in WM volumes.
- Musicians possessed greater cortical surface area of the left BA44.

8.2.2 STUDY 2- INVESTIGATING EFFECT OF MUSICAL EXPERTISE ON REGIONAL CALLOSAL MORPHOMETRY

- Professional musicians possessed greater cross-sectional area of CC1 and CC4 regions compared to amateur musicians and non-musicians. There were no significant group differences in CC2 and CC3 regions.
- There were no significant differences between amateur musicians and non-musicians.

8.2.3 STUDY 3- INVESTIGATING REGIONAL MACRO- AND MICROSTRUCTURAL PLASTICITY OF THE CC IN MUSICIANS

- Musicians possessed greater cross-sectional area, average FA, tract volume, number of streamlines, and reduced MD in CC4 region.
- There were no significant group differences in macrostructural and microstructural measures in CC1-CC3 regions.
- There was a negative correlation between age of starting musical training and cross-sectional area of CC4 region.
- There was a positive correlation between cross-sectional area and average FA of CC4 region in all subjects.

8.2.4 STUDY 4- INVESTIGATING STRUCTURAL PLASTICITY OF CEREBELLAR WM IN MUSICIANS

- Musicians possessed greater number of streamlines of the right SCP, tract volume of the right SCP, and tract volume of the right MCP.
- There were no significant group differences in FA values of SCP and MCP.
- Musicians possessed greater WM volume of the right cerebellum.
- There was no significant group difference in GM volume of cerebellar hemispheres.

I hypothesize that these findings represent use-dependent structural adaptations induced by enriched environment in the form of skilful musical performance. These structural adaptations were extensive enough to be observed at the macroanatomical level as revealed in MR images. They represent structural re-organization that is essential to support enhanced functional demands on musicians' brains. Although not yet resolved, possible microscopic changes underlying the observed findings include increased dendritic arborization, changes in synaptic bulk, formation of new axon collaterals, and generation of new neurons (Gould et al., 1999). More studies are required in the future to correlate MR-derived structural measures with histological data.

8.3 REGIOONAL CC MORPHOMETRY IN MUSICIANS, COMPARISON OF RESULTS OBTAINED IN COHORT 2 AND 3A

I have examined regional CC morphometry in two cohorts of musicians (cohort 2, study 2 and cohort 3A, study 3) using two methods: conventional morphometry to measure regional callosal macrostructure (cross-sectional areas) and DTI-based tractography to examine regional callosal microstructure (FA, MD, tract volume, and number of streamlines). Since an identical scheme was used to divide the CC, it is sensible to compare findings in both studies. Results obtained in both studies revealed that professional musicians had a greater cross-sectional area of CC4 region relative to non-musicians, which was corroborated and supported by microstructural findings (increased average FA, tract volume, number of streamlines, and reduced MD) in musicians in the same region in cohort 3A. In addition, there was a significant positive correlation between size and average FA value in CC4 region. Taken together, the above DTI-derived evidence may suggest that the increased CC4 size detected in both cohorts is associated with microscopic plasticity in the form of increased fiber density, fiber diameter, and thicker myelination (Mori and Zhang, 2006, Sun et al., 2006, Trivedi et al., 2009). Recent studies have suggested a novel technique that could measure axonal diameter and density in the CC (Alexander et al., 2010, Zhang and Alexander, 2010, Zhang et al., 2011), which could be used in future studies to further expand our knowledge about regional microstructural plasticity of the CC in musicians. In contrast to cohort 2, there was a significant negative correlation between CC4 size and age of starting musical tuition in cohort 3A. This discrepancy might be attributed to inter-individual variation in human anatomy. Furthermore, the effect of age of starting musical tuition is complex and the possibility of genetic/environmental interaction can not be excluded (to be discussed later).

The two cohorts showed contradicting results in measures of CC1 region. While professional musicians showed larger CC1 size in the second cohort, there was no significant group difference in CC1 size in the third cohort. Musicians of both cohorts were right-handed and had approximately equivalent demographics (age, age of starting musical training, and instrument specialty). Cohorts' differences in CC1 size could thus be attributed to differences in scanning parameters, influence of gender, and variability in cognitive abilities. Although subjects of both cohorts were examined using MPRAGE protocol, there were differences in magnetic field strength (cohort two, 1.5 Tesla; cohort three, 3 Tesla), and acquisition time (cohort two, 7.38 min.; cohort three, 4.14 min.), which might have different effects on regional cross-sectional area measurements. Variations in imaging parameters, such as through the use of different field strengths may result in image changes that are independent of the biological characteristics of the tissue, but rather reflect the physics of the imaging process (Fischl et al., 2004). It is important to rule out whether these changes could impact contrast properties of images thereby introducing differences and variability in morphometric brain measures that do not reflect biological effects, but rather technological ones and should thus be minimized. In a previous study performed at MARIARC, I specifically investigated the effect of scanning at different magnetic fields (1.5 Tesla versus 3 Tesla) on regional callosal morphometry in 10 healthy subjects (Abdul-Kareem et al., 2009) and found no significant effect of field strength on CC structure, hence this factor may be disregarded as a potential confounder in my results.

The second cohort included only male professional musicians, while the third included mixed gender. The morphology of the frontal lobe, which contains prefrontal and premotor regions, was shown to exhibit striking sex differences (Schlaepfer et al., 1995, Sowell et al., 2007, Taki et al., 2011). These gender-related structural differences in the frontal lobe were shown to be reflected on regional microstructural parameters of the CC. For example, a recent study revealed that males had significantly higher FA and lower MD compared to females in the genu and anterior body of the CC (Westerhausen et al., 2011), while no significant results detected in the isthmus and splenium. These findings may reflect higher axon density or stronger myelination in males. It is thus expected that males and females vary in the strength of inter-hemispheric callosal frontal connections. While these differences in inter-hemispheric connectivity might be related to gender differences in frontal lobe structure, it might also be linked to differences in frontal lobe functional asymmetry, such as memory function (Putnam et al., 2008), language lateralization (Josse et al., 2008) or executive functions (Huster et al., 2011). Cohorts' differences in CC1 size might thus be related to gender. Additionally, there are several subject's characteristics known to influence the size of the prefrontal cortex with consequent effect on CC1 size. For example, it has been shown that both the volume and GM density of the prefrontal cortex are correlated with scores of fluid intelligence tests (Gong et al., 2005); and a significant association was reported between total GM volume of the frontal cortex and a measure of general intelligence (Thompson et al., 2001). Likewise, the size (Narberhaus et al., 2008) and FA value (Salo et al., 2009) of the genu were shown to correlate with cognitive functions, such as attention and memory (Narberhaus et al., 2008), and intelligence quotient (IQ) level (Hutchinson et al., 2009). These parameters should have been taken into consideration when performing data analysis to verify any possible impact on CC1 size in both cohorts.

8.4 FACTORS CONTROLLING NEUROPLASTICITY IN MUSICIANS

Results of the present work revealed three factors that have potentially impacted structural plasticity in musicians' brains namely, duration of musical performance (which was positively correlated with left POP GM volume), musical expertise (professional musicians demonstrated larger cross-sectional areas of CC1 and CC4 regions compared to amateurs and non-musicians), and age of commencing musical tuition (which was negatively correlated with cross-sectional area of CC4 region).

Considering the different factors that could impact plasticity in musicians, the age of commencing musical tuition appears to be the most frequently implicated in previous studies. Mozart began piano training at the age of 3 and Beethoven before the age of 8. Many music programs for children emphasize that training should begin as early as possible for optimum musical skill development. The literature suggests the presence of a sensitive period during childhood when the network of the central nervous system is very plastic, and an intensive stimulation during this period would result in long-term structural-functional benefits. For example, increased cross-sectional area of the anterior CC (Schlaug et al., 1995), and increased MD in the corticospinal tract (Imfeld et al., 2009) were reported only in musicians who commenced training before age of seven years. Furthermore, stronger correlations between mean FA of the internal capsule and number of piano practicing hours were reported in children than in adults, although the number of hours practiced during childhood was much lower than in adulthood (Bengtsson et al., 2005). Behavioral studies verified the significance of early practicing through examining two groups of musicians (one group started musical training before age of seven years and the other started after that age) using a motor learning task. It was shown that musicians who commenced training before age of 7 performed better than the other group, although both groups were matched for years of musical training and experience (Hughes and Franz, 2007, Watanabe et al., 2007). In the auditory domain, behavioral studies comparing early- and late-trained musicians have shown that early training (around age of seven years) is essential for the development of absolute pitch ability (Baharloo et al., 1998, Costa-Giomi et al., 2001, Schlaug, 2001). Taken together, the above evidence emphasizes the significance of early practicing for inducing neuroplasticity and the existence of a critical period (possibly around the age of seven years) in the development of motor and auditory components of musical practice. The term ‘critical period’ was suggested after finding that certain behavior developed only if an appropriate stimulus is perceived during a specific period in development (Brainard and Knudsen, 1998, Knudsen, 2004). Therefore, one could propose that selecting a cohort of musicians who began their training early in life would probably result in more robust structural-functional differences compared to non-musicians. This assumption justified selecting cohorts of musicians (cohort 2 and 3) who commenced their training around age of seven years. The negative correlation between age of starting musical training and cross-sectional area of the isthmus and splenium reported in study 3 is in agreement with the ‘critical period’ assumption as 10/12 of recruited musicians had commenced their training before age of 7 years. It is important to note that despite the relatively older age of starting musical training in the first cohort, I could still detect significant results when comparing volume and cortical surface area of POP/BA44 (study 1). It appears that the effect of this factor is complex depending on the brain region examined. Consistent with my findings in study 1, several studies reported training-induced morphological brain changes even when the age of commencing skill acquisition was relatively old, such as in musicians (Bangert and Schlaug, 2006, Baumann et al., 2007), adult jugglers (Draganski, 2004), London taxi drivers (Maguire et al., 2000, Maguire et al., 2006), and musically naive stroke patients who undergone intonation-based rehabilitation therapy (Schlaug et al., 2009). The above evidence suggests that the assumption of the presence of a critical period around age of seven years should not be generalized, and that structural plasticity could still be seen even when subjects began their training at a relatively older age.

8.5 ISSUE OF BRAIN DEVELOPMENT

The GM and WM structures of the brain have distinct maturation patterns. The development of GM regions usually follows an inverted U-shaped curve with distinct rates in different regions. For example, in the frontal lobe (including Broca's area) cortical GM volume peaks at 9.5 years in girls and 10.5 years in boys. The development of WM, on the other hand, continues throughout childhood and adolescence (Ostby et al., 2009, Giedd et al., 2010). These developmental asynchronies among different brain regions and between GM and WM structures may indicate that in a single cohort, the effect of musical training is asynchronized among different brain regions.

8.5.1 THE CEREBELLUM

The cerebellum approaches its peak volume later than the cerebrum (Tiemeier et al., 2010). The total cerebellar volume follows an inverted U-shaped pattern of development compatible with the general growth pattern of GM peaking at age 11.8 years in females and 15.6 years in males (Tiemeier et al., 2010). The volume of the cerebellar cortex increases dramatically during the first two years of life reaching a lifetime maximum at that age, while the myelinated part of the cerebellum continues to increase in size into childhood and adolescence (Saksena et al., 2008, Ostby et al., 2009, Wu et al., 2011). It could thus be seen that the cerebellum has a longer development period relative to the cerebral hemisphere, which is mainly due to longer development of its WM possibly through the formation of new synapses, dendritic branching, axonal sprouting, and myelination. This would also mean that cerebellar WM is exposed to the effect of plasticity for a relatively longer period of time highlighting the significance of investigating plasticity in cerebellar WM and the major input/output cerebellar pathways (MCP and SCP) as that performed in the present work (study 4).

8.5.2 THE CORPUS CALLOSUM

The CC is one of the latest maturing neural networks of the brain as reported by MR and postmortem studies (Rakic and Yakovlev, 1968, Pujol et al., 1993). The CC size has been shown to increase up to the 4th decade (Hasan et al., 2008b) with maximal growth during childhood (Keshavan et al., 2002). A comparable development pattern in microstructural parameters was also shown (McLaughlin et al., 2007, Hasan et al., 2008a, Hasan et al., 2008b). Interestingly, structural maturation cycle within the CC shows a region-specific pattern with size increase slowing in an anterior to posterior direction meaning that the splenium would reach its maximum maturation later than the other parts (Giedd et al., 1999). In accordance, it has been shown that aging is associated with reduced FA in the genu but not in the splenium (Abe et al., 2002, Snook et al., 2007, Muetzel et al., 2008). This might imply that the splenium is exposed to environmental stimuli for a relatively longer duration compared to anterior CC regions, hence it may be more vulnerable to plastic effects. In two of the cohorts examined in the present work, musicians showed evidence of structural plasticity in the isthmus and splenium in the form of increased cross-sectional area (cohort 2 and 3A, study 2 and 3), increased average FA, tract volume, and number of streamlines (cohort 3A, study 3). The above findings could partly be explained by the theory of CC development.

8.6 ARE THESE STRUCTURAL CHANGES REPRESENT USE-DEPENDENT PLASTICITY OR GENETIC PREDISPOSITION FOR MUSIC?

It has been shown that hereditary factors can influence structural measures of brain regions, such as cortical surface area (Tramo et al., 1998), cortical volume (Joshi et al., 2011), and total brain volumes (Brun et al., 2009). Genetic influence appears to be region specific and vary between GM and WM structures. However, environmental factors do play a major role as can be reflected from the wealth of literature (Maguire et al., 2000, Draganski et al., 2004, Maguire et al., 2006, Aydin et al., 2007). The effect of environmental factors appears to be individual-specific and is not shared even between twins (Kremen et al., 2010).

It is still debatable whether the observed structural-functional changes in musician's brain result from intensive musical practice or merely represent an innate predisposition for music. After reviewing the wealth of literature and performing the current work, I would suggest the former explanation for the following reasons. First, structural and functional changes related to musical expertise were correlated both with the age of onset of musical training (Elbert et al., 1995, Schlaug et al., 1995, Amunts et al., 1997, Jancke et al., 1997, Pantev et al., 1998, Schlaug et al., 1998, Ohnishi et al., 2001, Schlaug, 2001, Lotze et al., 2003, Koeneke et al., 2004, Bengtsson et al., 2005, Imfeld et al., 2009) and duration of musical performance and practice intensity (Sluming et al., 2002, Hutchinson et al., 2003, Aydin et al., 2005, Abdul-Kareem et al., 2011). Second, structural and functional changes were correlated with the level of musical expertise (McAdams et al., 1995, Schneider et al., 2002, Gaser and Schlaug, 2003, Lotze et al., 2003, Shahin et al., 2008). Third, studies that compared two groups of instrumentalists have pointed out that differences were directly related to instrument type (Elbert et al., 1995, Pantev et al., 2001, Schneider et al., 2005, Bangert and Schlaug, 2006, Shahin et al., 2008). Fourth, evidence of structural-functional plasticity was shown even after short-term musical training in non-musicians yielding effects comparable to those found with long-term musical training (Pascual-Leone, 2001, Tremblay and Kraus, 2002, Lappe et al., 2008). Finally, Norton and colleagues (Norton et al., 2005) performed structural and functional MR study and applied a battery of tests (verbal, motor, musical, and visuospatial) to answer the question of whether pre-existing neural and cognitive markers exist in children who choose to participate in music training compared to children who were not planning to take music lessons. Authors reported no significant differences suggesting the lack of pre-existing markers between groups. Playing musical instrument, being a multimodal task that requires a host of skills, including motor, sensory, auditory, and visuospatial skills could thus be envisaged as a strong environmental factor that plays a major role in inducing structural plasticity in musicians' brains. Results of the present work are in line with this assumption as there was a correlation between structural measures and the duration of musical performance (study 1), musical expertise (study 2), and age of commencing musical training (study 3). It is important to realize that considerable inter-individual variations do exist in human anatomy, and that the interaction of environmental/genetic factors varies both among individuals and in different brain regions in the same individual. This might explain why the effect of factors, such as the duration of musical performance and age of commencing musical tuition was variable across studies conducted in the present work.

8.7 CONCLUSION

In conclusion, this work has extended the currently expanding literature through demonstrating novel evidence of structural plasticity in musician’s brain in regions known to subserve several musically relevant functions, including POP, CC, and cerebellum. The correlation of these findings with the duration of musical performance, musical expertise, and age of starting musical training may support the assumption that the observed structural changes in the brains of musicians represent use-dependent plasticity induced by musical performance rather than innate predisposition for being a musician. These structural changes would support enhanced functional demands on musicians’ brains. At the microscopic level, these macroanatomical findings may reflect increased dendritic arborization, thicker myelination, generation of new axonal projections and collaterals, and the formation of new neurons. However, more studies are required in the future to correlate MR-derived structural measures with histological data. Results of the present work can direct future structural-functional studies in musicians, and may be used as baseline data when designing rehabilitation programs for patients with neurological deficits.

8.8 RECOMMENDATIONS FOR FUTURE STUDIES

- More studies are required in the future to perform longitudinal investigations that may confirm results observed in cross-sectional studies. These studies may provide more valid results as they are more sensitive to individual differences in development. However, it is important to take into account standardization of scanning parameters, such as magnetic field strength, pulse sequence, and scanner manufacturer in addition to the use of unified measurement methods to allow unbiased data comparison.
- In line with my findings of structural enlargement of POP in musicians, future studies can examine cortical connections of this region in musicians. As the POP is considered the functional 'hub' of the mirror neuron system, it is reasonable to use DTI-based tractography to examine fiber connections between POP and other components of the mirror neuron system.
- It is interesting to investigate plasticity of POP and cerebellar peduncles in female subjects; insufficient number of females volunteered in the present work prohibited such investigation.
- Recent studies have proposed a technique to measure axonal diameter and fiber density in the CC (Alexander et al., 2010, Zhang and Alexander, 2010, Zhang et al., 2011). It is interesting to compare these measures between musicians and non-musicians, which could provide an additional support to the hypothesis of training-induced structural plasticity in musicians' brains.
- More studies are required in the future to examine functional plasticity of the cerebellum in line with results of the present work suggesting greater tract volume and number of streamlines of SCP and MCP, hence the possibility of widely distributed cerebellar networks through increased axonal projections and collaterals.
- Future studies should also explore the precise correlation between DTI-derived measures and histological properties of the nervous tissue. Although there were some studies that combined DTI with histological examination (Concha et al., 2010, Raya et al., 2011), the precise significance of DTI-derived quantitative

parameters, such as FA, MD, tract volume, and number of streamlines has not yet been resolved. Such exploration is essential for allowing valid interpretations of DTI results.

- It is important to apply our knowledge of training-induced structural-functional neuroplasticity in clinical fields, such as in rehabilitation medicine. A rehabilitation program based on musicians' studies would potentially promise an improvement of the functioning capabilities in subjects with neurological deficits. Few reports suggested that such approach is doable (Schneider et al., 2007, Schlaug et al., 2009) even following short-term musical training of musically naive patients. For instance, based on previously published functional data and the findings of increased GM volume and cortical surface area of the left POP reported here, researchers may selectively apply repetitive transcranial magnetic stimulation (a non-invasive procedure that utilizes magnetic fields to create electric currents in discrete brain areas) on the POP to help to restore function in patients with non-fluent aphasia.

  There are many neurodegenerative disorders, such as progressive supranuclear palsy, multiple system atrophy (Paviour et al., 2006), and Parkinson's disease that affect SCP and MCP, thereby producing a variety of motor, sensory, and cognitive deficits. In line with my findings of increased tract volume and fiber count of SCP and MCP, and the fact that the cerebellum subserves motor coordination and auditory discrimination functions, it might be feasible to design specific motor and/or auditory paradigms to provide means of inducing structural plasticity in these tracts. More studies would be needed; however, to determine whether rehabilitative changes induced by musical training are correlated with the duration of treatment and other factors that control this process, such as age and gender of patients, size and site of lesions.

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APPENDIX-

PUBLICATIONS AND AWARDS

Journal publications

1. **Abdul-Kareem IA & Sluming V (2008):** Heschl Gyrus and Its Included Primary Auditory Cortex: Structural MRI Studies in Healthy and Diseased Subjects. *JMRI*. 28(2):287-99.
2. **Abdul-Kareem IA, Stancak A, Parkes L & Sluming V (2009)**: Regional corpus callosum morphometry: Effect of field strength and pulse sequence. *JMRI*. 30(5):1184-90.
3. **Abdul-Kareem IA, Stancak A, Parkes L & Sluming V (2011)**: Increased gray matter volume of left pars opercularis in male orchestral musicians correlate positively with years of musical performance. *JMRI*. 33(1):24-32.
4. **Abdul-Kareem IA, Stancak A, Parkes L, Al-Ameen M, AlGhamdi J, Aldhafeeri F, Embleton K, Morris D & Sluming V (2011):** Plasticity of the superior and middle cerebellar peduncles in musicians revealed by quantitative analysis of volume and number of streamlines based on diffusion tensor tractography. *Cerebellum*. 10: 611-623*.*

Conference papers

1. **Abdul-Kareem IA & Sluming V (2009)**: Comparison of scanning at different field strengths and pulse sequences using voxel-based morphometry. *Proceedings* of *Salford Postgraduate Annual Research Conference, SPARC*. University of Salford, Manchester.

Abstracts presented at conferences and symposia (poster presentation)

1. **Abdul-Kareem IA, Stancak A, Parkes L & Sluming V (2009)**: Increased gray matter volume of left pars opercularis correlated positively with years of musical performance in male orchestral musicians. *British Neuroscience Association*, 20th National Meeting, Liverpool. Poster no. 4.14.
2. **Abdul-Kareem IA, Stancak A, Parkes L & Sluming V (2009)**: Regional corpus callosum morphometry: Effect of field strength and pulse sequence. *18th British Chapter ISMRM annual symposium for PhD students and Post-docs*. Imperial College, Hammersmith Hospital, London. Poster no. 1.
3. **Sluming V, Abdul-Kareem IA, Garcia-Finana M & Cowell PE (2009):** Morphometry of the corpus callosum: comparing male orchestral musicians and non-musicians. *International Society of Magnetic Resonance Imaging in Medicine, ISMRM conference*. Honolulu, USA. Poster no. 4956.
4. **Abdul-Kareem IA, Stancak A, Parkes L & Sluming V (2011):** Increased cross sectional area of genu and splenium of corpus callosum in professional musicians compared to amateur musicians and controls. *International Society*

of Magnetic Resonance Imaging in Medicine, ISMRM conference. Montreal, Canada. Electronic poster no. 7024.

5. **Abdul-Kareem IA, AlGhamdi J, Stancak A, Parkes L & Sluming V (2011)**: Plasticity of the superior and middle cerebellar peduncles in musicians revealed by DTI. *Organization of Brain Mapping Conference*, OHBM, Quebec, Canada, Poster no. 2234.

Abstracts presented at conferences and symposia (oral presentation)

1. **Abdul-Kareem IA & Sluming V (2009)**: Comparison of scanning at different field strengths and pulse sequences using voxel-based morphometry. *Salford Postgraduate Annual Research Conference, SPARC*. University of Salford, Manchester.
2. **Abdul-Kareem IA & Sluming V (2009)**: Increased cortical surface area of left Brodmann area 44 in orchestral musicians *Postgraduate Researchers in Science Medicine, PRISM conference*. University of Manchester, Manchester.

Prizes and awards

1. Poster prize winner (2nd prize), University of Liverpool poster day, 2010. Poster title: Increased gray matter volume of left pars opercularis in male orchestral musicians correlated positively with years of musical performance.
2. ISMRM award, Stockholm-Sweden, 2010.
3. ISMRM award, Montreal-Canada, 2011.