NEUROPLASTICITY OF IPSILATERAL CORTICAL MOTOR REPRESENTATIONS, TRAINING EFFECTS AND ROLE IN STROKE RECOVERY

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I, Damon Hoad confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

ABSTRACT

This thesis examines the contribution of the ipsilateral hemisphere to motor control with the aim of evaluating the potential of the contralesional hemisphere to contribute to motor recovery after stroke. Predictive algorithms based on neurobiological principles emphasize integrity of the ipsilesional corticospinal tract as the strongest prognostic indicator of good motor recovery. In contrast, extensive lesions placing reliance on alternative contralesional ipsilateral motor pathways are associated with poor recovery. Within the predictive algorithms are elements of motor control that rely on contributions from ipsilateral motor pathways, suggesting that balanced, parallel contralesional contributions can be beneficial. Current therapeutic approaches have focussed on the maladaptive potential of the contralesional hemisphere and sought to inhibit its activity with neuromodulation.

Using Transcranial Magnetic Stimulation I seek examples of beneficial plasticity in ipsilateral cortical motor representations of expert performers, who have accumulated vast amounts of deliberate practise training skilled bilateral activation of muscles habitually under ipsilateral control. I demonstrate that ipsilateral cortical motor representations reorganize in response to training to acquisition of skilled motor performance. Features of this reorganization are compatible with evidence suggesting ipsilateral importance in synergy representations, controlled through corticoreticulopropriospinal pathways.

I demonstrate that ipsilateral plasticity can associate positively with motor recovery after stroke. Features of plastic change in ipsilateral cortical representations are shown in response to robotic training of chronic stroke patients. These findings have implications for the individualization of motor rehabilitation after stroke, and prompt reappraisal of the approach to therapeutic intervention in the chronic phase of stroke.

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TABLE OF CONTENTS

ABSTRACT **5**

ACKNOWLEDGEMENTS **7**

LIST OF FIGURES **20**

LIST OF TABLES **23**

CHAPTER 1 INTRODUCTION **24**

1.1 **GENERAL INTRODUCTION** **24**

1.2 **THE IPSILATERAL HEMISPHERE IN MOTOR CONTROL** **26**

1.2.1 The Contralesional Hemisphere After Stroke and Ipsilateral Motor Pathways to Paretic Limbs 27

1.2.2 The Ipsilateral Hemisphere in Motor Control in Healthy Subjects 30

1.2.3 Excitatory and Inhibitory Neural Pathways Mediating Ipsilateral Activity 34

1.2.4 Descending Ipsilateral Motor Pathways in Skilled Motor Control 38

1.3 **NEUROANATOMY OF IPSILATERAL MOTOR PATHWAYS** **40**

1.3.1 Cortical Ipsilateral Motor Representations 41

1.3.2 Brainstem Ipsilateral Motor Pathways 45

1.3.3 Descending Ipsilateral Motor Pathways 46

1.4 **ANIMAL MODELS OF PLASTICITY IN IPSILATERAL MOTOR PATHWAYS** **51**

1.4.1 Translation of Animal Experimental Models to Stroke Rehabilitation 52

1.4.2 Animal Models of Cortical Reorganization 53

1.4.3 Animal Models of Plasticity in Brainstem Ipsilateral Motor Pathways 55

1.4.4 Animal Models of Plasticity in Segmental Ipsilateral Motor Pathways 56

1.4.5 Animal Models of Plasticity in Ipsilateral Motor Pathways Concurrently at Multiple Levels 58

1.5 **THE IPSILATERAL HEMISPHERE POST STROKE** **59**

1.5.1 Upregulation of Ipsilateral Motor Pathways After Stroke 60

1.5.2 Beneficial and Maladaptive Potential of Alternative Ipsilateral Motor Pathways After Stroke 62
1.5.3 Predicting Benefit from Ipsilateral Activity After Stroke 64
1.5.4 Specific Stroke Syndromes Demonstrating Beneficial Ipsilateral Plasticity 66
1.6 **USE OF CORTICAL MOTOR MAPPING AS A MARKER OF PLASTICITY IN IPSILATERAL MOTOR PATHWAYS 67**
1.6.1 Structural and Functional Organization of Cortical Motor Representations 68
1.6.2 Plasticity in Cortical Motor Representations 70
1.6.3 Deliberate Practise Effects on Cortical Synergy Representations 71
1.7 **BROAD HYPOTHESIS AND INTRODUCTION TO THE EXPERIMENTAL CHAPTERS 72**
CHAPTER 2 METHODS 74
2.1 **BASIC PRINCIPLES OF TMS 74**
2.2 **BIOPHYSICAL INTERACTIONS BETWEEN TMS AND CORTICAL MICROARCHITECTURE RELEVANT TO IPSILATERAL MOTOR MAPPING IN HEALTHY SUBJECTS AND AFTER STROKE 78**
2.2.1 Regional Variability of Induced Field Interactions with Neuronal Populations and Motor Map Outcome 78
2.2.2 Modelled Induced Field Interactions with Cortical Microcolumns 79
2.2.3 Macroscopic Factors Causing Variability in Induced Field Interactions with Neuronal Populations 81
2.2.4 Synchrony Effects on Ipsilateral Motor Mapping 83
2.2.5 Effect of Stroke Lesion on Induced Field Interactions with Neuronal Populations 84
2.3 **PRINCIPLES OF TMS APPLIED TO IPSILATERAL MOTOR PATHWAYS. THE FUNDAMENTAL CHARACTERISTICS OF IPSILATERAL MEPS 85**
2.4 **BACKGROUND ACTIVATION IS REQUIRED TO ELICIT IPSILATERAL MEPS 86**
2.4.1 Facilitation Profiles of Proximal and Axial Muscles 87
2.4.2 Timing TMS to Rise of Background Activation is Required to Evoke Ipsilateral MEPs 88

2.4.3 Task Dependency of Background Contraction Alters the Degree of Facilitation 89
2.4.4 Effect of Background Contraction on Motor Map Measures 90
2.5 **STIMULATION PARAMETERS FOR STUDYING IPSILATERAL MEPS..... 91**
2.5.1 Stimulation Intensity 91
2.5.2 Stimulation Intensity for Mapping Ipsilateral Motor Representations 92
2.5.3 Coil Orientation for Ipsilateral Motor Mapping 93
2.5.4 Coil Design for Ipsilateral Motor Mapping 94
2.5.5 Current Spread Considerations in Ipsilateral Motor Mapping 95
2.6 **RELIABILITY OF TMS MOTOR MAPPING 96**
2.6.1 TMS Motor Mapping Spatial Resolution 96
2.6.2 TMS Motor Mapping Temporal Reproducibility 97
2.7 **SENSORY AND ATTENTIONAL INFLUENCES ON IPSILATERAL MEPS 98**
2.8 **RECORDING IPSILATERAL MEPS 99**
2.8.1 Surface Electrodes and Composite Signals 99
2.8.2 Muscle to Electrode Distance Artefact 100
2.8.3 Stimulus Artefact Contaminating Short Latency Ipsilateral MEPs 101
2.8.4 Limitations of Using the MEP to Deduce the Nature of Ipsilateral Motor Pathways 103
2.9 **MEASURES OF IPSILATERAL MEPS AND TMS MOTOR MAPPING..... 106**
2.9.1 MEP Area 106
2.9.2 Normalized MEP 108
2.9.3 Ipsilateral to Contralateral Ratio 109
2.9.4 Laterality Index 110
2.9.5 Map Volume 111
2.9.6 Centre of Gravity 112
CHAPTER 3 EFFECTS OF DELIBERATE PRACTISE OF A BILATERAL MOTOR TASK ON IPSILATERAL CORTICAL MOTOR REPRESENTATIONS IN ELITE ATHLETES 113
3.1 **INTRODUCTION 113**

3.1.1 Hypothesis 115
3.1.2 Basis for Hypothesis 116
3.2 **METHOD 118**
3.2.1 Overview of Experimental Design 118
3.2.2 Subjects 119
3.2.3 Data Acquisition 121
3.2.4 Data Reduction and Analysis 126
3.2.5 LIMITATIONS OF METHOD 129
3.3 **RESULTS 136**
3.3.1 Strength of Cortical Representations 136
3.3.2 Asymmetry and Focality of Contralateral and Ipsilateral Motor Representations 143
3.3.3 Organization of Cortical Representations 145
3.3.4 Summary of Results 151
3.4 **DISCUSSION 152**
3.4.1 Main Findings 152
3.4.2 Overview of Unifying Interpretation of Findings Relating to Ipsilateral Motor Control 153
3.4.3 Task-Specificity of Observed Ipsilateral and Contralateral Effects 154
3.4.4 Relative Strength of Contralateral and Ipsilateral Representations 156
3.4.5 A Synergy Based Mechanistic Explanation 157
3.4.6 Possible Increased Direct Ipsilateral Corticofugal Output 159
3.4.7 Secondary Motor Areas as a Potential Explanation for Map Expansion – a Potential Role for SMA 160
3.4.8 Developing a Synergy Based Explanation 163
3.4.9 Supportive Context from TMS Studies 164
3.4.10 Supportive Context from Studies of Structural Plasticity 166
3.4.11 Deliberate Practise and Experience Dependent Plasticity 171
3.4.12 Genetic and Epigenetic Determinants of Ipsilateral Plasticity 173
3.4.13 Summary 174

CHAPTER 4 LONGITUDINAL EFFECTS OF TRAINING A BILATERAL MOTOR TASK ON IPSILATERAL CORTICOMOTOR CONNECTIVITY 176

4.1 **INTRODUCTION** **176**
4.1.1 Hypothesis 177
4.1.2 Basis for Hypothesis 178
4.2 **METHOD** **181**
4.2.1 Experimental Design 181
4.2.2 Subjects 182
4.2.3 Training 183
4.2.4 Data Acquisition 184
4.2.5 Data Reduction and Analysis 190
4.2.6 Methodological Limitations 195
4.3 **RESULTS** **199**
4.3.1 Compliance with Training 199
4.3.2 Functional Classification of Electrode Positions 200
4.3.3 Behavioural Measures 201
4.3.4 Analysis by Respiratory Phase and EMG Calculation of Patterns of Muscle Use 202
4.3.5 Change in patterns of muscle use over time with training 203
4.3.6 Change in Diaphragm Percentage Contribution to Total EMG is Driven by the Expiratory Phase 204
4.3.7 Change in MEPs Over Time 207
4.4 **DISCUSSION** **210**
4.4.1 Main Findings 210
4.4.2 Training Related Changes in EMG Calculated Patterns of Muscle Use 212
4.4.3 A Mechanistic Proposal to Explain why EMG Change was Limited to Diaphragm 214
4.4.4 Possible Non-Neural Alternative Explanations for the EMG findings 217
4.4.5 Temporal Evolution of EMG Change Between 0 and 6 Months, but not Between 6 and 12 Months 219
4.4.6 Why EMG Change Was Not Paralleled by MEP Change 220
4.4.7 Change in MEP Size With Training 223
4.5 **SUMMARY** **229**

CHAPTER 5 EFFECTS OF DELIBERATE PRACTISE OF A BILATERAL MOTOR TASK ON IPSILATERAL CORTICAL MOTOR REPRESENTATIONS IN ELITE SINGERS 231

5.1 **INTRODUCTION 231**

5.1.1 Hypothesis 233

5.1.2 Basis for Hypothesis 234

5.2 **METHOD 237**

5.2.1 Overview of Experimental Design 237

5.2.2 Subjects 238

5.2.3 Data Acquisition 240

5.2.4 Data Reduction and Analysis 244

5.2.5 Methodological Limitations 246

5.3 **RESULTS 247**

5.3.1 Cortical Motor Maps 247

5.3.2 Evidence of experience dependent plastic change in the size of the motor representation 250

5.3.3 Evidence of experience dependent plastic change in the organization of the motor representations – absolute differences in CoGx and CoGy coordinates 252

5.3.4 Evidence of experience dependent plastic change in the organization of the motor representations – differences in the position of ipsilateral representations relative to contralateral representations 254

5.4 **DISCUSSION 256**

5.4.1 Main findings 256

5.4.2 A mechanistic explanation of positive contralateral and negative ipsilateral findings based on limits of interaction between voluntary and involuntary pathways of respiratory motor control 259

5.4.3 Possible Corticocortical Mechanisms Underlying Contralateral Diaphragm Map Change 263

5.4.4 Cingulate Motor Area Contributions to Corticocortical Control of Diaphragm Motor Maps 264

5.4.5 Supplementary Motor Area Contributions to Corticocortical Control of Diaphragm Motor Maps 268

5.4.6 How study of plastic responses to lesions of voluntary and involuntary respiratory pathways contributes to interpretation of the findings 271

5.5 **SUMMARY** **273**

CHAPTER 6 LONGITUDINAL EFFECTS OF TRAINING A REACH TASK ON IPSILATERAL CORTICAL REPRESENTATIONS IN THE CONTRALESIONAL HEMISPHERE IN THE CHRONIC PHASE OF STROKE **274**

6.1 **INTRODUCTION** **274**

6.1.1 Hypothesis 276

6.1.2 Basis for Hypothesis 277

6.2 **METHOD** **279**

6.2.1 Overview of Experimental Design 279

6.2.2 Subjects 280

6.2.3 Training 282

6.2.4 Data Acquisition 283

6.2.5 Data Reduction and Analysis 288

6.2.6 Methodological Limitations 293

6.3 **RESULTS** **298**

6.3.1 Patient Baseline Characteristics 298

6.3.2 Grouping Patients According to Functional Response to Training 301

6.3.3 Change in Corticomotor Connectivity with Training 302

6.3.4 Change in Laterality Index with Training 303

6.3.5 Strength of Ipsilateral Cortical Motor Representations in Contralesional 305

6.3.6 Strength of Contralateral Cortical Motor Representations in Ipsilesional Hemisphere Pre and Post Training 308

6.3.7 Measures of Organization of Cortical Representation in the Contralesional and Ipsilesional Hemispheres Pre and Post Training 311

6.3.8 Associations Between Measures of Spasticity and Measures of Ipsilateral Activity 314

6.3.9 Associations Between Functional Upper Limb Performance and Measures of Ipsilateral Activity 316

6.3.10 Summary of Results 319

6.4 **DISCUSSION** **320**

6.4.1 Main Findings 320

6.4.2 Proposing Beneficial Ipsilateral Plasticity Requires Awareness of Maladaptive Potential 321
6.4.3 Interpreting Ipsilateral Findings in the Context of the Competitive Interference Model 322
6.4.4 Maladaptive Synergies and Ipsilateral Pathways 328
6.4.5 Beneficial Plasticity in Ipsilateral Pathways 334
6.5 **SUMMARY 338**

CHAPTER 7 ASSOCIATION BETWEEN FUNCTIONAL PERFORMANCE AND IPSILATERAL CORTICAL MOTOR REPRESENTATIONS IN THE CHRONIC PHASE OF STROKE 340

7.1 **INTRODUCTION 340**
7.1.1 Hypothesis 342
7.1.2 Basis for Hypothesis 343
7.2 **METHOD 345**
7.3 **RESULTS 346**
7.3.1 Characteristics of Low and High Performance Groups 346
7.3.2 Cortical Maps 347
7.3.3 Relationship Between Strength of Ipsilateral Cortical Representation of Paretic Muscles in the Contralesional Hemisphere and Functional Motor Recovery After Stroke 348
7.3.4 Relationship Between Size of Ipsilateral Representation and Upper Limb Function 349
7.3.5 Summary of Results 355
7.4 **DISCUSSION 356**
7.4.1 Main Findings 356
7.4.2 Corticocortical Connectivity Between Premotor Cortex and Primary Motor Cortex and Paretic Reach 358
7.4.3 Functional Aspects of Premotor Cortex Relevant to Control of Representation of Contralesional Paretic Reach – Action Encoding and Arm Selection 359
7.4.4 The Role of PMC in Encoding Synergies 360
7.4.5 Corticocortical Connectivity of PMC in Primates Demonstrates Intracortical Effects Capable of Influencing Primary Motor Cortex Motor Representations 361

7.4.6 Functional Connectivity of PMC and Primary Motor Cortex in Humans Demonstrates Intracortical Effects Capable of Influencing Primary Motor Cortex Motor Representations....362
7.4.7 Corticospinal Output from PMC Demonstrates Anatomical Organization Suited to Control of Ipsilateral Contribution to Synergies....363
7.4.8 PMC May Influence Ipsilateral Synergy Control Both by Corticocortical and Corticospinal Connectivity....365
7.4.9 Animal Models of the Role of PMC in the Post Stroke Motor Network....366
7.4.10 The Role of PMC in the Human Post Stroke Motor Network....368
7.4.11 Direct Demonstration That PMC Can Represent Ipsilateral Synergies in Humans....370
7.4.12 A Possible Explanation as to Why Ipsilateral Changes were Identified in Pectoralis Major in the Chronic Stage, but Emerged in Deltoid with Training....371
7.5 **SUMMARY....373**

CHAPTER 8 DISCUSSION....375

8.1 **A review of the main findings in relation to a hypothesis that the ipsilateral hemisphere contributes to skilled motor control through cortical synergy representations....376**
8.1.1 Athlete Study....376
8.1.2 Singers Study....379
8.1.3 Stroke Study....381
8.1.4 Pursuing a Unifying Explanation Based on Cortical Synergy Representations....384
8.2 **Basic functioning and hierarchical organization of synergy control....385**
8.2.1 Cortical, Subcortical and Spinal Synergy Modules....387
8.3 **Cortical representation of synergies....388**
8.3.1 Focal, Hard Wired and Widely Distributed, Dynamic Cortical Synergy Modules....389
8.3.2 Cortical Topography of Synergy Representations....391
8.4 **Cortical control of synergy representations....393**
8.4.1 Cortical Controllers Utilize Spatial and Temporal Summation of Synergy Modules....394

8.4.2 Corticocortical Control of Synergy Representations 395
8.4.3 Oscillatory Control of Cortical Synergy Representations 396
8.5 **Cortical fractionation and stabilization of synergy representations 398**
8.5.1 Functional and Anatomical Recurrent Intracortical Connectivity and Synergy Representations 399
8.5.2 Canonical Cortical Microcircuitry and Reorganization of Synergy Representations 400
8.6 **DELIBERATE PRACTISE EFFECTS ON CORTICAL SYNERGY REPRESENTATIONS 401**
8.7 **AXIAL AND RESPIRATORY SYNERGIES 402**
8.7.1 Axial Synergies 403
8.7.2 Respiratory Synergies 405
8.8 **PROPRIOSPINAL NEURONES AS EFFECTORS OF IPSILATERAL CORTICAL SYNERGY REPRESENTATIONS 406**
8.8.1 Propriospinal Neurones and Synergy Control. 407
8.8.2 Evidence of Propriospinal Activity in Humans 409
8.8.3 Evidence of Propriospinal Activity in Human Synergy Control 410
8.8.4 Functional Organization of Propriospinal Circuits 412
8.8.5 Propriospinal Activity After Stroke 415
8.9 **CORTICORETICULAR INPUTS TO PROPRIOSPINAL CIRCUITS ... 417**
8.9.1 Corticoreticulospinal Pathways and Ipsilateral Motor Control 418
8.9.2 Ipsilateral Reticulospinal Pathways After Stroke 419
8.9.3 Possible Ipsilateral Reticulospinal Pathways of Skilled Motor Control 420
8.9.4 Ipsilateral Cortical Origins of Corticoreticulospinal Pathways ... 421
8.10 **EVIDENCE FOR IPSILATERAL SYNERGY REPRESENTATIONS FUNCTIONING VIA CORTICORETICULOPROPRIOSPINAL PATHWAYS 422**
8.11 **THE IPSILATERAL HEMISPHERE IN STROKE REHABILITATION 425**
8.11.1 Proportional Recovery and Patient Selection According to Ipsilateral Neurophysiological Measures 426
8.11.2 Therapeutic Strategies Based on Ipsilateral Cortical Synergy Representations, Ipsilateral Corticoreticulopropriospinal Pathways and Proportional Recovery 428

8.11.3 Robotic Synergy Based Therapy as a Therapeutic Strategy to Optimize Ipsilateral Motor Control....430
8.11.4 Neuromodulation and Ipsilateral Cortical Synergy Modules....432
8.12 **RELEVANCE OF ACTIVITY DEPENDENT AND EXPERIENCE DEPENDENT PLASTICITY IN EXPERTS TO STROKE REHABILITATION....433**
8.12.1 The Natural History of Motor Recovery in the Acute and Chronic Post Stroke Phase....434
8.12.2 Relevance of Accumulated Deliberate Practise in Athletes to Motor Recovery in the Chronic Post Stroke Phase....436
8.12.3 Fast and Slow Plasticity of Cortical Reorganization....438
8.12.4 Immediate Early Genes and Late Genes of Long Term Potentiation and Cortical Representations....439
8.12.5 The Timing of Plasticity of Ipsilateral Cortical Representations....440
CHAPTER 9 CONCLUDING REMARKS....441
Glossary of Technical Terms....443
List of Abbreviations....453
REFERENCES....455
APPENDICES....530

LIST OF FIGURES

FIGURE 1.1 BILATERAL AXIAL MOTOR EVOKED POTENTIALS 37
FIGURE 1.2 IPSILATERAL MEPS ARE UPREGULATED IN THE PARETIC LIMB FOLLOWING STROKE 61
FIGURE 2.1 SAMPLE OF BILATERAL SCALENE MEPS AND ILLUSTRATION OF STIMULUS ARTEFACT 102
FIGURE 2.2 ILLUSTRATION OF CRITERIA FOR MEASUREMENT OF MEP AREA 107
FIGURE 3.1 MAPPING AXIAL MUSCLES. 137
FIGURE 3.2 BILATERAL ABDOMINAL OBLIQUE MEPS SHOWING DEVELOPING MOTOR MAP 138
FIGURE 3.3 NORMALIZED MEANS AND CI (BACK TRANSFORMED) FOR EACH MUSCLE IN EACH GROUP. 139
FIGURE 3.4 TRIPLE INTERACTION BETWEEN GROUP, SIDE AND MUSCLE (PLOTTED USING ESTIMATED MARGINAL MEANS) 140
FIGURE 3.5 INTERACTION BETWEEN GROUP AND SIDE FOR EACH MUSCLE 141
FIGURE 3.6 EFFECT OF GROUP ON SIDE IN PECTORALIS MAJOR AND ABDOMINAL OBLIQUE 142
FIGURE 3.7 NORMALIZED MAP VOLUME FOR EACH MUSCLE. 144
FIGURE 3.8 PLOTS OF COG. 146
FIGURE 3.9 EFFECT OF GROUP ON CONTRALATERAL COGY. 147
FIGURE 3.10 A SIGNIFICANT EFFECT OF MUSCLE ON IPSILATERAL COGY. 148
FIGURE 4.1 EMG RECORDING DURING VOLUNTARY RESPIRATORY MANOEUVRES187
FIGURE 4.2 ILLUSTRATION OF THE DIVISION OF PASSAGE OF SONG FOR EMG ANALYSIS 192
FIGURE 4.3 CHANGE IN PERCENTAGE CONTRIBUTION OF DIAPHRAGM TO TOTAL EMG ACTIVITY DURING SONG (AVERAGE OF ALL RECORDED PHASES) OVER TIME 203
FIGURE 4.4 CHANGE OVER TIME IN DIAPHRAGM CONTRIBUTION TO TOTAL EMG RECORDED IN EXPIRATORY PHASES OF SONG 205
FIGURE 4.5 CHANGE IN PERCENTAGE CONTRIBUTION OF DIAPHRAGM TO TOTAL EMG OVER TIME FOR EACH SUBJECT AT EACH TIME POINT FOR INSPIRATORY PHASES (LEFT HAND GRAPH) AND EXPIRATORY PHASES (RIGHT HAND GRAPH). 205
FIGURE 4.6 CHANGE IN MEAN NORMALIZED MEP AREA OVER TIME FOR CONTRALATERAL SCALENE (TOP GRAPH) AND IPSILATERAL SCALENE (BOTTOM GRAPH). 208
FIGURE 5.1 CORTICAL MAPS OF DIAPHRAGM REPRESENTATION FOR EACH INDIVIDUAL IN EACH GROUP 248
FIGURE 5.2 MAPPING AXIAL RESPIRATORY MUSCLES 249

FIGURE 5.3 GRAPHS OF MEAN MAP AREA FOR CONTRALATERAL (TOP GRAPH) AND IPSILATERAL (BOTTOM GRAPH) DIAPHRAGM REPRESENTATION 251
FIGURE 5.4 COG OF PECTORALIS MAJOR, SHOWN FOR TRAINING, INTERMEDIATE AND EXPERT GROUPS..... 253
FIGURE 5.5 THE RELATIONSHIP BETWEEN POSITION OF IPSILATERAL REPRESENTATIONS RELATIVE TO CONTRALATERAL REPRESENTATIONS IS SHOWN FOR DIAPHRAGM (TOP), SCALENE (MIDDLE) AND PECTORALIS MAJOR (BOTTOM). 255
FIGURE 6.1 SAMPLE OF BILATERAL MEPS IN BICEPS MUSCLES OF A STROKE PATIENT..... 300
FIGURE 6.2 MEAN NORMALIZED IPSILATERAL MEP AREA RECORDED FROM PARETIC LIMB TO STIMULATION OF THE CONTRALESIONAL HEMISPHERE, AVERAGED FROM ALL SUBJECTS IN THE IMPROVEMENT GROUP 302
FIGURE 6.3 CHANGE IN LATERALITY INDEX WITH TRAINING, EXPRESSED AS POST-PRE FOR EACH SUBJECT SHOWING IMPROVEMENT WITH TRAINING..... 304
FIGURE 6.4 CORTICAL MAPS OF THE IPSILATERAL REPRESENTATION IN THE CONTRALESIONAL HEMISPHERE FOR EACH MUSCLE IN EACH OF THE 9 SUBJECTS THAT SHOWED FUNCTIONAL IMPROVEMENT WITH TRAINING ... 306
FIGURE 6.5 STRENGTH OF THE IPSILATERAL REPRESENTATION IN THE CONTRALESIONAL HEMISPHERE OF EACH OF THE MUSCLES IN THE PARETIC LIMB PRE AND POST TRAINING. GROUP AVERAGES FOR THE 9 SUBJECTS IN THE IMPROVEMENT GROUP ARE SHOWN..... 307
FIGURE 6.6 CORTICAL MAPS OF THE CONTRALATERAL REPRESENTATION IN THE IPSILESIONAL HEMISPHERE FOR EACH MUSCLE IN EACH OF THE 9 SUBJECTS IN THE IMPROVEMENT GROUP 309
FIGURE 6.7 STRENGTH OF THE CONTRALATERAL REPRESENTATION IN THE IPSILESIONAL HEMISPHERE OF EACH OF THE MUSCLES IN THE PARETIC LIMB PRE AND POST TRAINING..... 310
FIGURE 6.8 THE SIZE AND DIRECTION OF CHANGE IN CENTRE OF GRAVITY X (TOP ROW) AND Y COORDINATES (BOTTOM ROW) OF IPSILATERAL REPRESENTATION OF PARETIC MUSCLES IN THE CONTRALESIONAL HEMISPHERE, PRE AND POST TRAINING..... 312
FIGURE 6.9 THE SIZE AND DIRECTION OF CHANGE IN CENTRE OF GRAVITY X AND Y COORDINATES OF CONTRALATERAL MUSCLES FROM BASELINE AFTER TWO WEEKS OF TRAINING IN THE IPSILESIONAL HEMISPHERE..... 313
FIGURE 6.10 PLOTS OF SPASTICITY AGAINST MEASURES OF IPSILATERAL ACTIVITY IN THE UNAFFECTED HEMISPHERE..... 315
FIGURE 6.11 RELATIONSHIP BETWEEN BASELINE DELTOID LATERALITY INDEX AND BASELINE FUGL-MEYER UPPER LIMB SUBSET SCORE 317

FIGURE 6.12 RELATIONSHIP BETWEEN BASELINE DELTOID LATERALITY INDEX AND CHANGE IN FUGL-MEYER SCORE WITH TRAINING 317
FIGURE 6.13 RELATIONSHIP BETWEEN CHANGE IN FUGL-MEYER UPPER LIMB SUBSET SCORE WITH TRAINING AND CHANGE IN LATERALITY INDEX WITH TRAINING 318
FIGURE 7.1 CORTICAL MAPS FOR IPSILATERAL AND CONTRALATERAL PECTORALIS MAJOR FOR EACH SUBJECT IN THE LOW AND HIGH PERFORMANCE GROUPS 347
FIGURE 7.2 THE MEAN SIZE OF THE IPSILATERAL CORTICAL REPRESENTATION OF PARETIC PECTORALIS MAJOR IN THE CONTRALESIONAL HEMISPHERE IN THE LOW PERFORMANCE AND HIGH PERFORMANCE GROUPS 348
FIGURE 7.3 RELATIONSHIP BETWEEN NORMALIZED MEAN MAP AREA AND FUGL-MEYER UPPER LIMB SUBSET FOR PECTORALIS MAJOR 349
FIGURE 7.4 PLOT OF MEAN CENTRE OF GRAVITY, AVERAGED ACROSS MUSCLES, FOR THE HIGH AND LOW PERFORMANCE GROUPS..... 350
FIGURE 7.5 DIFFERENCE IN MEAN COGX (TOP GRAPH) AND COGY (BOTTOM GRAPH) BETWEEN HIGH AND LOW PERFORMANCE GROUPS..... 351
FIGURE 7.6 CENTRE OF GRAVITY X COORDINATE (LEFT GRAPH) AND Y COORDINATE (RIGHT GRAPH) FOR PECTORALIS MAJOR 352
FIGURE 7.7 POSITION OF THE IPSILATERAL PECTORALIS MAJOR REPRESENTATION RELATIVE TO THE POSITION OF THE CONTRALATERAL REPRESENTATION IN THE CONTRALESIONAL HEMISPHERE 353
FIGURE 7.8 ASSOCIATION BETWEEN THE DISPLACEMENT OF PECOTRALIS MAJOR IPSILATERAL AND CONTRALATERAL COGX IN THE CONTRALESIONAL HEMISPHERE AND FUGL-MEYER UPPER LIMB SUBSET SCORE (LEFT GRAPH). 354

LIST OF TABLES

TABLE 4.1 COMPARISON OF ANATOMICAL AND FUNCTIONAL DESCRIPTIONS OF ELECTRODE POSITION, BASED ON EMG BEHAVIOUR IN QUIET AND VOLUNTARY BREATHING. 200
TABLE 4.2 CHANGE IN CONTRIBUTION OF EACH MUSCLE TO THE TASK, AVERAGED FOR THE GROUP, AT EACH TIME POINT, IN INSPIRATORY AND EXPIRATORY PHASES AND THE TOTAL OF ALL PHASES ACROSS THE SONG. 202
TABLE 6.1 PATIENT CHARACTERISTICS, BASELINE AND POST TRAINING MEASURES 299
TABLE 7.1 THE CHARACTERISTICS OF THE STROKE PATIENTS IN THE HIGH PERFORMANCE AND LOW PERFORMANCE GROUP 346

CHAPTER 1
INTRODUCTION

1.1 GENERAL INTRODUCTION

This thesis examines the contribution of the ipsilateral hemisphere to skilled motor control. Examples are sought of neuroplastic change in ipsilateral motor pathways driven by deliberate practise in experts. It is hoped that greater understanding of ipsilateral motor control may inform the development of therapeutic approaches to optimally utilize the plastic resources of the contralesional hemisphere in motor rehabilitation after stroke. The functional organization of cortical movement representations in the ipsilateral hemisphere lends itself to the use of Transcranial Magnetic Stimulation to describe ipsilateral neuroplastic change.

The contribution of the ipsilateral hemisphere to motor control was studied by the founding physicians of the National Hospital in the 1860's, influenced by two of Queen Victoria's physicians. Sir William Gull of Guy's Hospital noted that in complete and sudden paralysis of the limbs, muscles closest to the trunk will recover first. Sir William Broadbent of St Mary's Hospital wrote that in upper motor neurone lesions, muscles that are habitually bilaterally activated will be relatively spared. Both are statements to the effect that ipsilateral corticofugal output from the contralesional hemisphere serves motor recovery of the paretic limb after stroke. Archival records from nineteenth century Queen Square record the use of voltaic stimulation of the contralesional hemisphere in the treatment of spastic paresis after stroke.

A century and a half later and a series of studies using similar neuromodulation of the ipsilateral hemisphere propose an elucidated mechanism underpinning an important ipsilateral contribution to motor control. The timing of this coincides with a paradigm shift in approach to motor rehabilitation after stroke, driven by robust observations that upper limb motor recovery is reliably predicted by contralateral corticospinal tract integrity from the ipsilesional hemisphere. Within the algorithms that

formalize this contralateral imperative are measures that paradoxically incorporate some implicit degree of reliance on ipsilateral neural pathways. This ipsilateral aspect is subtle and does not detract from the robust simplicity of the contralateral prediction. To balance this, the potential for the contralesional hemisphere to be maladaptive in the process and impair the final quality of recovered movement has been well demonstrated clinically and experimentally. Examples of good motor recovery relying on contralesional ipsilateral pathways recur throughout the literature as notable exceptions, but are overshadowed by the weight of maladaptive potential. The notable exceptions recur with sufficient frequency to conclude subgroups of patients exist for whom the plastic reserve of ipsilateral alternative motor pathways from the contralesional hemisphere provide substrates to be therapeutically exploited. Identifying these subgroups is one challenge to which neurophysiology measures are clinically predisposed.

One objective is to place recognition of contralesional potential for harm or benefit within the broader movement toward individualizing stroke rehabilitation according to tract integrity. Another objective is to use knowledge of contralesional activity to inform development of therapeutic intervention. The experimental approach employed is structured towards this. Firstly, plastic change in ipsilateral motor pathways should be most evident in representations of muscles under strong innervation by ipsilaterally descending motor pathways. Secondly, activity in these pathways should be most evident when those muscles are engaged in forms of movement known to be represented in the ipsilateral hemisphere. Finally, neuroplastic change in ipsilateral motor pathways should be most evident in those who extensively train skilled bilateral movements. Studying representations of axial synergies in experts who have spent thousands of hours training should provide the clearest examples if ipsilateral plastic change is used to serve skilled motor control. Use of Transcranial Magnetic Stimulation allows cortical representations in the ipsilateral hemisphere and output through ipsilateral corticofugal pathways to be studied.

1.2 THE IPSILATERAL HEMISPHERE IN MOTOR CONTROL

Recent experimental evidence has prompted rethinking of long held views of basic functional organization of contralateral and ipsilateral motor pathways. Ipsilateral motor responses are easily observed in proximal muscles, becoming absent more distally where contralateral corticospinal and corticomotorneuronal control dominates. Two recent experimental observations prompt more careful consideration of this basic scheme of a gradient from unskilled proximal ipsilateral to skilled distal contralateral control. Firstly, evidence emerged to suggest highly skilled proximal corticomotorneuronal control with the finding in primates that proximal and distal motor representations are intermixed within New M1 regions originating corticomotorneurones (Rathelot and Strick, 2009). This may account for the early TMS observation of monosynaptic facilitation of deltoid (Colebatch et al., 1990) amidst the steep distal to proximal gradient of monosynaptic corticospinal facilitation (Palmer and Ashby, 1992). Secondly, at the same time that proximal corticomotorneuronal representations were described, the converse situation was reported, with ipsilateral motor pathways traditionally considered to provide unskilled proximal control shown to act on hand motorneurones in primates. Monosynaptic excitatory post synaptic potentials in cervical motorneurones innervating intrinsic hand muscles were produced in response to ipsilateral reticulospinal tract stimulation (Riddle et al., 2009).

These observations suggest the role of the ipsilateral hemisphere in skilled motor control is more complicated than the view traditionally offered in standard accounts. Revisiting the role of the ipsilateral hemisphere leads to the related question of the role of the unaffected contralesional hemisphere in recovery after brain injury.

1.2.1 The Contralesional Hemisphere After Stroke and Ipsilateral Motor Pathways to Paretic Limbs

Robust algorithms predicting motor recovery after stroke have been developed over the last decade. These incorporate clinical, neurophysiological and neuroimaging measures (Stinear et al., 2012). Accurate prognostication offers the ability to individualize therapeutic intervention and deploy rehabilitation resources efficiently. Integrity of the ipsilesional corticospinal tract is the strongest neurobiological predictor of good motor recovery of the paretic upper limb. The strongest clinical predictors of recovery of dexterity and ambulatory status are strength of finger extension, shoulder abduction and independent trunk control (Kwah and Herbert, 2016). Finger extension relies on contralateral corticospinal and corticomotorneuronal tracts, whereas shoulder abduction and trunk control draw heavily on ipsilateral motor pathways. The ipsilateral contribution to strength of shoulder abduction is estimated around 40% (Colebatch and Gandevia, 1989). For habitually bilaterally activated trunk muscles, ipsilateral and contralateral post-synaptic facilitation may be comparable in size and latency suggesting common drive (Carr et al., 1994). Without detracting from the emphasis placed on contralateral motor pathways in predictive algorithms, parallel balanced ipsilateral activity is likely to contribute to optimal motor recovery.

Balance of ipsilateral and contralateral activity supports skilled motor control in healthy subjects (Perez and Cohen, 2009). This balance is not yet fully characterized, and its behaviour varies between unimanual and bimanual tasks, and scales with motor task complexity. Imbalances occur after brain injury. Most experimental therapeutic interventions have focussed on correcting imbalance of Interhemispheric Inhibition (IHI) to free the ipsilesional hemisphere from excessive inhibitory influence driven by the contralesional hemisphere. Excessive IHI is thought to limit ipsilesional deployment of plastic reserve, limiting recovery. It is accepted that some patients with extensive ipsilesional corticospinal tract damage will rely on ipsilateral pathways, and this reliance is thought to be a marker

of poor recovery. Unrestrained contralesional activity has been associated with maladaptive plasticity, driving spasticity and movements with unwanted coactivation.

The ability to predict spontaneous neurobiological recovery now makes it vital to correctly classify patients according to the potential consequence of contralesional activity. In those with the potential to recover well, avoiding excessive contralesional activity may limit unwanted IHI and prevent maladaptive coactivation to improve the final quality of recovered movement. For those who will make poor recovery and rely on alternative ipsilateral motor pathways, optimizing contralesional control becomes important. There is a need for measures to assess balance of ipsilesional and contralesional activity and plastic potential to individualize therapeutic intervention. Inappropriately inhibiting the contralesional hemisphere may remove potentially useful contributions to motor control. Conversely, in other settings inappropriately engaging the contralesional hemisphere may impede recovery or reduce the quality of the final recovered movements. Across the spectrum of patients will be individuals who are not easily classified by the model. In this situation an ability to neurophysiologically characterize ipsilateral activity and estimate potential effects becomes important.

The ipsilateral hemisphere has received renewed attention in stroke rehabilitation because of technological advances. Neuromodulation, robotic therapy and brain machine interface all require understanding of ipsilateral activity for optimal benefit. With increased availability of neuromodulation comes the potential for it to be applied inappropriately. Where the effect of contralesional activity could be benefit or harm, it is important to be able to characterize this on an individual basis before seeking to modulate it. The increased use of robotic therapy provides a cost effective way of accumulating vast amounts of repetitive practise, whilst potentially sculpting optimal synergies to correct impairment. The role of the contralesional hemisphere in representing ipsilateral contributions to paretic limb synergies needs to be understood before attempting to over train them. The intact contralesional hemisphere is also a desirable site for decoding

neural signals for brain machine interfaces. It has been demonstrated with multiple modalities that it is possible to decode movement kinetics from ipsilateral cortical signals.

This work seeks to increase knowledge of the function of the ipsilateral hemisphere in healthy subjects and stroke patients. The starting point is that aspects of skilled motor control are encoded in the ipsilateral hemisphere. It is questioned whether these elements are responsive to deliberate practise in healthy subjects, and whether this can be translated to stroke rehabilitation.

1.2.2 The Ipsilateral Hemisphere in Motor Control in Healthy Subjects

Demand on ipsilateral motor networks varies depending on whether tasks are unimanual or bimanual, simple or complex, and focussed on distal muscles or requiring proximal and distal coactivation. For unimanual tasks ipsilateral activity depends on whether the dominant or non-dominant hand is used. For bimanual tasks ipsilateral activity depends on whether muscle activation is homonymous or heteronymous.

Ipsilateral activity can be considered according to the output generated, whether transcallosal, intracortical or corticofugal.

1.2.2.1 *Ipsilateral Activation Scales to Movement Complexity*

A role for the ipsilateral hemisphere in skilled motor control is demonstrated by activation in ipsilateral frontal motor areas scaling with task complexity, seen with neurophysiology and imaging measures. Event Related Depolarizations (ERD) are produced in ipsilateral motor cortex with finger movement (Rau et al., 2003), and the power spectra increases with complex, sequential movements (Hummel et al., 2003). fMRI activation is seen in ipsilateral motor cortex with finger movement (Cramer et al., 1999), and increases with sequential movements and coactivation (Verstynen, 2004). Facilitation of evoked responses in the contralateral finger scales with complexity of ipsilateral finger movement, associated with increasing ipsilateral activation on fMRI (Verstynen and Ivry, 2011). Precision grip, but not power grip produces ipsilateral fMRI activity (Ehrsson et al, 2000). Ipsilateral fMRI activity scales to target size in accuracy tasks (Butefisch, 2015). Ipsilateral finger contraction modulates contralateral MEPs (Liang et al., 2008; Stedman et al., 1998), but complex ipsilateral finger sequences are required for strong contralateral facilitation (Tinazzi and Zanette, 1998).

1.2.2.2 *Bilateral Temporal and Spatial Encoding in the Ipsilateral Hemisphere*

Further evidence of the involvement of the ipsilateral hemisphere in complex motor control is shown by the ability to encode temporal

parameters of ipsilateral and contralateral movement. TMS to ipsilateral motor cortex disrupts reaction time of the ipsilateral and contralateral hand (Foltys et al., 2001). Inhibitory neuromodulation of the ipsilateral motor cortex with repetitive TMS (rTMS) introduces timing errors to performance of a complex piano sequence (Chen et al., 1997), and disrupts temporal control of complex sequential finger tapping tasks (Avanzino et al., 2008). A similar study reported improved temporal performance in finger tapping (Dafotakis et al., 2008).

Temporal encoding is achieved through evolution of IHI from ipsilateral to contralateral hemisphere during preparation and execution of ipsilateral movements. IHI negatively correlates with reaction time and decreases most dramatically in the preparation of fast ballistic movements. During the execution phase, IHI from the ipsilateral hemisphere again builds, suggesting a contribution from the ipsilateral hemisphere to the timing of both onset and offset of movement (Tazoe and Perez, 2013).

The ipsilateral hemisphere is also involved in shaping the kinematics of contralateral and ipsilateral movements. Inhibiting the ipsilateral hemisphere with rTMS worsens finger-tracking accuracy (Carey et al, 2006). Training a unimanual finger task changes kinematics of TMS evoked movements in the trained hand contralateral to TMS, but also in the ipsilateral untrained hand. Untrained ipsilateral finger abduction was reduced in proportion to the trained increased finger abduction in the contralateral hand (Duque et al., 2005). This shows that the ipsilateral hemisphere encodes bilateral spatial information, and ipsilateral movement representations are directionally sensitive to training contralateral representations.

1.2.2.3 *The Ipsilateral Hemisphere in Synergy Representation*

The ipsilateral hemisphere has been strongly implicated in the control of synergy representations. Synergies are a feature of motor control that simplify the computational demands of programming movements by linking neuronal representations of muscles commonly coactivated. Synergies coordinate ipsilateral and contralateral, proximal and distal muscles. The

ipsilateral hemisphere has shown to be important in control of antagonist and coactivated muscles, whereas the contralateral hemisphere dominantly controls the agonist (McMorland et al., 2015). Different elements of ipsilateral synergy control may use transcallosal or corticofugal pathways.

Hand movement alters excitability of homologous ipsilateral representations, but also produces diffuse inhibition of adjacent muscles, stronger distally than proximally, compatible with synergy control (Sohn et al., 2003). Inhibiting ipsilateral motor cortex with inhibitory rTMS disrupts grip-lift and step-tracking kinematics. The disruption is to temporal sequencing of synergies, disturbing the timing of recruitment of proximal relative to distal muscles and agonist relative to antagonist and stabilizing muscles (Davare et al., 2007). Similarly, TMS to the ipsilateral hemisphere disrupts the triphasic EMG pattern of a ballistic wrist movement (Irlbacher et al., 2006). The timing of the disruption corresponds to that of the ipsilateral cortical silent period (iCSP), suggesting transcallosal IHI mediates the effect.

Other aspects of ipsilateral contribution to synergy control have a corticofugal basis. The synergy ratio is a measure of functioning of synergy control, expressed as the ratio of facilitation of biceps MEP when in agonist role compared to suppression of biceps MEP in antagonist role (Gerachshenko and Stinear, 2007). Inhibitory neuromodulation of the ipsilateral hemisphere with cathodal tDCS (McCambridge et al., 2011) and continuous theta burst (Bradnam et al., 2010) alters the synergy ratio. The same ipsilateral inhibition disrupts the modulatory effect of peripheral heteronymous afferent conditioning of the contralateral MEP (Bradnam et al., 2011). This demonstrates an ipsilaterally descending input onto spinal premotor circuits, presumably propriospinal, that function to distribute motor commands to multiple muscles engaged in a synergy. Further support for the idea of ipsilateral control of synergy representations is provided by the ability to decode the kinematics of ipsilateral reach from the local field potentials of widely distributed neuronal ensembles in the ipsilateral motor cortex in primates (Wang et al., 2012) and in humans using ECoG (Ganguly et al., 2009), EEG (Bundy et al., 2012), and MEG (Buch et al., 2008).

1.2.2.4 *The Ipsilateral Hemisphere Can Compensate Contralateral Motor Areas*

In some tasks the ipsilateral hemisphere is seen to increase its activity as contralateral activation declines. This may distribute the motor command to release the contralateral hemisphere to new functions when sustained activity is required. Incremental ipsilateral activation is seen late in a grip task, increasing as the early contralateral activity declines (Shibuya et al., 2016). Another example of compensatory activity is provided by inhibitory neuromodulation of ipsilateral motor cortex in ageing. Inhibitory cathodal tDCS applied during motor learning reduces later performance of rehearsed complex finger sequences. The disruption is greater in older subjects, suggesting increased reliance on the ipsilateral hemisphere to compensate age related motor decline (Zimerman et al., 2014). Contralateral rTMS disrupts controlled force finger tapping. Adding combined ipsilateral and contralateral rTMS produces disruption greater than the sum of individual effects (Strens et al., 2003). This demonstrates loss of the compensation provided by the ipsilateral hemisphere when it is inhibited.

1.2.3 Excitatory and Inhibitory Neural Pathways Mediating Ipsilateral Activity

The function of the ipsilateral hemisphere in motor control can be considered according to a framework of excitatory and inhibitory pathways with interhemispheric, intracortical and corticofugal trajectories.

1.2.3.1 *Transcallosal Pathways From the Ipsilateral Hemisphere*

The ipsilateral cortical silent period (iCSP) is an inhibitory response from the ipsilateral hemisphere thought to be mediated by transcallosal pathways (Wassermann et al., 1991). It has a gradient of duration from proximal to distal (Werhahn et al., 1995), is modulated by voluntary contraction of contralateral muscles (Giovannelli et al., 2009) and scales to task complexity (Tinazzi and Zanette, 1998). IHI suppresses unwanted contralateral motor activity during movement preparation. It is mediated by transcallosal pathways and also scales to force (Ferbert et al., 1992) and complexity (Perez and Cohen, 2009) of movement. Increasing IHI is associated with increased ipsilateral activation on fMRI (Talelli et al., 2008). It is maximal 100ms prior to movement and transitions to facilitation immediately prior to movement onset (Murase et al., 2004). IHI and iCSP are produced by distinct neural populations within ipsilateral motor cortex.

Different ipsilateral cortical representations are under different degrees of transcallosal influence. Use of Multi Voxel Pattern Analysis to measure activity in resting ipsilateral cortical representations quiescent under diffuse inhibition shows that ipsilateral finger representations mirror those of the contralateral hemisphere, and become active when driven by input from the contralateral hemisphere in bimanual tasks. Separate ipsilateral representations are identified with behaviour that is non-linear in relation to the contralateral activity (Diedrichsen and Classen, 2012). These functionally different ipsilateral representations are differentially modulated depending on the laterality of the task and vary in encoding bimanual movements.

1.2.3.2 *Ipsilateral Intracortical Circuits Mediated Bimanual Facilitation and Transcallosal Pathways*

Paired pulse TMS protocols can measure intracortical excitability. Ipsilateral finger movements increase Intracortical Facilitation (ICF) in homologous muscle representations (Rau et al., 2003), against a background of diffuse inhibition of adjacent muscles (Sohn et al., 2003). Short Intracortical Inhibition (SICI) within ipsilateral motor cortex scales to task complexity with dextrous tasks (Morishita et al., 2011), and when upper and lower limb movements are coordinated (van den Berg et al., 2011). Progressively increasing force of contraction reduces the strength of ipsilateral SICI in inverse correlation to strength of IHI (Perez and Cohen, 2008).

MEPs are suppressed by bilateral homonymous elbow flexion, and facilitated by heteronymous ipsilateral flexion and contralateral extension. The timing of the effect is incompatible with transcallosal pathways, and is thought to be mediated within ipsilateral intracortical circuits (Tazoe and Perez, 2014). The iCSP and IHI can be facilitated by conditioning stimuli with timings compatible with I-wave transmission, suggesting intracortical modulation of transcallosal outputs from the ipsilateral hemisphere. The ipsilateral CSP is also modulated by repetitive TMS protocols acting on inhibitory intracortical interneurons, again suggesting an intracortical contribution (Avanzino et al., 2007).

1.2.3.3 *Ipsilateral Corticofugal Output*

TMS generates ipsilateral corticomotor output. Ipsilateral Motor Evoked Potentials (MEPs) are elicited most frequently in axial and proximal muscles (MacKinnon et al., 2004; Strutton et al., 2004). The presence of distal ipsilateral MEPs is rare in healthy subjects, but has been reported with very strong background activation and stimulation (Ziemann et al., 1999). The proximal to distal gradient of activation reflects the distribution of spinal terminations of ipsilateral and contralateral pathways. Ipsilateral MEPs recorded throughout the arm demonstrate this gradient, easily elicited in axial muscles, variably elicited in proximal muscles, and absent distally

(Bawa et al., 2004). Peri Stimulus Time Histograms from individual motor unit recording of 14 arm muscles responding to contralateral TMS shows that monosynaptic corticospinal facilitation mirrors this gradient, prevalent in intrinsic hand muscles, becoming progressively less in forearm, then switching to absent facilitation or inhibition in proximal arm (Palmer and Ashby, 1992). This gradient shows inter-individual variation in terms of the distal extent of ipsilateral MEPs and the nature and strength of contractions required to produce them (Bawa et al., 2004). There is also inter-hemispheric variation independent of handedness between individuals that influences prevalence of ipsilateral MEPs (MacKinnon et al., 2004).

The proximal to distal gradient of ipsilateral corticomotor output is observed in primate studies which confirm it arises from the relative strength of corticospinal and reticulospinal inputs. Stimulus Triggered Averaging of Pontomedullary Reticular Formation neurones on hand, wrist, elbow, shoulder and trunk muscles show a similar proximal to distal gradient of post stimulus facilitation in primates, and sites producing ipsilateral responses are more frequent for axial muscles (Davidson and Buford, 2006). No hand or forearm contractions were seen to reticular formation stimulation (Buford and Davidson, 2004), with the distal extent of post-stimulus effects seen in wrist muscles (Davidson and Buford, 2006).

Common drive of homologous axial muscle pairs also illustrates the organization of ipsilateral motor pathways. In abdominal muscles habitually bilaterally activated, there is a common central peak in EMG cross-correlograms. Bilateral MEPs with the same latency are seen in homologous axial muscles displaying this common drive (see Figure 1.1). Neither feature is seen in arm muscles (Carr et al., 1994). It was proposed the common drive results from branched last-order presynaptic fibres, consistent with the bilateral action of reticulospinal fibres reported in primates (Davidson and Buford, 2006).

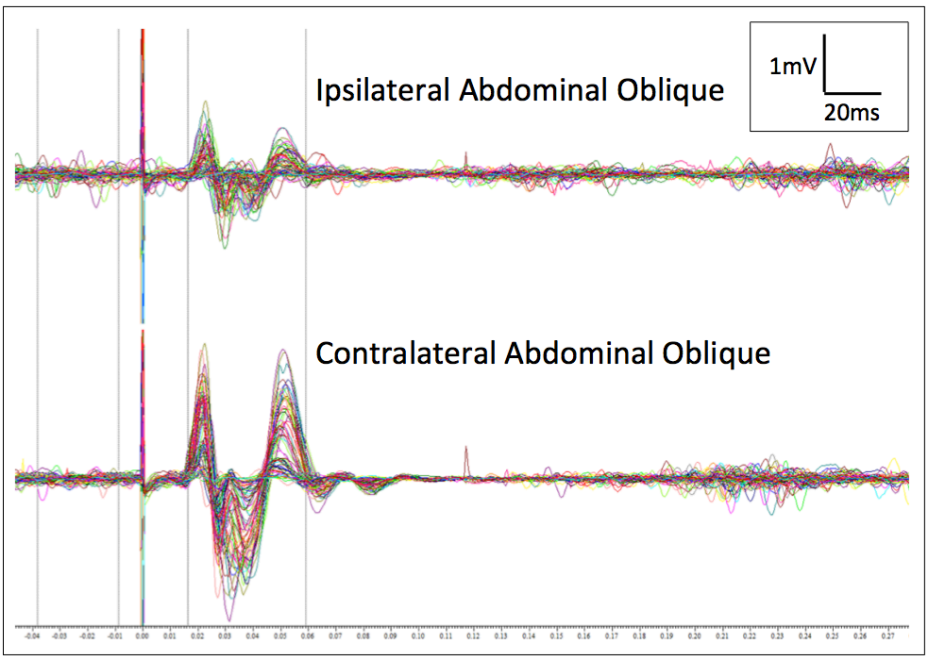

FIGURE 1.1 BILATERAL AXIAL MOTOR EVOKED POTENTIALS

Example of overdrawn Contralateral and Ipsilateral Motor Evoked Potentials recorded from Abdominal Oblique muscle. The development of the motor map can be appreciated within the overdrawn traces, with different grid points producing MEPs of different sizes. The ipsilateral MEPs can be noted to be smaller, of longer latency and more variable morphology than the contralateral.

1.2.4 Descending Ipsilateral Motor Pathways in Skilled Motor Control

The most commonly described role for activity in ipsilateral motor pathways is basic postural support. There are a number of experimental findings relating to ipsilateral motor pathways that suggest complex control functions not yet incorporated into standard accounts of ipsilateral motor control. Recording neuronal modulation in Pontomedullary Reticular Formation showed bilateral Spike Triggered Averaging (STA) effects, with preparatory activity and modulation throughout movement in response to cued reach and grasp (Buford and Davidson, 2004). This would suggest brainstem level premotor activity in ipsilateral pathways. Such premotor computation has been demonstrated in medullary reticular nuclei in mice, active in skilled motor learning of reach tasks (Esposito et al., 2014). Similar ipsilateral reticular modulation was observed in response to both active index finger movements and the afferent feedback generated by passive finger movements in primates (Soteropoulos et al., 2012). Propriospinal neurones are known to give Lateral Reticular Nuclei collaterals within a feedforward cerebellar loop, but this would not account for preparatory activity.

Wide Peak Width at Half Maximum of post-spike effects of Pontomedullary Reticular Formation neurones on ipsilateral arm muscles suggests summation of multiple weak inputs achieved by synchronous convergence on shared segmental interneurons (Davidson et al., 2007). This convergence has been quantified on cervical Intermediate Zone interneurones by stimulating the ipsilateral reticulospinal tract in the Medial Longitudinal Fascicle and the contralateral corticospinal tract in the contralateral pyramid. The degree of convergence was around 50% of all interneurons, and remained around 50% for sites where stimulation produced finger movements (Riddle and Baker, 2010). The functional significance is not yet established but again suggests importance of ipsilateral inputs to premotor circuits in distal motor control. In addition to the convergence on segmental interneurons, direct monosynaptic reticulomotorneuronal connections to

anterior horn cells of intrinsic hand muscles are demonstrated (Riddle et al., 2009).

1.3 NEUROANATOMY OF IPSILATERAL MOTOR PATHWAYS

Knowledge of the different cortical regions controlling ipsilateral output is central to the interpretation of motor mapping. The anatomy of ipsilateral motor control can be described using a simple framework of primary and secondary frontal motor cortical areas, their brainstem relays and descending spinal tracts. A small proportion of corticospinal tract fibres descend ipsilaterally in dorsolateral and ventromedial funiculi. Ipsilateral projections from bilateral brainstem motor pathways descend in ventromedial funiculi to influence proximal postural control and grasp, and ventrolaterally to influence axial and proximal movement (Lawrence and Kuypers, 1965). Interspecies differences are important in extrapolating primate experiments to man. For example, a major source of ipsilateral distal control in primates comes from rubrospinal pathways (Kuypers and Lawrence, 1967), which are vestigial in man (Nathan and Smith, 1982).

Within primate species the use of cervical premotor neurones with bilateral outputs varies with the degree of development of corticomotorneuronal projections (Lemon et al., 2004). The strength of compensation through ventromedial motor pathways in response to bilateral pyramidal lesions is very different in primates (Lawrence and Kuypers, 1968) compared to humans (Nathan, 1994).

1.3.1 Cortical Ipsilateral Motor Representations

1.3.1.1 *Primary Motor Cortex*

Around 10% of corticospinal tract fibres descend ipsilaterally, in lateral and ventromedial funiculi with much inter-individual variability (Nathan et al., 1990). There are focal populations of Pyramidal cells within primate primary motor cortex that are modulated by ipsilateral activity (Cisek, 2002; Tanji et al., 1988), and a region has been identified where stimulation produces bilateral hand movements (Aizawa et al., 1990). Lesions of primary motor cortex can produce ipsilateral deficits in primates (Kaeser et al., 2010) and ipsilateral primary motor cortex reorganizes in response to downstream contralateral corticospinal tract lesions (Schmidlin et al., 2005). However, ipsilateral post spike effects from primary motor cortex are weak (Messamore et al., 2016) and post-synaptic effects in cervical motorneurones are rare in response to ipsilateral corticospinal stimulation (Soteropoulos et al., 2011).

Primary motor cortex is also a source of ipsilateral inputs to bilaterally descending brainstem pathways (Lemon, 2016). Stimulation of motor cortex in primates produces intracellular responses in Pontomedullary Reticular Formation neurones (Fisher et al., 2012). The latency of the corticobrainstem responses varied from rapid monosynaptic to slow polysynaptic routes. In primates there are strong ipsilateral projections from primary motor cortex to Red Nucleus, some of which relay in parvocellular nucleus to originate ipsilateral ventromedial reticulospinal pathways of axial control (Kuypers and Lawrence, 1967).

1.3.1.2 *Supplementary Motor Area*

Supplementary Motor Area (SMA) is strongly concerned with control of axial movements (Weisendanger et al, 1973), and is the source of Anticipatory Postural Adjustments that accompany axial synergies (Massion et al., 1989; Massion, 1992). fMRI shows strong SMA activation in response to observation of axial movements (Sakreida et al., 2005). Stimulation of SMA in human intraoperative studies can produce ipsilateral

axial muscle contraction (Penfield and Boldrey, 1937; Woolsey et al., 1979). In primates, stimulation of SMA produces the highest proportion of ipsilateral excitatory responses of all of the cortical motor areas, around one quarter of all responses elicited (Montgomery et al., 2013).

SMA has corticospinal projections to ipsilateral anterior horn cells and Intermediate Zone interneurones (Dum and Strick, 2002). Around one quarter of SMA corticospinal projections are ipsilateral, and whilst the majority terminate in Lamina VIII, some terminate on anterior horn cells (Dum and Strick, 1996). SMA also has outputs to bilaterally descending bulbospinal pathways via brainstem relays (Keizer and Kuypers, 1989). Functional SMA output through descending ipsilateral reticulospinal pathways is demonstrated in humans by the interaction between auditory startle and axial Anticipatory Postural Adjustments (MacKinnon et al., 2007).

SMA has extensive corticocortical connectivity with primary motor cortex and other secondary motor areas (Narayana et al., 2012). Primate tracer studies reveal strong somatotopically arranged connections to proximal and distal representations of primary motor cortex. Intracortical connections are also seen between SMA and Premotor and Cingulate cortex (Luppino et al., 1993; Picard and Strick, 1996). Functional connectivity between primary motor cortex and SMA is demonstrated in humans by corticocortical evoked potentials from subdural electrodes (Matsumoto et al., 2007).

1.3.1.3 *Premotor Cortex*

Premotor Cortex (PMC) is strongly functionally implicated in guidance of reach movements, a motor task requiring ipsilateral control (Kantak et al., 2012). PMC has a strong hierarchical position within the motor network with a similar laminar distribution to primary motor cortex (Dum and Strick, 2002). PMC contribution to the corticospinal tract is weak compared to other motor areas, around 4% of the frontal motor area contributions (Dum and Strick, 1991). Human Diffusion Tensor Imaging (DTI) studies suggest PMC is a major source of corticoreticulospinal fibres (Yeo et al., 2012). Accordingly stroke patients with PMC lesions have Wallerian degeneration

of the corticoreticulospinal tract visible on DTI (Do et al., 2013). Double labelling of corticospinal fibres at cervical level and reticulopsinal fibres at brainstem level identifies PMC as a source of corticospinal fibres which give collateral branches to reticular nuclei relays (Keizer and Kuypers, 1989). Premotor projections to Parvocellular Red Nucleus contribute to ipsilaterally descending ventromedial pathways (Kuypers and Lawrence, 1967). In contrast to the human DTI studies, some primate stimulation studies do not report very high numbers of ipsilateral outputs from PMC (Montgomery et al., 2013).

The anatomical organization of PMC corticospinal terminations is unusual, and may reflect an important role in producing bilateral output to control synergies by relaying through reticulospinal and propriospinal neurones. PMC projects indirectly to proximal and distal motorneurones in upper and lower cervical segments, with terminations largely in Intermediate Zone (Dum and Strick, 2002; Galea and Darian-Smith, 1994).

Neurones projecting from PMC hand representations terminate on upper cervical segments C2-4, but not lower segments (He et al., 1993). The mismatch between the functional representation of the cortical origin and the segmental termination has been interpreted as evidence that PMC corticospinal output utilizes Propriospinal relays (Dum and Strick, 2002; He et al., 1993; Martino and Strick, 1987).

In contrast to the weak contribution to corticospinal tract, PMC is heavily interconnected with primary motor cortex (Dancause et al., 2006). Primary motor cortex mediates distal forelimb responses to PMC stimulation (Kraskov et al., 2011; Maier et al., 2013) in a manner that demonstrates dependence of PMC output on primary motor cortex integrity for distal effects (Schmidlin et al., 2008).

1.3.1.4 *Cingulate Motor Area*

Corticospinal outputs from Cingulate Motor Areas (CMA) include projections to ipsilateral cervical Laminae VII, VIII and IX (Dum and Strick, 1996). CMA involvement in control of axial movements is shown

with microstimulation in primates, evoking contraction of back muscles (Akazawa et al., 2000) and Latissimus Dorsi (Boudrias et al., 2010). The fastest CMA projections to axial muscles may be monosynaptic (Boudrias et al., 2010). Other studies have failed to observe axial responses from CMA at stimulation intensities that evoked axial movements from SMA (Luppino et al., 1991). In the limited number of human intraoperative studies, microstimulation of CMA produces contralateral distal arm (Chassagnon et al., 2008; Diehl et al., 2000; Lim et al., 1994) and contralateral leg (Diehl et al., 2000) movements, but no axial movements were reported. CMA neurones are modulated by ipsilateral unimanual movement (Nakayama et al., 2015; Yokoyama et al., 2016).

Strong homotopic arrangement is seen within corticocortical connections between CMA and primary motor cortex forelimb and hindlimb areas, but axial connections are more diffuse (Morecraft and Van Hoesen, 1992). Transcallosal projections from CMA to motor cortex are described as modest and heterotopic for hand area (Rouiller et al., 1994). Functional transcallosal projections from ipsilateral CMA are proposed to be active in humans during complex unimanual tasks, but with much inter-individual variability (Kobayashi et al., 2003).

1.3.2 Brainstem Ipsilateral Motor Pathways

Brainstem nuclei are a source of bilaterally descending motor pathways from Reticular and Vestibular Nuclei and bulbospinal motor neurones from Pontomedullary Respiratory Groups. Inputs to Reticular Nuclei may be direct corticobrainstem pathways, collaterals from corticospinal fibres, or relays via Red Nucleus. Brainstem strokes affecting reticulospinal fibres but sparing pyramidal tracts can produce proximal tetraparesis with preserved distal function (Chen et al., 2011).

Corticobrainstem pathways from motor cortex to Pontomedullary Reticular Formation vary in their transmission. Recording intracellularly from reticulospinal neurones in primate Pontomedullary Reticular Formation in response to TMS of motor cortex reveals a range of latencies for transmission of the cortical stimuli (Fisher et al., 2012). Some responses are as fast as the most rapid equivalent for corticospinal transmission to pyramids at the same level. There are also medium latency responses with low jitter suggesting a slower monosynaptic route through fibres with slower conduction velocity. For example, Premotor corticospinal neurones which give collaterals to reticular nuclei have slower conduction than corresponding fibres from primary motor cortex (Murray and Coulter, 1981). Later responses with high jitter suggest polysynaptic routes. Inputs from motor cortex to parvocellular Red Nucleus can relay to bilateral ventromedial brainstem pathways, but also supply a cortico-subcortical loop (Kuypers and Lawrence, 1967; Lemon, 2016). Such a circuit could explain late corticobrainstem inputs seen in response to motor cortex TMS. Cortical TMS in cats evokes MEPs in contralateral and ipsilateral forelimbs with near simultaneous latency. Lesions at cortical and collicular levels do not abolish the responses, but electrical stimulation of vestibular nuclei replicates them (Haghighi et al., 1995). This suggests acoustic stimulation was activating bilateral vestibulospinal output at brainstem level. A similar conclusion was suggested by primate TMS studies that noted some responses to TMS that could be produced by stimulation away from the skull, mimicked by vibratory otolith activation (Fisher et al., 2012).

1.3.3 Descending Ipsilateral Motor Pathways

1.3.3.1 *Ipsilateral Corticospinal Tracts*

One study quantified 11% of corticospinal fibres descending ipsilaterally to lumbar segments, of which 10% were in the lateral funiculus and 1% in ventromedial funiculus (Lacroix et al., 2004). In humans there is great variability and asymmetry of ipsilateral corticospinal fibres (Nathan et al., 1990). Another quantification of the ipsilateral corticospinal tract in primate describes greater variability, with 2 to 15% of corticospinal fibres descending ipsilaterally, of which 1 to 12% were in ipsilateral dorsolateral funiculus and 1 to 7 % in ipsilateral ventromedial funiculus. The ipsilateral corticospinal neurones in the dorsolateral funiculus decussate contralaterally through Lamina X without giving ipsilateral boutons. Those of the ventromedial funiculus make terminations in Lamina VIII, and therefore influence spinal reflexes and interneurons shared with reticulospinal fibres (Yoshino-Saito et al., 2010). Another study observed ipsilateral, contralateral and bilateral termination patterns, ipsilaterally dominantly to Lamina VIII (Rosenzweig et al., 2009). Ipsilateral terminations to Lamina IX with synaptic boutons closely apposed to alpha motor neurones have been reported (Lacroix et al., 2004).

The strength of the ipsilateral corticospinal tract is weak relative to the contralateral, and the majority of the ipsilaterally descending fibres will decussate without giving ipsilateral boutons. Ipsilateral corticospinal terminations are largely to Lamina VIII. The Lamina VIII interneurones that project to axial alpha motor neurones predominantly receive contralateral corticospinal terminations (Porter and Lemon, 1993), and act bilaterally through Lamina X decussations. There is some spinal level reorganization after experimental cortical injury in animal studies. Following experimental stroke of frontal motor areas in primates, terminal bouton density of ipsilateral corticospinal fibres increases in Lamina VII and VIII. With extensive cortical lesions, density also increases in Lamina IX (Morecraft et al., 2016). This situation is also seen in animal studies of plasticity promoting immunotherapy (Lindau et al., 2014). Although the increased

ipsilateral corticospinal synaptic boutons were seen in monkeys who had made motor recovery, other studies suggest the finding lacks functional correlate. Stimulation studies of the ipsilateral pyramid in the brainstem do not provide functional support for these anatomical observations. Ipsilateral pyramidal stimulation in healthy primates (Soteropoulos et al., 2011) and in monkeys recovered from contralateral corticospinal tract lesions only elicits rare monosynaptic Excitatory Post Synaptic Potentials (EPSPs) (Zaaimi et al., 2012).

1.3.3.2 *Reticulospinal Tracts*

The reticulospinal tract originates in pontine and medullary reticular nuclei. The medullary fibres from Nucleus Reticularis Gigantocellularis descend bilaterally in ventrolateral funiculi, whereas the pontine fibres from Nucleus Reticularis Pontis Caudalis and caudal Nucleus Reticularis Pontis Oralis descend ipsilaterally in the ventromedial funiculus (Brodal, 1969). Human post mortem series note much variability in the projections in man, but the ventrolateral and ventromedial courses are consistent. Reticulospinal terminations were most abundant in cervical regions, but reticulospinal fibres could be traced as far as sacral segments (Nathan et al., 1996).

Descending reticulospinal fibres do not travel in discrete tracts, vary their position within the cord as they descend and are not topographically organized. Respiratory bulbospinal motor neurones from Pontomedullary Respiratory Groups also descend close to ventrolateral reticulospinal pathways (Nathan, 1963). The pontine reticular pathways terminate predominantly on Lamina VIII and partly on Lamina VII, whereas the medullary pathways terminate predominantly on Lamina VII with few terminals on Lamina VIII and IX (Brodal, 1969). Monosynaptic and disynaptic Excitatory Post Synaptic Potentials have been recorded from intrinsic hand cervical motor neurones in response to reticulospinal stimulation in the Medial Longitudinal Fascicle (Soteropoulos et al., 2011).

Reticulospinal fibres give collaterals at multiple segmental levels, from cervical to lumbar (Peterson et al., 1975). There is bilateral branching at segmental levels (Matsuyama et al., 1993). Stimulation of the reticulospinal

tract in the Medial Longitudinal Fascicle produces a pattern of ipsilateral flexor facilitation and extensor inhibition, with the opposite pattern seen contralaterally (Davidson and Buford, 2006). This opposes the effect of vestibulospinal excitation, which facilitates ipsilateral extensors. The reticulospinal effect is more powerful, with stimulation of the medullary reticular nucleus sufficient to suppress decerebrate rigidity (Magoun and Rhines, 1946).

1.3.3.3 *Vestibulospinal Tracts*

The lateral vestibulospinal tract originates in Dieters Nucleus in the medulla and descends ipsilaterally to lumbar regions in the ventromedial funiculus. Inputs to Dieters Nucleus are largely peripheral vestibular and cerebellar, particularly from Fastigial Nucleus. The medial vestibulospinal tract originates from Schwalbes Nucleus and travels to the spinal cord within the Medial Longitudinal Fascicle to descend bilaterally in the ventromedial funiculus to cervical segments. The medial tract is predominantly concerned with head position. Medial tracts terminate in Lamina VII and VIII, and the lateral predominantly in VIII with bilateral action through decussating commissural interneurones. The Vestibular Evoked Myogenic Potential measurable in actively contracting neck muscles in response to otolith stimulation results from fast conducting ipsilateral monosynaptic inhibitory activity in this pathway (Colebatch et al., 1994). Stimulation of Dieter's Nucleus facilitates ipsilateral extensors and inhibits flexors (Walberg et al., 1962), and uncontrolled lateral vestibulospinal tract activity results in the extensor rigidity of the decerebrate posture (Pompeiano and Hoshino, 1976). Vestibulospinal fibres also terminate on gamma motorneurones.

1.3.3.4 *The Propriospinal Tract*

The propriospinal tract has a functional organization suited to control of synergies. Premotor interneurones provide spinal level computation, able to integrate bilateral input and output from corticospinal, reticulospinal and vestibulospinal tracts with peripheral afferent modulation. Short propriospinal neurones span few segmental levels, whereas long propriospinal neurones may connect cervical and lumbar segments. There is

anatomical abundance of propriospinal neurones in humans shown in post-mortem studies in cervical, thoracic and lumbar cord (Nathan et al., 1996).

Short propriospinal neurones in cervical segments receive contralateral and ipsilateral corticospinal and reticulospinal input, and give collaterals to alpha motor neurones, Lamina VII and VIII interneurons and inhibitory interneurons from Ia afferents (Alstermark et al., 1990a). Long propriospinal neurones additionally receive vestibulospinal inputs and may span from cervical to lumbar segments (Alstermark et al., 1987). Lamina VIII interneurons may decussate and respond to stimulation of contralateral motor nuclei and ipsilateral and contralateral Group I afferents (Harrison et al., 1986).

Propriospinal neurones innervating multiple motor nuclei at different segmental levels do not give the same pattern of collaterals to Ia inhibitory interneurons at each level, showing function beyond reciprocal inhibition (Alstermark et al., 1990b). Importantly for synergy organization, propriospinal terminations may be to both alpha motor neurones and Ia inhibitory interneurons, or just to Ia inhibitory interneurons (Alstermark et al., 1991). In humans homonymous and heteronymous muscle and cutaneous afferent input produces polysynaptic excitation of motor neurones with a central delay dependent on segmental distance from C3-4 short propriospinal neurones (Gracies et al., 1991). The pattern of afferent input determines propriospinal facilitation or inhibition (Nielsen and Pierrot-Deseilligny, 1991).

A single propriospinal neurone labelled in the cat projected to motor nuclei of muscles acting across hand, wrist, elbow and shoulder joints (Tantisira et al., 1996). In humans the functional relevance of this is shown by potentiation of infraspinatus MEPs by peripheral heteronymous afferent stimulation when the hand is functionally coupled with the shoulder in a reach grip-lift task (Roberts et al., 2008). Activity of propriospinal neurones depends on the balance of peripheral afferent and descending motor inputs. Strong corticospinal inputs produce feedforward disynaptic Inhibitory Post Synaptic Potentials, whereas weaker inputs can produce monosynaptic

Excitatory Post Synaptic Potentials (Isa, 2006; Nicolas et al., 2001). Propriospinal axons terminate on alpha motor neurones or Ia inhibitory interneurons, and project to control multiple muscles acting across multiple joints to control synergies. The afferent feedback functions to focus descending motor input to active muscles, and to terminate the movement. Propriospinal neurones send ascending collaterals to the ipsilateral Medullary Reticular Nucleus to function as a pre-cerebellar relay (Alstermark and Ekerot, 2015; Isa, 2006). Disrupting this system impairs ipsilateral reach and grasp (Kinoshita et al., 2012).

1.4 ANIMAL MODELS OF PLASTICITY IN IPSILATERAL MOTOR PATHWAYS

Animal experiments provide models of plasticity in ipsilateral motor pathways, with plastic change demonstrated at cortical, brainstem and spinal levels. Ipsilateral plasticity may occur concurrently at multiple levels. The importance of ipsilaterally descending motor pathways was demonstrated in a classical experiment, lesioning ipsilaterally descending motor tracts in the ventral funiculus in monkeys who had recovered from bilateral pyramidotomies (Lawrence and Kuypers, 1968). The monkeys had made good motor recovery from the corticospinal tract lesions, with loss of dexterity, but recovering a functional grasp within weeks. Subsequent lesions of ipsilaterally descending brainstem pathways produced marked disability, with impaired reach and grasp and impaired axial control.

1.4.1 Translation of Animal Experimental Models to Stroke Rehabilitation

Primate lesion studies illustrate the importance of ipsilaterally descending motor pathways, but clinical observations show that they are not directly translatable to humans. Where bilateral basis pontis infarcts mimic experimental bilateral pyramidotomy, no compensation through ipsilateral pathways is seen in tetraplegic Locked-In patients (Heywood et al., 1996). Late motor recovery from Locked-In syndrome has been reported to occur with compensation by corticospinal tract rather than ipsilateral motor pathways (Kwon and Jang, 2012). Similarly, bilateral surgical cordotomies producing complete corticospinal tract lesions in lateral funiculi but sparing ipsilateral motor pathways in ventromedial funiculi produce lasting paraplegia, with no ipsilateral compensation. In contrast a unilateral lesion of the lateral funiculus may be rapidly compensated within hours, suggesting upregulation of latent decussations from the intact contralateral lateral funiculus (Nathan, 1994 - cases 93 and 80). This is supported by another case where cordotomies were performed sequentially. After the first hemicordotomy lesioning the corticospinal tract in the lateral funiculus, the ipsilateral leg was paretic but recovered almost fully. The second cordotomy on the opposite side then resulted in recurrence of the weakness, the ipsilateral compensation from contralateral decussation had been removed (Nathan and Smith, 1973 - case 93). With complete corticospinal tract lesions, unbalanced activity in ipsilateral pathways may produce flexor or extensor synergies, rigidity or spasticity (Brown et al., 1994).

1.4.2 Animal Models of Cortical Reorganization

Animal models of cortical reorganization provide the basis for the experimental approach employed in this thesis. Cortical reorganization of motor representations is seen in primates in response to skilled motor training in a task specific manner, demonstrated with intracortical microstimulation (Nudo et al., 1996). Similar primate studies reveal the extent of reorganization of cortical motor representations in response to experimental ischaemic lesions. This provides a cellular correlate of human fMRI studies of motor network activation after stroke. The extent of fMRI activation is dependent on lesion extent and severity of functional deficit. Extensive bihemispheric networks incorporating secondary motor areas are recruited initially, and with motor recovery this normalizes to ipsilesional activation patterns. In patients with severe damage to ipsilesional corticospinal tracts, contralesional activation persists, associated with poor recovery (Ward and Cohen, 2004). Recent imaging studies have suggested that activity in ipsilateral motor pathways can beneficially associate with some functional measures (Jang, 2014; Rüber et al., 2012), but this does not generalize.

If distal forelimb representation of primary motor cortex is focally lesioned in primates the cortical response is influenced by motor training. Without rehabilitation the proximal representation expands and the distal representation contracts. The proximal representation reorganizes into perilesional cortex that produced hand movements prior to the lesion (Nudo and Milliken, 1996). A similar reorganization is seen in healthy primates following temporary limb immobilization (Milliken et al., 2013). With rehabilitative training the distal representations are preserved, and hand representations may even expand into regions previously producing shoulder movements prior to the lesion (Nudo et al., 1996). Large primary motor cortex forelimb representation lesions drive cortical reorganization increasing forelimb representation in Premotor cortex. The Premotor increase is in proportion to the extent of the primary motor cortex damage (Frost, 2003), and can be enhanced with immunomodulation (Barbay et al.,

2015). In contrast, small focal lesions within subregions of primary motor cortex forelimb representations result in a reduction of PMv forelimb representations. This occurs irrespective of the position of the lesion in relation to intracortical connections between primary motor cortex and premotor cortex (Dancause et al., 2006). The cortical reorganization is therefore driven by the extent of the lesion, rather than disruption of specific intracortical pathways. Although these studies only describe cortical reorganization in the ipsilesional hemisphere, they raise an important point relating to ipsilateral motor pathways. The reorganization in Premotor areas was only seen with extensive infarcts. Premotor areas are an important source of corticoreticulospinal fibres with bilateral effects at segmental level (Yeo et al., 2012). A similar situation is seen with reorganization of motor representations in SMA, a region with strong axial representation and bilateral motor output (Montgomery et al., 2013). Ischaemic lesions of primary motor cortex producing deficits of reach and grasp alter SMA wrist and forelimb representations in proportion to the size of the lesion (Eisner-Janowicz et al., 2008).

These studies demonstrate reorganization of cortical motor networks to increase the role of secondary motor areas that could generate bilateral motor output relevant to axial and proximal control. No studies have mapped the contralesional cortex in a similar longitudinal manner in response to focal motor cortex lesions. A mild effect on control of ipsilesional hand function from a similar focal forelimb area lesion was identified in monkeys (Bashir et al., 012). Deficits of motor planning in ipsilesional hand are also reported (Roitberg et al., 2003). Recovery of subtle ipsilesional deficits mirrors the course of contralateral recovery (Kaeser et al., 2010). Ipsilateral motor cortex reorganization has also been reported in response to cervical cord hemisection (Schmidlin et al., 2005).

1.4.3 Animal Models of Plasticity in Brainstem Ipsilateral Motor Pathways

Convergence of cortical inputs on brainstem nuclei originating ipsilateral motor pathways makes brainstem nuclei a site of potential plasticity. Animal experiments provide direct examples of ipsilateral plasticity at brainstem level. Immunomodulators promoting axonal growth improve functional recovery from experimental lesions through brainstem level plasticity, encouraging ipsilaterally descending corticofugal fibres from the contralesional hemisphere to decussate downstream. Treating rats with experimental Middle Cerebral Artery infarcts with amphetamine increases axonal growth cone activity. Improved forelimb function is associated with doubling of the number of descending fibres from contralesional motor cortex decussating within the Red Nucleus (Papadopoulos et al., 2009). This is only seen when combined with rehabilitation. A similar degree of amphetamine enhanced decussation of ipsilaterally descending fibres from contralesional motor cortex in Pontine nuclei has also been reported (Ramic et al., 2006). Decussation of intact contralesional fibres at multiple brainstem levels therefore has the potential to compensate damaged fibres from ipsilesional cortex. Recovery of skilled reach after bilateral pyramidotomies in rats can be served by extrapyramidal pathways combined with rehabilitation. Lesioned corticospinal tract neurones were seen to extensively sprout within the Red Nucleus. Blocking Red Nucleus activity then decompensated the recovered skilled reach (Mosberger et al., 2017). This suggests that ipsilateral brainstem relays are a potential route for plastic compensation of corticospinal lesions.

1.4.4 Animal Models of Plasticity in Segmental Ipsilateral Motor Pathways

Numerous studies in animals have used immunomodulatory treatments to promote plasticity driving decussation of ipsilaterally descending motor fibres into denervated cord. Response to lesions includes astrocytic upregulation of chondroitin sulphate proteoglycans in extracellular matrix that inhibit axonal growth (Yiu and He, 2006), and myelin associated axonal growth inhibitors such as NogoA, Myelin Associated Glycoprotein, Oligodendrocyte Myelin Glycoprotein, semaphorins and ephrins (Filbin, 2003). Release of axonal growth inhibition promotes plasticity. Antibodies against NogoA are successful in animal models in increasing the number of midline decussating fibres to reinnervate denervated anterior horn cells on the paretic side (Wahl and Schwab, 2014).

The Crossed Phrenic Phenomenon is a classical model of spinal ipsilateral plasticity relating to descending bulbospinal tracts. After cord hemisection above the phrenic nucleus, ipsilaterally descending bulbospinal phrenic motorneurones from ipsilateral and contralateral pontomedullary respiratory groups are interrupted. This results in hemidiaphragmatic paralysis, and a compensatory respiratory response. A further lesion of the contralateral phrenic nerve then briefly induces asphyxia from complete diaphragmatic paralysis. Rapidly after, recovery of the previous paretic ipsilateral hemidiaphragm is triggered. This results from a contralaterally descending motor pathway responding to increased respiratory drive by decussating through latent pathways. This acts to reinnervate ipsilateral phrenic motorneurones denervated by the original hemisection (Goshgarian, 2009). The decussating fibres producing the ipsilateral recovery are proposed to cross at the level of the phrenic nucleus (Moreno et al., 1992).

Recovery of respiratory muscle paralysis following surgical cordotomies in cancer patients was proposed to occur through similar unmasking of latent decussating pathways on the basis of the time course of recovery (Nathan, 1963). Although animal experiments give clear examples of plasticity using brainstem and spinal decussations, there is no direct evidence for this in

response to stroke, and clinical examples in humans are limited to these very specific surgical lesions.

1.4.5 Animal Models of Plasticity in Ipsilateral Motor Pathways Concurrently at Multiple Levels

Ipsilateral plastic processes may occur in parallel at cortical, brainstem and spinal levels. This makes it problematic to rely on temporal measures from ipsilateral MEPs to deduce the nature of the pathways used. Synaptic delays could accumulate at multiple levels. Amphetamine in rats was shown to promote plastic decussation of contralesional motor fibres at brainstem and spinal level, and is also reported to induce contralesional axonal sprouting in cortical regions (Stroemer et al., 1998). Similarly, with anti-NogoA immunomodulatory therapy after experimental strokes in rats, improved function is associated with both increased spinal level decussation, and cortical reorganization of contralesional forelimb representations (Lindau et al., 2014).

1.5 THE IPSILATERAL HEMISPHERE POST STROKE

A classical account of the importance of activity in ipsilateral motor pathways was provided by Brodal's self-observations after a stroke. He was aware of the impairment of postural control of his non-paretic dominant hand (Brodal, 1973). This latent ipsilateral motor function unmasked by lesions has been quantified experimentally. Measures of the strength of the non-paretic ipsilesional limb reveals significant weakness proximally, being around one third weaker than predicted, but no distal weakness, consistent with a reticulospinal basis (Colebatch and Gandevia, 1989). Latent ipsilesional lower limb weakness is also reported (Adams et al., 1990). The kinematics of grip and lift, pointing, and step-tracking tasks are impaired in the non-paretic ipsilesional limb (Hermsdörfer et al., 2003; Hermsdörfer and Goldenberg, 2002; Yarosh, 2004).

1.5.1 Upregulation of Ipsilateral Motor Pathways After Stroke

After stroke, monosynaptic EPSPs are not seen in the paretic arm in response to TMS of the contralesional hemisphere. This suggests that the unaffected ipsilateral corticospinal tract does not serve motor recovery after stroke (Palmer et al., 1992). The same conclusion is drawn from a primate study of recovery from a pyramidal lesion. Stimulation of the ipsilateral corticospinal tract does not produce monosynaptic EPSPs in cervical motor neurones innervating finger flexors. However mono and disynaptic EPSPs were seen in response to ipsilateral reticulospinal tract stimulation, with increased frequency relative to control animals. This suggests upregulation of activity in ipsilateral reticulospinal pathways in response to corticospinal tract lesions (Zaaimi et al., 2012) (see Figure 1.2).

This introduces some of the key issues relating to the role of the ipsilateral hemisphere after stroke. Reticulospinal fibres are organized to produce bilateral responses, with ipsilateral flexor facilitation and extensor suppression, and the reverse pattern contralaterally (Davidson and Buford, 2006). Reticulospinal fibres also give off multiple collaterals at multiple segmental levels (Matsuyama et al., 1993). This pattern explains the flexor and extensor emphasis to tendon stretch reflexes in paretic and unaffected limbs (Thilmann et al., 1990). These reticulospinal properties, combined with prominent gamma motor neurone terminations, predispose to mass movements with flexor coactivation and spasticity.

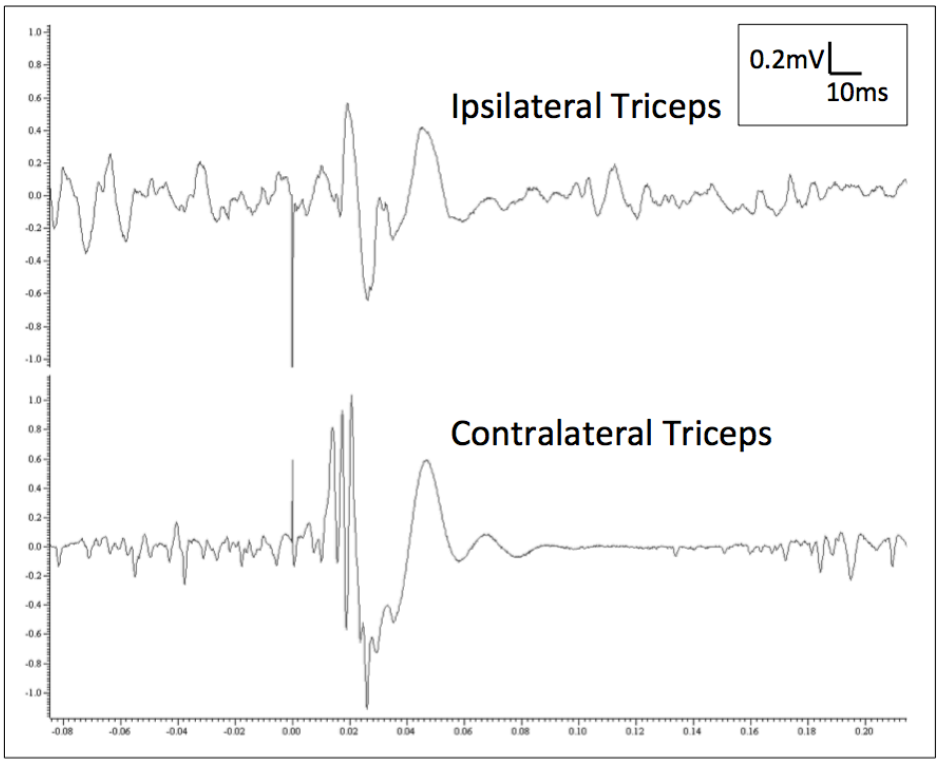

FIGURE 1.2 IPSILATERAL MEPS ARE UPREGULATED IN THE PARETIC LIMB FOLLOWING STROKE

MEPs recorded from the paretic ipsilateral Triceps and unaffected contralateral Triceps of a stroke patient from stimulation of the Contralesional hemisphere. The ipsilateral MEP is prominent, reflecting increased use of compensatory ipsilateral motor pathways. The background activity is more variable in the paretic limb reflecting more effortful control of voluntary movement.

1.5.2 Beneficial and Maladaptive Potential of Alternative Ipsilateral Motor Pathways After Stroke

The functional anatomy of the reticulospinal tract can be used to interpret the abnormal flexor and extensor synergies that develop after stroke as the consequence of reliance on ipsilateral reticulospinal pathways (Dewald et al., 1995). To support this, the presence of ipsilateral MEPs in proximal muscles has been associated with abnormal synergies and poor functional outcome after stroke (Schwerin et al., 2008). Allowing compensation through ipsilateral pathways may then occur at the expense of potential later recovery of better quality movement through crossed corticospinal pathways. An alternative view is provided by studies demonstrating upregulation of ipsilateral axial MEPs after stroke (Misawa et al., 2008) associating positively with measures of trunk function (Fujiwara et al., 2001), which in turn correlates with long-term functional outcome (Verheyden et al., 2006). Which situation is likely will depend on individual factors.

Whether ipsilateral corticofugal output is ultimately beneficial after stroke is largely determined by properties of the lesion. Inhibitory neuromodulation of the ipsilateral hemisphere results in deterioration of synergy function in patients with extensive ipsilesional corticospinal tract damage. These patients have functional reliance on ipsilateral pathways for their recovered motor function, and downregulating ipsilateral activity worsens control of the paretic limb. Conversely, those with mild and moderate motor deficits who have recovered due to preservation of sufficient ipsilesional corticospinal fibre numbers improve motor control when the contralesional hemisphere is inhibited (Bradnam et al., 2012). One possible explanation for the improvement is offered by the Competitive Interference model. This was proposed on the basis of fMRI studies demonstrating that contralesional activation was associated with poor recovery (Ward et al., 2003), and measures of IHI showing that in stroke patients, IHI failed to transition to facilitation prior to movement onset (Murase et al., 2004). In combination, these observations suggest that failure of the usual balance of excitation and

inhibition between ipsilesional and contralesional hemispheres results in an excess of inhibitory influence from the contralesional hemisphere. This is suggested to limit the plastic potential of the ipsilesional hemisphere and prevent optimal recovery.

1.5.3 Predicting Benefit from Ipsilateral Activity After Stroke

It is valuable to be able to differentiate patients for whom ipsilateral activity can usefully serve motor recovery, and those in which it could be maladaptive. A proportion of stroke patients can be predicted to make good distal upper limb motor recovery to around 70% of their potential based on early impairment scores (Prabhakaran et al., 2008). This prediction relies on a degree of spontaneous biological recovery which becomes possible if there are sufficient intact ipsilesional corticospinal tract fibres (Byblow et al., 2015). Using this principle, the PREP algorithm provides the ability to predict the extent of motor recovery on the basis of early clinical, neuroimaging and TMS measures (Stinear et al., 2012). It is expected that more severely impaired patients are those with most reliance on ipsilateral pathways. In the study demonstrating deterioration of paretic arm function with ipsilateral cortical inhibition, the functional behaviour of the patients could be predicted with the same measure of ipsilesional corticospinal tract fibre integrity used in the PREP algorithm (Bradnam et al., 2012).

Inherent within the predictive factors are positive prognostic features reliant to some degree on ipsilateral activity. This complicates straightforward division of patients into beneficial and maladaptive ipsilateral potential purely on ipsilesional corticospinal tract integrity. The PREP algorithm uses power of finger extension and shoulder abduction as the clinical measures. Finger extension relies on crossed corticospinal or corticomotorneuronal fibres. Although corticoreticulospinal fibres innervate cervical motor neurones to intrinsic hand muscles in primates, only flexor responses are seen (Riddle et al., 2009). Shoulder abduction however is under strong ipsilateral control from reticulospinal fibres (Davidson and Buford, 2006). Deltoid may also be innervated by corticomotorneuronal fibres (Colebatch et al., 1990; Rathelot and Strick, 2009), but the estimate of latent deltoid weakness in the ipsilateral non-paretic arm suggests a significant ipsilateral contribution to normal function (Colebatch and Gandevia, 1989). Despite the imaging and neurophysiological measures in the PREP algorithm focussing on crossed corticospinal pathways, the clinical measures may

include some element of reliance on ipsilateral activity important to good motor recovery. Further support for this idea is provided by studies of predictive factors for ambulatory status after stroke. Independent sitting balance in the acute stage is the strongest predictor of ambulatory status at 6 months (Kwah and Herbert, 2016).

Within the studies of Proportional Recovery there are patients who are exceptions, who go on to make good recovery despite reliance on ipsilateral pathways (Bigourdan et al., 2016). Similar examples of patients making good motor recovery through reliance on ipsilateral pathways recur frequently (Butefisch et al., 2005). This highlights the need to be able to individualize stroke rehabilitation according to their potential to benefit from ipsilateral activity.

1.5.4 Specific Stroke Syndromes Demonstrating Beneficial Ipsilateral Plasticity

There are specific clinical syndromes that also suggest a beneficial role for activity in ipsilateral motor pathways in some patients. A small infarct affecting motor pathways may prompt recovery through motor reorganization to the opposite hemisphere. A subsequent large Middle Cerebral Artery infarct to the same hemisphere may then produce Global Aphasia Without Hemiparesis, since the motor component is spared by the previous reorganization (Bang et al., 2004). If a subsequent large stroke affects the opposite hemisphere then the unusual situation of Ipsilateral Hemiparesis may be seen (Saada and Antonios, 2014). In patients who are Locked-In following bilateral basis pontis infarcts, emotional stimuli can produce bilateral axial movements, presumed to originate from Cingulate Motor Areas and relay through brainstem tegmental relays (Heywood et al., 1996), demonstrating a latent alternative ipsilateral motor pathway.

1.6 USE OF CORTICAL MOTOR MAPPING AS A MARKER OF PLASTICITY IN IPSILATERAL MOTOR PATHWAYS

The following experiments use ipsilateral motor maps as markers of plasticity in ipsilateral motor pathways. This approach is suited to detection of plastic change predicted by the known functions of the ipsilateral hemisphere in motor control. Of the functional roles described for the ipsilateral hemisphere, the most relevant to motor recovery after stroke is the contribution of ipsilateral motor output to synergy control. The ability to decode complex synergies in the ipsilateral hemisphere (Liu et al., 2010) and the impact of neuromodulation of the ipsilateral hemisphere on synergy expression (McCambridge et al., 2011) supports this. Neurophysiological indices describe deterioration in synergy functioning after stroke, and implicate involvement of ipsilateral pathways (Gerachshenko et al., 2008). Training tasks demanding of coactivation of multiple muscles expands the cortical representation of the synergy in animal studies (Nudo, 2013), suggesting that the intact cortical architecture of the contralesional hemisphere could be utilized to reorganize synergy representations in response to stroke impairments.

1.6.1 Structural and Functional Organization of Cortical Motor Representations

A key principle underlying motor mapping is that movements are multiply represented within a cortical region. These may be in adjacent or remote cortical columns, linked synaptically through pyramidal collaterals and interneurones (Huntley and Jones, 1991), and functionally through synchronization (van Wijk et al., 2012). Representations of the same muscle can be incorporated into multiple synergy representations. One microstimulation study in primates identified 7 sites within primary motor cortex representing the same movement (Gould et al., 1986). A TMS cortical mapping study in humans confirms that muscles are multiply represented, with proximal and distal muscle representations overlapping and having similar total areas of representation (Devanne et al., 2006). The representations to be linked in a synergy may be adjacent or remote. Depending on the nature of the movements, they may be represented by widely distributed neuronal ensembles, expansive across cortical regions (Nicolelis and Lebedev, 2009), or focal, using adjacent corticomotorneuronal cells within a small area of primary motor cortex (McMorland et al., 2015).

Synaptic connectivity within cortical representations can be achieved through Horizontal Fibres, pyramidal cell collaterals that travel horizontally allowing connectivity between adjacent or distant cortical columns (Huntley and Jones, 1991; Keller, 1993). These give synaptic boutons throughout their length. This produces a dense core of connectivity, measured to be 3mm2 from a single cortical point in the cat. Under conditions of balanced activity within intracortical circuits, activation of a single point spreads 1.5mm, but when inputs alter excitability this can be up to 7mm (Capaday et al., 2009). Horizontal Fibres synapse with pyramidal cells and inhibitory interneurons, controlling local intracortical excitation and inhibition. Theta burst stimulation of horizontal fibres in Layer III drives Long Term Potentiation (LTP) in animal studies, but synaptic connectivity is also controlled by stimulation of vertical pyramidal cells. Synaptic connectivity

in a cortical representation is therefore driven by the spatiotemporal pattern of firing in pyramidal cells and horizontal fibres (Hess et al., 1996; Hess and Donoghue, 1994).

Synchronous spike timing can also link populations of cortical motorneurones within a synergy representation. Pairs of motorneurones in primary motor cortex of primates linked within movement representations demonstrate increased synchrony of firing (Jackson et al., 2003). This property can be used to reorganize motor representations with stimulation to entrain synchrony in neuronal populations shown to alter synergy expression in monkeys (Jackson et al., 2006).

1.6.2 Plasticity in Cortical Motor Representations

Motor representations are functionally organized to provide stability of learnt skill, but with the flexibility to update with further learning. This stability is shown by measuring the motor output of pyramidal cells in a movement representation responding to changes in postural or skill demands of a reach movement in primates. Measuring post-spike effects from Layer V primary motor cortex pyramidal cells shows that the polarity of the output remains stable in nearly all cells responding to a postural challenge, but around one fifth alter their polarity to increased skill demands. The movement representation remained constant when the same synergies could be deployed to respond to postural challenge, but when new motor learning was required to meet increased skill demand, the representation was fractionated (Griffin et al., 2009). Varying stability according to motor learning is achieved by locally controlling the weight of synapses and through the balance of intracortical excitation and inhibition within the representation.

Horizontal Fibres are organized with densely, recurrently interconnected cortical points. Each fibre makes multiple synapses with inhibitory interneurons, controlling balance of excitation within the representation. Excitatory inputs are balanced by recurrent inhibition within the representation so that linear behaviour of inputs and outputs is maintained. This stabilizes the representation performing a motor task to a normal level of demand. When the demand increases through skilled motor learning, additional excitatory synaptic input alters inhibition at remote recurrently interconnected cortical points to allow new synaptic activity. In this way the map can be reorganized when demand is placed upon it, or stabilized to consolidate skilled motor learning. This property can be measured with cortical mapping using TMS. Pyramidal cells linked within a synergy representation will be brought closer to threshold when the movement is performed, such that the strength and extent of the synergy representation can be measured by MEPs in represented muscles.

1.6.3 Deliberate Practise Effects on Cortical Synergy Representations

Synergies requiring extensive coactivation of muscles under habitual bilateral control, such as axial and reach synergies, are most likely to be evident in the ipsilateral hemisphere. Reorganization of cortical motor representations should become most evident after extensive deliberate practise. Motor map reorganization studied longitudinally in animals is shown to continue with training over the course of one year (Nudo et al., 1996), and be measurably different between primates trained for one and five years (Schieber, 2002). Studies of expert performers with accumulation of vast amounts of deliberate practise show motor training becomes consolidated into structural grey matter (Wei et al., 2011) and white matter (Bengtsson et al., 2005) changes after at least 20,000 hours of training. Studying ipsilateral changes in experts who train skilled bilateral axial movements should optimize the chance of identifying beneficial ipsilateral change.

1.7 BROAD HYPOTHESIS AND INTRODUCTION TO THE EXPERIMENTAL CHAPTERS

The broad hypothesis is based on observations from a classical experiment. Ipsilateral motor pathways effectively compensate bilateral pyramidotomies in primates (Lawrence and Kuypers, 1968). The effectiveness of similar compensation is variable in humans where a spectrum of responses in ipsilateral motor pathways is seen in response to corticospinal tract lesions. The spectrum ranges from absence of compensation, through maladaptation to effective recovery. The starting point takes the broadest view that activity in ipsilateral motor pathways can be beneficial to motor control in health and following stroke. The experimental question asks whether deliberate practice can drive plasticity in ipsilateral cortical representations in healthy subjects, and whether this may be relevant after stroke.

The experimental approach is guided by two main pieces of experimental evidence. After stroke, reliance on alternative ipsilateral motor pathways overlaps with cortical mechanisms that normally contribute to synergy control (Bradnam et al., 2012). Mapping cortical synergy representations in the ipsilateral hemisphere is therefore an investigative approach suited to the experimental question. Another contributing observation is that accumulation of deliberate practise is associated with cortical reorganization in expert performers (Ericsson, 2014).

A series of experiments is used to study plasticity in ipsilateral motor pathways driven by deliberate practise. TMS is used to construct cortical motor maps in healthy subjects, expert performers and stroke patients. The measures are designed to allow interpretation in terms of neuronal populations to understand something of the mechanisms and pathways involved. Each experiment tests deliberate practise effects on cortical representations in different groups where practise focuses on different aspects of habitual activation of muscles under strong ipsilateral control. Findings are reported as measures of cortical motor representations. The MEPs used to construct the motor maps have features within their latency,

morphology and stimulation dependency that may influence interpretation of the motor map findings.

The motor tasks studied are chosen for a predicted link between motor behaviour and neural control. Axial, respiratory and reach synergies are studied. The motor networks engaged are predicted to vary in their relative contributions from different secondary motor areas and their access to different corticomotor outputs. Studying different levels of deliberate practise will allow findings to be interpreted as beneficially serving skilled motor control. Studying stroke patients determines the relevance of applying findings from healthy subjects to stroke rehabilitation.

Athletes up to Olympic standard are studied. They train movements combining axial and reach synergies, potentially with greater reliance on bilateral output from Supplementary Motor Area (SMA). Singers up to world-stage opera performers are studied, training respiratory synergies potentially with greater involvement Cingulate Motor Areas (CMA) within the singing motor network, and potentially with greater voluntary access to bilateral corticobulbospinal pathways. Stroke patients trained in reach tasks may use residual contralateral or intact ipsilateral pathways to perform the movement, and are expected to increase reliance on Premotor Cortex (PMC). The functional consequences of use of alternative ipsilateral motor pathways after stroke are tested.

CHAPTER 2
METHODS

2.1 BASIC PRINCIPLES OF TMS

Magnetic fields induce currents in conducting materials. Rapidly changing magnetic fields delivered across the scalp induce currents that alter transmembrane properties of axons. This may be sufficient to depolarize cortical neurones to produce action potentials. If sufficient numbers of pyramidal neurones are depolarized then a descending corticospinal volley is produced. This can produce post-synaptic potentials in spinal motor neurones with resulting measurable post-synaptic effects in innervated motor units. The post stimulus effects may be inhibitory or excitatory and can be measured by recording from single units or with surface electrodes. Inhibitory effects suppress ongoing EMG if the magnetic stimulus has activated low threshold inhibitory intracortical interneurones (Butler et al., 2007). Excitatory effects are seen either if low threshold intracortical interneurones summate trans-synaptic inputs to depolarize pyramidal neurones (indirect I-waves), or if the induced current is sufficient to depolarize pyramidal cell axons (direct D-waves) (Ziemann and Rothwell, 2000). A descending corticospinal volley represents the sum of output from the depolarized cortical neurones, and may be comprised of a number of D-waves and I-waves. The excitatory muscle response to a descending corticospinal volley is the Motor Evoked Potential (MEP).

The MEP is the basic unit of measurement from which all other TMS measures are derived. The size of the MEP depends on cortical and spinal factors. At a cortical level the number of neurones depolarized, the repetitive discharge of those neurones, and the synchrony of the repetitive outputs will influence MEP size. There is therefore inherent variability in MEP size to a given stimulation intensity. The number of cortical neurones depolarized will depend on regional cortical architecture and the interaction of the applied magnetic field with neural and non-neural structures. Repetitive discharge of pyramidal cells results from oscillatory activity of

excitatory connections within the cortical microcircuit (Di Lazzaro and Ziemann, 2013). At high stimulation intensities, such as those required to evoke ipsilateral MEPs, both D-waves and I-waves will result from increasing repetitive pyramidal cell discharge (Berardelli et al., 1990; Di Lazzaro et al., 1998). Desynchronized outputs may result in phase cancellation of different components of the descending volley. At a spinal level, summation of multiple components of the descending volley may produce polyphasic MEPs with variable morphology, typical of ipsilateral MEPs. The strength of the different components of the descending volley determines whether the alpha motor neurone will fire in response to early or late components, influencing MEP latency.

The cortical response to the applied magnetic field will depend on stimulation parameters and biological factors. The coil design, pulse waveform and stimulation intensity will influence the focality of the field, rate of change of field and depth of penetration. Induced current depends on the rate of change of the magnetic field. Stimulation is typically in the order of 2 Teslas, of duration around 0.1ms and penetrates to a depth of between 1 and 3cm depending on intensity. If greater intensity is used, depth of stimulation increases at the expense of focality. Attenuation of the field will be dictated by properties of overlying non-neural tissue. Charge accumulation occurs at each tissue interface, between skin, skull, cerebrospinal fluid, grey matter and white matter (Opitz et al., 2013). Conductance through cerebrospinal fluid spaces can alter current density and induce regional eddy currents (Stokes et al., 2013).

Neuronal interaction with the magnetic field will be determined by cortical architecture, from macroscopic gyral and sulcal morphology through to microscopic columnar orientation. The shape of gyri and sulci alter alignment of neurones within cortical columns relative to the magnetic field. Cortical columns are perpendicular to the surface, and pyramidal axons aligned longitudinally within columns. Stellate cells, interneurones and pyramidal horizontal fibres in superficial cortical layers have axons that travel horizontally, parallel to the cortical surface in lateral convexity gyral crowns, with axons that are densely radially interconnected without overall

directional emphasis. The magnetic field is perpendicular to the coil surface, and the induced electric field at right angles to this. Where gyral crowns are parallel to the scalp surface, the current may be expected to flow through superficial horizontal cortical layers, but this is highly dependent on coil position and the shape of gyri. Cortical microcolumns will be variably exposed to the influence of the magnetic field across the radius of the curvature between sulcal bank, gyral lip and gyral crown (Fox et al., 2004). If the orientation of the magnetic field favours depolarization of intracortical interneurones then I-waves will be preferentially activated, or if the magnetic field is orientated to depolarize the axon then D-waves may be produced. This is exploited experimentally by using different coil orientations known to preferentially activate different D and I waves (Di Lazzaro and Ziemann, 2013; Sakai et al., 1997).

Neurones are most sensitive to depolarization at the axon hillock, which has the greatest density of voltage gated sodium channels. There is a density gradient of voltage gated sodium channels along the axon, reducing with distance from soma. The angle of orientation of the axon relative to the magnetic field will influence exposure of the axon hillock, and therefore the likelihood of activating D-waves. The gradient of the magnetic field will change most sharply when part of the axon is aligned with the magnetic field, and the axon then bends sharply to produce a membrane gradient of polarized and non-polarized adjacent segments. Therefore, the induced current in a particular neurone depends on its orientation, the length of axon exposed to the field, and whether there is directional change along the course of the axon.

At a spinal level, MEP size depends on the number of alpha motor neurones recruited by the descending corticospinal volley. This depends on the excitability of alpha motor neurones at the time of the corticospinal input. Excitability of spinal circuits is controlled by balance of descending supraspinal and peripheral afferent inputs (Day et al., 1987). Spinal level facilitation may be produced by muscle pre-activation. This is exploited experimentally using background muscle activation to place the segmental alpha motor neurone pool closer to threshold, making MEPs more visible in

response to weak stimulation. Use of target muscle pre-activation to facilitate MEPs therefore needs to be controlled to consistent levels of contraction. The facilitatory effect of muscle activation on MEP size can be estimated so that contraction can be constrained within ranges where only small changes in MEP size will occur with minor fluctuations of background activation. For intrinsic hand muscles the facilitation saturates at low levels of contraction, whereas for proximal and axial muscles facilitation profiles remain more linear over wider ranges (Ravnborg et al., 1991).

2.2 BIOPHYSICAL INTERACTIONS BETWEEN TMS AND CORTICAL MICROARCHITECTURE RELEVANT TO IPSILATERAL MOTOR MAPPING IN HEALTHY SUBJECTS AND AFTER STROKE

2.2.1 Regional Variability of Induced Field Interactions with Neuronal Populations and Motor Map Outcome

The introduction of navigated TMS has greatly improved TMS motor mapping. Underlying cortex being stimulated can be visualized using stereotactic localization of coil position relative to 3D views of the cortical surface reconstructed from individual MRI scans. Computational modelling allows regional estimates of the magnetic field interaction with cortex to be projected onto the anatomical view. The estimate of field intensity can model attenuation by non-neural structures. This provides the operator with a predicted anatomical location of maximal stimulation for a particular combination of scalp stimulation site, coil orientation and stimulation intensity. Conversely a neural target of interest can be defined and the operator guided to the most effective scalp site to stimulate the target. Navigated TMS increases operator perception of the variable relationship between coil handling, stimulation parameters and the resulting cortical effect. Effectiveness of the modelled estimates of biophysical interaction will increase as computational capabilities improve. Predicted magnetic field interactions calculated with a Finite Elements Method incorporating factors of gyral folding and tissue conductivity can be compared to physiological MEP data. A large proportion of MEP variance arises from fluctuation in electric field magnitude (Opitz et al., 2013). Tailoring stimulation parameters to the biological properties of the underlying cortex could reduce MEP variability and increase accuracy of TMS motor mapping.

2.2.2 Modelled Induced Field Interactions with Cortical Microcolumns

Some fundamental electrical properties of neurones illustrate the importance of considering induced field biophysical interactions at a microscopic level. Voltage gradients applied across isolated axons produce depolarization when longitudinally aligned, but not when perpendicular. The threshold for depolarization is inversely proportional to the cosine of the angle between applied current and axon axis. Threshold also depends on the length of the axon exposed to the voltage gradient (Rushton, 1927). Pyramidal cells are aligned in columns perpendicular to the cortical surface, and will be preferentially polarized when the magnetic field is parallel to the axis of the pyramidal axons. Accordingly, stimulation with TMS is optimal when the coil orientation delivers the induced field at 45 degrees medial to the anteroposterior plane, perpendicular to central sulcus (Day et al., 1989; Mills et al., 1992).

Directional dependence of polarization means that although stellate cells, interneurones and horizontal fibres in superficial layers of gyral crowns are in closer proximity to the coil, depolarization may be stronger in the bank of the sulcus. Finite Element Method models show the potential variability of sites of maximal stimulation. With standard coil orientations, maximal stimulation may be within pyramidal cells or interneurones in the bank of sulcus, lip of gyrus or crown of gyrus depending on spatial variation (Silva et al., 2008). Greater predictive value can be achieved by comparing modelled data to physiological data. Use of PET to study TMS evoked finger movement shows that activation is strongest in the sulcus. The neuronal population activated by TMS was found to correlate to the angle of the cortical surface and the distance from the coil (Krieg et al., 2013). The direction of induced field was a stronger determinant of neuronal activation than stimulus intensity. Another PET study confirmed that TMS activations were maximally sulcal, and activation obeyed the angle of cortical columns relative to the induced field (Fox et al., 2004). This illustrates the importance of coil handling in constructing motor maps. Neuronal

populations that are potentially accessible to TMS at a given scalp site may only be effectively stimulated if the correct coil orientation, pitch and plane suits their spatial alignment within the cortical column.

2.2.3 Macroscopic Factors Causing Variability in Induced Field Interactions with Neuronal Populations

Consideration of macroscopic factors influencing induced field biophysical interactions is required to place the earlier discussion of microscopic factors into context. Depth and geometry of cerebrospinal fluid spaces, non-neural tissue conductance and conductance change at tissue interfaces have been mentioned as examples of macroscopic influences on cortical stimulation effects. These factors will clearly show marked inter-individual variability. Intra-individual hemispheric asymmetry in macroscopic tissue determinants of biophysical interaction has also been quantified. Estimates for commonly stimulated cortical regions show marked asymmetries of field interactions within hemispheres, and between homologous regions of opposite hemispheres (Bijsterbosch et al., 2012). This may be relevant to note when comparing ipsilateral and contralateral MEPs from different hemispheres, such as when stroke lesions affect either hemisphere. Inter-individual differences may further accentuate these hemispheric biophysical asymmetries, such as common polymorphisms that amplify inter-hemispheric cortical surface asymmetries (Duboc et al., 2015). The relevance of these considerations becomes heightened in ipsilateral motor mapping, since the hemisphere dominant for ipsilateral responses to TMS shows inter-individual variability in a manner unrelated to handedness (Ferbert et al., 1992; MacKinnon et al., 2004). Marked inter-individual variability of ipsilateral activation is also reported with fMRI (Kobayashi et al., 2003). In monozygotic twins of identical handedness, lateralization of ipsilateral cortical representations of bulbar muscles was seen on opposite hemispheres (Hamdy, 2006). The genetic basis for this is not clear (Klar, 2003). Variable hemispheric lateralization may explain variation in deficits between individuals with similar stroke lesions for muscles with strong bilateral innervation (Hamdy, 1998). Awareness of these factors allows conclusion that there may be unpredictable variability in ipsilateral motor maps between hemispheres. Some of this variability may be due to hemispheric asymmetry of macroscopic factors determining biophysical interactions of induced fields, and some to poorly understood factors

making lateralization of ipsilateral representations unpredictable. To add to this complexity, the ipsilateral dominant hemisphere may be different for different muscles in the same individual (Hamdy, 2006; MacKinnon et al., 2004).

2.2.4 Synchrony Effects on Ipsilateral Motor Mapping

Some inherent variability of MEPs arises from desynchronization of pyramidal cell outputs. This may lead to phase cancellation when summated at spinal level. Timing of TMS in relation to the spontaneous oscillatory activity of cortical neuronal populations may influence desynchronization and repetitive firing within the descending corticospinal volley. This can be noted in relation to ipsilateral MEPs that often arise from summation of multiple weak descending inputs. A relationship between oscillatory EEG activity and MEP amplitude is recognized (Mäki and Ilmoniemi, 2010; Sauseng et al., 2009), and the phase of oscillation rather than EEG amplitude determines the corticospinal excitability effect (Berger et al., 2014). Precisely how single TMS pulses interact with underlying cortical rhythms is incompletely understood (Pellicciari et al., 2017), but it may be possible to improve accuracy of TMS motor mapping by attending to underlying oscillatory activity. This is of interest in the study of ipsilateral motor maps, where control of synergy representations is an important feature. Recurrent loops of beta oscillations coherent between cortical motor representations and muscles may bind muscle representations linked in a synergy (Aumann and Prut., 2015). Resonance between the recurrence of the cortico-muscular loop and the underlying cortical oscillation may strengthen the ipsilateral representation. Combined TMS-EEG methodology could provide an opportunity to explore the functional basis of the motor map suggested by such a hypothesis. As TMS-EEG methods advance, TMS motor mapping could also provide information about the function of cortical points within large scale motor networks, for example by measuring perturbation of cortico-cortical coherence (Rogasch et al., 2017; Strens et al., 2004).

2.2.5 Effect of Stroke Lesion on Induced Field Interactions with Neuronal Populations

Consideration of macroscopic influences on induced field biophysical interactions becomes of great importance when stimulating the ipsilesional hemisphere after cortical stroke. In the chronic phase the lesioned brain becomes atrophic and gliotic (Huang et al., 2014). The damage to the brain surface may include irregular cerebrospinal fluid filled spaces. Applying high intensity TMS over such a region of damage can result in induced current being transmitted unpredictably to neighbouring neuronal tissue, carried by eddy-currents. In this situation spatial information within the motor map may not be reliable. One study using Finite Element Method modelling of induced field interactions in the region of infarcted parenchyma demonstrated that current density is unpredictably altered in its location, strength and orientation (Wagner et al., 2006). Another study also modelling with Finite Element Methods showed that spatial resolution of TMS was reduced when stroke damaged the cortical surface, but decay of field with depth was not altered (Minjoli et al., 2017). The unpredictable effect of cerebrospinal fluid spaces on cortical sites of maximal stimulation in pathological conditions was quantified by a neurosurgical study of brain tumour resection, comparing focality of TMS motor mapping to intracortical microstimulation. A cerebrospinal fluid void following tumour resection increased the focality discrepancy from a few millimetres to many centimetres (Kantelhardt et al., 2010). An awareness of the effect of the lesion on response to TMS is required to plan and interpret motor mapping studies in patients with cortical damage.

2.3 PRINCIPLES OF TMS APPLIED TO IPSILATERAL MOTOR PATHWAYS. THE FUNDAMENTAL CHARACTERISTICS OF IPSILATERAL MEPS

Bilaterally descending motor drive is predominantly concerned with postural stability therefore activity in ipsilateral motor pathways is most evident in proximal and axial muscles. Reflecting the distribution of bilaterally descending motor pathways, there is a proximal to distal gradient of synaptic facilitation in ipsilateral motor pathways. This reflects facilitation patterns seen in response to reticulospinal stimulation in primates (Davidson and Buford, 2006), and the inverse of the distal to proximal gradient of monosynaptic corticospinal facilitation in humans (Palmer and Ashby, 1992). Accordingly, ipsilateral MEPs are most evident in axial and proximal muscles, becoming scarce distally. The distal extent of ipsilateral responses varies between individuals (Bawa et al., 2004). Ipsilateral cortical motor output is relatively weak and therefore requires stronger stimulation intensity and background activation to make MEPs evident. The summation of multiple weak inputs required to produce ipsilateral MEPs makes them smaller than contralateral MEPs and of variable morphology. For this reason, MEP area is often a more suitable measure of ipsilateral MEPs than the peak-to-peak amplitude used for contralateral MEPs. Ipsilateral MEPs are of longer latency than contralateral, reflecting the polysynaptic nature of the pathways and reliance of multiple descending I-waves to summate to produce the MEP.

2.4 BACKGROUND ACTIVATION IS REQUIRED TO ELICIT IPSILATERAL MEPS

Strong background muscle activation is required to produce the spinal facilitation necessary to elicit ipsilateral MEPs. Combined with the high stimulation intensities required, study of ipsilateral MEPs can be demanding for subjects to tolerate. High levels of background activity can also make it effortful to accurately measure small MEPs, and automated analysis tools become of limited use. Some investigators have used paired pulse protocols with facilitatory interstimulus intervals to reduce the pre-activation requirements of ipsilateral MEPs. It has been demonstrated that intracortical facilitation can be measured within ipsilateral cortical representations (Schwerin et al., 2011). This approach can potentially improve subject tolerability, but introduces the complication of interpreting findings in terms of the additional variables within the intracortical component.

2.4.1 Facilitation Profiles of Proximal and Axial Muscles

MEP size is influenced by spinal level facilitation produced by background muscle contraction. This is exploited experimentally to make it practical to elicit ipsilateral MEPs within acceptable stimulation intensity ranges. Ensuring that fluctuations in MEP size are not driven by fluctuations in background activation requires both a knowledge of the facilitation profile for the muscle studied, and a means of controlling voluntary muscle pre-activation within an acceptable range identified according to the facilitation profile. Distally, such as in intrinsic hand muscles, the facilitation profile obeys a sigmoid function and plateaus between 5% and 10% of maximal voluntary contraction (Hess et al., 1987). This makes it easy to produce steady levels of background contraction that will not produce undue further facilitation with small fluctuations and will not rapidly fatigue. For proximal and axial muscles the facilitation profiles are more linear and plateau at higher levels (Ravnborg et al., 1991). For axial muscles the range at which facilitation plateaus is estimated to be between 20% and 40% of maximal voluntary contraction (Jaberzadeh et al., 2013; Nowicky et al., 2001; Tunstill et al., 2001). The manoeuvres and exertion required to pre-activate axial muscles for ipsilateral MEP recording makes it impractical to test at plateau level. Controlling the activation level with auditory and visual feedback, monitoring the background activity in the 50ms prior to contraction, and normalizing the MEP to background activity can all be used to safeguard against the effects of fluctuations in background activation.

2.4.2 Timing TMS to Rise of Background Activation is Required to Evoke Ipsilateral MEPs

The timing of TMS relative to the ramping of background activation also influences the effectiveness of the facilitation provided by voluntary muscle activation. Because of the relative weakness of ipsilateral MEPs, every facilitatory factor should be optimized to make weak post-synaptic effects most visible. TMS is most effective when delivered at the rise of background activation before reaching the steady value (Mills and Kimiskidis, 1996). Manual triggering of TMS timed to the rise of activity judged by auditory feedback is an effective means of delivering consistent optimal stimulation.

2.4.3 Task Dependency of Background Contraction Alters the Degree of Facilitation

Different methods of producing background activation can facilitate ipsilateral MEPs to a varying extent (Bawa et al., 2004). The same level of background activation may produce different amounts of facilitation depending on the task. For abdominal and back muscles, the facilitation is greater when the activation is produced by forced expiratory breath holding compared to voluntary trunk flexion (Jaberzadeh et al., 2013; Tunstill et al., 2001). Lateralization of voluntary contraction alters facilitation. Bilateral homonymous contractions of elbow flexors suppress MEPs, whereas heteronymous elbow flexor and elbow extensor contractions are facilitatory (Tazoe and Perez, 2014).

The nature of contraction becomes particularly important in relation to some specific features of ipsilateral motor pathways. Reticulopsinal and vestibulospinal contribution to ipsilateral MEPs is sensitive to modulation by tonic neck reflexes. The mass contraction required to activate axial muscles will inevitably include neck muscle activation. Cervical rotation (Tazoe and Perez, 2014) and cervical flexion (Fujiwara et al., 2009) facilitates ipsilateral MEPs. Head position should be kept constant, and visual feedback provided directly in front of the subject.

2.4.4 Effect of Background Contraction on Motor Map Measures

There is variability between motor mapping studies in the level of background activation used, commonly expressed as a percentage of maximal voluntary contraction. Different levels of activation may be required depending on muscle and task studied. Whilst varying background activation will result in different levels of facilitation, comparison of some motor map measures between studies using different background activation is possible. The influence of background activation on stability of motor mapping measures was studied by repeated motor mapping of finger muscle representations at 5%, 10%, 20% and 40% of maximal voluntary contraction. Increasing activation increased map area and map volume but topography and Centre of Gravity (CoG) were unchanged (van de Ruit and Grey, 2016).

2.5 STIMULATION PARAMETERS FOR STUDYING IPSILATERAL MEPS

2.5.1 Stimulation Intensity

High stimulation intensities are required to produce ipsilateral MEPs. As stimulation intensity increases a depth-focality trade off compromises spatial resolution in favour of increasing the chance of eliciting an MEP. Many studies of ipsilateral MEPs have used 100% of maximum stimulator output. At such high stimulation intensity the depth and spread of current flow challenges mechanistic interpretation in terms of discrete cortical neuronal populations. Very high stimulation intensities are also not practical for mapping experiments because repeated stimulation would not be tolerable. When mapping, care must be taken to optimize background activation and delivery of TMS to allow the lowest practical stimulation intensity to be used. Ensuring high quality mapping requires a stimulation intensity that balances spatial resolution against the likelihood of revealing ipsilaterally active cortical areas.

The development of controllable pulse width stimulators has the potential to improve the study of mapping ipsilateral cortical representations. Tailored pulse characteristics could potentially target specific neuronal populations and reduce the energy requirement of individual pulses. This reduces scalp sensation to increase tolerability and would allow higher density mapping (Peterchev et al., 2017). Current commercially available controllable stimulators lack the pulse energies required to elicit ipsilateral MEPs (Peterchev et al., 2014).

2.5.2 Stimulation Intensity for Mapping Ipsilateral Motor Representations

The optimal stimulation intensity for motor mapping with TMS is not defined, but consensus suggests 120% of the Active Motor Threshold (aMT) provides a compromise between thorough evaluation and focality. Comparing mapping at 110% and 120% of Resting Motor Threshold (rMT) shows larger maps at higher intensity (Kallioniemi and Julkunen, 2016). Another study repeated mapping at 110% and 120% aMT and also found larger representations at higher intensity, but no difference in topography (Devanne et al., 2006). Mapping at 110, 120 and 130% of rMT shows map area increases with intensity, but does not alter topography or Centre of Gravity (van de Ruit and Grey, 2016). Mapping finger muscles at rest and when active does not alter the Centre of Gravity (Ngomo et al., 2012). Applied to ipsilateral mapping studies, use of 120% aMT provides a reasonable balance between allowing reliable calculation of map topography and subject tolerability.

Hysteresis effects of successive high intensity single TMS pulses have been reported when producing stimulus-response curves (Möller et al., 2009). This may be relevant to TMS mapping studies, and can be avoided by pseudo-randomization of the stimulation order of cortical points. Avoiding sequential stimulation of adjacent points will avoid any potential hysteresis effects.

2.5.3 Coil Orientation for Ipsilateral Motor Mapping

Reports vary regarding the optimal coil orientation for evoking ipsilateral MEPs. Interpretation of the literature is complicated by older studies using circular coils, and stimulation at different intensities with different levels of background activation. Some studies have not identified a coil orientation preference for ipsilateral MEPs (Chen et al., 2003). One study that systematically mapped coil orientation preference at different cortical locations show that ipsilateral MEPs have a coil orientation preference distinct from contralateral MEPs. Furthermore, the ipsilateral coil orientation preference varied between cortical points within a map (Maskill et al., 1991). This is consistent with the results of a Finite Element Modelling study that calculated the optimal coil orientation for each cortical point based on gyral architecture and tissue conductance (Opitz et al., 2013). It is likely that this degree of guidance from computational models will become incorporated into navigated TMS mapping protocols as technology improves.

2.5.4 Coil Design for Ipsilateral Motor Mapping

The engineering of the TMS coil influences directionality, focality and depth of stimulation. The focality offered by Figure-of-Eight coils makes them a standard choice for TMS motor mapping. The magnetic field is maximally discharged at the intersection of the windings which makes focal positioning on the scalp straightforward. More powerful stimulation is provided by circular and double-cone coils and many studies of ipsilateral MEPs have used these for convenience, but at the expense of focality. H-coils are designed to have slower decay of field with depth, estimated to penetrate to 6cm, but also lack focality (Roth et al., 2007). Coil designs are yet to evolve to improve focality much beyond the Figure-of-Eight coil (Deng et al., 2013). Coils are being developed to reduce acoustic artefacts that may influence the vestibulospinal and reticulospinal components of ipsilateral MEPs (Peterchev et al., 2017).

2.5.5 Current Spread Considerations in Ipsilateral Motor Mapping

In addition to local spread of current at cortical points, another issue specific to mapping ipsilateral cortical representations with high stimulation intensity is the potential for stimulation to cross the midline to inadvertently stimulate the opposite hemisphere. In this situation, unintended stimulation of the contralateral motor cortex could elicit contralateral MEPs which could then be misinterpreted as ipsilateral MEPs. Studies quantifying the effect have suggested that this issue becomes relevant within 2cm of the midline. Systematically stimulating in 1 cm intervals from the midline laterally showed that the ipsilateral MEP latency changed within 2 cm of the midline, from a delay compatible with polysynaptic transmission to a fast latency suggestive of contralateral current spread (Tsao et al., 2008a). Awareness of this potential problem allows the experimenter to monitor responses from stimulation close to the midline. Cross-checking against the contralateral MEP latency, and persistence of the MEP with coil orientations that would not favour current spread are strategies to add certainty to the lateralization of the MEP origin.

2.6 RELIABILITY OF TMS MOTOR MAPPING

2.6.1 TMS Motor Mapping Spatial Resolution

Reliability of the spatial resolution of TMS motor maps has been evaluated experimentally through comparison with fMRI and intraoperative microstimulation studies. Comparison with fMRI suggests that the discrepancy between TMS and fMRI localization of an intrinsic hand muscle representation is in the order of approximately 1 to 2cm, averaged 13.9mm in one study (Lotze et al., 2003). The discrepancy becomes greater for muscles with a higher motor threshold, suggesting that absolute distance between scalp stimulation site and the neuronal population was a strong influence on TMS focality. A study that used PET to measure cortical activation during TMS confirms that focality of activation varies with the distance of the population of cortically active neurones from the scalp surface (Krieg et al., 2013). Comparing the resolution of pre-operative TMS localization with intraoperative cortical electrical stimulation reported a discrepancy of 5mm when localizing finger representations (Kantelhardt et al., 2010). The magnitude of these discrepancies needs to be considered when interpreting findings of TMS motor mapping studies in the context of neuronal populations in distinct anatomical structures.

2.6.2 TMS Motor Mapping Temporal Reproducibility

The use of infrared neuronavigation systems has transformed intersession reliability of TMS motor mapping (Julkunen et al., 2009; Ruohonen and Karhu, 2010). A longitudinal study of stability of navigated TMS motor maps repeated 6 times over 12 weeks showed that the Centre of Gravity (CoG) and mean of MEPs over all sites remained very stable. Map area however was unstable (Kraus and Gharabaghi, 2016). Robotic TMS mapping systems have the potential to increase the test-retest reliability of motor mapping. These techniques are reported to dramatically improve concordance between fMRI and TMS localization of finger representations (Kantelhardt et al., 2010). Using current standard manual navigated TMS methods to assess plasticity in motor maps at different time points, the choice of map measure used should have proven intersession reliability.

2.7 SENSORY AND ATTENTIONAL INFLUENCES ON IPSILATERAL MEPS

There are sensory and attentional influences on MEP size which may fluctuate through the course of a TMS motor mapping experiment. An awareness of potential sensory influences allows design of the experimental protocol to limit their effect. The acoustic effects of TMS coil discharge are reported to be sufficient to activate otoliths in the sacule and utricle of the inner ear. The resulting afferent input to the brainstem Vestibular Nuclei could modulate ipsilateral MEPs through descending vestibulospinal pathways. In primates this effect has been shown to influence cortical inputs to brainstem reticulospinal relays (Fisher et al., 2012). Novel coil designs reduce the sound output of TMS by changing the acoustic properties of the coil (Peterchev et al., 2015), and programmable stimulators may evolve to generate tailored pulse waveforms with sufficient energy to produce ipsilateral MEPs with quieter stimulation (Peterchev et al., 2014).

Attentional mechanisms are known to modulate MEP size in distal muscles (Ruge et al., 2012). This has also been demonstrated for visual attention effects on Trapezius EMG (Westgaard et al., 2006), and MEPs (Hiraoka et al., 2013). Directing the subject's attention to the auditory and visual feedback of background muscle activation ensures consistent attentional modulation of ipsilateral MEPs.

2.8 RECORDING IPSILATERAL MEPS

2.8.1 Surface Electrodes and Composite Signals

Surface electrodes provide the most practical way to study ipsilateral MEPs. The need for strong background contraction makes it problematic to record intramuscularly, with needle electrode movement being uncomfortable for subjects and requiring constant signal monitoring. The issue of signal cross-talk between muscles within the composite surface signal is a potential limitation in the use of surface electrodes, particularly as many axial muscles of interest overlie deep overlapping muscle layers. Identifying the motor point of the muscle of interest is relatively straightforward for most axial muscles, and positioning the recording electrode accordingly helps to reduce cross-talk. Checking that the recorded EMG signal responds maximally to movement requiring agonist activity of the muscle of interest can then be used to confirm optimal electrode placement. ECG contamination is an unavoidable consequence of recording with surface electrodes in some axial muscles. Where this situation may be encountered, individual assessment of each MEP is the safest way to avoid ECG contaminating MEP measures. Alternatively, an electrode positioned to record ECG can be used as a threshold for timing of stimulation to ensure TMS is triggered away from the QRS complex of the ECG.

2.8.2 Muscle to Electrode Distance Artefact

In contracting muscles, potential variations in electrode-to-muscle distance artefact can be anticipated. This may occur when surface electrodes are used to study diaphragm activity. Chest wall excursion could potentially move the electrodes away from the zone of apposition of the diaphragm. Studies comparing oesophageal and surface electrodes to quantify the artefact show this effect becomes relevant at high lung volumes (Luo et al., 2008). The effect of body habitus on electrode-to-muscle distance artefact must also be considered when studying axial muscles during certain movements. Where there is much subcutaneous tissue overlying the muscle, trunk flexion could dramatically alter the position of the surface electrode relative to the contracting muscle.

2.8.3 Stimulus Artefact Contaminating Short Latency Ipsilateral MEPs

Recording from muscles close to the TMS coil, such as neck muscles, places electrodes in positions prone to stimulus artefact. With the high stimulation intensities required for ipsilateral responses, the duration of the artefact can merge with short latency MEPs. For muscles such as scalene and sternocleidomastoid the MEP latency may be in the order of 8ms. The difficulty that artefact presents in measuring short latency MEPs is illustrated in Figure 2.1. Analysing each MEP individually allows assessment of whether stimulus artefact contamination is problematic. A primate study has used an artefact removal algorithm in this setting. Measuring the response after amplifier saturation allows EMG to be identified emerging on a shifted baseline, and the baseline subtracted (Fisher et al., 2012). Simple practical measures can alert the experimenter to potential artefact. Prior to mapping, electrodes can be checked for artefact by stimulating on or close to the scalp with coil positions or stimulation intensities that would not be expected to produce MEPs, but could interact with recording electrodes and cables.

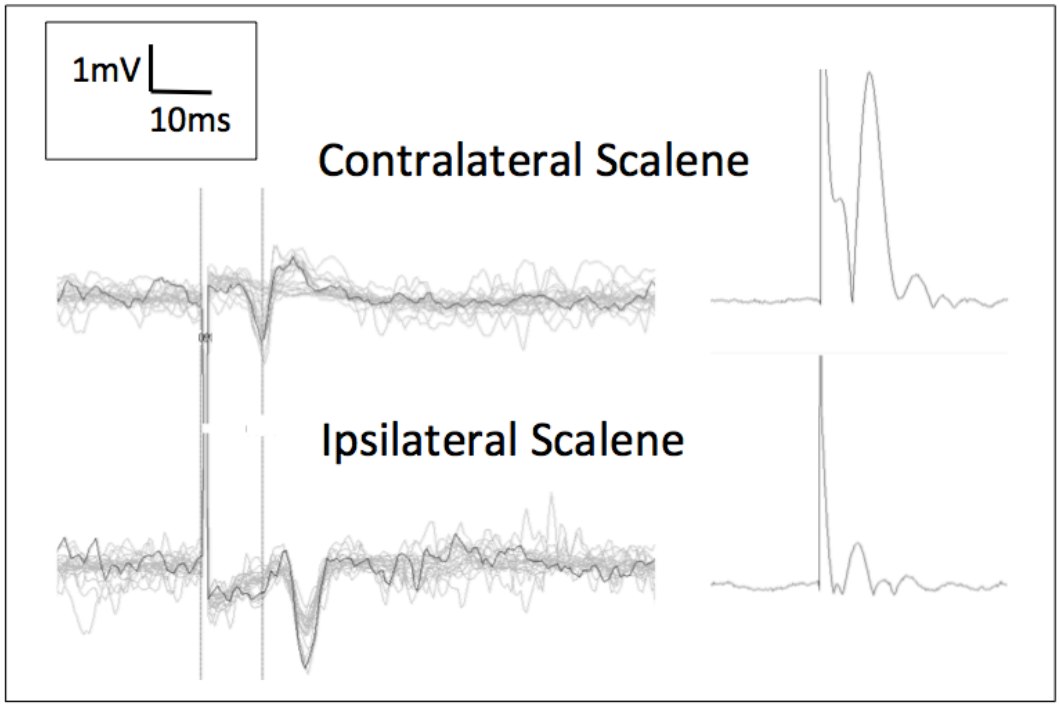

FIGURE 2.1 SAMPLE OF BILATERAL SCALENE MEPS AND ILLUSTRATION OF STIMULUS ARTEFACT

The left hand traces show overdrawn raw mapping data with short latency MEPs recorded without influence of Stimulus Artefact. The right hand traces show different, single MEPs rectified for analysis. The problem of contamination of Stimulus Artefact can clearly be seen in the upper trace, making the MEP unsuitable for analysis.

2.8.4 Limitations of Using the MEP to Deduce the Nature of Ipsilateral Motor Pathways

MEP latency can be used to deduce something of the route of motor pathways. The latency may reflect synaptic delays at any point in the pathway. In the simplest form, comparison between contralateral and ipsilateral MEP latencies can be used to suggest the number of additional synaptic delays in the ipsilateral route. Using an approach similar to the estimation of Central Motor Conduction Time, stimulating at cortical, brainstem and spinal levels can be used to give an indication of the level of additional synaptic relays in the ipsilateral pathway. The neurophysiological techniques best directed to study this question require single unit recording with needle electrodes. For reasons described above, single unit recording is rarely practical in the study of axial ipsilateral MEPs. Standard MEP recording can be used to deduce something of the polysynaptic nature of ipsilateral motor pathways, providing they are interpreted with awareness of the factors that influence MEP latency.

The latency delay between contralateral and ipsilateral MEPs has been reported to vary greatly within and between experiments. The reported range varies from near simultaneous to more than 10ms. Negligible latency difference has been reported in homologous axial muscle pairs in humans (Carr et al., 1994). This was suggested to result from common innervation from branched pre-synaptic fibres, such as reticulospinal axons. It is more usual for there to be a delay exceeding several milliseconds. The ipsilateral route may involve additional synaptic relays at many points in the pathway. Experiments using Central Motor Conduction Time and Peri Stimulus Time Histograms are discussed below as being useful in considering this issue.

2.8.4.1 *Use of Central Motor Conduction Time to Deduce Polysynaptic Ipsilateral Corticobrainstem Pathways*

Variation in timing of the corticobrainstem component of the cortical response to TMS has been quantified experimentally in primates. Cortical inputs to ipsilaterally descending brainstem pathways have a wide range of latencies (Fisher et al., 2012). This may reflect contribution from different I-

waves, or use of fast direct monosynaptic compared to slow indirect polysynaptic pathways. Variation within responses grouped as being monosynaptic may represent different conduction velocities of different groups of corticoreticular fibres. Polysynaptic routes incorporate synaptic delays within brainstem circuits or long delays in cortical-subcortical loops. Calculating the Central Motor Conduction Time may give useful information, if I-wave effects can be controlled by coil orientation specificity. Latency delay of many milliseconds is reported between the Central Motor Conduction Time of ipsilateral and contralateral diaphragm responses (Khedr and Trakhan, 2001).

2.8.4.2 *Use of Single Unit Recording to Deduce the Nature of Descending Ipsilateral Motor Pathways*

The course of ipsilaterally descending motor pathways may involve premotor relays at spinal level. Various neurophysiological techniques can be used to determine synaptic delays at spinal level. Combining TMS of motor cortex with peripheral nerve stimulation reveals cervical propriospinal premotor relays active in producing disynaptic EPSPs in contralateral upper limb muscles (Nicolas et al., 2001). Ipsilateral disynaptic EPSPs are seen in response to brainstem stimulation of reticulospinal fibres in primates (Zaimi et al., 2012). Decussation via commissural interneurons is a potential synaptic relay mediating ipsilateral responses (Nathan, 1963; Soteropoulos et al., 2013). In these examples, post-synaptic potentials from single unit recordings allow suggestion of the course of motor pathways. Single unit recordings of contralateral MEPs in proximal muscles reveal both short latency responses suggestive of monosynaptic corticomotorneuronal EPSPs and medium latency responses insensitive to coil orientation suggestive of bilateral reticulospinal activity (Colebatch et al., 1990). Single unit recording has been used to assess the facilitation of proximal muscles in response to TMS (Palmer and Ashby, 1992; Turton and Lemon, 1999) and coherence of common drive to axial muscles (Carr et al., 1994). Peri Stimulus Time Histograms and Peak Width at Half Maximum are measures derived from single unit recordings which allow comment on the synchronicity of summation of multiple weak inputs relevant to

ipsilateral MEPs. Single unit recordings can therefore be of value in the study of ipsilateral responses, but their application is limited by the technical demands of obtaining high quality single unit recordings in ipsilateral axial muscles.

2.9 MEASURES OF IPSILATERAL MEPS AND TMS MOTOR MAPPING

2.9.1 MEP Area

The variable morphology of ipsilateral MEPs requires that thought is given to the most appropriate measure. The conventional peak-to-peak amplitude measure employed in the majority of TMS studies may not be best suited when measuring MEPs without the characteristic morphology of distal, contralateral MEPs. Use of MEP area rather than amplitude has greater relevance to studies of ipsilateral MEPs in axial and proximal muscles. Ipsilateral MEPs often represent summation of multiple weak descending inputs producing polymorphic MEPs. Desynchronization of descending corticospinal output has less effect on area than amplitude (Rösler et al., 2002). Area is therefore better suited to the study of ipsilateral MEPs. An accepted criteria for including an ipsilateral response in MEP analysis requires occurrence within expected latency, compatible morphology, and duration exceeding the mean plus two standard deviations of background activity for at least 5ms (Schwerin et al., 2008). This is illustrated in Figure 2.2.

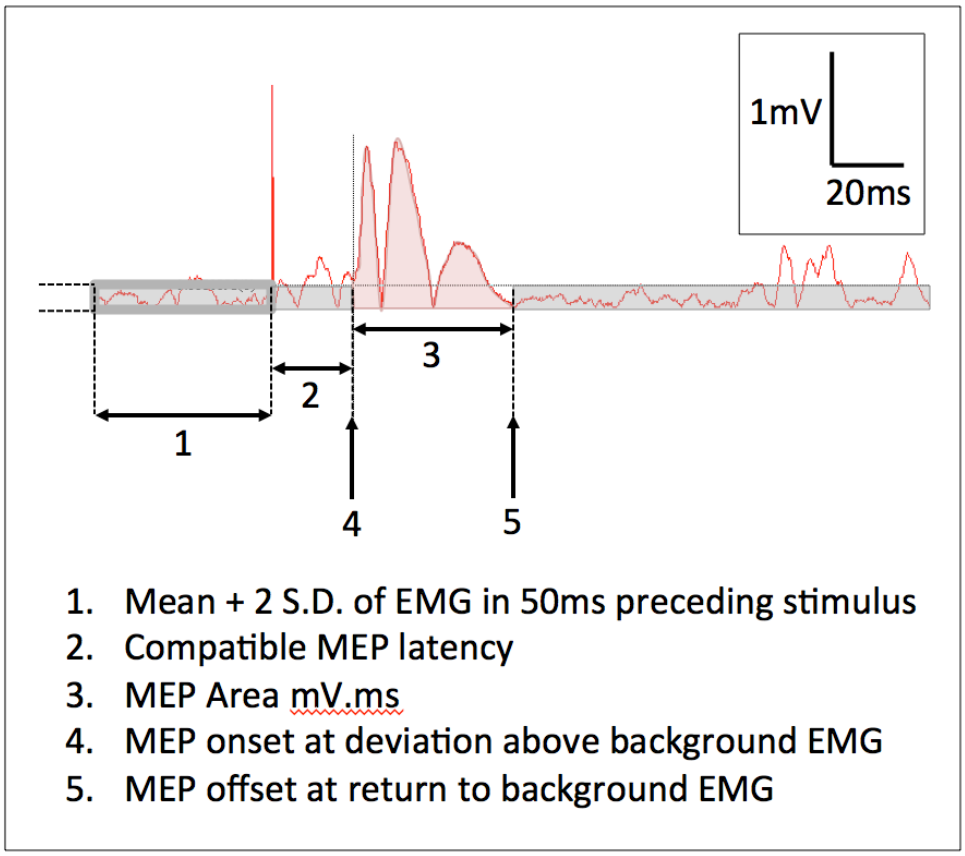

FIGURE 2.2 ILLUSTRATION OF CRITERIA FOR MEASUREMENT OF MEP AREA

MEP Area is useful in measuring ipsilateral axial MEPs which are typically small and of variable morphology. Mean background EMG is rectified in the 50ms preceding the stimulus. A threshold cursor is set at mean background EMG plus 2 Standard Deviations. An MEP is analysed if it occurs with an expected latency, has compatible morphology and exceeds the threshold level for more than 5ms. Cursors are set to mark the point of leaving and return to baseline EMG. The MEP Area is measured between these vertical cursors.

2.9.2 Normalized MEP

Normalization of MEP values is required to allow for inter-individual and inter-session differences in potential artefacts such as variations in electrode position. Normalization is also required to allow pooling of group data. Standard methods of normalization include to Maximum Voluntary Contraction (MVC), maximum MEP amplitude, or supramaximal Compound Motor Action Potential (CMAP). Some of these normalization methods are less reliable in the paretic limb after stroke, where voluntary contraction will have high inter-session variability with factors such fatigue, and MEP amplitude is more variable than in the normal population. Peripheral nerves are not always easily accessible for the stimulation required to produce supramaximal CMAPs in axial muscles. High intensity stimulation over spinal roots or Erb's point offers one potential approach, but at the expense of subject tolerability.

2.9.3 Ipsilateral to Contralateral Ratio

Ipsilateral to Contralateral MEP ratios have been used as an expression of the strength of activity in ipsilateral motor pathways. This is problematic to use in mapping studies since the measure can amplify very small ipsilateral responses from some map regions. For example, stimulating a cortical point that evokes no contralateral response and a very small ipsilateral response would give a high ipsilateral to contralateral ratio that could be misinterpreted as being important to the ipsilateral representation, even if the ipsilateral effect size is very small.

2.9.4 Laterality Index

The Laterality Index is a useful measure to express the balance of ipsilateral and contralateral drive to a muscle. The relative strength of ipsilateral to contralateral MEPs is expressed as (Contralateral MEP-Ipsilateral MEP-)/(Contralateral MEP+Ipsilateral MEP), (Schwerin et al., 2008).

2.9.5 Map Volume

Various definitions have been provided for Map Volume. It is variably described as the sum of relative MEP amplitudes from all excitable points (Gugino et al., 2008); the map area multiplied by the mean map MEP (Kraus and Gharabaghi, 2016); and the sum of the average MEP at each point stimulated normalized to the largest MEP in the map (Wittenberg, 2010). The latter definition is useful in combining information about spatial extent of the motor representation and strength of motor output. This allows the difference between focal and strong versus expansive but weak cortical representations to be contrasted.

2.9.6 Centre of Gravity

The Centre of Gravity (CoG) of a motor map is a robust measure that gives the response at each grid point a relative weighting, and provides a means of comparing regions of cortical representations between studies. The Centre of Gravity predicts the region of greatest excitability of corticomotor neurones projecting to the muscle studied. Centre of Gravity is calculated with the formula $Xcg = \Sigma xa/\Sigma a$ to determine the CoG along the mediolateral dimension and $Ycg = \Sigma ya/\Sigma a$ to locate the CoG along the anteroposterior dimension. The Centre of Gravity of the map is the point at which both coordinates intersect (Wassermann et al., 1992).

CHAPTER 3
EFFECTS OF DELIBERATE PRACTISE OF A BILATERAL MOTOR TASK ON IPSILATERAL CORTICAL MOTOR REPRESENTATIONS IN ELITE ATHLETES

3.1 INTRODUCTION

This is the first of a series of experiments that studies task-dependent and experience- dependent aspects of plasticity in ipsilateral motor pathways. Each experiment uses similar structure and measures but examines different motor tasks designed to challenge different aspects of ipsilateral motor control. The purpose of this chapter is to question whether accumulation of deliberate practise of a task highly demanding of skilled bilateral motor control in muscles strongly innervated by ipsilateral motor pathways can produce plastic change in ipsilateral cortical motor representations. Training a complex movement that demands bilateral coordination of distal, proximal and axial muscles is hoped to make evidence of ipsilateral activity serving skilled motor control most apparent. Training driven neuroplastic change should be most evident in athletes who have accumulated vast amounts of deliberate practise of the task. Comparing groups with different levels of deliberate practise, from trainees to Olympic athletes, will allow any observed differences in ipsilateral motor representations to be related to behavioural measures of skilled motor task performance.

The overall objective of the series of experiments is to understand the role of plasticity in ipsilateral motor pathways in skilled motor control in healthy subjects. This may enable better understanding of the role of the contralesional hemisphere in motor recovery after stroke. The first step is to demonstrate that ipsilateral plasticity can benefit skilled motor control. The next step is to understand the nature of the drive to plastic change in ipsilateral motor representations and the corresponding neural mechanisms.

In this first experiment of the series, TMS is used to map the cortical motor representations of key muscles in the trained movement. Canoeists grouped according to hundreds, thousands, and tens of thousands of hours of deliberate practise of the kayak paddle stroke are compared. Muscles are chosen to be equally task relevant but to vary in lateralization of activation, degree of coactivation and segmental separation. Comparison between groups and between muscles allows the experience dependency and task dependency of ipsilateral plastic change to be tested.

3.1.1 Hypothesis

1. Deliberate practise of a skilled movement producing habitual bilateral activation of muscles under control of ipsilateral motor pathways will drive plastic change in ipsilateral cortical motor representations.

2. Ipsilateral plastic change will manifest as differences in measures of corticomotor connectivity and organization of cortical motor representations of axial muscles.

3. Ipsilateral plastic change will be most evident in subjects with the greatest accumulation of deliberate practise of skilled bilateral axial movements, resulting in differences between groups.

3.1.2 Basis for Hypothesis

The experiment is designed to investigate two main potential mechanisms by which the ipsilateral hemisphere could contribute to skilled motor control. The measures may identify strengthened ipsilateral corticomotor connectivity, or reorganization of cortical ipsilateral movement representations.

The first possibility to consider is that deliberate practice may strengthen ipsilateral corticofugal output. This could be through corticospinal or corticobulbospinal pathways to alpha motor neurones or segmental interneurones. Motor output could originate from primary motor cortex or from secondary motor areas. Primary motor cortex has weak direct output to ipsilateral alpha motor neurones in Lamina IX and Intermediate Zone, and bilateral action via decussating fibres to Lamina VIII (Galea and Darian-Smith, 1994). Pyramidal tract fibres also give collaterals to corticobulbospinal projections to Lamina IX, VIII and Intermediate Zone (Keizer and Kuypers, 1989). Secondary motor areas also have weak but direct corticospinal output to Lamina IX, VIII and Intermediate Zone (Dum and Strick, 2002) and originate ipsilateral corticobulbospinal pathways through brainstem relays (Keizer and Kuypers, 1989). Increased ipsilateral corticofugal output driven by deliberate practise would be evidenced as focal strengthening within the motor map corresponding to an appropriate region of primary motor cortex or a secondary motor area.

Alternatively, in addition to the strength of the representation, the organization of cortical representations of synergies controlling the movement could be altered by deliberate practise. Where complex synergies require coactivation and inhibition of large numbers of muscles, deliberate practise can drive synaptic linkage of multiple movement representations. This may increase the excitability of linked neuronal populations and expand the spatial extent of the motor map. Widely distributed neuronal ensembles recorded in ipsilateral primary motor cortex can be shown to encode ipsilateral synergies of reach (Ganguly et al., 2009). Secondary motor areas are known to represent complex whole-body movements

involving coactivation of multiple interdependent joints. They also encode Anticipatory Postural Adjustments which utilize ipsilateral motor pathways and link multiple axial and proximal muscle representations (Massion, 1992). If deliberate practice reorganizes the motor representation by linking distributed neuronal populations, then the topography of the motor map would be expected to reflect this.

The synaptic mechanisms that underlie experience driven change in motor representations makes TMS suitable for studying this process. Deliberate practise generates excitatory inputs to existing representations, driving formation of new tentative synapses through dendritic spine extrusion and transient glial contact. If beneficial, further practise will reinforce new synapses by Long Term Potentiation. If not, dendritic spines retract and synapses are phagocytosed. Over time this process produces the most efficient neural networks. Repeated coactivation of groups of muscles leads to their representations being synaptically linked in adjacent and remote cortical columns through dense connections between pyramidal cell horizontal fibres and intracortical interneurones. The pattern of synaptic connectivity is governed by spatiotemporal patterns of pyramidal cell firing and synchrony within the cortical network. Whether a representation is stabilized or receptive to change will depend on the balance of intracortical excitation and inhibition, determined by the sum of training related afference, intrahemispheric corticocortical and interhemispheric transcallosal influences. The excitability of a cortical point on the motor map is measured by TMS. When stimulated during voluntary contraction, neurones synaptically linked within a movement representation will be closer to threshold and more likely to contribute to an MEP. The motor map measured by TMS may therefore reflect the degree of deliberate practise driven synaptic linkage within the movement representation.

3.2 METHOD

3.2.1 Overview of Experimental Design

The study of subjects highly trained in a task requiring strong bilateral activation of axial and proximal muscles is a practical choice given the difficulties presented in studying ipsilateral MEPs. High motor thresholds demand background muscle activation and high stimulation intensities. Ipsilateral MEPs are most evident in axial muscles, which are harder to activate under experimental conditions. Controlling consistent optimal background activation to allow more focal, lower intensity stimulation requires some skill and endurance on the subject's part. Athletes were a logical choice in terms of their endurance for repeated muscle activation. The combination of repeated, effortful pre-activation, and large numbers of stimuli at high intensity makes subject tolerability a central feature of experimental design of ipsilateral mapping experiments.

Dominant hemisphere motor maps of ipsilateral and contralateral representations of muscles relevant to the bilateral anti-phase kayak paddle stroke were constructed in competitive canoeists. MEPs in homologous pairs of axial muscles were recorded in response to TMS of frontal motor areas. The total strength of corticomotor output from the representation is expressed as the mean map area, the average response from all grid points stimulated. The organization of the representation is expressed in terms of the map volume and Centre of Gravity. The strength and organization measures of the contralateral and ipsilateral motor maps for each muscle are compared between groups of training, intermediate and expert athletes. These groups differ both in their accumulation of deliberate practice and behavioural motor skill level. Trapezius, Latissimus Dorsi, Pectoralis Major and Abdominal Oblique MEPs were recorded in a single session. An extensive cortical grid with 28 grid points over the dominant hemisphere was stimulated, guided by a neuronavigation system.

3.2.2 Subjects

30 subjects were recruited into three groups of different levels of deliberate practise and acquisition of expertise, Expert, Intermediate and Training. All subjects were right handed. Ages ranged from 18 to 55.

3.2.2.1 *Inclusion criteria*

All subjects were canoeists in regular training and competition. Subjects recruited to the Expert group had competed to World Cup or Olympic level. Their estimates of accumulated deliberate practise exceeded 10,000 hours. Subjects recruited to the Intermediate group were competitors in the First Division of the National League. Their estimates of accumulated deliberate practise exceeded 5000 hours. Subjects recruited to the Training group were competitors in the University team who had begun regular training and competition after the age of 18. They competed in the Fourth Division of the National League. Their estimates of accumulated deliberate practise were in the order of up to 1000 hours.

3.2.2.2 *Exclusion criteria*

Subjects were questioned to ensure that they had not trained to a level of expertise in any other activity that may have influenced findings.

Standard exclusion criteria for TMS were applied according to international consensus safety statements, i.e. history of epilepsy, vascular, traumatic, tumoral, infectious or metabolic brain lesions, medication that may lower seizure threshold, implanted intracranial metal devices, pacemakers, nerve stimulators, pregnancy, sleep deprivation, alcohol excess, heart disease (Rossi et al, 2009; Wassermann, 1998).

3.2.2.3 *Ethics Approval and Informed Consent*

All TMS was performed with local Ethics Committee approval and with the written, informed consent of each subject. Only single pulse TMS was performed. All subjects participated voluntarily without financial reward.

3.2.2.4 *Estimated Duration of Training*

Subjects were asked to make an estimate of their cumulative duration of training based on recollection of typical training schedules at different stages of their competitive careers.

3.2.3 Data Acquisition

3.2.3.1 *Overview of Data Acquisition*

MEPs were recorded from surface electrodes on homologous muscle pairs designated contralateral or ipsilateral to the dominant hemisphere stimulated by TMS. Trapezius, Pectoralis Major, Latissimus Dorsi and Abdominal Oblique MEPs were recorded bilaterally in response to TMS of each point on a scalp grid. In this way a cortical map of the representation in that hemisphere of each ipsilateral and contralateral muscle was constructed. Only the dominant hemisphere was mapped in a single session. TMS was performed during simultaneous background activation of all muscles studied, at 15% of Maximum Voluntary Contraction (MVC). TMS was performed at single stimulation intensity, 120% of active Motor Threshold (aMT) for the ipsilateral Abdominal Oblique.

3.2.3.2 *Background Contraction*

Subjects were seated in a semi-reclined position on a couch with armrests and headrest, legs extended and supported. The backrest was inclined to 45 degrees. Subjects were instructed when verbally cued to raise the back and head gently away from the supported position whilst raising both legs straight off the couch and abducting the arms against the armrests. Slow and small movements were trained to reduce head movement.

Subjects were asked to produce Maximum Voluntary Contraction (MVC) for a period of 3 seconds on 3 trials of Root Mean Square EMG recording. The average of the 3 trials was then used to give a mean value for MVC. A period of practise to make background contraction uniform then preceded the mapping. Subjects were provided with visual and auditory feedback of the EMG signal from each ipsilateral muscle in turn. Visual feedback was provided using a Cathode Ray Oscilloscope. Auditory feedback was through a loudspeaker amplifying rectified EMG signal. For each muscle a cursor was set at 15% of MVC and subjects practised producing controlled muscle contraction to this level. Subjects practised contracting each muscle in turn

with this feedback until consistent levels of contraction were easily repeated.

15% of MVC was chosen as providing a degree of contraction on a favourable position on the slope of the facilitation profile of the muscles studied to optimize MEP facilitation without small fluctuations in contraction levels being likely to unduly influence MEP size. 15% of MVC also provided a degree of contraction that is practical to produce repeatedly for the duration of the experiment.

During mapping it was only practical to provide visual and auditory feedback from one muscle being studied. Additional feedback would have imposed an unsatisfactory attentional demand. By practising the contraction in each muscle with designated feedback, subjects were later able to reliably produce correct levels of contraction in all muscles. The abdominal oblique was chosen as the source of feedback as experience in pilot experiments showed that it was somewhat harder to control low levels of activation in this muscle in this task.

3.2.3.3 *EMG Recording*

Self-adhesive F301 silver/silver chloride foam solid gel surface electrodes (Skintact, Innsbruck, Austria) were used. These electrodes were found to be convenient to place and resisted movement with repeated contractions.

Electrodes were positioned according to accepted belly-tendon placement positions, balancing proximity to motor points with a surface position resistant to movement. The Trapezius active electrode was placed on the upper fibres close to the angle of the border of the neck, and the reference at 3cm along the belly of the muscle (Berardelli et al., 1991). Latissimus Dorsi electrodes were positioned 5 cm inferiorly from the posterior axillary fold, and a further 3 cm distally (MacKinnon et al., 2004). The Pectoralis Major reference was on the central tendon, the active electrode 3 cm proximal on the axillary fold (MacKinnon et al, 2004). Abdominal Oblique electrodes were positioned at one third of the distance along a line from the anterior

superior iliac spine and the umbilicus, with the reference a further 3cm inferomedially (Strutton et al., 2004).

EMG was acquired through an 8-channel D-360 Headstage box (Digitimer, Welwyn, UK) and Power1401 amplifier (CED, Cambridge, UK). Spike software (CED, Cambridge, UK) was used for measuring EMG to calculate MVC and provide visual feedback of background contraction during the subject training. Signal software (CED, Cambridge, UK) was used to record MEPs.

Wide filter settings were used, Low-pass 2kHz, High-pass 10Hz. A sample rate of 10kHz was used. Standard gain of 100 was used. Raw EMG was recorded without notch-filters and rectified off-line using virtual channels in Signal. MEPs were recorded with Signal in Peri-trigger mode, with 200ms of EMG preceding the stimulus included in the frame. TMS was triggered manually with an Event trigger generated by a TTL pulse from the Stimulator.

3.2.3.4 *TMS*

The dominant hemisphere of each subject was mapped systematically with TMS using a scalp grid. TMS was delivered with a monophasic Magstim 2002 stimulator and figure-of-eight coil, Double 70mm Alpha Coil (Magstim, Whitland, UK). TMS was guided by a Brainsight stereotactic infrared navigation system (Rogue Resolutions Ltd, Cardiff, UK).

3.2.3.4.1 *Stimulation Intensity*

Mapping was performed at single stimulus intensity, 120% aMT of ipsilateral Abdominal Oblique. Use of 120% aMT is a conventional choice in cortical mapping as an accepted compromise between reliably eliciting MEPs whilst limiting loss of focality from current spread. The decision to determine the stimulation intensity from the measure of Abdominal Oblique threshold arose from the observation in pilot experiments that this muscle tended to have a slightly higher threshold than the other muscles studied.

Prior to mapping the aMT was determined. The dominant left hemisphere was stimulated in a preliminary search to identify a Hotspot for the ipsilateral Abdominal Oblique muscle. Stimulation began at the grid point 2cm anterior and 2 cm lateral to the vertex, frequently described as a common location for abdominal muscle representation. aMT was determined in the standard way, using the definition of the minimal intensity required to produce MEPs of 200 to 300 microvolt amplitude in 50% of trials. Stimulation intensities were generally in the range of 60 to 80% of Maximum Stimulator Output.

3.2.3.4.2 *Stimulation Technique*

The coil was held with the handle 45 degrees to the mid-sagittal line. A Brainsight neuronavigation system guided coil placement to map points. Stimulation was triggered manually using a foot pedal trigger. Stimulation was timed to the peak rise of background muscle activation, judged by the experimenter using auditory feedback. This ensured that each stimulation was consistently timed to the same level of activation, and minimized the duration of muscle contraction to limit participant fatigue. Delivering TMS in this way ensured stimulation was optimally timed to the rise of muscle activation, before reaching a steady state value (Mills and Kiniskidis, 1996). Interstimulus intervals were appropriately spaced to allow subjects to rest between contractions.

3.2.3.4.3 *TMS Mapping Technique*

A 28-point scalp grid was manually constructed and programmed into Brainsight using a surface pointer directed at points drawn on a tightly fitting skullcap. The vertex was marked at the intersection of the intertragal line and the nasion-inion line. Rows of grid points were marked over the dominant left hemisphere with 1 cm separation. The medial limit of the grid was 1 cm from the midline, the lateral limit 5 cm from the midline, producing 4 parallel rows. The posterior limit of the grid was 2 cm from the vertex, and the anterior limit 4 cm from the vertex. Manually inputting grid

points marked first directly onto the scalp was found to be more reliable than using the software generated automatic grids.

A pseudo-randomized stimulation order was used. Each point on the grid was stimulated 3 times and the average used to give a mean MEP response for that point.

3.2.4 Data Reduction and Analysis

3.2.4.1 *Data Quality*

Each frame was analysed separately with individual manual cursor placements used to measure each MEP and the preceding background EMG. Each frame was monitored following each stimulus to ensure that background activity had remained within acceptable limits, and rejected at the point of acquisition if there was variance.

3.2.4.2 *MEP measurement*

Raw EMG was recorded and virtual channels used off-line to rectify EMG for measurement. Rectified EMG in the 50msec preceding the stimulus was averaged, two standard deviations added and this value was used to set a horizontal cursor as part of the criteria for MEP measurement.

Criteria for analysing ipsilateral MEPs adopted by other investigators to allow for the typical small size and variable morphology were used. Inclusion criteria for a response to be measured as an MEP were occurrence with expected latency, compatible morphology, and exceeding baseline EMG plus two standard deviations for at least 5msec (Schwerin et al., 2008).

MEP area was measured from the rectified EMG using individual cursor placements to identify the MEP onset and offset, marked as the point of deflection above, and return to the baseline EMG. As discussed above, use of MEP area is more relevant to the study of ipsilateral axial MEPs than amplitude.

3.2.4.3 *MEP normalization*

Normalization was required to allow pooling of data for comparison between groups. Variations in electrode positioning between individuals with distance from the motor point in large muscles can contribute to variation in absolute MEP values. To control for this and for variations in background activity, normalization to background activity was chosen.

Other normalizations have been successfully used in similar experiments, such as to MVC, maximum MEP size or supramaximal Compound Motor Action Potential. An aim of this experiment was to allow later comparison to studies in stroke patients, and these alternative methods of normalization may not be reliable in the paretic limb of stroke patients.

3.2.4.4 *Construction of the cortical map*

Three stimuli were delivered at each grid point and the MEPs averaged to give a mean response for that grid point. Average responses were tabulated corresponding to the scalp location.

3.2.4.5 *Cortical Map Measurements*

The mean map area was calculated as the arithmetic mean of the MEP area from all grid points stimulated. The normalized map volume was used to give a measure of focality of the map, dividing the sum of mean MEP area from all grid points that produced responses by the largest MEP area from the map Hotspot. The Centre-of-Gravity (CoG) is a weighted-mean that gives each grid point a relative weighting and provides a robust and reproducible means of comparing maps. The CoG predicts the region of greatest excitability of corticomotor neurones projecting to the muscle studied. The CoG is calculated with the formula $Xcg = \Sigma xa/\Sigma a$ to determine the CoG along the mediolateral dimension and $Ycg = \Sigma ya/\Sigma a$ to locate the CoG along the anteroposterior dimension. The CoG of the map is the point at which both coordinates intersect (Wassermann et al., 1992). Subtracting the ipsilateral from the contralateral CoGx and CoGy gives a measure of the organization of the ipsilateral representation in relation to the contralateral.

3.2.4.6 *Comparison Between Groups*

Map measures were compared between the Expert, Intermediate and Training groups using Analysis of Variance (ANOVA) with initial factors of group, side and muscle to test the hypothesis that deliberate practise may alter the ipsilateral cortical representation. Post-hoc tests were then performed to further explore significant findings. Tests of Normality

revealed that the data was not normally distributed, so logarithmic transformation was used prior to performing ANOVA. Subsequent reporting is in original units using back transformed data.

3.2.5 LIMITATIONS OF METHOD

3.2.5.1 *Use of a Single Coil Orientation*

All mapping was performed with the coil orientated with the handle at 45 degrees to the mid sagittal line. This orientation was employed for each subject as a means of making testing uniform, without attempt at individualizing orientations according to response. This coil orientation was selected according to convention, shown to be effective in producing ipsilateral axial MEPs in numerous experiments. The biophysical rationale for conventional use of this orientation comes largely from the alignment of neurones in hand area of M1. When applied to axial muscle representations and secondary motor areas, the alignment of neuronal populations becomes less certain. It can be acknowledged that a systematic mapping study of axial muscles that included varying coil orientation reported different coil orientations influencing the MEPs (Maskill et al., 1991). Different coil orientation preferences were found at different grid points within the map. The limitation of the number of stimuli on subject tolerability meant that identifying optimal coil orientations for each grid point was not practical. Repeated mapping over multiple sessions each using different coil orientations would be required to test the influence of coil orientation on map stability. It is possible that technological improvements will improve mapping protocols through automated guidance to optimal coil orientations calculated on-line by predictive modelling based on gyral architecture and tissue conductance (Opitz et al., 2013).

3.2.5.2 *Medial Extent of the Map and Current Spread to the Contralateral Hemisphere*

Motor output from secondary motor areas is an important feature of the hypothesis, and it was therefore important that the map was extensive to cover all frontal motor areas involved in axial control. This includes stimulating close to the midline for axial regions of primary motor cortex, and more anteriorly Supplementary Motor Area. When stimulating close to the midline there is potential for current spread across the central sulcus to produce MEPs from the contralateral hemisphere. Without vigilance these

could be misinterpreted as ipsilateral responses. One study that sought to quantify the effect suggested that any stimulation within 2cm of the midline had potential to produce contralateral current spread (Tsao et al., 2008a).

It is suggested that the latency can be used to differentiate between faster responses evoked by fast-conducting contralateral corticospinal pathways and slower ipsilateral responses mediated by polysynaptic pathways. If MEP's are evoked from medial grid points, an ipsilateral origin can then be supported by searching for persistence with alternative coil orientations that would reduce current directed across the midline. This does not provide absolute certainty of a contralateral origin if the MEP does not persist, since the alternative coil orientation may also not be favourable for a discrete ipsilateral neuronal population with specific coil orientation dependence. Use of the lowest practical stimulation intensity is the most efficient way to limit potential current spread.

3.2.5.3 *Use of Single Stimulation Intensity to Map Multiple Muscles*

Mapping was performed at 120% aMT of the ipsilateral Abdominal Oblique muscle. 120% aMT has been widely used in mapping studies as providing a suitable compromise between preserving focality of stimulation by limiting current spread whilst effectively eliciting MEPs.

The choice of the muscles used to determine the stimulation intensity in these mapping experiments was based upon experience from pilot experiments, where Abdominal Oblique tended to have slightly higher thresholds than other recorded muscles.

It was not practical to accurately identify individual Hotspots for each muscle and accurately determine individual muscle thresholds. This would have added greatly to the total stimulation requirement of the experiment and limited subject tolerability. It should be considered that the stimulation intensity used may then have stated representations of the other muscles more strongly than the Abdominal Oblique. Whilst this may be relevant when interpreting between-muscle effects, it should not alter interpretation

of the between-group deliberate practise effects that formed the basis of the hypothesis.

It would have been ideal to map each muscle individually in separate sessions performed at a stimulation intensity calculated accurately for that muscle. This would have required 4 separate experimental sessions and was not practical due to resource and time constraints.

3.2.5.4 *Use of 15% Maximum Voluntary Contraction for Background Activation*

The mapping was conducted with brief muscle activation guided to be 15% of MVC. Background activation is necessary to elicit ipsilateral axial MEPs. The requirement for activation introduces the potential variable of differing degrees of facilitation with fluctuations in voluntary activation. Use of on-line feedback of strength of contraction is aimed to limit this fluctuation, but it is not possible for subjects to attend to feedback from all muscles studied during the brief contractions.

Plateau of MEP facilitation by background contraction occurs at higher values in axial muscles compared to distal muscles. Whilst a finger muscle may be fully facilitated around 10% of MVC, axial muscles may plateau in the range 20-40% (Jaberzadeh et al., 2013; Nowicky et al., 2001; Tunstill et al., 2001). Stimulating during activation levels that have plateaued removes potential for varying amounts of facilitation. It would not be practical to use high contraction levels to reach facilitation plateau in lengthy mapping experiments as subjects would rapidly fatigue.

15% of MVC was chosen as a suitable level of background activation for providing a balance between subject tolerability and facilitation of MEPs. This level of contraction occupies a linear portion of the facilitation profiles for the muscles studied, such that small variations in background activity would not unduly influence MEP size in the way that could be envisaged if the facilitation profile were sigmoid.

3.2.5.5 *Use of Auditory and Visual Feedback from a Single Muscle*

The ipsilateral Abdominal Oblique channel was used to source auditory and visual feedback to guide background activation. This was chosen as experience in pilot studies suggested that this was the hardest muscle in which to control low levels of contraction. Subjects had practised controlling the contraction of other muscles with feedback prior to mapping. It would not be practical to attend to feedback from multiple muscles. For the reasons discussed above, the choice of level of background contraction and the MEP normalization were aimed at minimizing the influence of small variations in background contraction.

Having simple, consistent feedback allowed the subjects to sustain the same level of attention and engagement with the task throughout the long experimental session. Providing feedback from additional muscles would have added attentional demand. High attentional demands have been shown to modulate ipsilateral MEP size (Hiraoka et al., 2013).

3.2.5.6 *Use of a Fixed Point Scalp Grid Positioned Relative to Vertex*

The maps were constructed by stimulating pre-determined points on a grid positioned in a uniform site on all subjects over the dominant hemisphere, marked relative to the vertex. This approach meant that stimulation was uniform between subjects to allow easy comparison between groups.

The decision to use an extensive fixed grid was made largely to ensure that all frontal motor regions including secondary motor areas had been investigated. The disadvantage of this approach is the requirement for a fixed high number of stimuli. An alternative approach is to identify the Hotspot and then progressively demarcate the anteroposterior and mediolateral limits of the map, ending the map at the point when no further responses are seen. This approach is effective when a single muscle is studied, but becomes harder in practise when multiple muscles are mapped simultaneously.

Positioning the grid relative to vertex uses a bony point as a reference. An alternative approach is to use the hotspot of an accessible distal hand muscle such as First Dorsal Interosseus to provide a functional neuroanatomical reference. An advantage of individualizing the grid according to a functional landmark is that it reduces the possibility that muscle representations extend outside of the region stimulated. This was however unlikely with the extensive grid used. A functional landmark can also be of use in attempting to extrapolate grid coordinates to anatomical locations. This consideration becomes less important with the capability of neuronavigation systems to identify the anatomical sites of stimulation more precisely from 3D reconstructions of structural MRI. I did not have the resources to obtain structural imaging on all participants, but this would have been of value in interpreting map measures in terms of distinct frontal motor regions.

3.2.5.7 *Mapping Confined to the Dominant Hemisphere*

Constraints of time and subject tolerability of repeated high intensity stimulation under background contraction limited the mapping session to one hemisphere. For axial muscles there may be a hemispheric dominance of bilateral representation independent of handedness (MacKinnon et al., 2004). It would have been ideal to map both hemispheres in separate sessions, but this was not practical as it would have doubled the time and resource requirements of the experiment.

3.2.5.8 *Limitations of Cross Sectional Studies of Deliberate Practise Effects*

The mapping experiments investigated cumulative deliberate practise effects on cortical movement representations. It was hypothesized that if the ipsilateral hemisphere contributed to skilled motor control of muscles habitually bilaterally activated then development of skill through training may manifest as change in cortical movement representations. This relies on the assumption that observed differences between groups are driven by training effects. The alternative explanation should also be considered, that athletes persevere in sports with movements that they are genetically

predisposed to excel in. It is difficult to separate cause and effect without longitudinal study. Longitudinal study of deliberate practise effects in the order of the 10,000 hours suggested to be required to obtain expert status would be resource intensive.

The cross-sectional approach I employed is a time-effective way of studying this issue. The choice of 3 groups with 1000 hour, 5000 hour and 10,000 hour milestones was aimed at demonstrating a gradation of effect that would offer support to the notion that observed changes were training effects. Whilst not absolute, a progressive change that scaled with hours of practise would offer stronger support to training effects rather than an inherited predisposition that may be evident earlier in the competitive career. These methods are unable to separate the possibilities entirely, particularly as a combined effect may be active, that a genetic predisposition confers an initial advantage later expanded through training.

3.2.5.9 *Limitations of Pooled Group Map Data*

Comparison between groups uses pooled map measures from each subject in the group. It should be appreciated that comparison of measures of mean map volume and Centre of Gravity does not imply a 'mean map' for the group. There is marked inter-individual variation in map topology as evident from visual inspection of the maps, and as would be anticipated from the normal variability in macroscopic brain anatomy. It was therefore not intended to create a distinct topographic representation of training effects. To attempt this with a merged map of all subject data would disregard inter-individual anatomical differences. In pooling data into groups the intention is to identify broad characteristics of the representations that provide information about training effects, such as size of representation, and the broadest information about patterns of organization in terms of anteroposterior and mediolateral emphasis that may suggest something of contributory activity from different brain regions.

3.2.5.10 *Limitations of Small Sample Size*

Convenience Sampling was necessary due to constraints of time, resources and availability of suitable participants. The specialist nature of the motor tasks studied limited the local pool of suitable subjects. Access to experts who had accumulated vast amounts of deliberate practise who were available and willing to participate on a voluntary basis further limited the potential subject pool. A larger sample size could have increased the scope of the work. The limited number of similar TMS studies reported on comparable sample sizes.

3.3 RESULTS

3.3.1 Strength of Cortical Representations

Maps are shown in the Appendix A. Example of typical MEPs obtained during mapping are shown in Figures 3-1 and 3-2.

Data collected from the three groups were not normally distributed and so were logarithmically transformed. Transformed data did not violate normality assumption and were subsequently entered in the 3x2x4 mixed effects factorial ANOVA. This test was conducted to explore the relationship between Group (Training / Intermediate / Expert), Muscle Side (Contralateral / Ipsilateral) and Muscle (LD / TRAP/ PM / AO) on the mean MEP area from all cortical points. All statistical analysis was performed using transformed data. Means and confidence intervals were back-transformed and reported further.

Significant triple interaction was observed between the three factors, $F(6, 48) = 3.927$, $p=0.003$, Figure 3.3 (back-transformed means with CI's) and Figure 3.4 (estimated marginal means). This result shows that depending on whether the muscles are ipsilateral or contralateral to the side of stimulation, the difference in mean responses varied between the four muscles. Additionally, relationships between side and muscle varied across the three groups.

Post hoc two-way ANOVAs were then tested in order to explore the effect of side (contralateral/ipsilateral) on each group in the four muscles separately (Figure 3.5). There was no statistically significant interaction between side and group in Latissimus Dorsi and Trapezius muscles, showing that the relationship between ipsilateral and contralateral did not differ across groups for these two muscles. Significant interaction between side and group was present in Pectoralis Major, $F(2, 17) = 9.638$, $p = 0.002$ and Abdominal Oblique muscles, $F(2, 18) = 8.318$, $p = 0.003$.

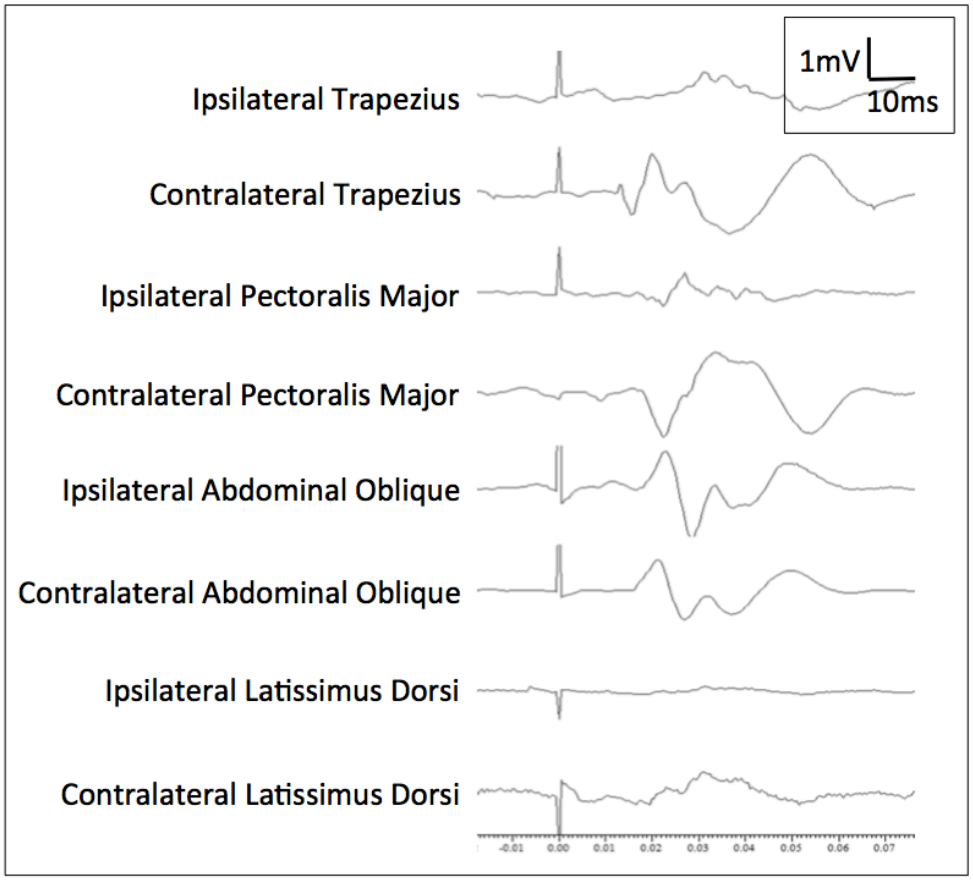

FIGURE 3.1 MAPPING AXIAL MUSCLES.

A sample of bilateral recording of MEPs in multiple axial muscles during motor mapping. MEPs are not elicited in every muscle with every stimulation as some grid points will be remote from some muscle representations. In this example prominent bilateral MEPs are obtained from Abdominal Oblique.

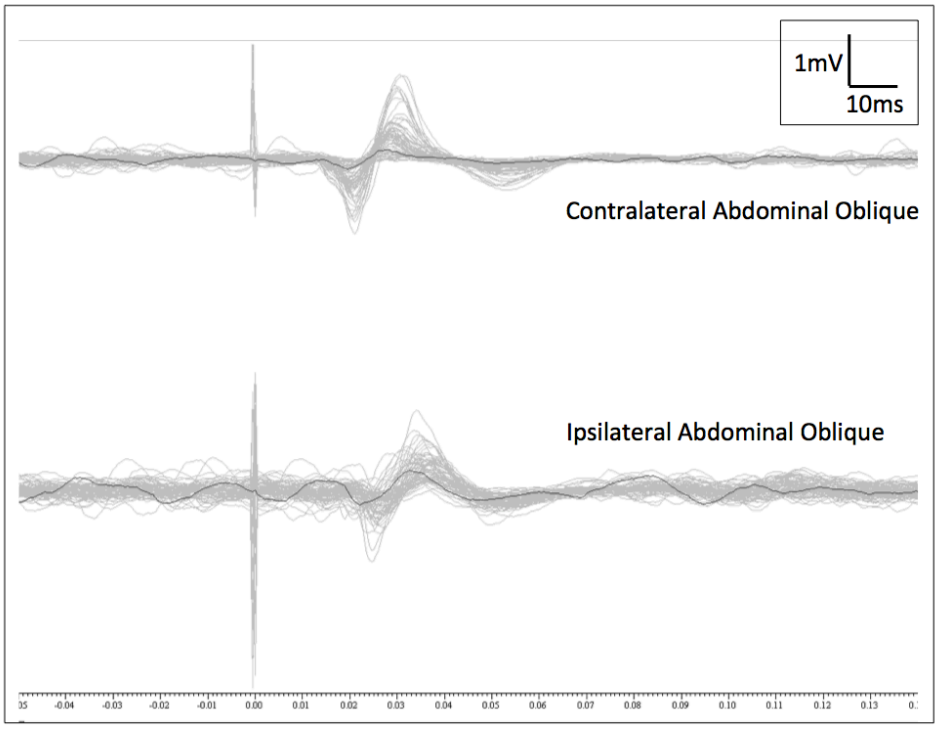

FIGURE 3.2 BILATERAL ABDOMINAL OBLIQUE MEPS SHOWING DEVELOPING MOTOR MAP

Example of overdrawn Contralateral and Ipsilateral Motor Evoked Potentials recorded from Abdominal Oblique muscles. The development of the motor map can be appreciated within the overdrawn traces, with different grid points producing MEPs of different sizes.

Further, four post-hoc one-way ANOVAs were performed to test for the effect of group on ipsilateral and contralateral side of the Pectoralis Major and Abdominal Oblique muscles. Results showed significant group dependent effect on responses only in the ipsilateral Pectoralis Major ($F(2, 17) = 15.012$, $p < 0.001$) and ipsilateral Abdominal Oblique muscles ($F(2,18) = 6.145$, $p < 0.009$) (Figure 3.6).

Tukey HSD post-hoc test, further addressing the results of the one-way ANOVA, verified that responses in the ipsilateral Pectoralis Major muscle were significantly lower in the training group (0.69, CI: 0.49 to 0.69) as compared with the intermediate (1.68, CI: 1.21 to 2.32, $p = 0.001$) and expert group (1.51, CI: 1.2 to 1.9), $p = 0.001$). There was no statistically significant difference between intermediate and expert groups ($p = 0.777$).

Similarly, responses in the ipsilateral Abdominal Oblique muscle were significantly lower in the training group (0.98, CI: 0.61 to 1.57) as compared with the intermediate (2.01, CI: 1.45 to 2.78), p = 0.032) and expert group (2.16, CI: 1.51 to 2=3.09), p = 0.009). Again, there was no statistically significant difference between intermediate and expert groups (p = 0.94).

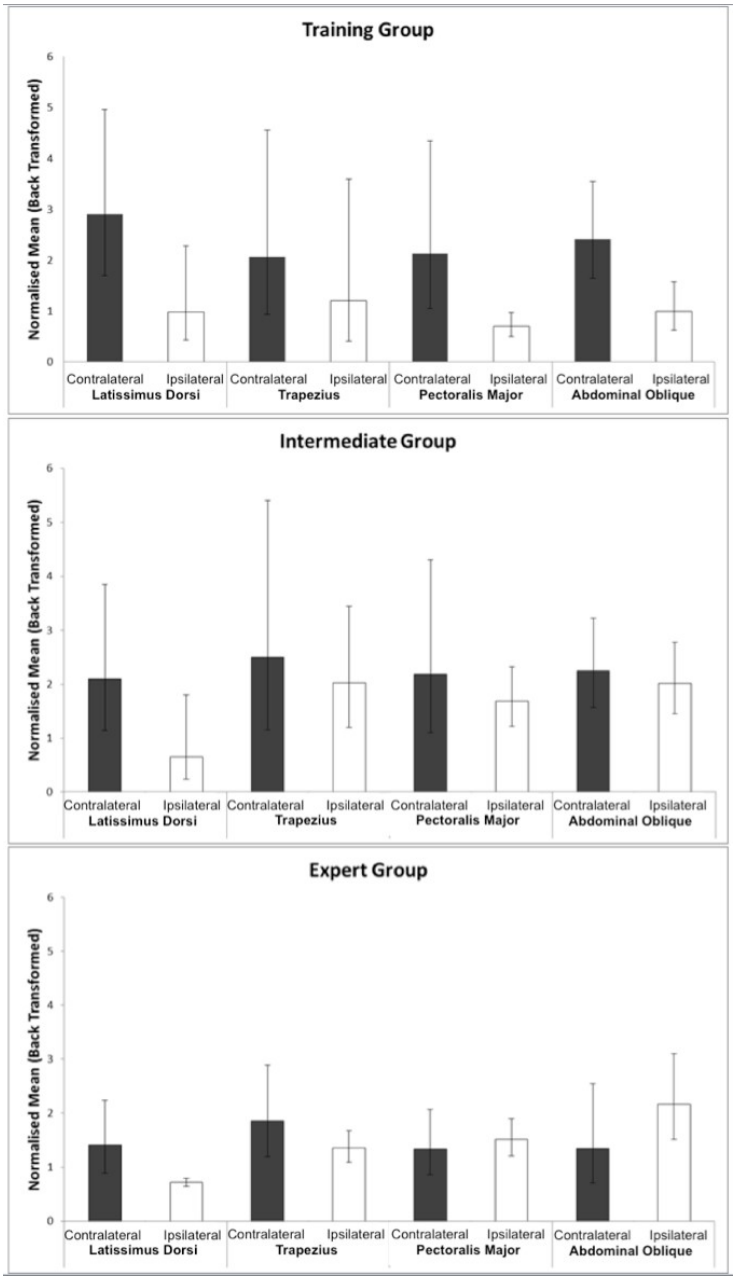

FIGURE 3.3 NORMALIZED MEANS AND CI (BACK TRANSFORMED) FOR EACH MUSCLE IN EACH GROUP.

Black bars – Contralateral muscles. White bars – Ipsilateral muscles.

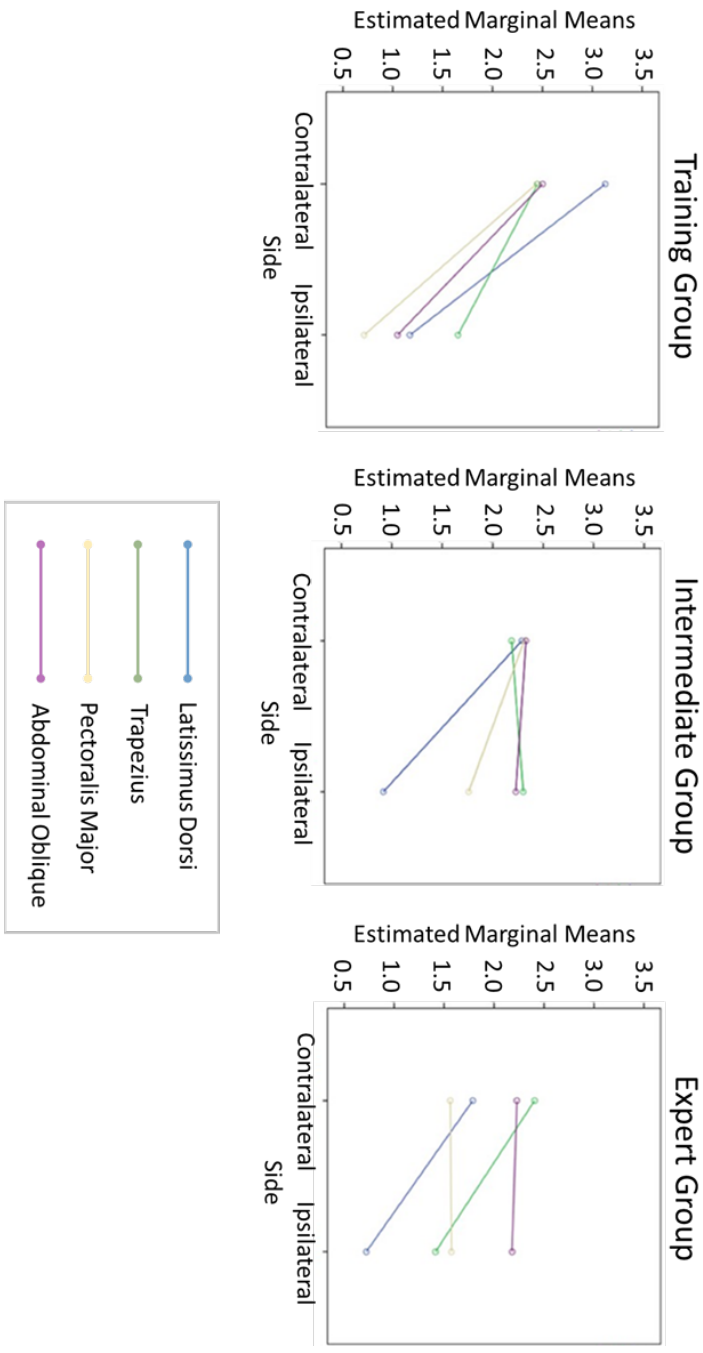

FIGURE 3.4 TRIPLE INTERACTION BETWEEN GROUP, SIDE AND MUSCLE (PLOTTED USING ESTIMATED MARGINAL MEANS)

The interaction between Side and Muscle differs in the three groups.

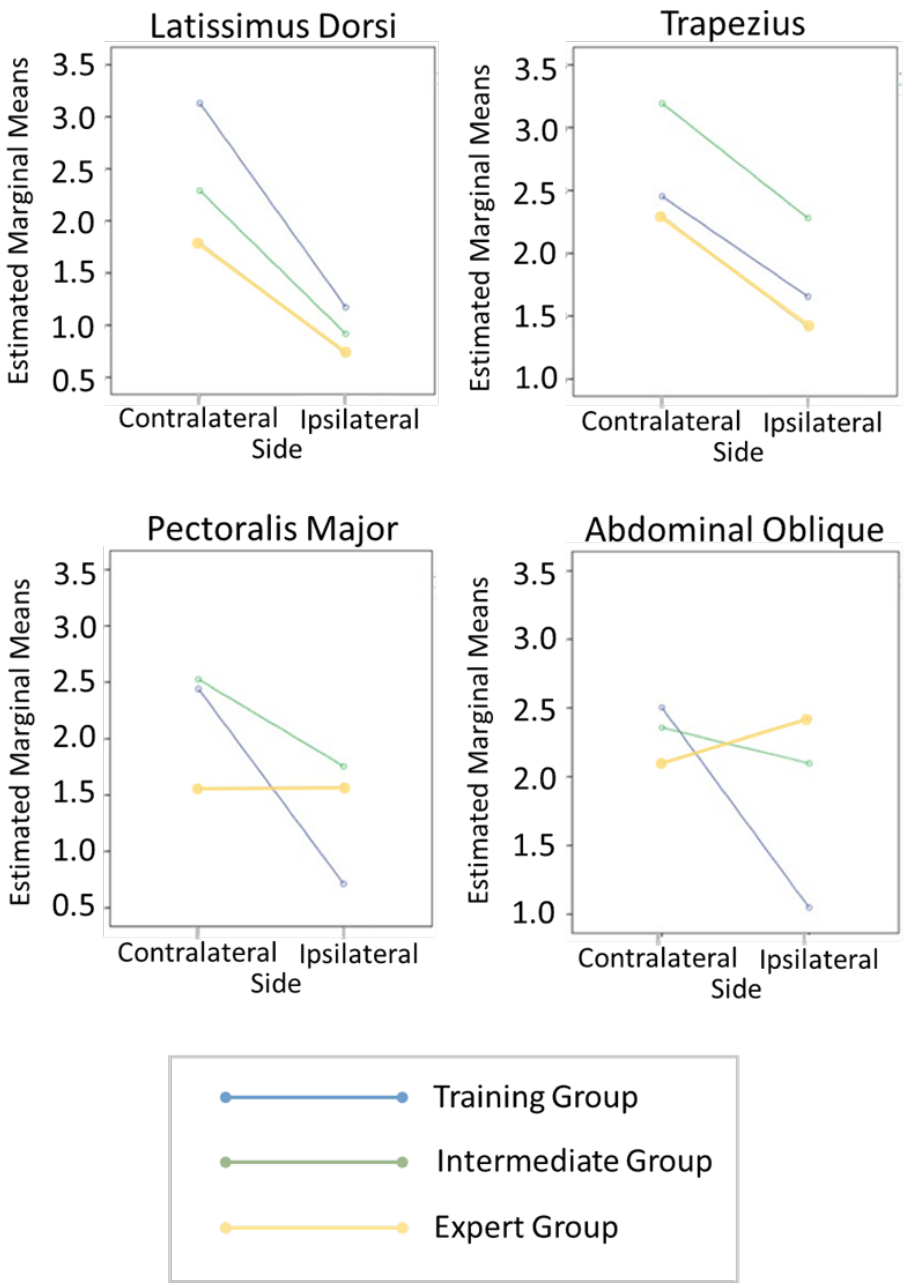

FIGURE 3.5 INTERACTION BETWEEN GROUP AND SIDE FOR EACH MUSCLE

Interaction is only significant in Pectoralis Major and Abdominal Oblique muscles.

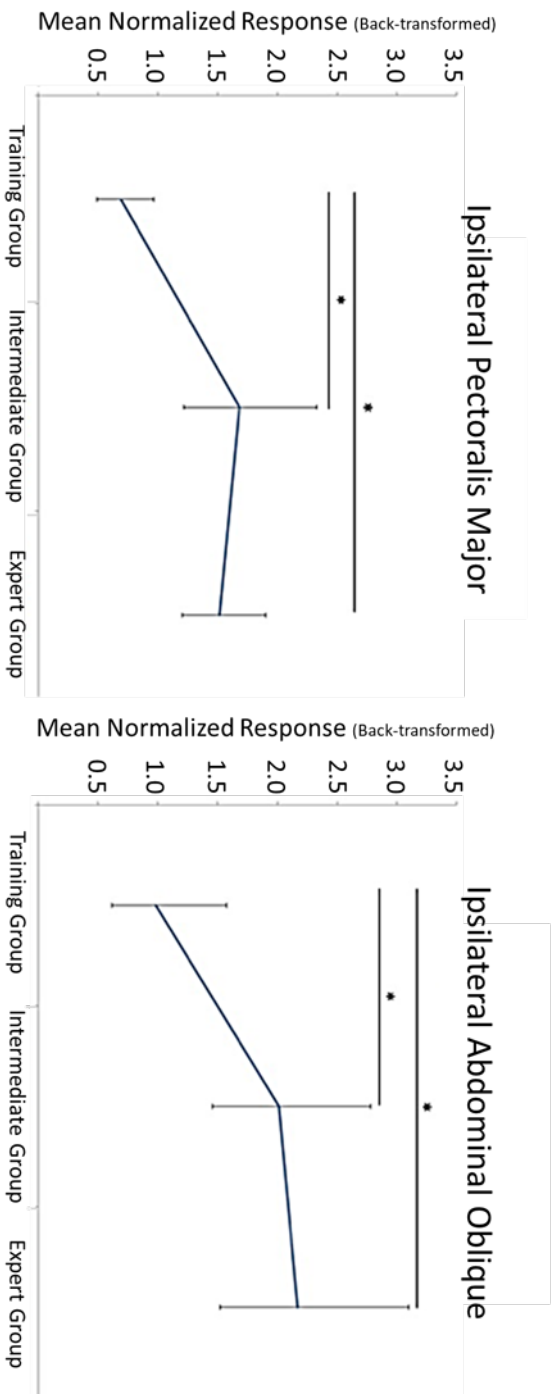

FIGURE 3.6 EFFECT OF GROUP ON SIDE IN PECTORALIS MAJOR AND ABDOMINAL OBLIQUE

3.3.2 Asymmetry and Focality of Contralateral and Ipsilateral Motor Representations

Comparison of the difference in the strength of contralateral and ipsilateral representations shows that for Latissimus Dorsi, contralateral responses are higher than the ipsilateral in all three groups. This is evident from inspection of the graph in Figure 3.3. Post-hoc paired t-test confirms statistically significant difference between contralateral and ipsilateral side in the training group p =.005, intermediate p=.023 and expert group p =.015. Under a conservative Bonferroni correction, the result in the intermediate group does not pass statistical significance, however a clear trend is obvious.

Using an adapted normalization, dividing the sum of MEPs from all excitable grid points by the number of excitable grid points allows an expression of focality. In this way a focal representation with few cortical points evoking large MEPs will have a higher value than an expansive representation with multiple cortical points evoking small MEPs. In each muscle in each group this pattern was consistently seen. Even when the total map volume was higher for the ipsilateral representation, this pattern of focality of representation was observed. The normalized volumes are shown for each muscle in Figure 3.7.

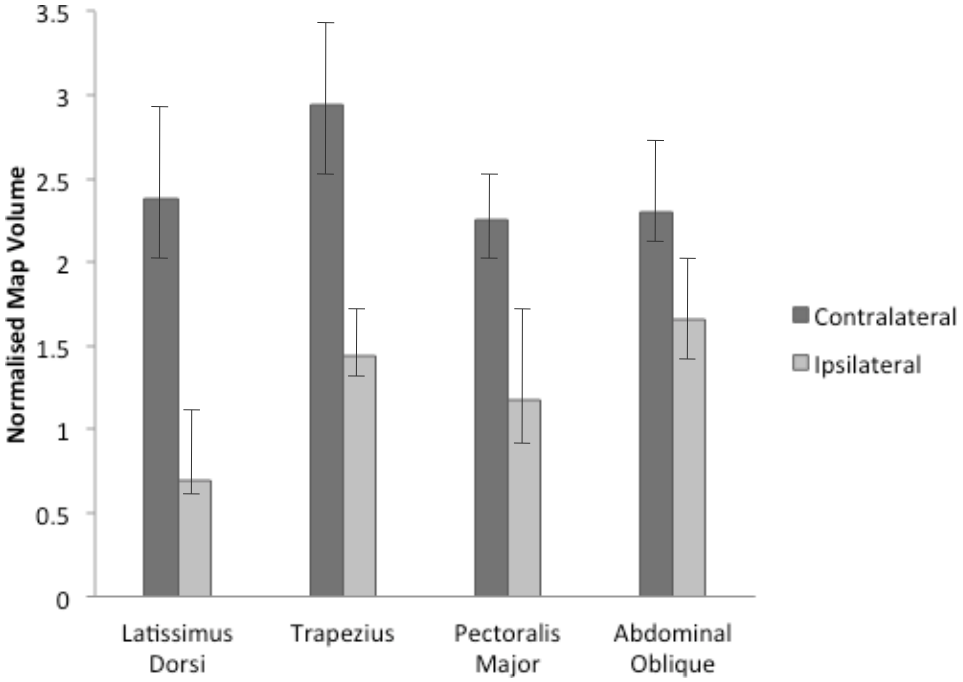

FIGURE 3.7 NORMALIZED MAP VOLUME FOR EACH MUSCLE.

Calculated as the sum of MEP Area from all excitable grid points divided between the number of excitable grid points.

3.3.3 Organization of Cortical Representations

The x and y coordinates of the Centre of Gravity (CoG) in ipsilateral and contralateral representations were compared for each muscle in each group. Plots of ipsilateral and contralateral CoG are shown for all subjects in each muscle in Figure 3.8.

A 3x4 ANOVA was performed with factors of group and muscle for ipsilateral and contralateral representations. Another pattern of organization was also tested, the relationship between location of ipsilateral representations relative to the contralateral. This was expressed by Ipsilateral CoGx- Contralateral CoGx and Ipsilateral CoGy- Contralateral CoGy.

A significant effect of group on contralateral CoGy is shown in Figure 3.9. A significant effect of muscle on ipsilateral CoGy is shown in Figure 3.10. There were no significant effects on CoGx, nor the ipsilateral CoG relative to the contralateral.

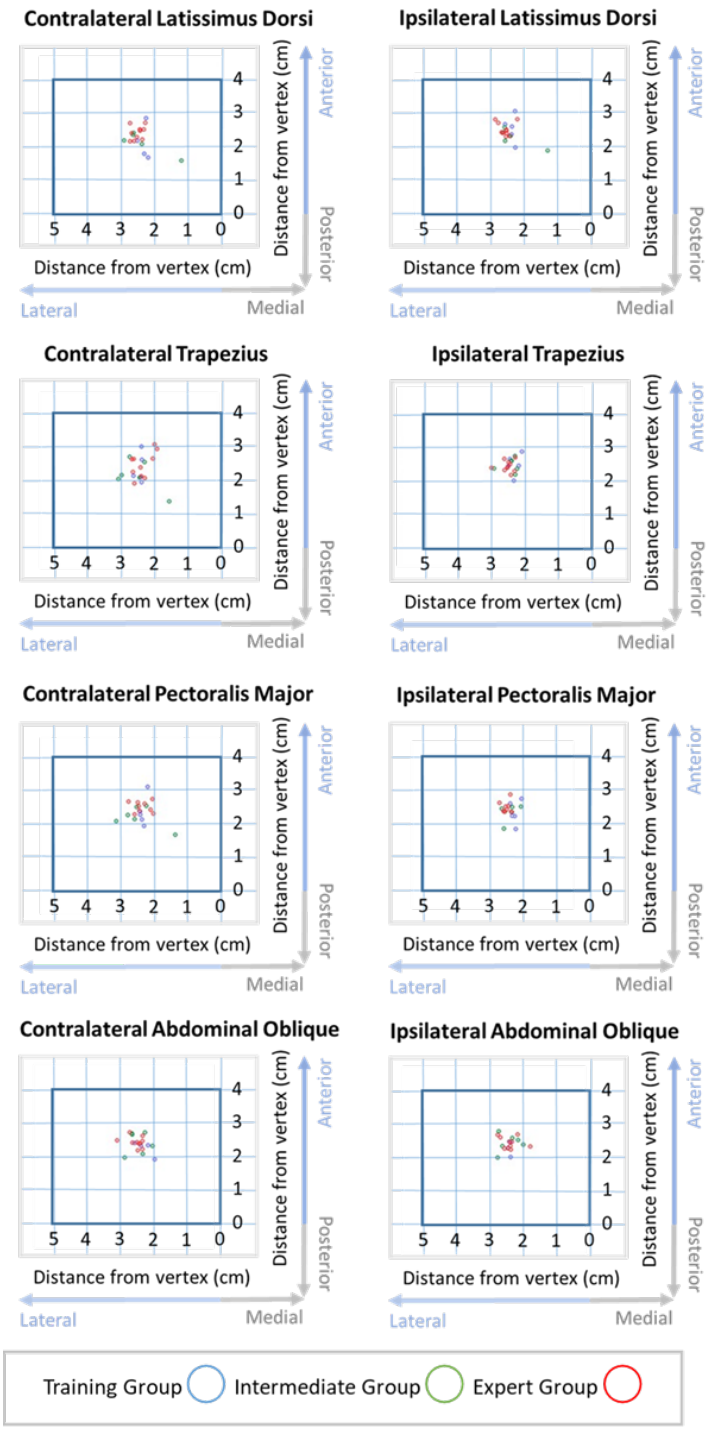

FIGURE 3.8 PLOTS OF COG.

Contralateral CoG (left graph of each pair) and Ipsilateral CoG (right graph of each pair) for each subject in each muscle, descending order in each column Latissimus Dorsi, Trapezius, Pectoralis Major and Abdominal Oblique.

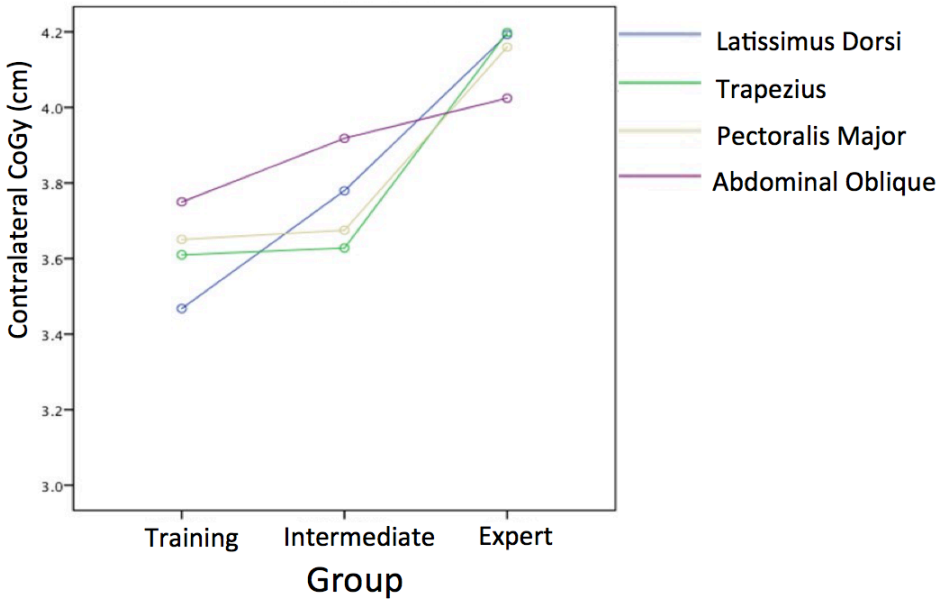

FIGURE 3.9 EFFECT OF GROUP ON CONTRALATERAL COGY.

Significant main effect of group on CoGy, $F(2, 14) = 5.546$, $p = 0.017$. Post hoc analysis (Tukey HSD) reveals the difference lies between expert and training groups ($p = 0.034$).

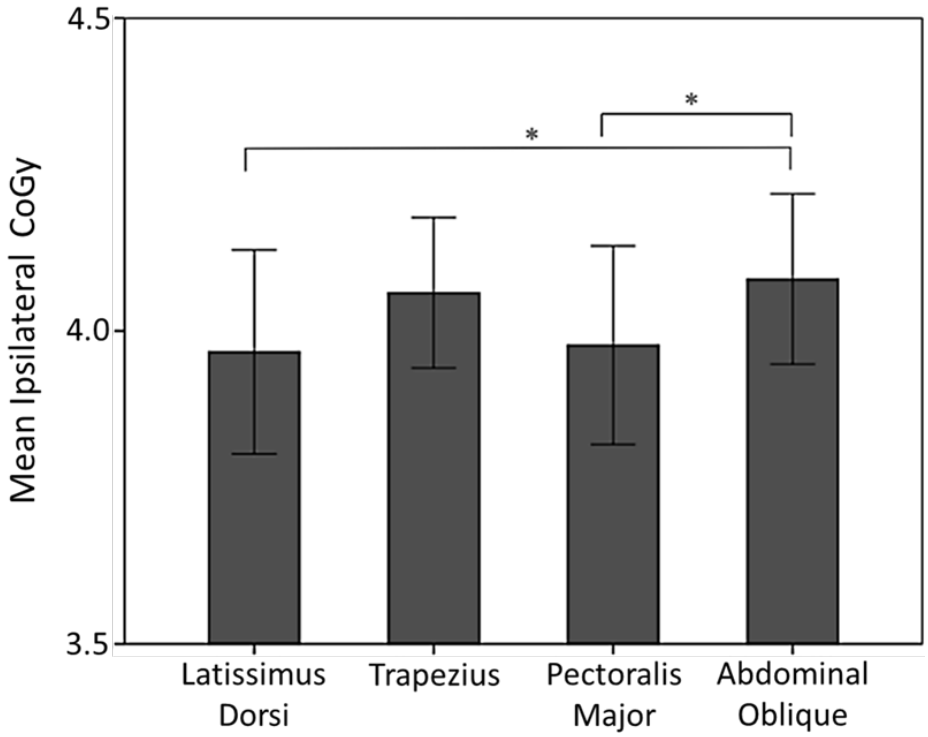

FIGURE 3.10 A SIGNIFICANT EFFECT OF MUSCLE ON IPSILATERAL COGY.

Post hoc tests show the difference lies between Latissimus Dorsi and Pectoralis Major and Pectoralis Major and Abdominal Oblique, but this does not withstand correction for multiple comparisons.

3.3.3.1 *CoGx Contralateral*

There was no significant effect effect of group on CoGx in contralateral muscles, $F(2, 14) = 0.098$, $p = 0.907$. There was no significant effect of muscle on CoGx in contralateral muscles, $F(3, 42) = 0.066$, $p = 0.319$, and there was no interaction between contralateral muscles and groups $F(6, 42) = 1.273$, $p = 0.290$.

3.3.3.2 *CoGx Ipsilateral*

There was no significant effect of group on CoGx in ipsilateral muscles, $F(2, 15) = 0.176$, $p = 0.515$. There was no significant effect of muscle on CoGx in ipsilateral muscles, $F(3, 45) = 0.057$, $p = 0.394$, and there was no interaction between ipsilateral muscles and groups $F(6, 45) = 1.305$, $p = 0.275$.

3.3.3.3 *CoGx Ipsilateral - Contralateral*

There was no significant effect of group on difference in CoGx between ipsilateral - contralateral muscles, F (2, 14) = 0.098, p = 0.907. There was no significant effect of muscle on difference in CoGx between ipsilateral - contralateral muscles, F (3, 42) = 1.699, p = 0.182, and there was no interaction between difference in CoGx between ipsilateral - contralateral muscles and groups F (6, 42) = 0.172 , p = 0.983.

3.3.3.4 *CoGy Contralateral*

There was a significant main effect of group on CoGy in contralateral muscles, F (2, 14) = 5.546, p = 0.017. Post hoc analysis (Tukey HSD) revealed that CoGy was significantly higher in expert group than in the training group (p = 0.034), and was almost significantly higher than in the intermediate group (p = 0.059).

There was no significant effect of muscle on CoGy in contralateral muscles, F (3, 42) = 0.324, p = 0.808, and there was no interaction between contralateral muscles and groups F (6, 42) = 1.290, p = 0.283.

3.3.3.5 *CoGy Ipsilateral*

There was no significant effect of group on CoGy in ipsilateral muscles, F (2, 15) = 0.306 , p = 0.323. However, there was a significant main effect of muscle on CoGy in ipsilateral muscles, F (3, 45) = 3.361, p = 0.027. Although post hoc paired- samples comparison showed significant difference in CoGy between Latissimus Dorsi and Abdominal Oblique (p = 0.03) and between Pectoralis Major and Abdominal Oblique (p = 0.029), the significance did not survive Bonferroni correction for multiple comparisons.

There was no interaction between ipsilateral muscles and groups F (6, 45) = 1.9, p = 0.102.

3.3.3.6 *CoGy Ipsilateral - Contralateral*

There was no significant effect of group on the difference in CoGy between ipsilateral - contralateral muscles, F (2, 14) = 0.775 , p = 0.479. There was no significant effect of muscle on the difference in CoGy between ipsilateral

- contralateral muscles, $F(3, 42) = 2.096$, $p = 0.115$, and there was no interaction between the difference in CoGy between ipsilateral - contralateral muscles and groups $F(6, 42) = 1.594$, $p = 0.173$.

3.3.4 Summary of Results

1. Significant differences are seen in the strength of the ipsilateral representations of Pectoralis Major and Abdominal Oblique between training and intermediate groups and training and expert groups.

2. There is a significant difference between the strength of the contralateral and ipsilateral representations of the Latissimus Dorsi muscle.

3. Normalized map volumes consistently show a more focal contralateral and more expansive ipsilateral representation.

4. Contralateral CoGy is more anterior in the expert group compared to the training group. The effect is not specific for different muscles.

5. There is a significant effect of muscle on ipsilateral CoGy, but not of group. Post-hoc tests suggest the difference lies between Latissimus Dorsi and Abdominal Oblique and Pectoralis Major, but the significance of this difference does not withstand scrutiny with correction for multiple comparisons.

6. There are no significant differences in the location of the ipsilateral representation relative to the contralateral representation

3.4 DISCUSSION

3.4.1 Main Findings

1. There was an increase in the strength of the ipsilateral representation of the Pectoralis Major and Abdominal Oblique muscles in the intermediate and expert athletes compared to the trainee athletes. There were no significant differences in the strength of the contralateral representations between the groups. The normalized map volumes suggest more focal contralateral and more expansive ipsilateral representations.

2. The ipsilateral difference was significant between training and intermediate and between training and expert groups. There was a further increase in the strength of the Abdominal Oblique representations between the intermediate and expert groups, but this was not significant.

3. Only in Latissimus Dorsi was there a significant difference between the strength of the ipsilateral and contralateral representations.

4. The only significant difference in organization of ipsilateral representations was seen with an effect of muscle but not with group.

3.4.2 Overview of Unifying Interpretation of Findings Relating to Ipsilateral Motor Control

There are a number of features identified within the results that relate to a described role of ipsilateral motor control. Strengthened ipsilateral representations were seen in muscles most strongly bilaterally activated. In contrast the asymmetry between a stronger contralateral and weaker ipsilateral representation was greatest in the muscle most unilaterally activated. The two muscles that demonstrated strengthened ipsilateral representations are coactivated but segmentally remote. Where ipsilateral representations were strengthened, the pattern of organization was that of a focal contralateral representation with few strong responses from strong cortical points, and expansive ipsilateral representation with multiple cortical points producing weak output.

The ipsilateral contribution to synergy control is compatible with each of these features. Ipsilateral pathways are functionally organized to control muscles coactivated in a task bilaterally with proximal and distal coordination. The contralateral hemisphere is concerned with agonist control, achieved by focal, powerful corticomotorneuronal and corticospinal output, whereas the ipsilateral hemisphere controls coactivated and antagonist muscles, linking multiple cortical movement representations in expansive maps. Fundamental motor control principles can explain how multiple weak ipsilateral outputs become effective through summation at the level of spinal Premotor interneurones.

3.4.3 Task-Specificity of Observed Ipsilateral and Contralateral Effects

An important part of pursuing this explanation is proving that the observed changes in cortical representations relate directly to the task, and truly reflect a task specific component of deliberate practise of skilled motor control.

The main finding was strengthened ipsilateral representations of muscles that are habitually bilaterally coactivated but segmentally distant. Pectoralis Major stabilizes, retracts and protracts the upper limb, and Abdominal Obliques stabilize and rotate the trunk.

Contraction of Internal Oblique produces trunk rotation and side flexion, bringing the shoulder towards the opposite hip, a movement fundamental to the kayak paddle stroke (Fleming et al., 2012). These muscles receive strong converging bilateral central motor drives from lateral vestibulospinal postural drive in ventromedial pathways and reticulobulbospinal output, including respiratory drive, from medullary nuclei in ventrolateral pathways. Bulbospinal drive converges with contralateral corticospinal and bilateral corticoreticulospinal pathways on Lamina VIII interneurones. These in turn project to axial alpha motorneurones and decussate through Lamina X to act bilaterally. This convergence of motor drive lends to the hypothesis that these muscles would be the site where ipsilateral change would be most evident. This contrasts with the lateralized activation of Latissimus Dorsi. Latissimus Dorsi is also of major importance to the skilled kayak stroke, the major agonist of the draw phase, but performs in the most lateralized way of all the muscles tested (Baker and Hardy, 1989). Therefore, it was the muscle predicted to show the least ipsilateral activity. Accordingly, this was the only muscle displaying significant difference between weaker ipsilateral and stronger contralateral representations.

This task specific interpretation of the findings is compatible with the proposed role of the ipsilateral hemisphere, but the lack of change in Trapezius must be noted as an exception. EMG analysis of the kayak paddle

stroke demonstrates bilateral activation of Trapezius (Trevithick et al., 2007). There are idiosyncratic neuroanatomical features of ipsilateral innervation of Trapezius that would have predicted ipsilateral change. In primates, stimulation of a specific region of Pontomedullary Reticular Formation produces powerful rapid facilitation of ipsilateral Trapezius. Of all the proximal and upper limb muscles studied in primates, this focal reticulospinal facilitation was only identified for Trapezius (Davidson and Buford, 2006). Despite this potential, no significant training effects were seen in Trapezius, and the reason for this is not clear.

3.4.4 Relative Strength of Contralateral and Ipsilateral Representations

The relationship between the strength of ipsilateral and contralateral representations provides useful information about ipsilateral motor control. Individually, contralateral MEPs are considerably larger than corresponding ipsilateral MEPs. When viewed collectively, the strength of ipsilateral and contralateral cortical representations may become more balanced. The mean representation values reported here are calculated from the summed responses of cortical points stimulated. Inspection of the map and the normalized map volume shows smaller, focal contralateral representations evoking large responses, whereas the ipsilateral representations contain numerous sites that individually evoke smaller responses.

This explains the apparent discrepancy between the extents of the cortical representations compared to the strength of the descending pathways. Descending contralateral output comprises around 90% of corticospinal fibres, with a large number producing monosynaptic excitatory post-synaptic potentials. In contrast the descending ipsilateral output is weaker in terms of fibre numbers and use of polysynaptic pathways. The ipsilateral motor output is able to convert weak ipsilateral input into effective motor control. Synchrony driven by interaction between cortical rhythms and those of corticomuscular loops allows the synchronous arrival of multiple weak inputs to summate (Aumann and Prut, 2015). Peripheral Ia afferent modulation of the spinal premotor circuits focuses multiple weak inputs toward relevant motor motorneurones (Gracies et al., 1991; Nielsen and Pierrot-Deseilligny, 1991). Feedforward inhibition sensitive to the strength of the corticospinal input gates the spinal premotor circuits, such that weak inputs become permissive for downstream facilitatory effects (Nicolas et al., 2001). These features allow weak ipsilateral motor output to become an effective distributed motor command controlling multiple muscles in a synergy.

3.4.5 A Synergy Based Mechanistic Explanation

Synergies function to reduce the computational demand of motor control of groups of muscles commonly coactivated in a task. Synergies may be organized in cortical modules. These can either directly pattern a motor command to spinal motor neurones, or provide output to downstream modules, such as spinal premotor interneurones, to perform further computation (McMorland et al., 2015). Combination of synergies becomes increasingly important with multiple interdependent joint movements. As permutations of joint angles and torques increase, synergies resolve computational complexity.

Movements such as the kayak stroke combining axial control with reach and precise distal movements therefore demand multiple linearly combined synergy modules. Accordingly, the cortical representation of the movement will become expansive, linking multiple synergy representations with proximal, distal and axial muscles.

A basic principle of synergy modular construction is bihemispheric organization. Contralateral output is most concerned with agonist control, whereas ipsilateral output is more concerned with control of antagonist and coactivated muscles (Gerachshenko et al., 2008). The extent of coactivation in the kayak-stroke movement should therefore be demanding of the ipsilateral hemisphere.

Another important function of synergies is to link control of proximal and distal muscles within a task (Roberts et al., 2008; Tantisira et al., 1996). In this task the finding of strengthened Abdominal Oblique and Pectoralis Major representations could reflect an expanded synergy representation driven to provide control across high cervical to low thoracic segmental separation. Although axial, the muscles become treated functionally as proximal and distal. Cortical axial synergy representations can control across segmental separation by utilizing the long propriospinal nerves of spinal synergy modules whose cell bodies are in the high cervical

Intermediate Zone but whose axons can project to Lumbar segment interneurones (Alstermark et al., 1987).

A synergy based explanation can account for the main findings presented. Association of asymmetry of contralateral and ipsilateral representation with lateralization of muscle activation, segmental distance between strengthened ipsilateral representations and expansive but weak ipsilateral representations are all compatible features. Before focussing on this explanation, it is worth considering whether alternative explanations could be equally plausible. The neuroanatomical basis for the hypothesis suggests another possibility to test would be direct corticofugal output from primary motor cortex or secondary motor areas.

3.4.6 Possible Increased Direct Ipsilateral Corticofugal Output

Before discussing the ipsilateral findings in terms of cortical synergy modules, alternative corticofugal explanations should be considered. A larger number of cortical regions generating direct ipsilateral corticospinal or corticobulbospinal output to spinal motor-neurones or segmental interneurons could also expand the map. The neuroanatomical basis for this has been established in primates from all frontal motor areas (Boudrias et al., 2010; Dum and Strick, 2002; Picard and Strick, 1996). However these pathways are weak when functioning directly and it is difficult to envisage from popular motor control theories how this would offer advantage in skilled motor control. Common drive could confer neural efficiency in axial movements requiring bilateral activation (Carr et al., 1994; Marsden et al., 1999). Such efficiency could only be valued in unskilled movements able to utilize the multisegmental branching of reticulospinal fibres (Peterson et al., 1975), and the ipsilateral flexor, contralateral extensor pattern of bilateral facilitation (Davidson and Buford, 2006). A bilateral, antiphase axial movement could potentially utilize this functional organization, but the level of skill offered would be inferior to propriospinally mediated control. Neural efficiency at the expense of fractionation seems unlikely in a movement trained by Olympic athletes.

If plastic reorganization occurred to favour direct ipsilateral corticofugal pathways, something of the regional cortical excitability could be predicted on the basis of anatomical origins of these ipsilateral pathways. For the main possibilities, an associated CoG change could be predicted, and this was not found.

3.4.7 Secondary Motor Areas as a Potential Explanation for Map Expansion – a Potential Role for SMA

If the representation were focussed toward a region generating ipsilateral corticofugal output then a change in CoG would have been anticipated. Ipsilateral corticospinal output from primary motor cortex and secondary motor areas were proposed as possibilities. The majority of corticospinal terminations on Lamina VIII interneurones projecting to axial anterior horn cells are contralateral (Porter and Lemon, 1993), but there are numerous projecting corticospinal fibres that give collaterals to reticular nuclei (Keizer and Kuypers, 1989; Kuypers and Lawrence, 1967). However, secondary motor areas have strong bilateral activity and are important in representing movements with multiple coactivations. These may be more likely to respond to axial training.

Of the secondary motor areas, Premotor Cortex (PMC) and Supplementary Motor Area (SMA) are potentially of interest as a source of corticoreticulospinal fibres. Premotor regions are reported to generate a large proportion of ipsilateral reticulospinal fibres in humans on the basis of imaging studies (Yeo et al., 2012), and primate tracer studies show SMA as a dominant source (Montgomery et al., 2013). SMA is most functionally relevant to this task, strongly concerned with control of axial movement (Weisendanger et al, 1973). Anatomical connectivity supports SMA involvement in this role. SMA has corticospinal projections to ipsilateral anterior horn cells and Intermediate Zone interneurones (Dum and Strick, 2002). Around one quarter of SMA corticospinal projections are ipsilateral, and whilst the majority terminate in Lamina VIII, some terminate on anterior horn cells (Dum and Strick, 1996). SMA also projects to bilaterally descending bulbospinal pathways via brainstem relays (Keizer and Kuypers, 1989). Ipsilateral activity through these pathways to axial muscles is demonstrated in humans by the interaction between auditory startle and axial Anticipatory Postural Adjustments (MacKinnon et al., 2007). In addition to corticospinal and corticobulbospinal outputs, SMA has extensive

corticocortical connectivity with primary motor cortex and other secondary motor areas (Narayana et al., 2012).

Functional studies of SMA also predict involvement in this role. Stimulation of SMA in human intraoperative studies can produce bilateral abdominal muscle contraction (Woolsey et al., 1979) and bilateral neck muscle contraction (Penfield and Boldrey, 1937). fMRI action observation studies show that SMA is most strongly activated in response to axial movements, compared to distal movements for PMv and proximal movements for PMd (Sakreida et al., 2005). SMA is the source of axial Anticipatory Postural Adjustments (APA), which utilize ipsilateral pathways. These accompany limb and trunk movements and are shown to be absent in patients with SMA lesions (Massion et al., 1989). Reduced subcortical inputs to SMA results in abnormal axial synergies, evident in Parkinsonian disorders (Vaugoyeau et al., 2006). The reverse situation is also demonstrated. Experience dependent structural change in SMA is seen with deliberate practise of skilled axial motor control in experts (Hänggi et al., 2010) and in response to training a whole-body balance task (Taubert et al., 2010).

Having presented anatomical and functional evidence supportive of potential ipsilateral corticofugal output from SMA, the CoG findings can be examined for features supportive of this suggestion. There were no significant differences in the CoGx in ipsilateral or contralateral hemisphere for any of the muscles. This perhaps argues against strengthened focal regions within primary motor cortex, where a medial shift could have been anticipated on the basis that deliberate practise would synaptically strengthen coactivated neuronal populations within proximal and axial regions. There is a difference in the position of the contralateral CoGy, which is more anterior in the expert group compared to the training group. The effect is seen in all muscles without specificity. This could be compatible with a greater use of PMC or SMA in experts, potentially encoding bilateral activity within contralateral representations. Within the ipsilateral representations there was no evidence of deliberate practise related reorganization to emphasize secondary motor areas, but differences in the functional organization were seen between muscles that altered extent

of representation with deliberate practise. The pattern was not entirely consistent with an explanation based on increased use of more anterior secondary motor areas. A more anterior position was seen for Abdominal Obliques compared to Latissimus Dorsi, which could be compatible with SMA involvement, but no anterior change was seen for Pectoralis Major. The CoG cannot be used to distinguish between mechanistic possibilities, only to suggest support. Synergy representations mapped with intracortical microstimulation in primates are seen to extend across primary motor cortex and PMC (Graziano et al., 2002), a situation where a more anterior CoG might be expected to reflect a strengthened PMC contribution.

3.4.8 Developing a Synergy Based Explanation

The idea that the observed cortical changes reflect experience dependent expansion of synergy representations can be explored further. Supportive context from TMS motor mapping studies is limited by the small number of studies performed with similar intent. I am not aware of any studies with any modality that have specifically sought to describe synergy control in terms of focality of contralateral and ipsilateral representations. The other aspect that must be reconciled with the proposed mechanism is the experience dependence of the deliberate practise effect. Imaging studies of structural plasticity in experts may offer useful context for discussion of size of cortical representations.

The proposed explanation includes the assertion that skilled motor performance can be achieved both by expansion and contraction of movement representations, depending on the motor task and the aspect of motor control being represented. Expansion implies increased synaptic connectivity between both adjacent and remote cortical columns, densely intercurrently connected through intracortical interneurones and Horizontal Fibres. Focality implies that neural efficiency has been achieved by synaptic strengthening and pruning to produce an optimal representation. Both processes can result in improved performance depending on the requirement of the motor output. Expanding motor representations will increase the density of neural, glial and vascular components in cortical layers, whereas pruning will reduce them. Measuring structural cortical change in response to deliberate practise with morphometric imaging provides another means of testing this explanation.

3.4.9 Supportive Context from TMS Studies

Plasticity of cortical motor representations has been demonstrated with TMS for both gain and loss of function, across short and long time scales. There is neurophysiological evidence of deliberate practise in elite athletes leading to demonstrable changes in action observation (Aglioti et al., 2008) and motor imagery (Fourkas et al., 2008; Wei and Luo, 2010). There are however very few TMS studies examining deliberate practise effects on cortical motor representations in athletes, and none of ipsilateral representations.

The few TMS studies of athletes report expansion of contralateral representations of distal muscles in tennis players (Pearce et al., 2000), and expansion of a contralateral proximal muscle representation in volleyball players (Tyč and Boyadjian, 2011). Motor map shifts with training to improved performance have been shown with musicians, in contralateral Abductor Digiti Minimi representation with 6 months of string instrument training (Kim et al., 2004) and in contralateral finger flexor maps with 5 days of piano training (Pascual- Leone et al., 1995).

Motor map responses to loss of function have been shown in studies of kinesophobia in pain states, limb immobilization and amputation. Lower back pain may cause a posterior shift in Rectus Abdominus representations (Tsao et al., 2008b). Amputation has been shown to produce motor map shifts for both upper and lower limb, both ipsilaterally and contralaterally to the amputated limb (Karl et al., 2001; Schwenkreis et al., 2003). Surgical restoration of the amputated part and regain of function then restores the motor map (Ni et al., 2010). Speed of map shifts with loss of function is illustrated with a study of proficient Braille readers, in whom cessation of their daily practise leads to rapid contraction of the digit representation (Pascual-Leone et al., 1995).

Mapping studies in normal subjects exist for the muscles studied. These support the findings reported here as task specific training effects. Bilateral responses to stimulation of both hemispheres were measured for Internal

Oblique (Strutton et al., 2004) and External Oblique (Wightman et al., 2011). There was some difference in background activation and stimulation intensity used. For Internal Oblique the Ipsilateral to Contralateral ratio was around 0.7 and for External Oblique the Laterality Index was 0.38, comparable to 0.42 in the training group here. The Laterality Index for Latissimus Dorsi was reported as 0.54, comparable to 0.5 in the training group here. Another study of Latissimus Dorsi reported similar magnitude of ipsilateral responses, the average being around a quarter of the size of the contralateral response (MacKinnon et al., 2004). The values reported from comparable studies are of similar magnitude to those of the training group here. This also validates the higher values seen in the intermediate and expert groups as reflecting deliberate practise effects.

3.4.10 Supportive Context from Studies of Structural Plasticity

Evidence from functional and structural imaging is more abundant. Structural imaging has been used to test theories on the requirement for long-term deliberate practise to acquire expert performance in cognitive and motor tasks (Ericsson, 2008), and elite athletic performance (Ericsson, 2013). Studies of structural plasticity demonstrate both increases and decreases in cortical thickness driven by deliberate practise, depending on context. Structural studies have been performed on experts with comparable levels of deliberate practise to the athletes tested here. Longitudinal studies of deliberate practise of skilled motor tasks in trainees also contribute information about the timing of plastic change.

3.4.10.1 *Cortical Thinning and Cortical Thickening*

Morphometric studies of deliberate practise report both grey matter thinning and thickening in response to deliberate practise. With some motor tasks, the direction of change is interpreted as task relevant. With others it is unclear what determines which process will dominate. Concurrent thickening and thinning within connected cortical regions is also reported.

Cortical thinning arises from pruning, the process of retraction of dendritic processes and phagocytosis of less functional synapses not reinforced by Long Term Potentiation (LTP). Once synaptically efficient, representations are stabilized if the balance of intracortical excitability favours that enduring structural arrangement. Synaptic efficiency reduces metabolic demand, accordingly non-neural elements become less dense, and grey matter thins. Cortical thinning is also observed pathologically with focal diaschisis of regions remotely connected to a lesion as functional deafferentation renders synapses non-functional (Duering et al., 2015). Cortical thickening occurs as neural networks are expanded with increased dendritic branching, increased synaptic density, and correspondingly increased glial activity. Cortical thickening can also occur in pathological states, where failure of synaptic pruning and other homeostatic mechanisms leads to inefficiently dense networks with an imbalance of excitation that resists further remodelling. Evidence to support pruning to improved performance is

provided by studies of plasticity in autism and expert musicians. Evidence for cortical thickening improving performance is provided by studies of musicians and athletes.

3.4.10.2 *Co-existence of focal and expansive cortical representations within a network.*

The idea that thickening and thinning of cortex equates to performance is an oversimplification. Cortical movement representations include multiple intermixed and overlapping muscle representations, which may be recruited multiply into networks with different functional demands. It is therefore difficult to envisage a cortical region where all microcolumns within a region measurable macroscopically by imaging voxels will behave in the same way with cortical thinning or thickening. Within the resolution of current imaging methods, variations are measurable that could represent a balanced average of these processes, dynamically evolving in an experience dependent way. The cortical surface architecture of hand area of expert professional musicians shows sufficient regional variation to allow string instrument players to be distinguished from pianists, suggesting that these structural variations can manifest very focally (Bangert and Schlaug, 2006). Dorsolateral frontal regions of expert musicians show cortical thickening, but within this group, those who possess Absolute Pitch show additional regional thinning of Planum Temporale (Bermudez et al., 2009). Training a balance task over short periods, simultaneous cortical thickening and thinning can be observed at different points in the motor network (Taubert et al., 2010).

Having presented the evidence that these structural changes have the potential to behave in the manner proposed, the next step is to establish a link between structure and function.

3.4.10.3 *Cortical Thickening Correlating With Improved Motor Performance*

Accepting structural change as a marker of plastic reorganization of movement representations is made difficult by the mechanistic challenge

presented by columnar cortical organization. Evidence of structure-function relationships needs to be sought to validate use of the structural comparison.

The field of structural plasticity developed from animal models where training in an enriched environment produced structural cortical change (Diamond et al., 1966; Rosenzweig and Bennett, 1972; van Praag et al., 2000). There are numerous structure function relationships studied for cognitive performance. A structural basis is proposed for common variant cognitive traits (Kanai and Rees, 2011). Grey matter changes occur as medical students revise for exams (Draganski et al., 2006), and as mathematicians progress through their academic career (Aydin et al., 2007). Structural changes are seen with memory and visuospatial training (Engvig et al., 2010; Haier et al., 2009) and when intensively learning a second language (Stein et al., 2014).

In motor tasks, learning juggling increases frontal, temporal and cingulate grey matter volume over 6 weeks (Boyke et al., 2008; Driemeyer et al., 2008), and training a whole-body balance task increases SMA and frontal cortex grey matter volume over 6 weeks (Taubert et al., 2010). Cortical volume changes in professional keyboard players (Gaser and Schlaug, 2003), and orchestral musicians (Sluming et al., 2002) correlate with extent of deliberate practise. In musicians structural changes were correlated to MEG activity during pitch processing (Schneider et al., 2002). The reverse situation, cortical thinning with loss of function is also shown by limb immobilizing thinning cortical representations (Langer et al., 2012).

Cross sectional studies of elite athletes with vast amounts of deliberate practise are limited, but useful for comparison to the findings in athletes reported here. Professional golfers with up to 27,000 hours of deliberate practise increase PMC and parietal grey matter thickness (Jancke et al, 2009). Divers with 23,000 hours of deliberate practise increase temporal and frontal thickness (Wei et al., 2011), with some structural changes correlating to task relevant fMRI activation (Wei and Luo, 2010).

3.4.10.4 *Cortical Thinning Correlating With Improved Motor Performance*

Cortical thinning has also been related to both training to improved performance and loss of function. The idea that pruning of cortical networks results in improved performance is best illustrated by pathological examples of the opposite situation, the consequence of failure of pruning. Developmental disorders can arise from deficient kinases involved in synaptic pruning. A cortical structural basis for autism is the most completely studied example.

The majority of excitatory synapses are on distal dendritic processes. Failure to prune excitatory synapses creates imbalance in intracortical circuits. Stability of cortical representations is compromised and noise added to input signals. There is an increased density of dendritic spines in Layer V temporal pyramidal cells of autistic patients (Tang et al., 2014). The genetic basis lies in expression of a kinase which increases glutaminergic synapse expression and inhibits synaptic pruning (Bourgeron, 2009; Kim et al., 2011). The cortical thickness resulting from failure of pruning is the basis of the cognitive impairments. The reverse situation of optimal pruning would therefore be expected to produce balanced, stabilized cortical representations sensitive to excitatory inputs.

Functional correlates of cortical thinning are provided by studies of cognitive and motor tasks in experts. Expert chess players show reduced grey matter in regions of fMRI activation (Hänggi et al., 2014), and have more focal activations measured with MEG (Amidzic et al., 2001). Professional ballet dancers have reduced grey matter volume in PMC and SMA (Hänggi et al., 2010).

The discussion above provides examples of structural plasticity driven by deliberate practise to expert level producing both thickening and thinning of grey matter. These processes can be driven simultaneously in opposite hemispheres with training of axial muscles. The study that longitudinally monitored response to axial training used a whole body balance task without lateralization, so can only be used to illustrate the principle rather than give

information about the ipsilateral hemisphere (Taubert et al., 2010). Animal models can complete the survey of experience dependent structural plasticity for the purpose of supporting a synergy based explanation of the observed ipsilateral changes.

3.4.10.5 *Animal Models of Differential Structural Plasticity Directly Relevant to Changes in Motor Map Reorganization Over Time.*

Animal experiments provide a direct link between structural and functional aspects of cortical reorganization driven by deliberate skilled motor practise. Training skilled reach in rats expands distal forelimb representations, and increases the number of synapses per neurone in Layer V (Kleim et al., 2002). Skilled motor training increases dendritic branching in Layer V, while reducing dendritic spine density in Layer III (Kolb et al., 2008). These are plastic changes of a different nature, concurrently active but developing independently in response to the same training. They represent different temporal and functional aspects of motor map reorganization, dynamic and stabilizing, which could potentially drive expansion, or stabilize and focus the representation.

These observations from animal studies add to the dual observations from human imaging studies showing cortical motor representations respond to deliberate practise with structural change consistent with both expansion and focussing of the representation.

Importantly in relation to the findings reported here, these processes may occur concurrently in different cortical regions. Whilst there is no direct evidence relating to ipsilateral expansion and contralateral focussing, there is a cellular basis to justify pursuing this explanation by seeking further evidence from the subsequent experiments.

3.4.11 Deliberate Practise and Experience Dependent Plasticity

Discussion so far has focussed on extrapolating evidence to support a mechanistic explanation. The findings provide evidence of ipsilateral activity contributing to expert performance, but there is a feature of the hypothesis that is only partially supported. Differences in the strength of ipsilateral representations were seen between training and intermediate groups, between training and expert groups, but not between intermediate and expert groups. A compatible but non-significant direction of change in Abdominal Obliques showed a further small increase between intermediate and expert groups. CoG differences were only seen between training and expert groups. This suggests that plastic changes driving expansion and reorganization of representations may occur with different time courses. It is possible that within thousands of hours of training the representations are strengthened and stabilized, and that further training to expert levels of skill drives more subtle reorganization. It is also possible that additional training from intermediate to expert level produces change beyond the resolution of these measures, or in systems outside of those measured.

The timing of these changes may be of relevance to stroke rehabilitation. Theories of motor learning predict that acquisition of expert performance in a motor skill requires over ten thousand hours of deliberate practise (Ericsson, 2008). The majority of studies of stroke rehabilitation report effects of interventions delivered over days or weeks. The need for greater knowledge of rehabilitation potential over long time periods has not been pressing, since resources limit the capacity to maintain sustained intervention. Increased availability of robotic therapy may change this by offering a resource efficient means of accumulating vast amounts of repetitive practise. Longitudinal studies of functional outcome after stroke suggest performance at 5 years is static or worse compared to 2 months (Meyer et al., 2015). Providing a neural mechanism for the effects of long term training is a necessary part of suggesting that these ideas could have a place in stroke rehabilitation.

Cortical structural change becomes evident after two sessions training axial synergies (Taubert et al., 2010). This speed suggests rapid remodelling of dendritic branching rather than processes requiring cellular expression and maturation occurring over weeks. Consistently, grey matter change is observed within 5 days of rTMS delivered to auditory cortex (May et al., 2006). Animal experiments produce similar findings. Altering sensory input to drive plasticity in cortical receptive fields in mice is associated with dendritic spine sprouting in Layer V pyramidal cells, changing over days and weeks, but not with large dendrite branching or axonal change (Trachtenberg et al., 2002). Motor task-specific cortical change after five days of training in mice is associated with markers of synaptic remodelling, but not neuronal or glial turnover (Lerch et al., 2011).

While early components of cortical reorganization are well described, the basis for later consolidation over months and years is less well studied. Training induced dendritic spines are mostly eliminated over weeks, but a small fraction persists, related to slow-phase learning and retention of improved performance (Yang et al., 2009). Cortical representations increase over one year of training in primates (Nudo et al., 1996) and differences between one and five years of training are evident as synchrony in cortical representations (Schieber, 2002). The findings of different deliberate practise effects after thousands of hours and tens of thousands of hours of training suggest different and specific processes, which must be an elaboration of the basic principles of cortical representation. The nature of additional factors involved in consolidating training over very long time periods is not clear.

3.4.12 Genetic and Epigenetic Determinants of Ipsilateral Plasticity

When attempting to translate findings from athletes to stroke patients, fundamental physiological differences must be acknowledged. The plastic environment in healthy and injured brain is dramatically different. Common polymorphisms in athletes that confer athletic advantage may be replaced by ones conferring cardiovascular risk in stroke patients. These genetic considerations also complicate interpretation of cross sectional studies of expert performers. It is difficult to separate cause and effect in cross sectional studies, unless a consistent graded relationship between practise and plastic change is observed. Athletes may self-select into groups on the basis of common polymorphisms that confer advantage in general athleticism or a particular motor skill.

Genetic factors may alter the accessibility of ipsilateral pathways, shown for strength of interhemispheric pathways (Gallea et al., 2013), and hemispheric asymmetry (Ocklenburg and Güntürkün, 2012).

There are also genomic predictors of athletic trainability (Bouchard, 2012), which may intersect with polymorphisms predicting plastic response. For example BDNF (Cheeran et al., 2010) and TRPV-1 polymorphisms (Mori et al., 2012) influence the response to TMS plasticity inducing protocols, but may also confer cardiovascular advantage (Knaepen et al., 2010; Randhawa and Jaggi, 2017). This is relevant to stroke rehabilitation, since it follows that absence of these polymorphisms could increase stroke risk, and limit the plastic response to ischaemic damage. It is likely that as knowledge of genetic determinants of plasticity and stroke risk increase and more intersections are identified, molecular factors will become incorporated into predictive algorithms. The finding of neuroprogenitor cells in the ipsilesional subventricular zone acutely after stroke (Martí-Fàbregas et al., 2010) will increase attention to epigenetic factors (Kawase-Koga et al., 2009). Applying findings from athletes to the post stroke situation may include genetic factors influencing plasticity.

3.4.13 Summary

Increased strength of the ipsilateral motor representation of two task relevant muscles was evident between trainee and intermediate and expert athletes. The ipsilateral representations were more expansive than the contralateral. This was interpreted as a role for the ipsilateral hemisphere in representing components of complex axial synergies. Consistent with this, the only muscle to show a significantly stronger contralateral representation relative to ipsilateral was most unilaterally activated in the task. The muscles that displayed ipsilateral plastic change were bilaterally coactivated in the task but segmentally remote, another feature compatible with ipsilateral involvement in synergy control.

Some features of the organization of contralateral motor representations were different between trainees and expert athletes, with experts tending anteriorly across muscles. Within ipsilateral representations, the muscle with the strongest habitual bilateral axial role had a more anterior representation. This anterior tendency was interpreted as reflecting increased involvement of secondary motor areas, important in the control of bilateral, multi-muscle movements.

There are no TMS studies of experience dependent plastic change in ipsilateral representations by which to make comparison. There is no direct evidence to support the idea that focal contralateral and expansive ipsilateral representations are a feature of cortical organization of synergy representations. This is compatible with functional descriptions of contralateral control of agonists and ipsilateral control of coactivated muscles within synergies. Studies of deliberate practise effects on structural cortical plasticity can be extrapolated to support this idea.

These findings provide support for the hypothesis that ipsilateral plasticity can beneficially serve skilled motor control. These ideas are now tested further by comparing to ipsilateral deliberate practise effects in expert singers and stroke patients. Singers provide a group with comparable practise of bilateral axial motor tasks. The difference in task predicts a

different degree of access to corticobulbospinal pathways, which may be reflected in the organization of ipsilateral representations. Comparison in stroke patients is required to establish whether the finding of ipsilateral activity serving skilled motor control can generalize to the contralesional hemisphere after brain injury.

CHAPTER 4
LONGITUDINAL EFFECTS OF TRAINING A BILATERAL MOTOR TASK ON IPSILATERAL CORTICOMOTOR CONNECTIVITY

4.1 INTRODUCTION

The purpose of this experiment is to seek further support for the hypothesis that plasticity in ipsilateral motor pathways may beneficially serve skilled motor control. This is sought by monitoring for changes in measures of ipsilateral motor control in muscles habitually bilaterally recruited in singing when driven by daily deliberate practise. Cortical regions with ipsilateral motor output known to exhibit experience dependent activity changes within the singing network are proposed as drivers of ipsilateral change. Trainee singers are trained for one year and measures of ipsilateral and contralateral corticomotor connectivity are compared to changes in patterns of muscle use and behavioural measures of singing performance.

4.1.1 Hypothesis

1. Daily deliberate practise of singing in trainees may drive plastic change in ipsilateral motor pathways that benefits acquisition of skilled motor performance.

2. Training skilled bilateral activation of muscles under the control of bilaterally descending motor pathways will increase corticomotor connectivity in ipsilateral pathways. This will manifest as increased ipsilateral MEPs in subjects who demonstrate improved singing performance.

3. Evidence of ipsilateral plastic change occurring in corticomotor pathways will be most apparent in muscles showing the greatest increase in contribution to the task, measured by changes in EMG pattern during singing.

4. If ipsilateral change occurs through corticobulbospinal pathways, then this will be most apparent in the obligate respiratory muscles most strongly under bulbospinal control.

5. Muscles displaying evidence of training related plasticity over this training period are most likely to display evidence of consolidated long term plastic change in the subsequent experiment comparing cortical representations of singers of different levels of experience and expertise.

4.1.2 Basis for Hypothesis

Singing requires skilled bilateral activation of muscles known to be under control of bilaterally descending bulbospinal pathways originating in pontomedullary respiratory centres. A number of specific plastic processes in brainstem circuits are described in relation to respiratory motor control, as outlined previously. This brainstem circuitry provides potential access to ipsilateral motor pathways from diverse cortical inputs. The many functional roles of these muscles place them under the control of multiple motor drives, converging in spinal distribution networks. This combination of ipsilateral innervation and segmental integration makes muscles under respiratory control of interest in studying ipsilateral plasticity. Singers provide a group comparable to athletes in terms of their duration of training and acquisition of expertise in skilled motor control, allowing mechanistic comparisons across different motor behaviours. A major animal model of ipsilateral plasticity, the Crossed Phrenic Phenomenon, also provides basis for supposing that pathways of respiratory control may be accessed for plastic change.

This study is of importance in developing upon the findings of the previous chapter. There it was discussed that expansion of the ipsilateral motor map could represent recruitment of corticospinal or corticobulbospinal output from secondary motor areas. If the corticobulbospinal route is accessed through experience dependent plasticity, then it is hypothesized that this should be more evident in subjects training a motor behaviour that interacts directly with bulbospinal motor drive.

A number of cortical plastic processes could potentially alter ipsilateral motor control through training in singers. Corticospinal output from secondary motor areas is one possibility. Animal and human evidence exists for direct ipsilateral corticospinal pathways from secondary motor areas to respiratory muscles. This is shown in response to stimulation of SMA and CMA in primates (Boudrias et al., 2010; Dum and Strick, 1996) and SMA in humans (Sharshar et al., 2004). These secondary motor areas are known

to increase their activity in the brains of expert opera singers (Kleber et al., 2010a).

Another possible mechanism is illustrated by the clinical observation of bilateral axial movements in response to emotional stimuli in otherwise tetraplegic Locked-In patients (Munschauer et al., 1991). Here basis pontine lesions make corticospinal and corticobulbar tracts unviable, so reveal an alternative bilaterally descending motor pathway relaying through brainstem tegmentum. This demonstrates a corticobulbospinal pathway, and with it the possibility of motor map reorganization to access latent brainstem relays. In these patients corticomotor output follows limbic system activation, with subsequent motor activity in Cingulate Motor Areas. This is a specific clinical example from a very particular pathological state, far removed from training skilled motor control in terms of both stimulus and output, but provides an illustrative example of the principle of accessing alternative corticobulbospinal pathways.

Implicit in this suggestion is the assumption that pathways of voluntary respiratory control of cortical origin can interact with the involuntary brainstem respiratory pathways. Evidence for voluntary-involuntary interaction is not secure. Supportive evidence is provided by animal experiments and observations from human physiology. These interactions are discussed more fully later, but two illustrative experiments outline the supportive case for the argument. In animals trained to breath-hold, intracellular recording of medullary respiratory neurones shows they are silenced during voluntary breath holding. These brainstem neurones should increase activity with increasing hypercapnia suggesting the silence reflects dominant cortical control (Oyer 1984). In humans the lung volume threshold for the Hering-Breuer reflex varies in sleep and wakefulness.

When awake the reflex is only active at high lung volumes, but in sleep states without cortical activity the reflex is active at tidal volumes (Hamilton et al., 1988). These provide examples of the voluntary respiratory system interacting with the involuntary respiratory drive, but there is much opposing evidence to counter the suggestion. Nevertheless, the clinical

observations of Locked-In patients are compelling and deserving of further investigation.

A corticocortical mechanism may also underlie ipsilateral plastic change through alteration of excitatory and inhibitory inputs to primary motor cortex. Stimulation of motor cortex produces ipsilateral responses in respiratory muscles with characteristics compatible with corticospinal transmission (Maskill et al., 1991). Training driven changes in inputs to motor cortex could allow motor map reorganization to link representations of muscles habitually co-activated in a skilled task. Experience dependent activation of secondary motor areas seen in singers supports this as a mechanism.

These main possibilities, ipsilateral corticospinal pathways from primary motor cortex, ipsilateral corticospinal pathways from SMA or CMA or corticobulbospinal pathways provide the mechanistic basis for the hypothesis. It can be mentioned that decussating interneurons at segmental level are proposed to restore ipsilateral respiratory weakness after surgical lesions in humans (Nathan, 1963), although the relevance of segmental plasticity in healthy subjects or after stroke is not clear.

There are some other important aspects of ipsilateral plasticity relevant to stroke recovery tested within the hypothesis. Studying the temporal evolution of plastic change with daily practise over one year is relevant to informing the timing of delivery of therapeutic intervention in stroke recovery. Comparison of the plastic effects of training singing allows task-dependency of plastic change to be studied through comparison to the findings of the athlete study and the later study of stroke patients trained in a reach task. Study of respiratory muscles is also relevant to stroke recovery because of the morbidity and mortality resulting from respiratory insufficiency from thoracic and diaphragmatic weakness. The effects of respiratory insufficiency also generalize to impact on plasticity in cortex through a BDNF dependent mechanism (Xie and Yung, 2012) and in brainstem circuitry (Mateika and Syed, 2013).

4.2.1 Experimental Design

4.2.1.1 *Overview of Experimental Design*

Subjects undertook daily practise of singing for one year. TMS measures, EMG measures and behavioural measures were obtained at baseline, 6 months and on completion of training at 12 months. Each experimental session consisted of EMG, TMS and audio measures. EMG patterns of recruitment of 8 homologous muscle pairs were recorded during an excerpt of song, and during performance of voluntary respiratory manoeuvres. Bilateral MEP size from stimulation of one hemisphere at 120% aMT for Diaphragm was averaged. Audio recordings of the same excerpts of song were taken.

Singing practise was directed and overseen by a professional singing teacher, using a task intended to alter the pattern of thoracic, abdominal and diaphragm muscle recruitment to optimize subglottal pressure support in singing. Twelve muscle pairs were studied, chosen to vary in their degree of obligate and accessory, inspiratory and expiratory actions. This strategy was aimed at optimizing the chance of revealing training effects. After 6 months of training some subjects underwent extensive cortical mapping as part of the experiment reported in the following chapter, comparing to singers of different duration of training and level of expertise.

4.2.2 Subjects

12 subjects were recruited, completed all baseline measures and embarked on the year programme of daily deliberate practise of singing. 10 Persisted to complete follow-up measures at 6 months and 5 completed 12 months of training and completed all measures after one year. Age ranges varied from 23 to 61. All subjects were right handed. All subjects were naïve to TMS.

4.2.2.1 *Inclusion criteria*

All subjects were novice singers who sang recreationally but had not undertaken any period of formal training and had not sung regularly within any organized structure or public performance.

All subjects undertook to comply with 15 minutes of daily deliberate practise guided by a professional singing tutor with weekly group lessons and weekly individual remote on-line video-call lessons.

4.2.2.2 *Exclusion criteria*

Subjects were questioned to ensure that they had not trained to a level of expertise in any other activity that may have influenced findings, for example playing string instruments requiring lateralized skilled control of axial muscles.

Standard exclusion criteria for TMS were applied according to international consensus safety statements, i.e. history of epilepsy, vascular, traumatic, tumoral, infectious or metabolic brain lesions, medication that may lower seizure threshold, implanted intracranial metal devices, pacemakers, nerve stimulators, pregnancy, sleep deprivation, alcohol excess, heart disease. (Rossi et al, 2009; Wassermann, 1998)

4.2.2.3 *Ethics Approval and Informed Consent*

All TMS was performed with local Ethics Committee approval and with the written, informed consent of each subject. Only single pulse TMS was performed. All subjects participated voluntarily without financial reward.

4.2.3 Training

4.2.3.1 *Training Exercises*

All subjects were instructed in a training technique aimed at optimizing the pattern of axial respiratory muscle recruitment for song. Training was based on 5 vocal exercises and diagrams with postural cues were provided as a visual reminder. Instruction was given in introductory tutorials with a professional singer teacher. Feedback was provided and the singing tasks repeated until correct engagement with the exercises was reliably and repeatedly demonstrated. Subjects were asked to perform the exercises for 15 minutes per day for 12 months.

4.2.3.2 *Monitoring of Training*

Subjects were asked to keep a diary record of their training schedule and record any variance from the daily routine. Progress was monitored in weekly group lessons and individual weekly on-line video-call lessons by the singing tutor.

4.2.4 Data Acquisition

4.2.4.1 *Overview of Data Acquisition*

All measures were taken at baseline, 6 months and 12 months.

EMG was recorded during performance of song from 8 homologous muscle pairs. Scalene, Sternocleidomastoid, Trapezius, Pectoralis Major, 2nd Parasternal Intercostal space, Transversus Abdominus, Lumbar Paraspinals and the 8th-9th Intercostal space in the mid-clavicular line were recorded. These muscles were chosen to provide a mix of respiratory functions - obligate and accessory, expiratory and inspiratory.

Prior to recording the song to be analysed, subjects were recorded during quiet breathing and a series of voluntary respiratory manoeuvres and vocal exercises. This allowed a functional characterization of electrode position to be added to the anatomical description.

EMG was recorded during performance of the same passage of song in each session. EMG was used to derive one outcome measure, the percentage contribution of each muscle to the total EMG recorded, a marker of change in pattern of muscle use. This combined with the auditory measures to confirm a behavioural effect of the deliberate practise. This also identified muscles where training effects might be anticipated to be reflected in TMS measures.

Audio recording of song was a behavioural measure used to ensure that any change in muscle patterning or MEP was associated with improved performance. These were presented in a blind, randomized manner to professional listeners for rating.

MEPs were recorded from surface electrodes on the homologous muscle pairs detailed above, designated contralateral or ipsilateral to the dominant hemisphere stimulated by TMS. MEPs were recorded bilaterally in response to TMS of a point identified as producing responses in the majority of the muscles recorded. Only the dominant hemisphere was stimulated. TMS was performed under conditions of quiet breathing with low-level background

activation of all muscles studied. TMS was performed at single stimulation intensity, 120% aMT for the electrode recording from the 8th-9th intercostal space.

4.2.4.2 *EMG Recording*

Self-adhesive F301 silver/silver chloride foam solid gel surface electrodes (Skintact, Innsbruck, Austria) were used. These electrodes were found to be convenient to place and resisted movement.

Electrodes were positioned according to accepted belly-tendon placement positions, balancing proximity to motor points with a surface position resistant to movement. Trapezius, Pectoralis Major and Abdominal Oblique positions were identical to those reported in the earlier experiment. Sternocleidomastoid electrodes were positioned with the active electrode on the lower third of the muscle, the reference 2cm above, the standard position used in recording of Vestibular Evoked Myogenic Potentials (Welgampola and Colebatch, 2005). Scalene electrodes were positioned with the active electrode posteroinferiorly to the midpoint of the posterior border of sternocleidomastoid and the reference electrode 2 cm above the clavicle (Luu et al., 2015). The Parasternal Intercostals were recorded in the second intercostal space with the active electrode close to the sternal edge and the reference electrode 2cm away over rib (Hudson et al., 2012). The Lumbar Paraspinal active electrode was positioned over the muscle bulk at the level of L3/4, determined by palpation of the anterior superior iliac spine, 3 cm lateral to the spinous process, and the reference 1 cm lateral to the spinous process of L5 (O'Connell et al., 2007). An electrode positioned in the 8th or 9th intercostal space in the mid clavicular line, with reference electrode 2cm away on overlying rib was used as a measure of costal Diaphragm activity (Maskill et al., 1991).

EMG was acquired through two 8-channel D-360 Headstage boxes (Digitimer, Welwyn, UK) and Power1401 amplifier (CED, Cambridge, UK). Spike software (CED, Cambridge, UK) was used for recording EMG.

Wide filter settings were used, Low-pass 10Hz-High-pass 2kHz. A sample rate of 10kHz was used. Standard gain of 100 was used. Raw EMG was recorded without notch-filters and rectified off-line using virtual channels in Spike.

EMG was recorded during the same passage of continuous song in each session. Subjects had completed a set series of vocal exercises each session before the song was measured. The total song duration was around 20 seconds. For analysis the song was later divided off-line into a series of short inspiratory and expiratory segments. EMG recording began with a period of quiet breathing before the onset of song, with measurement of at least 3 respiratory cycles prior to onset.

The onset of song was clear on the EMG record as the deep inspiration that precedes onset is easily recognizable as a characteristic burst pattern in inspiratory muscles. Manual keyboard markers were also placed on the EMG record to confirm onset and offset of song.

Subjects were recorded whilst standing. They were asked not to introduce additional head, trunk or limb movements and this was monitored for during the recording.

4.2.4.3 *Confirmation of Electrode Positioning*

Functional characterization of the electrode positions was used to confirm that the surface electrode position was recording activity from the intended muscle of interest, e.g. bursts of activity in inspiration in the electrodes intended to record diaphragm activity. This was required to ensure that crosstalk from other muscles was not significantly contributing to the signal. It also allowed observation of the effect of chest wall and muscle movement on the EMG signal, to observe for potential electrode-to-muscle distance artefact. The activity in each electrode was measured during quiet inspiration, quiet expiration, forced maximal inspiration over 1 second and forced maximal expiration over 1 second. This allowed a functional categorization as obligate, accessory, inspiratory and expiratory. A sample of representative EMG is shown in Figure 4.1.

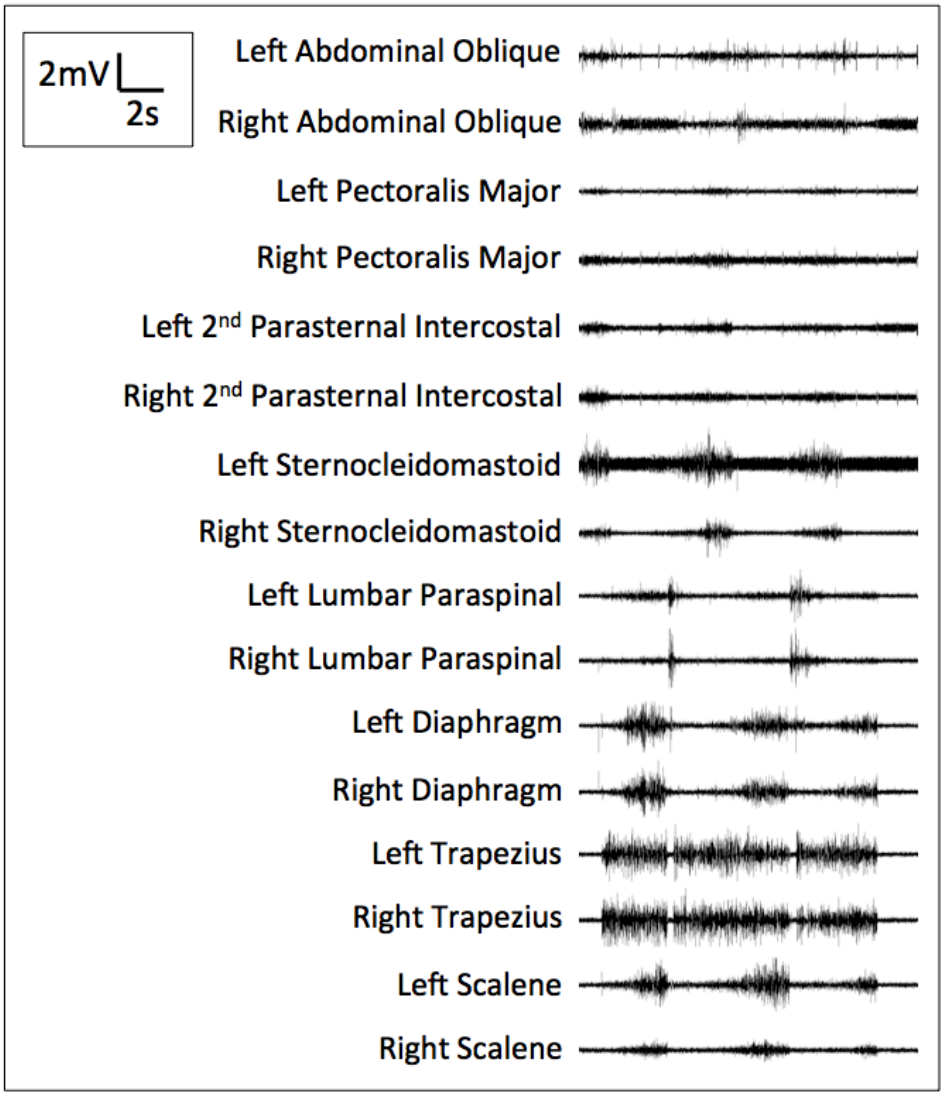

FIGURE 4.1 EMG RECORDING DURING VOLUNTARY RESPIRATORY MANOEUVRES

A sample of EMG is shown during performance of voluntary respiratory manoeuvres. This is used to characterize the function of the recorded muscle. It can be seen that different muscles display bursts of activity at different stages of respiration. In the lower pair of traces, bursts of scalene activity can be seen with inspiration. This is preceded by inspiratory activity in diaphragm.

4.2.4.4 *TMS*

TMS was delivered with a monophasic Magstim 2002 stimulator and figure-of-eight coil, Double 70mm Alpha Coil (Magstim, Whitland, UK). Consistency of coil placement was ensured with TMS guided by a Brainsight stereotactic infrared navigation system (Rogue Resolutions Ltd, Cardiff, UK).

Signal software (CED, Cambridge, UK) was used to record MEPs. Raw EMG was recorded without notch-filters and rectified off-line using virtual channels in Signal.

TMS was performed while seated on a stool without back support with the instruction to sit with the spine straight and head horizontal. This produced a constant low level of postural background activity in most muscles. Background respiratory facilitation was achieved by performing TMS in mid-inspiration of quiet breathing.

MEPs were recorded with Signal in peri-trigger mode, with 200ms of EMG preceding the stimulus included in the frame. An analogue trigger input was used with a threshold marker from a channel recording from the 2nd Parasternal Intercostal muscle. The EMG in this channel provided reliable change through the respiratory cycle and was convenient to use as a marker of a fixed level of inspiration. TMS was triggered at a mid-inspiratory point. It was necessary to consistently stimulate at a fixed point in the respiratory cycle partly to ensure that TMS coincided with the same descending respiratory drive, and partly to ensure that TMS was delivered with a degree of background facilitation in diaphragm. The inter-stimulus interval was variable with this method, depending on the respiratory cycle and manual arming of the stimulator after each stimulus. Occasional triggering of TMS by ECG rather than the intended level of inspiratory activity is unavoidable with use of a chest wall electrode. This was continually monitored for and any frames including ECG complexes were discarded.

TMS was performed with single stimulus intensity, 120% aMT of ipsilateral 8th-9th Intercostal Space. The motor threshold was generally higher for Diaphragm so provided a reasonable intensity to also obtain MEPs in other muscles that may have had hotspots in adjacent locations. Ideally detailed hotspot mapping and threshold measurement for each muscle recorded would have been performed, but this would have been an impractically long process.

aMT was determined in the standard way, using the definition of the minimal intensity required to produce MEPs of 200 to 300 microvolt

amplitude in 50% of trials. Stimulation intensities were generally in the range of 60 to 80% of Maximum Stimulator Output.

The coil was held with the handle 45 degrees to the mid sagittal line. A search was made for a stimulation site that could evoke MEPs in the majority of muscles. Particular focus was placed in ensuring that reliable MEPs were present in the main obligate respiratory muscles, Scalene and Diaphragm. The rationale for this focus of attention was that obligate inspiratory muscles are under stronger influence of ipsilateral bulbospinal pathways, and therefore of particular interest for displaying ipsilateral change. It would have been ideal to identify individual Hotspots for all muscles of interest but this would have been impractically time consuming. Identifying an optimal stimulation site to produce MEPs in as many muscles as possible was a practical compromise.

A Brainsight neuronavigation system was used to record the optimal stimulation site and ensure that coil position then remained constant throughout the experiment. Recording the stimulation position also allowed a reproducible stimulation site to be used in each of the three experimental sessions.

Once the stimulation site and intensity were established, 50 MEPs were obtained stimulating at one site in the dominant hemisphere under conditions of quiet breathing with gentle postural activation.

4.2.5 Data Reduction and Analysis

4.2.5.1 *EMG Analysis*

EMG was rectified off-line using virtual channels in Spike. The EMG recorded for each homologous muscle pair was averaged to give a single value for the muscle.

The record was divided into 4 inspiratory phases and 4 expiratory breath support phases (Figure 4.2). The division was necessary to avoid phase cancellation, but also to optimize the chance of measuring training induced change, since the pattern of muscle use may only be noticeably different in certain stages of song. This allowed inspiratory activity, expiratory activity and total activity to be analysed separately.

Manually positioned cursor settings divided the record and provided the Root Mean Square value of the EMG in each segment. Although the precise timing of these segments varied slightly between individuals, reliably reproducible division was straightforward as characteristic inspiratory bursts were easily recognizable and punctuated the transition between phases.

It was necessary to normalize data to allow comparison between individuals and between sessions, and to overcome the potential for variation in electrode position to alter EMG size. Root Mean Square EMG in each measured segment was normalized to the Root Mean Square EMG in one respiratory cycle of quiet breathing preceding song.

For each subject, the total normalized EMG activity for all muscles in inspiratory phases was summed, and the total normalized EMG activity in expiratory phases was summed. The contribution of each muscle to inspiratory or expiratory phases was then expressed as a percentage contribution to the total of all recorded EMG activity in that phase. Any change in patterns of muscle use with training over time could then be appreciated as an increase or decrease in that muscles percentage contribution to the total muscle activity in that phase of song. For example training an improved technique to increase contraction of diaphragm for breath support might increase the percentage contribution of diaphragm to

the expiratory phase, then allowing a reduction in upper intercostal muscle activity in that phase.

Measuring changes in percentage contribution of an individual muscle over time with training is intended to describe deliberate practise driven change in pattern of muscle use. It is hypothesized that muscles that show the greatest alteration in their contribution to the motor task are those most likely to display change in MEP.

Comparison of muscle use at different time points was made using ANOVA with factors of time and muscle. Post-hoc tests were then performed to further explore significant findings.

4.2.5.2 *MEP Analysis*

4.2.5.2.1 *Data Quality*

Each individual frame was analysed separately with manual individual cursor placements used to measure each MEP and the preceding background EMG. Each frame was monitored following each stimulus to ensure that background activity had remained within acceptable limits and that the MEP was not contaminated by stimulus artefact or ECG artefact. Stimulus artefact is problematic for neck electrodes with short latency MEPs. Attention to electrode cable positioning, coil position and minimizing contact with the subject helped to reduce stimulus artefact.

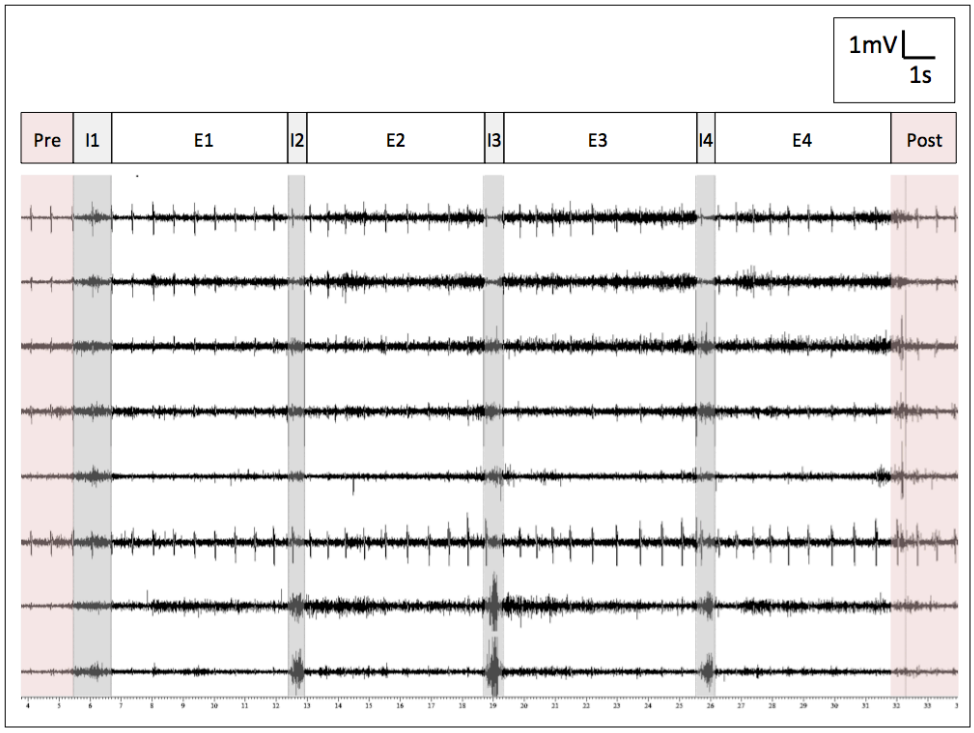

FIGURE 4.2 ILLUSTRATION OF THE DIVISION OF PASSAGE OF SONG FOR EMG ANALYSIS

Raw EMG recorded during the performance of a passage of song is shown. The record was divided into four brief inspiratory phases, I1-4, and four sustained expiratory breath support phases, E1-4.Quiet breathing was recorded for normalization prior to the onset of song. The lower pair of traces shows inspiratory bursting of diaphragm, then with sustained contraction into breath support phases.

4.2.5.2.2 *MEP measurement*

Raw EMG was recorded and virtual channels used off-line to rectify EMG for measurement. Rectified EMG in the 50msec preceding the stimulus was averaged, two standard deviations added and this value was used to set a horizontal cursor to function as part of the criteria for MEP measurement.

Criteria for analysing ipsilateral MEPs adopted by other investigators to allow for the typical small size and variable morphology were used. Inclusion criteria for a response to be measured as an MEP were occurrence with expected latency, compatible morphology, and exceeding baseline EMG plus two standard deviations for at least 5msec (Schwerin et al., 2008).

MEP area was measured from the rectified EMG using individual cursor placements to identify the MEP onset and offset, marked as the point of deflection above, and return to the baseline EMG. Use of MEP area was considered to be more relevant to the study of ipsilateral axial MEPs than amplitude. Amplitude is a conventional measure for more distal MEPs. However, axial MEPs are often of variable morphology and polyphasic, reflecting summation of multiple weak descending inputs. Change in MEP area can occur with little change in amplitude if multiple descending corticospinal outputs are desynchronized (Rosler et al., 2002).

4.2.5.2.3 *MEP normalization*

MEP Area was normalized to the mean background EMG area plus two standard deviations in the 50msec preceding the stimulus. Normalization was required to allow pooling of data for comparison between individuals and between time points. Absolute MEP size varies between individuals and is influenced between sessions by variations in electrode placement. To control for this and for small variations in background activity, normalization to background activity was chosen.

Other normalizations have been successfully used in similar experiments, such as normalizing to MVC, maximum MEP size or supramaximal Compound Motor Action Potential. However an aim of this experiment is to allow later comparison to studies in stroke patients, and these alternative methods may not be reliable in the paretic limb of stroke patients.

4.2.5.2.4 *Comparison Between Time Points*

The 50 MEPs obtained in each session were averaged to provide mean MEP Area at baseline, 6 months and 12 months for each subject. MEP size is then compared between time points using ANOVA with factors of muscle, side and time. Post-hoc tests were then performed to further explore significant findings.

4.2.5.3 *Audio Recording Analysis*

Audio recordings provided a behavioural measure to confirm that training, and any related MEP change, had resulted in improved performance. Furthermore, recording sound level ensured change in muscle use represented skilled control and not simply increased volume production.

Objective and automated measures of vocal performance are problematic in introducing many confounding factors. The simplest possible rating of singing performance was devised. Audio recordings of the song were presented as randomized, blinded pairs of sound clips to 3 expert listeners with a professional role in training world-stage singers. The pairs were the same excerpt of song for a single subject at baseline and 6 months, baseline and 12 months, or 6 months and 12 months. The instruction to the listener was simply “Please rate which clip is better. If no discernible difference state that they are the same.”

4.2.6 Methodological Limitations

Some broad limitations of TMS technique relevant to this chapter have been discussed earlier in Chapter 3. Further specific limitations relating to this recording are discussed below.

4.2.6.1 *EMG and MEP Recordings in Different Activity States*

The purpose of the EMG recording was to describe the pattern of muscle use during singing. TMS was performed in the quiet breathing state. The aim was to identify associations between the EMG changes and TMS measures. It would therefore have been preferable to study both under conditions of voluntary activation during song.

In pilot studies I explored the feasibility of performing TMS during song, but it was not possible to control for the effect of marked and continuous variations in background facilitation on MEP variability. It was also difficult to accurately stabilize the coil position and difficult for the subject to tolerate.

It was considered necessary to perform TMS in the quiet breathing state to be able to control a consistent degree of facilitation from background muscle activation. Stimulating at a consistent phase of the respiratory cycle ensured stimulation during a constant level of muscle activity and descending respiratory drive. There are specific aspects of respiratory neurophysiology that are only evident in voluntary or involuntary states, and the potential relevance to discrepancy between EMG and TMS findings are considered in detail in the discussion below.

4.2.6.2 *Influence of Muscle Selection and Recording from Multiple Muscles.*

The muscles studied were selected to provide a mix of obligate and accessory, inspiratory and expiratory muscles. This was hoped to optimize the chance of observing training effects, and to ease interpretation of any change in neural activity measured by TMS. The obligate and accessory respiratory muscles receive different degrees of voluntary and involuntary

central respiratory drive, and are served by different ipsilaterally descending pathways, making the contrast of interest to study.

The choice of muscles also had a biomechanical basis, with the training technique aimed at sustaining subglottal pressure through the breath support phase of song by altering axial muscle recruitment. The muscles selected are described to increase and decrease their activity with the acquisition of singing expertise. It was therefore hoped that any early changes identified in the Training group would later become consolidated and manifest as cortical changes in the Expert singers studied later.

Eight muscle pairs was the maximum number it was practical to study with the equipment available. Recording from many muscles increased the usefulness of the EMG findings but added technical challenge of ensuring sustained signal quality. Even with recording from the maximal number of muscles and making predictions about the muscles most likely to manifest deliberate practise effects, it is possible that practise effects lie outside of the muscle studied.

For TMS, simultaneously recording from a large number of muscles is more problematic. Reliance on a single stimulation point meant that it was harder to ensure high quality MEPs in each muscle recorded. Whilst this would have been problematic if the emphasis was on absolute MEP values, in this setting the intention was to demonstrate robust changes in corticomotor connectivity over time. This should have been evident if corticomotor excitability was driven to change over time even if the point of stimulation was not optimal for a given muscle. Recording from multiple muscles was a necessary compromise to answer the experimental question. This approach demanded rigorously constant stimulation technique in each session. Although dedicated studies for each muscle would have been preferable, this would have been unduly resource and time intensive.

4.2.6.3 *Low Level Background Contraction*

Achieving effective levels of background contraction in axial respiratory muscles with a single task was problematic. Using respiratory manoeuvres

for facilitation would introduce an inspiratory or expiratory bias. In pilot studies, stimulating during song provided effective but highly variable facilitation with constantly changing levels of facilitation in different muscles. This was also difficult for subjects to tolerate.

Use of an unsupported posture that required activation of trunk and neck muscles was the simplest way to provide low-level background contraction. Devising stronger contractions would have made MEPs easier to elicit but would have made the experiments much harder to tolerate for the subjects. In general this group of subjects were less tolerant of TMS than the subjects in the athlete study, and a more effortful subject experience would likely have increased the already high attrition rate over the 12-month period.

4.2.6.4 *Artefact Rate*

It was necessary to reject multiple frames because of contamination with ECG artefact, or in neck muscle electrodes, stimulus artefact. Stimulus artefact was frequently present but did not always converge with MEPs. Viewing return of EMG to baseline following the stimulus artefact prior to onset of the MEP was essential criteria for accepting a frame for analysis. Frames were rejected at the point of acquisition if contaminated by artefact. In some instances the artefact could be minimized through careful attention to electrode lead position. Others have used stimulus artefact removal techniques, but the problem was not so frequent as to require this.

Rejecting frames resulted in a higher total number of stimuli per experimental session. This was also a factor in using the simplest possible TMS measure, to keep the stimulation burden low in a subject group with comparatively low tolerance of TMS.

4.2.6.5 *Surface Electrode Recording*

Many of the electrode placements were in positions where there was potential for crosstalk from multiple muscles to produce a composite signal. This could have then lead to misinterpretation when interpreting findings in biomechanical terms. Adding a functional characterization to the anatomical description of the electrode position was aimed at limiting this possibility.

Other studies of respiratory muscles have used needle electrodes for muscles such as intercostals or swallowed oesophageal electrodes for diaphragm. This would have not been tolerated by this subject group, and would not have been practical due to the muscle movement during song.

Another potential issue with surface electrodes in this setting is variable muscle to electrode distance artefact. The varying chest wall movements through song may take the surface electrode further or closer from diaphragm muscle. This is considered in more detail in later discussion with attention to the biomechanics of the diaphragm zone-of-apposition.

4.2.6.6 *Describing Muscle Patterning by Percentage Contribution to Total Recorded EMG Activity*

Intersession variability of EMG recording makes use of absolute values unreliable to describe changes in muscle patterning over time. Expressing individual muscle contribution to total EMG was a simple way of describing change in muscle patterning with the advantage of the normalized value allowing direct comparison between individuals.

The disadvantage was that this makes an assumption that change will occur within the muscles studied for a reasonable effect size to be observed. This method becomes insensitive to small changes within the muscles recorded if these are part of wider training effects involving muscles outside those studied. The size of the effect is therefore dependent on the choice of muscles recorded. Significant changes in muscle patterning were observed with small percentage changes in muscle contribution to total EMG recorded.

4.3.1 Compliance with Training

12 subjects attended the first session and completed baseline measures. Of these 10 persisted to 6 months. 8 of the 10 subjects were able to complete all measurements at 6 months, and only 5 subjects completed 12 months of training. The attrition rate reflected the time demands of the project. Of those who completed, the only variance from the prescribed training programme was one subject missing a period of 4 weeks between months 9 and 10 due to a throat infection. They resumed the training schedule upon recovery and were included in the analysis.

4.3.2 Functional Classification of Electrode Positions

EMG activity was measured during quiet breathing and voluntary sharp inspiratory and expiratory manoeuvres. Muscles increasing activity in inspiration more than expiration were described as inspiratory, and vice versa. Muscles that displayed a proportionately greater contribution to voluntary manoeuvres compared to quiet breathing were described as accessory, whilst muscles with consistent bursts of activity with every respiratory cycle were described as obligate. The functional description of the muscles tested, and the corresponding anatomical description of the electrode position is given in Table 4.1. The description allows for the observation that muscles may perform both obligate and accessory, inspiratory and expiratory roles according to this classification. In that case the dominant function defined by the measurements is given first. The electrode between eighth and ninth intercostal space was inferred to be recording diaphragm activity, and EMG behaviour was concordant with this.

Anatomical Description	Functional Description
Scalene	Obligate Inspiratory. Accessory Inspiratory
Sternocleidomastoid	Accessory Inspiratory
Trapezius	Accessory Inspiratory
Second Parasternal Intercostal	Obligate Inspiratory. Accessory Inspiratory
Pectoralis Major	Accessory Inspiratory. Accessory Expiratory
Abdominal Obliques	Obligate Expiratory. Accessory Expiratory
Eighth - Ninth Intercostal Space	Obligate Inspiratory
Lumbar Paraspinals	Obligate Inspiratory. Accessory Expiratory

TABLE 4.1 COMPARISON OF ANATOMICAL AND FUNCTIONAL DESCRIPTIONS OF ELECTRODE POSITION, BASED ON EMG BEHAVIOUR IN QUIET AND VOLUNTARY BREATHING.

4.3.3 Behavioural Measures

All of the subjects were judged to have improved their singing performance over the course of the study using the assessment method described. This was true for comparison of song between baseline and 6 months, baseline and 12 months and 6 months and 12 months. The blinded ranking favouring improvement was consistent for each listener, at each time point for each subject.

There were no significant differences in the volume measurements of the audio recordings at different time points.

4.3.4 Analysis by Respiratory Phase and EMG Calculation of Patterns of Muscle Use

Table 4.2 gives the change in muscle use calculated from EMG measures over time for each muscle, averaged for the group. Values are shown for each muscle at each time point, in inspiratory and expiratory phases, and the total for the song, combining all phases. Values are the percentage contribution of that muscle to the total EMG recorded from all muscle pairs during that phase of song.

	Percentage Contribution to Total EMG								
Muscle	Inspiration Baseline	Inspiration 6 Months	Inspiration 12 months	Expiration Baseline	Expiration 6 Months	Expiration 12 Months	Total All Phases Baseline	Total All Phases 6 Months	Total All Phases 12 Months
Abdominal Obliques	11.7	11.5	12.1	11.8	12.1	12.8	11.7	11.8	12.4
Pectoralis Major	12.3	12.5	12.0	12.1	13.0	12.0	12.2	12.8	12.0
Second Parasternal Intercostals	11.9	11.7	11.9	11.8	12.2	12.0	11.9	12.0	12.0
Srenocleidomastoid	12.5	13.0	11.8	11.8	12.3	11.8	12.1	12.7	11.8
Lumbar Paraspinal	14.0	12.3	12.5	16.2	12.6	13.2	15.1	12.4	12.9
Diaphragm	11.9	13.1	13.1	11.7	13.4	13.2	11.8	13.3	13.2
Trapezius	12.9	11.6	12.4	12.7	11.8	11.9	12.8	11.7	12.2
Scalene	12.8	14.2	14.2	11.8	12.5	13.1	12.3	13.4	13.6

TABLE 4.2 CHANGE IN CONTRIBUTION OF EACH MUSCLE TO THE TASK, AVERAGED FOR THE GROUP, AT EACH TIME POINT, IN INSPIRATORY AND EXPIRATORY PHASES AND THE TOTAL OF ALL PHASES ACROSS THE SONG.

Values are the percentage that muscle contributes to the total of all EMG recorded from all muscles for that phase of song.

4.3.5 Change in patterns of muscle use over time with training

One-way ANOVA was performed on each muscle looking at the effect of time on the muscle's percentage of total EMG use. This showed a significant change in percentage of EMG use over time only in the diaphragm, F (2,81)=4.89, p=.01. The diaphragm activity expressed as percentage of the total EMG increased from 12 +/- 1.1 % at baseline to 13.1 +/- 1.7 % at 6 months and 12.8 +/- 1 % at 12 months. This is shown in Figure 4.3. Post-hoc analysis (Tukey HSD and Games-Howell) shows a significant difference between baseline and 6 months (p=.016) and baseline and 12 months (p=.049) but not between 6 months and 12 months.

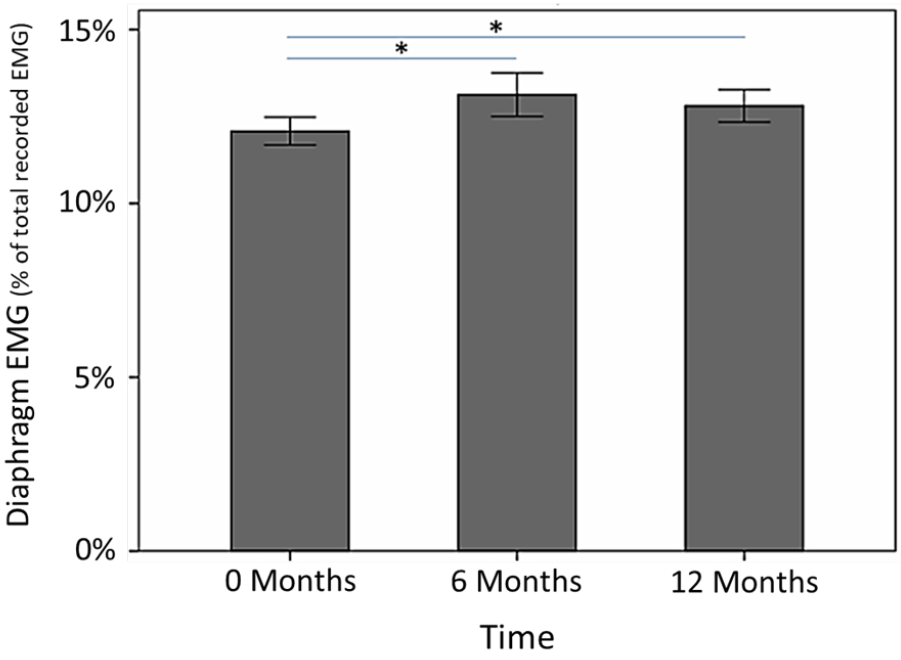

FIGURE 4.3 CHANGE IN PERCENTAGE CONTRIBUTION OF DIAPHRAGM TO TOTAL EMG ACTIVITY DURING SONG (AVERAGE OF ALL RECORDED PHASES) OVER TIME

A significant difference is seen between 0 and 12 months (p=.049), and 0 and 6 months (p=.016), but no additional change between 6 and 12 months.

4.3.6 Change in Diaphragm Percentage Contribution to Total EMG is Driven by the Expiratory Phase

Two further One-way ANOVAs were performed to look at the effect of time on diaphragm percentage contribution to total EMG use during inspiratory and expiratory phases. There was a significant effect of time on diaphragm percentage contribution to total EMG only during expiration. Post-hoc analysis (Tukey HSD and Games-Howell) showed significant difference for expiration between baseline and 6 months (p=.017) and baseline and 12 months (p=.044), but not between 6 and 12 months. This is shown in Figure 4.4. No significant differences were seen in inspiration. In inspiration the percentage contribution of diaphragm to total EMG was 12.2 +/- 1.3 % at baseline, 12.9 +/-1.8% at 6 months and 12.7 +/- 1.1 % at 12 months. In expiration the percentage contribution of diaphragm to total EMG was 11.9 +/- 0.9% at baseline, 13.3 +/- % at 6 months and 12.9% at 12 months.

Change in the percentage contribution of diaphragm to total EMG over time is shown for each subject in inspiration and expiration in Figure 4.5.

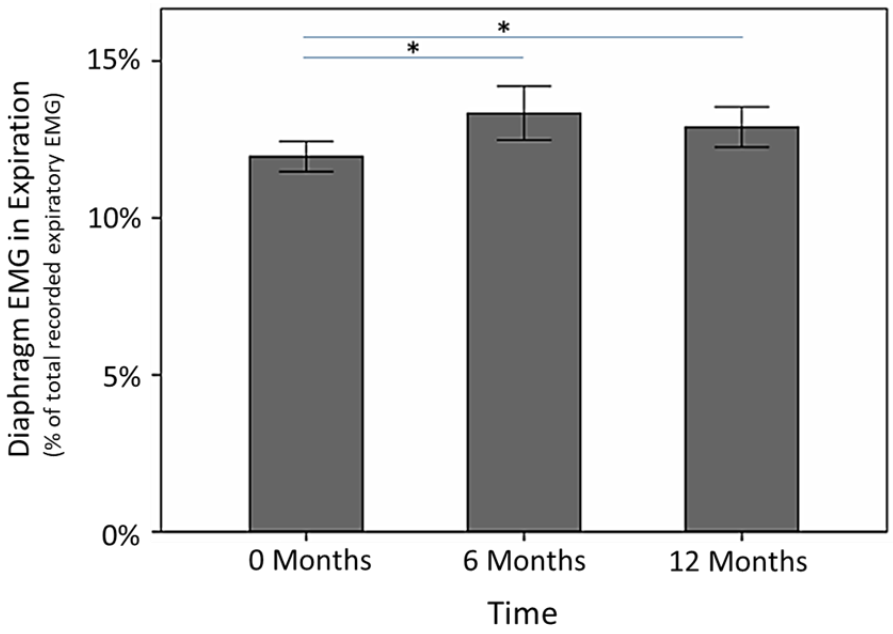

FIGURE 4.4 CHANGE OVER TIME IN DIAPHRAGM CONTRIBUTION TO TOTAL EMG RECORDED IN EXPIRATORY PHASES OF SONG

A significant difference is seen between 0 and 12 months (p=.044) and 0 and 6 months (p=.017), but no additional change between 6 and 12 months.

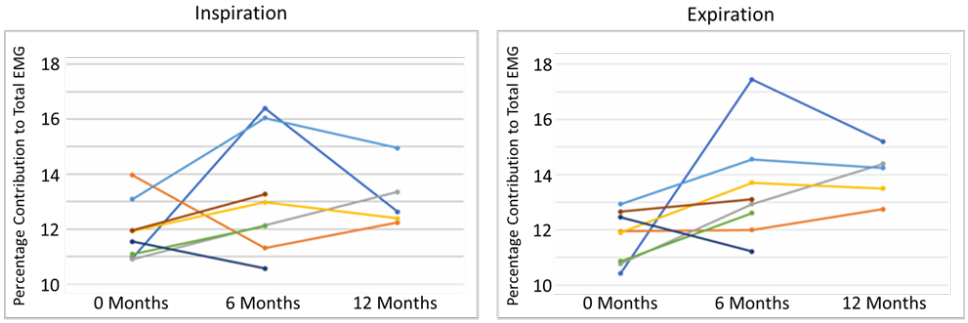

FIGURE 4.5 CHANGE IN PERCENTAGE CONTRIBUTION OF DIAPHRAGM TO TOTAL EMG OVER TIME FOR EACH SUBJECT AT EACH TIME POINT FOR INSPIRATORY PHASES (LEFT HAND GRAPH) AND EXPIRATORY PHASES (RIGHT HAND GRAPH).

These results show behavioural improvement in singing performance with training at 6 months and additional improvement at 12 months. The only change in the pattern of muscle detected with these EMG measures was a small increase in diaphragm activity. This change was seen during expiratory phases of song. This change was evident at 6 months and

persisted, but was not augmented by additional training between 6 and 12 months.

4.3.7 Change in MEPs Over Time.

MEPs were measured according to the method described in previous experiments, expressing the MEP area as a percentage of background activity. The average of 50 normalized MEPs produced by stimulation at 120% aMT for ipsilateral diaphragm was measured and compared at different time points.

A One-way ANOVA was performed to test for differences in MEP size over time. The only significant change was seen in the contralateral scalene muscle ($F(2,17) = 20.745$, $p < 0.001$) which became significantly different from baseline at 12 months (Tukey HSD post hoc - $p < .001$), and between 6 and 12 months (Tukey HSD post hoc - $p = .001$).

Scalene MEP was 77% +/- 17% at baseline, 166% +/- 68% at 6 months and 390% +/- 146% at 12 months. No significant differences were seen in the ipsilateral scalene. This is shown in Figure 4.6.

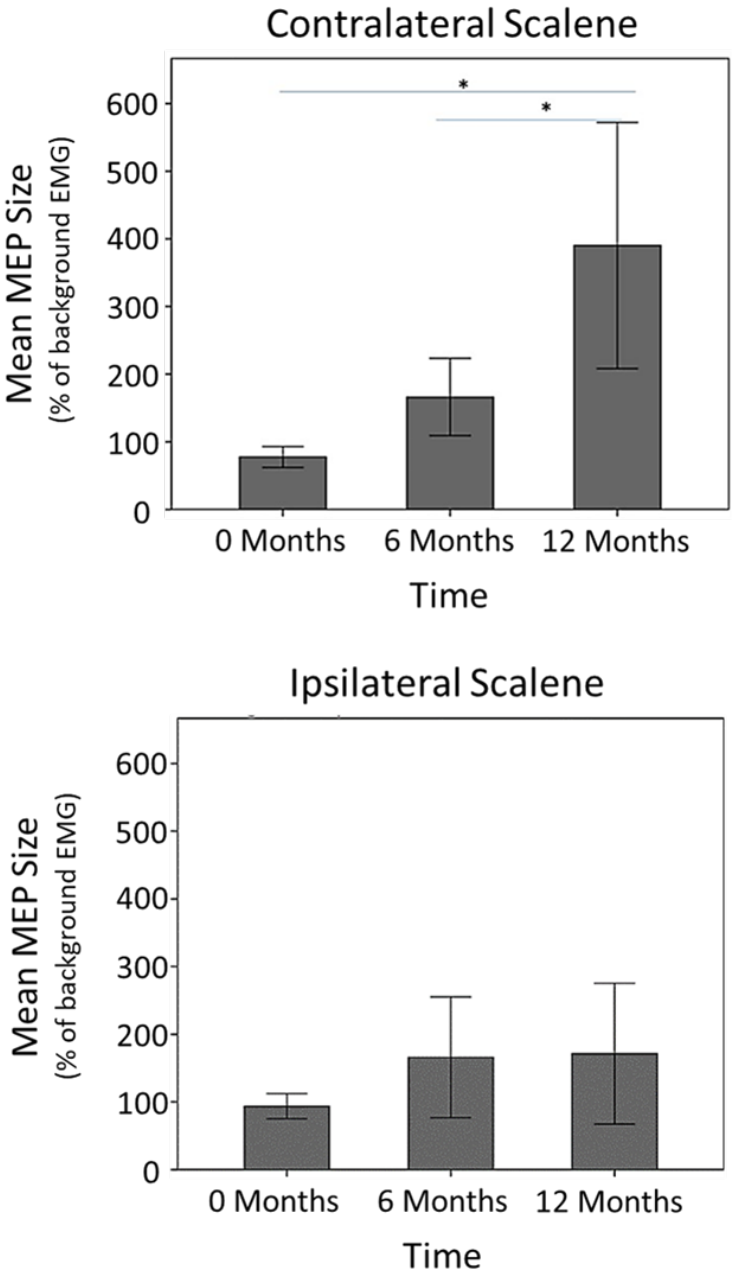

FIGURE 4.6 CHANGE IN MEAN NORMALIZED MEP AREA OVER TIME FOR CONTRALATERAL SCALENE (TOP GRAPH) AND IPSILATERAL SCALENE (BOTTOM GRAPH).

A significant difference in contralateral scalene MEP size is seen compared to baseline at 12 months, and between 6 months and 12 months.

There were no significant changes in MEP size for any of the other muscles. Diaphragm MEPs did not change, even though this was the only muscle in which a significant change in EMG use was seen. No significant changes

were seen in any of the ipsilateral MEPs. This does not support the hypothesis that deliberate practise of singing would increase ipsilateral corticomotor connectivity over time.

4.4 DISCUSSION

4.4.1 Main Findings

1. Daily deliberate practise of singing results in a change in pattern of muscle use to favour increased diaphragm activity in the expiratory phase of song. This change is evident at 6 months. Further training between 6 and 12 months does not produce additional change.

2. The fact that increased diaphragm activity in the expiratory phase was the significant change is notable as an example of an obligate inspiratory muscle recruited to a voluntary expiratory role. The change in scalene MEP also demonstrates change in control of an obligate inspiratory muscle through increased voluntary recruitment.

3. The only significant change in the strength of corticomotor connectivity seen with these measures was in contralateral scalene.

4. There were no significant changes to ipsilateral MEPs, arguing against the hypothesis that training uses activity in ipsilateral corticospinal or corticobulbospinal pathways.

5. There was no association between the change in pattern of muscle use and strengthening of corticomotor connectivity. EMG change occurred between 0 and 6 months, whereas MEP change occurred between 6 and 12 months.

These results do not support the hypothesis. There were no changes in ipsilateral corticomotor connectivity observed. The increased contralateral corticomotor connectivity in scalene muscle suggests that training induced improvement in singing performance is associated with increased activity of crossed corticospinal pathways. This is true for an obligate inspiratory muscle under strong ipsilateral bulbospinal control, where ipsilateral plasticity was hypothesized to be most evident by virtue of receipt of drive through these pathways. Scalene also performs accessory function to increasing inspiratory loads. The fact that contralateral change was observed

at sites hypothesized likely to reveal ipsilateral change adds emphasis to the negativity of this finding in relation to the hypothesis.

The other negative finding was that the change in MEPs was not seen in muscles that displayed greatest behavioural change in their contribution to the task. The prediction was based on basic cellular principles of plasticity, that corticomotor connectivity increases through formation of tentative synaptic connections which become strengthened by repetitive practise if functionally useful, and pruned if not. This simplistic interpretation may not describe practise effects in a task requiring complex skilled control, such as singing. Some of the skilled control is provided at segmental premotor level, with spinal distribution networks integrating voluntary and involuntary respiratory drives (Hudson et al., 2011). It is possible that practice effects occur in elements of motor control not apparent in simple measures of corticomotor connectivity.

Mechanistic interpretation of the results is required to validate the negative findings before applying them toward interpretation of the results in other chapters. The negative findings here do not favour interpretation of plastic change in ipsilateral motor maps in terms of access to direct ipsilateral corticobulbospinal pathways. Given the importance of this interpretation, consideration needs to be given to mechanistic validation of the findings. A mechanistic model integrating biomechanical and neural training effects can be proposed to account for the findings. This becomes particularly important given the temporal and spatial discordance of EMG and TMS findings. It is also possible that the sensitivities of the measures were not ideally suited to the experimental question, or that training effects occurred in muscles or sensorimotor systems outside of those studied. If this was the case then placing emphasis on the lack of ipsilateral change here may not be appropriate. Understanding how some of the specific biomechanical and neuromechanical processes of singing relate to the results is important to drawing more certain conclusions.

4.4.2 Training Related Changes in EMG Calculated Patterns of Muscle Use

For the EMG findings to be considered useful in interpreting ipsilateral plastic processes, task relevance of observed EMG change must be proven. Motor control of axial muscles in singing is concerned with control of subglottal pressure. Artificially producing subglottal pressure is sufficient for sound production in cadavers (Van den Berg, 1968). Subglottal pressure exerts horizontal and vertical forces on the vocal cords. Vocal cord abduction and adduction, elevation and descent establish basic vibratory patterns. Varying laxity and tension in the cords adds complexity through subglottic and supraglottic, horizontal and vertical vibratory phase differences (Sears and Davis, 1968). Professional singers master the control of subglottal pressure to change pitch and volume (Rubin et al., 1967) and phonation type (Herbst et al., 2015). Skilled control of subglottal pressure is used to accentuate tones (Sundberg et al., 1991) and reduce harmonic-to-noise ratio (Zhang, 2016). Singing training aims to improve control of laryngeal and articulator muscles for phonation, and control of axial muscles for subglottic pressure. In trainee singers, establishing basic subglottal support is required before developing finer articulatory control. It was anticipated that the early stage of training and the nature of the training exercise would develop control of abdominal, thoracic, neck and diaphragmatic muscles.

The finding of increased use of diaphragm over time is compatible with studies of muscle patterning in trained singers showing the relationship between diaphragm activation and subglottal pressure increases with expertise. In untrained singers the diaphragm is often relaxed, with inspiratory intercostals used to counteract negative pleural pressures. The higher rib cage volume from intercostal activity allows the relaxed diaphragm to ascend, and the reduced pleural pressure is balanced by abdominal wall muscle contraction (Bouhuys et al., 1966). In contrast, professional singers are seen to activate the diaphragm tonically, or with punctuated changes in transdiaphragmatic pressure corresponding with

sudden changes in subglottic pressure (Leanderson et al., 1987). In one study, providing on-line feedback to increase diaphragm use improved objective measures of song performance (Leanderson et al., 1987). Untrained singers use the same ratio of thoracic to abdominal movement employed for involuntary breathing, but expert singers use a highly fractionated combination with strong diaphragmatic component and much paradoxical movement (Salomoni et al., 2016).

The increased use of diaphragm reported with training is therefore consistent with observations of patterns of muscle use in expert singers. It is clear that increased diaphragm use requires coordinated change in muscle activity elsewhere, such as abdominal wall and intercostal muscles in the example given above. It can then be questioned why such coactivation was not manifest in the EMG results.

4.4.3 A Mechanistic Proposal to Explain why EMG Change was Limited to Diaphragm

There are muscular, neuromuscular and neuromechanical reasons that can explain why increase in EMG was seen in diaphragm but not in other muscles. Diaphragm has great fatigue resistance and redundancy of contractile ability, using less than a quarter of contractile reserve in normal ventilation (Mantilla et al., 2014). These properties dispose to additional recruitment and sustained contraction in singing, but supportive abdominal and thoracic activity is required to translate diaphragmatic tension to subglottal pressure in the expiratory phase. The diaphragm is habitually active in inspiration and passive in expiration. During quiet breathing, diaphragm descends upon contraction to reduce intrathoracic pressure for inspiration. Diaphragm recoils passively in expiration.

Voluntary control in singing sustains diaphragm activity in expiration, stabilized without descent. Studies of trained singers provide a biomechanical model that is useful to explain the observed change in diaphragm in expiration, and also the absence of identifiable change in other muscles.

4.4.3.1 *The Stabilized Central Tendon Model*

A biomechanical model constructed around sustained diaphragm expiratory activity has a main feature of stabilization of the diaphragm central tendon, supported by concerted thoracic and abdominal muscle activation. Professional singers rely on abdominal activity to provide 2.5 times greater contribution to lung volume than untrained singers, and spend more time with the rib cage in paradoxical movement, directionally opposite to that predicted by normal airflow principles (Salomoni et al., 2016). This pattern of abdominal activation, sustained diaphragm tension and skilled thoracic movement in expiration is the basic description of a more expert configuration of muscle use in singing. Immediately prior to onset of song there is inward movement of the abdominal wall, raising intra-abdominal pressure and stabilizing the central tendon of the diaphragm. Limiting diaphragm descent maintains an optimal position on the length- tension

curve, producing the most efficient changes in transdiaphragmatic pressure. In addition to explaining why diaphragm activity is seen to increase, the model also explains why other muscles may not display change despite having a coactivated role with diaphragm.

4.4.3.2 *Intercostal Length-Tension Relationships and Mechanical Advantages*

Mechanical advantages bestowed on other muscles by the stabilized diaphragm position may increase their efficiency, and in doing so reduce their visibility within pooled EMG from all recorded muscles. The chest wall configuration around the stabilized diaphragm amplifies the effect of diaphragmatic tension on subglottal pressure, but also has a facilitatory effect on other respiratory muscles. Small thoracic expiratory muscles are lengthened, improving their length-tension relationship (Salomoni et al., 2016). Intercostal muscles contribute significantly to pump muscle function (Gandevia et al., 1996), so may have been predicted to change activity with training. Controlled, graded transition of intercostal muscle activity from inspiratory to expiratory is required to maintain subglottal pressure through the breath support phase of song. Sharp inspiration precedes song, and inspiratory intercostal activity continues after onset of song to prevent chest wall recoil. Progressive reduction in inspiratory intercostal muscle activity occurs until lung volume is about half of vital capacity. The role transitions as expiratory intercostal muscles progressively activate, with increasing support from abdominal activity (Sears and Davis, 1968; Sundberg, 1993). The large rib cage area requires only small intercostal contractions to have a large effect on alveolar pressures, and in turn rapid and precise changes in subglottal pressure. Small contractions of muscles with optimal length-tension relationships could make intercostal effect sizes too small to manifest in mean group results.

4.4.3.3 *Abdominal, Chest Wall and Neck Muscle Activity in Singing in Relation to EMG Change*

Complex patterns of muscle use of abdominal, chest wall and neck muscles are employed by expert singers and may not become evident until

later stages of training. The biomechanical model proposed that abdominal muscle activity was important to stabilize the diaphragm and contribute to late intercostal contribution of subglottal pressure. Other observations predict increased abdominal activity with singing training. The importance of abdominal wall contraction in singing is well described in both amateur singers (Bouhuys et al., 1966) and professional opera singers (Watson and Hixon, 1985). Neural coupling of diaphragm and abdominal muscle contraction is seen in Anticipatory Postural Adjustments (Hodges et al., 1997). Greater reliance on abdominal muscle activity in professional singers is associated with a greater degree of fractionation of abdominal movements (Salomoni et al., 2016). Fractionated abdominal muscle control may be achieved late in the process of singing training, closer to the acquisition of expert performance.

A similar explanation may apply to Pectoralis Major. Sustained expiratory diaphragm activity allows the chest wall to be maintained in a position from which pectoral muscles can slowly descend the rib cage from a maximal inspiratory position against the contracted diaphragm (Sonninen et al., 2005). In a study of professional singers, Pectoralis Major was only activated by around half of singers during phonation, and then at low levels (Pettersen, 2006). It is likely that Pectoralis Major activity in expiration is a variable feature of advanced technique, and part of the higher inter-individual variability of muscle patterning seen with accumulated training.

Neck muscle activity may also be a feature of advanced technique in singing. Animal studies show the effect of artificially increasing abdominal and diaphragm support has less effect on upper compared to lower intercostal activity (Troyer and Wilson, 2016). There may then be a greater role for neck muscles in control of the upper rib cage. Sternocleidomastoid and scalene show activity during both inspiration and phonation in professional classical singers, and this activity increases when singing at the highest pitch (Pettersen and Westgaard, 2005). Trapezius has a synergistic activity on pectoral muscles and sternocleidomastoid. In professional singers, trapezius activity in expiratory phases contributes to movement of the upper thorax (Pettersen and Westgaard, 2004).

4.4.4 Possible Non-Neural Alternative Explanations for the EMG findings

Validating the interpretation of the EMG findings in the context of ipsilateral plasticity requires exclusion of non-neural factors that could contribute to the EMG changes.

4.4.4.1 *Composite EMG Signals and Muscle-to-Electrode Distance Artefact*

Lung volume change can potentially introduce muscle-to-electrode distance artefact of diaphragm activity recorded with chest wall electrodes (Gandevia and McKenzie, 1986). Artefactual explanations therefore need to be excluded given that only diaphragm EMG was seen to change. This artefact is measured as only being particularly problematic at high lung volumes, exceeding 60% of vital capacity, when the site of peak voltage moves down one intercostal interspace (Lansing and Savelle, 1989). Observation of the EMG pattern during voluntary inspiratory and expiratory manoeuvres ensured that chest wall movement did not cause variation from the expected EMG burst pattern. If training altered chest wall configuration rather than diaphragm use, this could potentially also alter diaphragm EMG signal. The model proposed above would result in a longer duration of lower rib cage incursion and closer apposition of muscle to electrode. However the model also requires a higher, more active diaphragm to accompany lower rib cage incursion, so any artefact would only have amplified a real effect rather than generated a false effect.

It should also be remembered that the electrode position interpreted as representing diaphragm activity will also record intercostal activity. It is possible that training increased intercostal activity, interpreted in the composite signal as diaphragm activity. Again, observation of the behaviour of the EMG burst in voluntary and involuntary breathing was used to ensure the majority of the signal was compatible with diaphragm activity.

4.4.4.2 *Muscular Plasticity*

The EMG findings are interpreted as a training related change in number of motor units recruited, or their firing frequency. Some muscular properties specific to diaphragm could also potentially influence the EMG signal. Related to its high activity cycle and need for fatigue resistance, diaphragm has the ability to alter its innervation ratio of muscle fibres per motor unit, and muscle fibre type. Fibre type is demonstrated to alter frequency characteristics of EMG, with fast fibres generating higher frequencies (Kupa et al., 1995), and fatigue resistant fibres showing lower torque to power frequency relationships (Gerdle et al., 1991). The diaphragm is fatigable with exercise (Johnson et al., 1993) so it is possible that training alters muscle fibre type composition. This is seen in animal models (Powers et al., 1992), and in response to inspiratory loading (Levine et al., 1997), and inspiratory training (Ramírez-Sarmiento et al., 2002), in COPD patients. This could alter the EMG characteristics. It is not demonstrated that fibre type composition changes in response singing training, but this may be another possible contributor to EMG change in the diaphragm that would not be seen in other muscles studied.

4.4.4.3 *Inter-individual Heterogeneity*

The absence of EMG change in other muscles may reflect inter-individual heterogeneity of muscle recruitment, which becomes more pronounced with training in singing. Anatomical differences independent of neuromuscular control, such as nasal cavity volume, alter sound production. Individual anatomical influences on singing style may lead to individual patterns of muscle use. Individuality becomes more pronounced with training, with trained singers showing greater variance of muscle use than untrained singers (Salomoni et al., 2016). Increased diaphragm activity may be fundamental to facilitating gains through other muscles. Smaller changes in other muscles may then have greater inter-individual variability and not be apparent as group effects.

4.4.5 Temporal Evolution of EMG Change Between 0 and 6 Months, but not Between 6 and 12 Months

Changes in the pattern of muscle use were seen between 0 and 6 months, with no further change between 6 and 12 months. There were further improvements in behavioural measures between 6 and 12 months, without parallel EMG change. Factors discussed above are relevant to this observation. It could be that the major changes in diaphragm activity are established in the first 6 months, establishing basic subglottal support, which then allows later fine control between 6 and 12 months. This fine control could occur in the muscles studied, but with small effect sizes due to optimal length-tension relationships and mechanical advantages requiring only small contractions. Alternatively, later change may occur in systems outside of those tested here, such as laryngeal and articulator muscles. fMRI studies show that only these muscle representations are increased in motor cortex of professional opera singers (Kleber et al., 2010a). Improving vocal cord adduction by lateral cricoarytenoid, interarytenoid and thyroarytenoid muscle reduces phonation threshold pressure, reduces noise and increases pitch. Increasing anteroposterior stiffness by the cricothyroid and thyroarytenoid increases harmonic-to-noise ratio and improves higher-order harmonics (Zhang, 2016). It may be that training to a certain level at 6 months then facilitates changes of greater inter-individual heterogeneity later, meaning varied, small changes are not apparent in group results between 6 and 12 months.

4.4.6 Why EMG Change Was Not Paralleled by MEP Change

There are specific features of diaphragm neurophysiology that could explain why recording EMG in the voluntary state did not translate to increased MEPs tested in the involuntary state.

4.4.6.1 *Neuromechanical Matching and Henneman's Size Principle*

Out of necessity, the EMG and TMS measures were obtained in different states, EMG in the active, voluntary state compared to TMS in the quiet, involuntary state. There is a specific feature of neuromechanical matching related to voluntary recruitment of phrenic motor units that may explain why EMG changes in the voluntary state do not translate to MEPs recorded in the involuntary state.

In animal experiments phrenic motor neurones are recruited according to Henneman's Size Principle. Recruitment of diaphragm motor units in response to hypercapnia is ordered by axon size in cats (Dick et al., 1987), and in rats across a range of voluntary and involuntary respiratory motor behaviours (Seven et al., 2014). In humans the Size Principle applies under involuntary breathing conditions, and largely applies in voluntary breathing with the majority of motor units recruited at a threshold volume irrespective of airflow rate (Butler et al., 1999). However, a small percentage of units show some change in recruitment order with voluntary tasks, particularly at high airflow rates. Recruitment order is therefore not identical for voluntary and involuntary breathing, reflecting different descending drives. Voluntary control of diaphragm is able to demonstrate a degree of task-specific motor unit recruitment that is not accessible to the involuntary system. The observation that dorsal root lesions do not alter recruitment order in animals suggests there is no segmental role of pulmonary afferent feedback, therefore ordering of recruitment is a feature of the descending drives (Dick et al., 1987). This means there is limited potential for the involuntary system to access recruitment outside of size order, a mechanism accessed in a task-specific way by the voluntary system. The involuntary adherence to the Size Principle optimizes fatigue resistance, but the freedom to recruit outside of this order may be required for the sudden changes in transdiaphragmatic

pressure used by opera singers to produce abrupt change in subglottic pressure (Leanderson et al., 1987).

Since the voluntary recruitment is seen only in a small percentage of motor units at high airflow, this activity may be very task specific. When active during song it may manifest as increased EMG, particularly as activating a greater proportion of large fibres will favour a higher innervation ratio (Sieck, 1988) and higher median frequency (Kupa et al., 1995). This task specificity could mean it is not apparent in MEPs obtained in quiet breathing. To counter this thought, one study that trained increased diaphragm use with a voluntary inspiratory task and monitored MEP changes over one week identified post-training changes in MEP characteristics obtained both during quiet breathing and active inspiration (Demoule et al., 2008). This suggests that strengthened corticomotor connectivity to phrenic motor neurones trained by a voluntary task remains evident when tested in the involuntary state.

4.4.6.2 *Innervation Ratio*

The Size Principle reveals another task-specific feature of diaphragm neurophysiology relevant to the discrepancy between the EMG and TMS results. Recruitment of motor units of different types according to the Size Principle results in a non-linear force generation curve (Mantilla et al., 2014). The Innervation Ratio of muscle fibres per motor unit varies with muscle fibre type (Sieck, 1988). Different respiratory motor tasks will activate different muscle fibre types (Mantilla et al., 2014). Rapidly fatiguing and intermediately fatiguing fibre types are required to produce sudden powerful contractions. Rapid fibres have a higher Innervation Ratio, with more muscle fibres innervated per motor neurone than slow and fatigue resistant motor units. Singing requiring more sustained or dynamically varied subglottal pressure will recruit more fatigable units, resulting in higher numbers of fibres being activated in response to motor neurone activation. In contrast, the Size Principle recruits fatigable units innervated by fast, large fibres late, so they do not contribute to quiet breathing, and may not be evident in the MEP. Since the largest fibres will amplify the

EMG pattern but may not contribute to the MEP with stimulation at 120% aMT, this could potentially produce differential detection by EMG compared to MEP. This task specific access to recruitment of units with the highest Innervation Ratios may explain why high levels of EMG generated during singing is not reflected in MEPs recorded in the quiet state.

4.4.7 Change in MEP Size With Training

Only contralateral scalene showed a significant change in MEP size over the period of training, increased at 12 months compared to baseline and 12 months compared to 6 months. The notable points for discussion are that MEP change was only seen in one muscle, was only seen contralaterally, and significant changes only occurred in the 6 to 12 month period. This is in contrast to the EMG changes which occurred between 0 and 6 months, but not further between 6 and 12 months. It is also notable that the scalene MEP change occurred without accompanying changes in scalene EMG patterns. There are no other studies of ipsilateral scalene MEPs with which to make comparison. The limited number of studies that have investigated scalene MEPs have only recorded contralaterally with non-focal stimulation.

4.4.7.1 *Temporal Evolution of Change in Scalene MEPs*

Knowledge of the time course of evolution of plastic change induced by this training is useful to inform approach to training and physical therapeutic approaches after stroke. Values of scalene MEPs at baseline are small, reflecting use of the measurement MEP area normalized to baseline EMG plus two standard deviations. This normalization can result in small values when MEP area is used, even when MEP amplitudes meet aMT criteria. The stimulation intensity used was calculated from diaphragm aMT which tends to have a higher threshold than scalene. Despite this the scalene MEPs are smaller than diaphragm MEPs at baseline. The fact that scalene MEPs become larger than diaphragm at 12 months reassures that the observed effect is not due to threshold artefacts. Neck muscles and diaphragm share a similarly linear facilitation curve over this range of stimulation (Sharshar et al., 2004), so small threshold differences should not unduly influence MEP size. If intersession threshold artefacts influenced the scalene MEP size, then diaphragm would have been facilitated to a similar degree, but this was not the case.

Both ipsilateral and contralateral Scalene MEPs showed a non-significant increase between 0 and 6 months. Although not significant, this change is relevant to mention since it positions the 6 month change mid-way between

the significant change seen between 0 and 12 months. Observing an appropriately graded, directionally concordant change at the mid-point adds strength to the argument that the observed changes were training effects.

MEP changes became significant at 12 months. EMG changes were seen between 0 and 6 months, but not further between 6 and 12 months. A trend for MEPs to increase was seen at 6 months, but didn't become significant until 12 months. This may indicate something of the nature of the time course of consolidation of practise effects into corticomotor change. Supportive context from TMS studies for comparison is limited. A number of studies report MEP changes accompanying training in skilled motor function after single sessions, multiple days, multiple weeks and multiple months. These vary greatly in training tasks and outcome measures, but none are directly comparable. The closest in study in methodology reported MEP changes after one week of training in diaphragmatic control (Demoule et al., 2008). Studies of operant conditioning give more detailed temporal information about the evolution of plastic change with training, but this applies to unskilled motor control, cortical modulation of spinal excitability. In one operant conditioning study with 10 weeks of training, a rapid plastic effect could be appreciated after early training sessions, with the size of the sessional effect increasing week upon week. A slower plastic effect emerged half way through the training, consolidating gains from individual sessions. This later plastic effect peaked at 10 weeks and persisted at 3 months after cessation of training (Thompson et al., 2009). Although not directly comparable, this provides an example of plastic effects of daily practise being consolidated over months to support the observation of the temporal evolution of MEP changes seen here as being compatible with experience dependent plastic change.

4.4.7.2 *Features of the Involuntary and Voluntary Control of Scalene, Which Explain Why MEP Changes Were Only Observed in Scalene.*

There are functional properties of scalene related to its voluntary and involuntary respiratory functions that may explain why it was a target for MEP change.

4.4.7.3 *Involuntary Control of Scalene*

The involuntary respiratory role of scalene is well described, and contains features relevant to discussion of the MEP findings. Scalene shows phasic EMG activity corresponding to the respiratory rhythm in quiet breathing (Chiti et al., 2008; Hug et al., 2006) and recruitment closely follows diaphragm activity with pre-inspiratory activity (Raper et al., 1966) and near simultaneous peak frequency time (Saboisky et al., 2006). Respiratory drive to scalene increases with hypercapnia (Campbell, 1955), and with respiratory effort in healthy subjects (Chiti et al., 2008; Raper et al., 1966). Cervical spinal cord injury patients (Short et al., 1991), and COPD patients (Gandevia et al., 1996), increase respiratory drive to scalene to compensate diaphragm insufficiency.

Segmental features of involuntary control of scalene are important to interpretation of the MEP findings. Scalene recruitment profile is not altered by lung volume at different inspiratory pressures, showing that pulmonary afferent feedback at segmental level does not modulate the involuntary respiratory drive (Hudson et al., 2007). This inaccessibility of the involuntary system to segmental influence questions the likelihood of ipsilateral MEP change being mediated by decussation at segmental level. In contrast, other segmental inputs are able to powerfully modulate bilateral motor output to scalene. Sudden inspiratory loading produces a short latency reflex inhibition in scalene. The segmental basis of this reflex is proven by its absence in spinal cord injury patients (McBain et al., 2016). Unilateral intercostal nerve stimulation is able to mimic this reflex bilaterally (McBain et al., 2016), showing that this segmental circuit

involves decussating interneurons and therefore a potential site of ipsilateral plasticity.

Accessing such examples of bilateral involuntary control systems through training was a feature of the hypothesis. Together these examples show that segmental level modulation of the involuntary drive is possible, but access to bilateral motor output is governed by neuromechanical principles rather than voluntary motor control.

4.4.7.4 *Voluntary Control of Scalene*

There are features of the voluntary control of scalene that also explain why contralateral control could be expected to dominate in this task. Understanding control of scalene in voluntary movement is not straightforward from existing accounts, but there are sufficient examples of lateralized training effects to demonstrate skilled crossed corticospinal control arising from deliberate practise. There is disagreement about the dominant voluntary movement of scalene. EMG generated in voluntary neck movement is much greater than that generated in maximal respiratory manoeuvres (Gandevia et al., 1996). In humans, contribution to cervical flexion (Falla et al., 2003), and extension (Siegmund and Blouin, 2009) is demonstrated with EMG. Selectively stimulating scalene in monkeys results in ipsilateral cervical rotation (Buford et al., 2002) and mechanically contracting scalene in human cadavers produces ipsilateral cervical lateral flexion (Olinger and Homier, 2010). There are numerous examples of scalene being recruited through deliberate practise or providing compensatory movements in pathological states. Chronic neck pain increases scalene contribution to cervical flexion (D. L. Falla et al., 2004) and stabilization of arm movements (D. Falla et al., 2004). In the Scalenus Anteriorus Syndrome, vascular impingement results from scalene hypertrophy arising from deliberate practise (Ochsner et al., 1935). A series of sportsmen including Olympic athletes treated for brachial plexus impingement from scalene hypertrophy is reported (Baltopoulos et al., 2008). Scalene hypertrophy is frequently seen when overhead arm action is trained such as in baseball (Ligh et al., 2009) and swimming (Katirji and

Hardy, 1995). Musicians who train specific neck postures, such as cellists (Yan et al., 2008) and violinists (Demaree et al., 2016) can develop scalene hypertrophy. These examples prove lateralized voluntary control of scalene across a variety of behavioural tasks.

In discussion of the involuntary role of scalene, an example was given where dominance of the involuntary system limited access to bilateral output. The voluntary control of scalene provides an example of dominance of the voluntary system overriding involuntary control. In wakefulness the Hering-Breuer reflex is normally only evident at very high lung volumes, but in sleep the reflex becomes active at lower volumes (Hamilton et al., 1988), demonstrating cortical control over the involuntary brainstem reflex. An experiment studying the effect of lung volume on scalene MEPs showed that higher lung volumes, which increase pulmonary afferent feedback to activate the Hering-Breuer reflex, facilitated MEPs (Hudson et al., 2012). The effect was opposite to the expected inhibitory action from the brainstem reflex, and demonstrates voluntary drive dominating over the involuntary system. This was proposed to provide a means for voluntary control to override ongoing involuntary drive that would otherwise prevent some respiratory manoeuvres. This dominance would favour strengthening of contralateral rather than ipsilateral MEPs.

In addition to pulmonary afferents, hypercapnia is a dominant drive to the involuntary respiratory system. Scalene EMG is increased by hypercapnia (Campbell, 1955), but TMS measures of cortical control of scalene, both Cortical Silent Period (Luu et al., 2015), and EMG suppression to sub-threshold TMS (Petersen et al., 2011) are not altered by hypercapnia. The EMG suppression in response to TMS activating inhibitory intracortical circuits is however enhanced by increasing voluntary drive with static inspiratory efforts (Petersen et al., 2011). Again this absence of interaction between the voluntary and involuntary systems supports the finding of contralateral change in MEPs. The failure of sub threshold TMS to supress scalene EMG under hypercapnic conditions confirms the expected dominance of involuntary drive under these conditions, in contrast to the lung volume effect.

Taken collectively this evidence validates the finding of contralateral MEP change. It substantiates the interpretation of the contralateral MEP change as indicating that this training does not drive plasticity in ipsilateral pathways. In this setting, interaction between involuntary and voluntary pathways of respiratory motor control was not accessible to training induced plasticity.

4.5 SUMMARY

This discussion has largely been aimed at validating the findings of increased diaphragm EMG and increased contralateral scalene MEP size by incorporating them into a unifying biomechanical model that explains how changes in patterns of thoracic, abdominal and diaphragmatic muscle use can improve singing performance.

This model describes how increased diaphragm activity in the expiratory phase can support sustained control of subglottal pressure for singing. The model also provides an explanation as to why only small changes in muscle activity would be anticipated in muscles other than diaphragm. Small effect sizes may not manifest in group results, or training effects may occur outside of the systems studied or outside of the time frame of the experiment.

Neurophysiological explanations were provided as to why the EMG and TMS findings were discordant. The voluntary respiratory systems access to motor unit recruitment outside of the neuromechanical Size Principle, and ability to recruit motor units with higher Innervation Ratios may explain why EMG changes were not reflected in MEP size. Accounting for this discrepancy was required to allow these findings to be included in the discussion of features of ipsilateral plasticity identified in other chapters.

Discussion of the nature of involuntary and voluntary control of scalene provided explanation as to why only contralateral changes were seen in MEPs. The discussion provided examples of how principles governing involuntary respiratory control limited voluntary system access to bilateral segmental outputs. Conversely, there were examples of how dominance of the voluntary system is able to override involuntary control to allow crossed corticospinal pathways to dominate.

Although much of the discussion relates peripherally to plasticity in ipsilateral pathways, it was essential to establish that the findings had a valid mechanistic explanation before using them as evidence for interpretation of the findings of ipsilateral plasticity in other chapters. A

conclusion is drawn that training subjects at this level of expertise, with this task and duration does not produce evidence of plasticity in ipsilateral pathways. Emphasis was placed on the bulbospinal control of these muscles and the proposal that if ipsilateral training effects occurred through corticobulbospinal pathways, they would be most visible here. The fact that muscles under strong obligate respiratory control did not display ipsilateral change argues against this.

These ideas will now be elaborated upon in the next chapter by comparing cortical motor maps of ipsilateral and contralateral muscle representations in these trainee singers, intermediate and expert singers. The following experiment also tests the hypothesis that deliberate practise of singing drives ipsilateral plastic change to access corticobulbospinal pathways, but over a much longer time frame.

CHAPTER 5
EFFECTS OF DELIBERATE PRACTISE OF A BILATERAL MOTOR TASK ON IPSILATERAL CORTICAL MOTOR REPRESENTATIONS IN ELITE SINGERS

5.1 INTRODUCTION

The purpose of this study is to investigate whether deliberate practise of singing results in reorganization of cortical motor maps controlling muscles bilaterally activated in skilled motor tasks. The specific aim is to test the possibility arising from the findings in previous chapters that ipsilateral motor map reorganization may represent increased activity in cortical regions with access to corticobulbospinal pathways. As described in the discussion of the previous chapter, the reason for examining this in singers is firstly that muscles habitually bilaterally activated in singing are under strong bulbospinal control, and secondly that singers are a group with a comparable duration of deliberate practise to athletes. The neuroanatomical substrates for the proposed plastic change were outlined in the previous chapter and are reiterated briefly in the context of motor mapping below.

The findings of the preceding chapter failed to provide evidence to support corticobulbospinal training effects in trainees trained over one year. The question is now asked whether access to corticobulbospinal pathways is a phenomenon of cumulative practise, occurring over longer time periods in the order of thousands of hours of practise. Another feature of the previous experiment was that EMG and MEP changes were identified in obligate inspiratory muscles that were functioning to provide stability of diaphragm and chest wall configuration in the expiratory phase. This is an example of training obligate inspiratory muscles to perform accessory expiratory roles, integrating control from involuntary and voluntary respiratory pathways. It may be relevant that the only significant neural changes identified in that

experiment were in muscles behaving in this way. If so, it can be hypothesized that these neural changes will be consolidated into movement representations in the brains of expert singers, and plastic change will be most evident in these muscles.

To examine this, ipsilateral motor maps are studied in singers of different duration of training, from trainees with hundreds of hours of training, through to university choir singers with thousands of hours of training, and world-stage opera singers with tens of thousands of hours of training.

5.1.1 Hypothesis

1. Ipsilateral plastic change can beneficially serve the acquisition of skilled motor performance through deliberate practise.

2. Consolidated deliberate practise of singing will result in plastic reorganization of ipsilateral cortical representations of muscles that are habitually bilaterally activated in singing.

3. Plastic change will be experience dependent, so increasingly evident in the groups of singers with the greatest duration of deliberate practise.

4. Singers are more likely to display directional components of cortical reorganization that reflects access to corticobulbospinal pathways.

5. Long term plastic change is most likely to be seen in the representations of those muscles which displayed change over one year of singing training in the preceding experiment.

5.1.2 Basis for Hypothesis

The hypothesis is based on the potential for training driven plasticity to access the neural pathways serving the three main systems of control of muscles performing respiratory function, those of voluntary cortical control, involuntary brainstem control, and limbic pathways (Howard et al., 2001; Polkey et al., 1999).

5.1.2.1 *Corticospinal Pathways of Respiratory Motor Control*

The existence of fast conducting pathways from primary motor cortex to phrenic motor neurones is demonstrated in humans by intraoperative microstimulation (Foerster, 1931), Transcutaneous Electrical Stimulation (TES) (Gandevia and Rothwell, 1987) and TMS (Maskill et al., 1991). The similarity of latency and Central Motor Conduction Time (CMCT) of diaphragm MEPs to those of limb muscles supports monosynaptic corticospinal transmission (Gandevia and Rothwell, 1987). Ipsilateral diaphragm MEPs are smaller and of longer latency than contralateral MEPs (Maskill et al., 1991). These studies demonstrate ipsilateral corticospinal output from primary motor cortex to respiratory muscles. The longer ipsilateral latency suggests a polysynaptic course with brainstem or spinal relays.

There are also ipsilateral corticospinal connections from secondary motor areas to respiratory muscles. Supplementary Motor Area (SMA) involvement in voluntary respiratory movements is evidenced from the localization of Pre-Inspiratory Potentials (Raux et al., 2007), Pre-Expiratory Potentials (Morawiec et al., 2015), and Respiratory Related Evoked Potentials to SMA (von Leupoldt et al., 2010). Patients with increased reliance on voluntary respiratory drive due to deficient central respiratory drive show increased Pre-Inspiratory Potentials in SMA (Tremoureux et al., 2014). Intraoperatively stimulating SMA can produce bilateral respiratory muscle activation and vocalization (Ikeda et al., 1992). Neuromodulation of SMA activity with repetitive TMS can alter breathing parameters (Nierat et al., 2015). SMA is known to originate ipsilateral corticobulbospinal pathways in primates (Montgomery et al., 2013). In humans, stimulation of

SMA produces diaphragm responses distinct from those obtained by stimulation of primary motor cortex, but of similar latency. This suggests rapid corticospinal transmission from SMA, comparable to that from motor cortex (Sharshar et al., 2004). In mapping diaphragm representations, bilateral MEPs can be obtained from stimulation of regions overlying SMA (Maskill et al., 1991).

5.1.2.2 *Limbic Pathways of Respiratory Motor Control*

A pathway serving respiratory motor control is known to originate from the limbic system, and can produce bilateral axial motor output in the presence of complete lesions of the corticospinal and corticobulbar tracts in Locked-In patients (Heywood et al., 1996; Munschauer et al., 1991). This limbic output integrates emotional and autonomic influences on respiration, and voluntary access to its pathways is not easy to demonstrate. In contrast, increasing involuntary drive results in increased CMA activity without accompanying activity in cortical regions concerned with voluntary respiratory control (Corfield et al., 1995). The pathways serving limbic-involuntary interaction are not clear. Motor output of the limbic system derives from Cingulate Motor Areas (CMA), which have extensive intrahemispheric and transcallosal corticocortical connectivity with primary and secondary motor cortical areas. This connectivity could influence cortical reorganization of motor maps.

5.1.2.3 *Corticobulbospinal Pathways of Respiratory Motor Control*

Interpretation of the animal evidence relating to interaction between voluntary and involuntary pathways of respiratory motor control is complex and sufficiently inconclusive to allow the suggestion that cortical inputs may influence ipsilateral respiratory drive at brainstem level (Orem and Netick, 1986). Anatomical evidence in humans shows a bulbospinal pathway of ipsilateral involuntary respiration anatomically separate from the ventromedial anterior column (Nathan, 1963; Severinghaus and Mitchell, 1962). This may represent a corticoreticulospinal route with cortical inputs distinct from ventromedial reticulospinal pathways. In animals, the functional effects of this pathway are distinct from pyramidal corticospinal

effects (Aminoff and Sears, 1971; Andersen and Sears, 1970). Primary motor cortex inputs to the reticular origins of this pathway have been identified in animals (Lipski et al., 1986). In humans, ipsilateral diaphragm MEPs have a latency delay suggesting a polysynaptic pathway (Urban et al., 2002), and a slower CMCT has been observed for ipsilateral diaphragm responses, suggestive of a corticobulbospinal route (Khedr and Trakhan, 2001). Whilst evidence for interaction between voluntary and involuntary pathways in humans remains inconclusive, it is reasonable to investigate whether cortical mapping can reveal responses consistent with ipsilateral corticobulbospinal pathways.

These mechanistic possibilities are now explored with cortical maps made anteriorly extensive to adequately investigate potential involvement of secondary motor areas.

5.2 METHOD

5.2.1 Overview of Experimental Design

Motor mapping of ipsilateral and contralateral axial respiratory muscle representations was performed in the dominant hemisphere of 3 groups of singers. One ipsilateral and one contralateral map for each of the 8 muscles studied in the preceding chapter was constructed for each subject in a single session. TMS was performed during quiet breathing with low-level postural background muscle activation.

Singers were of different duration of experience of deliberate practise of singing and different levels of acquisition of singing expertise. Classification into training, intermediate and expert groups on the basis of experience was similar to the athlete study to allow comparison.

5.2.2 Subjects

15 subjects were recruited into three groups of different levels of deliberate practise and acquisition of expertise, Expert, Intermediate and Training. All subjects were right handed. Ages ranged from 19 to 56.

5.2.2.1 *Inclusion criteria*

Subjects recruited to the Expert group were world-stage level opera singers, regular performers at the Royal Opera House. Their estimates of accumulated deliberate practise exceeded 10,000 hours.

Subjects recruited to the Intermediate group were singers in Oxford University choir. They had undertaken regular singing practise from an early age and participated in regular public performance. Their estimates of accumulated deliberate practise exceeded 5000 hours.

Subjects recruited to the Training group were the singers undertaking training as part of the longitudinal study of the previous chapter. Their estimates of accumulated deliberate practise were in the order of up to 500 hours.

5.2.2.2 *Exclusion criteria*

Subjects were questioned to ensure that they had not accumulated deliberate practise in any other activity that may have influenced axial muscle representations, for example practise of string instruments requiring lateralized axial muscle activation.

Standard exclusion criteria for TMS were applied according to international consensus safety statements, i.e. history of epilepsy, vascular, traumatic, tumoral, infectious or metabolic brain lesions, medication that may lower seizure threshold, implanted intracranial metal devices, pacemakers, nerve stimulators, pregnancy, sleep deprivation, alcohol excess, heart disease (Rossi et al, 2009; Wassermann, 1998).

5.2.2.3 *Ethics Approval and Informed Consent*

All TMS was performed with local Ethics Committee approval and with the written, informed consent of each subject. Only single pulse TMS was performed. All subjects participated voluntarily without financial reward.

5.2.2.4 *Estimated Duration of Training*

Subjects were asked to make an estimate of their cumulative duration of training based on recollection of typical practise schedules at different stages of their singing careers.

5.2.3 Data Acquisition

5.2.3.1 *Overview of Data Acquisition*

MEPs were recorded from surface electrodes on homologous muscle pairs designated contralateral or ipsilateral to the dominant hemisphere stimulated by TMS.

MEPs were recorded bilaterally in response to TMS of each point on a scalp grid. In this way a cortical map of the representation of each ipsilateral and contralateral muscle was constructed. Only the dominant hemisphere was mapped in a single session.

TMS was performed during simultaneous low-level background activation of all muscles studied, achieved through postural activation in an unsupported upright sitting posture and stimulation in mid-inspiration.

TMS was performed at single stimulation intensity, 120% of active Motor Threshold (aMT) for the ipsilateral 8th-9th intercostal space.

5.2.3.2 *Background Contraction*

Subjects were seated without back support with the instruction to sit with the spine straight and head horizontal. This produced a constant low level of postural background activity in most of the muscles studied.

Background respiratory facilitation was produced by performing TMS in mid-inspiration during quiet breathing. This was achieved by using a trigger based on a threshold level of EMG in a channel that displayed reliably cyclical EMG activity through the inspiratory phase of the respiratory cycle.

5.2.3.3 *EMG Recording*

Self-adhesive F301 silver/silver chloride foam solid gel surface electrodes (Skintact, Innsbruck, Austria) were used. These electrodes were found to be convenient to place and resisted movement through the duration of repeated contractions.

Electrodes were positioned according to accepted belly-tendon placement positions, balancing proximity to motor points with a surface position resistant to movement. Trapezius, Pectoralis Major and Abdominal Oblique electrode positions were identical to those describe in Chapter 3. Sternocleidomastoid, Scalene, Parasternal Intercostal, Lumbar Paraspinal, and 8th-9th Intercostal space electrode positions were identical to those described in Chapter 4.

EMG was acquired through an 8-channel D-360 Headstage box (Digitimer, Welwyn, UK) and Power1401 amplifier (CED, Cambridge, UK). Signal software (CED, Cambridge, UK) was used to record MEPs.

Wide filter settings were used, Low-pass 10Hz-High-pass 2kHz. A sample rate of 10kHz was used. Standard gain of 100 was used. Raw EMG was recorded without notch-filters and rectified off-line using virtual channels in Signal. MEPs were recorded with Signal in peri-trigger mode, with 200ms of EMG preceding the stimulus included in the frame.

5.2.3.4 *TMS*

The dominant hemisphere of each subject was mapped systematically using a scalp grid. TMS was delivered with a monophasic Magstim 2002 stimulator and figure-of-eight coil, Double 70mm Alpha Coil (Magstim, Whitland, UK). TMS was guided by a Brainsight stereotactic infrared navigation system (Rogue Resolutions Ltd, Cardiff, UK).

5.2.3.4.1 *Determination of Stimulation Intensity*

Mapping was performed at single stimulus intensity, 120% aMT of ipsilateral 8th-9th Intercostal space. Use of 120% aMT is accepted as standard practise in cortical mapping as an accepted compromise between reliably eliciting MEPs whilst limiting loss of focality from current spread. The decision to determine the stimulation intensity from the measure of 8th-9th Intercostal space threshold arose from the observation in pilot experiments that this muscle tended to have a slightly higher threshold than the other muscles studied.

Prior to mapping the aMT was determined. The dominant left hemisphere was stimulated in a preliminary search to identify a Hotspot for the Diaphragm. aMT was determined in the standard way, using the definition of the minimal intensity required to produce MEPs of 200 to 300 microvolt amplitude in 50% of trials. Stimulation intensities were generally in the range of 60 to 80% of Maximum Stimulator Output.

5.2.3.4.2 *Stimulation Technique*

The coil was held with the handle 45 degrees to the mid sagittal line. A Brainsight neuronavigation system guided coil placement to map points.

Stimulation was triggered using an analogue trigger with a threshold marker from a channel recording from the 2nd Parasternal Intercostal muscle. The EMG in this channel provided reliably cyclical activity through the respiratory cycle and was convenient to use as a marker of a fixed level of inspiration. TMS was triggered at a mid-inspiratory point. It was necessary to consistently stimulate at a fixed point in the respiratory cycle partly to ensure that TMS coincided with the same descending respiratory drive, and partly to ensure that TMS was delivered with a degree of background facilitation in Diaphragm.

The inter-stimulus interval was variable with this method, depending on the respiratory cycle and manual arming of the stimulator after each stimulus. Occasional triggering of TMS by ECG rather than the intended level of inspiratory activity is unavoidable with use of a chest wall electrode. This was continually monitored for and any frames including ECG complexes were discarded.

5.2.3.4.3 *Mapping Technique*

A 28-point scalp grid was manually constructed and programmed into Brainsight using a surface pointer directed at points drawn on a tightly fitting skullcap. The vertex was marked at the intersection of the intertragal line and the nasion-inion line. Rows of grid points were marked over the dominant left hemisphere with 1 cm separation. The medial limit of the grid

was 1 cm from the midline, the lateral limit 5 cm from the midline, producing 4 parallel rows. The posterior limit of the grid was 2 cm from the vertex, and the anterior limit 4 cm from the vertex. Manually inputting grid points marked first directly onto the scalp was found to be more reliable than using software generated automatic grids.

A pseudo-randomized stimulation order was used. Each point on the grid was stimulated 3 times and the average used to give a mean MEP response for that point.

5.2.4 Data Reduction and Analysis

5.2.4.1 *Data Quality*

Each individual frame was analysed separately with individual manual cursor placements used to measure each MEP and the preceding background EMG.

Each frame was monitored during acquisition following each stimulus to ensure that background activity had remained within acceptable limits and that ECG and stimulus artefacts had not contaminated MEPs.

5.2.4.2 *MEP measurement*

Raw EMG was recorded and virtual channels used off-line to rectify EMG for measurement. Rectified EMG in the 50msec preceding the stimulus was averaged, two standard deviations added and this value was used to set a horizontal cursor to function as part of the criteria for MEP measurement.

Criteria for analysing ipsilateral MEPs adopted by other investigators to allow for the typical small size and variable morphology were used. Inclusion criteria for a response to be measured as an MEP were occurrence with expected latency, compatible morphology, and exceeding baseline EMG plus two standard deviations for at least 5msec (Schwerin et al., 2008).

MEP area was measured from the rectified EMG using individual cursor placements to identify the MEP onset and offset, marked as the point of deflection above, and return to the baseline EMG. The choice of MEP area as a suitable measure for ipsilateral axial MEPs has been detailed in the previous mapping chapter. This provides a more robust measure for MEPs composed of multiple weak, desynchronized descending inputs.

5.2.4.3 *MEP normalization*

Normalization was required to allow pooling of data for comparison between groups. Variations in electrode positioning between individuals with distance from the motor point in large muscles can contribute to

variation in absolute MEP size. To control for this and for small variations in background activity, normalization to background activity was chosen. The choice of this method of normalization has been discussed in the previous mapping chapter.

5.2.4.4 *Construction of the cortical map*

Three stimuli were delivered at each grid point and the MEPs averaged to give a mean response for that grid point. Average responses were positioned in a table corresponding to the scalp location.

5.2.4.5 *Cortical Map Measures*

The mean map volume was calculated as the arithmetic mean of the MEP area from all grid points stimulated. The normalized map volume is used to give a measure of focality of the map, dividing the sum of mean MEP area from all grid points that produced responses by the largest MEP area from the map Hotspot. The Centre-of-Gravity (CoG) is a weighted-mean that gives each grid point a relative weighting and provides a robust and reproducible means of comparing maps. The CoG predicts the region of greatest excitability of corticomotor neurones projecting to the muscle studied. The CoG is calculated with the formula $Xcg = \Sigma xa/\Sigma a$ to determine the CoG along the mediolateral dimension and $Ycg = \Sigma ya/\Sigma a$ to locate the CoG along the anteroposterior dimension. The CoG of the map is the point at which both coordinates intersect (Wassermann et al., 1992). Subtracting the ipsilateral from the contralateral CoGx and CoGy gives a measure of the organization of the ipsilateral representation in relation to the contralateral.

5.2.4.6 Comparison Between Groups

Map measures are then compared between the Expert, Intermediate and Training groups using Analysis of Variance (ANOVA) with initial factors of group, side and muscle to test the hypothesis that deliberate practise may alter the ipsilateral cortical representation. Post-hoc tests were then performed to further explore significant findings.

5.2.5 Methodological Limitations

The limitations of the previous cortical mapping experiment were discussed in Chapter 3 and apply equally to this experiment.

5.3.1 Cortical Motor Maps

A representative sample of MEPs obtained during mapping is shown in Figure 5.2. Only the cortical representation of contralateral diaphragm in the expert group showed evidence of plastic change. There was an enlargement of the mean map area of the contralateral diaphragm representation in the expert group that was significantly different from that of the training group. The cortical maps of ipsilateral and contralateral diaphragm representations are shown for each subject in each group in Figure 5.1.

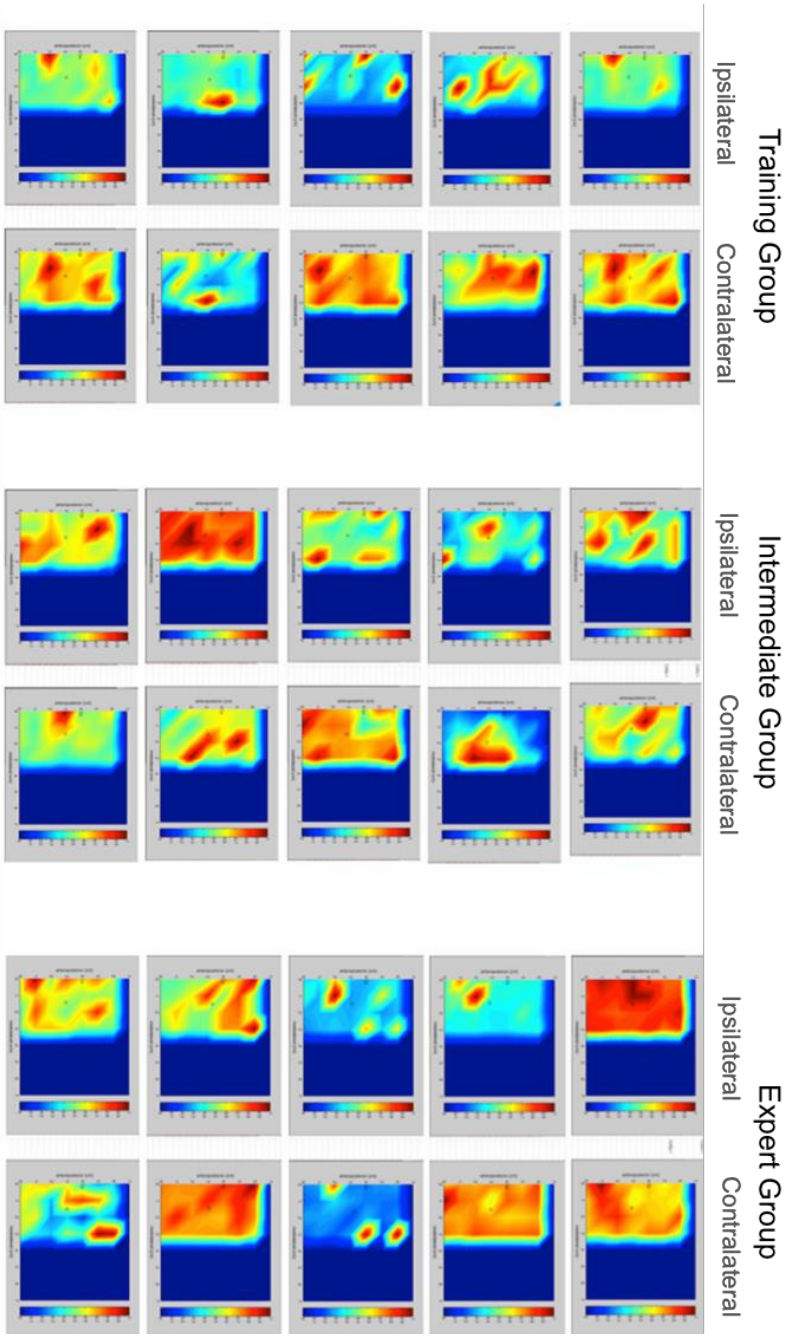

FIGURE 5.1 CORTICAL MAPS OF DIAPHRAGM REPRESENTATION FOR EACH INDIVIDUAL IN EACH GROUP

In each pair of columns, the ipsilateral representation is shown in the left column, the contralateral representation on the right. The left pair of columns shows the training group, the middle pair of columns shows the intermediate group and the right pair of columns shows the expert group.

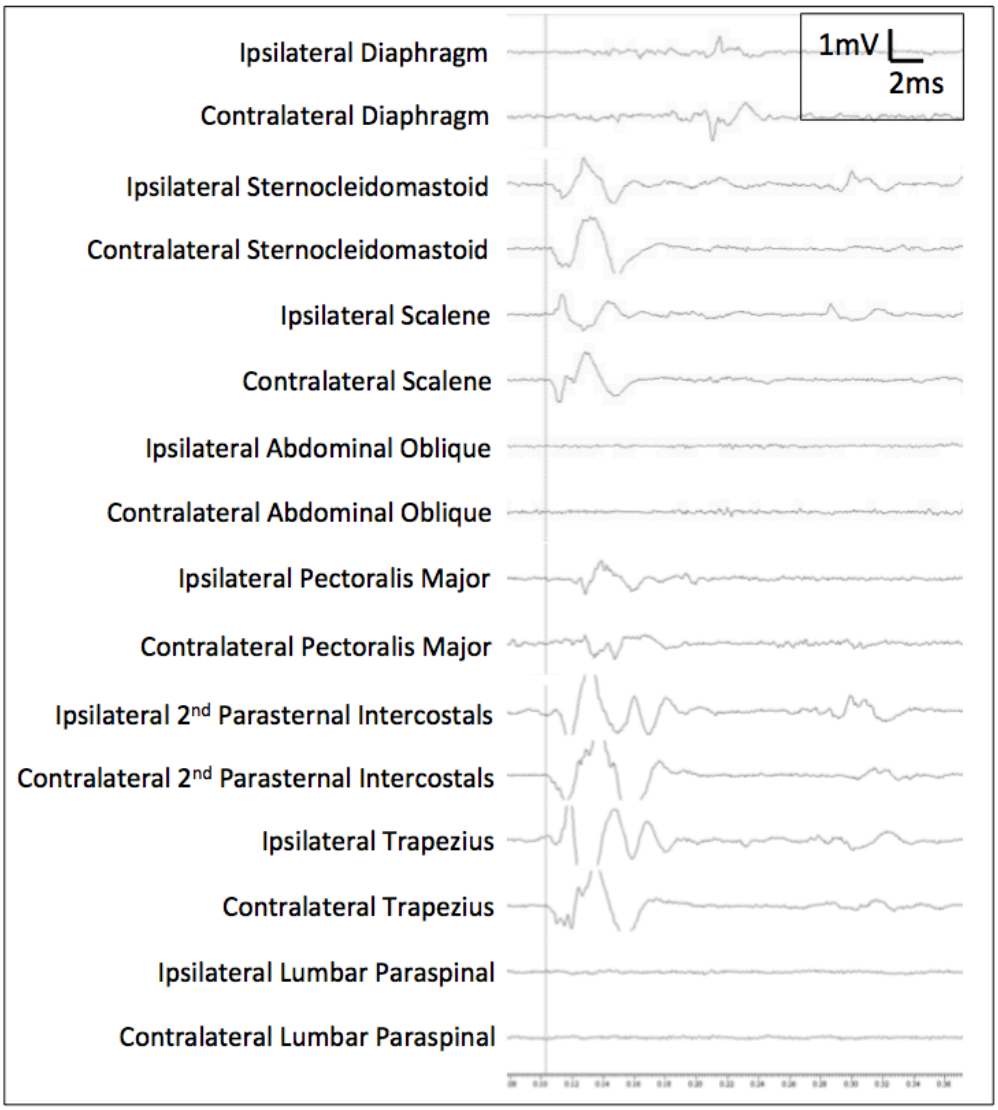

FIGURE 5.2 MAPPING AXIAL RESPIRATORY MUSCLES

A sample of bilateral recording of MEPs in multiple axial respiratory muscles during motor mapping. MEPs are not elicited in every muscle with every stimulation as some grid points will be remote from some muscle representations.

5.3.2 Evidence of experience dependent plastic change in the size of the motor representation

Graphs of mean map size for each group are shown for diaphragm in Figure 5.3. Each bar represents the average of all maps in each group, with each individual subject's data pooled into a group average. Differences in mean map size between groups were analysed with One Way ANOVA.

There was a significant difference between groups ($F(2, 65) = 4.679$, $p = .013$) and Tukey HSD post hoc revealed that the only significant difference is seen between the training group and expert group for contralateral diaphragm representation ($p = .013$).

Although there were no significant differences between the training and intermediate groups ($p = .051$), or between the intermediate and expert groups ($p = .896$), the value for the intermediate group lies between that of the training and expert group. This shows a direction and gradation of change that is compatible with the suggestion that the significant difference between the training and expert groups is the result of a cumulative practise effect.

There were no significant differences in the size of the ipsilateral maps between training, intermediate and expert groups. The hypothesis that deliberate practise of singing would increase cortical representation of ipsilateral muscles is not supported by these results. No significant difference in cortical representations was seen between groups for any of the other muscles tested.

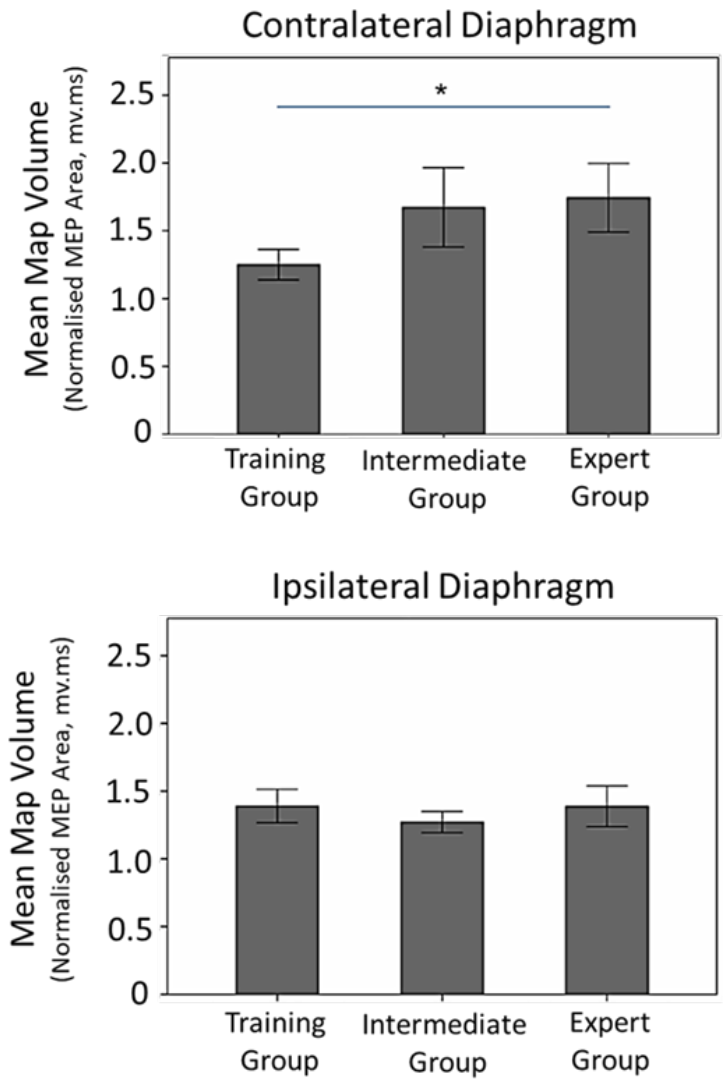

FIGURE 5.3 GRAPHS OF MEAN MAP AREA FOR CONTRALATERAL (TOP GRAPH) AND IPSILATERAL (BOTTOM GRAPH) DIAPHRAGM REPRESENTATION

Each graph gives the mean map area, with each individual subject's map pooled into an average value for the group. Each graph shows values for training (left), intermediate (middle), and expert groups (right). There is a significant difference between the size of the training and expert contralateral diaphragm representations ($p = .013$).

5.3.3 Evidence of experience dependent plastic change in the organization of the motor representations – absolute differences in CoGx and CoGy coordinates

The mean Centre of Gravity for each group was calculated from the pooled map data. One-way ANOVA was performed for each muscle with factors of group and CoGx- coordinate, and group and CoGy-coordinate. The only significant effect of group on CoG was seen for the CoGx-coordinate of ipsilateral Pectoralis Major CoG, $F=(2,14)$ 4.849 $p=0.29$. Post-hoc analysis (Tukey HSD) looking at the effect of group showed that the difference was found between the expert and intermediate groups $p=0.23$. The lower CoGx value in the expert group translates to a more medial representation of Pectoralis Major.

Of all the map measures, this was the only significant result relating to ipsilateral representations. However, the result is problematic to interpret as representing an experience dependent plasticity effect. Inspection of the plot of CoG data in Figure 5.4 reveals that the Training group CoGx value lies between that of the Intermediate and Expert groups. This lacks the directional gradation of effect needed to relate the finding to experience dependent plasticity, and is therefore difficult to interpret in terms of the presented hypothesis.

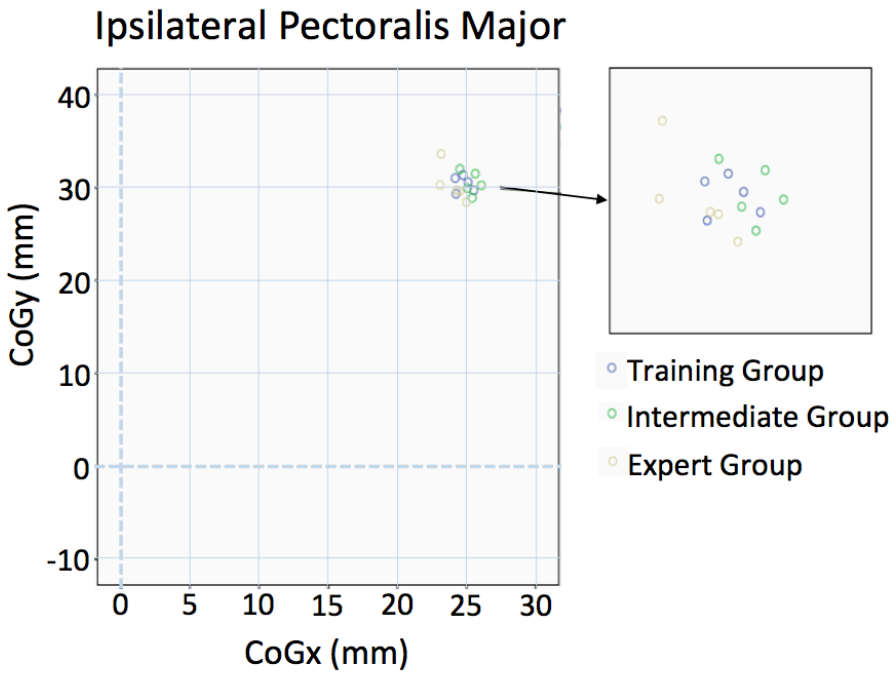

FIGURE 5.4 COG OF PECTORALIS MAJOR, SHOWN FOR TRAINING, INTERMEDIATE AND EXPERT GROUPS

A significant difference is seen with One-way ANOVA with factors of CoGx and group, F=(2,14) 4.849 p=0.29. Post-hoc analysis (Tukey HSD) shows the difference is between the Expert and Intermediate groups p=0.23. The expanded view of the plot shown within the square emphasizes the values for the Training group lie between those of the Expert and Intermediate group.

5.3.4 Evidence of experience dependent plastic change in the organization of the motor representations – differences in the position of ipsilateral representations relative to contralateral representations

The graphs in Figure 5.5 examine whether there is a training effect on the position of the ipsilateral representation relative to the contralateral representation. By expressing the relationship as (contralateral CoGx – ipsilateral CoGx) and (contralateral CoGy – ipsilateral CoGy), positive values show a more medial or anterior ipsilateral representation, whereas negative values show a more lateral or posterior ipsilateral representation. One-way ANOVA of group (contralateral CoGx – ipsilateral CoGx) and group (contralateral CoGy – ipsilateral CoGy) shows no significant group effects in any of the muscles tested.

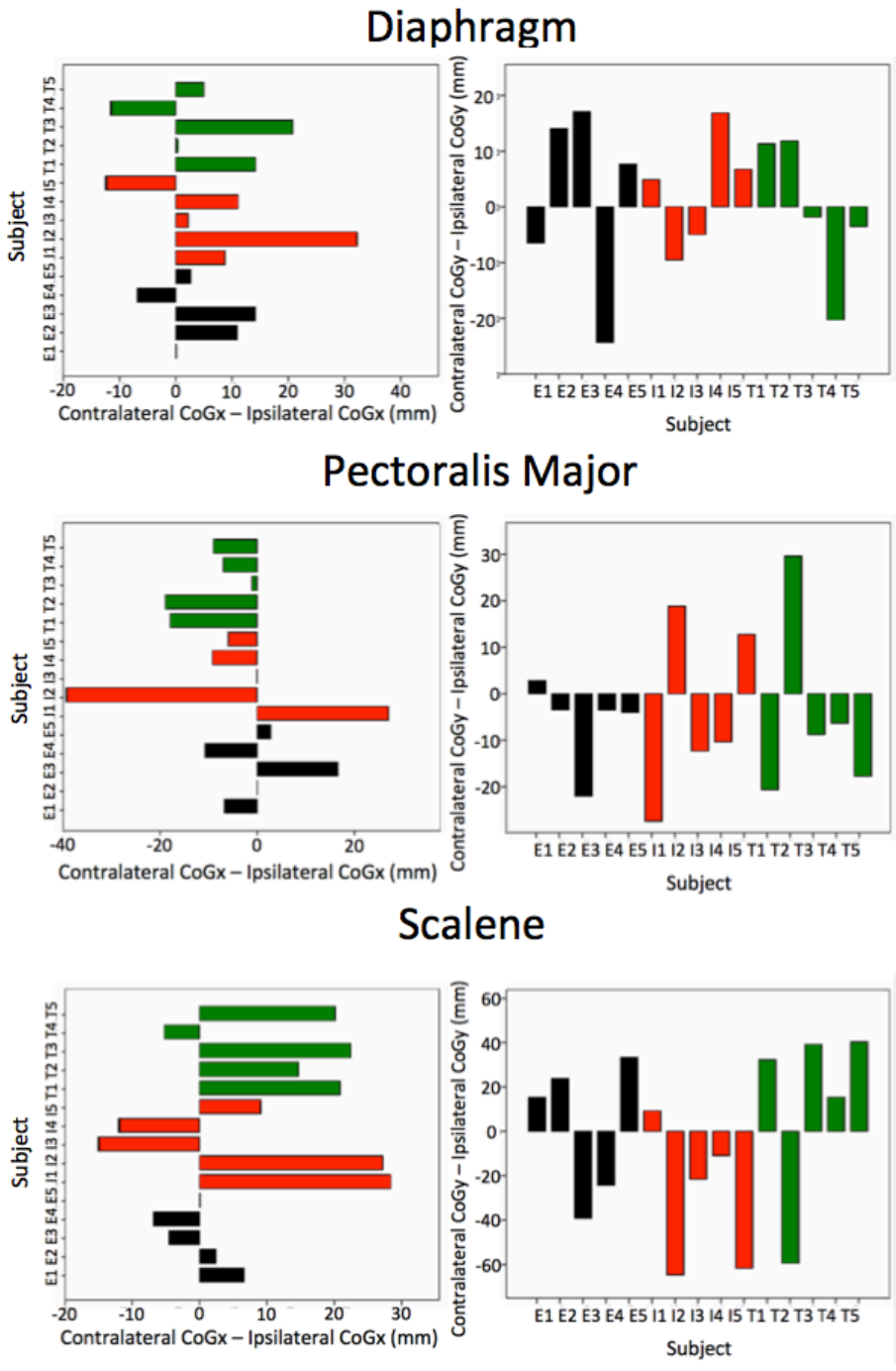

FIGURE 5.5 THE RELATIONSHIP BETWEEN POSITION OF IPSILATERAL REPRESENTATIONS RELATIVE TO CONTRALATERAL REPRESENTATIONS IS SHOWN FOR DIAPHRAGM (TOP), SCALENE (MIDDLE) AND PECTORALIS MAJOR (BOTTOM).

In each pair the left hand graph describes the relationship between the contralateral and ipsilateral CoGx, expressed as contralateral CoGx – ipsilateral CoGx. The right hand graph in each pair is equivalent for CoGy. Each bar represents an individual subject, arranged according to group, (training = green, intermediate = red, expert =black).

5.4 DISCUSSION

5.4.1 Main findings

1. There were no significant differences in the size of the cortical representations of ipsilateral muscles between groups of different durations of accumulated deliberate practise of singing.

2. There were no significant differences in the location of the Centre-of-Gravity for the ipsilateral cortical muscle representations between groups of different durations of accumulated deliberate practise of singing.

3. There was a significant difference in the size of the contralateral diaphragm representation between the training and expert groups. The size of the intermediate group map lay midway between the training and expert groups, concordant with the suggestion that the significant findings represent experience dependent plastic change.

There is no support for the hypothesis that deliberate practise of singing drives plastic change in ipsilateral motor pathways. The hypothesis was based on the idea that experience dependent plasticity in singers may utilize ipsilateral motor control through corticobulbospinal pathways or corticospinal pathways from secondary motor areas, but this was not the case. These negative results are consistent with the findings from the previous study of singers training over one year. There, the only evidence of plastic change was seen in contralateral scalene MEPs. Here the only relevant significant change was seen in contralateral diaphragm representation.

It may be of relevance that in the previous study, only diaphragm was identified as changing its pattern of muscle use with training. This was taken to predict that evidence of plastic change in diaphragm representations would be seen in expert singers. It was also predicted that scalene representation would display plastic change in experts on the basis of the previously observed MEP change over time with training, but this was not seen. The paired observations of change in diaphragm EMG with training in

trainees and change in contralateral diaphragm representation in expert singers suggest a mechanistic link. This pairing could arise from a plastic process that progressively consolidates control of a motor behaviour functionally relevant to improved singing performance from early to advanced stages of training. Accordingly, the biomechanical model used to explain the EMG change in the previous chapter incorporated observations from expert singers which confirm a persisting role for increased diaphragm use in expert performance. The lack of diaphragm MEP change in the previous experiment is the missing link in proving a stepwise plastic process, but it is unlikely that plastic change would obey such a simple scheme according to the chosen experimental time points. The biomechanical model also proposes how the function of diaphragm in singing makes it the muscle most likely to display map change. The high degree of coactivation of diaphragm with multiple muscles in this model increases the extent of its potential connectivity within a motor map. Participation of diaphragm in Anticipatory Postural Adjustments adds to this potential for expansive cortical representation (Hodges et al., 1997).

Discussion of these negative ipsilateral findings is required to reconsider whether interpretation of the findings of the athlete study becomes narrowed in the light of these results. It was hypothesized that a cortical reorganization based on access to ipsilateral corticobulbospinal pathways from secondary motor areas would become less likely if not observed in singers. This hypothesis was reliant on proposed interaction between voluntary and involuntary motor pathways of respiration and a plastic ability to exploit greater convergence of descending motor drives. However, the evidence supporting these assumptions was equivocal and some of the putative pathways have only been evidenced in the pathological state. Arguing against access to involuntary brainstem and segmental ipsilateral motor output narrows the possible mechanistic explanations of motor map change to other aspects of motor control. To use these negative results toward this conclusion requires validation of the findings with mechanistic principles. This is also required to exclude the possibility that the measures

used here were either not sensitive enough or not directed appropriately to detect ipsilateral change.

5.4.2 A mechanistic explanation of positive contralateral and negative ipsilateral findings based on limits of interaction between voluntary and involuntary pathways of respiratory motor control

To understand why the hypothesis was disproven and only contralateral map change was seen, it is necessary to consider voluntary corticophrenic control, potential sites of interaction of voluntary and involuntary systems in the corticophrenic pathway, and why interaction was not seen. This is important in view of animal evidence that suggests that both crossed corticospinal and crossed corticobulbospinal pathways to phrenic motor neurones may both be used naturally, but with task specific selection. If this is accepted, then these results would need to be reviewed with the question of whether testing the system in the active rather than quiet state would have produced a different outcome. Testing in the active state would not be possible with these measures. Evidence from studies of stroke patients will be discussed later that makes it unlikely that corticobulbospinal findings in animal studies apply directly to humans.

A crossed corticospinal pathway from primary motor cortex to phrenic motor neurones is well established. Intraoperative stimulation of human motor cortex reveals a small area close to the convexity adjacent to the thoracic homuncular region that contracts the diaphragm (Foerster, 1936). Transcutaneous electrical stimulation (TES) and TMS over vertex produce diaphragm MEPs with latency and Central Motor Conduction Time (CMCT) compatible with monosynaptic corticospinal transmission (Gandevia and Rothwell, 1987; Murphy et al., 1990). Lateralized focal TMS over motor cortex shows that fast contralateral MEPs dominate, with smaller ipsilateral MEPs occurring at longer latency (Urban et al., 2002).

The monosynaptic, crossed corticospinal pathway to diaphragm is well described in humans, but animal evidence suggests a crossed corticobulbospinal pathway from primary motor cortex to phrenic motor neurones, possibly supported by some neurophysiological observations in humans. This possibility requires exclusion to ensure the interpretation of

the contralateral map change as evidence against use of corticobulbospinal pathways is correct. Details of the potential corticobulbospinal route can be constructed from human and animal evidence. In humans ipsilateral diaphragm MEPs have a longer latency to suggest polysynaptic pathways (Urban et al., 2002), which could include brainstem bulbospinal relays or segmental premotor relays. Diaphragm CMCT was measured as being twice as long for ipsilateral compared to contralateral responses (Khedr and Trakhan, 2001), suggesting relays with either medullary premotor neurones, medullary bulbospinal motorneurones or cervical interneurons above the phrenic nucleus. Each of these levels of interaction has a supportive correlate in animal studies, with midline medullary lesions (Duffin and Li, 2006; Gromysz and Karczewski, 1981), and phrenic nucleus lesions (Janczewski and Karczewski, 1990) influencing phrenic motorneurone output bilaterally in rats and rabbits.

These bulbospinal pathways accessed by ipsilateral motor cortex inputs in humans have been shown to be activated by stimulation of contralateral primary motor cortex in animals. Phrenic motor neurone output in response to focal microstimulation of contralateral motor cortex in cats contains both excitatory and inhibitory components. This suggests the presence of Central Respiratory Drive Potential, and therefore brainstem relay (Colle and Massion, 1958; Lipski et al., 1986). Further support for brainstem relays comes from an experiment that observed that stimulating the pyramidal tract in the medulla does not replicate the phrenic motorneurone response to primary motor cortex stimulation, but stimulating the reticulospinal tract in the medulla elicits fast monosynaptic phrenic EPSPs (Lipski et al., 1986). The ability of primary motor cortex stimulation to entrain the respiratory frequency in cats also suggests a corticobulbospinal pathway (Bassal and Bianchi, 1982). However, under similar experimental conditions no coactivation was seen in medullary respiratory neurones or phrenic motor neurones in response to the cortical stimulation, questioning the nature of the interaction (Bassal et al., 1981).

A pair of related microstimulation studies in cats is useful in reconciling the conflicting observations. Stimulation of motor cortex when involuntary

drive is diminished by hypocapnia results in facilitation of expiratory intercostal motor neurones. This effect is remarkably similar to the reflex effect of intercostal nerve stimulation, suggesting a common segmental interneurone mechanism. Repeating the cortical stimulation in the presence of involuntary drive in normocapnia then additionally resulted in reciprocal inhibition between expiratory and inspiratory motorneurones, an effect suggesting the segmental influence of the Central Respiratory Drive Potential. Repeating stimulation in hypercapnia when involuntary drive dominates abolished the cortical effect. Depending on the interstimulus interval, cortical stimulation could facilitate at short intervals, or inhibit at long intervals the intercostal reflex (Aminoff and Sears, 1971). All of these observations are compatible with the cortical stimulation interacting with the Central Respiratory Drive Potential at segmental interneurone level, rather than brainstem level. The demand for very focal stimulation of motor cortex and the short latency of the responses suggested the cortical effect was produced through corticospinal pathways. They went on to lesion the corticospinal tract in the dorsolateral column and this abolished all effects of cortical stimulation but preserved the respiratory rhythm.

Conversely, lesioning the reticulospinal pathways in the ventrolateral column abolished the respiratory rhythm but did not alter the cortical stimulation effects (Aminoff and Sears, 1971). These lesions make the conclusion that the stimulation effects of primary motor cortex have a corticospinal and segmental basis very clear. Further support is provided by a similar experiment with stimulation of the pyramidal tracts in the medulla. The effect on the expiratory and inspiratory motor neurones was similar with cortical or brainstem pyramidal stimulation, confirming that the cortical stimulation was transmitted through pyramidal tracts (Sears, 1966).

Having presented examples of corticospinal and possible corticobulbospinal respiratory pathways from primary motor cortex, the balance of evidence is equivocal for brainstem relays, but compelling for the corticospinal route. These experiments also show the extent of voluntary influence on respiratory motorneurone output is modulated at a segmental level by the strength of accompanying involuntary central respiratory drive. From this,

the interpretation of the enlarged contralateral diaphragm map as being evidence of strengthened crossed corticospinal control and evidence against training resulting in access to corticobulbospinal pathways is valid.

5.4.3 Possible Corticocortical Mechanisms Underlying Contralateral Diaphragm Map Change

A corticocortical basis for the increased contralateral diaphragm representation can be proposed. Map expansion is seen through formation of new connections between cortical regions representing movements relevant to the task. Muscles trained to be coactivated will be linked within a motor map. Diaphragms extensive coactivation within the biomechanical model of singing anticipates motor map expansion with training. The mechanism of formation of new synaptic connections between non-continuous cortical regions requires changes in the balance of activity in excitatory and inhibitory intracortical circuits. Altered input from secondary motor areas may control this balance. Imaging and neurophysiological studies point to experience dependent increases in activation of SMA and CMA within the singing network as being relevant to the control of intracortical circuits governing the motor map in primary motor cortex.

5.4.4 Cingulate Motor Area Contributions to Corticocortical Control of Diaphragm Motor Maps

CMA is involved in voluntary respiratory control, shown in functional imaging studies of the singing network (Zarate, 2013). Cingulate cortex is modulated by involuntary respiratory drive in cats (Frysinger and Harper, 1986), and strongly interconnected to primary motor cortex in humans (Beckmann et al., 2009; Habas, 2010). TMS and functional imaging studies provide indirect evidence for CMA involvement in respiration in a manner that may link voluntary and involuntary respiratory control. Diaphragm MEPs obtained from stimulation of primary motor cortex were seen to be facilitated by hypercapnia (Murphy et al., 1990). Hypercapnia is the dominant drive to the involuntary system, but is not expected to have cortical effects. Studies of hypercapnia on scalene responses to TMS found no effect (Luu et al., 2015; Petersen et al., 2011) and hypocapnia does not alter diaphragm MEPs (Corfield et al., 1998). Three observations confirmed a cortical basis to the hypercapnic facilitation of diaphragm MEPs. Firstly, the CMCT was consistent with a monosynaptic corticospinal route, ruling out brainstem level interaction. Secondly, there was no facilitation of phrenic nerve stimulation, ruling out a spinal level facilitation. Thirdly, the only subject who did not display hypercapnic facilitation had fallen asleep, thereby inactivating cortical activity. The proposed basis of the cortical facilitation was increased CMA input to primary motor cortex secondary to limbic activation by the dyspnoea, induced by hypercapnia. Hypercapnia induced dyspnoea produces Anterior Cingulate Cortex activation on fMRI (Binks et al., 2014), and subsequent increase in CMA motor output (Nishino, 2011). Another possibility with less supporting evidence in humans is coupling between involuntary drive and Cingulate activity, suggested by modulation of Cingulate neurones by the respiratory rhythm in cats (Frysinger and Harper, 1986). Increased Cingulate activity seen with increasing involuntary drive on functional imaging is difficult to interpret because of inability to separate sensorimotor components of nociception of dyspnoea from other motor activity (Corfield et al., 1995). The primary

motor cortex facilitation could have arisen from altered corticocortical inputs from CMA.

These findings are made relevant to this study by functional imaging findings identifying a potential link between CMA response to experimental hypercapnia and neural correlates of singing expertise. Acquisition of expert singing performance is associated with changes to the cortical singing network measured by fMRI (Kleber et al., 2010b; Wilson et al., 2011). Cingulate cortex activation is see in singing in professional opera singers (Kleber et al., 2007). An experience dependent behavioural correlate of this Cingulate activity was shown to reflect acquisition of expertise in a study using pitch-shifted auditory feedback. Experts employed compensatory strategies including activation of Anterior Cingulate Cortex, not seen in less trained singers (Zarate and Zatorre, 2008). Taken together, the hypercapnic facilitation of primary motor cortex output to diaphragm, hypercapnic activation of Cingulate Cortex, and the experience dependent activation of Cingulate Cortex in expert singers strengthens the suggestion that cortico-cortical pathways from CMA to primary motor cortex may have contributed to reorganization of the diaphragm motor map.

5.4.4.1 *Functionally Relevant Corticocortical Pathways from CMA to Primary Motor Cortex*

There is variable anatomical evidence to support this proposed CMA to primary motor cortex connectivity as a basis for axial motor map reorganization. Discrete corticocortical connections from CMA to primary motor cortex limb representations are demonstrated in primate studies, but are less distinct to axial representations. Strong homotopic arrangement is seen within corticocortical connections between CMA and primary motor cortex forelimb and hindlimb areas, but axial connections are more diffuse (Morecraft and Van Hoesen, 1992). Other studies fail to identify clear somatotopy of CMA projections to primary motor cortex (Tokuno and Tanji, 1993). Transcallosal projections from CMA to primary motor cortex are described as modest and heterotopic for hand area (Rouiller et al., 1994). An fMRI and TMS study in humans has proposed transcallosal connections

between CMA and contralateral primary motor cortex being active in complex unimanual tasks of the non-dominant hand, but with great inter-individual variability (Kobayashi et al., 2003).

To support this proposed behavioural role for CMA, involvement of CMA in the control of axial movements and respiration is shown in primate studies. CMA microstimulation in primates evokes movement of axial muscles in some studies (Akazawa et al., 2000; Boudrias et al., 2010). Other studies have failed to observe axial responses from CMA at stimulation intensities that evoked axial movements from SMA (Luppino et al., 1991). In the limited number of human intraoperative studies, microstimulation of CMA produces contralateral distal arm (Chassagnon et al., 2008; Diehl et al., 2000; Lim et al., 1994) and contralateral leg (Diehl et al., 2000) movements, but no axial movements were reported. There are Anterior Cingulate Cortex neurones with correlation of their discharge frequency to the respiratory rhythm in cats (Frysinger and Harper, 1986). Collectively, the available evidence demonstrates that CMA represents axial movements and shows respiratory modulation, but CMA corticocortical projections to axial regions of primary motor cortex may be diffuse and heterotopic.

5.4.4.2 *Corticospinal Output from CMA*

The discussion above sets out the case for CMA corticocortical inputs to primary motor cortex as a basis for the contralateral diaphragm map change seen in expert singers. The negative finding of absence of ipsilateral change can also be discussed briefly in the context of these studies of CMA functional connectivity. It was outlined in the introduction that corticospinal outputs from CMA include projections to ipsilateral cervical Laminae VII, VIII and IX (Dum and Strick, 1996), and the fastest CMA projections to axial muscles may be monosynaptic (Boudrias et al., 2010). CMA also has subcortical relays that overlap those of primary motor cortex, with CMA terminations in rostral striatum intermixed with those from motor cortex (Takada et al., 2001). A functional role for CMA in ipsilateral movements is shown neurophysiologically in primates by modulation by ipsilateral unimanual movement (Nakayama et al., 2015; Yokoyama et al., 2016). This

evidence of functional anatomy for ipsilateral corticospinal output from CMA prompted features of the hypothesis, but no corresponding activity was seen experimentally.

5.4.5 Supplementary Motor Area Contributions to Corticocortical Control of Diaphragm Motor Maps

SMA is another potential source of corticocortical control of the extent of the diaphragm representation. SMA activation is seen in functional imaging studies of voluntary breathing (Ramsay et al., 1993) and the singing network (Brown et al., 2004; Perry et al., 1999; Zarate, 2013). There is strong neurophysiological evidence for the involvement of SMA in voluntary control of respiratory movements. SMA is the optimal site to detect Pre-Inspiratory Potentials (Tremoureux et al., 2010) and Pre-Expiratory Potentials (Morawiec et al., 2015) during voluntary breathing tasks. Dipole source localization maps Respiratory Related Evoked Potential generation to SMA (von Leupoldt et al., 2010). This evidence for the role in SMA in both voluntary respiration and singing lend support to the idea that deliberate practise might alter SMA connectivity with primary motor cortex.

5.4.5.1 *Corticocortical Pathways from SMA to Primary Motor Cortex*

Demonstration by TMS of a tonic excitatory pathway between SMA and the diaphragm representation of primary motor cortex adds further strength to the argument for a role of SMA in the control of diaphragm motor maps. In one study the balance of Short Intracortical Inhibition and Intra Cortical Facilitation between SMA and primary motor cortex sites producing diaphragm MEPs suggested tonic excitatory connectivity from SMA to primary motor cortex (Sharshar et al., 2004). This was confirmed with neuromodulation of SMA, with inhibitory rTMS of SMA resulting in reduction of diaphragm MEPs obtained from primary motor cortex (Raux et al., 2010). The reverse is also true, with excitatory rTMS to SMA increasing diaphragm MEPs from primary motor cortex (Laviolette et al., 2013).

There is both primate and human evidence providing anatomical substrates to support the observation of functional connections between SMA and primary motor cortex revealed by neuromodulation. In primates injection of tracer to SMA reveals extensive connectivity, with strong somatotopically

arranged connections to proximal and distal representations of primary motor cortex (Luppino et al., 1993; Picard and Strick, 1996). Intracortical connections are also seen between SMA and Premotor and Cingulate cortex. Evidence for functional connectivity between SMA and primary motor cortex is provided in humans by Diffusion Tensor Imaging (Guye et al., 2003), and intraoperative studies. Use of subdural electrodes intraoperatively in epilepsy patients reveals both pre movement ECoG coherence between SMA and primary motor cortex (Ohara et al., 2001) and Corticocortical Evoked Potentials recorded at primary motor cortex in response to stimulation of SMA electrodes (Matsumoto et al., 2007). Subdurally stimulating SMA produces blood flow changes measured with NIRS in arm and trunk regions of primary motor cortex (Fukuda et al., 2015).

5.4.5.2 *Corticospinal and Corticobulbospinal Output from SMA*

The existence of potential corticospinal and corticobulbospinal outputs from SMA is relevant to the negative findings here. Corticospinal output from SMA is suggested by TMS studies. The only study to systematically map both ipsilateral and contralateral diaphragm representations with focal TMS also examined the regional effect of coil orientation. Grid points with a location overlying SMA were seen to produce diaphragm MEPs with an optimal coil orientation distinct from that of primary motor cortex regions. This implies a discrete neuronal population representing diaphragm within SMA was being stimulated (Maskill et al., 1991). This finding was confirmed in another TMS study by stimulating from vertex anteriorly along the midline. Diaphragm MEPs were seen from two distinct stimulation sites, one consistent with primary motor cortex representation, and another anterior site identified as SMA when co-registered with MRI (Sharshar et al., 2004). Anatomical evidence supports the possibility of a direct corticophrenic pathway from SMA. Termination of SMA neurones in Lamina IX of the cervical spinal cord is demonstrated in primates. For SMA corticospinal terminations in Intermediate Zone, three quarters are contralateral, and one quarter ipsilateral (Dum and Strick, 1996). The possibility of direct ipsilateral corticophrenic output from SMA was a factor

motivating study of the cortical maps of singers. The failure of the CoG of the maps to move anteriorly in this study argues against direct SMA output as contributing to the observed map change.

Another TMS study raises the possibility of there being corticobulbospinal pathways of respiratory control originating from SMA. Excitatory rTMS protocols applied to SMA shortened inspiratory time, reduced tidal volume and increased end-tidal C02. The opposite effects were seen with inhibitory rTMS protocols (Nierat et al., 2015). This suggests strong influence of SMA over brainstem respiratory bulbospinal pathways, a claim that would require further evidence to substantiate. The lack of ipsilateral change in this experiment or the previous chapter does not support the suggestion that corticobulbospinal pathways are involved in any of the observed training effects.

5.4.6 How study of plastic responses to lesions of voluntary and involuntary respiratory pathways contributes to interpretation of the findings

Diaphragm MEPs are reduced or absent in hemiplegic stroke patients with respiratory weakness (Khedr et al., 2000) Ipsilateral MEPs are not seen to compensate, even if cortex is intact for reorganization in subcortical stroke (Similowski et al., 1996). This situation occurs in spite of bilateral diaphragm MEPs being seen commonly to motor cortex stimulation in healthy subjects (Maskill et al., 1991). It appears that redundant corticophrenic pathways are not upregulated after stroke. This clinical observation is compatible with the finding here, that ipsilateral diaphragm representation does not show evidence of training related plastic change.

Despite clear anatomical potential for segmental plastic change, compensatory spinal reorganization also does not occur. This is surprising in the light of animal evidence from the Crossed Phrenic Phenomenon (Goshgarian, 2009), and observations following lesions in humans (Severinghaus and Mitchell, 1962). When surgical spinal lesions disrupt descending respiratory pathways, rapid and near complete recovery of respiratory muscle weakness can occur within weeks of surgery (Nathan, 1963). The speed of this recovery is evidence of latent connections or structural plasticity within segmental interneurone circuits. The fact that descending motor drives cannot access such plasticity after stroke makes it likely that local factors which are a neurobiological feature of response to surgical lesions allow that particular ipsilateral plastic response. Attempts have been made to drive decussation of neurones at cortical, brainstem and spinal levels after experimental ischaemic lesions in animal studies using antibodies against neurite growth inhibiting proteins. Whilst animal studies have had some success when combined with appropriate physical therapeutic interventions at appropriate time intervals (Wahl and Schwab, 2014), this has not translated to clinical success in humans.

It is not clear why after stroke ipsilateral plasticity does not occur in a pathway that would seem anatomically predisposed, under conditions where

plasticity promoting mediators should be abundant. These negative findings in singers are consistent with this and an explanation that specific neurobiological permissions may be required to reorganize involuntary respiratory pathways otherwise tightly organized according to neuromechanical respiratory principles.

5.5 SUMMARY

Experience dependent plasticity from singing training results in increased cortical representation of contralateral diaphragm, but does not alter measures of cortical representation of ipsilateral muscles.

The failure to observe ipsilateral map changes argues against access to corticospinal or corticobulbospinal pathways from secondary motor areas as a mechanism of ipsilateral plasticity. This is true for muscles under strong bulbospinal control in subjects who have undergone tens of thousands of hours of training, making the absence of ipsilateral map change here an important negative finding.

The discussion has included examples of task specific dominance of voluntary and involuntary respiratory systems, which may explain the lack of interaction required for ipsilateral change. In addition, different ipsilateral plastic responses to different lesion types suggests there may be neurobiological control of these processes that is not active in healthy subjects.

These findings are now combined with those of the athlete study in the interpretation of evidence of ipsilateral plastic change related to recovery of upper limb function after stroke. The negative findings prompt exploration of the idea that ipsilateral plasticity in athletes and stroke patients may have a basis in contribution to control of synergies, rather than direct corticomotor output.

CHAPTER 6
LONGITUDINAL EFFECTS OF TRAINING A REACH TASK ON IPSILATERAL CORTICAL REPRESENTATIONS IN THE CONTRALESIONAL HEMISPHERE IN THE CHRONIC PHASE OF STROKE

6.1 INTRODUCTION

The purpose of this chapter is to investigate whether plasticity in ipsilateral motor pathways can be beneficial to motor recovery after stroke. Despite much accumulated evidence, there is no consensus on this.

It is acknowledged that individual factors are important in determining the effect of activity in ipsilateral motor pathways after stroke. Conclusions from related experiments vary depending on lesion extent, lesion location and time since stroke. These factors combine with variation in pre-stroke pathology and genotypic determinants of plasticity to produce a spectrum of associated outcomes from excellent recovery to harmful maladaptation. The only singular conclusion from current evidence is that individualization is required to estimate the effect of driving contralesional plasticity through ipsilateral pathways.

At present there is no practical clinical measure by which to perform this individualization. The Proportional Recovery model (Byblow et al., 2015), and PREP algorithm (Stinear et al., 2014) have accelerated clinical acceptance of the necessity for a greater degree of structured individualization according to neurobiological and neurophysiological principles in planning therapy after stroke. The PREP algorithm utilizes simple neurophysiological metrics to augment clinical assessment of contralateral corticospinal pathways. By further characterizing the role of ipsilateral activity after stroke, an equivalent metric accounting for

ipsilateral activity could be engineered to guide therapeutic intervention and prognostication.

The potential for maladaptive change through ipsilateral processes stresses the importance of this issue. The increasing use of neuromodulation and robotic assisted therapy in the subacute phase of stroke carries the risk of driving maladaptive change if these principles are not observed. Altering the balance of contralateral and ipsilateral activity without truly understanding the effects on that patient, or repetitively training movement without knowledge of the neural pathways being activated could be counter productive. Conversely, recent evidence identifying a pivotal role for early ipsilateral activity in predicting late functional recovery needs to be recognized in a clinical approach that acknowledges the beneficial potential of ipsilateral activity. Shoulder movements and trunk control are early predictors of recovery of distal arm function and ambulatory status in the chronic stage. Both of these movements require strong ipsilateral motor inputs. Failure to harness the plastic potential of the contralesional hemisphere through undue cautionary avoidance of maladaptive aspects would also fail to optimize recovery. The balance of ipsilateral and contralateral activity therefore needs to be found to tailor therapy. This balance will show individual variation and temporal evolution and therapeutic approach should be reactive to this. Any measure needs to be capable of describing both balance and evolution to guide appropriate physical, robotic and neuromodulatory intervention.

Here I question whether training in a reach task alters the balance of ipsilateral and contralateral motor output or cortical motor representations in stroke patients. The aim is to identify features of beneficial training driven ipsilateral plasticity, and potential parallels with the findings from the athlete study that could be relevant to post stroke motor rehabilitation.

6.1.1 Hypothesis

1. Training chronic stroke patients in a reach task with the paretic limb can drive beneficial ipsilateral plastic change in the contralesional hemisphere.

2. Beneficial ipsilateral plasticity will associate with improved functional upper limb performance in patients responding to the therapeutic intervention. Ipsilateral plasticity will be evident as training related strengthening of ipsilateral corticomotor connectivity in task relevant muscles, or changes in the cortical representations of ipsilateral muscles in the contralesional hemisphere.

3. Training will result in a greater contribution of ipsilateral activity in muscles habitually bilaterally innervated. This will be evident as a change in the Laterality Index reflecting the balance of corticomotor output from the ipsilesional and contralesional hemisphere.

4. Implicit with the association with improved motor performance is the prediction that any increased activity in ipsilateral pathways will not drive maladaptive synergies or increase spasticity. This will be evident from association between the Laterality Index and functional motor improvement, and Laterality Index and measures of spasticity.

6.1.2 Basis for Hypothesis

Whilst the crossed corticospinal tract provides the main control of agonist movements, the ipsilateral hemisphere contributes to control of complex unimanual tasks (Perez and Cohen, 2009), and control of synergies (McCambridge et al., 2011). Neurophysiological changes in the ipsilateral hemisphere accompany training to improved performance in unimanual tasks with high speed and accuracy demands (Christiansen et al., 2016). Despite a role in skilled motor control, there is also potential for ipsilateral activity to have a detrimental effect on motor function. Transcallosal projections mediating Interhemispheric Inhibition (IHI) normally function to limit unwanted coactivation, such as mirror movements (Hubers and Ziemann, 2006). If disturbed balance of excitatory and inhibitory activity favours increased ipsilateral excitability then increased IHI may impair function of the contralateral motor cortex. Also, extrapyramidal pathways providing ipsilateral motor output contain neurons with flexor or extensor bias with terminations across multiple segmental levels (Davidson and Buford, 2006) and extensive terminations on gamma motor neurones (Mukherjee and Chakravarty, 2010). Increasing activity in these pathways therefore has the potential to drive unwanted flexor or extensor synergies and spasticity.

These few examples of ipsilateral contribution to skilled motor control, twinned with the potential for ipsilateral activity to impede motor control provide context to illustrate the difficulty in approaching the subject of ipsilateral activity post stroke. In certain settings ipsilateral activity can provide useful plastic reserve through alternative motor pathways. In other settings such activity may limit recovery. There are examples of increased ipsilateral activity serving excellent motor recovery after stroke and persisting into the chronic stage (Butefisch et al., 2008; Nair et al., 2007). As expected from the outline above, there are also numerous examples of ipsilateral activity associated with poor motor recovery (Ward et al., 2003) through increased IHI of the recovering ipsilesional hemisphere (Murase et

al., 2004), increased expression of abnormal synergies (Schwerin et al., 2008) and increased spasticity (Bradnam et al., 2010).

Determining which situation will dominate in the individual is therefore crucial but not straightforward. The aim of this experiment is to add to the knowledge of factors which determine when ipsilateral plasticity could be beneficial after stroke. TMS measures of strength of corticomotor connectivity, the balance of ipsilateral and contralateral activity, and strength and organization of cortical representation are compared before and after a period of upper limb reach training in chronic stroke patients.

6.2 METHOD

6.2.1 Overview of Experimental Design

Chronic stroke patients were studied at baseline and after two weeks of robotic training in a reach task. TMS measures were taken from both the ipsilesional and contralesional hemisphere.

Baseline measures of ipsilateral and contralateral MEPs were used to construct cortical maps of the representation of the paretic Pectoralis Major, Deltoid, Biceps and Triceps. Measures of the cortical representations were used to identify features of ipsilateral activity that were associated with good recovery of motor function in the chronic stage of stroke.

Baseline MEPs were also used to calculate the Laterality Index, a measure of balance of activity in contralateral and ipsilateral pathways. This was used to search for associations between reliance on ipsilateral activity and measures of recovery of function.

The cortical mapping was repeated after the period of robotic reach training. Changes in ipsilateral activity associated with improvement of upper limb function through training were sought.

Measures of upper limb function and measures of spasticity, weakness and sensory impairment were taken. Training in a robotically assisted reach task with varying speed and accuracy requirements was delivered over two weeks.

6.2.2 Subjects

19 chronic stroke patients were recruited. Ages ranged from 47 to 74. The side of the lesioned hemisphere and cortical or subcortical extent of the lesion varied. Only patients with ischaemic strokes were studied. Record was made of age, handedness, months since stroke, and lesion location.

6.2.2.1 *Inclusion criteria*

Chronic stroke patients who were at least 12 months following stroke were recruited. Patients with both cortical and subcortical ischaemic lesions were included.

To participate patients needed to have weakness of shoulder abduction measured as less than MRC grade 4 and upper limb spasticity had to be less than Modified Ashworth Scale grade 2. Subjects had to be able to reach 15cm with partial weight support.

Patients had to have sufficient comprehension and communication abilities to demonstrate clear ability to engage with the consent process.

6.2.2.2 *Exclusion criteria*

Patients were excluded if there was severe sensory involvement. No patients with haemorrhagic stroke, known haemorrhagic transformation of an ischaemic stroke or cerebellar involvement were studied.

Patients were excluded if there was history of post-stroke seizures or epilepsy, traumatic, tumoral, infectious or metabolic brain lesions, medication that may lower seizure threshold, implanted intracranial metal devices, pacemakers, nerve stimulators, pregnancy, sleep deprivation, alcohol excess or heart disease.

6.2.2.3 *Ethics Approval and Informed Consent*

Local Ethics Committee approval was granted for the study. Written, informed consent was obtained for each subject. Written information clearly explaining the study was provided and it was made clear that patients were free to withdraw from the study at any time. Only single pulse TMS was

performed. All subjects participated voluntarily without financial reward but transport and meals were provided or reimbursed.

6.2.3 Training

After obtaining baseline functional and TMS measures, patients underwent a two week programme of robotic reach training of the paretic upper limb. Following the training all measures were repeated in a second session, 2 weeks after the initial measures.

Patients were trained in a reach task using a robotic manipulandum. The speed and accuracy demands of a reach-to-target task were varied. Patients performed reach whilst seated with trunk movement constrained.

Each patient performed 420 reaches per day of training in 7 blocks of 60. Four days of training were completed over 2 weeks. Patients were instructed to perform reach at either fast or slow speeds, scored for accuracy to target and given feedback to improve their scores.

6.2.4 Data Acquisition

6.2.4.1 *Overview of Data Acquisition*

Two mapping sessions were performed, at baseline and at two weeks after robotic training. Both hemispheres were mapped in both sessions.

MEPs were recorded from surface electrodes on homologous muscle pairs relevant to reach. Muscles were designated contralateral or ipsilateral to the hemisphere stimulated by TMS.

Anterior Deltoid, Biceps, Triceps and Pectoralis Major MEPs were recorded bilaterally in response to TMS of each point on a scalp grid. In this way a cortical map of the representation of each ipsilateral and contralateral muscle was constructed. TMS was performed during background activation by reach at 120% of aMT for the ipsilateral deltoid.

6.2.4.2 *Upper Limb Function, Power, Spasticity and Sensory Measures*

Functional measures were made at baseline and 2 weeks later, following the robotic upper limb training. The upper limb subsets of the Fugl-Meyer test were used (Fugl-Meyer et al., 1975).

The Modified Ashworth Scale was used to measure spasticity at baseline and after two weeks of training. For analysis the Modified Ashworth Scale was transformed into a six-point scale (Bohannon and Smith, 1987).

MRC grading of power of shoulder abduction was measured at baseline and after two weeks of training.

Distal light touch sensation was measured with a 1g monofilament applied to the dorsum and palm of the hand. Patients were classified as moderate (<80% accuracy) or mild (>80% accuracy) sensory impairment depending on number of errors of 10 consecutive stimuli to the dorsum of the hand, and 10 to the palm.

6.2.4.3 *EMG Recording*

Self-adhesive F301 silver/silver chloride foam solid gel surface electrodes (Skintact, Innsbruck, Austria) were used. These electrodes were found to be convenient to place and resisted movement.

Electrodes were positioned according to accepted belly-tendon placement positions, balancing proximity to motor points with a surface position resistant to movement. Pectoralis Major electrodes were positioned with the reference electrode close to the tendon insertion to the humerus and the active electrode approximately 3 cm lateral to the midpoint of the anterior axillary fold. The Deltoid reference electrode was positioned close to the insertion on the humeral deltoid tuberosity, and the active electrode 3 cm superiorly over the body of Anterior Deltoid. The Biceps reference electrode was positioned over its distal tendon and the active 3cm superiorly over the bulk of the muscle belly. Triceps reference electrode was positioned over its distal tendon and the active electrode superiorly and laterally over the bulk of the lateral muscle belly.

EMG was acquired through an 8-channel D-360 Headstage box (Digitimer, Welwyn, UK) and Power1401 amplifier (CED, Cambridge, UK). Signal software (CED, Cambridge, UK) was used to record MEPs.

Wide filter settings were used, Low-pass 10Hz-High-pass 2kHz. A sample rate of 10kHz was used. Standard gain of 100 was used. Raw EMG was recorded without notch filters and rectified off-line using virtual channels in Signal.

MEPs were recorded with Signal in peri-trigger mode, with 200ms of EMG preceding the stimulus included in the frame. An Event trigger was used with a TTL pulse from the stimulator as the input with TMS triggered manually using a foot pedal.

6.2.4.4 *TMS*

TMS was delivered with a monophasic Magstim 2002 stimulator and figure-of-eight coil, Double 70mm Alpha Coil (Magstim, Whitland, UK). TMS

was guided by a Brainsight stereotactic infrared navigation system (Rogue Resolutions Ltd, Cardiff, UK).

6.2.4.4.1 *Background Contraction*

Subjects were seated with armrests and instructed to make a steady reach forwards with both hands. Elastic resistance bands (Thera-Bands, UK) fixed behind the chair were used to provide a constant resistance to control the degree of background contraction. This also made it easier to produce smoother movements in the paretic limb. Balancing the activity in the paretic and unaffected upper limb required trialling the reach with different levels of resistance whilst monitoring the patient's ease of ability to perform the reach, and the level of EMG generated. Subjects were given verbal feedback on the quality of the movement as required.

In pilot studies, asking patients to achieve a set level of background activity, such as percentage of MVC used in the earlier experiment, proved too demanding. The attentional demands were fatiguing and patients found it very difficult to voluntarily control contractions to a set level. In practise the use of resistance bands was a very effective way of achieving steady levels of background activation in a way that was straightforward for patients to engage with.

The TMS was performed with long inter-stimulus intervals to limit subject fatigue. The length of time required by each subject to recover between reaches varied so TMS was triggered manually to allow stimulus frequency to be paced on a stimulus-by-stimulus basis.

6.2.4.4.2 *Stimulation*

Both hemispheres of each subject were mapped systematically using a scalp grid. Hemispheres are additionally referred to as ipsilesional or contralesional. All MEPs are referred to as being ipsilateral or contralateral to the side of stimulation.

6.2.4.4.3 *Stimulation Intensity*

Mapping was performed at 120% aMT of ipsilateral deltoid. The decision to determine the stimulation intensity from the measure of deltoid threshold was a practical one. With reach of the paretic upper limb the deltoid reached a steady level of activation earlier in the task. Using deltoid allowed briefer contractions for the stimuli determining threshold and therefore the overall subject burden was reduced. In practise all muscles tested had thresholds within a comparable range and MEPs were elicited in all muscles using this intensity.

Prior to mapping, preliminary stimulation to determine the aMT was performed. Both hemispheres were stimulated to identify a Hotspot for the ipsilateral deltoid muscles. A record was made of the Hotspot with a Brainsight marker for reference in the subsequent post-training mapping session. aMT was determined in the standard way, using the definition of the minimal intensity required to produce MEPs of 200 to 300 microvolt amplitude in 50% of trials. Stimulation intensities were generally in the range of 70 to 85% of Maximum Stimulator Output.

6.2.4.4.4 *Stimulation Technique*

The coil was held with the handle 45 degrees to the mid sagittal line. A Brainsight neuronavigation system guided coil placement to map points. Stimulation was triggered manually using a foot pedal trigger. Manual stimulation carried the advantage of being able to optimally time stimulation, and constant adjustment of inter-stimulus interval to avoid fatigue. Allowing subjects to adequately rest between stimuli was essential to ensure that data remained of good quality throughout the mapping session and to maintain patient comfort. Patients reported finding both the repetitive movements and the experience of TMS to be fatiguing. A long rest period was provided between mapping each hemisphere.

Stimulation was timed to the peak rise of background muscle activation, judged by the experimenter using auditory feedback. Auditory feedback was produced by a loudspeaker amplifying rectified EMG signal split from the

channel recording the paretic triceps. Triceps was used based on observation in pilot experiments that patients who found it effortful to produce reach tended to reach plateau of contraction marginally later in triceps. Use of the triceps signal to guide triggering therefore ensured all muscles were adequately activated at the point of stimulation.

6.2.4.4.5 *Mapping Technique*

A 9-point scalp grid was manually constructed for each hemisphere and programmed into Brainsight. A surface pointer was directed at points drawn on a tightly fitting skullcap. The vertex was marked at the intersection of the intertragal line and the nasion-inion line. Rows of grid points were marked over the dominant left hemisphere with 1.5cm separation. The medial limit of the grid was 1 cm from the midline, the lateral limit 4.5cm from the midline, producing 3 parallel rows. The posterior limit of the grid was 1cm from the vertex, and the anterior limit 3.5cm from the vertex. Manually inputting grid points marked first directly onto the scalp was found to be more reliable than using the software generated automatic grids.

This grid was more limited with wider spacing between grid points than the maps constructed in previous chapters. The selection of the total number of stimuli tolerable was based on experience from pilot experiments with stroke patients. The reduced number of cortical points was a compromise to provide the desired information whilst maintaining patient comfort and tolerability. Attempting to record greater numbers of MEPs in a single session would likely have compromised the quality of the data as patients fatigued.

A pseudo-randomized stimulation order was used. Each point on the grid was stimulated 3 times and the average used to give a mean MEP response for that point.

6.2.5 Data Reduction and Analysis

6.2.5.1 *Overview of Analysis*

The baseline and post training MEPs and map measures were used to answer a series of experimental questions. Associations were sought between baseline markers of ipsilateral activity and baseline upper limb function, and baseline spasticity measures. Association between baseline ipsilateral activity and subsequent response of upper limb function to training was investigated. Change in ipsilateral representations in the contralesional hemisphere with training was sought.

In addition to seeking correlation between markers of ipsilateral activity and aspects of upper limb performance, comparison of ipsilateral representations between groups was made. Ipsilateral representations in the contralesional hemisphere were compared between groups of patients who had made good recovery and limited recovery, and between groups of patients who had improved with training and patients who had not.

6.2.5.2 *Data Quality*

Each individual frame was analysed separately with manual individual cursor placements used to measure each MEP and the preceding background EMG. Each frame was monitored following each stimulus to ensure that background activity had remained within acceptable limits. Any frames with excessive variation were discarded during the mapping process.

6.2.5.3 *MEP Measurement*

Raw EMG was recorded and virtual channels used off-line to rectify EMG for measurement. Rectified EMG in the 50msec preceding the stimulus was averaged, two standard deviations added and this value was used to set a horizontal cursor to function as part of the criteria for MEP measurement.

Criteria for analysing ipsilateral MEPs used by other investigators to allow for the typical small size and variable morphology were adopted. Inclusion criteria for a response to be measured as an MEP were occurrence with

expected latency, compatible morphology, and exceeding baseline EMG plus two standard deviations for at least 5msec (Schwerin et al., 2008).

MEP area was measured from the rectified EMG using individual cursor placements to identify the MEP onset and offset, marked as the point of deflection above, and return to the baseline EMG. Use of MEP area was considered to be more relevant to the study of ipsilateral axial MEPs than amplitude. Amplitude is a conventional measure for more distal MEPs. However, axial MEPs are often of variable morphology and polyphasic, reflecting summation of multiple weak descending inputs. Change in MEP area can occur with little change in amplitude if multiple descending corticospinal outputs are desynchronized (Rosler et al., 2002).

6.2.5.4 *MEP Normalization*

Normalization was required to allow pooling of data for comparison between groups, since absolute MEP areas varied between individuals. Variations in electrode positioning between individuals with distance from the motor point in large muscles can contribute to this variation. To control for this and for small variations in background activity, normalization to background activity was chosen.

The rationale for use of this normalization has been discussed in previous chapters. It is suited to the study of stroke patients since other methods of normalization can produce values that are highly variable between subjects with different degrees of weakness of voluntary activation.

6.2.5.5 *Laterality Index*

The Laterality Index is useful as an expression of the balance of contralateral to ipsilateral activity in a muscle. It is a useful measure in stroke patients to reflect the extent of reliance of the recovered motor function on ipsilateral pathways.

Laterality Index is calculated using (Contralateral MEP - Ipsilateral MEP)/(Contralateral MEP + Ipsilateral MEP).

6.2.5.6 *Construction of the Cortical Map*

Three stimuli were delivered at each grid point and the MEPs averaged to give a mean response for that grid point. Average responses were positioned in a table corresponding to the scalp location.

6.2.5.7 *Cortical Map Measurements*

The mean map volume was calculated as the arithmetic mean of the MEP area from all grid points stimulated. The normalized map volume is used to give a measure of focality of the map, dividing the sum of mean MEP area from all grid points that produced responses by the largest MEP area from the map Hotspot. The Centre-of-Gravity (CoG) is a weighted-mean that gives each grid point a relative weighting and provides a robust and reproducible means of comparing maps. The CoG predicts the region of greatest excitability of corticomotor neurones projecting to the muscle studied. The CoG is calculated with the formula $Xcg = \Sigma xa/\Sigma a$ to determine the CoG along the mediolateral dimension and $Ycg = \Sigma ya/\Sigma a$ to locate the CoG along the anteroposterior dimension. The CoG of the map is the point at which both coordinates intersect (Wassermann et al., 1992). Subtracting the ipsilateral from the contralateral CoGx and CoGy gives a measure of the organization of the ipsilateral representation in relation to the contralateral.

6.2.5.8 *Comparison of Map Measures Between Groups*

The subjects were divided into groups to allow comparison of measures of ipsilateral activity in the contralesional hemisphere of patients who had good compared to limited motor recovery in the chronic stage, and patients who had improved with the robotic training compared to those who did not.

6.2.5.9 *Analysis by Group According to Improvement With Robotic Upper Limb Training*

The first division into groups discussed in Chapter 6 tests the hypothesis that change in the strength or organization of the representation of paretic ipsilateral muscles in the contralesional hemisphere was associated with

functional improvement from robotic upper limb training. All patients who had shown an improvement in their Fugl-Meyer upper limb score post-training were grouped into an Improvement group, the remainder grouped as No-improvement.

In the Improvement group one-way ANOVA was used to test for differences pre and post training in each muscles Laterality Index; mean ipsilateral MEP area; mean ipsilateral map volume in response to stimulation of the contralesional hemisphere; and mean contralateral map volume in response to stimulation of the ipsilesional hemisphere. Paired t-tests were used to test for difference in CoGx and CoGy of each muscle in the contralesional and ipsilesional hemisphere pre and post training. Differences in the ipsilateral CoG relative to the contralateral CoG were also tested with independent samples t-tests (expressed as ipsilateral CoGx – contralateral CoGx and ipsilateral CoGy – contralateral CoGy).

6.2.5.10 *Analysis by Group According to Baseline Upper Limb Function*

The second division into groups discussed in Chapter 7 investigates association between the strength or organization of the representation of paretic ipsilateral muscles in the contralesional hemisphere and upper limb function in the chronic stage of stroke. The patients were divided into High Performance and Low Performance groups according to their baseline Fugl-Meyer score. The median value was used as the split to divide the groups.

Difference between the groups in the mean map volume was tested with one-way ANOVA, and between CoGx and CoGy of the ipsilateral representation of each muscle in the contralesional hemisphere tested with independent samples t-tests. Differences in the ipsilateral CoG relative to the contralateral CoG were also tested with independent samples t-tests (expressed as ipsilateral CoGx – contralateral CoGx and ipsilateral CoGy – contralateral CoGy).

6.2.5.11 *Testing Correlation Between Baseline Function and Markers of Ipsilateral Activity*

Correlation coefficients were calculated using the data of all patients. Correlations were sought between the baseline Fugl-Meyer upper limb scores and ipsilateral MEP area from stimulation of the contralesional hemisphere; mean ipsilateral map volume in the contralesional hemisphere; ipsilateral map CoGx and CoGy in the contralesional hemisphere; Laterality Index.

6.2.5.12 *Testing Correlation Between Change in Ipsilateral Measures with Training and Change in Upper Limb Function with Training*

Correlation coefficients were calculated between the change in the Laterality Index with training and the change in Fugl-Meyer upper limb scores with training.

6.2.6 Methodological Limitations

Some limitations of TMS technique equally relevant to this chapter have been discussed above.

6.2.6.1 *Limited Number of Cortical Points Mapped*

The experimental question sought to determine if the contralesional hemisphere contributed usefully to skilled motor control of the paretic limb after stroke. To answer this it was also necessary to be able to comment on ipsilesional activity. The potential for maladaptive plasticity as a consequence of increased ipsilateral activity emphasizes the importance of being able to comment on balance of ipsilesional and contralesional activity in recovery of motor function. Mapping both hemispheres was therefore an important feature of the experiment in relation to the hypothesis. The expense of mapping both hemispheres was a reduction in the detail of the map.

In previous mapping studies only the dominant hemisphere was mapped to produce one map covering all frontal motor regions with reasonable detail. Map quality would increase with more data points, but the total number of stimuli was selected as a compromise between resolving sufficient detail and tolerability for subjects. Each stimulus required muscle contraction, and high intensity stimulation can produce unpleasant scalp sensations. These two factors limited the total number of stimuli that it was reasonable to request subjects to tolerate. This issue is of great importance in testing stroke patients, where fatigue may be prominent. The physical deficits limit the number of repeated voluntary muscle contractions that it is reasonable to expect a patient to perform in a given experimental session. Structural brain lesions also place stroke patients at a higher risk of experiencing adverse side effects from TMS. Although the risks from single pulse TMS with long inter-stimulus intervals are very low, this is another reason for wishing to minimize the total number of stimuli.

The use of a 3 by 3 grid with 1.5cm spacing of grid points was judged to be an acceptable compromise between obtaining useful maps whilst

maintaining subject tolerability. Adding more grid points by performing the mapping of each hemisphere in separate sessions would have been ideal but would have doubled time and resource requirements, and was not practical with the resources available.

6.2.6.2 *Choice of Muscles*

MEPs were measured in Biceps, Triceps, Pectoralis Major and Anterior Deltoid. It was only possible to map a limited number of muscles. The muscles studied were selected for their contribution to reach, a movement of functional relevance post stroke to obtain optimal workspace for dexterity (Ellis et al., 2007).

In addition to their biomechanical contribution to the reach movement, the muscles were selected for their suitability in neuroanatomical terms, having the balance of ipsilateral and contralateral innervation required to demonstrate changes in Laterality Index (Bawa et al., 2004). The task relevance of the muscles studied is well described both in terms of triphasic activation patterns (van der Fits et al., 1998) and synergies (d'Avella and Lacquaniti, 2013). Deltoid or Pectoralis Major may function as the prime movers (Hiengkaew et al., 2003; Reed et al., 2013).

Selection of appropriate muscles likely to display plastic change is important to the validity of the hypothesis. The biomechanics and neuroanatomy of the muscles selected were considered to place them well to manifest change in ipsilateral activity. The possibility that ipsilateral activity may be usefully active in muscles outside of those studied should be acknowledged in interpreting negative results.

6.2.6.3 *Analysis by Division into High and Low Performance Groups*

Some aspects of the hypothesis could be tested by correlation using all patients. Other aspects were tested dividing the patients to allow comparison between groups. This approach offered numerous advantages. It provides an easy means of testing the hypothesis that ipsilateral plastic change is beneficial by comparing its presence in patients with good and limited

motor recovery. Evidence of increased ipsilateral activity in the group judged to have made good recovery of upper limb motor function could be taken as evidence of beneficial ipsilateral change. The reverse situation could be seen if ipsilateral activity was increased in patients with limited recovery. This approach also allowed more direct comparison with the earlier studies of deliberate practise in expert performers.

Dividing the stroke patients into High and Low Performance groups according to the median Fugl-Meyer upper limb scores was a convenient way of grouping according to functional performance at the post-recruitment stage. To recruit patients according to functional scores would have added complexity to the recruitment process and introduced the issue of classifying according to arbitrary cut-off values. Some investigators have used a cut-off value of Fugl-Meyer scores to designate severe motor impairment (eg, <25/66 in Roh et al., 2013). There is less agreement about suitable cut-off values to stratify higher levels of motor recovery.

It is possible that selecting higher performing patients would favour a particular lesion type or younger age to bias the groups. Inspection of the table detailing the patient characteristics shows that in this case this was not an active problem, but this potential should be acknowledged. Use of t-tests to check that there were no significant differences in age, time since stroke, spasticity and strength between the High and Low Performance groups was a simple way of checking for bias between groups. In this sample, the simple means of division was effective in producing groups with suitable difference in mean Fugl-Meyer scores without other bias. The method may not generalize usefully in less favourably distributed patient samples.

Another analysis by group was made to compare subjects who had and had not improved with robotic training in the upper limb reach task. This division was made based on whether there had been any improvement in functional measures, i.e. an increase in the Fugl-Meyer upper limb scores of 1 or more. The intention was to identify changes in ipsilateral activity in response to training over short time periods that could be associated with improved function. Comparison between groups focussed attention to the

relevant subgroup that had improved with training, allowing any beneficial change in ipsilateral activity to be identified. Analysing the group as a whole may have concealed subgroup effects. The distribution of change in functional scores within this sample avoided the issue of imbalanced group sizes that could have been problematic with this small sample size. Again, this method may not generalize to samples with less favourable distribution.

6.2.6.4 *Current Spread in Cortical Mapping Post Stroke*

The patient sample included both cortical and subcortical strokes. Lesions close to the cortical surface could lead to gliosis formation and widened cerebrospinal fluid filled spaces beneath the point of stimulation. This makes current spread through eddy currents a possibility. The result could be that the cortical point maximally stimulated is remote from the intended site. An attempt was made to take the stroke lesion into account when interpreting MEPs. Efforts were made to obtain structural imaging to create a 3D reconstruction in Brainsight to be able to visualize regions where this may potentially be a problem. In this way any area of potential artefact can be marked on the scalp grid. MEPs elicited from these sites can then be monitored. If MEPs appear discordant a decision must then be made whether it is justified to include this data in the analysis.

The patients were recruited from a wide geographical area so it was not possible to obtain structural MRI scans in each case. A minimum requirement was a copy of the written imaging report to satisfy exclusion criteria. Ideally structural MRI scans would have allowed 3D reconstructions in each case, and the method could then have been adapted to allow individualized grid planning in patients with large surface cortical lesions.

6.2.6.5 *Timing of Stimulation to Peak Rise of Activity in the Paretic Limb*

Timing stimulation to the peak rise in muscle activity before reaching steady state optimizes the chance of eliciting ipsilateral MEPs. This was attempted in this experiment using auditory feedback of EMG activity to

guide manual triggering of stimulation. The quality of movement in the paretic upper limb performing reach was variable between stroke patients. There was a tendency for biceps and triceps to become fully activated later than deltoid. On this basis the auditory feedback was sourced from triceps, with the rationale that it was important to ensure adequate pre-activation in all muscles at the expense of losing a small facilitatory effect in the earliest rising muscles.

It would have been ideal to perform the experiment as a dedicated study for each muscle, using muscle-specific thresholds, Hotspots and perfectly timing the delivery of stimulation. This would have greatly increased the resource and time requirements to an impractical level.

6.3.1 Patient Baseline Characteristics

19 subjects completed all training and TMS sessions. Table 6.1 details their age, time since stroke and predominant location of lesion. Baseline and post training measures of upper limb function, spasticity, sensory impairment and strength are shown.

A sample of representative MEPs is shown in Figure 6.1.

Patient ID	Group	Age	Weak UL	Months since onset	Arm function (UL FM pre)	Arm function (UL FM post)	Change in arm function (UL FM post - pre)	Spasticity (MAS-pre)	Spasticity (MAS-post)	Change in spasticity (MAS post-pre)	Sensory impairment	Shoulder abduction strength	Lesion location
sbj 1	Improvement	60	L	36	48	54	6	3	3	0	Mild	3	Subcortical
sbj 2	Improvement	47	L	72	55	57	2	3	3	0	Mild	4	Subcortical
sbj 3	Improvement	58	R	42	49	52	3	1	1	0	Mild	3	Cortical
sbj 4	Improvement	74	L	15	63	64	1	0	0	0	Moderate	3	Cortical
sbj 5	Improvement	49	L	52	39	43	4	3	2	-1	Mild	3	Subcortical
sbj 6	Improvement	69	L	24	58	62	4	0	0	0	Moderate	4	Cortical
sbj 7	Improvement	52	R	184	34	38	4	0	0	0	Moderate	3	Cortical
sbj 8	Improvement	52	R	100	28	32	4	3	3	0	Mild	3	Cortical
sbj 9	Improvement	61	R	96	37	42	5	1	1	0	Mild	3	Mixed
Mean		**58**		**69**	**45.7**	**49.3**	**3.7**	**1.6**	**1.4**	**-0.1**		**3.2**	
sbj 10	No improvement	52	L	32	38	37	-1	3	3	0	Mild	3	Cortical
sbj 11	No improvement	49	L	15	32	32	0	3	2	-1	Moderate	3	Cortical
sbj 12	No improvement	54	L	12	51	49	-2	3	3	0	Moderate	3	Cortical
sbj 13	No improvement	88	R	84	61	60	-1	1	1	0	Moderate	3	Cortical
sbj 14	No improvement	49	R	28	49	49	0	3	3	0	Mild	4	Mixed
sbj 15	No improvement	56	R	48	41	37	-4	3	3	0	Mild	3	Subcortical
sbj 16	No improvement	54	L	20	64	64	0	0	0	0	Moderate	4	Subcortical
sbj 17	No improvement	57	R	60	38	34	-4	3	1	-2	Mild	3	Cortical
sbj 18	No improvement	63	R	18	58	56	-2	2	0	-2	Moderate	4	Subcortical
sbj 19	No improvement	71	R	17	62	57	-5	0	0	0	Mild	4	Cortical
Mean		**59.3**		**33.4**	**49.4**	**47.5**	**-1.9**	**2.1**	**1.6**	**-0.5**		**3.4**	

TABLE 6.1 PATIENT CHARACTERISTICS, BASELINE AND POST TRAINING MEASURES

Average values are shown for the Improvement and No-improvement groups, grouped according to change in Fugl-Meyer Upper Limb Subset score with training.

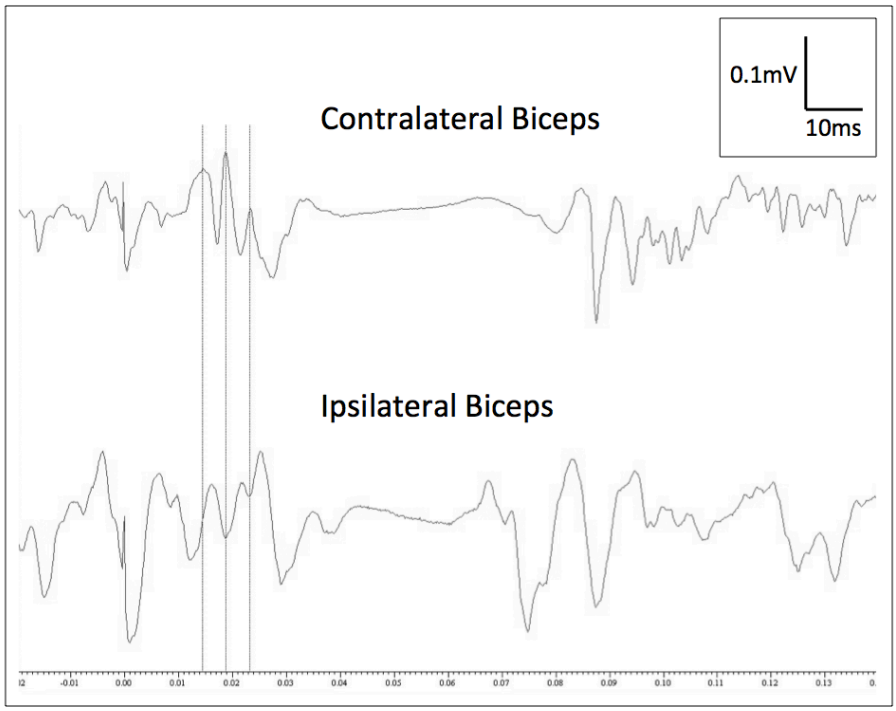

FIGURE 6.1 SAMPLE OF BILATERAL MEPS IN BICEPS MUSCLES OF A STROKE PATIENT

Biceps MEPs are shown in the paretic limb ipsilateral to the stimulated contralesional hemisphere, and the unaffected limb contralateral to the side of stimulation. The Cortical Silent Periods can be clearly appreciated but MEPs are harder to discern amid the heavily contracting background activity. Difficulty controlling background activation is a challenge in studying ipsilateral MEPs in stroke patients.

6.3.2 Grouping Patients According to Functional Response to Training

To assess for beneficial plastic change associated with training to improved performance, measures were compared between groups of patients who showed improvement in upper limb function with training and patients who did not improve. The change in Fugl-Meyer Upper Limb Subset score was used to divide patients into improvement and no improvement groups. 9 Patients showed a positive change in functional score, and 10 patients did not improve.

Baseline characteristics between the groups were not significantly different, tested with t-tests comparing age, time since stroke, baseline Fugl-Meyer Upper Limb score, baseline spasticity and baseline strength. In patients who responded to training, the mean increase in Fugl-Meyer Upper Limb Subset score was 3.7. Comparing baseline and post training measures there were no significant changes in spasticity, sensation or strength measures.

6.3.3 Change in Corticomotor Connectivity with Training

There was no evidence of change in corticomotor connectivity with training. No significant differences were seen between baseline and post-training average MEP size in the paretic limb. This is true for both ipsilateral MEPs from the contralesional hemisphere and contralateral MEPs from the ipsilesional hemisphere. This is true in both Improvement and No-improvement groups.

The average Improvement group ipsilateral MEP sizes for each muscle in the paretic limb are shown in Figure 6.2, at baseline and after training. The average values are the normalized ipsilateral MEP size recorded from stimulation of the contralesional hemisphere. There are no significant differences. This negative result does not support the hypothesis that training in a reach task strengthens ipsilateral corticomotor projections to proximal muscles.

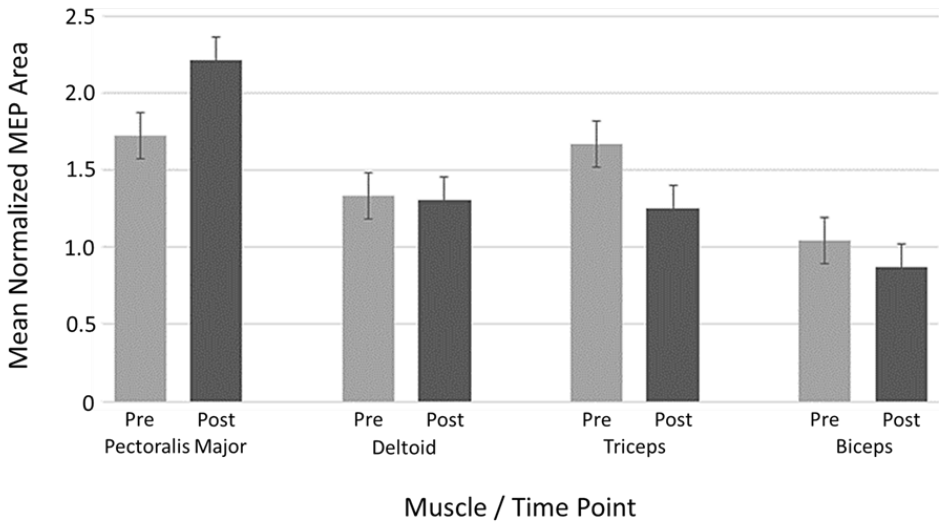

FIGURE 6.2 MEAN NORMALIZED IPSILATERAL MEP AREA RECORDED FROM PARETIC LIMB TO STIMULATION OF THE CONTRALESIONAL HEMISPHERE, AVERAGED FROM ALL SUBJECTS IN THE IMPROVEMENT GROUP

No significant differences were seen between pre and post values for any of the muscles.

6.3.4 Change in Laterality Index with Training

There were no significant differences in Laterality Index with training. This demonstrates that training does not alter the balance of ipsilateral and contralateral activity. Combined with the lack of change in ipsilateral MEP size, this confirms absence of training effect on ipsilateral corticomotor output. The values for change in Laterality Index are shown for each subject in the Improvement group in Figure 6.3. This expresses the change in Laterality Index as pre-post training values for each muscle. A positive value shows a tendency to change to favour increased contralateral activity, whereas negative values show increased ipsilateral activity. It is clear there is no consistent pattern of change, either within muscles, or within individual subjects between muscles. A single subject may have a positive change in one muscle showing change favouring contralateral control, but a negative change in another muscle, favouring ipsilateral control.

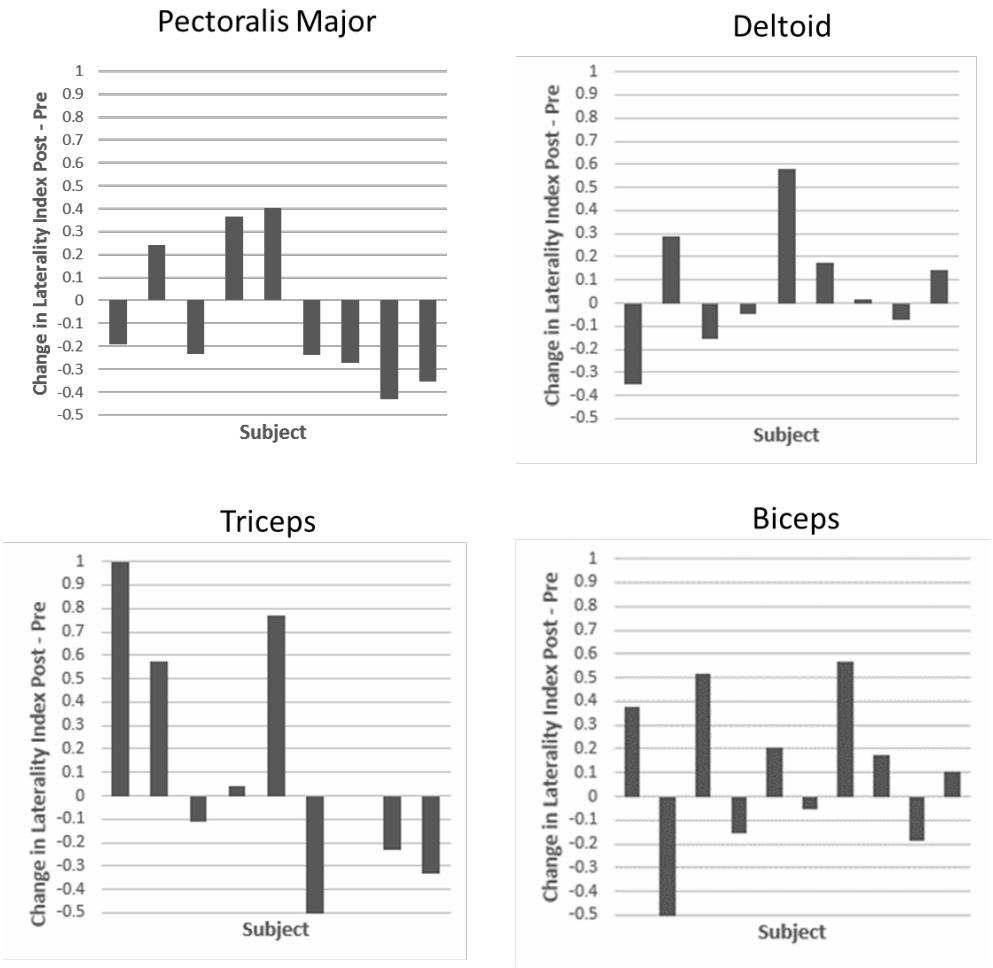

FIGURE 6.3 CHANGE IN LATERALITY INDEX WITH TRAINING, EXPRESSED AS POST-PRE FOR EACH SUBJECT SHOWING IMPROVEMENT WITH TRAINING

There are no significant differences between Laterality Index pre and post training for any of the muscles.

6.3.5 Strength of Ipsilateral Cortical Motor Representations in Contralesional

6.3.5.1 *Hemisphere Pre and Post Training*

Individual maps of ipsilateral cortical representations of paretic muscles in the contralesional hemisphere are shown pre and post training, for each muscle in each subject in the Improvement group in Figure 6.4. The mean values for the strength of the cortical representation from group data are shown in Figure 6.5. This shows the mean value of responses to all grid points in the map. One-way ANOVA shows no significant differences between pre and post training values in any of the muscles, in either group. This result does not support the hypothesis that training improves functional performance by strengthening ipsilateral cortical representation of paretic muscles in the contralesional hemisphere.

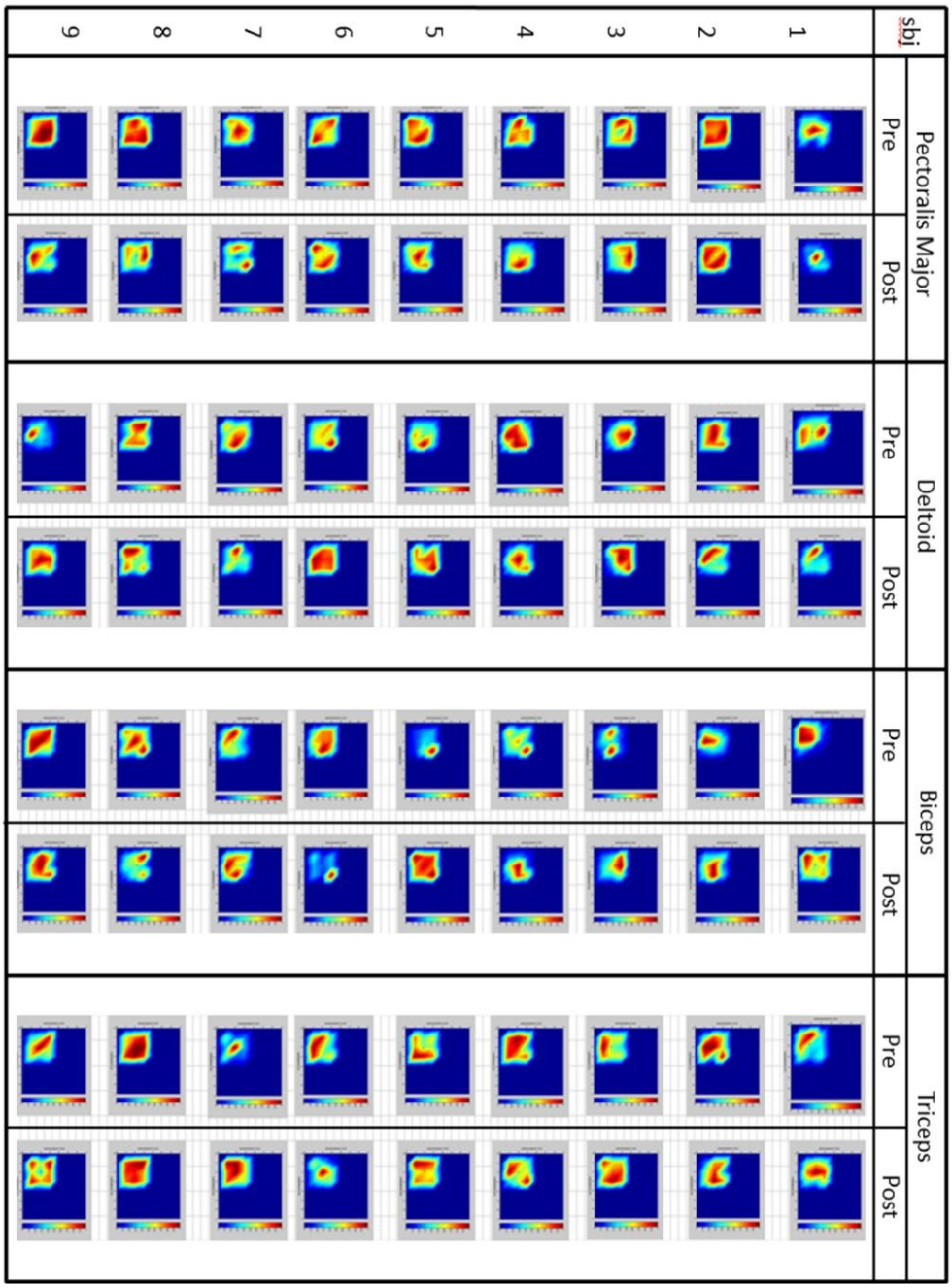

FIGURE 6.4 CORTICAL MAPS OF THE IPSILATERAL REPRESENTATION IN THE CONTRALESIONAL HEMISPHERE FOR EACH MUSCLE IN EACH OF THE 9 SUBJECTS THAT SHOWED FUNCTIONAL IMPROVEMENT WITH TRAINING

Pairs of maps pre (left) and post (right) training are shown for each subject.

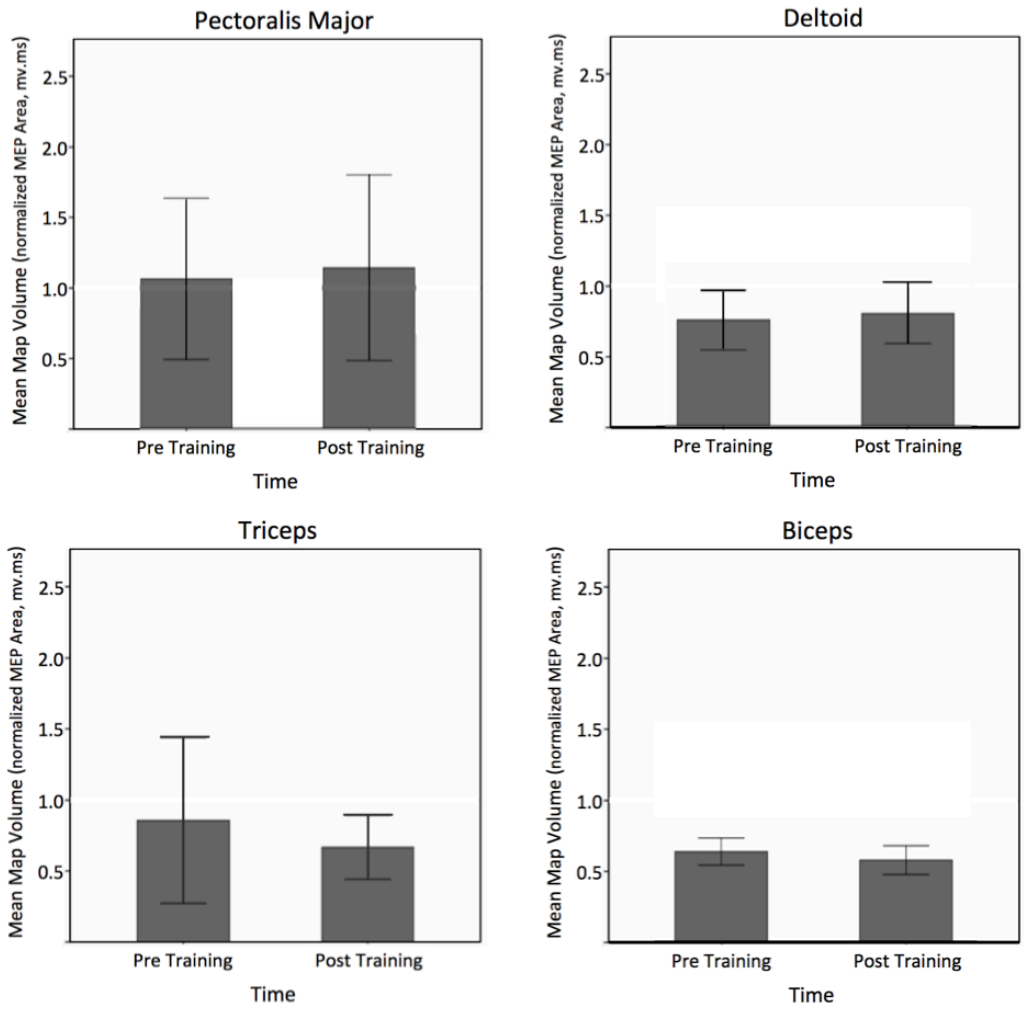

FIGURE 6.5 STRENGTH OF THE IPSILATERAL REPRESENTATION IN THE CONTRALESIONAL HEMISPHERE OF EACH OF THE MUSCLES IN THE PARETIC LIMB PRE AND POST TRAINING. GROUP AVERAGES FOR THE 9 SUBJECTS IN THE IMPROVEMENT GROUP ARE SHOWN.

Values on y-axis are Mean Map Volume, averaging the normalized MEP Area in mv.ms of all grid points stimulated. One-way ANOVA comparing pre and post training data reveals no significant differences (Pectoralis Major ($p = .837$), Deltoid ($p = .731$), Biceps ($p = .334$), Triceps ($p = .495$))

6.3.6 Strength of Contralateral Cortical Motor Representations in Ipsilesional Hemisphere Pre and Post Training

Individual maps of contralateral cortical representations of paretic muscles in the ipsilesional hemisphere are shown pre and post training, for each muscle in each subject in the Improvement group in Figure 6.6. The mean values for the strength of the cortical representation from pooled group data are shown in Figure 6.7. This shows the mean value of responses to all grid points in the map. One-way ANOVA shows no significant differences between pre and post training values in any of the muscles, in either group.

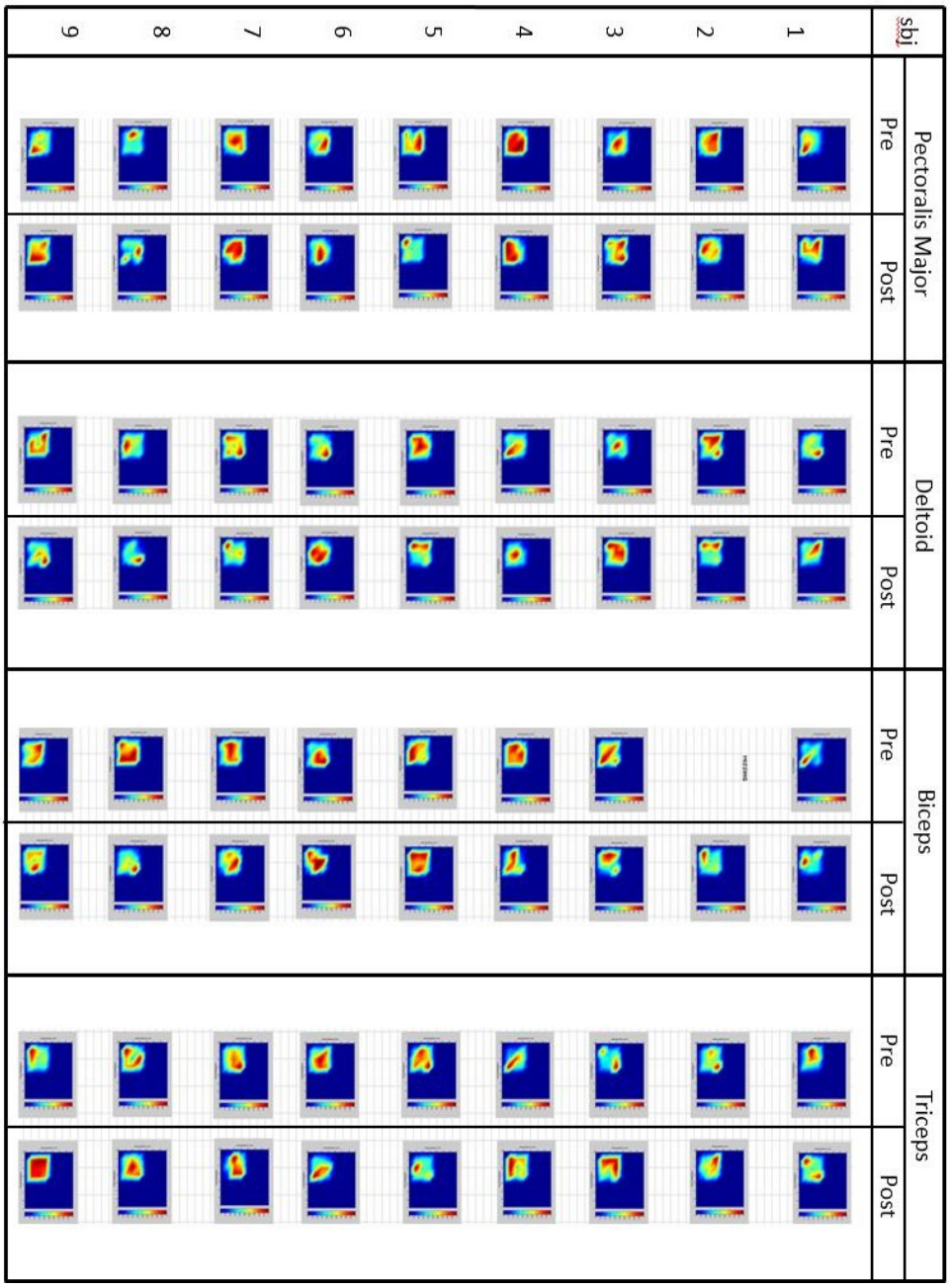

FIGURE 6.6 CORTICAL MAPS OF THE CONTRALATERAL REPRESENTATION IN THE IPSILESIONAL HEMISPHERE FOR EACH MUSCLE IN EACH OF THE 9 SUBJECTS IN THE IMPROVEMENT GROUP

Pairs of maps pre (left) and post (right) training are shown for each subject.

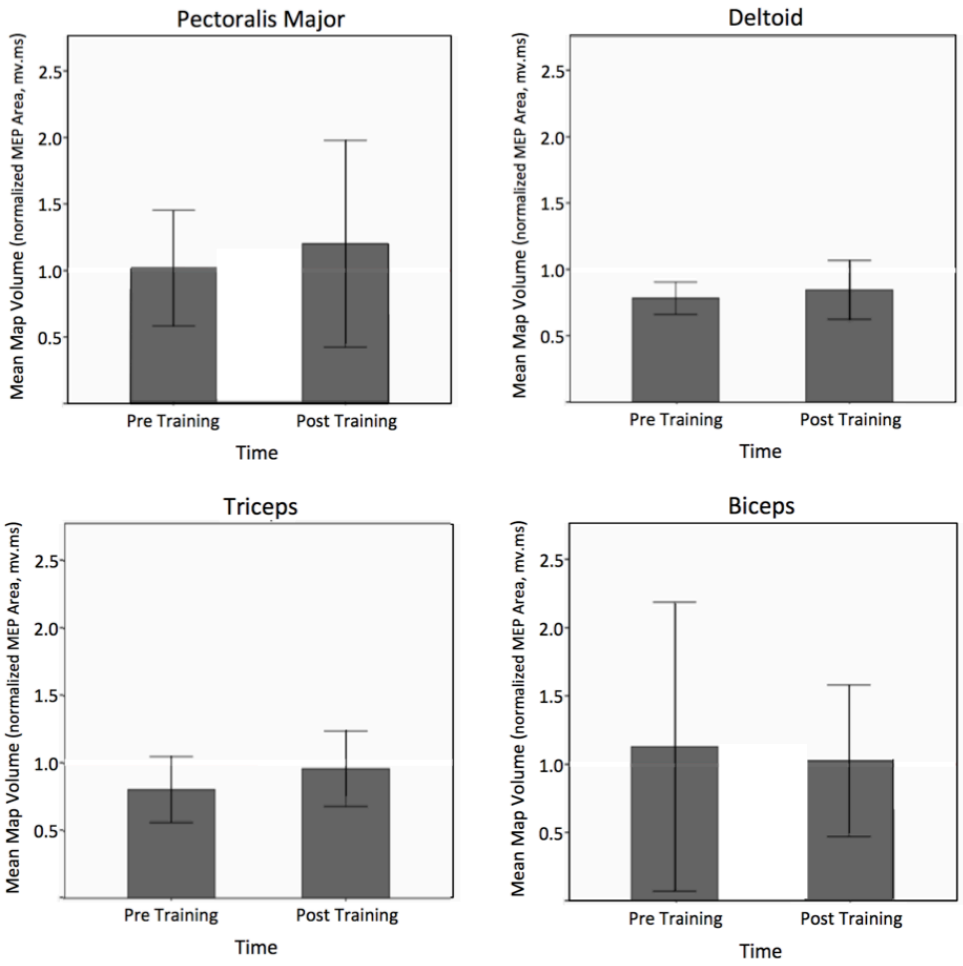

FIGURE 6.7 STRENGTH OF THE CONTRALATERAL REPRESENTATION IN THE IPSILESIONAL HEMISPHERE OF EACH OF THE MUSCLES IN THE PARETIC LIMB PRE AND POST TRAINING

Group averages for the 9 subjects in the Improvement group are shown. Values on y-axis are Mean Map Volume, averaging the normalized MEP Area in mv.ms of all grid points stimulated. One-way ANOVA comparing pre and post training data reveals no significant differences (Pectoralis Major ($p = .644$), Deltoid ($p = .575$), Biceps ($p = .845$), Triceps ($p = .355$))

6.3.7 Measures of Organization of Cortical Representation in the Contralesional and Ipsilesional Hemispheres Pre and Post Training

Figures 6.8 and 6.9 show the displacement of Centre of Gravity coordinates in the cortical representation of the paretic limb in the contralesional (Figure 6.8) and ipsilesional (Figure 6.9) hemispheres with training for each patient in the Improvement group. For CoGx coordinates, a positive value shows a shift in CoGx coordinate to a more lateral position, whereas a negative value shows a more medial shift. For CoGy coordinates a positive value shows a shift to a more anterior location, whereas a negative value shows a more posterior shift. Paired samples t-tests show a significant difference between CoGx pre and post for ipsilateral deltoid representation in the contralesional hemisphere only, $p= 0.017$. No significant differences were seen in the ipsilesional representations, or in the No-improvement group. This finding supports the hypothesis by demonstrating a feature of ipsilateral cortical reorganization associated with improved functional performance resulting from training.

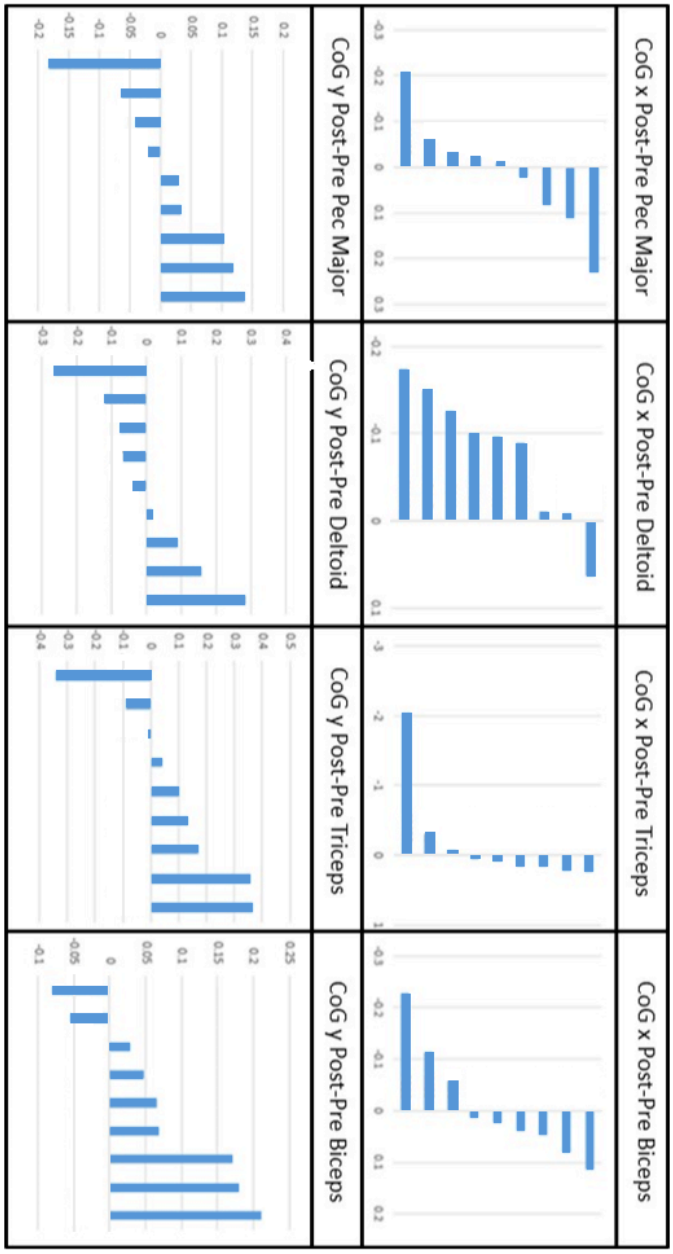

FIGURE 6.8 THE SIZE AND DIRECTION OF CHANGE IN CENTRE OF GRAVITY X (TOP ROW) AND Y COORDINATES (BOTTOM ROW) OF IPSILATERAL REPRESENTATION OF PARETIC MUSCLES IN THE CONTRALESIONAL HEMISPHERE, PRE AND POST TRAINING.

X-axis for CoGx and y-axis for CoGy show displacement in cm. Each bar represents an individual subject, ordered according to size and magnitude of change in each graph, therefore individual subjects are represented by bars in different positions in each graph. Values are post training – pre training. Paired samples t-tests show a significant difference between CoGx pre and post for ipsilateral Deltoid representation in the contralesional hemisphere only, p= 0.017.

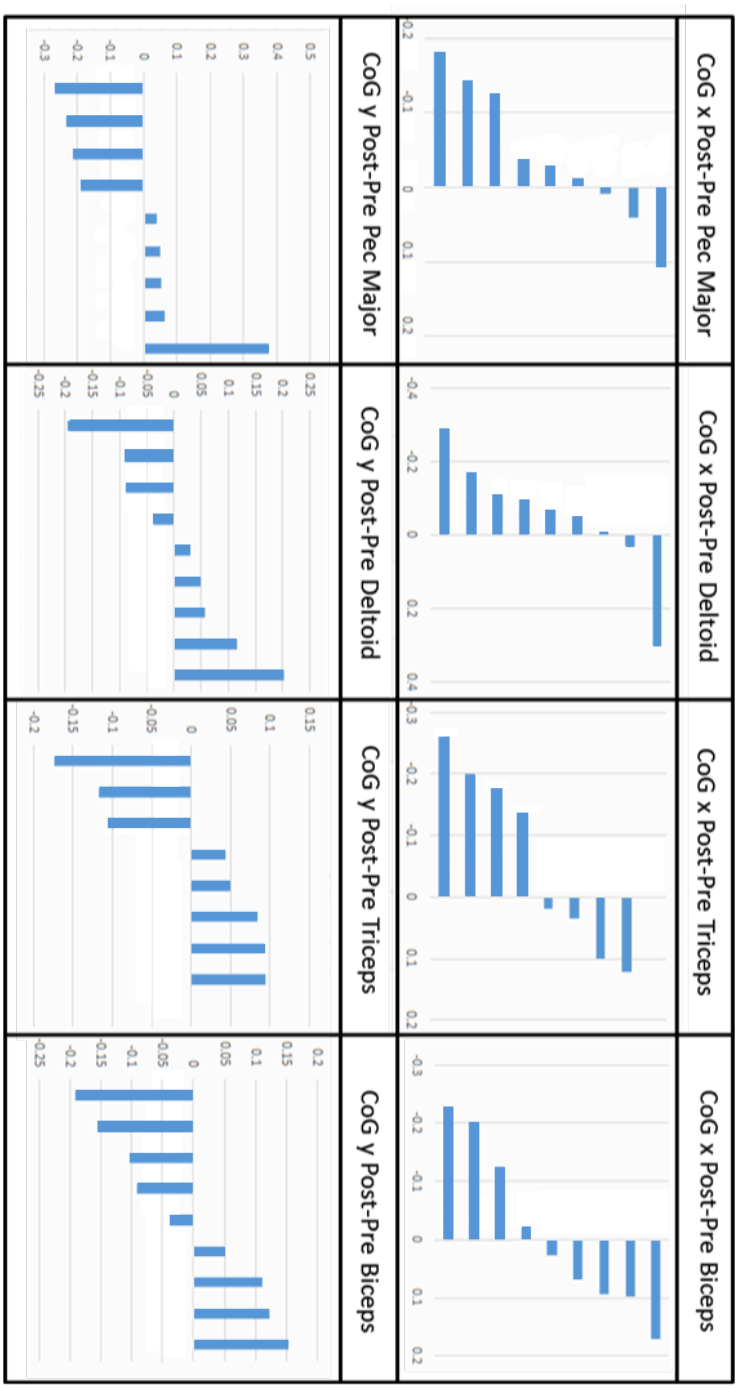

FIGURE 6.9 THE SIZE AND DIRECTION OF CHANGE IN CENTRE OF GRAVITY X AND Y COORDINATES OF CONTRALATERAL MUSCLES FROM BASELINE AFTER TWO WEEKS OF TRAINING IN THE IPSILESIONAL HEMISPHERE

Axes and data points are the same as for Figure 6-8. Paired t-tests show no significant differences between the pre and post positions of either CoGx or CoGy coordinates.

6.3.8 Associations Between Measures of Spasticity and Measures of Ipsilateral Activity

There is no association between spasticity and any of the measures of ipsilateral activity. Plots of spasticity measures and ipsilateral MEP area (r=-0.07), ipsilateral map size (r = -0.29), and Laterality Index (r=0.17) are shown in Figure 6.10 (critical value for 0.05, 2-tailed level of significance for 17 degrees of freedom = 0.455). In the bottom graph of Figure 6.10, although not significant, it can be noted that all subjects with negative Laterality Index values all had higher spasticity scores. The negative Laterality Index shows a greater reliance on ipsilateral pathways.

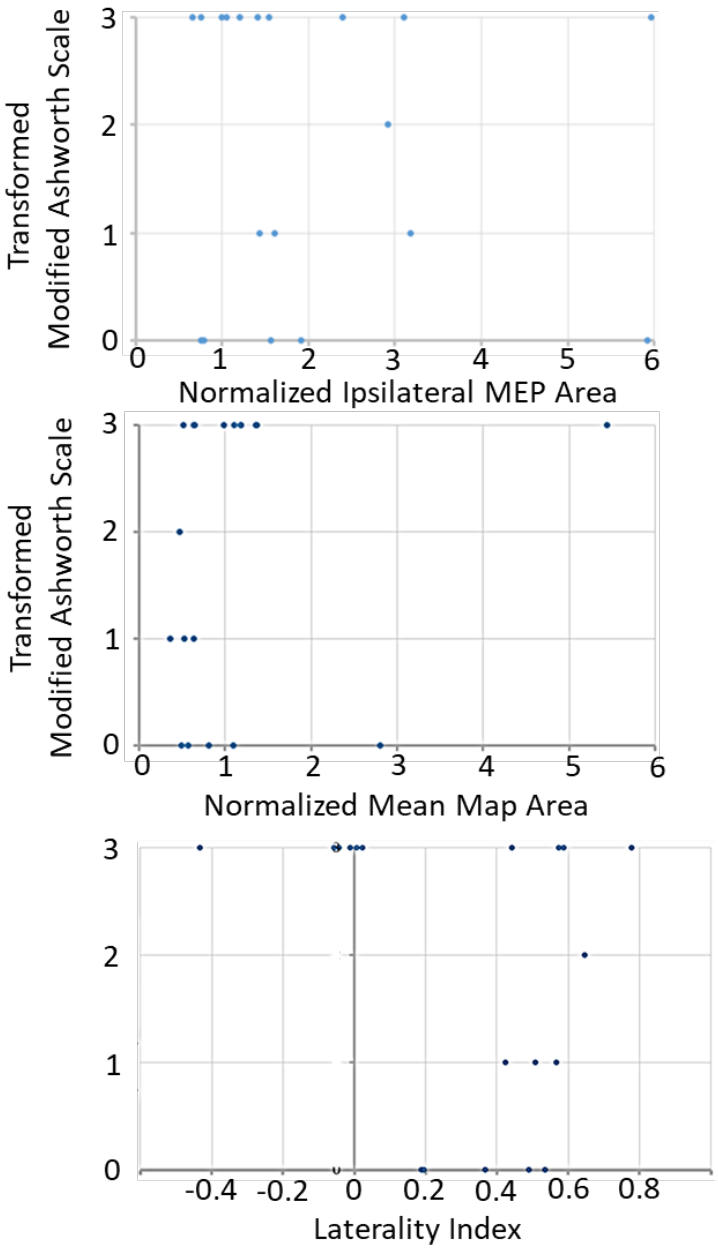

FIGURE 6.10 PLOTS OF SPASTICITY AGAINST MEASURES OF IPSILATERAL ACTIVITY IN THE UNAFFECTED HEMISPHERE

Each data point represents a patient (all patients from both Improvement and No-improvement groups included). In each graph y-axis is a measure of spasticity, transformed from Modified Ashworth Scale. In the top graph the x-axis is the normalized MEP, middle graph normalized mean map area, and bottom graph Laterality Index.

6.3.9 Associations Between Functional Upper Limb Performance and Measures of Ipsilateral Activity

To further test relationships between ipsilateral activity and functional performance, for each muscle in all patients, relationships were sought between measures of ipsilateral activity and functional upper limb performance. For each muscle the baseline ipsilateral MEP size, Laterality Index and size of ipsilateral map were correlated with Fugl-Meyer Upper Limb Subset score.

Figure 6.11 shows a significant correlation (r=0.65 at significance level 0.482) between baseline Deltoid Laterality Index and baseline Fugl-Meyer Upper Limb Subset score, showing that patients with increased use of contralateral pathways from the ipsilesional hemisphere make better functional recovery. This is consistent with the data from longitudinal functional imaging studies, and the predictive algorithms of upper limb recovery, that integrity of the contralateral corticospinal tract is the major determinant of good arm recovery. No associations were found in other muscles.

It is relevant in Figures 6.12 and 6.13 that baseline Laterality Index does not predict functional response to training, and the change in Laterality Index with training score does not correlate with functional improvement in training. This shows that increased use of ipsilateral pathways is still compatible with further improvement in the chronic phase of stroke.

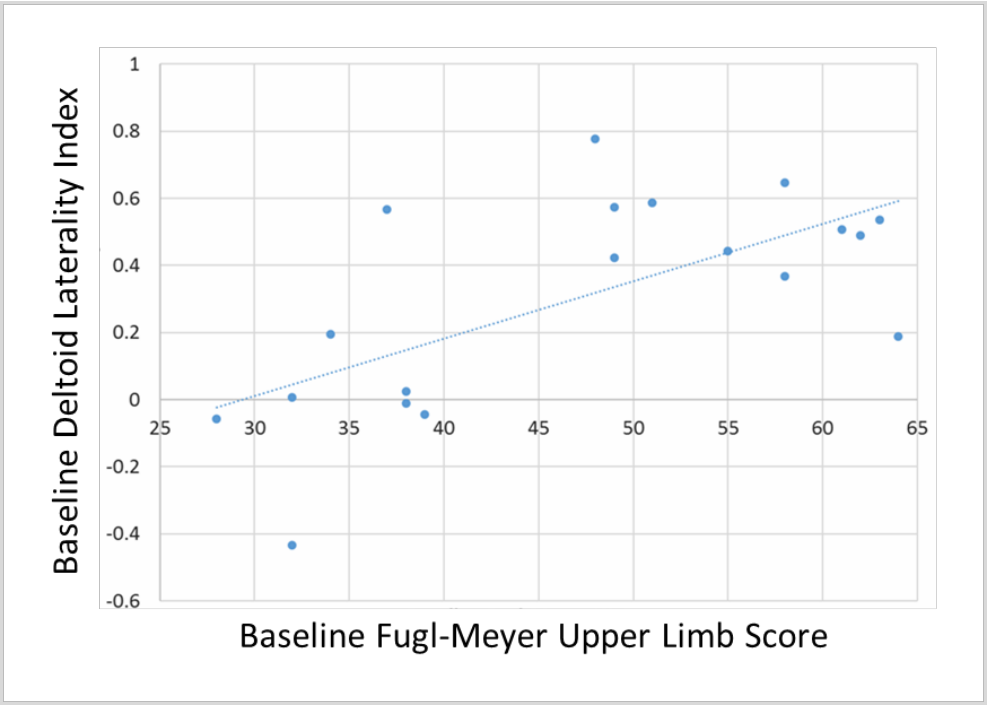

FIGURE 6.11 RELATIONSHIP BETWEEN BASELINE DELTOID LATERALITY INDEX AND BASELINE FUGL-MEYER UPPER LIMB SUBSET SCORE

There is a positive correlation. Increased use of contralateral pathways is associated with improved functional performance (r=0.65, significance level at 0.05 level for 17 (n=19) degrees of freedom = 0.482).

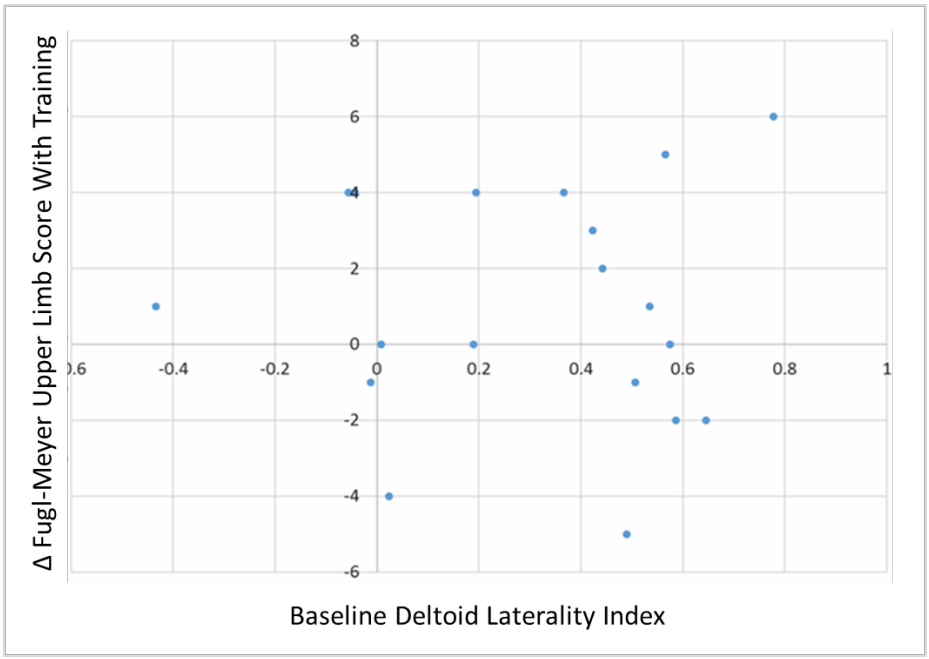

FIGURE 6.12 RELATIONSHIP BETWEEN BASELINE DELTOID LATERALITY INDEX AND CHANGE IN FUGL-MEYER SCORE WITH TRAINING

There is no significant correlation (r=0.17).

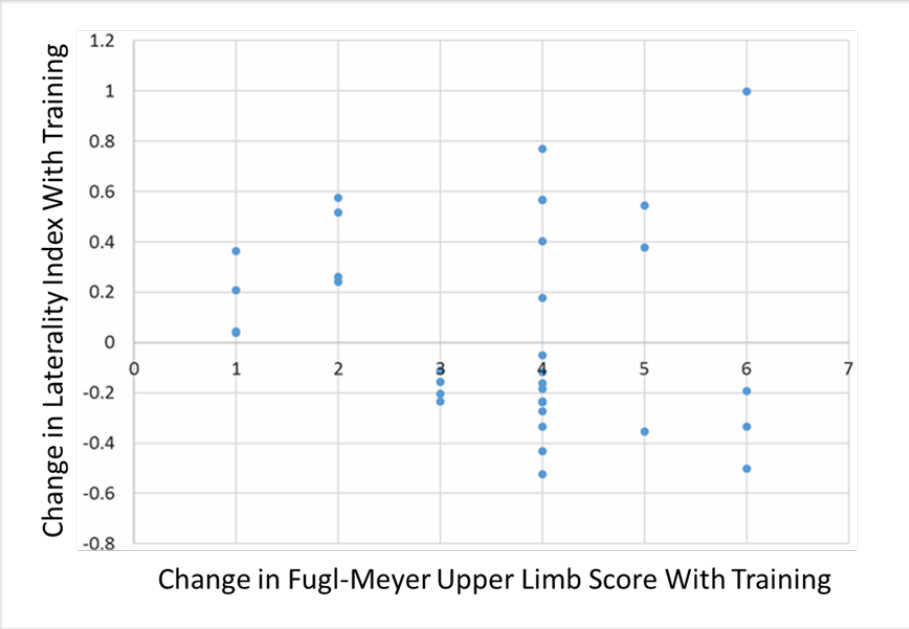

FIGURE 6.13 RELATIONSHIP BETWEEN CHANGE IN FUGL-MEYER UPPER LIMB SUBSET SCORE WITH TRAINING AND CHANGE IN LATERALITY INDEX WITH TRAINING

There is no significant correlation (r=-0.17).

6.3.10 Summary of Results

1. Of the patients who improved with two weeks of training in a reach task the only significant changes identified with these measures was a change in the organization of the ipsilateral representation of paretic deltoid in the contralesional hemisphere. This is seen as a medial displacement of the Centre of Gravity.

2. There were no changes in the strength of ipsilateral corticomotor connectivity or the strength of the ipsilateral representation in the contralesional hemisphere arising from training.

3. There is no association between any measure of activity in ipsilateral pathways and spasticity.

4. There is a positive correlation between baseline deltoid Laterality Index and baseline functional performance. This is consistent with the view that intact contralateral corticospinal tract will best serve post stroke recovery. Importantly the Laterality Index did not predict response to training, showing that reliance on ipsilateral pathways, (which is generally taken to represent exhaustion of ipsilesional plastic reserve), does not limit further functional improvement.

6.4 DISCUSSION

6.4.1 Main Findings

1. These measures provide no evidence that training in a reach task increases corticomotor connectivity or strength of ipsilateral cortical representation of paretic muscles in the contralesional hemisphere in chronic stroke patients trained over short time periods. This remains true when only patients who demonstrate functional improvement as a result of the training are considered.

2. There is evidence of cortical reorganization of ipsilateral motor representations in the contralesional hemisphere in response to training in patients who show functional improvement. The ipsilateral representation of paretic deltoid moves to a more medial location. This effect is not observed in patients who do not improve with training.

3. Baseline functional performance is better in patients with greater reliance on contralateral pathways. However, improved performance with training remains possible in patients with greater reliance on ipsilateral pathways.

These findings offer some weak support to the hypothesis that beneficial plastic change can occur through ipsilateral pathways from the contralesional hemisphere, and this can be driven by training to support motor recovery in chronic stroke patients. The clearest demonstration would have been strengthened corticomotor connectivity or strengthened cortical representation, but this was not seen. This is perhaps not surprising with training over short time periods in the chronic stage, where it could be anticipated that any plastic change evident with these measures would be subtle. The suggestion can be made that the observed reorganization of the cortical representation of the ipsilateral deltoid represents early plastic change, but placing such emphasis on a small change requires a supportive broader context. The following discussion relating to beneficial and maladaptive aspects of ipsilateral post stroke plasticity considers whether there is sufficient supportive evidence to permit this interpretation.

6.4.2 Proposing Beneficial Ipsilateral Plasticity Requires Awareness of Maladaptive Potential

Demonstrating avoidance of maladaptive associations is an essential feature of proving beneficial ipsilateral plasticity. Here the lack of association with spasticity, and observation of ipsilateral change in patients showing functional improvement is supportive of this. The Laterality Index finding of correlation between improved function and use of contralateral pathways has two possible interpretations, that ipsilateral activity is associated with poor recovery implying maladaptive activity, or that these patients have more extensive loss of ipsilesional plastic reserve. The fact that a Laterality Index showing greater reliance on ipsilateral pathways does not limit response to training argues against a maladaptive process.

The importance of making the correct interpretation of the finding of ipsilateral reorganization is made clear by models of stroke recovery that describe the maladaptive potential. The Competitive Interference model asserts that imbalanced excitability between ipsilesional and contralesional hemispheres allows excessive Interhemispheric Inhibition (IHI) to preside over the ipsilesional hemisphere, limiting deployment of plastic resources for dextrous recovery mediated by crossed corticospinal pathways. The Maladaptive Synergies model asserts that upregulating ipsilateral activity results in increased dependence on neurones that branch across multiple segmental levels, including prominent gamma motor neurone terminations, therefore driving spasticity and unwanted synergies. The Proportional Recovery model suggests a period when crossed corticospinal activity is quiescent but undergoing spontaneous recovery according to a time course constrained by the neurobiology of post ischaemic glial activity. If early reliance on ipsilateral activity developed before contralateral tracts had sufficient opportunity to fulfil their plastic potential, then the warnings from Competitive Interference and Maladaptive Synergies models would predict a spastic, coactivated arm at the expense of return of selective activation.

6.4.3 Interpreting Ipsilateral Findings in the Context of the Competitive Interference Model

6.4.3.1 *How the Neurophysiological Basis of Competitive Interference Relates to Ipsilateral Activity After Stroke*

Competitive Interference arises from disordered IHI, secondary to excitability changes in the motor network after stroke. IHI is mediated transcallosally (Hanajima et al., 2001; Rothwell et al., 1991) through somatotopic anterior callosal fibres (Hubers and Ziemann, 2006; Meyer et al., 1995), and mediated by low threshold inhibitory intracortical circuits (Chen, 2004; Daskalakis et al., 2002; Di Lazzaro et al., 1999). Strength of IHI normally scales to voluntary contraction in unimanual tasks (Ferbert et al., 1992; Hubers et al., 2008), in a task dependent manner (Bloom and Hynd, 2005). IHI functions to prevent unwanted contralateral coactivations, such as mirror movements (Li et al., 2007). IHI is also affected by integrity of callosal white matter after stroke (Demirtas-Tatlidede et al., 2015).

Excitability changes in the post-stroke motor network underlie the disturbance in IHI. Longitudinal fMRI studies describe the evolution of these excitability changes. Bilateral activation is seen acutely, with contralesional activity in both primary and secondary motor areas (Cramer et al., 1997; Tombari et al., 2004; Ward et al., 2003; Weiller et al., 1992). Contralesional activity subsides over time with recovery (Feydy et al., 2002; Foltys et al., 2003; Takeda et al., 2007), and with good recovery may come to resemble that of healthy subjects (Ward et al., 2003). Progressive normalization of excitability continues for up to 12 months, even in the absence of further functional improvement (Tombari et al., 2004). In patients with poor recovery secondary to extensive cortical infarcts, contralesional activity may reflect complete reliance of paretic movement on contralesional areas (Cao et al., 1998). Numerous studies report persistence of contralesional activity associated with poor functional outcome, described with PET (Calautti et al., 2001), NIRS (Takeda et al., 2007), and fMRI (Ward et al., 2003; Ward and Cohen, 2004). Some notable exceptions report chronic persistence of increased contralesional activity in

patients with excellent motor recovery (Butefisch et al., 2005; Cicinelli et al., 2003).

Competitive Interference is proposed when contralesional activity associates with poor recovery. Ischaemic lesions acutely reduce ipsilesional intracortical excitability (Blesneag et al., 2015; Koski et al., 2004; Traversa et al., 1998), which later normalizes with recovery in subacute (Traversa et al., 2000), and chronic stages (Delvaux et al., 2003). Acute reduction in ipsilesional excitability reduces ipsilesional to contralesional IHI, although directional measures show IHI does not explain all of the observed contralesional excitability change (Butefisch et al., 2008; Shimizu et al., 2002).

Contralesional excitability also increases acutely (Blesneag et al., 2015) and may normalize progressively with time (Catano et al., 1996; Lamola et al., 2016; Shimizu et al., 2002). Reduction in contralesional excitability has been shown to correlate with functional recovery in some studies (Manganotti et al., 2008), but others report persistence of increased contralesional excitability with good recovery (Butefisch et al., 2008).

Following stroke, IHI from ipsilesional to contralesional hemisphere is reduced, but at rest IHI from contralesional to ipsilesional hemisphere is not increased (Butefisch et al., 2008). However, the normal transition from IHI to facilitation that is seen prior to movement onset may be absent in stroke patients (Murase et al., 2004). Persistence of IHI could interfere with the movement and limit motor recovery from the ipsilesional motor cortex (Duque et al., 2005). Abnormal IHI from the contralesional hemisphere is associated with increased contralesional motor network activation on fMRI (Nair et al., 2007).

This abnormal IHI is the basis of the Competitive Interference model. Within the discussion above were examples of persisting contralesional activity associated with good motor recovery. These examples are important in relation to the finding of ipsilateral cortical reorganization, but need to be taken in context. Extensive testing of the Competitive Interference principle with neuromodulation studies provides useful further context.

6.4.3.2 *How Neuromodulation Based on the Competitive Interference Principles Contributes to Knowledge of Ipsilateral Activity After Stroke*

The Competitive Interference model has been extensively evaluated with neuromodulation studies, observing the motor effect on the paretic hand of either increasing ipsilesional excitability, or reducing contralesional excitability.

Various rTMS protocols have been used to excite the ipsilesional hemisphere. Excitatory intermittent theta burst increasing ipsilesional excitability (Di Lazzaro et al., 2008) improved paretic hand function in some studies (Ackerley et al., 2010; Talelli et al., 2007), but not others (Talelli et al., 2012). Excitatory 10Hz rTMS to the ipsilesional hemisphere has been shown to improve paretic hand function (Guo et al., 2016; Khedr et al., 2005). Improvement in paretic hand performance has also been reported with ipsilesional 3Hz rTMS (Khedr et al., 2010) and anodal tDCS (Fregni et al., 2005; Hummel, 2005; Hummel and Cohen, 2005). One study using 10Hz ipsilesional rTMS improved paretic hand movement kinematics in subcortical stroke, associated with reduced contralesional fMRI activity, but this led to worsening of paretic hand performance in cortical stroke patients (Ameli et al., 2009).

The alternative approach of inhibiting the contralesional hemisphere has also been studied. Inhibitory continuous theta burst to the contralesional hemisphere was shown to reduce excitability (Di Lazzaro et al., 2010), but did not improve paretic hand function (Talelli et al., 2007, 2012). In one study contralesional continuous theta burst deteriorated performance of the paretic hand, suggesting the contralesional activity was important for distal function (Ackerley et al., 2010). Numerous studies of inhibitory 1Hz rTMS to contralesional hemisphere have reported positive functional improvement of the paretic hand (Blesneag et al., 2015; Liepert et al., 2007; Mansur et al., 2005; Takeuchi et al., 2005). Some have reported that the rTMS effect correlated with measures of IHI (Takeuchi et al., 2005) and callosal integrity assessed with Fractional Anisotropy (Demirtas-Tatlidede et al., 2015).

Others have shown that contralesional inhibitory rTMS altered motor network connectivity, with increased coupling between ipsilesional SMA and motor cortex (Grefkes et al., 2010). Other combined rTMS fMRI studies failed to find any motor network effects associated with the paretic hand functional change (Nowak et al., 2008). One study found that there was only an rTMS effect for more complex tasks in the paretic hand, suggesting task dependency of the effect (Tretriluxana et al., 2015), consistent with task-dependency of IHI (Bloom and Hynd, 2005). Inhibitory cathodal tDCS to contralesional motor cortex has also been shown to improve paretic hand function (Fregni et al., 2005).

Collectively the majority of the neuromodulation studies broadly support the Competitive Interference model, and this must be acknowledged in interpreting the ipsilateral CoG shift as beneficial plasticity. However, the notable exceptions to the model are useful in this regard. Interpreting the relevance of studies of distal muscles to a study of proximal muscles also requires consideration.

6.4.3.3 *Within Evidence Relating to Competitive Interference There are Elements That Can be Reconciled With Beneficial Ipsilateral Plasticity*

Within the neuromodulation studies there were exceptions to the Competitive Interference model that showed deterioration in hand function with increased ipsilesional excitability (Ameli et al., 2009) and reduced contralesional excitability (Ackerley et al., 2010), suggesting reliance on ipsilateral activity to support dexterity. Other studies showed a lack of effect, which could suggest the model is not active (Talelli et al., 2012). Individual factors to explain this variance are not immediately identified in those experiments. Where deterioration was seen with increased ipsilesional excitability, cortical and subcortical strokes were tested. Subcortical strokes behaved according to the model, whereas 7 of 13 cortical strokes deteriorated, associated with increased bilateral primary and secondary motor area fMRI activation (Ameli et al., 2009). Where deterioration was seen following reduced contralesional excitability, all were subcortical

strokes, and ipsilesional excitability also decreased (Ackerley et al., 2010). Therefore, even where neuromodulation findings appear to support a beneficial role of ipsilateral activity, some elements of the Competitive Interference model persist. Variation in individual responsiveness to neuromodulatory protocols may also be relevant to the interpretation of these studies, although most confirm the intended intracortical excitability changes.

6.4.3.4 *The Relevance of Competitive Interference to Proximal Motor Control*

To relate this discussion of Competitive Interference to the reach task requires consideration of how studies of distal muscles relate to proximal muscle control. The neuromodulation studies discussed above considered distal paretic hand function, using measures of dexterity. Behaviour of proximal muscles habitually under stronger ipsilateral control could be expected to be different to that of distal muscles in this model. Studies that have sought to produce similar effects on axial and proximal muscles are limited, but also broadly concur in their support for the Competitive Interference model. Increasing ipsilesional excitability of trunk area with 10Hz rTMS (Choi et al., 2016) and anodal tDCS (Sohn et al., 2013), was seen to improve balance measures in hemiplegic stroke patients. Cathodal tDCS to the contralesional hemisphere improved proximal arm movements (Bradnam et al., 2011).

Knowing in which patients and at which time point Competitive Interference is active, and therefore the appropriateness of promoting ipsilateral plasticity, provides another argument for developing neurophysiological metrics incorporating ipsilateral measures to guide motor recovery after stroke. Markers of ipsilateral plasticity, estimates of ipsilesional plastic reserve, and knowledge of the time course of normalization of hemispheric excitability imbalances, when combined could tailor individualized therapy. It is again clear from discussion of Competitive Interference that there are individuals who do not behave as the

model would predict, and usefulness of ipsilateral plasticity post stroke will depend on individual factors.

6.4.4 Maladaptive Synergies and Ipsilateral Pathways

Another possible detrimental effect of plasticity in ipsilateral pathways is the driving of spasticity and unwanted synergies.

Synergies are spatiotemporal patterns of muscle activation that are stable across movements, achieved by coactivation or reciprocal inhibition of groups of muscles. Synergies are constructed through linearly recruited modules, each defining a pattern of muscle activation. Time varying and combining modules results in a small number of synergies being able to account for large variations of patterns of muscle activation (d'Avella and Bizzi, 2005; Tresch et al., 1999). In the arm, 5 time varying synergies account for all muscle activation patterns in fast reach (d'Avella et al., 2006; d'Avella and Lacquaniti, 2013), and in the leg 5 synergies account for muscle activation patterns in walking across a range of speeds and loads (Ivanenko et al., 2004). Use of synergies reduces the computational demand of motor programming, an economy required when programming vast numbers of motor units to control vast permutations of joint range and torque. Modules may be organized at cortical, brainstem and spinal level. Utilizing modules at spinal premotor interneurone level reduces computational requirements of descending inputs (Bizzi et al., 2008). Modular organization at cortical level is shown with intracortical recording and microstimulation in primates (Overduin et al., 2014, 2015), and EEG in humans (Yao et al., 2009).

6.4.4.1 *Cortical Representation of Synergies*

The cortical component of synergy representation is sensitive to pathology in a number of ways. Cortical modules may use direct corticospinal output to multiple muscles, or control descending inputs to downstream brainstem or spinal modules (McCambridge et al., 2014). The effect of cortical lesions on expression of synergies is apparent after stroke, most commonly abnormal flexor synergies with elbow flexion coactivated with shoulder abduction (Dewald et al., 1995; Dewald and Beer, 2001). The abnormal synergies may arise through a number of possible mechanisms. Increased use of alternative motor pathways may alter the descending command

(Bradnam et al., 2012), or modular reorganization may occur in residual intact cortex (Garcia-Cossio et al., 2014; Yao et al., 2009). Notably, work on healthy subjects demonstrates an important role for the ipsilateral motor cortex in control of synergies (McCambridge et al., 2011), making expression of synergies after stroke sensitive to altered balance of ipsilesional and contralesional activity.

6.4.4.2 *Impact of Stroke on Expression of Synergies, Merging, Fractionation and Preservation*

The impact of the stroke lesion on synergies depends on the location (Garcia-Cossio et al., 2014), and extent (Bradnam et al., 2012) of the lesion and severity of deficit (Cheung et al., 2009). Mild and moderately affected patients use the same number of synergies that are very similar to those of the non-paretic limb and healthy controls, despite greatly varying motor impairments (Cheung et al., 2009; Tropea et al., 2013). This similarity suggests that residual descending motor commands are sufficient to activate spinal modules. In severely affected patients, abnormal synergies are seen (Ellis et al., 2009; Kisiel-Sajewicz et al., 2011; Viau et al., 2004), but are not thought to emerge until after the time period for realizing the potential recovery predicted by the Proportional Recovery model (McMorland et al., 2015). Patients with subcortical lesions, where intact cortex allows compensatory reorganization, are thought more likely to develop abnormal synergies (Garcia-Cossio et al., 2014). This point is important, since in healthy subjects ipsilateral reorganization could be expected to optimize synergies, whereas this observation in stroke suggests that cortical reorganization driven by some other purpose may secondarily worsen synergy control.

In severely impaired stroke patients abnormal synergies are seen in the paretic arm (Cheung et al., 2012; Roh et al., 2013). At a late stage flexor synergies are seen with elbow flexion coactivating with shoulder abduction (Dewald et al., 1995). This is particularly problematic since it impacts on distal function by limiting effective workspace of the hand (Ellis et al., 2007) and increases with increasing effort of shoulder abduction (Sukal et

al., 2007). More subtle abnormal coactivations in reach are described in deltoid and pectoralis major at an earlier stage after stroke (Roh et al., 2013; Tropea et al., 2013). The abnormal synergies seen in the arms of severely affected stroke patients arise through a combination of some previous synergies merging together, some separating into fractionated components, and these combining with residual preserved synergies (Cheung et al., 2012). Merging of previous synergies has also been demonstrated to account for the fewer number of synergies in the paretic leg in walking (Clark et al., 2010). This merging, fractionation and preservation has been proposed to relate to disorganization of cortical representations with increased overlapping of cortical activities (Yao et al., 2009) and altered descending motor commands (Cheung et al., 2012). This link between cortical reorganization and synergy representation is important to the finding of the medial CoG shift, particularly since it occurred in deltoid, the prime mover in the task. The importance of the ipsilateral contribution to synergy control is further shown by neurophysiology experiments.

6.4.4.3 *The Contribution of the Ipsilateral Hemisphere to Synergy Control Revealed by TMS and Neuromodulation*

Neurophysiological evidence implicates the contralesional hemisphere in the development of abnormal synergies. A useful tool for investigating the balance of ipsilateral and contralateral contribution to synergies is the Synergy Ratio, measured with biceps MEPs. Contralateral MEP size prior to forearm pronation (when the biceps should be inhibited as an antagonist, most strongly under ipsilateral control), is divided by the MEP size measured prior to elbow flexion, (when the biceps should be facilitated as an agonist, most strongly under contralateral control). The Synergy Ratio is a measure of correct functioning of a synergy, in healthy subjects around 0.3 (Gerachshenko and Stinear, 2007) but in stroke patients around 1 (Gerachshenko et al., 2008), reflecting loss of the normal control relying on balance between ipsilateral and contralateral components of the synergy. A similar attempt, but with less eloquent measures, was made to describe abnormal synergies in the leg post stroke using ipsilateral and contralateral MEPs in muscles synergistic to hip adduction (Tan et al., 2016). It was

found that ipsilateral MEPs from the contralesional hemisphere of stroke patients obey activation more normally predicted by the synergy pattern than the ipsilesional contralateral MEPs.

The view that abnormal synergies arise through increased activity in ipsilateral pathways is supported by studies in healthy subjects using neuromodulation to inhibit the ipsilateral hemisphere. Inhibitory cathodal tDCS to the ipsilateral hemisphere reduced the Synergy Ratio by increasing inhibition of the biceps MEP in antagonist role. In this case inhibiting ipsilateral activity improved synergy performance by enhancing appropriate antagonist inhibition. This proves an important ipsilateral contribution to control of arm synergies, but implies it interferes with optimal function. The authors proposed the tDCS effect resulted from further downregulation of a tonic inhibitory pathway mediated by reticulospinal neurones (McCambridge et al., 2011). A similar experiment used inhibitory continuous theta burst. In this case Synergy Ratios increased bilaterally. In the contralateral arm this was driven by reduced biceps activity in the agonist role due to reduced excitability of contralateral motor cortex. In the ipsilateral arm the change was driven by increased biceps activity in the antagonist role. Inhibiting the ipsilateral hemisphere led to inappropriate facilitation of the antagonist. This demonstrates the ipsilateral hemisphere normally contributes to effective inhibition of antagonists, and is therefore important in the correct functioning of synergies (Bradnam et al., 2010).

The two experiments of ipsilateral cortical inhibition performed in a very similar way reported opposite polarities of effect and this may be of vital importance to interpretation of the CoG findings. Both clearly establish a role for the ipsilateral hemisphere in the control of synergies. In the tDCS experiment reducing ipsilateral influence improved synergy function, suggesting interference, compatible with the Maladaptive Synergies model. In the theta burst experiment reducing ipsilateral influence worsened synergy function, suggesting an important constituent role in optimal control of synergies. These findings of opposite polarity can be noted together with parallel observations made in upper and lower limb after stroke. Measuring synergistic activity in upper limb described the ipsilateral

contribution as maladaptive, but in the lower limb the ipsilateral hemisphere was maintaining synergies closer to normality. Just as exceptions to the Competitive Interference model were identified to allow interpretation of the CoG findings as supportive of the hypothesis, here are exceptions to the Maladaptive Synergies model that also permit this interpretation. A potential link to reconcile these conflicting tDCS and theta burst results becomes pivotal in interpreting ipsilateral map reorganization when viewed together with the findings linking post-stroke cortical reorganization and synergy expression (Yao et al., 2009).

6.4.4.4 *A Proposed Reticulospinal-Propriospinal Basis for Ipsilateral Contribution to Synergy Control*

The reason for the opposite effects on synergy seen with the two inhibitory protocols is not clear. A neuronal level difference in mechanisms of tDCS and theta burst that takes interpretation beyond transient reduction in excitability is possible but not obvious.

Both authors propose tonic corticoreticulospinal to propriospinal circuits as a mechanistic explanation, and differential activation of these circuits could allow both observations. Cortical reorganization could interact with this mechanism by altering inputs to these spinal modules. A further experiment supports this by combining inhibitory cathodal tDCS of the ipsilateral hemisphere with peripheral modulation of MEPs at cervical spinal level using median nerve stimulation at interstimulus intervals that activate propriospinal neurones (Bradnam et al., 2011). Altering propriospinal activity can modulate the tDCS effect on synergies. This supports potential interaction between ipsilaterally descending pathways and propriospinal circuits as a basis for abnormal synergies after stroke. This, together with the observation of increased propriospinal activity in the subacute stage of stroke recovery (Mazevet, 2003), will form the basis of later discussion.

6.4.4.5 *Ipsilateral Cortical Reorganization and Synergy Control*

Neurophysiological evidence in stroke patients also supports altered balance of ipsilesional and contralesional activity as a basis for abnormal synergies.

Mapping EEG current density shows that greater overlap of cortical regions controlling elbow flexion and shoulder abduction in stroke patients associates with abnormal joint-torque coupling (Yao et al., 2009). The strength of ipsilateral MEPs in Pectoralis Major was shown to correlate with abnormal secondary joint torque at the elbow during shoulder abduction (Schwerin et al., 2008). Strength of ipsilateral MEPs correlated negatively with abnormality of synergy and functional impairment. These observations support the reticulospinal component of the mechanistic proposal, since ipsilateral reticulospinal output facilitates flexor activity over multiple segmental levels (Davidson and Buford, 2006).

The discussion above relating ipsilateral activity to development of abnormal post stroke synergies shows again that ipsilateral activity may be both potentially harmful (Gerachshenko et al., 2008; Schwerin et al., 2008; Yao et al., 2009) or potentially beneficial (Bradnam et al., 2010, 2012). Increased use of abnormal synergies in more severely impaired patients (Cheung et al., 2009), and a greater reliance on ipsilateral contribution to synergies in more severely impaired patients (Bradnam et al., 2012; Schwerin et al., 2008) leaves the question of whether this represents represent maladaptive plasticity or use of the best compensatory strategy available. Demonstration of ipsilateral contribution to synergies in healthy brain (McCambridge et al., 2011), allows an explanation for the ipsilateral CoG changes based on synergy representations to be pursued. This will form the basis of unifying discussion later. Having presented precautionary examples of potentially harmful effects of ipsilateral plasticity, it remains to develop the argument that plasticity in ipsilateral pathways can be of potential benefit in the post stroke setting.

6.4.5 Beneficial Plasticity in Ipsilateral Pathways

Neuroimaging, neurophysiological and clinical evidence exists for a positive role for ipsilateral cortical plasticity supporting motor recovery after stroke. Establishing this is essential to provide supportive context to the interpretation of the findings of this study as being consistent with the hypothesis.

6.4.5.1 *Beneficial Ipsilateral Activity in Healthy Brain*

Studies in healthy subjects show a spectrum of usefulness of ipsilateral activity across motor tasks and individuals. Examples exist of both beneficial and unhelpful ipsilateral activity in unilateral skilled tasks. Ipsilateral activity accompanies skilled motor learning in unimanual tasks, with increased involvement seen with increasing task complexity. Training unimanual finger movements over many weeks to increasing speed and accuracy demands progressively increases excitability of ipsilateral motor cortex (Christiansen et al., 2016). Similar ipsilateral excitability changes are seen with training of leg muscles (Goodwill et al., 2012). Examples of useful ipsilateral contribution to synergy control have been provided above. Neuromodulation studies frequently counter this view, arguing inhibitory neuromodulation of the ipsilateral hemisphere improves unimanual task performance, mediated by reduced IHI (Avanzino et al., 2008; Kobayashi et al., 2009; Kobayashi, 2010).

6.4.5.2 *Beneficial Ipsilateral Activity After Stroke*

The most direct demonstration of beneficial ipsilateral activity is provided by TMS experiments of ipsilateral responses in proximal muscles post stroke. Extensive infarcts may reduce viable crossed corticospinal fibres to insufficient levels to support voluntary movement (Stinear et al., 2014). These patients may rely on ipsilateral pathways from contralesional hemisphere for paretic movement (Cao et al., 1998), sacrificing selective muscle activation for basic compensatory strategies to allow movement, albeit of poor quality. Beyond this simplest example, there are sufficient examples of good motor recovery associated with continued ipsilateral

activity to propose that factors other than residual ipsilesional corticospinal tract integrity determine whether ipsilateral plasticity will be beneficial.

6.4.5.3 *Beneficial Ipsilateral Activity After Stroke in Relation to Proximal Muscles*

Ipsilateral MEPs become upregulated after stroke. These are particularly evident in axial muscles due to the proximal to distal gradient of bilateral innervation (Bawa et al., 2004; Palmer and Ashby, 1992). One study observed proximal ipsilateral MEPs in three quarters of stroke patients, compared to one fifth of normal subjects (Misawa et al, 2008). Another study observed ipsilateral MEPs from the contralesional hemisphere in 19 of 20 stroke patients (Fujiwara et al., 2001). The presence of ipsilateral MEPs correlated with increased shoulder strength (Misawa et al., 2008), highly relevant given the importance of shoulder abduction in predictive algorithms of upper limb recovery (Beebe and Lang, 2009; Katrak et al., 1998; Nijland et al., 2010; Stinear et al., 2014).

Abdominal ipsilateral MEP size correlates with Trunk Control Test score (Fujiwara et al., 2001). Again this is of great importance since Trunk Control Test score predicts ambulatory status at 6 months (Veerbeek et al., 2011). Extrapolating from this further emphasizes the importance of ipsilateral activity in this setting, since ability to sit out predicts overall degree of functional independence at 3 months (Askim et al., 2014; Hokstad et al., 2016), and Trunk Control Test at admission predicts Functional Independence Measure at discharge (Duarte et al., 2002; Franchignoni et al., 1997).

6.4.5.4 *Beneficial Ipsilateral Activity After Stroke in Relation to Distal Muscles*

Studying proximal function provides compelling examples of beneficial ipsilateral activity, but as expected from the proximal to distal gradient of ipsilateral innervation, this is not so evident from studies of distal function. Return of contralateral activity from the ipsilesional hemisphere is shown to be of greatest importance to recovery of distal function in imaging studies

(Calautti et al., 2001; Loubinoux et al., 2007; Tombari et al., 2004). Cortical representation of the paretic hand in the ipsilesional hemisphere strengthens over time (Cicinelli et al., 1997; Foltys et al., 2003; Traversa et al., 1997), correlating with functional recovery (Bastings et al., 2002; Koski et al., 2004). Anterior displacement of ipsilesional FDI CoG was seen over 1 year of recovery, but no distal map reorganization was identified in the contralesional hemisphere (Delvaux et al., 2003). Ipsilateral hand MEPs are not seen in well recovered stroke patients (Foltys et al., 2003), and TMS to contralesional motor cortex fails to disrupt preparation of paretic hand movement in well recovered stroke patients (Werhahn et al., 2003). This generally argues against a functional role for ipsilateral activity in distal control of the paretic arm. Furthermore, others report association of distal ipsilateral MEPs from contralesional cortex with poor recovery (Turton et al., 1996), and mirror movements (Netz et al., 1997).

However, exceptions can again be noted, and ipsilateral MEPS in hand muscles of well recovered stroke patients have been reported (Alagona et al., 2001), associated with increased contralesional regional blood flow (Caramia et al., 2000). Distal ipsilateral MEPs were seen from stimulation of sites more anterior than would be anticipated for hand muscles, interpreted as representing bilateral output from secondary motor areas (Alagona et al., 2001). Consistent with this, TMS to contralesional Premotor areas disrupts motor behaviour of the paretic hand (Fridman, 2004). Persisting increased contralesional excitability (Butefisch et al., 2008; Cicinelli et al., 2003) and persisting bilateral fMRI activation (Foltys et al., 2003; Nair et al., 2007) have been shown in stroke patients who make excellent distal functional recovery. Again, amidst the body of evidence favouring contralateral activity for optimal recovery, there are examples of beneficial ipsilateral plasticity contributing to good motor recovery.

6.4.5.5 *Clinical Examples of Beneficial Ipsilateral Plasticity After Stroke*

Compelling evidence for beneficial ipsilateral plasticity in stroke recovery is provided by clinical observation of patients who mount a powerful plastic

response to a minor ischaemic insult, then later reveal the extent of plastic change following subsequent lesions. Patients are recognized who recover well from a first stroke by reorganizing motor control to the contralesional hemisphere, then develop severe motor deficits in response to a second stroke to the previously unaffected hemisphere. The second stroke may decompensate ipsilateral motor output that has arisen from ipsilateral cortical reorganization, resulting in unusual ipsilateral hemiparesis (Ago et al., 2003; Saada and Antonios, 2014; Song et al., 2005; Yamamoto et al., 2007). A prospective study of 8360 stroke patients identified 13 such patients with ipsilateral hemiparesis in response to second strokes. Combined fMRI and TMS analysis of these patients demonstrated earlier motor reorganization in the ipsilateral hemisphere (Inatomi et al., 2016). Similar examples are provided by cases of Global Aphasia Without Hemiparesis where lesion territory predicts motor deficit, but motor function is spared if previous subcortical lesions have prompted motor reorganization to the contralesional hemisphere. Subsequent extensive MCA territory infarcts then do not produce the anticipated Total Anterior Circulation Syndromes since motor function has reorganized and aphasia is seen in isolation (Bang et al., 2004). A similar reorganization of motor control to rely on ipsilateral pathways for distal upper limb function is measurable with intraoperative cortical stimulation and fMRI when contralateral corticospinal tracts are damaged by brain tumours (Roux et al., 2000). A wealth of literature exists for similar ipsilateral reorganization in patients with motor deficits from cerebral palsy and callosal agenesis or dysgenesis. Whilst useful in illustrating the principle, developmental plastic processes cannot be translated directly to plastic responses to pathology in the adult brain.

Overall, these clinical examples are compelling arguments for the beneficial role of ipsilateral plasticity in stroke recovery, but again illustrate how individual factors relating to lesion and plastic potential will dictate such responses.

6.5 SUMMARY

This discussion acknowledges the potentially harmful effects of ipsilateral activity, but emphasises clinical, neurophysiological and neuroimaging examples of ipsilateral plasticity serving useful motor recovery after stroke. This can be seen both convincingly in severely affected patients with focus on axial and proximal function, but also in a more subtle way in moderately affected patients with focus on distal function. In these moderately affected patients, ipsilateral activity has synergistic effects in optimizing positioning of the paretic hand in ideal workspace, and in providing the core activation to support ambulation. There is also evidence that the ipsilateral hemisphere contributes to complex skilled distal motor control in healthy subjects.

These examples provide a supportive context to allow interpretation of the finding of training driven medial displacement of the deltoid Centre of Gravity as a marker of beneficial plasticity. It is notable that the only change was seen in deltoid, a prime mover in reach in healthy subjects (van der Fits et al., 1998), and hemiparetic stroke patients (Wagner et al., 2007). Use of deltoid as the prime mover requires deployment of an Anticipatory Postural Adjustment (van der Fits et al., 1998), which will also draw on activity in ipsilateral pathways, and potentially increase the extent of deltoid motor map. The source of associated Anticipatory Postural Adjustments may be of relevance in interpretation of the medial shift of CoG, discussed further later. Deltoid is frequently observed to contribute to abnormal synergies in post-stroke reach (Cheung et al., 2009; Roh et al., 2013; Tropea et al., 2013), which could result from untrained contralesional motor maps representing suboptimal compensatory strategies rather than optimal movements. It may be that the therapeutic intervention here drove reorganization of the motor map to connect deltoid more optimally within cortical modules expressing synergies. This explanation is compatible with the associated observation that the strength of the ipsilateral representation and the Laterality Index were not influenced by training. This suggests that the map reorganization was concerned with some aspect of motor control other than direct corticomotor output. A role in synergies would fit with this observation.

Having proposed potentially beneficial ipsilateral plastic change driven by training over short time periods, it will now be questioned in the following experiment whether ipsilateral plasticity can be identified in patients who make good motor recovery with standard physical therapeutic intervention over long time periods.

This discussion has revealed the potential for ipsilateral involvement in synergies to serve as a mechanistic link, unifying these findings of ipsilateral change with those of beneficial ipsilateral plasticity in athletes, and the potentially beneficial ipsilateral contribution to stroke recovery predicted by Proportional Recovery algorithms. Further evidence to support this proposal will be sought in the following chapter.

CHAPTER 7
ASSOCIATION BETWEEN FUNCTIONAL PERFORMANCE AND IPSILATERAL CORTICAL MOTOR REPRESENTATIONS IN THE CHRONIC PHASE OF STROKE

7.1 INTRODUCTION

The purpose of this chapter is to investigate whether activity in ipsilateral motor pathways can serve useful motor recovery in chronic stroke patients. This aims to develop on the findings of the previous chapter. The same patients that participated in the previous training task are studied further for associations between measures of ipsilateral activity and level of functional recovery. The previous experiment questioned whether training in a motor task can drive plasticity in ipsilateral pathways. This tested task-dependent plastic response to training over a short time period. An experience dependent element is added by examining for change in neural organization that may have developed over years. The experimental question is now rephrased to ask whether ipsilateral activity associates with upper limb functional performance in patients who have undergone standard post-stroke physical therapy, tested at least one year after the stroke.

Quantifying experience dependence of drivers of plasticity is problematic when studying stroke patients. The varying effect of lesion, genotype, pre-stroke pathology and the variation in intensity, type and duration of therapeutic intervention makes any retrospective study of experience dependent plasticity impossible in all but the most general terms. The most effective way to derive measures from stroke patients that will allow analogy to the accumulation of deliberate practise in elite performers is with the simplest measures. A cross-sectional approach is used to circumvent confounding variations in accumulated experience of physical therapeutic intervention. Dividing the patients into those who have made good recovery

and poor recovery on the basis of their functional upper limb performance produced groups that did not differ in age, but differed in their duration since stroke. The low performance group were later after stroke, which removed potential confound from cumulative therapeutic intervention.

The experimental approach is otherwise identical to the preceding experiment. The ipsilesional and contralesional hemispheres of each patient are mapped. MEPs are recorded in the same muscles. These muscles are both practically suited to study of ipsilateral activity, due to their gradient of ipsilateral and contralateral innervation, and functionally relevant to motor recovery due to their role in reach movements. Comparing measures of ipsilateral activity between High and Low Performance groups provides further information about the usefulness of ipsilateral plastic change acquired over years to complement the information about ipsilateral plasticity trained over short time periods.

7.1.1 Hypothesis

1. Ipsilateral motor pathways from the contralesional hemisphere can serve beneficial motor recovery of the paretic limb after stroke.

2. This will be manifest as differences in ipsilateral corticomotor connectivity, or differences in the organization of the ipsilateral cortical representation of paretic muscles in the contralesional hemisphere between the High Performance compared to the Low Performance groups of chronic stroke patients.

7.1.2 Basis for Hypothesis

The discussion from the previous chapter has detailed the basis of the hypothesis. It is necessary to reconcile evidence relating to maladaptive plasticity (Jones and Adkins, 2015) with the potential pivotal role of ipsilateral activity in optimal recovery identified by predictive algorithms of upper limb function and ambulatory status (Kwah and Herbert, 2016). The previous chapter showed that training in the chronic phase after stroke can produce additional functional improvement, and that features of ipsilateral plasticity accompany this improvement. A relevant finding from the previous experiment was an association between baseline functional performance and baseline Laterality Index. This showed patients with greater reliance on ipsilateral pathways to proximal upper limb muscles had lower upper limb functional scores. This has alternative possible interpretations. One interpretation is that it is evidence against this hypothesis. The other interpretation is that it represents use of alternative ipsilateral motor pathways when contralateral reserve has been exhausted by extensive lesions. This version would be taken as a form of beneficial plasticity, the other reading taken as maladaptive activity. It is hoped that examining this further in this experiment will add evidence to delineate these possibilities with more certainty. The question asked is whether features of ipsilateral plasticity can be identified in the High Performance group that are absent in the Low Performance group to further demonstrate a beneficial role for ipsilateral beyond merely functioning as the last reserve in a damaged system.

Another finding from the previous chapter that requires development is that of the deltoid CoG shift to a medial position without accompanying change in map strength or strength of corticomotor connectivity. This combination of findings limits potential mechanistic explanations. The possibilities of increased corticospinal output, either secondary to altered inputs to primary motor cortex or from secondary motor areas, are not supported by the lack of corticomotor change. Proposing a mechanism whereby cortical reorganization improves motor performance without directly increasing

corticomotor output leads towards the suggested role of the ipsilateral hemisphere in contributing to synergy control.

This idea is compatible with what is known about the plasticity of reorganization of cortical maps, and importantly, by studying muscles of reach a link can be made to the major animal models of cortical reorganization after stroke. Premotor Cortex is of major importance in the control of reach movements. There are specific aspects of Premotor functional behaviour and anatomical organization that are ideally suited to ipsilateral contributions to synergies controlling reach. This is supported by animal and human experimental evidence demonstrating ipsilateral PMC activity encoding reach synergies. Experimental ischaemic lesions of primary motor cortex lead to alteration of corticocortical connectivity between primary motor cortex and secondary motor areas (Nudo, 2013). Premotor Cortex is heavily interconnected with primary motor cortex and serves a prominent role in the motor network hierarchy after primary motor cortex lesions. Corticocortical activity in the contralesional hemisphere orchestrated by Premotor Cortex could underlie the ipsilateral map shift seen in the previous chapter. The support offered to this idea from the considerable evidence from animal models deserves further study.

7.2 METHOD

The patients, TMS, cortical mapping technique and measures are identical to those described in the preceding chapter.

The division of patients into High and Low Performance groups was achieved by dividing the group according to the median Fugl-Meyer Upper Limb Subset score.

7.3.1 Characteristics of Low and High Performance Groups

Subject	Performance Group	Age	Paretic limb	Months since stroke	Fugl-Meyer UL Score	Spasticity (MAS)	Sensory impairment	Strength of Shoulder	Lesion Location
1	high	69	L	24	58	0	mod	4	cortical
2	high	54	L	12	51	3	mod	3	cortical
3	high	54	L	20	64	0	mod	4	cortical
4	high	88	R	84	61	1	mod	3	cortical
5	high	47	L	72	55	3	mild	4	sub-cort
6	high	63	R	18	58	2	mod	4	sub-cort
7	high	74	L	15	63	0	mod	3	cortical
8	high	71	R	17	62	0	mild	4	cortical
9	high	58	R	42	49	1	mild	3	sub-cort
10	high	49	R	28	49	3	mild	4	mixed
Mean		62.7		33.2	57	1.3		3.6	
11	low	49	L	15	32	3	mod	3	cortical
12	low	52	R	184	34	0	mod	3	cortical
13	low	60	L	36	48	3	mild	3	sub-cort
14	low	57	R	60	38	3	mild	3	cortical
15	low	52	R	100	28	3	mild	3	cortical
16	low	52	L	32	38	3	mild	3	cortical
17	low	65	L	82	32	1	mild	3	sub-cort
18	low	49	L	52	39	3	mild	3	sub-cort
19	low	61	R	96	37	1	mild	3	mixed
Mean		55.22		73	36.22	2.22		3	
p Value		0.13		0.04	<0.001	0.09		0.003	

TABLE 7.1 THE CHARACTERISTICS OF THE STROKE PATIENTS IN THE HIGH PERFORMANCE AND LOW PERFORMANCE GROUP

Subjects were divided according to the median Fugl-Meyer Upper Limb Subset score.

The characteristics of individual patients and the average values for the group are shown in Table 7.1. The method of dividing the groups according to the median Fugl-Meyer Upper Limb Subset score was effective in producing groups that were significantly different in their functional ability (unpaired t-test, p= <.001). The Low Performance group were also of longer duration after stroke, (mean 73 months compared to 33 months). This removes concern over the potential confound of any ipsilateral plastic change identified in the High Performance group as representing increased duration of therapeutic intervention, assuming treatment with standard physical therapy provision in all patients.

7.3.2 Cortical Maps

Representative cortical maps are shown for ipsilateral and contralateral Pectoralis Major for each subject in the Low and High Performance groups in Figure 7.1.

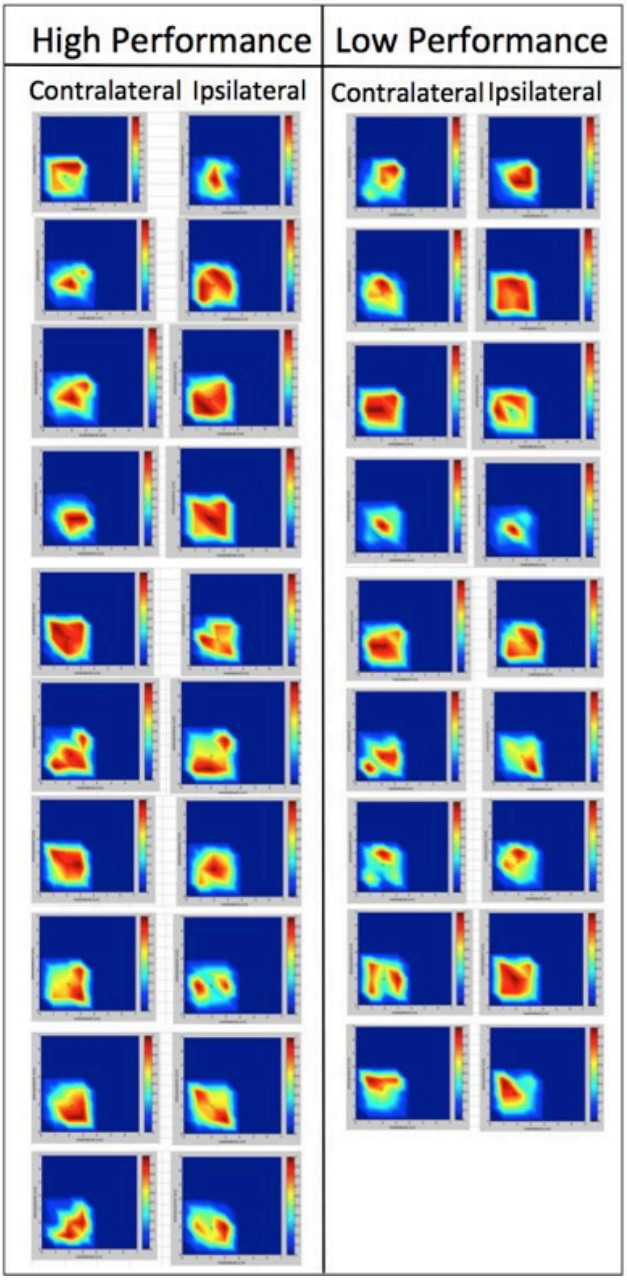

FIGURE 7.1 CORTICAL MAPS FOR IPSILATERAL AND CONTRALATERAL PECTORALIS MAJOR FOR EACH SUBJECT IN THE LOW AND HIGH PERFORMANCE GROUPS

7.3.3 Relationship Between Strength of Ipsilateral Cortical Representation of Paretic Muscles in the Contralesional Hemisphere and Functional Motor Recovery After Stroke

No significant differences were seen in the strength of the ipsilateral cortical representation between groups in any of the muscles tested as measured by the independent sample t-test ($p = 0.372$). The mean normalized MEP area, averaging each grid point stimulated on the map, is used as a measure of the size of the representation. No significant differences were seen in the strength of the ipsilateral cortical representation between the High and Low Performance groups in any of the muscles.

A representative graph showing size of the ipsilateral cortical representations of the paretic Pectoralis Major is shown for the Low Performance and High Performance groups in Figure 7.2.

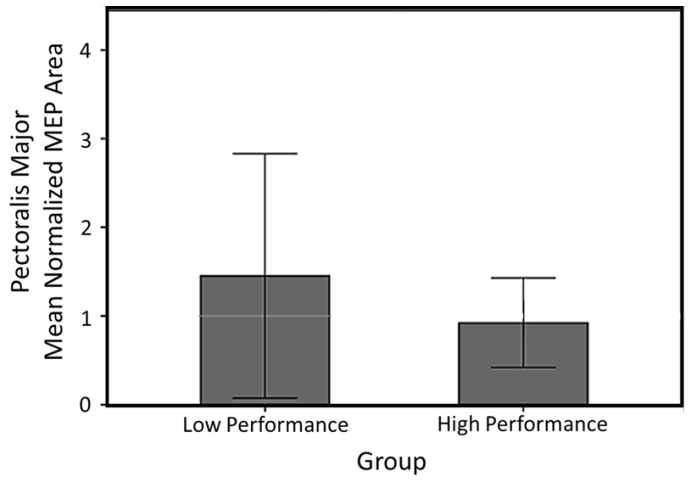

FIGURE 7.2 THE MEAN SIZE OF THE IPSILATERAL CORTICAL REPRESENTATION OF PARETIC PECTORALIS MAJOR IN THE CONTRALESIONAL HEMISPHERE IN THE LOW PERFORMANCE AND HIGH PERFORMANCE GROUPS

Y-axis is the mean normalized MEP area of all grid points stimulated in the map. There are no significant differences between the groups ($p= 0.237$).

7.3.4 Relationship Between Size of Ipsilateral Representation and Upper Limb Function

There are no significant associations between size of ipsilateral cortical representation and functional performance of the paretic arm for any of the muscles. These results do not support the hypothesis that a strengthened ipsilateral cortical representation is associated with good motor recovery after stroke.

Figure 7.3 shows a representative plot of the size of ipsilateral cortical representation against Fugl-Meyer Upper Limb Subset score for each patient, for Pectoralis Major. There are no significant associations.

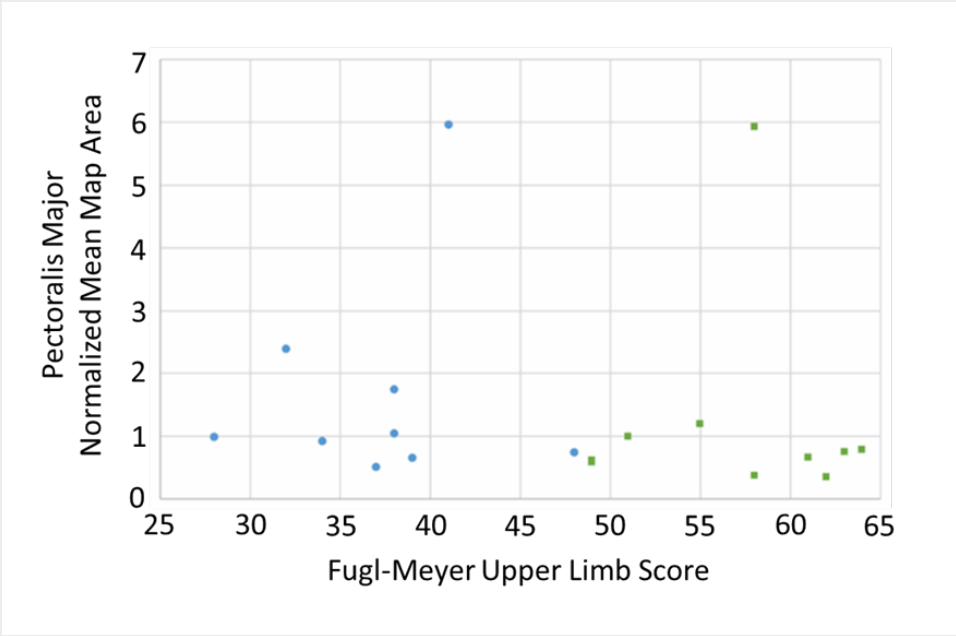

FIGURE 7.3 RELATIONSHIP BETWEEN NORMALIZED MEAN MAP AREA AND FUGL-MEYER UPPER LIMB SUBSET FOR PECTORALIS MAJOR

Each data point represents an individual patient. High performance patients are shown with green circles, Low Performance patients with blue circles. There is no significant correlation between size of cortical representation and arm function ($r = <0.01$ for Deltoid and Pectoralis Major).

7.3.4.1 *Relationship Between Organization of Ipsilateral Cortical Representation and Upper Limb Function*

Independent samples t-tests revealed no significant differences in the mean Centre of Gravity values between the High and Low Performance groups for any of the muscles (Figures 7.4 and 7.5).

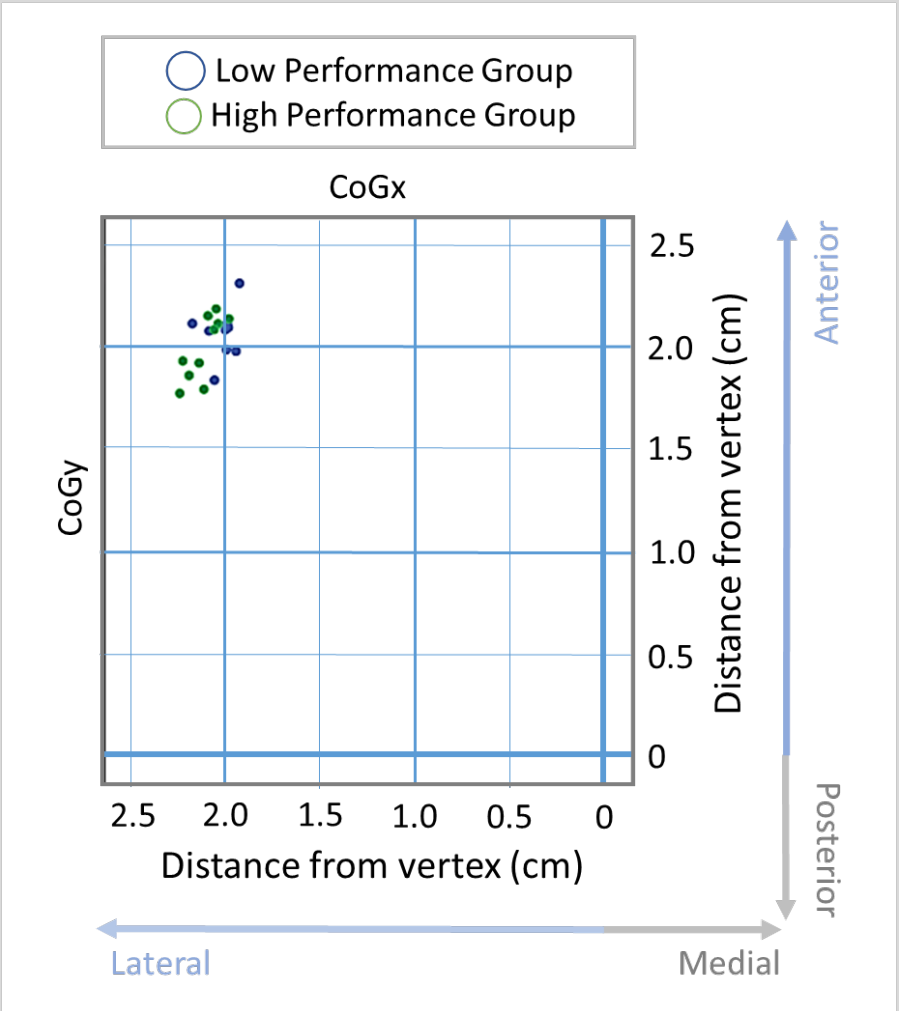

FIGURE 7.4 PLOT OF MEAN CENTRE OF GRAVITY, AVERAGED ACROSS MUSCLES, FOR THE HIGH AND LOW PERFORMANCE GROUPS

There is no significant difference between Groups.

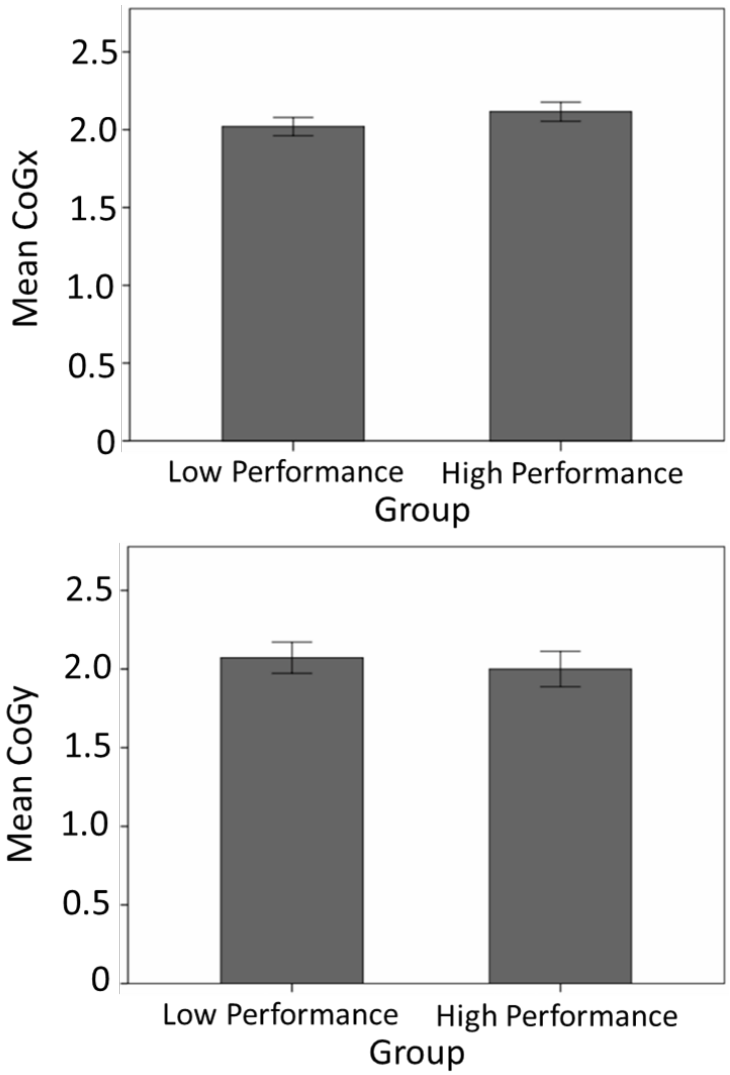

FIGURE 7.5 DIFFERENCE IN MEAN COGX (TOP GRAPH) AND COGY (BOTTOM GRAPH) BETWEEN HIGH AND LOW PERFORMANCE GROUPS

Centre of Gravity coordinates are plotted against Fugl-Meyer Upper Limb Subset scores to further test for association between CoG and functional performance. There are no significant associations between Centre of Gravity coordinates and Fugl-Meyer Upper Limb Subset scores for either muscle.

A representative plot is shown for Pectoralis Major in Figure 7.6. There is no significant association (r=0.11 for CoGx, 0.062 for CoGy).

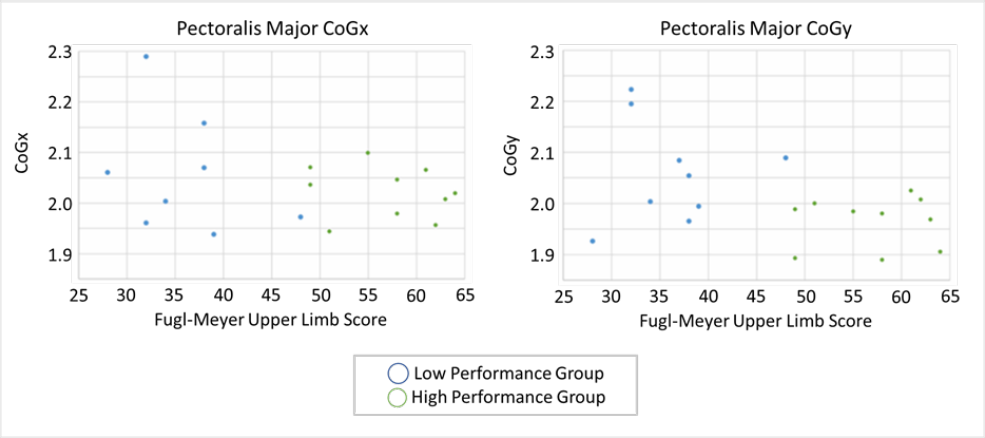

FIGURE 7.6 CENTRE OF GRAVITY X COORDINATE (LEFT GRAPH) AND Y COORDINATE (RIGHT GRAPH) FOR PECTORALIS MAJOR

Each data point is an individual patient. High Performance group green dots, Low Performance blue dots. There are no significant associations.

7.3.4.2 *Organization of Ipsilateral Cortical Representation Relative to Contralateral Representation*

Although there are no significant differences in the mean CoG values between groups and no significant correlations when CoG is plotted against functional scores, a tendency for a medial and posterior position could be seen for Pectoralis Major. This is examined further with a more sensitive measure of cortical organization, expressing the ipsilateral CoG relative to the contralateral CoG within the contralesional hemisphere. This uses a functional reference for the map rather than the anatomical reference used in calculating the absolute coordinates.

For Pectoralis Major a significant difference is seen in the mean displacement between Ipsilateral and Contralateral CoGx between the High and Low Performance groups (p=0.017, unpaired t-test). There are no significant differences in the displacement between Ipsilateral and Contralateral CoG positions for any of the other muscles tested.

Figure 7.7 shows the position of the CoG of the ipsilateral Pectoralis Major representation in the contralesional hemisphere relative to the position of the contralateral representation in the contralesional hemisphere. This is expressed by subtracting the ipsilateral CoGx value from the contralateral CoGx value and ipsilateral CoGy from contralateral CoGy. In this way, negative CoGx values show a more medial ipsilateral representation. Values are shown for each patient in the High and Low Performance groups.

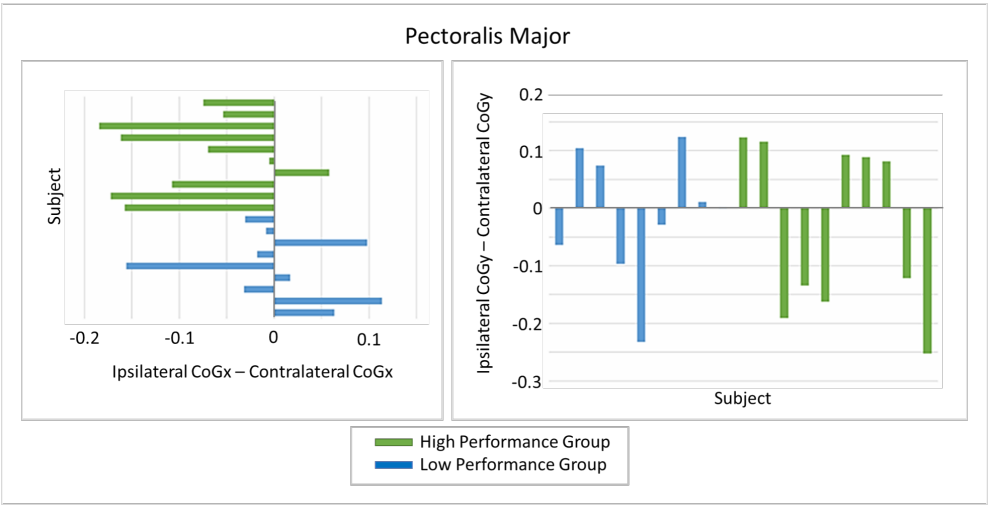

FIGURE 7.7 POSITION OF THE IPSILATERAL PECTORALIS MAJOR REPRESENTATION RELATIVE TO THE POSITION OF THE CONTRALATERAL REPRESENTATION IN THE CONTRALESIONAL HEMISPHERE

The left graph shows Ipsilateral CoGx – Contralateral CoGx. The right graph Ipsilateral CoGy – Contralateral CoGy. Each bar represents an individual patient. High-Performance group are shown in green, Low Performance group in blue. For CoGx, negative values show that the ipsilateral representation is positioned more medially than the contralateral, and positive values more laterally. For CoGy, negative values show that the ipsilateral representation is positioned more posteriorly than the contralateral, and positive values more anteriorly.

7.3.4.3 *Functional Correlates of a More Medial Ipsilateral Pectoralis Major Representation Relative to the Contralateral Representation*

The relevance of the displacement between ipsilateral and contralateral CoGx for Pectoralis Major is tested by plotting the displacement measured

in each patient against functional upper limb score. This is shown in Figure 7.8.

For Pectoralis Major a more medial position of ipsilateral CoGx relative to contralateral CoGx in the contralesional hemisphere is associated with higher functional performance. There is a significant correlation, r=0.51, (significance value for 17 d.f. (n=19) at 0.05 level of significance is 0.48). No significant associations were seen in any of the other muscles tested.

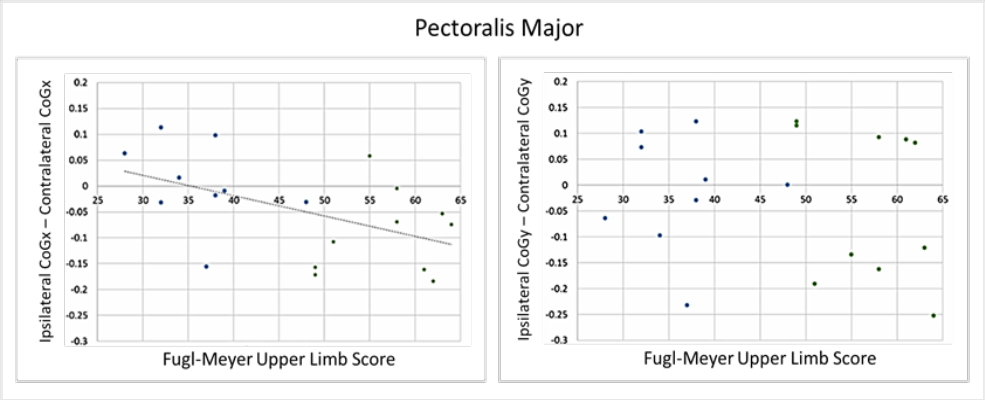

FIGURE 7.8 ASSOCIATION BETWEEN THE DISPLACEMENT OF PECOTRALIS MAJOR IPSILATERAL AND CONTRALATERAL COGX IN THE CONTRALESIONAL HEMISPHERE AND FUGL-MEYER UPPER LIMB SUBSET SCORE (LEFT GRAPH).

The same plot for CoGy is shown in the right graph. There is a significant correlation between displacement between Ipsilateral and Contralateral CoG x and Fugl-Meyer Upper Limb Score.

7.3.5 Summary of Results

1. There were no significant differences identified in the strength of the ipsilateral cortical representations of the paretic limb in the contralesional hemisphere between the Low and High Performance groups.

2. There were no significant correlations identified between the strength of the ipsilateral representation and arm function.

3. There were no significant differences in the absolute CoG coordinates of the ipsilateral representation of the paretic limb in the contralesional hemisphere between the Low and High Performance groups.

4. There were no significant correlations between absolute CoG coordinates of the ipsilateral representation of the paretic limb in the contralesional hemisphere and arm function.

5. There is a significant difference in the displacement between ipsilateral CoG x of Pectoralis Major and contralateral CoGx of Pectoralis Major in the contralesional hemisphere between the Low and High Performance groups.

6. Functional relevance of this medial CoGx displacement was shown by significant correlation to measures of upper limb function.

7.4 DISCUSSION

7.4.1 Main Findings

1. A medial displacement of the Pectoralis Major ipsilateral CoG relative to the contralateral CoG was associated with improved upper limb functional performance in chronic stroke patients. In healthy subjects, ipsilateral Pectoralis Major representation is posterior relative to the contralateral (MacKinnon et al., 2004). This observation permits discussion of this finding as representing post-stroke plastic reorganization.

2. There was no difference in the strength of the ipsilateral cortical representation between stroke patients who had made good motor recovery and patients with poor motor recovery.

These findings are weakly supportive of the hypothesis that ipsilateral plasticity in the contralesional hemisphere can support motor recovery after stroke. The most direct demonstration would have been a strengthening of the ipsilateral cortical representation, or a significant correlation between the absolute ipsilateral CoG and arm function, but this was not seen. It is notable that in the previous chapter a medial displacement of deltoid CoG was seen in response to training. Both deltoid and pectoralis major can function as prime movers in reach. Although reach was not trained in this study, the TMS was performed with background activation achieved during a reaching movement. Facilitation of the muscles as part of reach synergies would be active in both studies, and therefore relevant to the interpretation of both sets of results.

To interpret the two findings as being mutually supportive manifestations of beneficial ipsilateral plastic change, an explanation must account for why the change is seen in deltoid in response to training, but in pectoralis major in the chronic stage. A task specific training effect favouring deltoid use, not routinely employed by chronic stroke patients could explain the difference.

A mechanistic explanation can be proposed to allow consideration of the medial CoG displacement as a marker of beneficial ipsilateral plastic

change. The medial location may reflect either increased excitability of a region of motor cortex providing ipsilateral corticospinal output to the paretic limb, or increased activity in a neuronal population concerned with synergy control through ipsilateral pathways. Potential ipsilateral contribution to synergies was outlined in the previous chapter. Both of these possibilities would rely on altered intracortical connectivity, driven by changes in balance of inputs from contralesional secondary motor areas through corticocortical connections, or transcallosal inputs from ipsilesional motor areas. The lack of corticomotor change again favours reorganization to optimize some aspect of motor control other than direct output to motor neurones. The more medial position is presumably driven by multiple representations within motor cortex becoming linked through coactivation within a motor task such as reach. The medial shift may represent more extensive linkage with axial representations, with coactivation with trunk muscles improving performance in reach.

Altered corticocortical connections between secondary motor areas and primary motor cortex can be pursued as the basis for motor map reorganization. Animal models of the post-stroke motor network point to the interconnectivity between Premotor areas and primary motor cortex as being of vital importance in this role. Given the role of Premotor Cortex in control of paretic reach, it should be examined whether the medial displacement could be explained in terms of Premotor corticocortical connectivity.

7.4.2 Corticocortical Connectivity Between Premotor Cortex and Primary Motor Cortex and Paretic Reach

Changes in corticocortical connectivity between primary motor cortex and secondary motor areas can alter motor representations. Secondary motor areas with corticospinal output are activated during hand and shoulder movements (Fink et al., 1997). The total neuronal representation of arm movements in these areas exceeds that of primary motor cortex (Dum and Strick, 1991). Premotor Cortex (PMC), Supplementary Motor Area (SMA) (Ghosh and Porter, 1988; Stepniewska et al., 2006) and Cingulate Motor Area (CMA) (Pandya et al., 1981), have corticocortical projections to primary motor cortex pyramidal cells. There are also direct corticospinal outputs from these regions (Boudrias et al., 2010; Galea and Darian-Smith, 1994; He et al., 1993). Animal models of ischaemic primary motor cortex lesions demonstrate increased activity in secondary motor areas to serve functional recovery (Murata et al., 2015). The same is demonstrated in humans by mapping the post stroke network with fMRI (Ward et al., 2003). Regional excitability of motor cortex can be modulated by changes in the strength of connectivity with secondary motor areas, and there are neurophysiological demonstrations of this following stroke (Johansen-Berg et al., 2002). Paired pulse and neuromodulation studies demonstrate functional, task specific connectivity between PMC and primary motor cortex relevant to the interpretation of these findings.

7.4.3 Functional Aspects of Premotor Cortex Relevant to Control of Representation of Contralesional Paretic Reach – Action Encoding and Arm Selection

PMC has less direct corticospinal output than other secondary motor areas but occupies a position within motor network hierarchy that places it well to orchestrate cortical reorganization after damage to primary motor cortex. PMC receives strong input from prefrontal and sensory regions and is strongly interconnected with primary motor cortex (Katanak et al, 2012). The laminar distribution of the corticocortical connections between PMC and primary motor cortex is very similar, suggesting a similar functional hierarchical position (Dum and Strick, 2005).

PMC is known to have a dominant role in visuomotor transformation for reach and grasp (Rizzolatti and Luppino, 2001). Broadly, Ventral Premotor Cortex (PMv) is more concerned with visuospatial processing, selecting target location for grasp, whereas Dorsal Premotor Cortex (PMd) is concerned with integration for action selection and execution, more involved in reach (Hoshi and Tanji, 2004; Kurata, 1994). Disruption of PMv leads to amplitude and velocity errors, whereas disrupting PMd leads to direction and arm selection errors (Kurata and Hoffman, 1994; Kurata, 2010). Selection of the effector arm for a planned reach is an important function of PMd (Hoshi and Tanji, 2000). PMC encodes proposed actions rather than proposed movements. Proposed actions can then be lateralized to either arm depending on the sensory context, such as features of the target object (Rizzolatti et al., 1988). Around two thirds of PMC neurones recorded in a single unit study were modulated by activity in the contralateral or ipsilateral upper limb, compared to 8% in primary motor cortex (Tanji et al., 1988). These points are important in relation to ipsilateral synergy control, that PMC is strongly modulated by ipsilateral activity, and can encode action at an abstract level without specifying effector lateralization (Cisek et al., 2003).

7.4.4 The Role of PMC in Encoding Synergies

There is evidence from primates and humans to support synergies being expressed in PMC. Microelectrode recording from PMv and PMd shows neuronal activations predicting synergies in primates performing reach and grasp (Overduin et al., 2015).

Human fMRI studies identify PMC voxels whose activation predicts synergies controlling grasp (Leo et al., 2016). There is anatomical evidence with functional correlates in primates and humans to support PMC to primary motor cortex connections as a pathway by which synergies controlled by ipsilateral PMC can be effected to motor output in a way that would shape ipsilateral motor cortex maps. There is some experimental evidence to suggest that PMC action encoding is transformed to movement preparation in Pre-SMA (Rizzolatti and Luppino, 2001), but Pre-SMA lacks reciprocal output to PMC or output to primary motor cortex (Dum and Strick, 2005). A PMC to primary motor cortex route is demonstrated to function in humans.

7.4.5 Corticocortical Connectivity of PMC in Primates Demonstrates Intracortical Effects Capable of Influencing Primary Motor Cortex Motor Representations

PMC is heavily interconnected with primary motor cortex (Godschalk et al., 1984), providing around 70% of corticocortical input to primary motor cortex hand area (Darian-Smith et al., 1993). In primates microstimulation of PMC monosynaptically activates Lamina III and V pyramidal neurones in primary motor cortex (Ghosh and Porter, 1988). Stimulation of grasp modulated neurones in primate PMC results in excitatory, inhibitory and mixed responses in primary motor cortex hand area in primates, with reciprocal responses in PMC neurones seen to primary motor cortex stimulation (Kraskov et al., 2011). Observed motor output from PMC depends on stimulation parameters. Intracortical microstimulation of primate PMv with few pulses does not produce direct responses in hand muscles, but facilitates output from primary motor cortex (Cerri et al., 2003). High frequency repetitive stimulation of PMv can produce hand responses, but only if M1 is functionally intact (Schmidlin et al., 2008). The facilitation of primary motor cortex output by PMC stimulation occurs within M1, through late I-waves (Cerri et al., 2003; Shimazu, 2004). This information is proof that PMC output can alter intracortical circuits of primary motor cortex, a necessary condition to proposing that PMC can control cortical movement representations.

7.4.6 Functional Connectivity of PMC and Primary Motor Cortex in Humans Demonstrates Intracortical Effects Capable of Influencing Primary Motor Cortex Motor Representations

TMS studies confirm relevance of these primate studies to human brain, in healthy subjects and after stroke. Paired pulse TMS over PMv at appropriate interstimulus intervals facilitates MEPs from primary motor cortex hand area during grasp (Davare et al., 2009), but inhibits at rest (Davare et al., 2008). Using corticocortical Paired Associative Stimulation strengthens this pathway, again with a task specific polarity of effect. At rest, tonic inhibitory activity is enhanced, but the effect becomes facilitatory when PAS is combined with a visuomotor task (Buch et al., 2011; Ruge et al., 2012). Neuromodulation with rTMS and tDCS to PMv also alters primary motor cortex activity. Excitatory rTMS to PMv increases primary motor cortex activation on fMRI (Bestmann et al., 2005). Inhibitory 1HZ rTMS to PMv has been shown to either increase Intracortical Facilitation (Münchau et al., 2002), or Intracortical Inhibition (Gerschlager et al., 2001).

All of these studies further demonstrate that PMC has the ability to modulate primary motor cortex intracortical circuits, and can therefore shape motor maps.

7.4.7 Corticospinal Output from PMC Demonstrates Anatomical Organization Suited to Control of Ipsilateral Contribution to Synergies

Features of PMC corticospinal output are important to proposing a potential role for PMC in ipsilateral control of synergies. It was mentioned that in primates, motor output from PMC was dependent on primary motor cortex integrity to produce hand responses. The anatomical organization of PMC corticospinal terminations does not support direct motor output. The same features of spinal termination are suited to the proposed role in ipsilateral control of synergies, namely prominent reticulospinal and propriospinal terminations.

The strength of the PMC corticomotor output is weak relative to other secondary motor areas. PMC contributes only 4% of the frontal motor area contributions to corticospinal tract, compared to around half from primary motor cortex, and one-fifth from SMA and CMA (Dum and Strick, 1991). PMC corticospinal neurones are also smaller and slower than those from primary motor cortex (Murray and Coulter, 1981). Despite this relatively weak output, within the organization of this are features critical for synergy control in reach. PMd is more concerned with reach than PMv (Kurata and Hoffman, 1994), and accordingly has greater numbers of corticospinal neurones. In primates, PMd has projections to upper and lower cervical segments corresponding to regions of proximal and distal forelimb representation (He et al., 1993) but the majority of terminations are in the Intermediate Zone, rather than to hand alpha motor neurones (Galea and Darian-Smith, 1994). Corticospinal neurones from PMv have an unusual pattern of termination in that regions identified as hand area by microstimulation terminate on upper (C2-4) but not lower (C7-T1) cervical segments (He et al., 1993). This mismatch between the functional representation of the origin and the segmental termination has been interpreted as evidence that PMC corticospinal output utilizes propriospinal relays (He et al., 1993; Martino and Strick, 1987). This should be interpreted with the caution that propriospinal effects to hand muscles are

thought to be weak in monkeys (Cerri et al., 2003). Acknowledging this, there is a link to ipsilateral reach synergy representation, for which there is evidence in humans that it is mediated by propriospinal relays (Bradnam et al., 2010).

The other related factor from the observation of Intermediate Zone terminations of PMC corticospinal neurones (Dum and Strick, 2002) is the potential use of corticoreticulospinal pathways. There is anatomical evidence in primates for corticoreticulospinal fibres originating from PMC (Keizer and Kuypers, 1989). Ipsilateral synergies in humans are thought to be effected through corticoreticulospinal inputs onto propriospinal circuits (Bradnam et al., 2010; McCambridge et al., 2011; Schwerin et al., 2008). Therefore, the anatomical connectivity of PMC fulfils requirements for a role in shaping ipsilateral reach synergies both through interconnectivity with primary motor cortex, and with corticospinal output to spinal modules.

7.4.8 PMC May Influence Ipsilateral Synergy Control Both by Corticocortical and Corticospinal Connectivity

From this discussion it follows that the ability of PMC to shape synergies may take the form of both corticocortical and corticospinal change. Having stated the anatomical and functional potential for PMC to control synergies through corticospinal output to segmental modules, the finding of absence of corticomotor change, and absence of change in the CoG anterior coordinate in this experiment argues against this route being most active in these experiments. The interpretation of a potential role for PMC in explaining the observed medial CoG shifts is one of enhanced corticocortical connectivity from PMC to axial regions of primary motor cortex involved in reach.

7.4.9 Animal Models of the Role of PMC in the Post Stroke Motor Network

Numerous fMRI studies demonstrate increased PMC activity to compensate control of the paretic hand after damage to primary motor cortex. Animal models of ischaemic lesions of primary motor cortex provide important information about neural level plastic change that accompanies this observation.

7.4.9.1 *Lesion Studies Reveal the Relationship Between Primary Motor Cortex Lesions and PMC Plastic Response*

Following ischaemic primary motor cortex lesions in monkeys, return of hand function is seen in 1 to 2 months, with further improvement in skilled grip over 3 to 4 months to 30% of baseline functional scores. Cortical mapping at 9 months showed that no hand responses were seen to stimulation of ipsilesional or contralesional primary motor cortex, but were produced by stimulation of ipsilesional PMC. Furthermore, pharmacologically disrupting ipsilesional PMC resulted in loss of the recovered paretic hand function (Liu and Rouiller, 1999). The extent of this PMC compensatory activity is proportional to the extent of the primary motor cortex lesion. A similar study quantified the relationship between size of lesion and plastic response in PMC. When mapped at 12 weeks after the lesion, PMC forelimb maps had increased in size between 7 and 53% with PMC map expansion associated with the extent of the primary motor cortex lesion (Frost, 2003).

7.4.9.2 *Structural Plasticity of Primary Motor Cortex Intracortical Neurones in Response to Increased PMC Activity Post Stroke*

Structural plastic change accompanying PMC map expansion in response to ischaemic primary motor cortex lesion was investigated histologically 5 months after the lesion. Anatomical reorganization included altered axonal orientation of intracortical neurones in primary motor cortex (Dancause, 2005). This effect on intracortical circuits at a structural level is further evidence to support the proposed PMC to primary motor cortex

corticocortical pathway as a factor controlling the reorganization of the ipsilateral representation of muscles involved in paretic reach. Another observation from that experiment supports this corticocortical basis. Plastic reorganization included axonal sprouting and new terminations from PMC to sensory cortex. The distances involved lead to the suggestion that these connections were established through relays on existing intracortical circuits (Dancause, 2005).

7.4.9.3 *Lesion Studies Suggest a Role for Contralesional Cortex in Synergy Control*

A lesion study that combined functional imaging and focal pharmacological disruption provides a vital link between these examples of structural plasticity and ipsilateral representation of synergies. At a time when there was meaningful behavioural recovery of the paretic hand, bilateral activation of PMv was seen on PET in response to hand movements. Focal pharmacological disruption of GABAergic intracortical circuits in ipsilesional PMv lead to severe impairment of the recovered precision grip in the paretic hand, but disruption of contralesional PMv had variable effects, with no effect in one monkey (Murata et al., 2015). This particular task was precision grip with the arm supported. The lack of contralesional PMv effect on skilled dextrous function, despite strong PET activation, could be consistent with the contralesional hemisphere providing ipsilateral contributions to synergies required for reach, and therefore not manifest when grip is measured.

These animal studies provide a model of plastic change in response to stroke that is compatible with PMC actions on primary motor cortex intracortical circuits. This is supportive of the interpretation of the CoG change as representing a PMC driven reorganization of cortical circuits controlling ipsilateral synergies. It remains to relate this to studies of post stroke plastic reorganization in humans.

7.4.10 The Role of PMC in the Human Post Stroke Motor Network

Series of patients with lesions involving PMC but sparing primary motor cortex report predominant proximal impairment (Freund and Hummelsheim, 1984), particularly shoulder abduction in the upper limb (Freund and Hummelsheim, 1985). These observations are compatible with the observation of axial and proximal muscle responses to microstimulation of these regions in primates (Bucy, 1933) and humans (Woolsey, 1952). When primary motor cortex is lesioned but PMC is spared, then PMC performs compensatory recovery of distal function. Sparing of PMC by the stroke lesion has also been associated with a better prognosis for lower limb and ambulatory function (Miyai et al., 1999). Longitudinal fMRI studies show increased bilateral PMC activity which reduces over time following stroke (Ward et al., 2003). In contrast to progressive normalization of PMC activity, some studies report persisting bilateral PMC activation with excellent recovery of hand function after MCA stroke (Seitz et al., 1998).

Other studies show increased PMC activation in response to therapeutic interventions in subacute and chronic stroke patients. Training a wrist task in the subacute phase after stroke resulted in increased bilateral PMv activation on fMRI (Horn et al., 2016), and in ipsilesional PMC measured with NIRS after gait training (Miyai et al., 2003). Use of anodal tDCS to increase ipsilesional excitability, combined with upper limb physical therapy increased the strength of connectivity between contralesional PMv and ipsilesional primary motor cortex (Chen and Schlaug, 2016).

Following stroke, MEPs produced by stimulation of ipsilesional PMd were faster and larger than those from stimulation of ipsilesional M1, suggesting that some control of the paretic hand had reorganized to PMC (Fridman, 2004). If the lesion is small and sparing ipsilesional PMC, then TMS disruption of reaction time is only seen when applied to ipsilesional PMd. However, larger lesions also involving ipsilesional PMC result in the contralesional PMC becoming involved in control of the paretic hand (Fridman, 2004). Similarly, in patients with large infarcts, TMS to contralesional PMv 100ms before a go cue disrupted reaction time of the

paretic hand, and the size of the effect correlated with the degree of motor impairment, suggesting a greater reliance on contralesional PMv in the most impaired patients (Johansen-Berg et al., 2002). Using paired pulse TMS to probe connectivity between contralesional PMd and ipsilesional M1, the strength of facilitation in the pathway was seen to increase with functional impairment, as did fMRI activation of contralesional PMd (Bestmann et al., 2010).

Collectively these studies demonstrate a role for PMC in compensating primary motor cortex damage in control of axial, proximal and distal movements. The extent of PMC activation in these human studies is in agreement with the animal models, with the degree of activation scaling to the degree of functional impairment. Evidence for contralesional PMC activation and a role for PMC in reach and proximal movements in stroke patients is supportive of suggesting that PMC corticocortical connections influenced the medial CoG shift seen in the patients studied.

7.4.11 Direct Demonstration That PMC Can Represent Ipsilateral Synergies in Humans

Further supportive evidence of the role of the ipsilateral motor cortex and PMC in ipsilateral contributions to reach synergies is provided by studies of ECoG in epilepsy patients undergoing surgical evaluation. Recording during performance of reach tasks shows that spatial and temporal features of ECoG spectral patterns are able to describe ipsilateral movement parameters (Wisneski et al., 2008). Compatible with the idea that ipsilateral representation of synergies is reflected in changes in motor maps is the observation that both spiking and Local Field Potentials of widely distributed neural ensembles can accurately predict ipsilateral limb kinematics in reach. Furthermore recording sites with the strongest predictive value included primary motor cortex and PMC (Ganguly et al., 2009).

7.4.12 A Possible Explanation as to Why Ipsilateral Changes were Identified in Pectoralis Major in the Chronic Stage, but Emerged in Deltoid with Training

The experiment in the preceding chapter trained a skilled reach task and observed medial displacement of deltoid CoG. This experiment obtained MEPs using background activation produced by reach and observed a medial displacement of pectoralis major CoG relative to the contralateral CoG. Both experiments tested MEPs during reach, so would have activated reach synergies.

The robotic reach task placed demands on speed and accuracy of reach to target. Both pectoralis major and deltoid are variably employed as prime movers in hemiplegic reach. The additional skill requirement in the reach training may have favoured use of a synergy where deltoid is the prime mover. If deltoid is used as used as prime mover then additional Anticipatory Postural Adjustments are required to accompany the movement (Wagner et al., 2007). This may have increased the number of movement representations to be linked within the map and made map shifts apparent over short time periods. It may be that in daily life, without specific training stroke patients may preferentially use a computationally simpler reach strategy that has pectoralis major as the prime mover.

Experimental evidence relating to stability of maps and task complexity may also be relevant to the change in emphasis from pectoralis major to deltoid CoG observed with training over short time periods. Increasing complexity of synergies demands greater fractionation of the cortical motor map. In a primate study recording Stimulus Triggered Averaged EMG from forelimb muscles and intracellularly from primary motor cortex pyramidal cells during upper limb tasks of varying complexity, polarity of cortical output from facilitation to suppression varied with task complexity. The polarity of motor cortex output was generally stable across joint range changes and whole arm postural changes. However, synergies associated with more complex tasks resulted in instability of cortical motor output effect. This would drive change in the cortical map to provide greater

fractionation (Griffin et al., 2009). Related to these findings, the more complex robotic training could have driven a change evident in deltoid when task complexity required greater fractionation of a synergy representation that had previously reorganized to favour pectoralis major as the prime mover.

7.5 SUMMARY

The main conclusions are:

1. A medial displacement in the CoG of the ipsilateral Pectoralis Major representation relative to the contralateral representation in the contralesional hemisphere was associated with improved functional performance in chronic stroke patients who had made good recovery in comparison to those with poor recovery.

2. A medial CoG shift of ipsilateral Deltoid representation in the contralesional hemisphere was seen in response to training in a skilled reach task in chronic stroke patients who showed functional improvement with training.

3. These changes in ipsilateral cortical organization were not associated with changes in the size of the cortical map or the strength of corticomotor connectivity. This suggests that the ipsilateral cortical changes are concerned with some other aspect of motor control of reach, such as contribution to synergy control.

4. The mechanism proposed by animal models of increased corticocortical connectivity between PMC and primary motor cortex in the post stroke motor network is compatible with these observations, but not directly testable with these measures. The functional and anatomical properties of PMC described make it a strong candidate for controlling ipsilateral contribution to synergy expression in the contralesional hemisphere after ischaemic damage to primary motor cortex.

5. TMS paired pulse studies provide a tool to probe this potential connectivity, and it would be of interest to experiment further to test this possibility.

A more detailed neural basis for a synergy based explanation for the cortical map changes will now be developed in further discussion in terms of potential anatomical substrates and the cellular level plastic processes that

could produce the map changes. The relevance of these findings in the context of current models of stroke recovery will be explored.

CHAPTER 8
DISCUSSION

The main findings of the experimental chapters are now summarized, in order to identify common features which are then linked through a proposed unifying mechanism. The findings from the athlete study provide the first demonstration of experience dependent plasticity driven cortical reorganization of the ipsilateral hemisphere. The lack of ipsilateral change in the singers study argues against the ipsilateral reorganization favouring direct corticobulbospinal output. The finding of training related and functional performance related ipsilateral reorganization in chronic stroke patients suggests the findings in the athletes study are relevant to post stroke motor recovery. The challenge is to link the process of driving ipsilateral plastic change to improved skilled motor performance in athletes to therapeutic intervention in the chronic phase after stroke. A mechanism that explains the findings at synaptic, neuronal, cortical, corticofugal and spinal premotor levels is proposed. Evidence to support this mechanism is presented to develop the argument. Finally the place of the proposed mechanism in stroke rehabilitation is discussed in terms of patient selection, timing and nature of interventions.

8.1 A REVIEW OF THE MAIN FINDINGS IN RELATION TO A HYPOTHESIS THAT THE IPSILATERAL HEMISPHERE CONTRIBUTES TO SKILLED MOTOR CONTROL THROUGH CORTICAL SYNERGY REPRESENTATIONS

8.1.1 Athlete Study

Study of elite athletes revealed specific patterns of differences between ipsilateral and contralateral cortical representations of axial muscles. The patterns were explained in terms of the task specificity of the motor skill trained. Muscles habitually bilaterally activated in axial synergies and under continual direct influence of ipsilateral motor pathways conveying central postural drive displayed significant ipsilateral differences in cortical motor representations. In contrast the muscle most unilaterally activated displayed contralateral representation changes.

Features of the ipsilateral motor representations were different between groups of different levels of deliberate practise and skill acquisition. Compared to trainee athletes, intermediate and expert athletes had a stronger, more expansive ipsilateral representation. This was evidenced by a higher total mean MEP area for the stimulated map, combined with low Map Volume measures. This change with experience is interpreted as demonstrating a beneficial ipsilateral contribution to skilled motor control. There was no further increase in the extent of the representation between intermediate and expert groups, showing that the ipsilateral change is not simply a feature of repetition. However, there was a difference in the organization of the ipsilateral representation, suggesting that once an extensive cortical network has been established, another plastic process refines the cortical representation in acquisition of higher levels of skill. These finding are informative of both the nature and the time course of training required to alter ipsilateral representations of axial muscles performing skilled movements.

Two main features of the cortical representations suggested observed differences could be explained in terms of cortical representations of axial

synergies. Cortical control of synergy representations is distributed between ipsilateral and contralateral hemispheres. The ipsilateral hemisphere is most concerned with coactivated muscles and antagonists, controlled through corticospinal and corticoreticular pathways to spinal premotor networks. The contralateral hemisphere controls agonist muscles through corticomotorneuronal and corticospinal pathways. The observation of expansive ipsilateral and focal contralateral representations is compatible with this scheme. Synergies also function to coactivate segmentally distant muscles, through reticulospinal pathways with axonal branching over multiple levels and propriospinal circuits with long axonal projections. The observation of change in a proximal and distal muscle representation is therefore also compatible with a synergy based explanation.

There is no other direct experimental evidence to support either experience dependent plasticity altering ipsilateral cortical representations, or a differential ipsilateral expansion and contralateral focussing of motor maps in synergy representations. Supportive evidence is extrapolated from studies of experience dependent structural cortical change in response to deliberate practise. These provide a synaptic, neuronal and network level mechanistic hypothesis by which to develop discussion of the findings. The cortical synergy based explanation for these findings remains resilient when tested against fundamental principles of synaptic plasticity and canonical principles of cortical circuitry.

A stepwise progression from trainee, through intermediate to expert in the ipsilateral findings is useful in considering other plasticity defining features relevant to stroke recovery. This progression adds certainty that the ipsilateral findings represent true deliberate practise effects, rather than study of a group who self-select toward a pursuit because they possess a genetic predisposition through ipsilateral motor pathway variants which confer a performance advantage. Possible inter-individual factors to explain variation leads to discussion of how neurophysiological metrics of ipsilateral motor pathways can be usefully employed in patient selection for therapeutic approaches after stroke.

The findings from the athlete study provided the foundation for further work. Evidence for a synergy based mechanism was then tested in singers who had accumulated a comparable level of deliberate practise in the acquisition of a similar level of skilled motor control, training similar muscles but in a very different way. It was hypothesized that there would be task-specific differences in the movement representations, and that singers would be more likely to utilize direct ipsilateral corticobulbospinal pathways.

8.1.2 Singers Study

The hypothesis of deliberate practise driven access to ipsilateral corticobulbospinal pathways was tested longitudinally in trainees, and cross-sectionally in trainee, intermediate and expert singers. This was motivated by knowledge of the potential for ipsilateral output through alternative motor pathways from secondary motor areas upregulated in the singing motor network in expert singers.

Longitudinal effects of daily deliberate practise on changes in patterns of muscle use and corticomotor connectivity were monitored over one year in trainee singers. It was hypothesized that skilled bilateral activation of axial muscles would lead to strengthened ipsilateral corticomotor connectivity. It was also hypothesized that corticomotor change would be most evident in muscles that most altered their contribution to performance of the motor task. Neither of these predictions were supported. Only strengthened contralateral corticomotor connectivity was seen over time in scalene, and this was not associated with increased scalene use in the task.

Cortical mapping was performed in groups of singers with comparable levels of deliberate practise and expertise to the corresponding groups of athletes. No ipsilateral differences in cortical axial movement representations were seen. The contralateral representation of diaphragm was increased in the expert singers compared to the trainees. Diaphragm was the muscle that had displayed the greatest increase in contribution to the task in the longitudinal training of trainee singers, allowing this cortical finding to be interpreted as task-relevant.

It is notable that in both longitudinal and cross sectional studies only contralateral changes were observed. The location of contralateral change emphasises the relevance of this in relation to the hypothesis. Both changes occurred in obligate inspiratory muscles. These muscles are under strong control of Central Respiratory Drive Potentials, distributed by ipsilateral bulbospinal pathways. As such, these were hypothesized to be the sites where ipsilateral corticobulbospinal activity would be most evident if

trained by the task. The failure to observe any ipsilateral change, and in fact to find the strongest contralateral change, at sites where ipsilateral activity was perhaps most likely argues against the hypothesis that singers train skilled axial motor control through ipsilateral pathways. The purely bilateral nature of the task and abundant ipsilateral innervation of the muscles emphasizes the negative finding. It was concluded that the voluntary contralateral and involuntary ipsilateral pathways of respiratory motor control remain functionally distinct, and governed by neuromechanical principles which do not lend themselves to plastic interaction.

The investigation had been prompted largely by clinical observations of Cingulate Motor Area output producing axial motor output in tetraplegic patients with bilateral corticospinal tract lesions, linked with the observation of ipsilateral output from Ventral Respiratory Group bulbospinal motorneurones contracting axial muscles. The lack of evidence of corticobulbospinal activity being trained in expert singers is taken as tacit support for the synergy based interpretation of the athlete study. If the athlete findings had a direct corticobulbospinal basis, then this would be anticipated in the singers also. The absence favours an alternative explanation for the athlete findings. Respiratory synergies are known to be active in controlling posture during voluntary respiratory manoeuvres, so the counter argument could be applied, that if the athletes were using ipsilateral cortical synergy control then this should also be evident in the singers. The task specificity of the different motor tasks explains why this does not follow. The respiratory synergies predominantly control lower body segments, and would not have been so active during TMS performed during quiet breathing in a seated position.

The study of singers contributed little to advance the theory of ipsilateral motor output contributing usefully to synergy control, but also did not detract from it. Having demonstrated that ipsilateral motor output can usefully serve skilled motor control, evidence was sought that it may do so after stroke.

8.1.3 Stroke Study

Associating ipsilateral plastic change with improved functional performance in chronic stroke patients was a pivotal test of the concept of beneficial ipsilateral plastic change because of the potential for ipsilateral activity to be maladaptive in this setting. Stroke patients were also studied longitudinally and cross-sectionally.

The effect of a short period of training in a skilled robotic reach task on ipsilateral motor representations was monitored. The training was of a skill level, duration and intensity comparable to those shown to induce structural plasticity over similar time periods in longitudinal studies of skilled motor training. There was no change in the extent or strength of the ipsilateral motor representation. A medial shift in the Centre of Gravity of the cortical motor representation of deltoid was seen after training, only in patients who achieved functional improvement. The restriction of change to deltoid demonstrated task-specificity as deltoid can function as a prime mover in the task.

A cross sectional approach was used to assess experience dependent plastic change in ipsilateral representations. Chronic stroke patients were grouped into those who had shown good and poor motor recovery based on functional measures of upper limb performance. Motor mapping was again performed during active reach of the paretic arm. A medial displacement of the ipsilateral Pectoralis Major representation relative to the contralateral representation was seen in those patients who had made good functional recovery. Again this finding demonstrates task-specificity since Pectoralis Major can also function as a prime mover in this role.

The finding of a medial shift in Centre of Gravity in the patients with the best functional measures in both studies was interpreted as evidence of ipsilateral plasticity in the contralesional hemisphere contributing to improved motor performance. The medial displacement was interpreted according to the synergy based explanation, proposed to represent increased synaptic connectivity between proximal and axial cortical representations,

essential for stabilizing the upper limb during reach. Whilst evidence shows that cortical representations are overlapping, intermixed movement representations, the general principle holds that axial representations are more medially located than upper limb representations.

An expansion of the ipsilateral representation analogous to the athlete study was not found in the stroke patients. There may again be a task-specific or experience-dependent explanation for this. The athletes trained a complex whole-trunk combined with reach task and were measured with TMS during whole-body activation. The stroke patients were trained in skilled reach and measured with TMS when performing reach. The number of muscles coactivated in the movement was therefore greater in the athlete study, which would have predicted a more expansive synergy representation. It is also hypothesized that the expansive ipsilateral representation seen in athletes was the result of deliberate practise accumulated over thousands of hours. It is not possible to make direct comparison between athletes and stroke patients who rely on very different plastic processes, but it is unlikely that the time course of plastic change with standard post-stroke therapy approached that of the athletes.

The change in ipsilateral cortical reorganization was observed in deltoid and pectoralis major representations in the chronic and trained state. It can be suggested that the added skill element of the trained task introduced an advantage to increased use of synergies incorporating deltoid. Use of deltoid as the prime mover places a higher demand on accompanying Anticipatory Postural Adjustments, which may have also amplified the ipsilateral change through increasing demand on axial synergies. This difference raises an important point in relation to stroke therapy. The use of pectoralis major in the chronic stage may represent use of compensatory movement in reach, whereas the change in deltoid a correction of impairment. Being able to describe this through measurable cortical movement representations introduces the idea of neurophysiological measures of ipsilateral activity to plan and monitor therapeutic approaches.

Reorganization of ipsilateral cortical representations was associated with improved function after stroke. This provides a common link to suggest that the findings from the athlete study can be extrapolated for developing post-stroke therapy. Another important aspect of the stroke study was exclusion of maladaptive consequences of ipsilateral activity. The Laterality Index was used to measure reliance on ipsilateral pathways, tested against spasticity and upper limb functional measures. There was no association with spasticity, but greater reliance on ipsilateral pathways associated with poorer function. This is consistent with predictive algorithms which show integrity of ipsilesional corticcospinal tract determines good recovery. The Laterality Index finding here reflects reliance on residual pathways rather than maladaptation, evidenced by the parallel finding that reliance on ipsilateral pathways does not limit further improvement with training.

8.1.4 Pursuing a Unifying Explanation Based on Cortical Synergy Representations

Expansion of the ipsilateral motor map results from increased connectivity of multiple overlapping movement representations synaptically linked through coactivation in the task. The ipsilateral hemisphere controls coactivated and antagonist muscles in a synergy with corticofugal output integrated by spinal segmental and premotor interneurons. A greater number of coactivated muscles will require a larger cortical network to represent the synergy, seen with the axial-reach synergy in the athlete study. The anterior shift in Centre of Gravity reflects linking of secondary motor areas involved in axial control, a source of corticoreticular output to propriospinal premotor circuits coordinating synergy output. The medial shift of Centre of Gravity in the stroke study reflects strengthened connectivity between coactivated axial and proximal muscles. Synergy representations become apparent in TMS motor mapping since activation of synergistic movement brings a greater population of widely distributed cortical motorneurones closer to threshold.

This review of findings has identified features compatible with an explanation based on cortical synergy representations. This theory is now developed through discussion of supportive evidence. Basic operational principles of cortical, microcortical and corticofugal processes need to be shown to be compatible with this interpretation. Motor control theories and human neurophysiological evidence that describes the cortical control and neural pathways of axial synergies are presented as the foundations of this theory.

8.2 BASIC FUNCTIONING AND HIERARCHICAL ORGANIZATION OF SYNERGY CONTROL

Contraction of muscles spanning multiple interdependent joints will produce secondary torque in remote joints. This will degrade the quality of the agonist movement unless stabilized by co-contraction of associated muscles acting on related joints. Agonist and stabilizing muscle activation must be controlled in parallel with antagonist inhibition. Feed forward motor programs need to be continually updated by afferent feedback. Dynamic integration of activation of agonists, inhibition of antagonists and coactivation of stabilizers would be computationally exhausting for vast permutations of joint angles and torques. Computational demands of execution of movements are added to by the need for additional accompanying feedforward motor programs in the form of Anticipatory Postural Adjustments. Synergies provide computationally efficient coding of spatial or spatiotemporal patterns of muscle co-activation for common movement. Coordinated activation patterns are encoded in synergy modules, which can be linearly combined to construct complex movements from basic modules. Synergy modules may be organized at cortical, brainstem or spinal levels. Feedforward cortical output is used to generate an internal copy via a brainstem-cerebellar relay, and further computation and afferent updating is performed at spinal premotor level.

Motor cortex can control synergies in a number of ways. Cortical synergy modules are described in caudal primary motor cortex with direct distribution of corticomotorneuronal output to cervical Lamina IX motorneurones (Rathelot and Strick, 2009). Corticofugal output to premotor interneurons in downstream synergy modules is a more prevalent method of cortical synergy control. Spinal modules may be premotor interneurons in segmental intermediate zone modules, or propriospinal neurones. Inputs may be corticospinal or corticoreticulospinal (McMorland et al., 2015). Impairment of synergies after stroke is therefore dependent on the level of the lesion and levels of sparing synergy modules. Cortical damage, which spares downstream modules, leaves them sensitive to abnormal recruitment by alternative corticofugal commands. Conversely, subcortical lesions leave

cortical synergy modules sensitive to compensatory reorganization by intact cortex. Motor cortex can represent and control synergies in a number of ways amenable to plastic reorganization, sensitive to training and pathology.

8.2.1 Cortical, Subcortical and Spinal Synergy Modules

A basic operational principle is that cortical, spinal or brainstem synergy modules can be linearly combined to construct complex movement. Some of the synergy modules encode only temporal, and others spatiotemporal parameters of movement. Temporally sequential combination of synergy modules is also used. Awareness of downstream computation is important to understanding the organization at cortical level.

Synergies are encoded at spinal premotor level. Post spike effects generated by a single cervical premotor interneuron are seen in multiple hand muscles, revealing spinal synergy modules encoding coactivation for pinch and grasp in primates (Takei and Seki, 2010). Spatial and temporal encoding by Intermediate Zone premotor modules is seen (Hart and Giszter, 2010). Stimulation of these interneurones produces coactivation patterns resembling natural movements (Bizzi et al., 2008). Stimulation of just two Intermediate Zone sites was sufficient to produce synergistic patterns of muscle activation used in reach (Zimmermann et al., 2011). Animal studies also suggest brainstem level synergy modules. Multiple level brainstem transections reveal the level at which the repertoire of natural motor behaviours is reduced, showing dependence on synergy modules upstream of the spine (Roh et al., 2011).

These studies illustrate the principle of multi-level organization of synergy control. The emphasis in interpreting the mapping studies is on the cortical control of these downstream modules. There is evidence that human synergy control makes use of the complex premotor circuitry of propriospinal systems. Principles of cortical control of synergies can be discussed toward developing the idea of a control system based on corticofugal output to propriospinal circuits.

The nature of cortical synergy representation is variable. Synergy modules may encode time-invariant or time-varying synergies, and the cortical features of the representation can vary accordingly. Some synergy representations of common movements such as pinch and grasp are focal and relatively hard wired. Other synergy modules are widely distributed and dynamically fractionated. This difference is important when considering plasticity driven motor map reorganization.

8.3.1 Focal, Hard Wired and Widely Distributed, Dynamic Cortical Synergy Modules

8.3.1.1 *Focal, Hard Wired Corticomotorneuronal Synergy Modules*

In primates performing wrist movements, a single primary motor cortex pyramidal neurone has post-spike effects in multiple intrinsic hand muscles. Cortical motorneurones with muscle fields greater than 2 produced monosynaptic post-spike facilitation profiles, showing the synergy representation was controlling synergies corticomotorneuronally (Fetz and Cheney, 1980). A similar experiment examining precision grip confirmed similar post-spike effects, with a mean muscle field of 3 (Bennett and Lemon, 1994).

Retrograde labelling of primate primary motor cortex pyramidal cells shows that corticomotorneuronal cells are confined to caudal regions, the anterior bank of the sulcus. Corticomotorneurone cells project proximally and distally. Sites where stimulation produces shoulder, elbow and hand movements are intermixed within the upper limb representation. This anatomical arrangement of multiple, intermixed representations within a focal area allows for direct control of synergies. Accordingly, groups of these neurones are shown to display activity synchronous with coactivation in skilled motor tasks (Rathelot and Strick, 2009).

This situation is relevant to the finding of focussed, powerful contralateral representations in the athlete study with high mean map responses, but focal map volumes. The finding that proximal muscles are equally represented by this system is relevant, and adds to the historical view that fractionated finger movements were the dominant focus of this system. It could be anticipated that once optimal representations are established, a corticomotorneuronal system would be more resistant to motor representation reorganization.

8.3.1.2 *Dynamic, Widely Distributed Corticofugal Synergy Modules*

Other synergy representations are more widely distributed across motor cortex, and with properties that lend to dynamic reorganization. Recording

from primary motor cortex in primates identifies single pyramidal cells that produce coactivation, are activated by different synergies and are linked in ensembles that could construct complex movements when stimulated (Holdefer and Miller, 2002). This demonstrated individual neurones participating in multiple synergy representations to suggest multiple overlapping synergy representations and maps of movements. Such organization was confirmed in a primate mapping experiment.

Six synergies were identified in primates that described the patterns of forelimb muscle activity in natural behaviour. Microstimulation of motor and premotor cortex identified regions which evoked synergies resembling those of natural behavioural.

Stimulation sites that reproduced the six natural synergies were mapped. A non-uniform pattern of representations across motor cortex extending to premotor regions was found (Overduin et al., 2012). A similar experiment confirmed and extended these findings.

Recording from motor cortex pyramidal cells showed that natural use of the synergies in freely behaving monkeys generated patterns of neural activity corresponding to the neural populations mapped by microstimulation. The cortical recording showed that the neural activity encoded both spatial and temporal patterns of synergistic muscle activity (Overduin et al., 2014). This demonstrates widely distributed, overlapping synergy representations over broad cortical regions extending anteriorly from primary motor cortex, equivalent to the findings in the ipsilateral hemisphere in the athlete study. This pattern of cortical organization allows well trained movements to be efficiently programmed from few cortical synergy modules linked within a circuit, requiring few cortical controllers to generate complex commands (Diedrichsen and Classen, 2012).

8.3.2 Cortical Topography of Synergy Representations

The topographic organization of synergy representations is important to interpretation of the findings of the mapping studies. In humans, kinematic and EMG analysis of hand movements evoked by TMS shows similarity to those used naturally in grasp. Stimulation of 30 cortical sites revealed a topographic organization to hand synergies within motor cortex (Gentner and Classen, 2006). A subsequent study showed this topography is sensitive to deliberate practise in experts (Gentner et al., 2010).

Primate studies identify a feature of mapping synergy representations important to the findings of the stroke study. Tetanic cortical microstimulation can produce complex multi-joint movements with coordinated muscle coactivation. Stimulating one cortical site evoked synergies that produced grip, forearm supination, elbow flexion and shoulder rotation to bring hand to mouth. Irrespective of the starting position of the limb, the end position was constant each time. When adjacent cortical sites were stimulated, synergies were constant but the end position of the hand changed. Within the forelimb representation a map could be constructed of the final spatial target of the evoked synergies, with a gradient of contralateral to ipsilateral end positions mapped along an axis (Graziano et al., 2002). This demonstrates that the cortical representation of synergies is organized to encode complex properties of the movement beyond spatial and temporal activation patterns. The implication for interpretation of the mapping studies is that increasing the skill requirement of the practised movement would be anticipated to alter the cortical synergy representation. This is particularly relevant to the stroke study, where the robotic training included a requirement to correct movement perturbation in reaching to a target.

A similar study confirmed a topographic map in primate motor cortex of final spatial targets of movements irrespective of starting position (Griffin et al., 2014). Topographic maps of synergy representations can also be mapped in rat motor cortex for reach, grasp and forelimb retraction. Stimulation of a focal area can produce sequences combining these

synergies to produce complex movements (Ramanathan et al., 2006). These studies suggest that within widely distributed representations are cortical controllers that can link synergy representations for complex movement.

8.4 CORTICAL CONTROL OF SYNERGY REPRESENTATIONS

Cortical synergy modules are organized to simplify control of complex movements by fewer cortical controllers. The nature of the controllers is not clear. Animal studies identify cortical points that can combine synergies into complex movements with tetanic stimulation (Ramanathan et al., 2006). The human equivalent would be observation of natural movements evoked by focal epileptogenic zones, such as the 'fencing posture' in SMA seizures. The limited resolution of many millimetres for subdural electrodes in epilepsy patients does not allow the suggestion that the trigger is a discrete cortical point (Sato et al., 2013). Overlap of propagation of ictal bursts and synergy activation through recurrent intracortical circuits in animal studies suggests the analogy is valid (Capaday, 2004; Capaday et al., 2009). Some principles of the cortical control are useful in progressing discussion of a synergy-based explanation for the findings since it introduces validation according to canonical cortical principles.

8.4.1 Cortical Controllers Utilize Spatial and Temporal Summation of Synergy Modules

The cortex can control movements by spatial and temporal summation of synergy modules (Yakovenko et al., 2011). Simultaneously stimulating separate but remotely interconnected cortical synergy representations produces linear summation of the muscle activation patterns (Ethier, 2006). Reliably linear behaviour of summation simplifies calculation of the effect of combining synergies in constructing complex movements. This reliability is shown when the combined post-spike effects of two cortical motorneurones representing different synergies combined in a movement are considered. The summative effects of double spike-triggered averaging always behave linearly (Jackson et al., 2003; Sanes and Truccolo, 2003). This principle is relevant to the interpretation of training effects in terms of synergy representation expansion.

8.4.2 Corticocortical Control of Synergy Representations

Corticocortical networks are active in the control of synergy representations. The network between primary motor cortex, premotor and parietal cortex is well characterized for the control of expression of reach synergies in primary motor cortex. Transient pharmacological manipulation of the corticocortical connections from these areas alters the distribution of the synergy representation in primary motor cortex (Stepniewska et al., 2014). This is relevant to interpretation of the mapping studies, where changes in Centre of Gravity may be influenced by balance of corticocortical inputs.

8.4.3 Oscillatory Control of Cortical Synergy Representations

There is evidence that synchronization of widely distributed neuronal ensembles is used to control synergy representations, with consequences for the synaptic activity stabilizing the representation. Synchrony is important to synergy control. In humans, coactivating distal muscles synchronizes their cortical representations (Brown and Marsden, 2001). In primates, remote pairs of motor cortex pyramidal cells linked in synergy representations by common muscle fields show synchronous spiking in cross correlation studies. However, if pairs of neurones linked in the synergy control are agonist and antagonist, synchrony becomes less than in unrelated pairs (Jackson et al., 2003).

Synchrony provides a means of amplifying motor output and weighting strength of inputs to the synergy representation (van Wijk et al., 2012). A diffuse neuronal population that discharges synchronously would summate output effect at spinal level. This is relevant to the suggestion that the ipsilateral representation in the athlete study was an expansive population of numerous points each individually with relatively weak output. If governed by synchrony, post-synaptic potentials would summate and the collective strength of the synergy representation output would increase. Interaction of motor cortex beta-oscillation and the oscillatory activity of a cortico-muscular-cortical loop from synergy representations has been proposed to produce resonance to control synaptic stability in the representation (Aumann and Prut, 2015).

Enhanced synchrony is recognized to be a product of deliberate practise. Increased corticocortical synchrony is seen with motor learning in primates (Laubach et al., 2000), and humans (Serrien and Brown, 2003). A primate experiment allows the importance of deliberate practise developing synchrony in these systems to be extrapolated to the athlete study. Comparing monkeys trained in complex motor tasks for 5 years compared to 1 year, there is an increase in motor cortex pyramidal cells with multiple muscle fields, showing increased synergy representation. The Peak Width at Half Maximum (PWHM) of the post spike histogram differentiates narrow

monosynaptic corticomotorneuronal inputs from wider corticospinal inputs to the interneurone pool. The monkeys trained for 5 years had much wider PWHM showing synchronization of multiple weak descending inputs (Schieber, 2002). This situation is directly equivalent to that proposed to explain the findings in the ipsilateral mapping study in the athletes, increased synergy representation expanding the map, and use of synchrony to bind multiple small motor output effects to summate at spinal level allowing diffuse weak representations to have a powerful effect.

8.5 CORTICAL FRACTIONATION AND STABILIZATION OF SYNERGY REPRESENTATIONS

The basis of cortical representations of synergies is synaptic linkage of multiple, intermixed movement representations into widely distributed neuronal ensembles. To relate TMS measures of synergy representations to their function requires knowledge of the cortical circuitry involved. To respond to training the circuits must be fractionated to incorporate new motor learning. Once optimal motor control has been achieved the circuits must be stabilized. The balance of excitation and inhibition in intracortical circuitry determines conditions for development of Long Term Potentiation, Long Term Depotentiation and synchrony. The weight of short and long range inputs in turn determines regional intracortical excitability.

8.5.1 Functional and Anatomical Recurrent Intracortical Connectivity and Synergy Representations

Physical rules govern the behaviour of recurrent intracortical circuits. The findings of reorganization of motor representations in athletes and stroke patients are compatible with these rules, which also support the proposed explanation in providing a mechanism of map expansion. Injecting tracer to layer III and V pyramidal cells in cat reveals anatomical connectivity, and microstimulation reveals functional connectivity. Anatomical connections extend 7mm through Horizontal Fibre axon collaterals, with extensive synaptic boutons across their length. Superimposing functional connectivity maps shows strongly recurrently interconnected representations of a variety of proximal and distal muscles (Capaday et al., 2009). The extensive synaptic interconnection allows any cortical point to influence another. There is a dense core of connectivity seen around 3mm2 from a cortical point. Under conditions of normal balance of excitation and inhibition, current spread from activation of a single point is around 1.5mm, but when inputs to the system alter, excitability spread can be up to 7mm. This is the basis of reorganizing cortical representations. Inputs to the system alter excitability, which allows the synaptic strength of connections at remote but recurrently interconnected points to be adjusted to incorporate coactivated neurones into the representation.

8.5.2 Canonical Cortical Microcircuitry and Reorganization of Synergy Representations

The canonical cortical circuit is based on recurrent excitation and inhibition within cortical microcircuits. These balance inputs to layer III pyramidal cells with output from Layer V cells (Beul and Hilgetag, 2014). The cortical network will be balanced by changes in inhibitory activity in response to excitatory input. Output is a linear function of inputs despite non-linear properties of the cortical circuit. Balance is achieved by the recurrent interconnectivity described above, and the principle that every neurone in a target population receives synaptic input from projection axons (Capaday et al., 2013). The effect of an excitatory input will be balanced by recurrent changes in cortical points linked by Horizontal Fibres. Experimental proof of linear behaviour of inputs and outputs is provided by pharmacologically releasing inhibition from one of two cortical points linked in a synergy representation. Linear summation persists even when non-linear behaviour is artificially encouraged (Ethier, 2006). The network recurrently balances this perturbation to preserve linear behaviour.

These principles can be related to the proposed interpretation of ipsilateral map expansion. Excitatory input to a cortical point could result in a remote reduction of inhibition, allowing the spread of excitation to interconnected points, so expanding the map.

8.6 DELIBERATE PRACTISE EFFECTS ON CORTICAL SYNERGY REPRESENTATIONS

There is very limited evidence from comparable studies of practise effects on synergy use by which to compare my findings. Short term training studies, animal studies and the limited studies on expert athletes and musicians are not directly comparable, but are broadly supportive and do not counter the argument. A single study on cortical synergy representations in experts confirms a deliberate practise cortical reorganization of synergy representations.

Studies of training complex whole body tasks demonstrate that improvement is achieved by increasing use of synergies that stabilize variance toward the desired outcome (Asaka et al., 2008; Danna-dos-Santos et al., 2008). This motor behaviour follows predictions according to the Uncontrolled Manifold Hypothesis of motor control (Latash et al., 2005). These observations emphasize importance of feedforward internal copies of motor programmes, a feature of the corticoreticulopropriospinal pathways of ipsilateral synergy control proposed as interpretation of the map findings.

Training skilled reach and grasp in rats changes the synergies used. This associates with increased neuronal populations in motor cortex with shared muscle fields and the synchrony between them (Kargo and Nitz, 2003). Similar findings with skilled motor training over many years are seen in primates (Schieber, 2002).

The findings of studies of synergies in expert athletes vary depending on the complexity of the motor task. In unskilled tasks such as cycling the synergies used by experts are similar to untrained subjects (Turpin et al., 2011). In complex whole body tasks that can only be performed with deliberate practise, expert athletes with very different biomechanical profiles use the same synergies (Frère and Hug, 2012). There are no studies of the cortical representation of synergies in expert athletes. Expert musicians alter cortical synergy representations controlling hand movements. The kinematics and EMG patterns of TMS evoked finger movements are altered by violin and piano training (Gentner et al., 2010).

8.7 AXIAL AND RESPIRATORY SYNERGIES

The observed differences in ipsilateral cortical synergy representations between the groups have been explained in terms of task specific plasticity. It should also be questioned whether there are fundamental differences in the organization of axial, reach and respiratory synergy representations that could provide alternative explanations. Given the lack of comparable studies in expert performers, studies of these synergies in normal subjects may provide useful context for interpreting findings.

8.7.1 Axial Synergies

Forward movements of the upper trunk are stabilized by backward movements of lower body segments (Alexandrov et al., 1998). As speed of movement increases, synergistic muscle activation becomes earlier in relation to the prime movers (Crenna et al., 1987). As amplitude increases, distal synergistic muscles become activated earlier (Oddsson and Thorstensson, 1987). This demonstrates axial synergies are generated centrally as a feed-forward component of the motor programme. Cortical origin is also suggested by the persistence of unaltered axial synergies when trunk movements are performed without postural equilibrium constraints, in zero-gravity on parabolic flights (Vernazza- Martin et al., 2000), and underwater (Massion et al., 1995).

8.7.1.1 *Evidence for a Cortical Controller of Axial Synergies*

Evidence for SMA as a cortical controller of axial synergies is suggested by abnormal axial synergies in patients with deficient subcortical inputs to SMA in Parkinsonian diseases (Massion, 1992). Use of fMRI to identify cortical regions activated by voluntary contraction of muscles habitually coactivated in axial synergies (Asavasopon et al., 2014) and locomotor synergies (Rana et al., 2015) identifies SMA as a possible controller. SMA is functionally linked within an extensive motor network concerned with axial synergy control. This finding is consistent with the known functional involvement of SMA in axial control and production of Anticipatory Postural Adjustments. This is also consistent with the finding of anterior displacement of Centre of Gravity in the ipsilateral representation of the expert athletes.

8.7.1.2 *Deliberate Practise Effects on Axial Synergies*

Further support for the idea that deliberate practise can increase a role for SMA in control of axial synergies is provided by studies of short-term training and accumulated deliberate practise. Training a throwing task that demanded both postural stability and arm control showed that improved throwing accuracy related to change in the axial synergy (Yang and Scholz,

2005). Expert gymnasts develop an axial synergy with a distal anticipation pattern of muscle activation. Earlier coactivation of gastrocnemius, erector spinae and biceps femoris is used to reduce centre of gravity ground displacement across different movement velocities (Pedotti et al., 1989).

8.7.2 Respiratory Synergies

Respiratory synergies are rhythmic trunk movements which provide postural compensation for the effects of rib cage movement in breathing to stabilize centre of gravity (Massion, 1992). They rely predominantly on hip movements (Bouisset and Duchêne, 1994). Measuring coactivation patterns between ankle, knee, hip, trunk and neck movements during different patterns of respiration reveals synergies controlling postural responses to respiration (Kuznetsov and Riley, 2012).

There are no direct demonstrations of cortical representations of respiratory synergies, but this can be inferred from their disruption following cortical infarcts (Manor et al., 2012). There is no direct evidence of training effects altering respiratory synergies, but one experiment provides evidence to suggest this is possible. When the highest demands of accuracy are placed on a pointing task, respiratory synergies modulated to provide increased postural support (Kuznetsov et al., 2011). This suggests that respiratory synergies could be trained to improve skilled motor control.

There are similarities between respiratory synergies and axial synergies, which raises the question of why respiratory synergy representations were not seen in the singers study. Respiratory synergies are less effective in the sitting position and rely largely on hip movements (Bouisset and Duchêne, 1994; Massion, 1992), so may not be apparent in the muscles recorded in the singers study.

8.8 PROPRIOSPINAL NEURONES AS EFFECTORS OF IPSILATERAL CORTICAL SYNERGY REPRESENTATIONS

The discussion above has drawn on evidence from human neurophysiology and imaging studies suggesting motor output from ipsilateral cortical synergy representations is conveyed by corticoreticulopropriospinal pathways. There are organizational and functional properties of this system that are central to permitting a synergy-based explanation for the findings of ipsilateral cortical reorganization. Activity in these neural pathways is also compatible with clinical observations after stroke, and has significant therapeutic implications.

8.8.1 Propriospinal Neurones and Synergy Control.

Propriospinal circuits provide a means of focusing and distributing motor output to multiple muscles engaged in a synergy. Descending long propriospinal neurones can distribute motor output across multiple remote segmental levels (Roberts et al., 2008).

Propriospinal neurones receive converging inputs from corticospinal, reticulospinal and vestibulospinal pathways (Alstermark et al., 1987; Jankowska and Edgley, 2006), and have bilateral outputs equally distributed contralaterally and ipsilaterally in animals (Mitchell et al., 2016). There are contralateral (Boudrias et al., 2010) and ipsilateral (Catsman-Berrevoets and Kuypers, 1976; Illert et al., 1981) hemispheric inputs into propriospinal circuits. Descending corticofugal fibres travel diffusely through reticular formation to relay via reticular nuclei (Matsuyama et al., 2004). Propriospinal output is to multiple muscles, proximal and distal, agonist and antagonist (Tantisira et al., 1996). These properties are ideally suited to synergy control.

Propriospinal circuits play an important part in the dynamic control of synergies through generation of internal copies of motor programmes, relaying through a cerebellar loop (Azim et al., 2014). These motor programmes are continually updated by afferent feedback which focuses activity within the propriospinal circuits to excitation of agonists and inhibition of antagonists (Nicolas et al., 2001). Motor units in arm muscles modulated by peripherally stimulated propriospinal neurones alter their excitability according to afferent input added by cutaneous stimulation (Nielsen and Pierrot- Deseilligny, 1991). This ability to focus distribution of propriospinal drive according to afferent feedback is important to amplification of weak ipsilateral drive in synergy control. The contribution of propriospinal neurones to skilled motor control is demonstrated in monkeys by transiently inhibiting their activity using viral vector guided toxins. This disrupts upper limb synergies required for skilled reach and grasp (Kinoshita et al., 2012). A similar contribution to skilled reach and grasp in humans is shown by dynamic modulation of neurophysiological

measures of propriospinal activity throughout the course of the movement (Giboin et al., 2012).

Neural organization of propriospinal circuits is central to the synergy-based explanation of the mapping study findings. Converging inputs from multiple corticofugal pathways from both hemispheres, and divergent excitatory and inhibitory, contralateral and ipsilateral output across segmental levels are key features compatible with the proposed mechanism. Positioning the system under the control of an internal copy, feed-forward motor programme is an important part of this function.

8.8.2 Evidence of Propriospinal Activity in Humans

Experimental evidence of activity in propriospinal circuits in humans is limited. Experimental use of stimulation intensities that could have maintained propriospinal activity under feedforward inhibition may have contributed to this. Collision experiments using central and peripheral stimulation in healthy subjects, and observations from stroke and Parkinson's patients provide knowledge of propriospinal function in humans (Pierrot- Deseilligny, 2002). In healthy subjects, propriospinal facilitation of cortical excitation of arm muscle motor units is concluded when peripheral inputs too weak to alter the H-reflex are delivered to coincide with descending corticospinal input to propriospinal circuits. The effect is highly dependent on stimulation intensities and timings.

Recording from single units shows features of the Peri-Stimulus Time Histogram that support a propriospinal basis. Stimulation of musculocutaneous nerve ,which lacks monosynaptic inputs to cervical motorneurones, facilitates the MEP in wrist flexors. The interstimulus interval required for facilitation showed a central delay of 4 to 6ms was needed for the peripheral stimulus to collide with the central stimulus. This suggests multiple synaptic relays through interneurons, consistent with the propriospinal circuit. Furthermore, the facilitation produced by musculocutaneous stimulation was greater than expected from the sum of the cortical and peripheral components, showing that multiple premotor inputs to the motor neurone had been activated, again consistent with a propriospinal circuit (Pauvert et al., 1998). Also, the first 0.7-0.9ms of the PSTH is not influenced by peripheral stimulation, but shows preservation of the corticospinal component in this early period. The interval is consistent with the synaptic delay of a single interneurone activation, again supporting a propriospinal relay (Pierrot- Deseilligny and Marchand-Pauvert, 2002).

8.8.3 Evidence of Propriospinal Activity in Human Synergy Control

Two studies of upper and lower limb synergies in chronic stroke patients unmask the normal functioning of propriospinal circuits in synergy control. Flexor synergy coactivation is seen in the paretic arm during locomotion and voluntary leg movement.

Coactivation of leg muscles is seen with finger movements (Kline et al., 2006). These segmentally distant effects were interpreted as loss of the usual feedforward corticospinal inhibition of propriospinal circuits, unmasking ungated propriospinal activity in generating abnormal synergies. This was examined in more detail in a similar study of stroke patients, studying leg synergies and response to femoral nerve stimulation. Stroke patients had abnormal synergies, coactivating ankle plantarflexors and knee extensors. Proximal femoral nerve stimulation produced an abnormal distal pattern of facilitation and inhibition of soleus EMG (Dyer et al., 2011). These two observations provide evidence of neural circuits linking remote segmental levels, heteronymous activity consistent with propriospinal circuits unmasked by loss of corticospinal inhibition.

Similar evidence is provided by Parkinson's patients. During locomotion an abnormal coactivation of ipsilateral leg muscles is seen with arm swing. Therapeutic interventions in the form of L-Dopa and Subthalamic Nucleus deep brain stimulation have differential effects on this abnormal synergy, improving leg kinematics more than arm, but to a different degree. Combining both therapeutic interventions improved the leg, but not the arm. The arm and leg synergies would be expected to be tightly coupled in locomotion. The demonstration of a differential effect of basal ganglia output on arm and leg synergies suggests involvement of a heteronymous circuit, such as propriospinal neurones (Crenna et al., 2008).

Another clinical observation compatible with propriospinal function in humans is propriospinal myoclonus. Alternating arrhythmic contractions of axial muscles were first described in patients with cervical spine injuries, thought to relate to release of uncontrolled transmission in propriospinal

circuits (Brown et al., 1994). The frequency, velocity of propagation, and agonist and antagonist coactivation is compatible with propriospinal properties (van der Salm et al., 2014).

The evidence from human experiments appears sufficiently consistent, and the observations of abnormal synergies after stroke frequent enough to pursue the idea of a propriospinal basis for ipsilateral synergy control. The human experiments are few in number, and certainty in the human evidence becomes important in view of conflicting findings in primate studies. This conflict can be understood on the basis of disynaptic inhibition that provides continuous gating of propriospinal activity. Crossed corticospinal input is a vital component in control of this gating. Understanding this aspect of the propriospinal circuit is critical to interpreting the most directly relevant human experiments on propriospinal contribution to ipsilateral cortical synergy control.

8.8.4 Functional Organization of Propriospinal Circuits

There are conflicting views about the importance of the propriospinal tract in primates and humans. One view is that strong corticospinal output, as used in most stimulation studies, produces disynaptic IPSPs and monosynaptic EPSPs. Under conditions where disynaptic inhibition is removed, propriospinal neurones are released from inhibition and their functional contribution to synergy control becomes measurable as polysynaptic EPSPs (Isa et al., 2007; Nicolas et al., 2001). An opposing view is that propriospinal effects are weak in primates and humans, suggested by studies that did not see strong emergence of propriospinal effects when disynaptic inhibition was removed with corticospinal lesions above propriospinal circuits (Kirkwood et al., 2002; Maier et al., 1998).

8.8.4.1 *Disynaptic Inhibition Modulates Propriospinal Output in Primate Studies*

In primate studies, stimulation of the pyramidal tract under normal conditions produces monosynaptic EPSPs and disynaptic IPSPs in forelimb muscles. Disynaptic EPSPs emerge when inhibition is removed by Strychnine, revealing propriospinal effects facilitated by disinhibition. Corticospinal tract lesions immediately below cervical propriospinal circuits result in abnormal compensatory synergies with coactivated finger movements and disynaptic EPSPs, suggesting upregulated propriospinal activity (Isa et al., 2007).

Other experiments have failed to replicate the results. Similar stimulation and lesions in monkeys only produced a small propriospinal effect, with disynaptic EPSPs recorded in 3% of recorded motor neurones prior to lesion and 14% after. The presence of monosynaptic EPSPs from stimulation of the Lateral Reticular Nucleus can also be used as a marker of propriospinal facilitation, since propriospinal neurones send collaterals there. There was no change in amplitude or frequency of reticular evoked EPSPs (Maier et al., 1998). They concluded that propriospinal effects in monkeys and humans are weak, and cautioned against extrapolating findings of animal studies to human physiology (Kirkwood et al., 2002).

8.8.4.2 *Disynaptic Inhibition Modulates Propriospinal Output in Human Studies*

Disynaptic inhibition in propriospinal circuits would be a key feature to allow explanation of the measured asymmetries of ipsilateral and contralateral cortical representations in terms of synergy control. It is therefore important to demonstrate its existence in humans. Cortical Transcranial Electrical Stimulation with single unit recording from arm muscles produces almost exclusively monosynaptic EPSPs which would suggest propriospinal transmission is rare (de Noordhout et al., 1999). However, propriospinal effects are highly sensitive to cortical and peripheral stimulation intensities. Stimulation parameters determine whether propriospinal neurones will be inhibited with strong input or facilitated by weak inputs. With interstimulus intervals timed to collide peripheral and cortical stimulation on propriospinal neurones, propriospinal activation can facilitate the MEP if weak stimulation intensities are used (Nicolas et al., 2001). The weak corticospinal volley is insufficient to activate the disynaptic feedforward inhibition of the propriospinal neurone. The propriospinal neurones then summate weak inputs to produce a motor output. The relevance to the ipsilateral representation in the athlete study can be appreciated. A large number of weak outputs concerned with control of multiple muscles in a synergy can converge on propriospinal neurones which summate and distribute the motor drive to synergistic muscles. The principle of this amplification is similar to the effect described for synchrony of neural populations within the cortical representation.

Peripheral facilitation supporting propriospinal output is provided by afferent feedback from contracting muscles as the synergy is executed. Experimental propriospinal facilitation of MEPs is strengthened by co-contraction of the target muscle and the muscle innervated by the peripherally stimulated nerve. This peripheral facilitation focuses weak descending cortical inputs to relevant muscles by directing it toward subsets of propriospinal neurones highlighted by afferent feedback (Nicolas et al., 2001). This is another propriospinal feature that makes it ideally suited to synergy control, and is consistent with the idea that the ipsilateral

hemisphere can exert skilled motor control through weak corticofugal outputs. The combination of disynaptic inhibition, premotor summation and afferent focussing shows that the propriospinal system is designed to produce fine, distributed motor control from weak cortical inputs.

These propriospinal functions are central to the mechanistic explanation proposed. Findings in humans are based on very few experiments, but all report consistently.

Experiments on leg muscles identify similar activity. Quadriceps EMG responds to common peroneal nerve stimulation with biphasic EMG facilitation. The heteronymous circuit is presumed to be propriospinal, structured to control locomotor synergies.

Strong TMS disrupts this activity, consistent with corticospinal input activating disynaptic feedforward inhibition of propriospinal neurones (Iglesias et al., 2008). The convergent, divergent, and gain properties of the system make it probable that propriospinal neurones function in the axial and reach synergies tested, and neurophysiological evidence supports this. The functional properties are compatible with the measured asymmetries of ipsilateral and contralateral representations. Disynaptic inhibition functions to gate activity of the propriospinal circuits to weak inputs such as those received from the ipsilateral hemisphere in control of coactivated muscles.

8.8.5 Propriospinal Activity After Stroke

Observations of propriospinal activity after stroke are informative of corticofugal output from secondary motor areas. This is relevant to interpretation of the Centre of Gravity shifts seen in the mapping studies.

Neurophysiological evidence points to an increased reliance of propriospinal neurones to serve motor recovery after stroke, in a manner that reduces over time as motor function improves. In primates, pyramidal tract lesions below cervical propriospinal circuits result in increased disynaptic EPSPs, showing compensatory upregulation of propriospinal activity (Isa et al., 2007). A similar propriospinal effect can be demonstrated neurophysiologically in stroke patients. Weak radial stimulation suppresses wrist extensor EMG in the paretic limb, without altering H-reflex. The lack of H-reflex effect shows a premotor rather than segmental interneurone basis, compatible with propriospinal activity (Mazevet, 2003). This demonstrates an increased use of propriospinal pathways after stroke. The afferent radial stimulation activates inhibitory interneurons. This inhibition replaces the disinhibition of propriospinal neurones that results from loss of corticospinal feedforward disynaptic inhibition. Replacing inhibition suppresses the EMG activity. In combination with the corticospinal related disinhibition, the increased propriospinal activity likely also reflects increased extrapyramidal inputs compensating corticospinal damage. Corticoreticular fibres from secondary motor areas upregulated in the post-stroke motor network are likely to provide this compensation.

Another experiment furthered this observation by measuring the effect of collision of peripheral and cortical stimuli on MEPs in the paretic limb. Delivering heteronymous musculocutaneous afferent stimuli to collide with ipsilesional TMS facilitated wrist extensor MEPs (Stinear and Byblow, 2004). The facilitation in this experiment contrasts with inhibition in other experiments, likely due to polarity effects of stimulation intensities (Nicolas et al., 2001).

Increased ipsilateral propriospinal or reticulospinal activity after stoke can be indirectly inferred by associating neurophysiology measures with abnormal synergies. The presence of proximal upper limb MEPs after stroke has been correlated with abnormal upper limb flexor synergies (Schwerin et al., 2008). Inappropriate coactivation of biceps during forearm supination after stroke is evidence of increased abnormal synergy activity (Gerachshenko et al., 2008). Abnormal coactivation of hip adductors and knee flexors is reflected by patterns of MEP facilitation (Tan et al., 2016).

These experiments provide further evidence for reticulopropriospinal activity in control of synergies by revealing the effect of ungated activity in these circuits. They suggest that uncontrolled activity can be maladaptive after stroke. However, in patients who make good motor recovery, longitudinal studies show that propriospinal activity reduces over time as recovery occurs (Mazevet, 2003). The transition from propriospinal to corticospinal control shows that the propriospinal circuits can provide transient beneficial compensation to support recovery in some patients. This again highlights the importance of patient selection, and a potential role for neurophysiological measures of ipsilateral activity to monitor and guide intervention in stroke recovery.

8.9 CORTICORETICULAR INPUTS TO PROPRIOSPINAL CIRCUITS

Propriospinal neurones receive inputs from corticospinal, reticulospinal and vestibulospinal tracts, from both hemispheres, relaying through reticular nuclei (Jankowska and Edgley, 2006). Propriospinal neurones give collaterals to the Lateral Reticular Nucleus. Direct evidence from primate studies and indirect evidence from human studies supports a role for corticoreticulospinal tract in linking cortical representations to the propriospinal circuits. The source of corticoreticulospinal neurones is relevant to interpretation of the findings of the mapping studies. Corticoreticular inputs originate dominantly from Premotor areas in man (Yeo et al., 2012), and from primary motor cortex, Premotor areas and SMA in primates (Keizer and Kuypers, 1989).

8.9.1 Corticoreticulospinal Pathways and Ipsilateral Motor Control.

Corticoreticulospinal activity is discussed with emphasis on their function of relaying cortical output to propriospinal circuits, but reticulospinal properties are also relevant to ipsilateral motor control in their own right. Although lacking the computational capabilities of propriospinal circuits, some intrinsic reticulospinal properties are suited to axial control, such as bilateral descent and branching across multiple segmental levels. It must be considered whether the observed differences in ipsilateral cortical representations in the athletes and stroke patients are driven to utilize propriospinal circuits, or whether the final ipsilateral motor output is a combination of reticulospinal and propriospinal output. Polysynaptic EPSPs persisting in forelimb motorneurones after lesions of the contralateral pyramidal tract in primates could represent propriospinal or reticulospinal activity (Alstermark et al., 1999; Isa et al., 2007).

Traditionally the reticulospinal tract has been considered to perform basic postural and locomotor functions rather than skilled motor control. Recent findings challenge that view by reporting a role in hand muscle control. Intrinsic properties predispose to unskilled ipsilateral motor control. The reticulospinal tract descends bilaterally, with equal or ipsilateral bias of lateralization (Sakai et al., 2009). Reticulospinal fibres project to lumbar segments, and give off bilateral axonal branches at multiple segmental levels (Peterson et al., 1975). Post-spike effects recorded in primates from brainstem reticular neurones and arm muscles demonstrate ipsilateral flexor facilitation and extensor inhibition, and the reverse situation in the contralateral arm (Buford and Davidson, 2004; Davidson et al., 2007). In combination the multi-level, bilateral action with flexor or extensor bias produces simple mass movements without fractionation. This explains the flexor coactivation seen after stroke. Whilst in general these features would argue against a skilled motor role, in axial synergies this might provide computational efficiency for basic axial support.

8.9.2 Ipsilateral Reticulospinal Pathways After Stroke

Recovery of the paretic limb is served by ipsilateral reticulospinal pathways after corticospinal lesions in primates, where stimulating reticulospinal pathways in the Medial Longitudinal Fascicle produces disynaptic EPSPs in cervical motor neurones, but no response is seen to stimulation of ipsilateral medullary pyramids (Zaaimi et al., 2012). Increased reticulospinal activity in stroke patients may underlie abnormal flexor and extensor synergies (Dewald et al., 1995), and spasticity (Brown et al., 1994). Activating ipsilateral reticulospinal pathways with tonic neck reflexes increases abnormal coactivation in arm flexor synergies (Ellis et al., 2012)

8.9.3 Possible Ipsilateral Reticulospinal Pathways of Skilled Motor Control

The blunt flexor or extensor activation makes it unlikely that the differences seen in the athletes, or the improvement in skilled reach in the stroke patients arose from use of this pathway. A role for reticulospinal fibres in relaying to propriospinal circuits, and participating in control loops updating internal models of the motor command is a more likely contribution to skilled movement. Recent experimental findings suggest a more skilled reticulospinal role that is not yet completely defined. A high degree of convergence is seen between reticulospinal and corticospinal inputs to cervical Intermediate Zone interneurons concerned with reach, grasp and finger movements (Riddle and Baker, 2010). Single unit recording in the Pontomedullary Reticular Formation of monkeys shows reticular neurones modulate their activity in response to index finger extension (Soteropoulos et al., 2012), and in planning cued reach movements (Buford and Davidson, 2004). Stimulation of reticulospinal neurones in the Medial Longitudinal Fascicle can produce monosynaptic EPSPs in cervical motor neurones innervating hand muscles (Riddle et al., 2009). These findings provoke thought that reticulospinal pathways may serve some other aspect of ipsilateral control relevant to the findings.

An important reticulospinal function in skilled synergy control is participation in a cerebellar-propriospinal-spinal loop that updates motor commands. Propriospinal neurones bifurcate and send a collateral to the Lateral Reticular Nucleus which functions as a pre-cerebellar relay, providing a feedforward internal copy of the motor command. Reticulospinal neurones activate spinal motor neurones and propriospinal neurones. Cerebellar input to the reticular nucleus then provides a feedback loop. Selective disruption of the propriospinal collateral branch to the cerebellar circuit disrupts reach synergies in mice (Azim et al., 2014). The function of this pathway in humans is shown by cerebellar tDCS modulating the extent of propriospinally mediated MEP facilitation (Chothia et al., 2016).

8.9.4 Ipsilateral Cortical Origins of Corticoreticulospinal Pathways

Primate maps of cortical synergy representations extend from motor cortex anteriorly to Premotor cortex (Graziano et al., 2002). Premotor areas are an important source of corticoreticulospinal fibres in humans. A DTI study of the corticoreticulospinal tract showed dominant origin in Premotor areas (Yeo et al., 2012). Primate tracer studies also show contributions from primary motor cortex and SMA (Keizer and Kuypers, 1989). The extent of the map and anterior CoG in the athlete study makes a corticoreticular output from Premotor cortex or SMA a possible contributor to the ipsilateral cortical reorganization. Possible Premotor involvement in the corticoreticulospinal pathway of synergy control was investigated with inhibitory cathodal tDCS to ipsilateral Premotor Cortex. The Premotor cathode position did not modulate the reticulopropriospinal pathway, but positioning the cathode over primary motor cortex did (McCambridge et al., 2014). It is not possible to draw conclusions on the basis of one experiment, but a greater role for output from primary motor cortex is compatible with the findings of the ipsilateral representations in stroke patients. Following stroke Premotor areas are strongly upregulated in compensating primary motor cortex damage. If a Premotor source was active it should have been most evident as an anterior Centre of Gravity shift in the stroke patients, but this was not seen.

8.10 EVIDENCE FOR IPSILATERAL SYNERGY REPRESENTATIONS FUNCTIONING VIA CORTICORETICULOPROPRIOSPINAL PATHWAYS

The above discussion summarizes the evidence behind the theoretical basis used to interpret mapping findings as ipsilateral contributions to skilled motor control through synergy representation. Deliberate practise related cortical reorganization of synergy representations is then effected through corticopropriospinal pathways. Having presented evidence to support involvement of the various components of this system, it remains to consider the evidence that these are unified into a functional system in humans.

There is direct neurophysiological evidence in humans of ipsilateral cortical representation of synergies. Ipsilateral local field potential patterns studied intraoperatively with ECoG can accurately and reliably encode the kinematics of reach synergies of the ipsilateral arm (Ganguly et al., 2009). Ipsilateral synergy representations can also be decoded from contralesional EEG after stroke with sufficient resolution to control basic Brain Machine Interface functions (Bundy et al., 2012). The ipsilateral synergy representations in the contralesional hemisphere are known to undergo reorganization after stroke, including increased activity in Premotor regions known to source corticoreticulospinal output (Yao et al., 2009).

The most direct proof available that the ipsilateral hemisphere contributes to skilled motor control through propriospinal neurones comes from a study that combines TMS, peripheral stimulation and neuromodulation. The established peripheral and cortical collision protocol was used to probe propriospinal effects on MEPs evoked from the contralateral hemisphere. Addition of inhibitory cathodal tDCS to downregulate the cortical contribution from the ipsilateral hemisphere demonstrates its contribution. Inhibition of the ipsilateral hemisphere abolished both the inhibitory and excitatory effects of propriospinal activation at different stimulation intensities (Bradnam et al., 2011). This demonstrated descending ipsilateral motor cortex output modulates the excitability of propriospinal neurones.

A contribution of the ipsilateral hemisphere to synergy control is demonstrated further with neuromodulation. Inhibiting ipsilateral motor cortex output with cathodal tDCS (McCambridge et al., 2011) or continuous theta burst rTMS (Bradnam et al., 2011) alters the synergy ratio, measured as the ratio of facilitation of biceps MEP when it is performing an agonist, compared to MEP suppression when it is acting as an antagonist. Following inhibitory theta burst to the ipsilateral hemisphere there is reduced inhibition of the antagonist, demonstrating a beneficial contribution to normal synergy control. A conflicting finding was observed with cathodal tDCS, which produced more marked antagonist suppression. These findings are difficult to reconcile, since both are inhibitory protocols yet produced effects of opposite polarity.

An important point arises from the tDCS study. Inhibiting the ipsilateral hemisphere improved the functioning of the synergy, but the size of the change depended on the individual's baseline synergy ratio. Those with high baseline synergy ratios, showing poor baseline synergy control, were those who improved with neuromodulation. This is relevant to interpreting the findings of the athletes and stroke study since it identifies inter-individual variability in the baseline activity in these pathways. This variability is measurable, so could be useful in selection of training or neuromodulatory interventions. The ability to improve synergy ratios by modulating regional cortical excitability is also compatible with proposing this system as a target for training effects.

Variable polarity of effect was also seen in a similar study of stroke patients. In this case the variability could be explained by the degree of damage to the ipsilesional corticospinal tract, in accordance with Proportional Recovery models. Inhibitory cathodal tDCS to the contralesional hemisphere worsened the synergy ratio in the most severely impaired. These patients had the most damage to ipsilesional corticospinal tracts and the strongest ipsilateral MEPs, demonstrating reliance on ipsilateral motor pathways for control of the paretic arm. In contrast, those with the least crossed corticospinal damage improved their synergy ratios following contralesional cathodal tDCS. In these patients, the ipsilateral output was

hindering optimal motor control of the paretic limb (Bradnam et al., 2012). This study perfectly illustrates the need to individualize stroke rehabilitation according to an assessment of the state of the motor network guided by clinical, neurophysiology and neuroimaging measures. It importantly provides a link to the PREP algorithm, since it incorporated the same imaging measure of Fractional Anisotropy of the Posterior Limb of the Internal Capsule. The principles of the PREP algorithm may inversely apply to motor output from the ipsilateral hemisphere. In those not predicted to make good recovery, therapeutic intervention to optimize ipsilateral output could become appropriate.

Taking this finding that ipsilesional corticospinal tract integrity can predict responsiveness to therapeutic intervention directed at ipsilateral motor pathways, discussion will now consider how the findings from the athlete and stroke study can inform stroke rehabilitation.

8.11 THE IPSILATERAL HEMISPHERE IN STROKE REHABILITATION

A mechanistic explanation was proposed to account for ipsilateral map changes as synergy representations reorganized to control skilled movement through corticoreticulopropriospinal circuits. Examples were provided to demonstrate relevance of this pathway to stroke recovery. Its transient upregulation in subacute stages may support good eventual motor recovery with graded return to crossed corticospinal control if the corticospinal tract repairs to resume gating (Mazevet, 2003). Alternatively this pathway may provide the only residual control of the paretic limb in severely impaired patients (Bradnam et al., 2012). Constituent activity in this pathway can be unmasked in healthy subjects and inter-individual variability in the strength of its activity appreciated (McCambridge et al., 2011). There are no reports of deliberate practise training activity in this pathway in healthy subjects. The findings in the athlete study are the first demonstration of beneficial ipsilateral cortical reorganization driven by deliberate practise. This positive finding in expert athletes, combined with the evidence from the stroke study that ipsilateral change can associate with improved function establishes the basis for wishing to pursue ipsilateral plasticity in stroke rehabilitation. The suitability of this approach will be confined to a subset of patients, identifiable with clinical and neurophysiological measures.

8.11.1 Proportional Recovery and Patient Selection According to Ipsilateral Neurophysiological Measures

The Proportional Recovery model identifies stroke patients with mild to moderate motor deficits who will regain around 70% of their potential improvement based on impairment scores, and patients with severe impairments who will not make good recovery (Buch et al., 2016; Prabhakaran et al., 2008; Winters et al., 2015). Having sufficient residual intact ipsilesional corticospinal fibres allows spontaneous recovery dictated by neurobiological factors and insensitive to intensity of therapy (Byblow et al., 2015). Upregulating ipsilateral activity before corticospinal recovery in these patients could compromise the quality of recovered movement. Passage of time alone explains 25% of the variance of functional improvement, largely complete between 6 and 10 weeks (Kwakkel et al., 2006). Later functional improvement beyond 3 months largely occurs through compensatory strategies, rather than correcting impairment (Kwakkel et al., 2004). The PREP algorithm formalizes identification of patients who will recover according to the Proportional Recovery principle, by combining early clinical measures with imaging and TMS measures of ipsilesional corticospinal tract integrity (Stinear et al., 2012).

These findings have been replicated by multiple studies. Within neuroimaging studies that agree with the PREP algorithm (Buch et al., 2016; Byblow et al., 2015), there are notable exceptions who are difficult to classify according to the model. Some patients have sufficient residual ipsilesional corticospinal fibres to recover well but have discordantly low initial functional scores. They later make good recovery (Bigourdan et al., 2016). This description recalls the Competitive Interference model, whereby excessive contralesional excitability limits deployment of ipsilesional plastic resources. In this situation neurophysiological measure of ipsilateral activity would contribute usefully to the assessment. These patients could also represent those identified as transiently using upregulated propriospinal pathways until corticospinal recovery is complete (Mazevet, 2003). The process that guides the transition from propriospinal to corticospinal

transmission in these patients is not known and has not been studied at frequent time intervals. Neurophysiological measures of ipsilateral activity could help characterize this process. If these patients are misclassified by the algorithm, and inappropriate treatment pathways applied, persistence of ipsilateral activity could limit the quality of motor recovery.

Patients are also identified with low residual ipsilesional corticospinal fibre numbers and low initial functional scores who go on to make a better motor recovery than the model predicts (Bigourdan et al., 2016). In this situation analogy to the findings of the athlete study is appropriate. There are numerous other examples of similar patients who demonstrate beneficial use of ipsilateral pathways with good motor recovery (Butefisch, 2015). Identifying such patients clinically is not straightforward. There is a need to identify this group according to the neurophysiology of their ipsilateral motor pathways. The difficulty in identifying this group means their reorganization of contralesional motor representations has not been studied. Inter-individual difference in baseline synergy ratios in healthy subjects may be relevant here (McCambridge et al., 2011). It is possible that athletes self-select because of a genetically conferred advantage, in the case of the canoeists this may be common polymorphisms relating to bilateral motor pathways that produces optimal synergy ratios. It is possible that a similar advantage of ipsilateral plastic substrates determines which patients are able to use ipsilateral pathways beneficially after stroke. This may also apply to those patients who are able to reorganize motor representations to the opposite hemisphere in cases of Global Aphasia Without Hemiparesis and Ipsilateral Hemiplegia (Bang et al., 2004).

Analogous to the PREP algorithm, imaging measures of ipsilaterally descending motor pathways could be combined with TMS measures of ipsilateral motor activity to more completely characterize those patients who fall between predicted outcomes. The corticoreticular pathway has been imaged with DTI (Yeo et al., 2012). Connectivity of bilateral alternate motor pathways was reported to correlate with function in stroke patients (Rüber et al., 2012), and numbers of contralesional corticoreticulospinal fibres correlates with ambulatory function (Jang et al., 2013).

8.11.2 Therapeutic Strategies Based on Ipsilateral Cortical Synergy Representations, Ipsilateral Corticoreticulopropriospinal Pathways and Proportional Recovery

Once patients who may benefit from activity in ipsilateral motor pathways are identified, there is the challenge of knowing how to apply targeted therapeutic interventions. The findings of the athletes study raise some important points. An approach that seeks specifically to train optimal synergies may be the most effective way to utilize ipsilateral neural substrates. Many current therapeutic approaches accept compensatory strategies without regard to the motor control of impairment correction. Robotic therapy provides a means of continuously monitoring kinematics and EMG and applying perturbation or actuation to ensure synergies are progressively optimally trained.

Conversely, robotic treatments applied without sufficient regard to these principles could drive maladaptive synergies. Implicit in the suggestion of focus on training synergies is an understanding of the effect of the lesion on synergy representations. Cortical and subcortical strokes will differentially influence synergy modules at different levels (Garcia-Cossio et al., 2014). An awareness of the potential influence of the lesion on synergies is also required for monitoring. Abnormal synergies are not thought to emerge until after the period over which spontaneous recovery may occur, and then post stroke synergies combine preservation, fractionation and merging of existing synergies (Cheung et al., 2012).

The other factor identified in the athlete study is the time course of training. Vast amounts of deliberate practise may be required to drive ipsilateral cortical reorganization. The different plastic environment after stroke may alter this time course. If extensive repetition and duration of practise is required, then robotic therapy could again facilitate the necessary accumulation of practise. To advocate this, a greater understanding of how within-session plastic gains are consolidated over time is required. Embarking on extensive practise equivalent to athletes may be counter productive without correct off-line consolidation. These issues are discussed

in relation to the available evidence on robotic therapy and deliberate practise in motor learning.

8.11.3 Robotic Synergy Based Therapy as a Therapeutic Strategy to Optimize Ipsilateral Motor Control

Benefits of robotic training after stroke are widely reported, but rarely with a focus on synergies. Improved synergy control has been reported with robotic training (Tropea et al., 2013), and robotically driven synergy improvement can generalize to improved functional upper limb scores (Barker et al., 2009). The heterogeneity of stroke patients enrolled in robotic trials means this does not yet translate to a clinically relevant benefit in meta-analysis (Veerbeek et al., 2017). The strong appeal of robotic therapy is the combination of facilitating vast amounts of practise in a resource and cost efficient way with the ability to guide therapy according to principles of motor control. On-line kinematic and EMG analysis to describe the synergies employed (Ellis et al., 2016), and the ability to manipulate these through actuators driven by motor control algorithms (Huang and Krakauer, 2009), could efficiently train optimal synergies.

Findings from the stroke study are relevant to considering the role for robotic therapy in developing ipsilateral motor control of synergies. It was demonstrated that robotic training over short time periods alters ipsilateral synergy representations. At baseline, improved functional performance in chronic stroke patients was associated with a difference in the ipsilateral organization of the pectoralis major representation. When robotically trained in skilled reach, ipsilateral change was seen in anterior deltoid. It is possible that in the chronic state patients employed a compensatory strategy for paretic reach with pectoralis major as the prime mover, but the robotic training taught a reach that corrected the motor impairment by incorporating deltoid more prominently into synergies. Training to correct impairment rather than develop compensation is an important principle of robotic therapy. Selection of the correct robotic intervention and attention to evolving synergies is vital in this regard. Increasing resistance to direct joints towards ideal inter-joint patterns resulted in use of ideal synergies, whereas preventing error by constraining to an ideal trajectory encouraged compensatory strategies and abnormal synergies (Brokaw et al., 2013).

Robotically assisted reach supported on the same horizontal level encouraged abnormal synergies, whereas elevated reach improved synergies (Lum et al., 2004). Training robotic reach with full or varying support of the paretic limb showed that variation of weight support was required to encourage beneficial synergies (Ellis et al., 2009).

It is clear from these examples that robotic intervention has the potential to help optimize motor control of the paretic limb by training synergies, utilizing the neural pathways of the contralesional hemisphere. By analogy to the athlete study, producing optimal, stable ipsilateral cortical synergy representations may require an extent of practise only realistic with robotic assistance. The examples provided also show that success of this approach requires individualization of patient selection and robotic protocol.

8.11.4 Neuromodulation and Ipsilateral Cortical Synergy Modules

Another therapeutic intervention that has obvious relevance to training ipsilateral synergy representations is neuromodulation. Neuromodulation of the contralesional hemisphere has been extensively trialled with protocols guided by the Competitive Interference model. Studies examining neuromodulation effects on contralesional synergy representations are limited to those few discussed above. The potential of ipsilateral inhibition by cathodal tDCS to improve or worsen synergy control depending on the stroke lesion is of vital importance. No studies have selected patients according to their potential to benefit on the basis of ipsilateral MEPs, and no studies have examined the effect of upregulating the ipsilateral hemisphere on corticoreticulopropriospinal pathways.

Other neuromodulation modalities with specificity to exploit features of the corticoreticulopropriospinal pathways can be proposed as exploratory treatments. Vestibular inputs to reticular nuclei have the potential to be modulated peripherally. MEPs may be modulated by tonic neck reflexes (Tazoe and Perez, 2014), caloric stimulation (Guzman-Lopez et al., 2011), acoustic startle (Fisher et al., 2004) and optokinetic stimulation (Sakihara et al., 2007). Spinal tDCS has been proposed to modulate propriospinal circuits (Roche et al., 2012), although manipulating the sensitivity of the inhibitory gating of these circuits is complicated and problematic.

8.12 RELEVANCE OF ACTIVITY DEPENDENT AND EXPERIENCE DEPENDENT PLASTICITY IN EXPERTS TO STROKE REHABILITATION

The time course of plastic changes reported in the experts and stroke patients can be examined for relevance to planning stroke therapy. In the athlete study, a significant difference in the extent of the ipsilateral representation was seen in the intermediate group and expert groups compared to trainees, but had not shown further improvement between the intermediate and expert groups. The difference in cortical organization was only seen between the expert and training group. In the singers study a difference in the contralateral representation of diaphragm was seen between the expert and training group. The time course of plastic change driven by thousands of hours of deliberate practise in experts may be relevant to stroke recovery.

8.12.1 The Natural History of Motor Recovery in the Acute and Chronic Post Stroke Phase

The period of most rapid spontaneous recovery occurs in the first 2 to 3 months after stroke (Prabhakaran et al., 2008). Longitudinal study of this process shows it may continue at a lesser rate for up to 6 months (Kwakkel et al., 2006). Functional scores plateau around 3 months, whether distal, proximal or axial measures are used (Verheyden et al., 2008). Little further improvement is seen between 3 and 6 months (Jørgensen et al., 1995). Therapeutic focus on the first month has been accentuated by studies of the Critical Period for plasticity in the first weeks (Dromerick et al., 2015). A number of influential trials of plasticity modulating therapies have supported this idea. Studies of early combined rehabilitation and immunomodulation in animal studies of anti-NoGo (Wahl and Schwab, 2014) and early pharmacological intervention with Fluoxetine (Chollet et al., 2011; Cramer, 2011) support the idea of the Critical Period.

Sub-acutely there is evidence that Constraint-Induced Movement Therapy (Wolf et al., 2010) and robotic intervention (Krakauer et al, 2012), are most effective in the 9 months after stroke. Less has been studied about effectiveness of long term intervention in the chronic phase, although numerous short term interventions with neuromodulation report further functional gains after short periods of intervention in chronic patients (Grefkes and Fink, 2012).

A meta-analysis of the effect of augmenting therapeutic intervention found a benefit of additional treatment in the first 6 months, but no benefit from the few studies performed in the chronic phase (Kwakkel et al., 2004). Long term follow up of 238 patients over 5 years, shows that measures of arm, leg, trunk and overall function did not improve further between 2 months and 5 years, and deteriorate most for severe strokes (Meyer et al., 2015). The deterioration could result from inactivity after initial periods of intensive rehabilitation, or the natural history of the neurobiology of stroke pathology.

Training reach in the chronic stroke patients demonstrated further functional improvement is achievable with short periods of intensive rehabilitation in the chronic stage. Others report similar improvements training over similar time courses, but show that ipsilesional corticospinal tract integrity measured by MRI and TMS predicts potential for chronic stage gains (Stinear et al., 2006). Severely impaired stroke patients would therefore not be expected to improve with further training in the chronic stage. This situation was not seen in the stroke patients studied here. The patients with the greatest reliance on ipsilateral pathways measured by the Laterality Index were able to make functional gains with training. These patients were presumably those with the most damaged ipsilesional corticospinal pathways, and accordingly had the lowest baseline functional scores.

Of interest, a meta-analysis of postural training after stroke showed that postural intervention was beneficial in chronic stroke patients (Chen and Schlaug, 2016). This training differed in use of axial synergies. It may be that tasks that rely more on ipsilateral pathways remain receptive to training for longer periods by virtue of intact bilateral motor pathways from the contralesional hemisphere. The study showing trunk function reaching plateau at 3 months (Verheyden et al., 2008) questions this.

8.12.2 Relevance of Accumulated Deliberate Practise in Athletes to Motor Recovery in the Chronic Post Stroke Phase

There is evidence that accumulated repetitive practise of motor tasks is of benefit in stroke rehabilitation, but no consensus on the dose and duration of repetition (French et al., 2016). The finding from expert athletes and singers suggests that vast accumulation of deliberate practise is required for cortical reorganization of motor representations.

Repetition alone is not sufficient for skilled motor learning, but is known to be necessary to achieve expert control (Ericsson, 2017). The nature of the task practised also drives an activity dependent component that dictates the cellular nature of the plastic response. Skill rather than strength and endurance drive synaptogenesis in cortical reorganization (Adkins et al., 2006). Understanding how deliberate practise in experts relates to cortical reorganization could help to predict the usefulness in chronic stroke patients.

The concept of 10 years, or 10,000 hours of practise being required to achieve expertise in skilled motor control was developed from common findings of multiple studies of training to expert status (Ericsson et al., 1993). Recent interpretations of the rule have expanded on the training conditions required to achieve expertise, and this has implications for stroke rehabilitation. Training becomes most effective with immediate feedback (Ericsson, 2017), similar but mixed skill requirements that do not compete for neural representations (Been et al., 2011), and timing to incorporate optimal periods of consolidation (Barakat et al., 2013).

Dendritic change and synaptogenesis between intracortical Horizontal Fibres has been described as the basis of reorganization of cortical synergy representations. The same neural processes explain deliberate practise effects over tens of thousands of hours. Stabilization of repetitively practised movement representations is achieved by strengthening synapses once neural efficiency has been optimized. Structural change is driven by deliberate practise in experts, seen in the grey matter of divers with 27,000

hours of practise (Wei et al., 2011) and in the white matter of pianists with 23,000 hours of training (Bengtsson et al., 2005). Structural change reflects the accumulation over time of fast and slow plastic processes.

8.12.3 Fast and Slow Plasticity of Cortical Reorganization

Experimental studies of consolidation of skilled motor practise identify fast and slow phases of motor training. Few experiments study the process beyond weeks. A fast stage of learning occurs within a training session. Initial learning is rapid for novel tasks, and quickly increases the cortical representation as the motor network is explored for possible control solutions (Korman et al., 2003). After a single training session, improved performance can be seen the following day without further practise. This reflects neural consolidation of the learnt skill which may occur in neuronal populations distinct from those active during the movement. Consolidation correlates to sleep architecture (Barakat et al., 2013). Improvement in motor performance can be measured within each session, but also with a distinct slow component that accounts for progressive incremental gains over days and weeks (Thompson et al., 2009). Different cellular processes and cortical-subcortical networks control the fast and slow components (Karni et al., 1998). Fast and slow processes use separate, parallel cortical-subcortical loops, with the fast learning incorporating a larger attentional component. SMA, preSMA and Premotor areas control transition between fast and slow motor learning networks (Dayan and Cohen, 2011). Changes in task-specific (Yang, 2015) and resting state (Taubert et al., 2010) functional networks are seen to precede structural imaging changes (Debarnot et al., 2014) with motor training. In animal studies the speed of synaptogenesis in response to training precedes the reorganization of cortical movement representations by several days (Kleim, 2004).

8.12.4 Immediate Early Genes and Late Genes of Long Term Potentiation and Cortical Representations

Fast within-session effects represent unmasking of latent synaptic connections within the cortical network. The synaptic activity drives expression of Immediate Early Genes (Okuno, 2011), which produce transcription factors for Late Gene expression for consolidation and slow phases. The synaptic activity of fast learning activates NMDA receptors, and subsequent calcium flux stimulates post-synaptic AMPA receptor expression (Kandel et al., 2014) and BDNF release (Lu et al., 2008) to control late-LTP stabilizing the synaptic connections of the early stage (Richter and Klann, 2009). A number of late genes controlling the development of Late-LTP respond to NMDA receptor activation (Hong et al., 2004). This process can occur in dendrites, allowing local control over synaptic strength (Bradshaw et al., 2003). Accordingly, injecting protein synthesis inhibitors to motor cortex blocks consolidation and late phase of motor learning in animals (Luft, 2004).

The relationship of late gene stabilization of LTP to continued deliberate practise is not clear. Mapping cortical reorganization driven by skilled motor practise in monkeys over 500 days showed that representations of muscles coactivated in the task were strengthened. The extent of the representation increased from baseline until 8 months when training was stopped. Between 8 and 12 months the size of the representation dropped toward baseline. When training resumed over 4 months later, the monkey required less training time to expand the representation beyond the area achieved previously (Nudo et al., 1996). This shows that cortical representation of synergies respond dynamically to training, but the temporal relationship is not straightforward and is influenced to some degree by previous experience. A similar experiment with over 10,000 repetitions of reach training but without a skilled component to the task did not produce the same cortical representation changes (Plautz et al., 2000).

8.12.5 The Timing of Plasticity of Ipsilateral Cortical Representations

Cortical representation changes are therefore not merely a function of repetitive practise, they vary dynamically with temporary interruption of practise and the skill requirement of training. There is no evidence to suggest a different cellular basis for motor representations resulting from vast deliberate practise. The study of primate reaching over 500 days is the longest duration of frequent longitudinal cortical mapping to study skilled motor training, and this remained dynamic as expected over that time period. It is likely that cortical synergy representations developed over years in experts obey the same rules governing fast and slow motor learning. There are no studies that test change of cortical representations after cessation of vast amounts of deliberate practise.

It is likely that stroke rehabilitation to optimize ipsilateral synergy control will require vast amounts of repetitive practise with the correct skill requirement and mix, feedback and consolidation. This time investment may be the price of correcting impairment rather than accepting compensatory strategies. Such dedicated training may become possible with robotic assistance. Before investing time and resources in such an endeavour there would need to be some certainty that the training would generalize to improved function, since many repetitively trained motor skills remain specific without generalizing (Korman et al, 2003). Training axial synergies has been shown to translate to improved general functional measures (Chen and Schlaug, 2016). There must also be some certainty that accumulated practise effects can be consolidated in the chronic post stroke phase, and this has not been tested.

CHAPTER 9
CONCLUDING REMARKS

The objective of this thesis was to provide examples of beneficial plasticity in ipsilateral motor pathways that could be used to support a role of the contralesional hemisphere in the motor recovery from stroke. I have identified experience dependent plastic change associated with skilled motor control in the ipsilateral cortical motor representations of athletes. Features of these plastic changes are compatible with a role for the ipsilateral hemisphere in contributing to synergy control. I have also demonstrated that ipsilateral plasticity in the contralesional hemisphere can be associated beneficially with motor recovery after stroke, and this can be driven by robotic rehabilitation. These are novel findings.

This work may contribute to the body of evidence moving towards increased individualization of stroke rehabilitation based on knowledge of motor tract integrity. These neurophysiological means of assessing this integrity can be practically applied in the clinical setting. The synergy based mechanistic explanation can be tested further in healthy subjects and stroke patients. Combining measures of ipsilateral motor representations with neurophysiological measures of efficiency of synergy control could provide a functional correlate to test the conclusions of these studies. The idea that measures of balance of activity in contralateral and ipsilateral motor pathways to paretic arm will predict response to neuromodulation of the contralesional hemisphere can also be tested.

The introduction drew comparison between the voltaic neuromodulation of the contralesional hemisphere at the National Hospital in the 1860's guided by Broadbent's Law and the cathodal Transcranial Direct Current Stimulation study of 2012 on which the unifying explanation presented here is based. Throughout the intervening century and a half, knowledge of the neurophysiological mechanisms underpinning ipsilateral motor control steadily accumulated. The rate of progress of work on all fronts relating to this field has become rapid during the period of undertaking this thesis. Significant neuroanatomical findings emerged with the delineation of New

M1 and the finding of proximal monosynaptic corticomotorneuronal pathways and distal ipsilateral monosynaptic corticoreticulospinal pathways. The first evidence of an ipsilateral corticoreticulopropriospinal pathway of synergy control active in humans was described, and the first decoding of synergy representations in widely distributed neural ensembles in the ipsilateral hemisphere became possible. The development and widespread testing of predictive algorithms to inform approach to stroke rehabilitation has renewed interest in neurophysiological measures of motor tract integrity. Advances in brain machine interface technology have brought focus to neuronal activity in the contralesional hemisphere after stroke. The rise of robotic therapy has made the study of extensive deliberate practise relevant to stroke rehabilitation.

At the point of proposing further experimental work to test these conclusions, technological advances look set to transform the capabilities of TMS for motor mapping of the ipsilateral hemisphere. Improved computational modelling of induced field interactions with neuronal populations, the ability to tailor pulse waveforms, and to integrate TMS within study of cortical oscillatory activity will all make TMS motor mapping more effective. Just as advances in electrical engineering improve the ability to measure ipsilateral cortical activity, advances in robotics and motor control theory will make the information more clinically useful. The ability to correct motor impairment through extensive repetitive motor rehabilitation delivered efficiently according to motor control principles is a goal of robotic therapy. This work goes some way toward understanding how to individualize this prescription.

GLOSSARY OF TECHNICAL TERMS

Active Motor Threshold (aMT)

Active Motor Threshold is a term used in Transcranial Magnetic Stimulation experiments to reproducibly quantify the level of stimulation. The Active Motor Threshold is measured as the stimulation intensity required to produce Motor Evoked Potentials in pre-activated muscle. It is the percentage of Maximum Stimulator Output required to produce Motor Evoked Potentials of 200 to 300 microvolt amplitude in 50% of trials.

Central Respiratory Drive Potential (CRDP)

Central Respiratory Drive Potential the output from the pontomedullary respiratory central pattern generators to respiratory motor neurones. The Central Respiratory Drive Potential rhythmically alternates excitation and inhibition, distributed to respiratory motor neurones at segmental level through spinal distribution networks.

Centre of Gravity (CoG)

The Centre of Gravity of a motor map is a robust measure that gives the response at each grid point a relative weighting. It provides a means of comparing regions of cortical representations between studies. The CoG predicts the region of greatest excitability of corticomotor neurones projecting to the muscle studied. Centre of Gravity is calculated with the formula $Xcg = \Sigma xa/\Sigma a$ to determine the CoG along the mediolateral dimension and $Ycg = \Sigma ya/\Sigma a$ to locate the CoG along the anteroposterior dimension. The centre of gravity of the map is the point at which both coordinates intersect.

Common Drive

Common Drive describes motor control by a descending command that can control motor units exhibiting different firing patterns within a contracting muscle through a common command signal. Common Drive is evident in muscles that are habitually bilaterally activated.

Compound Motor Action Potential (CMAP)

The Compound Motor Action Potential is recorded in muscle EMG as the sum of potentials from all activated motor units. Summated action potentials carried in the motor nerve are desynchronized from passage in axons of different physical properties. The Compound Motor Action Potential obtained from supramaximal peripheral stimulation is frequently used as a control measure in TMS experiments.

Cross-Correlogram

Cross-correlograms may be used to describe synchrony of spike discharge between neuronal populations. Peaks in a cross-correlogram of motor unit discharge in homologous muscle pairs can be used to investigate bilaterally descending motor commands. In this setting a central peak can be interpreted as evidence of bilateral motor control and is evident in homologous axial muscle pairs.

Excitatory Post Synaptic Potential (EPSP)

Action potentials arriving at the pre-synaptic terminal activate voltage sensitive calcium channels triggering release of synaptic vesicles into the synaptic cleft. Excitatory neurotransmitter release increases permeability of the post-synaptic membrane. Sodium and potassium flux across the post-synaptic membrane results in post-synaptic depolarization producing Excitatory Post Synaptic Potential.

Inhibitory Post Synaptic Potential (IPSP)

Action potentials arriving at the pre-synaptic terminal activate voltage sensitive calcium channels triggering release of synaptic vesicles into the synaptic cleft. Inhibitory neurotransmitter release increases permeability of the post-synaptic membrane. Potassium and chloride flux across the post-synaptic membrane results in hyperpolarization, the Inhibitory Post Synaptic Potential.

Inter Hemispheric Inhibition (IHI)

Inter Hemispheric Inhibition is a Transcranial Magnetic Stimulation measure of activity in transcallosal interhemispheric pathways. A Conditioning pulse delivered to one hemisphere is timed to precede a Test pulse in the opposite hemisphere. The interstimulus interval of 6 to 15 ms aims to allow collision of the Conditioning pulse travelling transcallosally with activity of the homologous motor region from the Test hemisphere. IHI normally functions to prevent unwanted release of movement from the contralateral hemisphere during skilled motor tasks.

Intracortical Facilitation (ICF)

Intracortical Facilitation is a Transcranial Magnetic Stimulation measure of excitability in intracortical circuits. It is measured from primary motor cortex using a paired pulse protocol. A subthreshold Conditioning pulse is used to probe effect on corticomotor output from a subsequent suprathreshold Test pulse. Interstimulus intervals are timed to reveal activation of low threshold interneurons in intracortical circuits. For Intracortical Facilitation interstimulus intervals in the order of 10 to 15 ms are used.

Laterality Index (LI)

The Laterality Index is a TMS measure that expresses the relative strength of ipsilateral to contralateral innervation to a muscle. The strength of ipsilateral and contralateral MEPs are compared. Laterality Index is given as (Contralateral MEP-Ipsilateral MEP-)/(Contralateral MEP+Ipsilateral MEP).

Long Intracortical Inhibition (LICI)

Long Intracortical Inhibition is a Transcranial Magnetic Stimulation measure of activity in GABAergic inhibitory intracortical circuits. It is measured from primary motor cortex using a paired pulse protocol. A suprathreshold Conditioning pulse is used to probe effect on corticomotor output from a subsequent suprathreshold Test pulse. Interstimulus intervals are timed to reveal activation of inhibitory interneurons in intracortical circuits. For Long Intracortical Inhibition interstimulus intervals in the order of 50 to 200 ms are used.

Map Volume

The Map Volume is a measure used in Transcranial Magnetic Stimulation studies of cortical motor maps. Map Volume is calculated as the sum of relative MEP amplitudes from all excitable points. The Mean Map Volume is calculated from the Map Volume using the number of points stimulated to evoke responses.

Maximum Stimulator Output (MSO)

Maximum Stimulator Output is the strongest magnetic field that can be generated by the stimulator for Transcranial Magnetic Stimulation. Intensity of stimulation is always referred to as a percentage of the Maximum Stimulator Output.

Motor Evoked Potential (MEP)

The Motor Evoked Potential is the Compound Motor Action Potential evoked in response to cortical Transcranial Magnetic Stimulation. The Motor Evoked Potential is the basic unit of measurement for the majority of Transcranial Magnetic Stimulation experiments.

Motor Map

The motor map is a description of the cortical representation of a muscle. The map is constructed by recording the size of potentials evoked in the muscle studied in response to cortical stimulation at different sites. Muscles have multiple, overlapping cortical representations. Output properties of motor maps are described using measures derived from the evoked potentials, such as Map Volume and Centre of Gravity. Motor maps can be constructed non-invasively using Transcranial Magnetic Stimulation or invasively using microstimulation or subdural stimulating electrodes.

Muscle Field

The output of a single cortical pyramidal cell can diverge segmentally to excite multiple alpha motorneurones supplying multiple muscle groups. This is an important principle in relation to synergy organization. The Muscle Field describes the muscles facilitated by output from a single cortical pyramidal cell.

Normalized Map Volume

The normalized map volume is a motor map measure that can be used to infer something of the focality of the map. The normalized volume is obtained by dividing the sum of MEPs from all grid points that produced

responses by the largest MEP from the map Hotspot. An extensive map evoking multiple small responses will contrast from a small, focal map producing limited large responses.

Peak Width at Half Maximum (PWHM)

Peak Width at Half Maximum is a measure from the Peri Stimulus Time Histogram that allows inference of the synaptic nature of the descending motor commands. The width of the histogram at half of the peak value is measured. Strong, direct, monosynaptic corticomotor connectivity will produce a tall, narrow peak in the histogram. Histograms measuring multiple, polysynaptic, desynchronized, weak inputs will produce a shorter, wider histogram.

Peri Stimulus Time Histogram (PSTH)

The Peri Stimulus Time Histogram plots magnitude of evoked responses against time from stimulation. A narrow, unimodal histogram suggests rapid monosynaptic transmission whereas a wide, polymodal pattern suggests a branched polysynaptic pathway.

Post Spike Effects

Post Spike Effects are the influence of discharge of a single cortical pyramidal cell on spinal alpha motor neurones measured in limb muscle EMG using Spike Triggered Averaging. Clear Post Spike Effects result from direct monosynaptic connectivity. Post Spike Effects can be excitatory, Post Spike Facilitation, or inhibitory, Post Spike Suppression.

Post Spike Facilitation (PSF)

Post Spike Facilitation is an increase in post spike EMG recorded by Spike Triggered Averaging in response to discharge of a cortical pyramidal cell. Post Spike Facilitation suggests an excitatory synaptic connection between the cortical pyramidal cell and the spinal alpha motorneurone, the strength of which can be explored using the latency, duration and amplitude of the Post Spike Facilitation. Strong Post Spike Facilitation suggests direct excitatory monosynaptic connectivity.

Post Spike Suppression (PSS)

Post Spike Suppression is a decrease in post spike EMG recorded in limb muscle by Spike Triggered Averaging in response to discharge of a cortical pyramidal cell. Post Spike Suppression suggests direct inhibitory synaptic connection between the cortical pyramidal cell and the spinal alpha motorneurone.

Pre Inspiratory Potentials (PIP)

Pre Inspiratory Potentials are premotor potentials recorded over Supplementary Motor Area in advance of voluntary respiratory activity. They signify preparatory activity in the planning of voluntary respiratory movement.

Repetitive Transcranial Magnetic Stimulation (rTMS)

Repetitive Transcranial Magnetic Stimulation is delivery of repetitive, patterned non-invasive stimulation to cortical neurones with the aim of modulating underlying cortical excitability. Repetitive stimulation may be patterned to promote an excitatory or inhibitory neuromodulation.

Respiratory Related Evoked Potentials (RREP)

Respiratory Related Evoked Potentials are potentials recorded in EEG from cortical regions responding to sensory afferent information related to respiration, such as inspiratory airflow resistance.

Resting Motor Threshold (rMT)

Resting Motor Threshold is a term used in Transcranial Magnetic Stimulation experiments to reproducibly quantify the level of stimulation. The Resting Motor Threshold is measured as the stimulation intensity required to produce Motor Evoked Potentials in muscle at rest. It is the percentage of Maximum Stimulator Output required to produce Motor Evoked Potentials of 0.5mV amplitude in 50% of trials.

Short Intracortical Inhibition (SICI)

Short Intracortical Inhibition is a Transcranial Magnetic Stimulation measure of activity in inhibitory intracortical circuits. It is measured from primary motor cortex using a paired pulse protocol. A subthreshold Conditioning pulse is used to probe effect on corticomotor output from a subsequent suprathreshold Test pulse. Interstimulus intervals are timed to reveal activation of low threshold inhibitory interneurons in intracortical circuits. Short Intracortical Inhibition uses interstimulus intervals in the order of 1 to 5 ms.

Spike Triggered Averaging (STA)

Spike Triggered Averaging is a method of averaging EMG from muscle in response to spiking of a single cortical pyramidal cell. Implanted cortical intracellular electrodes trigger averaging of rectified limb muscle EMG across a time window with prespike and postspike activity. Cortical

pyramidal cells directly facilitating alpha motor neurones innervating the limb muscle studied will produce post spike effects.

Synergy

Synergies are spatiotemporal patterns of muscle activation that are stable across movements, achieved by coactivation or reciprocal inhibition of groups of muscles. Synergies are constructed through linearly recruited modules, each defining a pattern of muscle activation. Time varying and combining modules results in a small number of synergies being able to account for large variations of patterns of muscle activation. Synergies provide a computationally efficient means of motor control.

Synergy Module

A Synergy Module is an organizational unit playing a role in the motor control of synergies. Neuronal ensembles of motor and premotor neurones organize motor commands into basic patterns of coactivated or inhibited muscle groups. Synergy modules may be present at cortical, brainstem or spinal levels.

Synergy Ratio

The Synergy ratio is a TMS measure of the effectiveness of corticomotor synergy control. The Synergy Ratio compares the MEP evoked when the biceps is recruited to a task in agonist role to the MEP evoked in antagonist role. The Synergy Ratio is expressed as MEP agonist / MEP antagonist. Correctly functioning motor control produces a Synergy ratio in the order of 0.3. The ratio may become closer to 1 when the corticospinal tract is damaged in stroke patients.

Theta Burst Stimulation (cTBS/iTBS)

Theta Burst Stimulation is a plasticity inducing neuromodulatory Transcranial Magnetic Stimulation protocol. Theta Burst Stimulation patterns bursts of repetitive Transcranial Magnetic Stimulation at theta frequency. This seeks to mimic neuronal firing patterns producing Long Term Potentiation and Long Term Depotentiation. When delivered continuously an inhibitory modulation can be achieved (cTBS). When delivered with intermittent patterning an excitatory modulation can be achieved (iTBS).

Transcranial Direct Current Stimulation (tDCS)

Transcranial Direct Current Stimulation is a non-invasive neuromodulatory tool with low temporal and spatial resolution. Delivery of continuous current across the scalp and through underlying brain parenchyma is used to modulate activity of cortical neurones. Current flow beneath the anode aims to increase neuronal excitability. Conversely, current flow beneath the cathode seeks to enhance inhibition.

Transcranial Magnetic Stimulation (TMS)

Transcranial Magnetic Stimulation provides a non-invasive means of stimulating the brain with high temporal resolution. A brief, focal, high intensity electromagnetic pulse is delivered across the scalp using a coil held to the scalp surface. The induced electric field interacts with underlying cortical neurones. Direct depolarization, or summation of multiple synaptic inputs from interneurons may produce corticomotor output from pyramidal cells. Transcranial Magnetic Stimulation can be used to investigate corticomotor output measured Motor Evoked Potentials, or to alter cortical excitability using neuromodulatory protocols.

LIST OF ABBREVIATIONS

aMT	Active Motor Threshold
CMA	Cingulate Motor Area
CMAP	Compound Motor Action Potential
CoG	Centre of Gravity
CRDP	Central Respiratory Drive Potential
cTBS	Continuous Theta Burst Stimulation
EEG	Electroencephalogram
EMG	Electromyogram
EPSP	Excitatory Post Synaptic Potential
ICF	Intracortical Facilitation
IHI	Inter Hemispheric Inhibition
IPSP	Inhibitory Post Synaptic Potential
iTBS	Intermittent Theta Burst Stimulation
LI	Laterality Index
LICI	Long Intracortical Inhibition
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MCA	Middle Cerebral Artery
MRI	Magnetic Resonance Imaging
ms	Millisecond
MSO	Maximum Stimulator Output

mv	Millivolt
MVC	Maximum Voluntary Contraction
MVPA	Multivoxel Pattern Analysis
NRG	Nucleus Reticularis Gigantocellularis
NRPC	Nucleus Reticularis Pontis Caudalis
NRPO	Nucleus Reticularis Pontis Oralis
PEP	Pre Expiratory Potential
PET	Positron Emission Tomography
PIP	Pre Inspiratory Potential
PMC	Premotor Cortex
PSF	Post Spike Facilitation
PSS	Post Spike Suppression
PSTH	Peri Stimulus Time Histogram
PWHM	Peak Width at Half Maximum
rMT	Resting Motor Threshold
RREP	Respiratory Related Evoked Potentials
rTMS	Repetitive Transcranial Magnetic Stimulation
SICI	Short Intracortical Inhibition
SMA	Supplementary Motor Area
SR	Synergy Ratio
STA	Spike Triggered Averaging
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation

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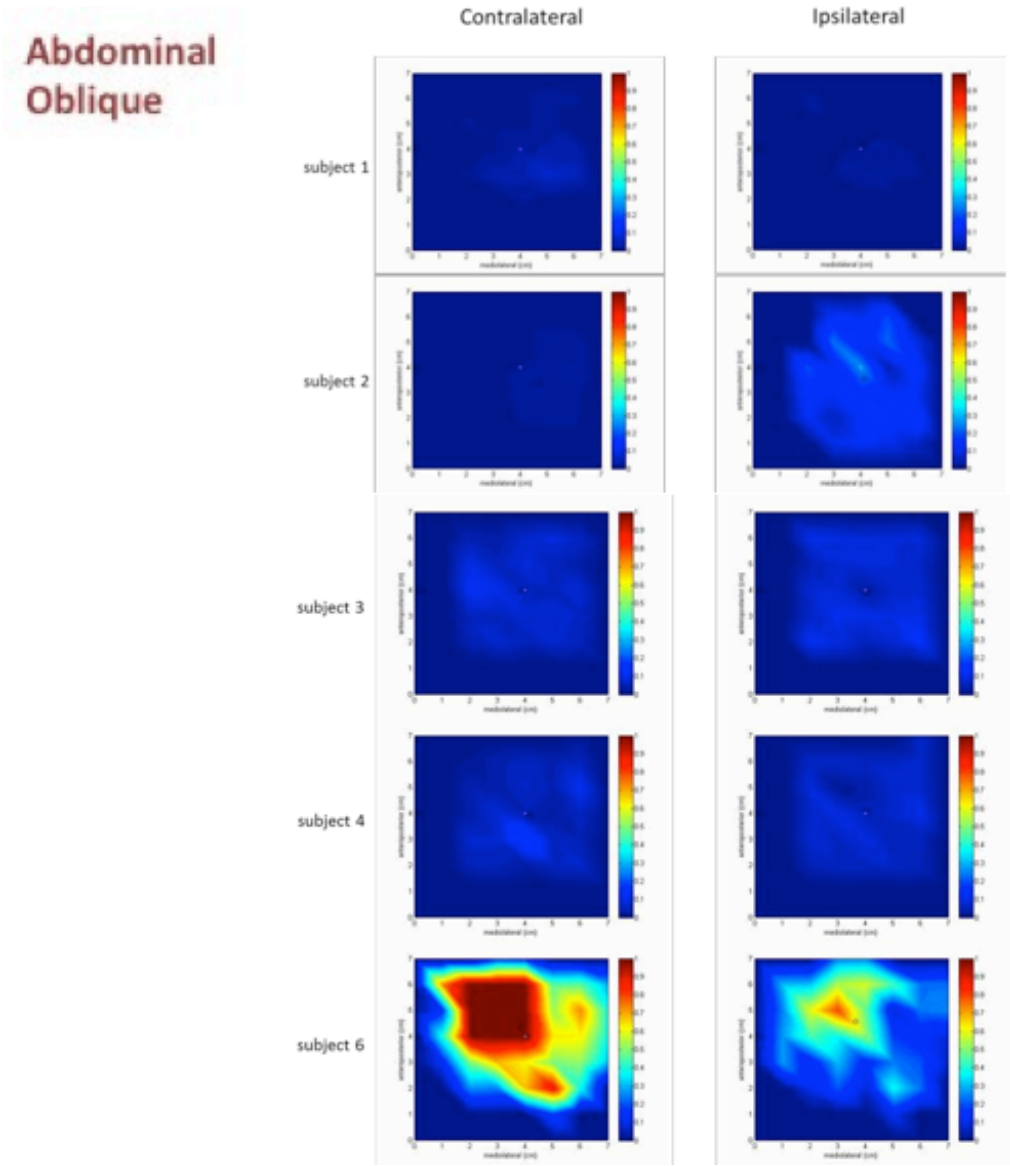
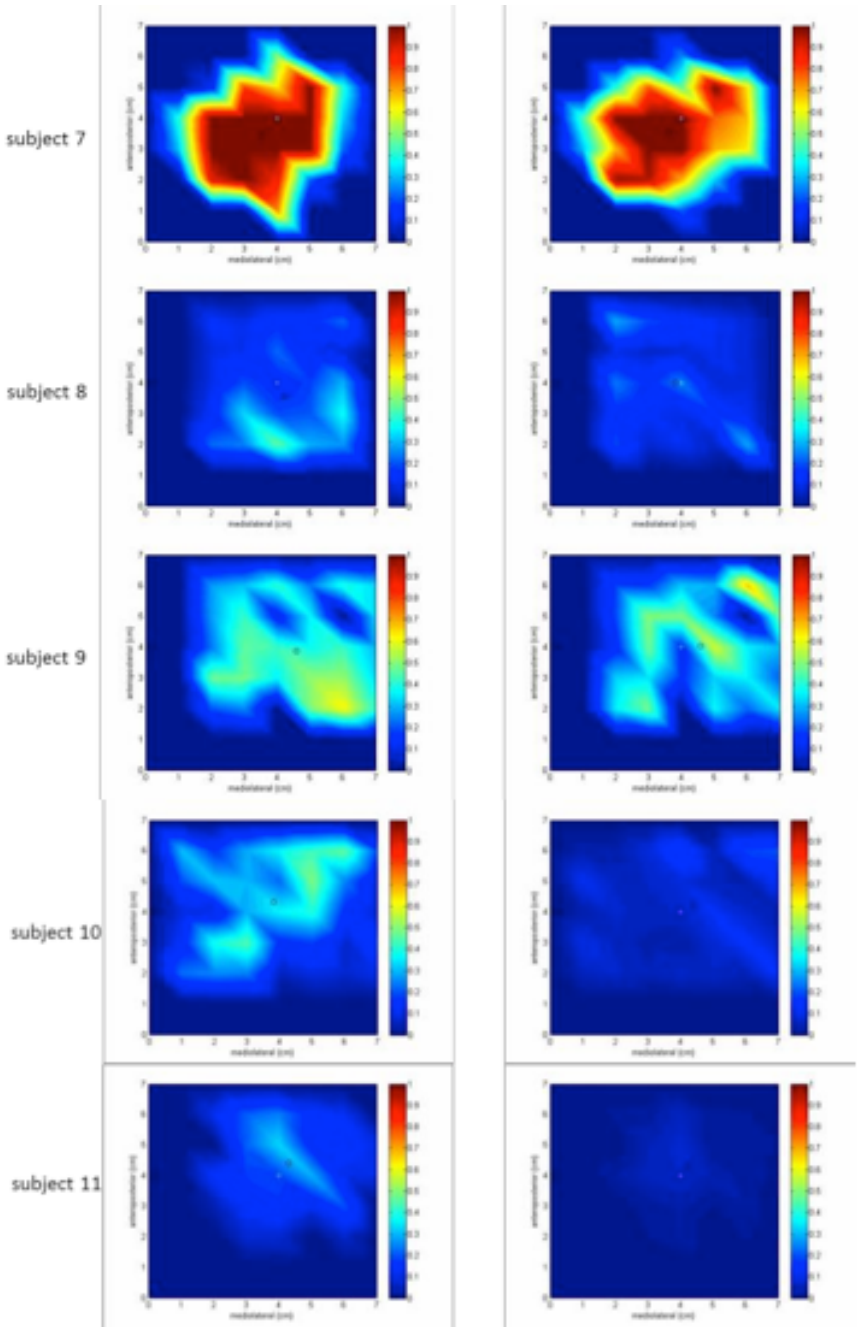
APPENDICES

APPENDIX A

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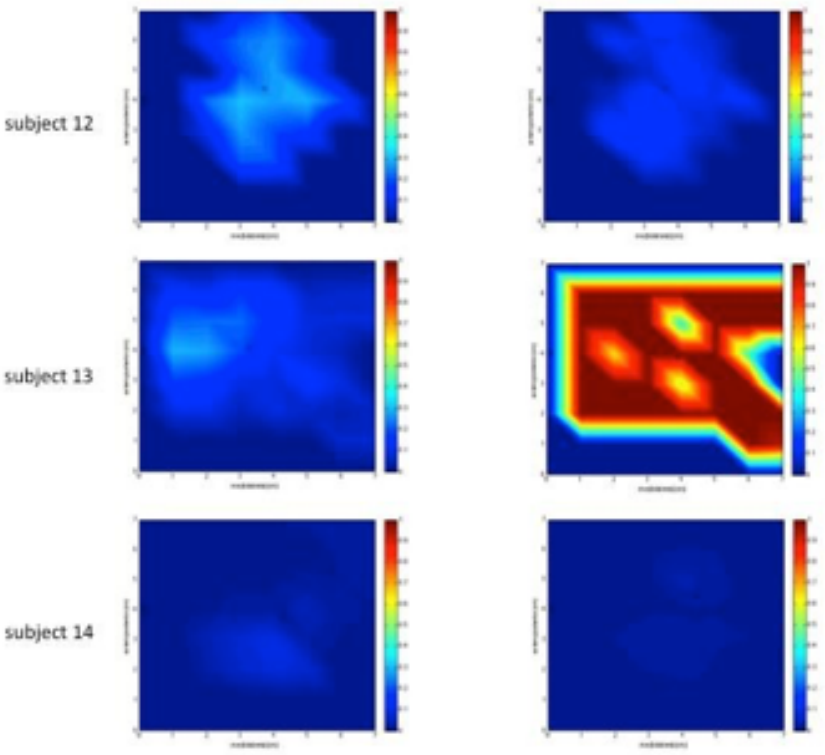
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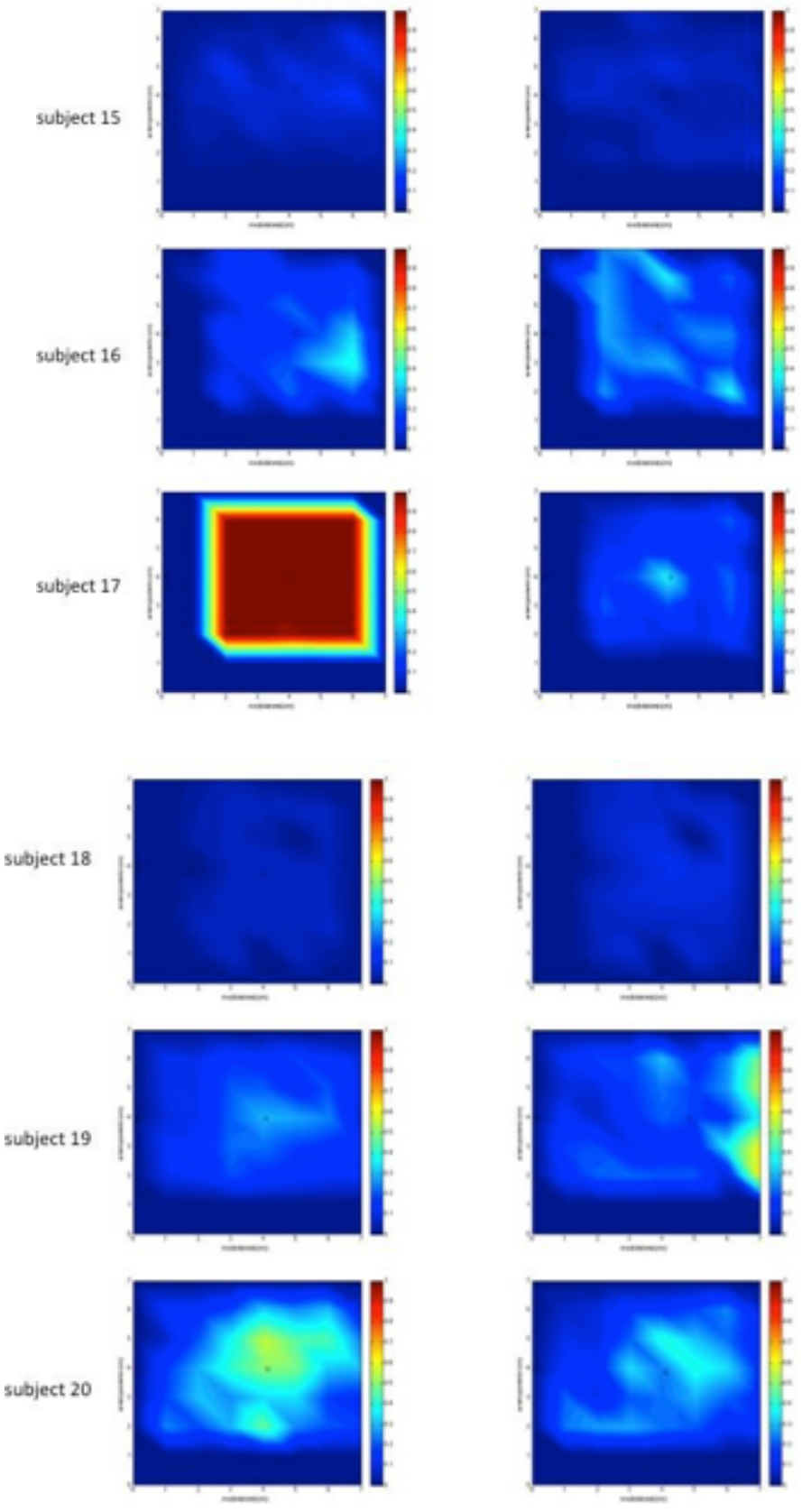
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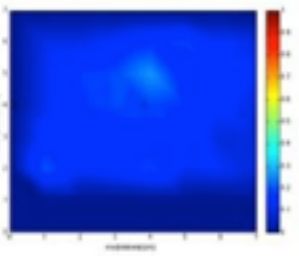

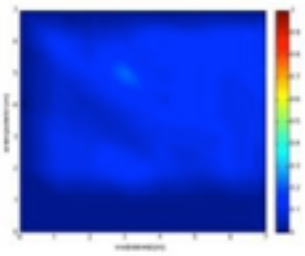

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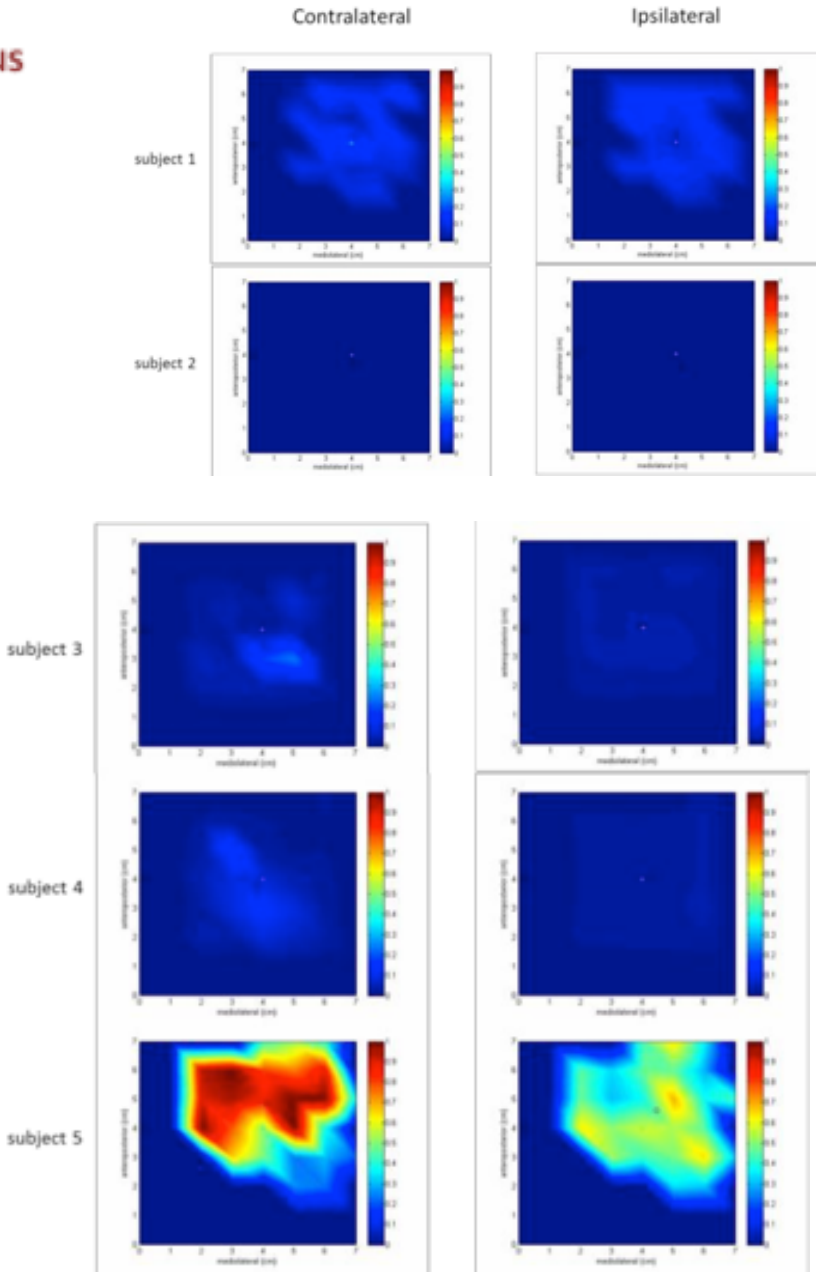
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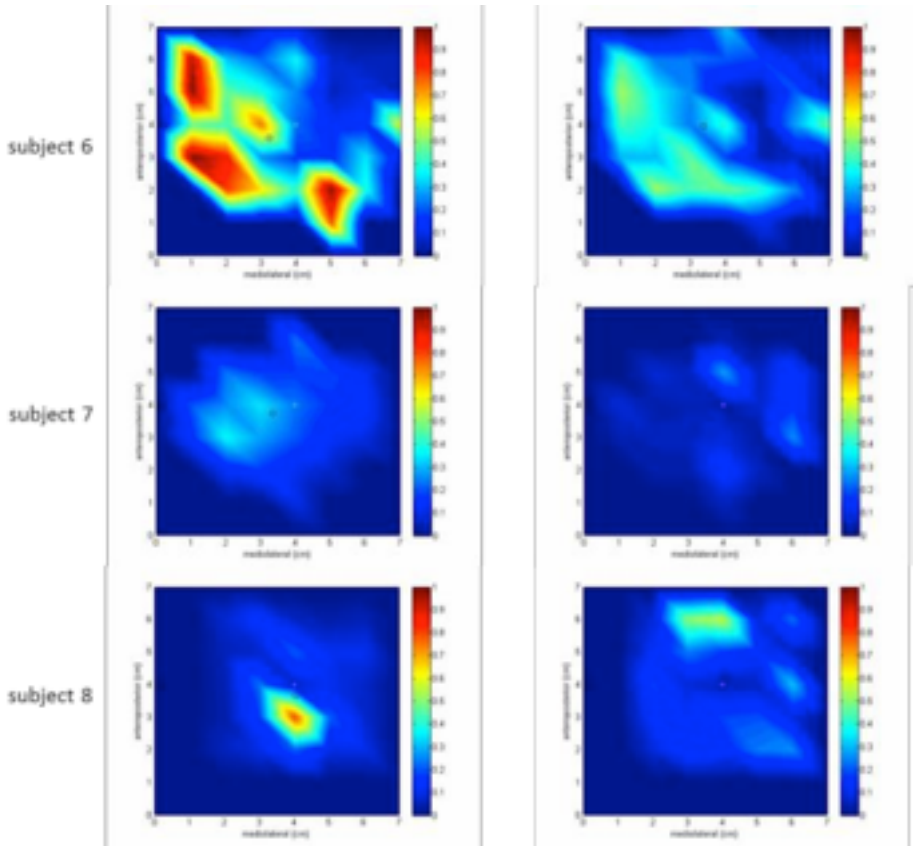
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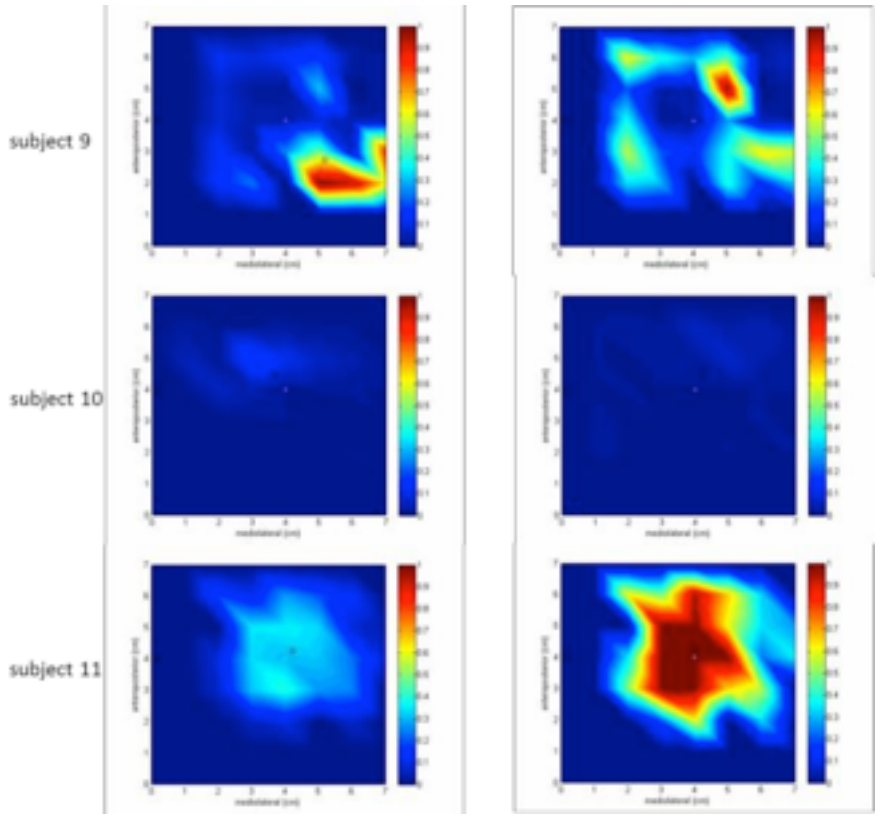
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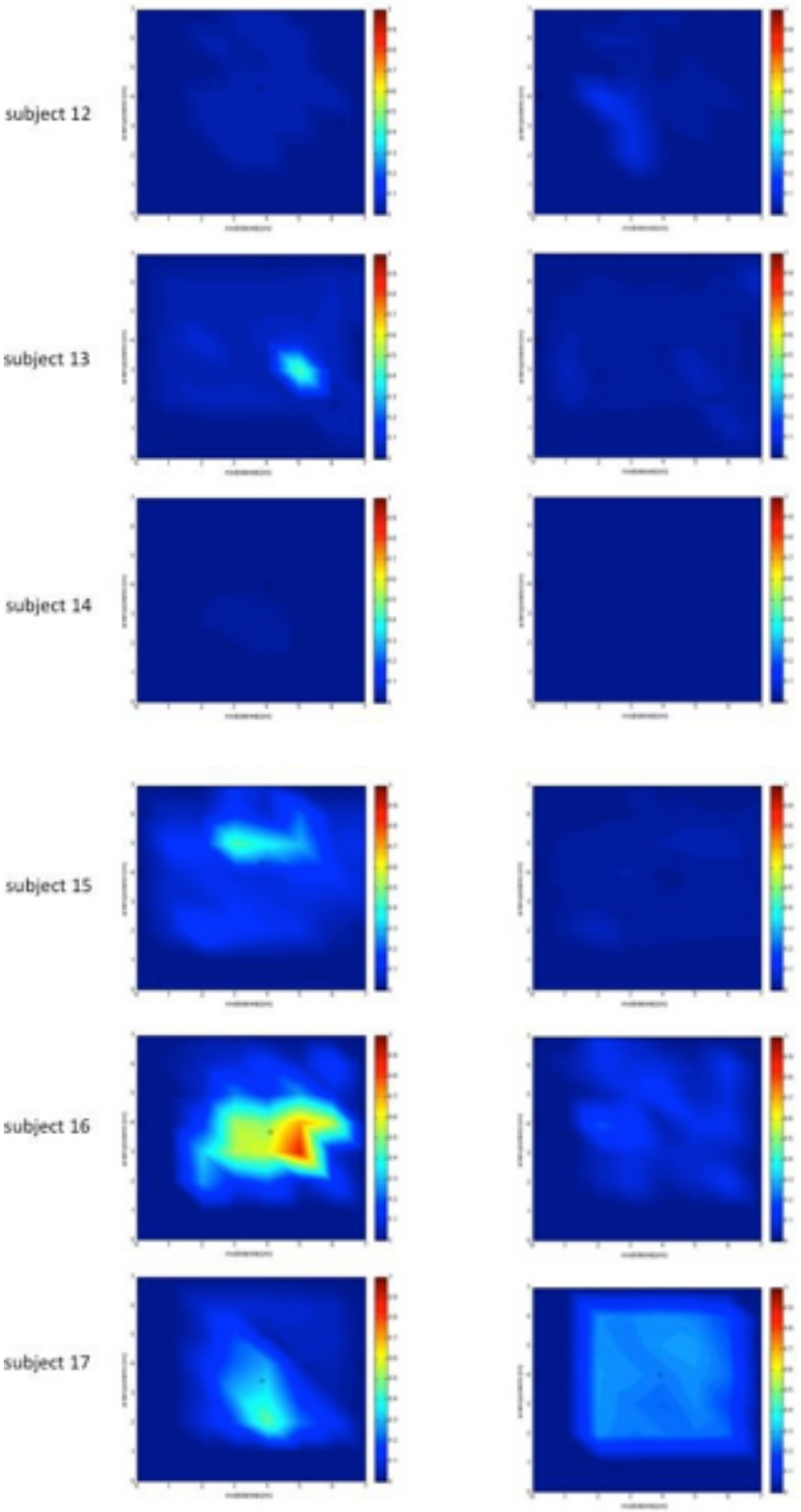
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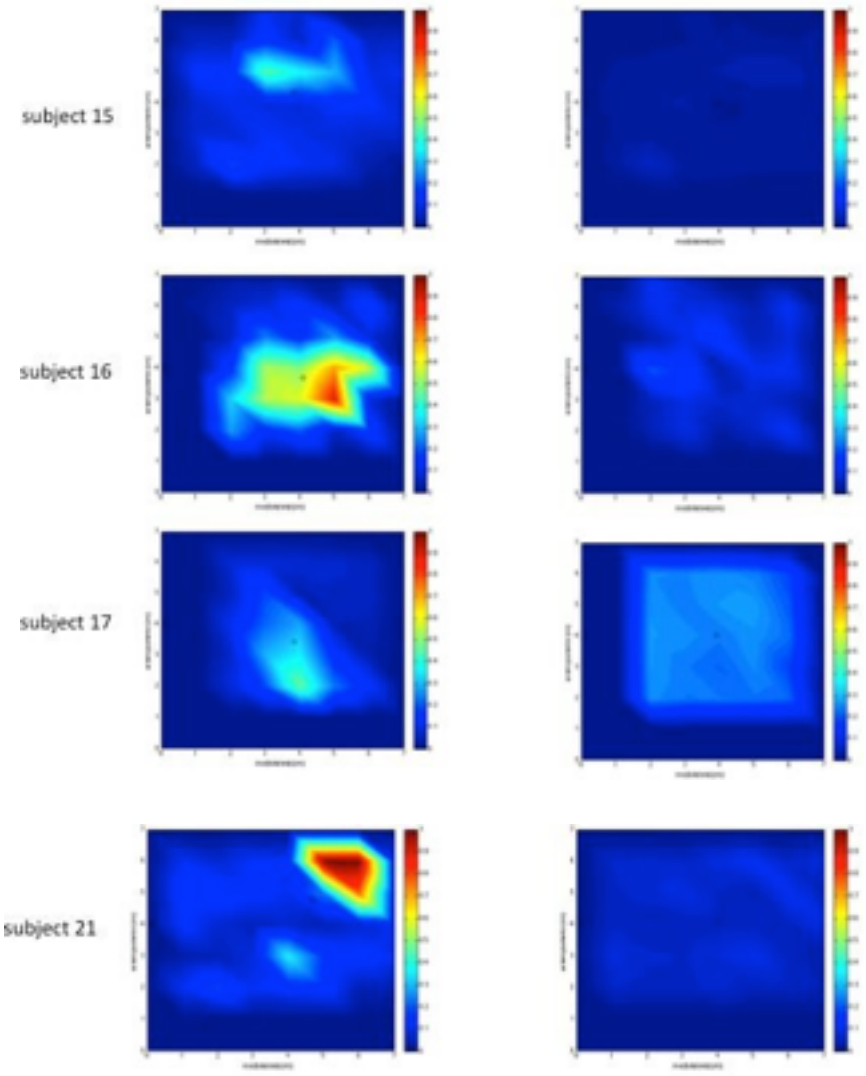
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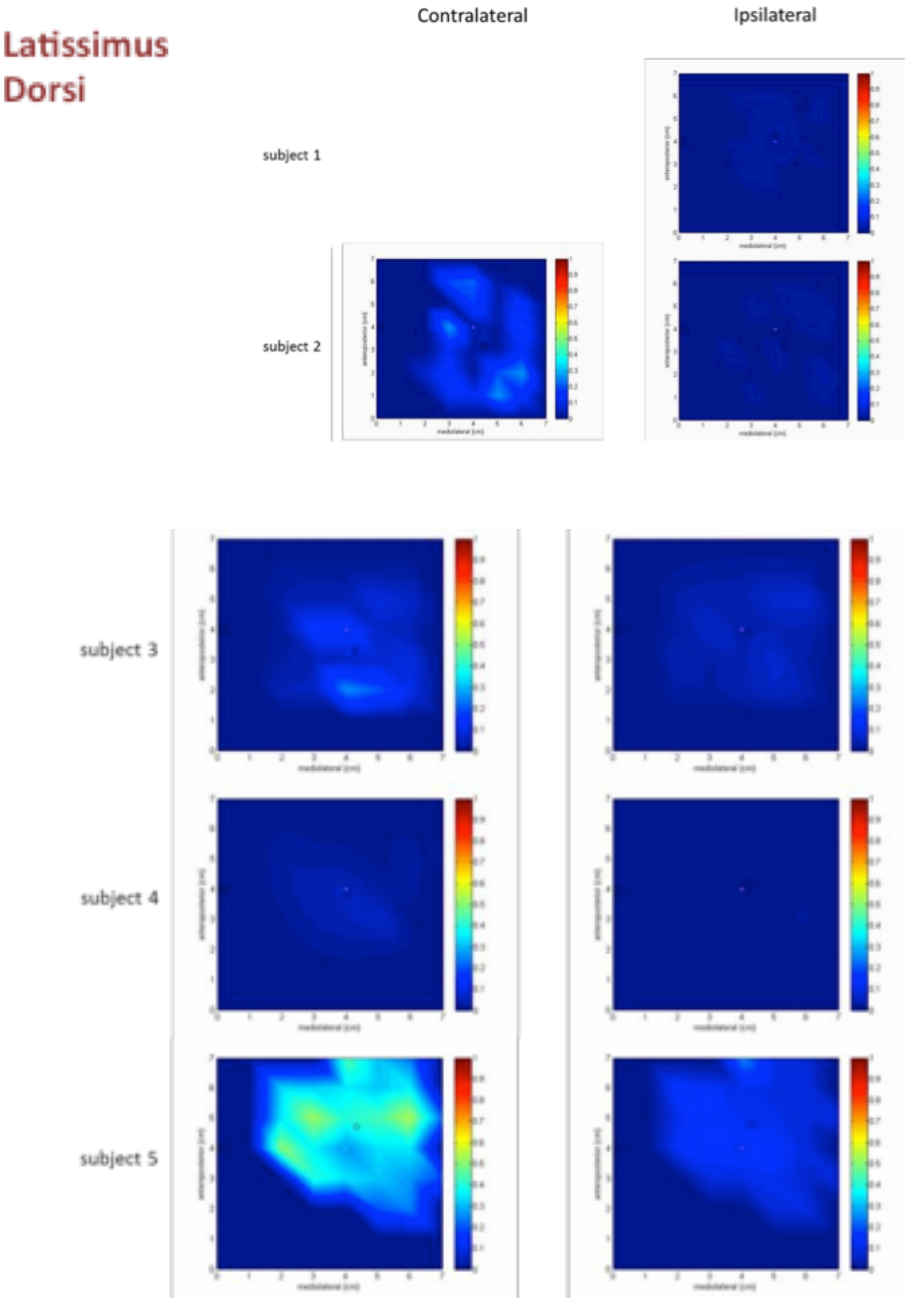
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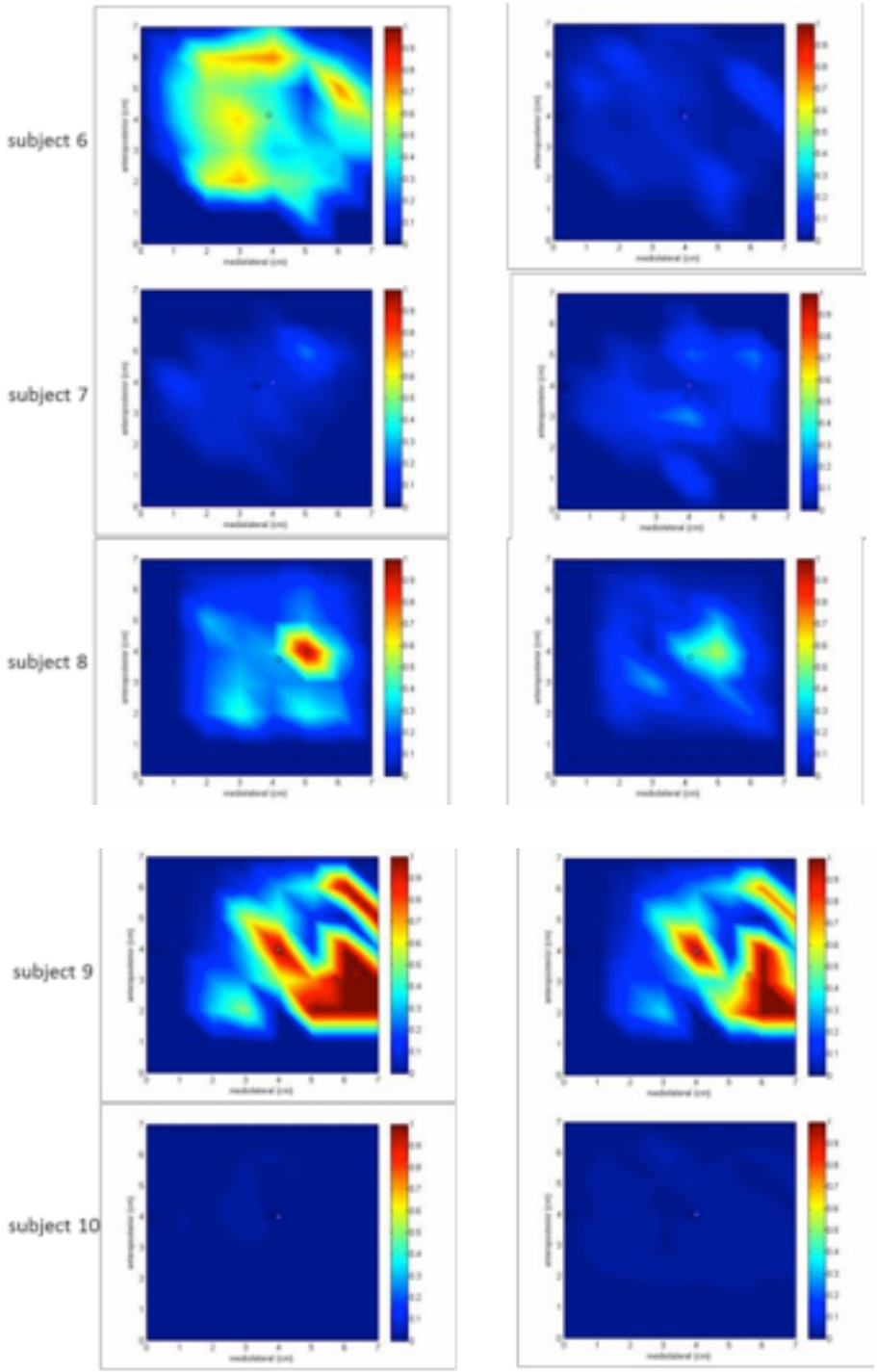
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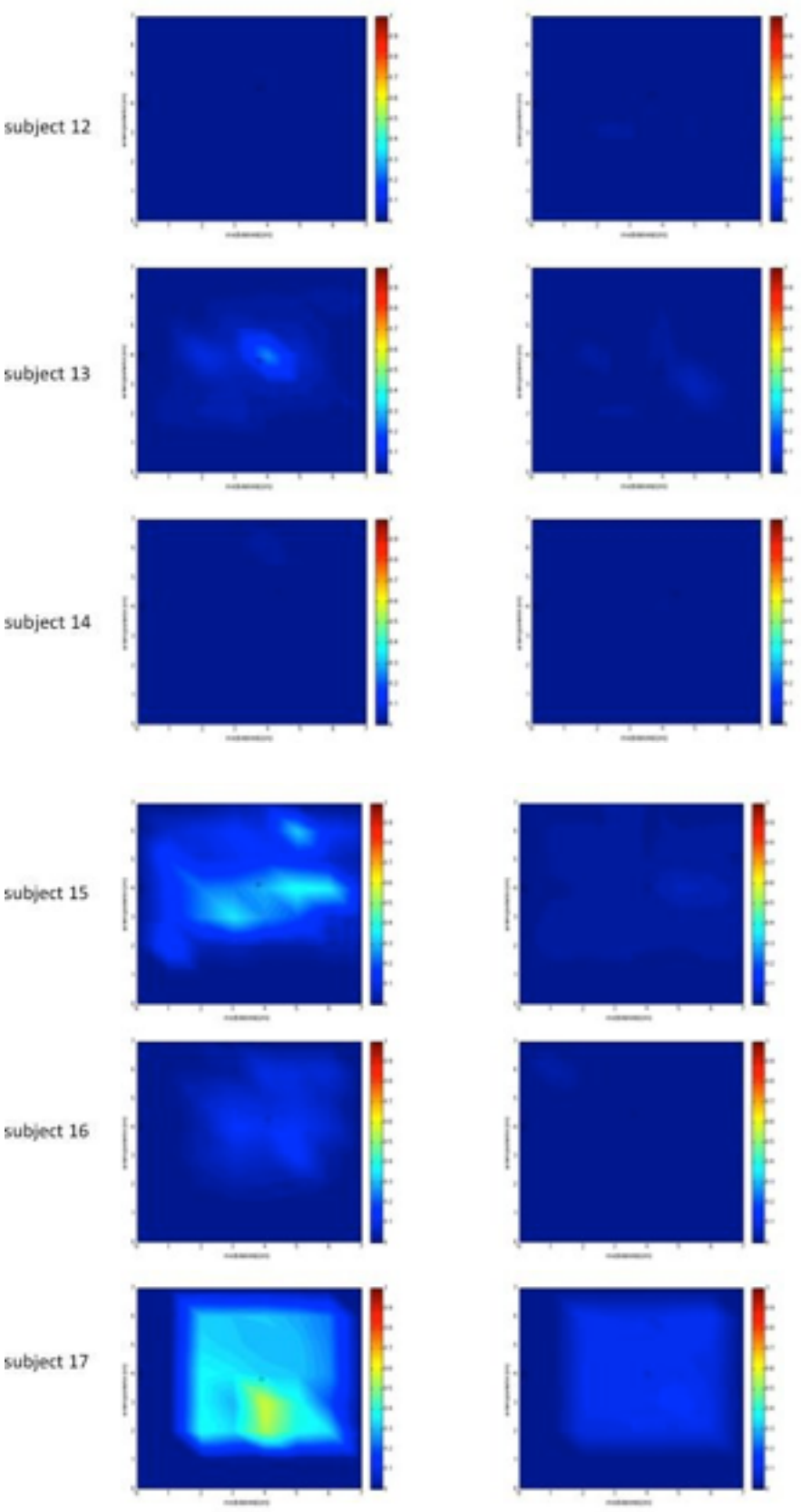
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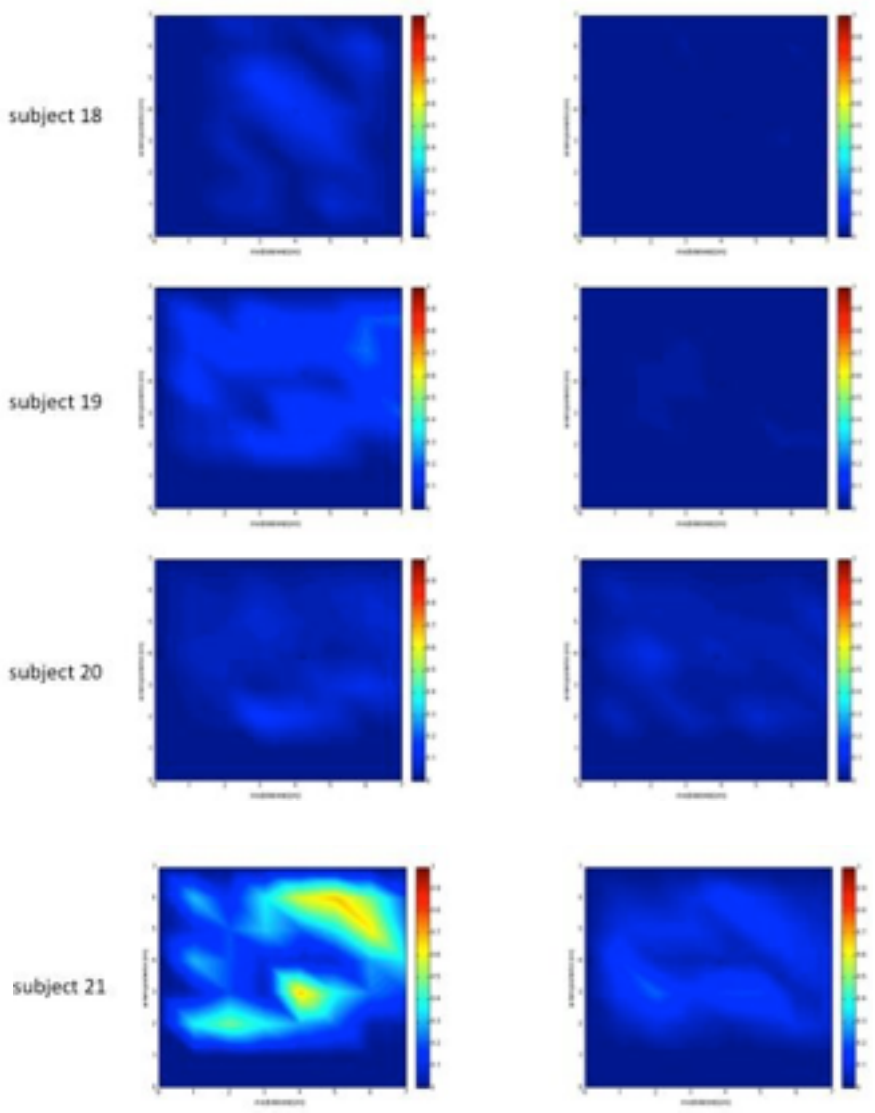
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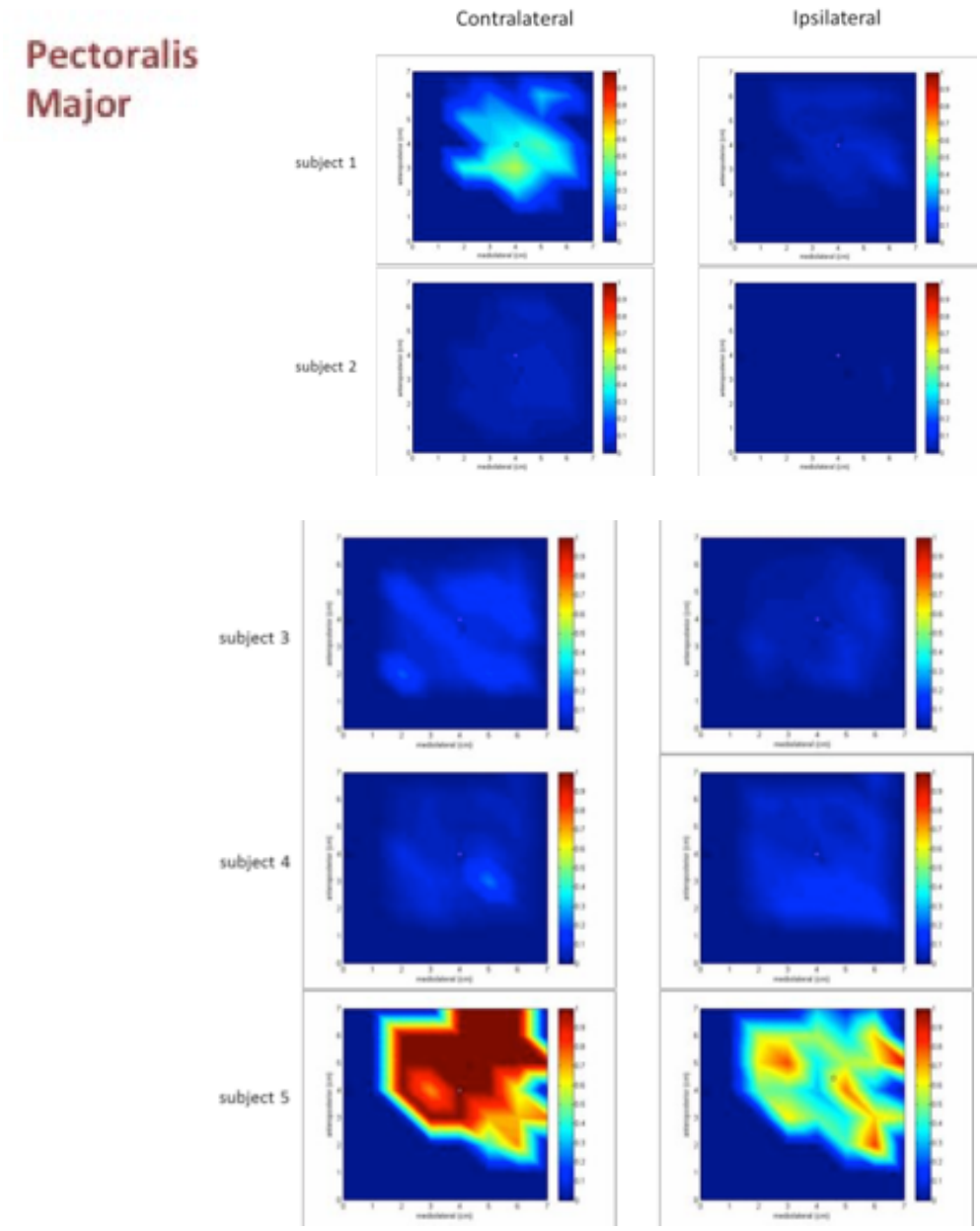
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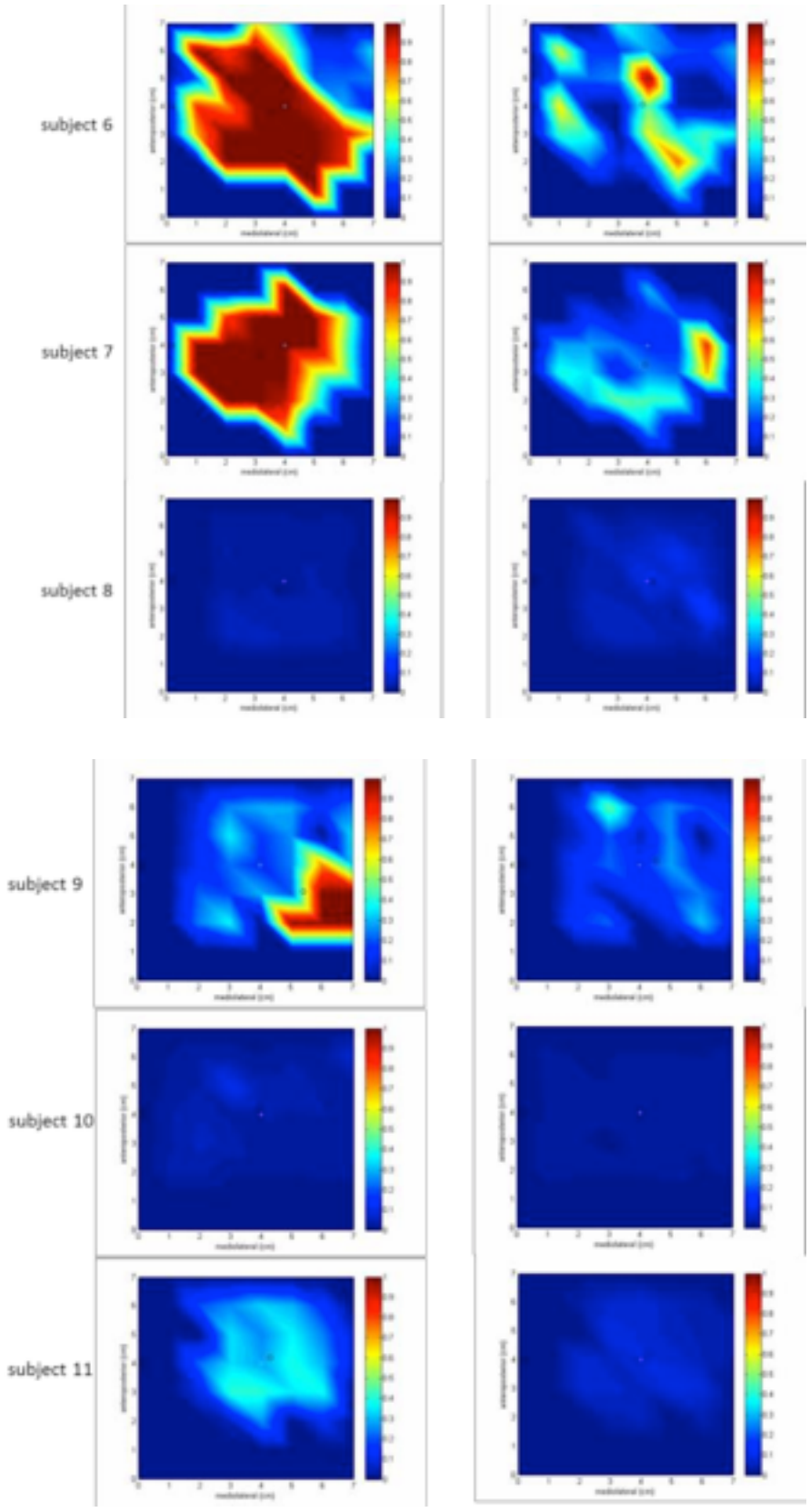
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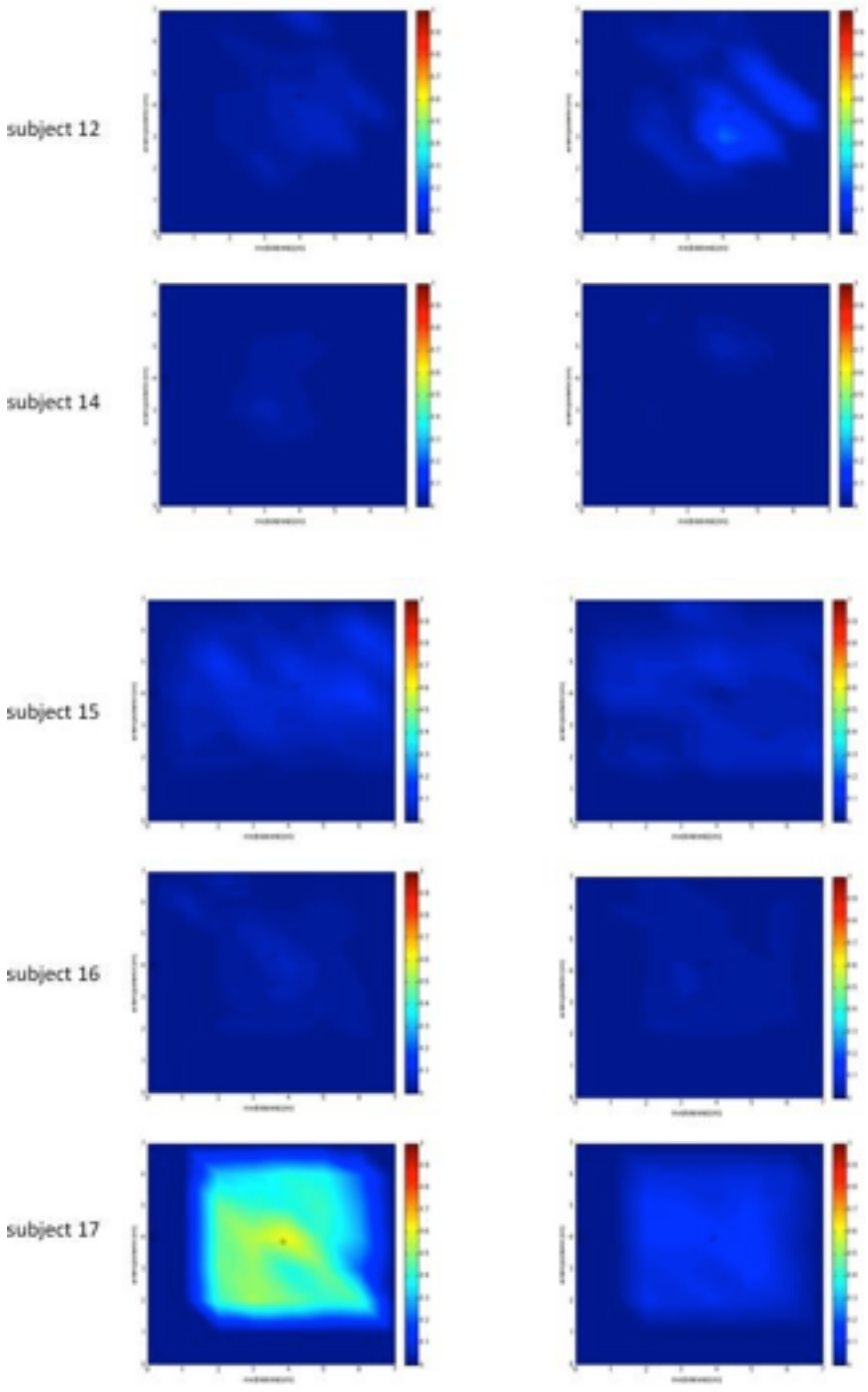
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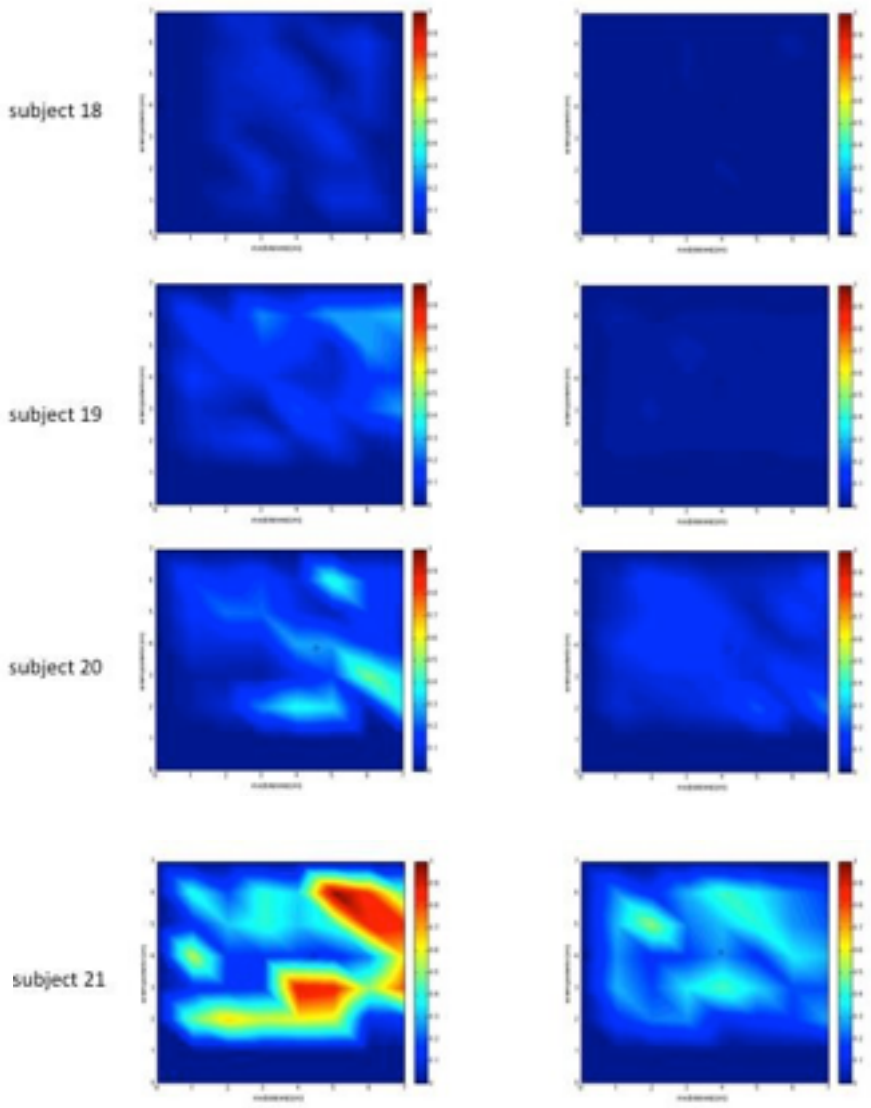
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