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Neurophysiological effects of ischaemia

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Submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy of Imperial College London, March 2022

Contents

Declaration of Originality ..... **xi**

Copyright Declaration ..... **xii**

Abstract ..... **xiii**

Acknowledgements ..... **xv**

Table of Figures ..... **xvii**

List of Tables ..... **xxv**

Abbreviations ..... **xxvi**

Aims and Outline of Thesis ..... **xxix**

Chapter 1: Introduction and Background **1**

Thoraco-abdominal Aortic Aneurysms 1

Epidemiology & Aetiology of TAAA 1

Classification 2

Spinal Cord Injury following TAAA Repair 3

Anatomy of Spinal Cord Blood Supply 6

Pathophysiology of Ischaemic SCI 11

Management of SCI following TAAA repair 13

Augmentation of spinal cord perfusion 14

Increasing tolerance to ischaemia 14

Intra-operative neuromonitoring of the Spinal Cord 14

Transcranial Magnetic Stimulation 16

Principles of TMS 16

Stimulation Coils 18

TMS-induced Motor Evoked Potentials 20

MEP Characteristics 21

Modulation of Cortical Plasticity using an Ischaemic Nerve Block 24

Motor Cortical Plasticity 25

Mechanisms of Cortical Plasticity 25

Near Infrared Light Spectroscopy 26

Physical Principles of NIRS 26

NIRS in Thoraco-abdominal Aortic Aneurysm Surgery 27

Chapter 2: Participants & General Methods 29

Introduction 29

Participant Recruitment 29

Healthy Participants 30

Patients 31

Neurophysiological Assessments 31

Transcranial Magnetic Stimulation 31

Somatosensory Evoked potentials 39

Quantitative Sensory Testing 39

Functional Assessments 40

Near Infrared Light Spectroscopy 40

Clinical Assessments 41

Patient Disease Severity Questionnaire (VascuQol) 41

Pain Scores 42

Experimental Intervention 42

Ischaemic Nerve Block 42

Statistical Analysis 43

Chapter 3: Survey of Intra-operative Neuromonitoring during TAAA Surgery in the United Kingdom and Ireland 45

Introduction 45

Aim 46

Methods 46

Data Analysis 47

Results 47

Questions 1 and 2 47

Question 3 48

Question 4 48

Question 5 48

Questions 6 and 7 49

Question 8 49

Question 9 50

Question 10 50

Discussion 51

Cost of Intra-operative Neuromonitoring 51

IONM is not beneficial and unlikely to change outcome 52

Awareness of IONM 53

Interventions designed to prevent or treat SCI 53

Increasing accessibility of IONM in TAAA surgery 54

Limitations 56

Conclusions & Recommendations 56

Chapter 4: An investigation into the variability of MEPs in patients with peripheral vascular disease 57

Introduction 57

Aim 58

Methods 59

Participants 59

Patient Disease Severity Questionnaire (VascuQol) 59

Surface EMG 59

Transcranial Magnetic Stimulation 60

Experimental Protocol 61

Data analysis 61

Statistical analysis 62

Results 63

VascuQol Score in PVD Group 63

MEP Amplitude 64

Correlations 65

CV of avMEP amplitude 69

Mean CV of MEP amplitude at each time point 70

avMEP Latency 72

Discussion 73

MEP Amplitude and Latency 73

Reliability 75
Limitations 77
Conclusions 78
Chapter 5: Cortical excitability changes induced by a novel low-pressure INB 79
Introduction 79
Aim 80
Methods 81
Participants 81
Pre-experiment Vascular Assessments 81
Surface EMG 81
Motor Evoked Potential (MEPs) 82
Somatosensory Evoked Potentials (SSEPs) 83
Near-infrared Spectroscopy (NIRS) 84
Occlusion and pain 85
Experimental Protocols 85
Data analysis 87
Results 88
Experiment 1 88
Experiment 2 95
Discussion 97
Low-Pressure Ischaemic Nerve Block 98
Cortical Excitability 99
Effect of pain 102

Limitations 103

Conclusion 104

Chapter 6: An investigation of deafferentation induced by ischaemic nerve block 105

Introduction 105

Aim 106

Methods 106

Participants 106

Pre-experiment blood pressure assessment 106

Surface EMG 106

Motor evoked potential 107

Quantitative Sensory Testing (QST) 108

Pain score 109

Experiment Protocol 109

Data Analysis 110

Statistical analysis 111

Results 111

Normalised MEP Amplitudes 111

Pain scores 115

Quantitative Sensory Testing 116

Hypersensitive vs Hyposensitive Response to Punctate Sensation 118

Discussion 121

Changes in corticospinal excitability 122

Sensory Changes 122

Differing Sensory Responses 123
Effects of Pain and Hypersensitivity 124
Potential Mechanism for Hypersensitive Response 125
Conclusion 126
Chapter 7: Exploration of mechanisms causing changes in cortical excitability in the motor cortex ipsilateral to an INB 127
Introduction 127
Aim 127
Methods 128
Participants 128
Pre-experiment blood pressure assessment 128
Surface EMG 128
Motor evoked potential 129
Experiment Protocols 130
Data Analysis 132
Statistical analysis 133
Results 134
Experiment 1 134
Experiment 2 138
Discussion 143
Conclusion 152
Chapter 8: TMS and NIRS in Theatre 153
Introduction 153

Experiment 1: TMS in Orthopaedic Surgery 154
Patient A 154
Patient B 157
Discussion 160
Limitations and TMS in Theatre 167
Experiment 2: NIRS in TAAA Surgery 168
Patient Demographics 169
Setup and intra-operative protocol 169
Results 171
Discussion 189
Future perspectives and clinical use 194
Conclusion 196
Chapter 9: Conclusions 197
Appendix 1: classification of TAAAs 201
Appendix 2: Methods to reduce SCI 203
Overview 203
Maintaining spinal cord perfusion: 203
Mean arterial pressure 203
CSF Pressure 204
Hypothermia 205
Surgical techniques 205
Appendix 3 – Sample Patient information sheet and consent form 208
Appendix 4 – TMS Safety Questionnaire 217

Appendix 5 – VascuQol Questionnaire 219

Appendix 6 – Papers & Abstarcts 229

Papers 229

Abstracts 229

References 230

Declaration of Originality

I hereby declare that this thesis and the work reported herein was composed by and originated entirely from me. Information derived from the published and unpublished work of others has been appropriately referenced. Any contribution made to this research by others is explicitly acknowledged in the text.

The author of this thesis has been the principal investigator in the work described in it, and has been involved in the planning, experimenting and analysis of all studies. A number of experiments were offered as laboratory projects for the Neuroscience BSc and Anaesthesia and Surgery BSc, and the Masters in Research MSc at Imperial College London; students undertaking these projects assisted in data collection and analysis. The experiments were conceived and executed by the author under the supervision of Dr Paul Strutton, with clinical advice for experiments provided by Mr Colin Bicknell.

Original investigations described in this thesis have been published in abstract form and presented in oral and poster formats at national and international scientific meetings. Copies of the published abstracts are provided in the appendices.

Copyright Declaration

Abstract

Spinal cord injury (SCI) and paralysis remains a tragic complication of thoraco-abdominal aortic aneurysm (TAAA) surgery, despite advances in surgical and medical management. A survey of vascular anaesthetists showed availability of intra-operative spinal cord monitoring to detect an injury and subsequently guide remedial interventions, is variable across the United Kingdom and Ireland, despite clear evidence of its benefit. This research sought to explore the potential benefits of transcranial magnetic stimulation (TMS) and near infrared spectroscopy (NIRS) as alternative, more accessible monitors of ischaemic SCI.

TAAA surgery has several nuances that required greater investigation if TMS was to be utilised in theatre. Firstly, the motor evoked potentials (MEPs) of peripheral vascular disease (PVD) patients were characterised. PVD is the primary pathology underlying TAAA and the MEPs of this cohort of patients showed no difference beyond that which would accountable by aging compared to healthy, younger controls. Also, it was demonstrated that over an hour of repeated single-pulse TMS, a time-frame similar to when the spinal cord is at greatest risk intra-operatively and a need for intense monitoring, the variability of the MEPs was no different to controls.

A second feature of TAAA surgery is the need to render the surgical field bloodless, thus providing a clear operative space for the surgeons to work in. This is achieved using arterial clamps, the unintended consequence of which is an ischaemic nerve block (INB). An INB has been used as a research tool to initiate changes in cortical excitability. Deafferentation of distal limb structures and subsequent disinhibition of the motor cortical output to non-ischaemic muscles ipsilateral to the INB, manifested as increased MEPs. Through the use of a novel, low pressure INB applied to the lower limb, an increase in MEP amplitude in muscles proximal to the INB occurred. It was further shown that this increase in cortical excitability extended to the contralateral legs muscles and to arm muscles. Simultaneous recordings of somatosensory evoked potentials (SSEP) from stimulation of the tibial nerve, also distal to the INB, demonstrated a reduction in SSEP amplitude but not a complete deafferentation as previously assumed.

Investigations into the mechanisms underlying these finding was then performed. Using quantitative sensory testing whilst an INB was performed, the loss of Aβ and Aδ indicated the deafferentation required to initiate changes in motor cortical excitability. The preservation of C-fibre function could account for the unexpected finding where participants with exaggerated punctate sensation had greater increases in MEPs and possible cortical excitability. Paired-pulse TMS paradigms explored the potential neuronal networks responsible for the increase in MEPs of the contralateral muscles. A reduction in interhemispheric inhibition was seen from the deafferented motor cortex to the intact motor cortex, whilst no change in intrahemispheric pathways was seen.

The final chapter of this thesis explores the use of TMS and NIRS under surgical conditions. Despite numerous obstacles to patient recruitment, not withstanding a pandemic, a case series is presented with meaningful data which can be used to guide future study. Under the correct anaesthetic regimen, TMS induced MEPs can be recorded. The limited sample size was unable to determine if changes in cortical excitability occur in these conditions during surgery utilising a thigh INB however. In the second clinical investigation, NIRS was used to measure paraspinal muscle oxygen saturations levels (rO_2), believed to correlate with intra-spinal oxygenation. This was performed alongside traditional intraoperative neuromonitoring of spinal cord with transcranial electrical stimulation (TES) MEPs. Paraspinal rO_2 appeared to follow changes in the haemodynamic status of the patients, where a low rO_2 would reflect a low blood pressure. One patient experienced a paraparesis, with a recoverable reduction in MEP amplitude and paraspinal rO_2. Another patient who later died without clinical confirmation of paralysis, had a precipitous and permanent reduction in both MEPs and rO_2, likely reflecting a SCI. A third patient where a decrease in MEPs and paraspinal rO_2 was seen had remedial interventions initiated to prevent a possible SCI, which resulted in a return of both measures close to baseline.

Future work should look to explore the changes in cortical excitability secondary to iatrogenic limb ischaemic during TAAA surgery and how this impacts TMS-induced MEP characteristics and their interpretation in detecting a SCI. It should also explore their use alongside NIRS to detect both intra-operative and post-operative SCI and to guide their management.

Acknowledgements

I will be forever grateful to my main supervisor, Dr Paul Strutton, for the tireless support and guidance provided to me to complete this PhD. His dedication to rigorous and noble research in the pursuit of knowledge, and for the betterment of patients, is inspirational. I want to thank him for the many academic and social conversations over the years, for being not only a great supervisor but also, I hope, a lifelong friend. This is just the start of our academic journey, and I am looking forward to the many more chats over MS Teams to come (possible even in person now, in the pub!).

I am also grateful to Mr Colin Bicknell, my second supervisor, for the clinical advice and guidance he has provided for this thesis. He has opened many clinical and academic opportunities to further both my future academic and clinical careers, and I look forward to working with him during a ruptured abdominal aortic aneurysm at 2am soon.

There are so many people who have assisted in some way during this PhD journey, it is impossible to list them all. However, I want to make a special mention of some notable individuals.

I would like to thank Prof. Alison McGregor for her candid and no-nonsense advice about all things in life. Thanks for her support at the height of the pandemic, her kind words and thoughts really kept me going. General advice on good research practices, ethics applications and data analysis was provided by Dr Sam Hughes and Dr Chris Mullington. Without their day-to-day guidance, I would probably still be writing my ethics proposals and failing to make my graphs look pretty.

The clinical studies would not have been possible without Mr Gareth Jones, who allowed to undertake the clinical research during his surgical theatre lists, and to Ms Louise Allen, vascular nurse specialist, who assisted with patient recruitment, both at Imperial College Healthcare NHS Trust. I am also thankful for the support and assistance of all the anaesthetic consultants at Imperial College Healthcare NHS Trust. I particularly would like to mention Dr Benjamin Graham, for giving me the opportunity to work in his department, and to Dr Nathalie Courtois and Dr Sadie Syed for their infectiously passionate support of clinical research and vascular anaesthesia. I would like to thank Alex Woodhams, Clinical Neurophysiologist, who acquired all the intraoperative motor evoked potential

data for the patient studies. He also provided valuable insight into the conduct of intraoperative neuromonitoring and advice for data interpretation.

Many of the studies in this thesis were conducted with BSc and MSc students at Imperial College London. I am incredibly grateful for their hard work in participant recruitment and data collection, but also for their endless enthusiasm and intellectual intrigue, which kept my research progressing.

Without the funding provided from the Association of Anaesthetists of Great Britain and Ireland I would not have been able to purchase necessary equipment and consumables required to undertake this research. I thank them for both their financial and moral support of my work. I also thank the Vascular Anaesthetic Society of Great Britain and Ireland for their assistance in conducting the survey described in this thesis.

I need to thank all the patients and participants who generously gave up their time to take part in the research studies outlined in this thesis. Without their altruism, advances in science and medicine could not continue.

Finally, I would like to apologise to my wife and children for being an absent husband and father, to apologise to my friends for missing all those social events and apologise to my cat for not feeding it occasionally – I was too busy writing this PhD.

Dr Pawandeep Sarai

Table of Figures

Figure 1: Intraoperative image of an aortic aneurysm, or aneurysmal sac (left), having been partially excised (right; white arrow). 1

Figure 2: Crawford classification of TAAA 4

Figure 3: Kaplan-Meir estimate for survival after TAAA repair stratified by all patients who presented with (dashed line) and without (dotted line) SCI. Reproduced from Conrad MF et al, 2015 (15). 5

Figure 4: Diagram of traditional model of arterial blood supply to the spinal cord, with one anterior and two posterior spinal arteries. Reproduced from Gray's Anatomy for Students, 2nd Edition (Copyright Churchill Livings, 2009). 7

Figure 5: Epoxy resin mould of showing the fine interconnecting blood vessels or "collateral circulation" (black arrows) between the paraspinal muscles (white stars) and the intraspinal arterial vasculature (white arrow). Reproduced from Etz CD et al, 2011 (21). 10

Figure 6: Schematic diagram illustrating the complex, intertwined mechanisms by which spinal cord ischaemia can occur on placement of an aortic clamp, occlusion of intercostal and segmental arteries by an endograft and on release of the aortic clamp. Adapted from Archer et al, 2012 (30). 12

Figure 7: In its most basic form, a transcranial magnetic stimulator consists of a power source, a capacitor, a switch that can be manually or digitally activated by a computer, and a copper coil with low impedance 17

Figure 8: Circular coil. A) 90mm circular stimulating coil. B) Schematic diagram showing a circular coil with an anti-clockwise coil current flow (white arrow) inducing a clockwise current flow with the brain (blue arrow), which in turn preferentially activates the left hemisphere (orange burst). 18

Figure 9: Figure-of-8 coil. Comprises of two circular coils with current flowing in opposite directions. At the central function, this flow is uni-directional, resulting in a stronger magnetic field and stronger induced electric current. 19

Figure 10: A cone coil. Consist of two circular coils which are then angled in to a cone shape, which generates a stronger magnetic field capable of stimulating deeper in the motor cortex. 20

Figure 11: Representative MEP showing measures of latency and peak-to-peak amplitude in a biphasic EMG recording. 22

Figure 12: Equipment used in the experiments outlined in this thesis. Under the monitor is the Magstim 200 and Bistim (black arrow), with the ISO Dam amplifiers (white arrows) and CED data acquisition device (red arrow) 32

Figure 13: Schematic diagram of view from top of head showing, Nasion-Inion and Inter-tragal lines, with the inter-section point (red dot) indicating the vertex. 34

Figure 14: Somanetics INVOS^{TM} 5100C Cerebral/Somatic Oximeter with 4 channels and 4 corresponding optodes attached. 40

Figure 15: Overview of the experimental set-up for the study described in Chapter 5; (i) Sphygmomanometer cuff providing ischaemic block (ii) SSEP anode (iii) Cathode placed over tibial nerve (iv) NIRS optode placed on sole of foot (gastrocnemius optode not visible) (v) Electrodes placed bilaterally on Vastus Lateralis muscle belly (vi) Electrodes placed bilaterally on Abductor Hallucis muscle belly. The ground electrode was placed on the elbow (not visible). 41

Figure 16: A: Automated sphygmomanometer, B) Manual sphygmomanometer and blood pressure cuff, C) Arterial doppler 43

Figure 17: Answers to Question 5 – Where IONM was not used, what do you think the reasons are? Answers given as total number of responses. 49

Figure 18: Question 8 –Which interventions were performed to prevent or manage cases of spinal cord ischaemia. Answers as a % of 59 respondents. 50

Figure 19: Experimental set up. Electrode position for healthy participants (A) and PVD patients (B)., LBR = left brachioradialis, LABP = left abductor pollicis brevis, , LVL and RVL = left and right vastus lateralis, , LTA and RTA = left and right tibialis anterior, LAH and RAH = left and right abductor hallucis. The cone coil is shown, although both cone and circular coils were used. 60

Figure 20: Amplitude of healthy participant and patient muscles MEPs. Data expressed as mean ±SEM. BR = brachioradialis, APB = abductor pollicis brevis, VL = vastus lateralis, TA = tibialis anterior, AH = abductor hallucis. * denotes significant difference between healthy and patient APB muscles ($P < 0.05$); ** denotes significant difference between healthy and patient BR muscles ($P < 0.01$). 64

Figure 21: Correlations between individual patient MEP amplitudes and Vascuuol score for each muscle. LBR = left brachioradialis,LAPB = left abductor pollicis brevis, LVL = left vastus lateralis, LTA = left tibialis anterior, LAH = left abductor hallucis, RVL = right vastus lateralis, RTA = right tibialis anterior, RAH = right abductor hallucis. 68

Figure 22: Coefficient of variation for patient and healthy participant muscles. Data expressed as mean ± SEM. BR = brachioradialis, APB = abductor pollicis brevis, VL = vastus lateralis, TA = tibialis anterior, AH = abductor hallucis. * denotes a significant difference between patient and healthy participant APB muscles (P<0.05) 70

Figure 23: Coefficient of variation of MEP amplitude at each time point for each muscle. LBR = left brachioradialis, LAPB = left abductor pollicis brevis, VL = vastus lateralis, TA = tibialis anterior, AH = abductor hallucis. For graphs for VL, TA and AH, left and right refers to side of body. * denotes a significant difference between healthy participant and patients' LAPB muscles (P<0.05) 71

Figure 25: Latencies of healthy participant and patient muscles MEPs. BR = brachioradialis, APB = abductor pollicis brevis, VL = vastus lateralis, TA = tibialis anterior, AH = abductor hallucis brevis. Data expressed as mean ± SEM. * denotes a significant difference between patient and healthy participant muscles (P<0.05). 72

Figure 26: Experimental set up. Electrode position for Experiment 1 (A) and Experiment 2 (B) are shown. LVL and RVL = left and right vastus lateralis, RAH = right abductor hallucis, LBR and RBR = left and right brachioradialis, and LABP and RAPB = left and right abductor pollicis brevis. Red burst indicates tibial nerve stimulation..... 82

Figure 27: Schematic diagram showing the location of the CPz and Fz positions for EEG electrode placement 84

Figure 28: Experiment 1 protocol. Black arrows indicate where MEPs and SSEPs were sampled. The dashed lines indicate where only MEPs were recorded. Numbers along the top represent protocol time in minutes, numbers on the bottom indicate start and end of occlusion. 86

Figure 29: Experiment 2 protocol. Black arrows indicated where MEPs were recorded. Numbers along the top represent protocol time in minutes, numbers on the bottom indicate start and end of occlusion. 87

Figure 30: Single participant representative MEPs (averaged) from A) right abductor hallucis brevis, B) right vastus lateralis, C) left abductor hallucis and D) left vastus lateralis at baseline (BL), early occlusion (EO), late occlusion (LO), early post-occlusion (EPO) and late post-occlusion (LPO). The first vertical deflections indicate time of TMS. Note the disappearance of the MEP in trace A during LO and the increase in amplitude seen in traces B-D..... 88

Figure 31: Mean (±SEM) MEP amplitudes as a percentage of baseline (BL) in left vastus lateralis (LVL), left abductor hallucis (LAH), right vastus lateralis (RVL) and right abductor hallucis (RAH), during

experiment 1. The shaded box represents the occlusion period. *** denotes a significant reduction in RAH amplitude compared to BL during LO period (40, 45 and 46 minutes) (P<0.001). 89

Figure 32: Mean (±SEM) MEP amplitudes as percentage of baseline (BL) for left vastus lateralis (LVL), left abductor hallucis (LAH), right vastus lateralis (RVL) and right abductor hallucis (RAH), during early occlusion (EO), late occlusion (LO), early post-occlusion (EPO) and late post-occlusion (LPO) time periods. * denotes a significant increase compared to baseline for that muscle, P<0.05). 90

Figure 33: Mean (±SEM) pain scores (out of 10) during experiment 1. Shaded area represents duration of INB. ** denotes significantly different from baseline of pain scores from 15 to 51 minutes, as indicated by the bracket (P<0.01). 91

Figure 34: Mean (±SEM) MEP amplitudes at the end of occlusion (45 minutes) as a percentage of baseline for left vastus lateralis (LVL), left abductor hallucis (LAH) and right vastus lateralis (RVL), grouped according to average low pain (1 to 5) (black bars) or high pain (6 to 10) (grey bars). * denotes a significant difference between low and high pain (P<0.05). 92

Figure 35: Single participant data for averaged SSEP amplitudes following tibial nerve stimulation at baseline, during late occlusion (LO) and late post occlusion (LPO). The traces begin at the time of nerve stimulation. 93

Figure 36: Mean (±SEM) SSEP P37-N45 peak-to-peak amplitudes as percentage of baseline, following right tibial nerve stimulation. ** denotes significance at 45 minutes compared to baseline (P<0.01). 93

Figure 37: Mean (±SEM) rSO_2 near infrared spectroscopy for the right calf. Shaded area represents time of INB. ** denotes significant difference compared to mean baseline values, between 15 and 57.5 minutes (P<0.01) 94

Figure 38: Temporal relationship of right abductor hallucis brevis (RAH) MEPs, right tibial nerve SSEPs and calf muscle regional oxygenation (rO_2) for the assessment of the effectiveness of the low-pressure ischaemic nerve block model. Values are normalised to baseline for each modality and plotted against time. SSEP data adjusted by +1minute for graphical clarity. Data are presented as mean (±SEM). Grey shaded area represents time of INB. 95

Figure 39: Mean (±SEM) MEP amplitudes as percentage of baseline (BL) for left abductor pollicis brevis (LABP), left brachioradialis (LBR), right abductor pollicis brevis (RABP) and right brachioradialis (RBR), during experiment 2. Grey shaded area represents time of INB. 96

Figure 40: Mean (±SEM) MEP amplitude as percentage of baseline for left abductor pollicis brevis (LABP), left brachioradialis (LBR), right abductor pollicis brevis (RABP) and right brachioradialis (RBR), during early occlusion (EO), late occlusion (LO), early post-occlusion (EPO) and late post-occlusion time (LPO) time periods. * denotes a significant increase in LAPB compared to baseline during the EO and LO period (P<0.05). 97

Figure 41: Experiment set. TMS Coil used was the 'figure-of-8' coil. RBR = right brachioradialis, RAPB = right abductor pollicis brevis, LBR = left brachioradialis, LAPB = left abductor pollicis brevis. The red star on the right hand represents the area where sensory testing was performed. 107

Figure 42: Experimental protocol diagram. Shaded box (top) showing the 3 phases of the experiment (baseline, occlusion and post-occlusion) with the black arrows showing the time (in minutes) at which testing occurred, with respect to the occlusion period. The white box (bottom) shows which tests were performed at each time point..... 110

Figure 43: Normalised RAPB MEP amplitude. Shaded area represents the occlusion period. Data expressed as mean ± SEM. ** denotes significance at 29 and 31 minutes compared to baseline (BL) (P<0.01). 112

Figure 44: Normalised RAPB MEP amplitudes grouped into time periods. MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Date expressed as mean ± SEM. ** denotes a significant reduction compared to baseline (P < 0.01). 113

Figure 45: Normalised mean MEP amplitudes for lleft abductor pollicis brevis (LAPB),right brachioradialis (RBR) and left brachioradialis (LBR) muscles. MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM; * denotes a significant difference compared to baseline (P<0.05)..... 115

Figure 46: Pain under the cuff. Scores range from 0 – 10, where 10 is most pain experienced and 0 is no pain (baseline). BL = baseline, MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM, * denotes a significant difference compared to baseline (P<0.05). Note: some error bars too small to be visible. 115

Figure 47: Dynamic sensation as compared to the contralateral side. Score range from 0 – 10, when 10 is dynamic sensation pre-occlusion. BL = baseline, MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM. * denotes a significant difference compared to baseline (P<0.05). Note: some error bars too small to be visible..... 116

Figure 48**:** Normalised heat pain thresholds (HPT) compared to the contralateral side (n = 16). BL = baseline, MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM. Note: error bars too small to be visible. 117

Figure 49**:** Punctate sensation as compared to the contralateral side, in 10 participants who experienced a reduction in sensation (n = 10). Score range from 0 – 10, where 10 is sensation pre-occlusion. BL = baseline, MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM; *** denotes a significant difference compared to baseline (P<0.001). Note: some error bars too small to be visible. 118

Figure 50: Normalised MEP amplitudes for hypersensitive (black bars) and hyposensitive (grey bars) responders shown for each muscle. (LAPB = left abductor pollicis brevis, RBR = right bracioradialis, LBR = left brachioradialis). MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM. 121

Figure 51: Experimental setup. TMS Coil used was the 'figure-of-8' coil. RAPB = right abductor pollicis brevis, LAPB = left abductor pollicis brevis. A single coil is shown in the diagram, as would be employed in experiment 1. For experiment 2, two coils placed on both ABP hotspots would be seen. 129

Figure 52: Protocol diagrams for experiment 1 (top) and experiment 2 (bottom). Shaded box showing the 3 phases of the experiment (baseline, occlusion and post-occlusion) with the black arrows showing the time (in minutes) at which testing occurred, with respect to the occlusion period. 131

Figure 53: Normalised MEP amplitudes for test alone states. Black bars represent MEP amplitudes from the occluded left abductor pollicis brevis (LAPB) muscle and the white bars represent the non-occluded right abductor pollicis brevis (RAPB). EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values are mean ± SEM. *denotes P<0.05 with respect to baseline. 134

Figure 54: Normalised RAPB MEP amplitudes over time for Experiment 1. BL = baseline. Data represented as mean ±SEM 135

Figure 55: Graph showing correlation between raw MEP amplitudes for RAPB test-alone state and time. Data represented as mean ± SEM. 136

Figure 56: Ratio of cMEP/tMEP for each of the paired-pulse paradigms at baseline. SICI = short interval intracortical inhibition, SICI = short interval intracortical facilitation, LICI = long-interval intracortical inhibition, ICF = intracortical facilitation. Data represented as mean ± SEM. 137

Figure 57: Conditioned MEP/test MEP amplitude showing SICI, SICF, LICI and ICF over the course of the experiment. BL = baseline, PO = post-occlusion. Data shown as mean ± SEM. 138

Figure 58: Normalised MEP amplitudes for the LABP test alone state during experiment 2. BL = baseline, EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values represented as mean ± SEM. *denotes P<0.05 with respect to baseline.....139

Figure 59: Normalised MEP amplitudes in RABP during experiment 2. BL = baseline, EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values represented as mean ± SEM. *denotes P<0.05 with respect to baseline.....140

Figure 60: Raw MEP amplitudes in RABP during experiment 2. BL = baseline, EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values represented as mean ± SEM. *denotes P<0.05 with respect to baseline.....141

Figure 61: Extent of interhemispheric inhibition, normalised to baseline, present during Experiment 2. EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values represented as mean ± SEM. *denotes a significant change with respect to baseline, P<0.05.....142

Figure 62: Graphs showing the correlation between RABP MEP amplitudes against the amount of IHI present at (A) -5mins and (B) -10mins baseline. As shown by the r^2 values, there is no correlation present over the range of RAPB MEP amplitudes generated in experiment 2. 143

Figure 63: Representative MEPs at baseline from patient A's left abductor pollicis brevis (LAPB), right abductor pollicis brevis (RAPB) and left abductor hallucis (LAH) (top to bottom). Black arrow indicates time of TMS stimulus. 156

Figure 64: Representative MEPs post-induction from patient A's left abductor pollicis brevis (LAPB), right abductor pollicis brevis (RAPB) and left abductor hallucis (LAH) (top to bottom). Black arrow indicates time of TMS stimulus.....157

Figure 65: Representative MEPs at baseline from patient B's left abductor pollicis brevis (LAPB), right abductor pollicis brevis (RAPB) and left tibialis anterior (LTA) (top to bottom). Black arrow indicates time of TMS stimulus. 158

Figure 66: Representative post-induction MEPs from patient B's left abductor pollicis brevis (LAPB), right abductor pollicis brevis (RAPB) and left tibialis anterior (LTA) (top to bottom). Black arrow indicates time of TMS stimulus.....159

Figure 67: Representative MEPs from patient B's B's left abductor pollicis brevis (LAPB) and left tibialis anterior (LTA) (top to bottom) following 30mins of tourniquet inflation. MEPs from the RAPB were no longer measurable due to interference. Black arrow indicates time of TMS stimulus. 160

Figure 68: Graphs displaying the MEP amplitude (μV) and paraspinal rO_2 (%) against time of surgery (hh:mm) for A) left APB, B) left TA, C) left AH, D) right APB and E) right AH for Patient 1. Upper limb muscles plotted with thoracic rO_2 and lower limb muscles with lumbar rO_2..... 173

Figure 69: Systolic blood pressure (mmHg) and rO_2 (%) from the 4 optodes plotted against time (hh:mm) for patient 1; A) whole surgery, B) from 13:00 to 15:00 showing close relationship between variables, and C) from 15:30 to 18:30 where a fall is seen, particularly in right lumbar rO_2 (blue line). 176

Figure 70: MEP amplitude (μV) and rO_2 (%) across time (hh:mm) for left A) ABP, B) VL, C) TA and D) AH muscles for patient 2. 179

Figure 71: MEP amplitude (μV) and rO_2 (%) across time (hh:mm) for right A) ABP, B) VL, C) TA and D) AH muscles for patient 2..... 181

Figure 72: Graph showing the paraspinal rO_2 (%) from 4 optodes and the systolic blood pressure (mmHg) plotted against time (hh:mm) for patient 2..... 182

Figure 73: Graph showing the paraspinal rO_2 (%) from 4 optodes and the systolic blood pressure (mmHg) plotted against time (hh:mm) for patient 3..... 183

Figure 74: MEP amplitude (μV) and rO_2 (%) across time (hh:mm) for left A) ABP, B) TA and C) AH and right D) ABP, E) TA and F) AH muscles for patient 3. 186

Figure 75: Graph showing the paraspinal rO_2 (%) from 4 optodes and the systolic blood pressure (mmHg) plotted against time (hh:mm) for patient 4..... 187

Figure 76: MEP amplitude (μV) and rO_2 (%) across time (hh:mm) for left A) ABP, B) TA and C) AHB and right D) ABP, E) TA and F) AHB muscles for patient 4. 189

List of Tables

Table 1: Risk factors associated with SCI following TEVAR. Reproduced from Awad H et al, 2017 (18). AAA = abdominal aortic aneurysm. 6

Table 2: Patient specific inclusion and exclusion criteria 31

Table 3: Experiment 1 single and paired pulse TMS paradigms. SICI = short interval intracortical inhibition, SICF = short interval intracortical facilitation, LICI = long interval intracortical inhibition, ICF = intracortical facilitation 36

Table 4: Survey questions. TAAA = thoracoabdominal aortic aneurysm, CSF = cerebrospinal fluid, IONM = intra-operative neuromonitoring 47

Table 5: Question 5 free text comments, "If not, why do you not use intra-operative neuromonitoring?" 49

Table 6: Question 8 free text comments: What interventions does your Trust use to prevent or manage spinal cord ischaemia? 50

Table 7: Correlation coefficients (r) for MEP amplitudes vs VasQuol score for each muscle. 67

Table 8: Demographic data of patients enrolled in experiment 2. M = male, F = female, Open = open TAAA repair. Hybrid = open and endovascular TAAA repair, ICU = intensive care unit. 166

Table 9: Modified Crawford Classification of TAAA 190

Abbreviations

ADM	Abductor digiti minimi	
AH	Abductor hallucis	
APB	Abductor pollicis brevisBB	Biceps brachii
BR	Brachioradialis	
CS	Conditioning stimulus	
CST	Corticospinal tract	
EMG	Electromyography	
GABA	Gamma-aminobutyric acid	
ICF	Intracortical facilitation	
INB	Ischaemic nerve block	
IONM	Intraoperative neuromonitoring	
LAH	Left abductor hallucis	
LAPB	Left abductor pollicis brevis	
LBR	Left brachioradialis	
LICI	Long-interval intracortical inhibition	
LTA	Left tibialis anterior	
M1	Motor cortex	
MEP	Motor evoked potential	
MSO	Maximum stimulator output	
NIRS	Near infrared spectroscopy	

PL	Peroneus longus
RAH	Right abductor hallucis
RAPB	Right abductor pollicis brevis
RBR	Right brachioradialis
RTA	Right tibialis anterior
QST	Quantitative sensory testing
rO_2	Regional tissue oxygen saturation
rTMS	Repetitive transcranial magnetic stimulation
SCI	Spinal cord injury
SEM	Standard error of the mean
SICF	Short-interval intracortical facilitation
SICI	Short-interval intracortical inhibition
SSEP(s)	Somatosensory evoked potential(s)
TA	Tibialis anterior
TAl	Test alone state
TAAA	Thoraco-abdominal aortic aneurysm
TIVA	Total intravenous anaesthesia
TMS	Transcranial magnetic stimulation
TS	Test stimulus
VL	Vastus lateralis
VM	Vastus medialis

Aims and Outline of Thesis

Spinal cord ischaemia (SCI) and subsequent paralysis following repair of thoracic-abdominal aortic aneurysm (TAAA) is a devastating complication. It is associated with significant morbidity and an increased risk of death for the patient. Current methods for monitoring and therefore mitigating for SCI have limitations, and the principal aim of this PhD was to explore novel methods to detect this complication. The two potential monitoring methods explored are transcranial magnetic stimulation (TMS) and near infrared spectroscopy (NIRS), both of which are well established technologies, but have not been widely explored for this potential application. In exploring the feasibility of TMS, the effects on cortical excitability of an ischaemic nerve block (INB) on TMS induced motor evoked potentials, as would be encountered iatrogenically during TAAA surgery, were also further explored.

The background to TAAA and SCI is discussed in chapter 1, as are the potential novel monitoring techniques of TMS and NIRS. In addition, current knowledge of the effects of INB on cortical excitability and proposed mechanism are also explored. In chapter 2, the neurophysiological tools, functional assessments and experimental interventions used throughout this thesis are fully described.

Chapter 3 contains a survey of practice of intraoperative neuromonitoring for TAAA surgery currently employed in the United Kingdom and Ireland, and identifies the need for improvements in monitoring for SCI.

The MEPs of patients with peripheral vascular disease (PVD), the predominant patient cohort susceptible to a TAAA, had not previously been characterised, nor had the reliability of TMS induced MEPs over a prolonged period of time. In chapter 4, the MEPs of PVD patients are recorded over a period of 1 hour, and their neurophysiological measures and variability compared to disease-free controls.

During TAAA repair, one or both lower limbs are rendered ischaemic by deliberate surgical occlusion of the arteries, allowing for a bloodless field to operate in. As a consequence, there is ischaemia of the limbs, including the nerves, resulting in deafferentation. In chapter 5, this is modelled with the use of

a novel low-pressure INB, which is first validated and then the effects of the subsequent deafferentation on motor cortical excitability are interrogated with TMS.

In chapters 6 and 7, the mechanisms underlying changes in cortical excitability are explored. Using quantitative sensory testing (QST), specific nerve fibre types which undergo INB-mediated deafferentation and initiate excitability changes are first characterised. Paired-pulse TMS paradigms are then employed to investigate the role of different intracortical and interhemispheric pathways may play in any of the observed changes.

TMS and NIRS are introduced to the surgical setting in chapter 8. In a series of case studies, the feasibility of using both technologies are explored within the confines of limited theatre availability during a pandemic. TMS is employed during orthopaedic surgery where a tourniquet is used, both to elucidate if MEP generation is possible, but also to observe any changes in cortical excitability. NIRS is utilised during TAAA surgery alongside traditional intraoperative neuromonitoring, where changes in NIRS values are compared to fluctuations in transcranial electrical stimulation evoked MEPs.

Finally, chapter 9 contains a summary of findings and concluding remarks.

Chapter 1: Introduction and Background

Thoraco-abdominal Aortic Aneurysms

A thoraco-abdominal aortic aneurysm (TAAA) is defined as a 50% increase in diameter of the thoracic or abdominal aorta, compared to normal (for age, sex, patient height and weight, as well as aortic level) (1) (see Figure 1). It is often found incidentally, such as on a chest X-ray investigating for an unrelated pathology, or by surveillance imaging where there is a strong family history or if the patient is known to have a connective tissue disorder (2).

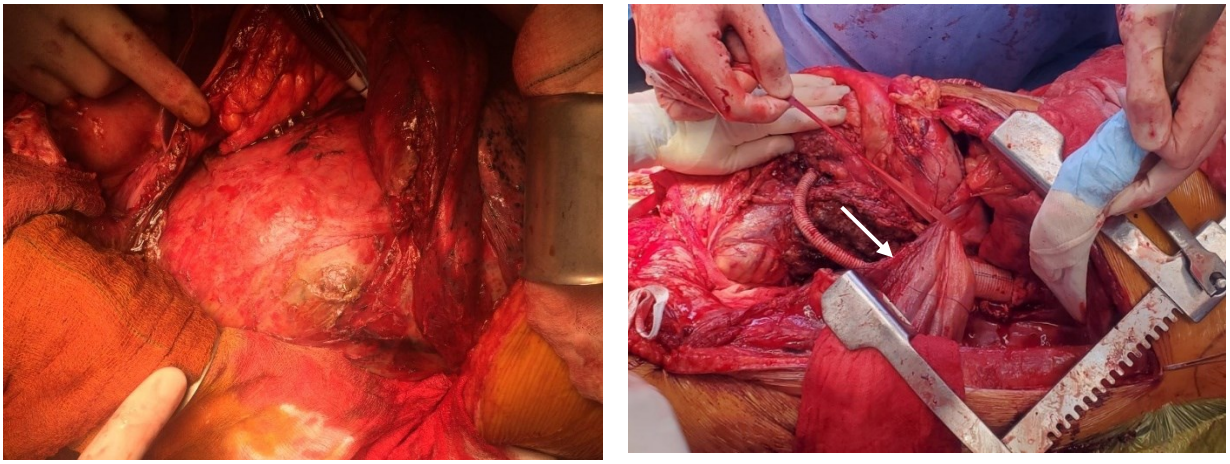

Figure 1: Intraoperative image of an aortic aneurysm, or aneurysmal sac (left), having been partially excised (right; white arrow).

Epidemiology & Aetiology of TAAA

The estimated incidence rate of thoracic or thoraco-abdominal aortic aneurysms is 6 per 100,000 person years (the number of years a person has a TAAA, multiplied by the number of people in the population who have been affected) (3). Although difficult to quantify in the United Kingdom, approximately 1000 admissions a year are related to the TAAA and 650 deaths per year are attributed to thoracic or thoraco-abdominal aortic aneurysms (3).

The causes of all aneurysms are broadly divided in to 3 categories, although some overlap can exist:

- Degenerative: atherosclerotic disease of the arterial wall
- Genetic: connective tissue disease of the arterial wall, effecting components of collagen

- Inflammatory: chronic infections of the arterial wall (e.g. tuberculosis) or autoimmune arteritis

Sixty per cent of thoraco-abdominal aortic aneurysms are due to atherosclerotic disease. Chronic dissection, where blood tracks between the arterial wall layers and subsequently expands, represents 30%. The remainder are due to connective tissue disorders, such as Marfan's Disease or Ehlers-Danlos, and are seen in younger patients with a family history of the disorder (4, 5).

Risk factors for degenerative TAAA are the same as other atherosclerotic processes such as coronary heart disease, cerebrovascular disease and peripheral vascular disease, namely hypertension, hypercholesterolaemia and smoking. Although the exact mechanisms are not entirely clear, atherosclerotic disease of the aorta results from luminal wall damage, exposure of collagen and the initiation of the inflammatory cascade, with subsequent loss of arterial wall (intimal) integrity. It is then susceptible to aneurysm formation(6).

Regardless of initiating cause, weakening of aortic wall integrity renders it susceptible to further damage over time, with resulting dilatation and further weakening. If left untreated, or undiagnosed, the aortic aneurysm will eventually rupture, with mortality following emergency surgical repair ranging from 30-46% at 30 days, depending on the location and size of the aneurysm (7).

Classification

Aortic aneurysms are classified based on their anatomical location:

- Thoracic
- Thoraco-abdominal
- Abdominal

The focus of my PhD is on thoraco-abdominal aortic aneurysms. TAAAs comprise 10% of all aneurysms involving the thoracic aorta.

See Appendix 1: classification of TAAAs for greater detail on the classification of TAAA.

Spinal Cord Injury following TAAA Repair

Epidemiology & Aetiology

Ischaemic spinal cord injury (SCI) is a devastating complication of thoraco-abdominal aortic aneurysm surgery. The overall incidence of open repair is approximately 11%, but as high as 22% for extensive aneurysms (8). Even with the use of endovascular techniques and improvements in protective strategies, paraplegia and paraparesis can still affect up to 19% of patients (9, 10).

Intuitively, one would think the introduction of minimally invasive, endovascular (via a blood vessel, usually an artery) repair of TAAA would result in a reduced incidence of SCI. The incidence of morbidity and length of hospital stay has certainly fallen (11). However, frustratingly the incidence of SCI has not declined as hoped (12). For TAAAs with the greatest involvement of the thoraco-abdominal aorta (whole of descending thoracic aorta to infra-renal arteries, known as 'type' or 'extent' 2, see Figure 2), the incidence of SCI from an endovascular repair is 10% versus up to 22% for an open repair (9). Vascular centres with high patient throughput and complex case mixes have the lowest SCI figures, as would be expected due to increased knowledge and skills base; a series with 2286 patients undergoing open repair, the largest to date, reported a SCI incidence rate of 3.3% for Type I, 6.3% for Type II, 2.6% for Type III and 1.4% for Type IV TAAAs (13); this down from 5% of Type I, 31% of Type II, 7% of Type III and 4% of Type IV in 1993 (14).

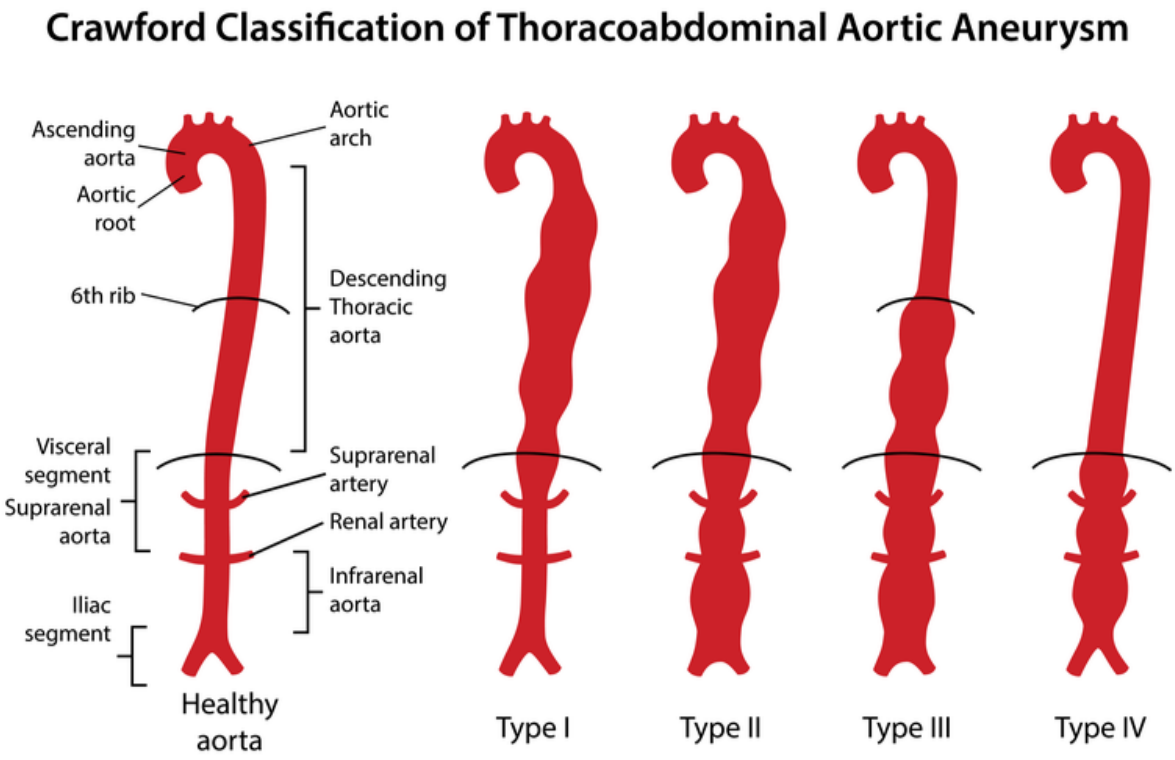

Figure 2: Crawford classification of TAAA

Survival of patients with a SCI versus those without a SCI following a TAAA repair is significantly worse; 25% at 4-5 years compared to 51%, respectively. At 30 days, mortality is significantly higher in the patients with SCI, being, 23.4% versus 8% with no post-operative neurological deficit (see Figure 3) (15).

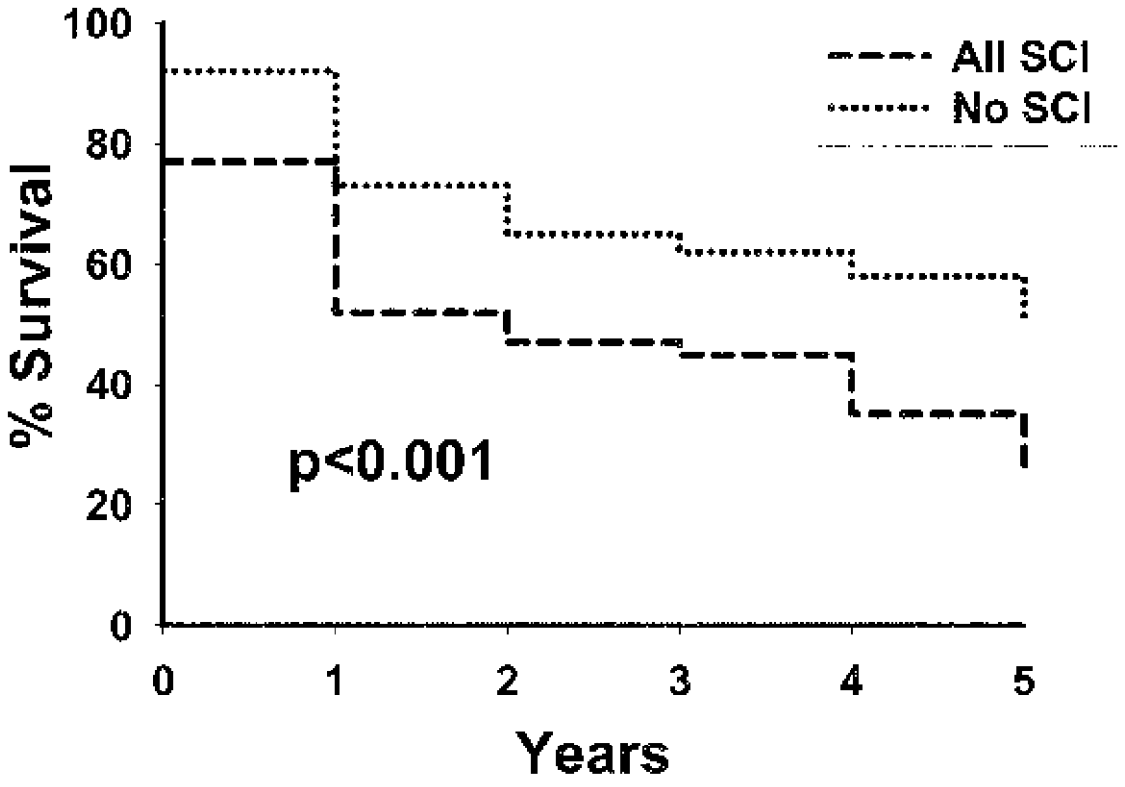

Figure 3: Kaplan-Meir estimate for survival after TAAA repair stratified by all patients who presented with (dashed line) and without (dotted line) SCI. Reproduced from Conrad MF et al, 2015 (15).

Risk Factors for Spinal Cord Injury

Due to the differing surgical insults and likely aneurysm suitability for each type or repair, open and endovascular repair of TAAAs are associated with different risk factors for SCI.

Open Repair

A prospective study investigating risk factors for SCI with 233 thoracic and TAAA repairs found the type or 'extent' of the aneurysm was the best risk predictor for SCI. An objective measure of the type which is superior to simply its length (length being determined by patient height) was the number of segmental arteries sacrificed during the procedure. If 10 or more pairs of arteries were excluded from the aneurysm (or "sac"), the odds ratio was 29. The only other significant risk factor was smoking, with an odds ratio of 21 (16).

Endovascular Repair

Endovascular repair (thoracic endovascular aortic repair, TEVAR) is more commonly associated with late SCI (17), which is discussed further below. A number factors have been identified as increasing the risk of late SCI (see Table 1)(18).

Table 1: Risk factors associated with SCI following TEVAR. Reproduced from Awad H et al, 2017 (18). AAA = abdominal aortic aneurysm.

Patient Related	Surgery Related
Increasing age (>70yr)	Total aortic coverage >205mm
Peri-operative hypotension (MAP <70mmHg)	Concomitant AAA/previous AAA repair
Hypertension	Coverage of 2 more vascular territories
Chronic Obstructive Pulmonary Disease	Left subclavian artery coverage
Renal disease	Urgency
	Adjunct procedure
	Use of 3 or more stents
	Procedure duration
	Increased blood loss

The most compelling risk factor appears to be the percentage of thoracic aorta covered by the stent (odds ratio 1.03) (17).

Anatomy of Spinal Cord Blood Supply

To appreciate the aetiology, pathophysiology and the measures designed to prevent ischaemic damage to the spinal cord, it is necessary to outline the anatomy of the spinal cord arterial system. This is also pertinent to the novel monitoring techniques being proposed.

Traditional Model

The blood supply of the cord is traditionally described as being via the longitudinal and segmental arteries (see Figure 4)(19).

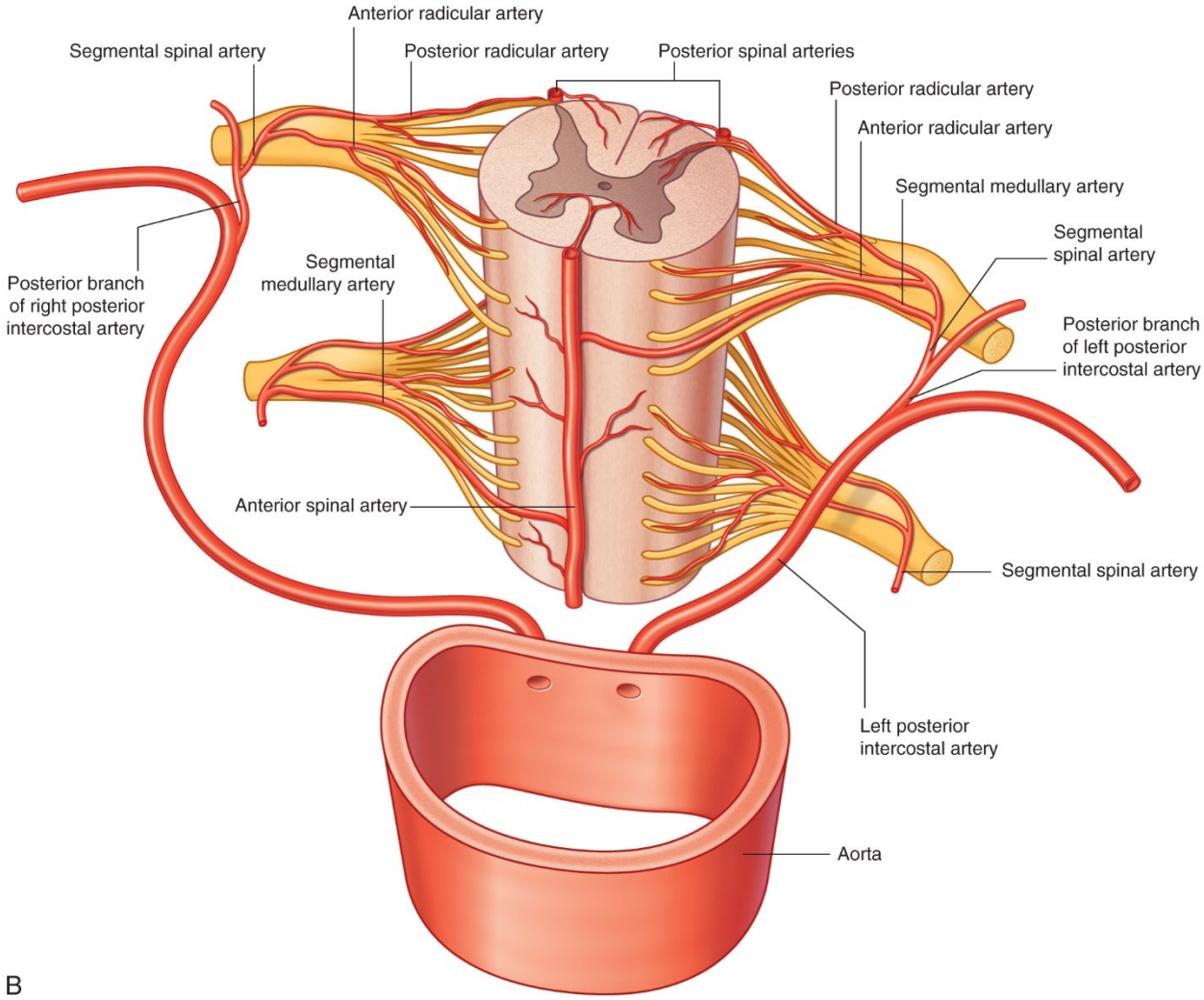

Figure 4: Diagram of traditional model of arterial blood supply to the spinal cord, with one anterior and two posterior spinal arteries. Reproduced from Gray's Anatomy for Students, 2nd Edition (Copyright Churchill Livings, 2009).

There are 3 longitudinal arteries, a single anterior spinal artery and two posterior spinal arteries, connected by anastomoses throughout the length of the spinal cord (20).

Paired segmental arteries arise directly from the aorta and the subclavian arteries and divide in to muscular and spinal branches. Spinal branches arise from vertebral, subclavian, deep cervical, intercostal, lumbar and internal iliac segmental arteries in a highly variable manner. These then divide to form radicular arteries, which anastomose with similar branches of the three longitudinal vessels

to make plexuses which supply the spinal cord. The radicular arteries can also supply to cord directly (19).

Most radicular arteries are small. Some however, are large enough to reach and supply the spinal cord around the anterior median sulcus, called anterior medullary feeder arteries. They often anastomose directly with the anterior spinal artery, the largest and most important being a dominant branch of a segmental artery in the lower thoracic region (T9-12), the Artery of Adamkiewicz (21). It was traditionally thought this artery is the dominant supply to the lower anterior two thirds of the spinal cord (22).

The anterior spinal artery, its intra-medullary branches and anastomoses supply the anterior two thirds of the cord when viewed in cross-section and, importantly, the corticospinal tracts responsible for voluntary motor control. The posterior spinal arteries supply the remaining posterior third.

Disruption of this traditional circulation, and especially the loss of flow from the artery of Adamkiewicz, is likely the dominant cause of early SCI following TAAA repair (23).

New Model: Collateral Network Concept

Despite surgical preservation of artery of Adamkiewicz and many segmentals, and with the use of newer endovascular techniques, SCI still occurs, often presenting later after surgery is complete. Therefore, the traditional model is likely a simplistic view of the spinal cord blood supply.

Until recently, it was felt re-implantation of individual segmental arteries during TAAA repair was vital to preserving blood supply to the cord and preventing ischaemic spinal cord injury and paraplegia. Segmental artery re-implantation can be found on the protocol of many centres to prevent SCI (24). However, SCI injury was still observed; it had been noted that in many TAAA patients, the vast majority of their intercostal and lumbar arteries were occluded with thrombus, aortic wall degeneration and plaque formation already (25). These patients had minimal segmental artery blood flow to their cords and yet had no features of a SCI. This questioned the traditional model of cord blood flow. This,

coupled with SCI rates still being greater than anticipated, led many to postulate alternative hypotheses to explain the pathogenesis of SCI.

The Collateral Supply Network was first proposed in 2011 (21) . Juvenile pigs had their aortas cannulated and subsequently infused with an acrylic resin at physiological pressures, allowing identification of an intricate blood supply in and around the spinal cord (see Figure 5)(21). Using this model, the investigators found that the thoracic and lumbar segmental arteries produced an extensive arterial network feeding the paraspinal muscles (PSM). The blood volume within this paraspinous network was 25 times that of the circular epidural arterial network and anterior spinal artery combined. They also observed extensive arterial collateralization between the intraspinal network arising from the longitudinal arteries and this paraspinous networks. Another finding contrary to traditional belief, only three quarters of segmental arteries provided direct branches to the anterior spinal artery or its tributaries. This led to the idea that the spinal cord supply was provided not only by the longitudinal and segmental arteries but also by an extensive, intricate collateral supply.

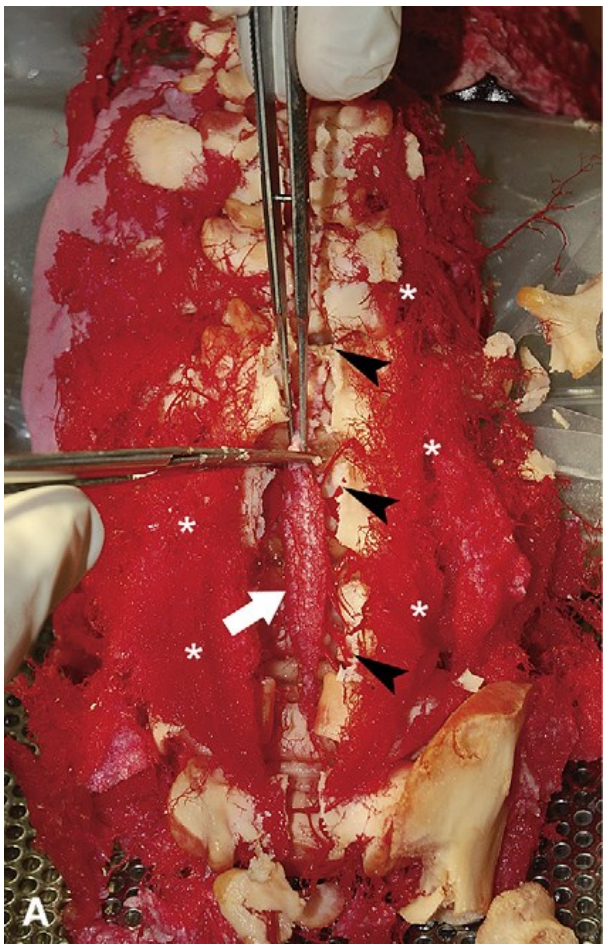

Figure 5: Epoxy resin mould of showing the fine interconnecting blood vessels or "collateral circulation" (black arrows) between the paraspinal muscles (white stars) and the intraspinal arterial vasculature (white arrow). Reproduced from Etz CD et al, 2011 (21).

It is postulated that this is the reason why spinal cord integrity is maintained in some patients and not others; those left paralysed lacked the development of this collateral network, despite what seemed to be appropriate preservation of segmental arteries. This was supported by further porcine modelling in which 8 pigs had all segmental arteries sacrificed, whilst a further 2 did not. Of those 8 where the artery was ligated, 2 pigs each had acrylic resin perfused either immediately, at 6 hours, at 24 hours and 5 days after ligation. Analysis of anterior spinal artery at 5 days showed that it had significantly increased in size by 80-100%, as had the epidural network and density of the PSM vessels. Changes were noted at 24hrs (26). This ‘remodeling’ due to progressive ischaemia, such as would be seen by the buildup of thrombi in segmental and radicular arteries of patients, likely prevents SCI through the development of a collateral network.

Pathophysiology of Ischaemic SCI

Ultimately, the pathophysiology of early or late SCI is simple: a lack of blood supply to the spinal cord, leads to lack of oxygen delivery with a resultant imbalance between oxygen supply and demand, followed by ischaemic cell death (27).

However, it can present differently depending on the repair technique, reflecting differences in the aetiology (28).

There are two main causes of insufficient blood supply. Firstly, a reduction in blood supply can occur due to artery ligation, coverage of apparently insignificant arteries with the graft and blood vessel blockage by atheroembolisation (20, 29). Systemic hypotension (low blood pressure) will exacerbate the effects of these significantly. The second newer theory is inadequate remodeling of the collateral spinal cord circulation, where new collateral vessels do not or have not had the opportunity to form; this is most likely to be the cause of late SCI (21, 26).

Early Spinal Cord Injury

Early SCI is more likely with an open TAAA repair, where SCI occurs intra-operative and paralysis presents immediately post-surgery (9). With an open repair, an aortic cross clamp is placed prior to excision of the aneurysmal sac and graft placement. This clamp results in hypoperfusion and reduced oxygen delivery with subsequent ischaemia to structures distal to the clamp, including variably to abdominal organs, lower limbs and potentially the spinal cord. In addition to the reduction in blood flow due to the clamp, ligation of intercostal and segmental arteries can contribute to hypoperfusion, as can a secondary 'ischaemia-reperfusion' insult on clamp release. Ischaemia results in cell injury and death which then exacerbates the ischaemia itself through the release of neurotoxins and pro-inflammatory mediators, creating a vicious feedback loop. The complex, intertwined mechanisms are simplified in the schematic diagram in Figure 6.

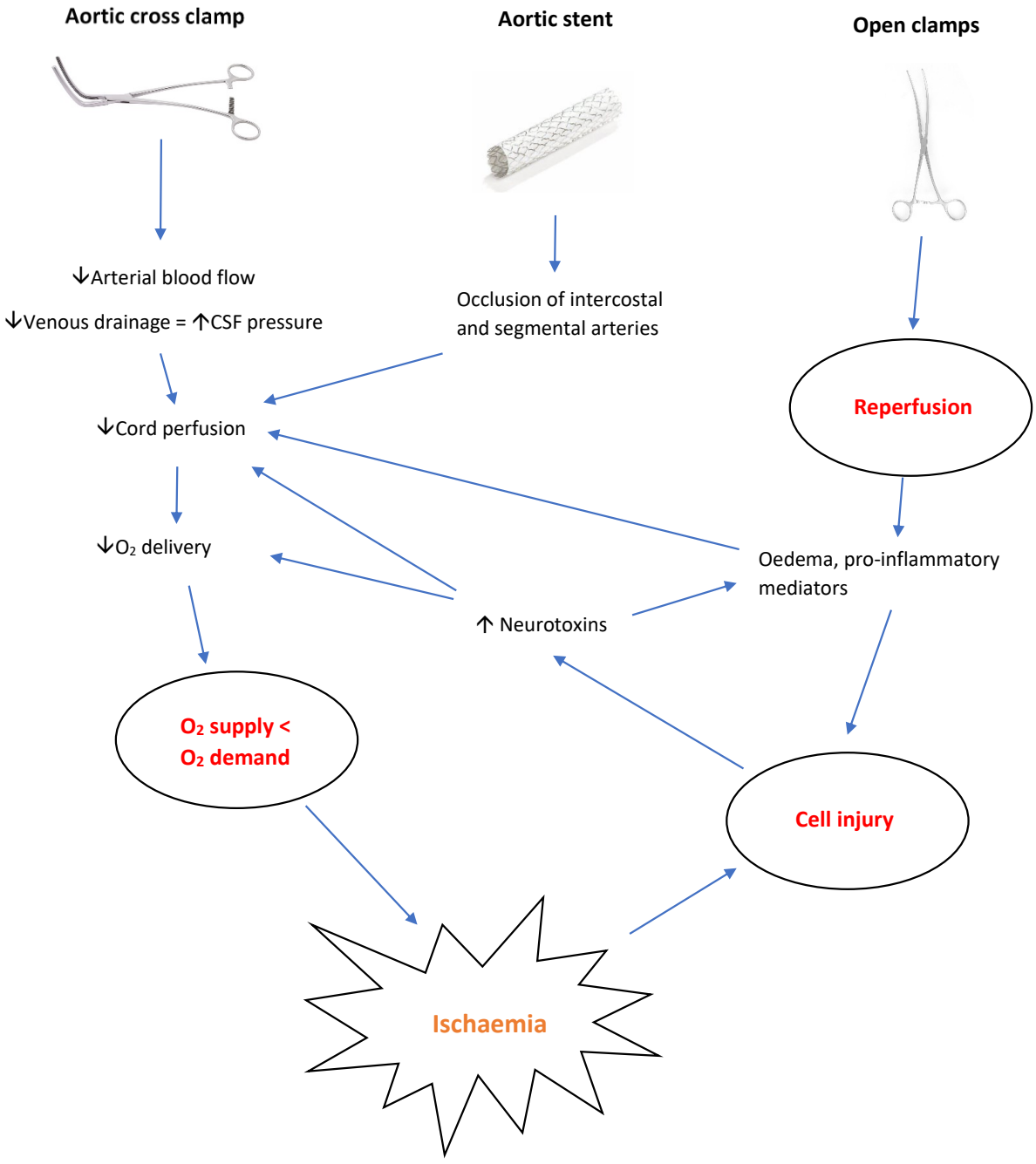

Figure 6: Schematic diagram illustrating the complex, intertwined mechanisms by which spinal cord ischaemia can occur on placement of an aortic clamp, occlusion of intercostal and segmental arteries by an endograft and on release of the aortic clamp. Adapted from Archer et al, 2012 (30).

Late Spinal Cord Injury

TEVAR is associated with a delayed presentation of SCI, with paralysis presenting 24hrs or more after surgery (9). Endovascular repair does not use a classic aortic cross clamp in the same way as open repairs and therefore there is no sudden loss of spinal cord perfusion and no significant ischaemia-reperfusion

injury. The hope was TEVAR would not see the high rates of SCI seen with open repair. As previously mentioned, this has frustratingly not been the case and late SCI can occur (17).

The graft may cover segmental arteries which are vital to spinal cord perfusion, where in rare situations and SCI can occur (as shown in Figure 6 above). However, usually fewer arteries are covered and there is no sudden, complete loss of blood flow as seen with an aortic clamp, hence likely no immediate SCI. In addition, as the aneurysmal sac is not excised (it is excluded from the circulation with a graft, but not cut out), in some cases it will still receive a small, insidious blood supply, which in turn will continue to supply the intercostal arteries and spinal cord. When this 'endoleak' eventually ceases, SCI develops (18). However, should there be an adequate collateral network, which can maintain spinal cord perfusion by redistributing blood from the paraspinal muscles, late SCI is avoided (18).

Management of SCI following TAAA repair

Several interventions can be introduced to reduce the likelihood of SCI where there is clinical suspicion (or objective diagnosis from neuromonitoring) (31).

These interventions are based on the understanding of SCI pathophysiology and have contributed to reducing the incidence of SCI. In most centres, these remedial measures are based upon expert clinical suspicion, rather than objective measures or monitoring (32), and are not without risk.

Methods to reduce spinal cord injury have been divided into 3 categories (27, 33):

1. Amelioration of the effects of spinal cord injury by augmentation of spinal cord perfusion pressure
2. Increasing tolerance to ischaemia
3. Detection of intra-operative spinal cord injury

Implementation of these measure requires a multidisciplinary approach. A more comprehensive overview is provided in Appendix 2: Methods to reduce SCI.

Augmentation of spinal cord perfusion

Interventions aim to increase perfusion to the spinal cord by increasing the perfusion gradient across the spinal cord. This can be achieved by increasing the driving pressure, the mean arterial pressure, so that more blood flows into the remaining intact arteries; or by reducing the venous pressure, which being analogous to the cerebrospinal fluid (CSF) pressure, means draining the CSF using a spinal drain (34).

Increasing tolerance to ischaemia

Studies have demonstrated reduced rates and severity of SCI using staged surgeries, such as a technique called temporary aneurysmal sac perfusion. Here, a small deliberate "leak" of blood within the graft permits some blood flow to the aneurysm but a predominantly ischaemic state occurs. The leak prevents early SCI, but the reduced supply to the cord stimulates new collaterals to form. The leak is then later corrected to prevent the aneurysm continuing to expand (35, 36). Other procedures such as progressive segmental artery dissection are thought to work via a similar basis (37).

Intra-operative neuromonitoring of the Spinal Cord

Methods to monitor perfusion of the spinal cord during TAAA repair surgery include testing the integrity of the ascending and descending neural pathways between the brain and the periphery, using stimulation of peripheral nerves or the brain respectively (38). Assessment of ascending pathways is undertaken using recordings of somatosensory evoked potentials (SSEPs) and of the descending pathways by recording motor evoked potentials (MEPs) (38). Detection of a SCI can guide targeted interventions.

Somatosensory Evoked Potentials

SSEPs were the first electrophysiological monitoring technique used in spinal cord surgery (39-41). They interrogate the dorsal (sensory) columns, hence their use alone to detect motor deficits resulted in a high false negative rate in paraplegia detection (42). SSEPs are commonly used as an adjunct to MEPs.

Motor Evoked Potentials

MEPs offer a direct assessment of the corticospinal tracts, which is at greatest risk from ischaemic injury and demonstrate a higher specificity to diagnose post-operative motor defects (41, 43). A reduction in MEP size by 50% (amplitude) or delayed onset (latency) by 10% during TAAA surgery suggests spinal cord ischaemia, and initiates interventions to reverse it (24, 37, 44).

MEPs can be generated by transcranial electrical stimulation (TES), which involves the application of large electric currents to the motor cortex of the brain(24). This in turn stimulates the corticospinal tracts which induces responses in muscles, the MEP, measured by electromyography (EMG). Large electrical currents are required to traverse the cranium and stimulate the underlying motor cortex, which are delivered using electrodes screwed into the scalp. This is extremely painful for the patient, hence can only be performed intra-operatively, when the patient is anaesthetised, and will therefore only detect early SCI. Patients who develop late SCI have no spinal cord monitoring unless they are kept anaesthetised. In addition, TES can be difficult to set up and hence requires a dedicated neurophysiologist to interpret responses, and therefore is not universally utilised (24).

In the following two sections, technologies that have the potential to overcome the limitations of current intraoperative neuoromonitoring techniques will be discussed.

Transcranial Magnetic Stimulation

The application of electricity to a peripheral nerve, resulting in a muscle contraction, had been noted since the discovery of electricity. Work in the 1980s had shown application of electricity to the motor cortex of non-human primates and humans could be used to manipulate movement and motor control (45). However, in vivo human research into the pathways of cortical control of movement was limited by the intensely painful nature of transcranial electrical stimulation (TES).

In 1985, a greater understanding of the pathways was made possible by the invention of transcranial magnetic stimulation (TMS)(46). This provided a painless, non-invasive tool to interrogate the many complex interneuronal connections responsible for the movement in man.

Principles of TMS

Transcranial magnetic stimulation is based on the principles of Faraday's law of electromagnetic induction, which states that a magnetic field will interact with an electric circuit to produce an electromotive force. Further work by James Clerk Maxwell showed that if this magnetic field varies, either in time or space, then so does the induced electric field, thus resulting in the flow of charge or electricity within this electrical circuit. Charging the TMS stimulators results in the storage of electrical charge within the capacitor. When the switch is activated, the capacitor releases this charge and there is current flow through the copper coil, producing a temporary magnetic field (in the region of 2 Tesla) (see Figure 7). The turning on and off of the coil current results in a changing magnetic field, i.e. a magnetic field which varies spatially as described by the Maxwell-Faraday equation, generating an

electrical field. This in turn produces a secondary current within conductive materials, such as excitable nervous tissue (47).

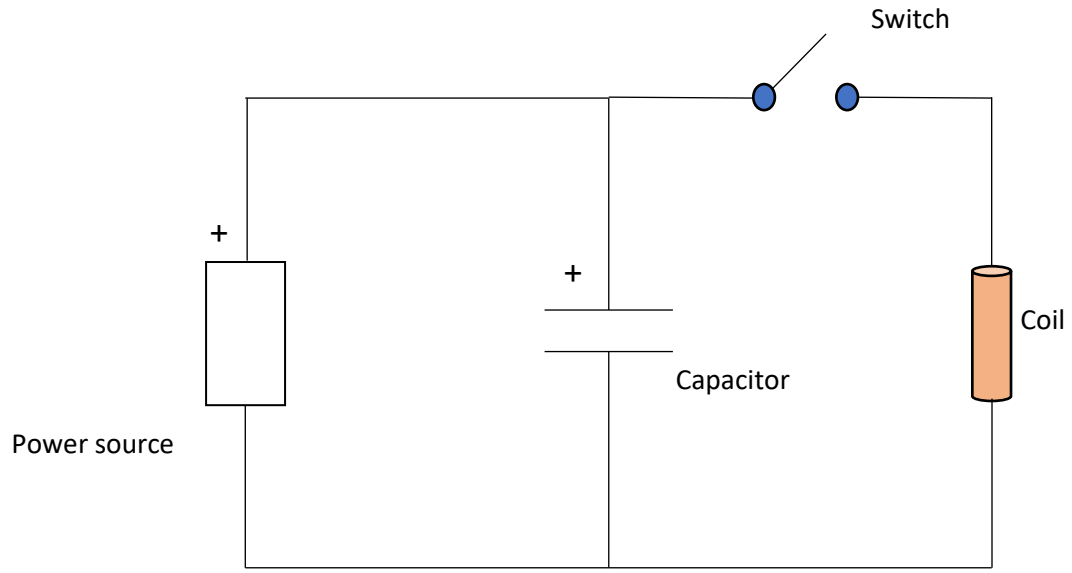

Figure 7: In its most basic form, a transcranial magnetic stimulator consists of a power source, a capacitor, a switch that can be manually or digitally activated by a computer, and a copper coil with low impedance

In the case of TMS, this 'electrical circuit' is the motor cortex. A changing electrical current in a tightly wound copper coil, in turn generates a changing magnetic field, which in induces an electrical current within the underlying motor cortex (M1). The flow of charge into these cells causes a change in the membrane potential, usually a depolarization, producing an action potential. This action potential is propagated down to corresponding skeletal muscle motor units, which subsequently cause the muscle to contract and from which a motor evoked potential (MEP) can be measured using electromyography (EMG) (48).

The frequency at which the magnetic field changes means it can pass through the skin, skull and meninges without attenuation and only the excitable brain tissue below is activated. Therefore, painless brain stimulation can be produced, making TMS now the primary research tool for investigating cortical control of movement (48, 49).

Stimulation Coils

Stimulation coils are so called because they are essentially a tightly wound coil of copper wiring in an insulated casing. Different coils generate unique EMFs and thus have different targeting properties. The following coils were used in various investigation in this thesis.

Circular coil

The copper wiring is coiled into a circle. They can vary in size from 7-15cm and have up to 20 turns of the wire (Figure 8). Electrical current flow within the wire produces a magnetic field and subsequently an electrical field in conductive material. The current flow in the conductive material is in the opposite direction to the electrical current flow in the coil, which allows for preferential hemispheric activation of the motor cortex. For example, if one is looking down at the head of a participant whilst standing from behind, and current flow within the coil is in the anti-clockwise direction, this orientation will result in clockwise current flow within the brain. Thus, the current is "towards" and activating the right hemisphere and motor cortex (which produces left sided muscle MEPs). Circular coils have an evenly distributed field and when placed on the vertex, they lack focality to specific muscles and depth of penetration. Although they can activate both hemispheres simultaneously, particularly at high stimulator intensities, one side is preferential due to the reasons outlined above and this makes them ideal for generating MEPs from many muscles on one side of the body (50).

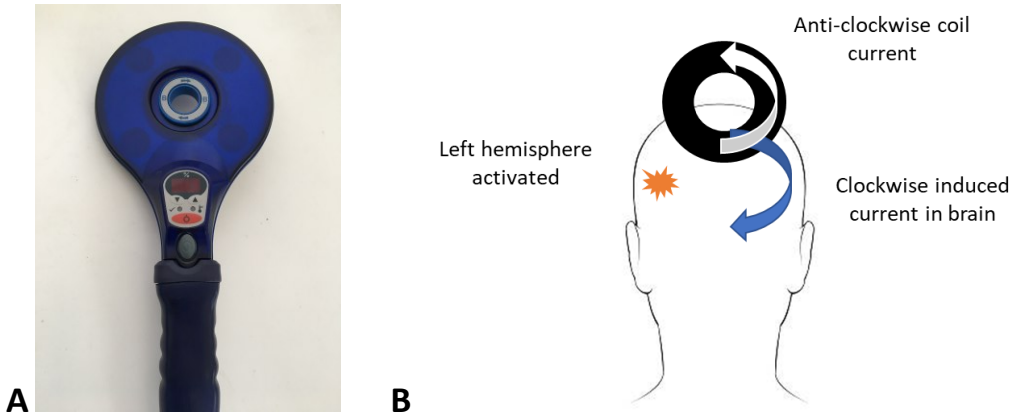

Figure 8: Circular coil. A) 90mm circular stimulating coil. B) Schematic diagram showing a circular coil with an anti-clockwise coil current flow (white arrow) inducing a clockwise current flow with the brain (blue arrow), which in turn preferentially activates the left hemisphere (orange burst).

Figure-of-8 Coil

The figure-of-8 coil is comprised of two smaller circular coils attached together with the current in each coil flowing in opposite directions. However, at the junction between the two copper coils the current flows in the same direction (see Figure 9). The induced electric field here is additive, as is the subsequent magnetic field, resulting in a focal point of maximum stimulation (51). Thus, with the figure-of-8 coil, it is possible to target a small area of the motor cortex and its corresponding muscle.

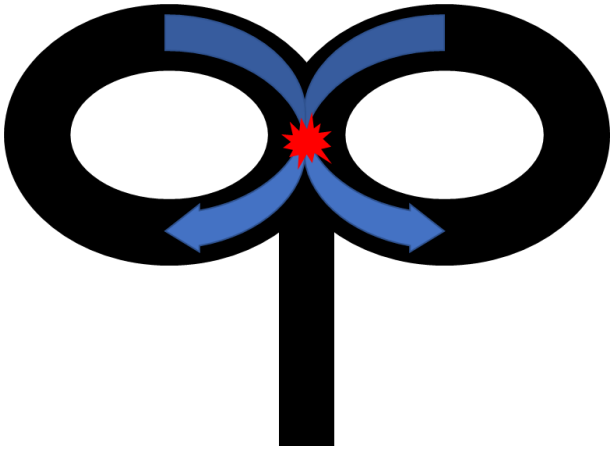

Figure 9: Figure-of-8 coil. Comprises of two circular coils with current flowing in opposite directions. At the central function, this flow is uni-directional, resulting in a stronger magnetic field and stronger induced electric current.

Cone Coil

The cone coil is a figure-of-8 coil which has been bent at the middle junction (see Figure 10). The electric field generated at the lateral parts of the figure-of-8 coil is small and considered to be insignificant. However, when a bend is introduced and a cone shape is produced, the side fields now become larger and can stimulate the motor cortex in addition to the central junction, allowing for greater stimulation intensity with greater cortical penetration, thus stimulating the M1 regions responsible for lower limb muscle control (50, 52).

Figure 10: A cone coil. Consist of two circular coils which are then angled in to a cone shape, which generates a stronger magnetic field capable of stimulating deeper in the motor cortex.

TMS-induced Motor Evoked Potentials

A TMS pulse can produce both an excitatory and inhibitory response from a muscle, proportional to the stimulus intensity. Muscle responses are measured by surface EMG, with skin surface electrodes placed over the muscle of interest. Where it is inhibitory, a silent period is seen (a period of almost no EMG activity following stimulation onset) and where it is excitatory, a motor evoked potential is observed.

A key difference noticed early on in the research with TMS was that the latency of MEPs was approximately 2ms longer when compared to the same muscles measured with TES (49, 53). This difference underlies a fundamental difference between the 2 modalities which is pertinent to this PhD. When a magnetic stimulus is given to M1, multiple spikes or volleys are generated and can be recorded in the epidural space of the cervical cord (54). The first spike is due to the direct activation of the corticospinal tract (CST), called the D-wave. The D-wave is then followed later by a series of volleys, with longer latencies separated by 1-2ms (55). These are believed to arise from the depolarisation of the same pyramidal cells, but instead due to synaptic activation (trans-synaptic) via cortical interneurons; hence they are activated indirectly and are called I-waves (56). Stimulation of the motor cortex with TMS at

threshold produces a series of waves with similar latencies to these I-waves and therefore, TMS is believed to work trans-synaptically to activate the CST(49).

When a single TMS pulse activates the CST, although this can be synchronous, it is usual to see more than one descending volley and therefore more than one deflection in the EMG recording. At higher intensities, TMS can also produce D-waves (57). Since TMS activates the CST trans-synaptically, later activation of the same CSTs, by multiple I-waves, is observed (58). The complex EMG trace produced has multiple deflections which likely reflect activation of motor axons with differing conduction speeds and spread of muscle action potentials across multiple motor units.

The synapses can be influenced by external factors and hence are modifiable, or plastic, in their function. As will become discussed in greater detail below, understanding this is vital to using TMS as an intra-operative monitor during TAAA surgery.

MEP Characteristics

Single Pulse TMS

When a single TMS pulse is delivered to M1 above threshold, the MEP that is generated provides information on motor cortical excitability and the integrity of the spinal cord. The two measures from single pulse TMS used in this thesis are latency and peak-to-peak amplitude (see Figure 11). The latency is the time from the when the stimulus of provided to the first deflection of the MEP. It gives an indication of the conduction velocity, synaptic transmission, and propagation within peripheral nerves. The biphasic response from bipolar EMG allows for the measurement of the peak-to-peak amplitude. It is influenced by the area of motor cortical representation of a muscle, density of CST projections and the strength of projections, the latter being indicative of the state of cortical excitability.

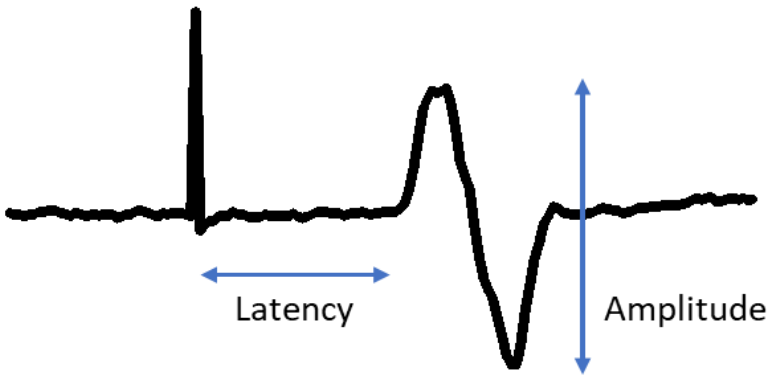

Figure 11: Representative MEP showing measures of latency and peak-to-peak amplitude in a biphasic EMG recording.

Paired Pulse Paradigms

The MEP amplitude determined from single pulse TMS is a measure of the strength of synaptic transmission within the motor cortex (59). It is, as a result, modifiable by interneurons that act on the synapses. Intracortical and interhemispheric TMS paradigms, which consist of two stimuli of specific intensities (as a proportion of resting threshold) and separated by specific time intervals, measure the activity of these interneurons and each represent distinct neuronal populations which can be measured independently (60).

Short-interval intracortical inhibition (SICI) is measured using a sub-threshold conditioning stimulus (CS) (0.7x resting motor threshold (RMT) in this investigation) followed by a suprathreshold test stimulus (TS) (1.2 x RMT), with a short interval of 2-5ms. The CS produces an inhibitory post-synaptic potential (IPSP) at the corticospinal neurones via activation of a low-threshold cortical inhibitory circuit. This ISPS in turn inhibits action potential generation by excitatory post-synaptic potentials (EPSPs) generated by the supra-threshold TS (61, 62). SICI is most likely mediated via $GABA_A$ transmission, with GABA being the most abundant inhibitory neurotransmitter in the central nervous system (63). Indeed, administration of lorazepam, a benzodiazepine that augments GABAergic

transmission, reduced the increases in cortical excitability secondary to an INB and enhanced SICI (64, 65).

Intracortical facilitation (ICF) can be explored with a similar paradigm as SICI, but with an ISI of 7-20ms (66). This increase in ISI stimulates neuronal pathways distinct from SICI, where the CS instead primes the synapse and thus facilitates transmission. The mechanism however is less clear, as SICI and ICF have a similar pharmacological profiles. The prevailing theory is ICF is the sum total of more dominant facilitatory pathways and weaker facilitatory pathways (66). The CS-induced IPSP seen in the SICI paradigm is the same as in the ICF paradigm, and thus contributes to the inhibition; whereas the excitatory component is most likely via glutamatergic neurones. This is supported by evidence that ICF was reduced when a NMDA-receptor antagonist is administered (67, 68).

Short-interval intracortical facilitation (SICF) differs from SICI and ICF as the CS is suprathreshold, usually 1.2 x RMT, followed by subthreshold TS (0.9 x RMT in our study). SICF is not a single entity; it can be seen with ISIs of 1.1–1.5 ms, 2.3–2.9 ms and 4.1–4.4 ms (69). It is believed the CS activates excitatory neurones, generating an EPSP; however not all of these will necessarily generate an action potential. The TS, despite being subthreshold, is able to activate these already depolarised excitatory neurones directly rather than trans-synaptically, which in turn generates EPSPs which can summate to produce action potentials (70).

Long-interval intracortical inhibition (LICI) uses two suprathreshold stimuli separated by an ISI of between 50-200ms; it is a long lasting inhibition and distinct from SICI (71). Studies have shown that LICI can reduce the effects of SICI as well as reduce MEP amplitude, (72, 73). It is believed this double inhibitory action must be through activation of $GABA_B$-receptors, which unlike $GABA_A$-receptors, are found both pre- and postsynaptically (74). Further credence to this theory is given by a study where it was demonstrated that administration a $GABA_B$-reuptake inhibitor increased LICI (75).

Unlike the intracortical paradigms above, the activity in one motor cortex can influence the motor cortical output of the other. Here, a CS is delivered by TMS to one motor cortex followed by a TS to the opposite M1, and the effects of the CS on the MEP that is generated by the TS is measured. If the

ISI is 7ms or more, then then CS has a negative influence, this is termed interhemispheric inhibition (IHI) (76). When the test stimulus is generated by electrical stimulation, the inhibitory effects of the CS are not observed, thus the IHI occurs transynaptically. Studies in stroke patients with an intact corpus callosum have a normal IHI, whereas those with a callosal lesion do not (77). In addition, children under 5 years old have an immature corpus callosum and do not exhibit IHI (78). Thus, IHI must be mediated via transcallosal fibres. At shorter ISIs, interhemispheric facilitation can be seen, but this is weak due to the greater effects of inhibition within the motor cortex (79).

Interrogation of the activity of these interneuronal pathways and how they change as the level of cortical excitability changes, can provide mechanistic insights into the effects of an intervention, such as an INB, on cortical excitability.

Modulation of Cortical Plasticity using an Ischaemic Nerve Block

A feature of many arterial surgeries, including TAAA repair, is the cessation of blood flow to the lower limbs through the use of a clamp or an arterial balloon. Amongst other reasons, this minimises blood loss and improves the visual surgical field. A consequence is ischaemia of the tissues distal to the site of the clamp, including the nerves, which lose their ability to function; there is a predictable loss of sensation and muscle contraction; neurophysiologically, an increase in MEP and SSEP latency is seen with progressive decrement in amplitude (80).

The clamp therefore can act as an INB, a tool which classically has been used to explore and modulate cortical plasticity. Neurophysiological changes in muscle MEPs at locations proximal to the surgical clamp however, may also be observed due to alterations in cortical excitability should TMS be employed as a intraoperative monitor of spinal cord function.

Motor Cortical Plasticity

The cortex can alter its output in response to both training and injury, including peripheral nerve ischaemia, in order to maintain or improve its function (81). This ability, known as plasticity, has been defined as: “any enduring changes in cortical properties like strength of internal connections, representational patterns, or neuronal properties, either morphological or functional” (82).

Where this functional modulation is rapid, such as immediately following a peripheral nerve injury or INB, the plastic changes involve the exposure of pre-existing latent mechanisms; the time over which the modulation occurs is too short for structural changes in the cortex to have occurred. The most favourable mechanism by which short-term plasticity can occur is through the removal of inhibitory mechanisms. This disinhibition exposes functionally inactive, excitatory horizontal interconnections and most strongly underlies the acute effects seen following an INB of a limb (83, 84).

Mechanisms of Cortical Plasticity

Peripheral nerve lesions and amputation of a limb results in increased motor cortical representation of muscles proximal to the injury, compared to the contra-lateral side. Lower intensities are also required to generate MEPs in these muscles (85). The increased responses of the proximal muscles is not accompanied by changes in spinal motor neurone excitability, indicating the changes are cortical in origin (86). Experimental studies employing an INB have supported these findings. An INB of the forearm for example, is associated an enlargement of the cortical representation of the muscles proximal to the ischaemic part of the arm (59). An increase in the amplitude of muscle MEPs proximal to an INB is observed with associated improvements in muscle function (80, 87).

This cortical plasticity is thought to be secondary to the loss of sensory input from areas distal to the lesion or INB, deafferentation, with a subsequent increase in cortical output to the proximal muscles via disinhibition (81). Animal studies have shown that a reduction in inhibition via a reduction in gamma-aminobutyric acid (GABA) transmission is crucial in deafferentation mediated corticospinal

excitability (83). Human studies support the above findings, where the increase in MEP amplitude secondary to INB are suppressed by administration of $GABA_A$ receptor modulators (88).

Many studies have explored how the activity of intracortical pathways change in experimental models of deafferentation, to explain the changes observed in proximal muscles (see discussion in Chapter 6 – An investigation of deafferentation induced by ischaemic nerve block). Studies contained in this thesis will build upon these findings to further characterise and explore changes in both proximal and contralateral muscles, which may be utilised in the development of a TMS-based neuromonitor for TAAA surgery.

Near Infrared Light Spectroscopy

Near infrared light spectroscopy (NIRS) is a non-invasive method that can determine the oxygenation level of tissues. Using the absorption ratio of red and infrared light by haemoglobin found in red blood cells, NIRS can be used to measure regional oxygen saturation (rO_2) of specific tissues underneath the NIRS probe (optode), placed on the skin surface. NIRS-based monitors for cerebral oxygenation are in widespread use (89-91). They have demonstrated post-operative cognitive decline for example is associated with a reduction in cerebral oxygen saturation (92). The optode can be used continuously to monitor oxygenation levels and hence used both intra-operatively and post-operatively. As they can measure the oxygenation of any tissue beneath the optode, NIRS could measure paraspinal muscle oxygenation as a surrogate for intra-spinal oxygenation and there is emerging evidence for use during TAAA.

Physical Principles of NIRS

A pulse oximeter is arguably the first and most well-known clinical monitor to use reflectance NIRS. It was first proposed to measure cerebral oxygenation however, by Jobsis in 1977 (93).

A chromophore is a molecule that can absorb light in the near-infrared spectrum. Haemoglobin found in red blood cells is the chromophore of interest in clinical oximetry monitors as it delivers oxygen to tissues. It can absorb a different range of light wavelengths depending on its oxygenation state; when in its deoxygenated state (Hb), it absorbs red light more than infrared light; when oxygenated (HbO_2), it absorbs infrared light more, particularly at a wavelength of 920nm, whilst reflecting the red light (hence oxygenated blood appears red)(94). The principles of Beer- Lambert Law determines how much near infrared light is absorbed and can be used to calculate how much HbO_2 is present under an optode. Since the amount of Hb and HbO_2 can alter depending on the oxygenation of a tissue, so the ratio of red to infrared light that is absorbed or reflected changes. Therefore an estimation of Hb versus HbO_2 present, expressed as a percentage, in that tissue can be made (95).

In the case of cerebral oximetry, it is not possible to have diodes and photodetectors on either side of the skull, like with a finger pulse oximeter – the light would not penetrate through. Photons in the near infrared spectrum can, however, reflect from the surface of chromophores taking an arc shaped path from emitter to receiver (93). With a cerebral oximeter, photons in the near infrared range with sufficient intensity pass through skin and skull and reach the cerebral tissues; some of this light will be absorbed whilst some will be scattered or reflected back to the scalp's surface and the overlying sensors, where the ratio of red:infrared light returning is proportional to the Hb to HbO_2 concentration in the brain.

NIRS in Thoraco-abdominal Aortic Aneurysm Surgery

Much of the clinical use and related research has focused on the use of cerebral NIRS in cardiac surgery. However, there is an increasing evidence base for the potential use for NIRS in TAAA surgery as a predictor of SCI. As discussed previously, the emerging importance of the paraspinal collateral network of blood supply to the spinal cord has driven research into paraspinal muscle oxygenation as a potential indicator of impending spinal cord injury.

Animal Vascular Studies of TAAA

Early animal studies have shown ligation of increasing numbers of segmental intercostal arteries is associated with a progressive desaturation of paraspinal muscles. Histologically, a greater number of ischaemic areas were seen in the lumbar spinal cord compared to the thoracic cord, and functional paralysis was observed in the hind legs of pigs (96).

Human Vascular Studies of TAAAThe use of NIRS to detect spinal cord ischaemia during TAAA repair in humans has been described in a small number of case reports and studies. In one such case, following the deployment of the thoracic endograft, a rapid unilateral and persistent absence of MEPs was observed, followed by a progressive reduction in rO_2 at the lower thoracic level, associated with a dense, unresolving paraplegia (97). Similar findings were observed in a study with 20 patients undergoing a TAAA repair; 3 patients developed a significant reduction in the paraspinal rO_2 at the lumbar level following aortic cross clamping compared to the thoracic, and all experienced a spinal cord injury (98). These changes have been demonstrated in conjunction with a reduction in MEPs from lower limb muscles, some of which recover to baseline alongside improvements in the rO_2 (99). Interpretation of both these studies is limited by small sample size; however, they demonstrate NIRS could be used as a simple, non-invasive monitor of ischaemic SCI.

Employed together, NIRS and TMS-induced MEPs have the advantage of being non-invasive and comparatively simple to set up. They therefore have the potential to detect both early and late injury in the post-operative period, whilst increasing the accessibility of IONM in TAAA surgeries where it may otherwise be unavailable.

Chapter 2: Participants & General Methods

Introduction

This chapter contains an outline of the general methods and participant selection used in the investigations contained within this thesis. Where a protocol deviates from these general principles, the differences will be described in methods section of the relevant chapters.

Participant Recruitment

Ethical approval for healthy volunteer studies was approved by the Imperial College Research Ethics Committee (number: 16IC3553). For investigations involving patients, ethical approval was granted by the Health Research Authority (HRA) Research Ethics Committee (REC) (number: 17/LO/1034). All studies contained within this thesis were performed with adherence to the Declaration of Helsinki guidance on conducting research in human participants.

All participants were provided with written information in layman's terms detailing the study protocol, potential risks, benefits, and their right to withdraw their voluntary participation. Verbal information was also provided to address and clarify any specific issues. Participants were given at least 24 hours to consider their decision and could withdraw at any time during the experimental process.

Written consent was obtained from all participants before commencement of any experiment.

Appendix 3 – Sample Patient information sheet and consent form, contains an example of the written information provided to patients and the corresponding consent form.

Healthy Participants

A TMS safety questionnaire was undertaken prior to inclusion in each study (100) . A sample checklist can be found in the Appendix 4 – TMS Safety Questionnaire; the main exclusion criteria were:

- Any metal implants in the head or cranium e.g. artery clip
- An artificial cardiac pacemaker
- History of epilepsy, neurological disorder and psychological disorder, unexplained loss of consciousness, head injury
- Pregnancy
- Age <18years

In addition to the above, experiments where an ischaemic nerve block was employed had additional exclusion criteria, based on guidelines for the use of surgical tourniquets (101):

- Open fractures
- Medical history of severe hypertension, angina, diabetes mellitus, sickle cell anaemia or peripheral artery disease
- Recent skin grafts
- Malignant tumours

Healthy participant experiments were conducted at The Nick Davey Laboratory, Human Performance Group, Imperial College London, Charing Cross Hospital, London, which later moved to the Sir Uren Building, White City Campus, Imperial College London, London.

Patients

In addition to the TMS checklist, inclusion and additional PVD-specific exclusion criteria for patients undergoing laboratory experiments are listed in Table 2.

Table 2: Patient specific inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Experience symptomatic lower limb claudication pain (cramping in lower leg), not currently suitable for surgery	Age < 18yrs
Under regular review by the Vascular Surgery Department at Imperial College Healthcare NHS Trust for peripheral vascular disease (the build-up of fatty deposits in the arteries (blood vessels) that restrict blood supply in the lower legs) and not currently suitable for surgery.	Diabetes Mellitus (high blood sugar level) with known microvascular complications e.g. peripheral neuropathy
	Confirmed or possibility of pregnancy
	Open wounds or skin lesions, including wet gangrene (dry gangrene permitted)
	Inability to speak and understand English fluently
	Patients with known thoracic or thoraco-abdominal aneurysm awaiting repair

Where patients participated in intra-operative studies, specific criteria are discussed in the relevant chapters.

Laboratory studies were undertaken at The Nick Davey Laboratory, Human Performance Centre, Imperial College London, Charing Cross Hospital, London. Intra-operative studies were performed at Charing Cross Hospital or St. Mary's Hospital, Imperial College Healthcare NHS Trust, London.

Neurophysiological Assessments

Transcranial Magnetic Stimulation

Magnetic Stimulators

TMS was delivered using the Magstim 200² magnetic stimulator for the studies in Chapters 4-6, with the addition of the Bistim for the studies included in Chapter 7 (Magstim Company, Dyfed, UK), connected to different stimulating coils.

The Magstim 200^2 has a voltage of 2.8kV, a maximum current of 10,000A and a magnetic field of 2 Tesla; the capacitor is rated at 185 µF, providing a maximum energy storage of 725 J. It has a maximum repetition rate of 1Hz. The output is determined by the user by adjusting a dial to choose 1-100% of the maximum stimulator output (%MSO, commonly referred to as the stimulator "intensity"). The magnetic field generated by the Magstim 200^2 is monophasic with a rise time of 100µs and a duration of 1ms approximately (102).

Being monophasic allows the Magstim 200^2 to be paired with another magnetic stimulator using the Bistim module, thus producing two stimulation pulses of different intensities, separated by an interstimulus interval (ISI) determined by the operator. Altering the ISI permits the exploration of intra-cortical and interhemispheric neural mechanisms using paired-pulse paradigms (as described in Chapter 1 and employed in Chapter 7). Using this module however reduces the strength of the magnetic field by approximately 10% and as such the %MSO needs to be adjusted to account for this, should baseline measurements be made with an independent stimulator.

See Figure 12 for an image of the equipment used.

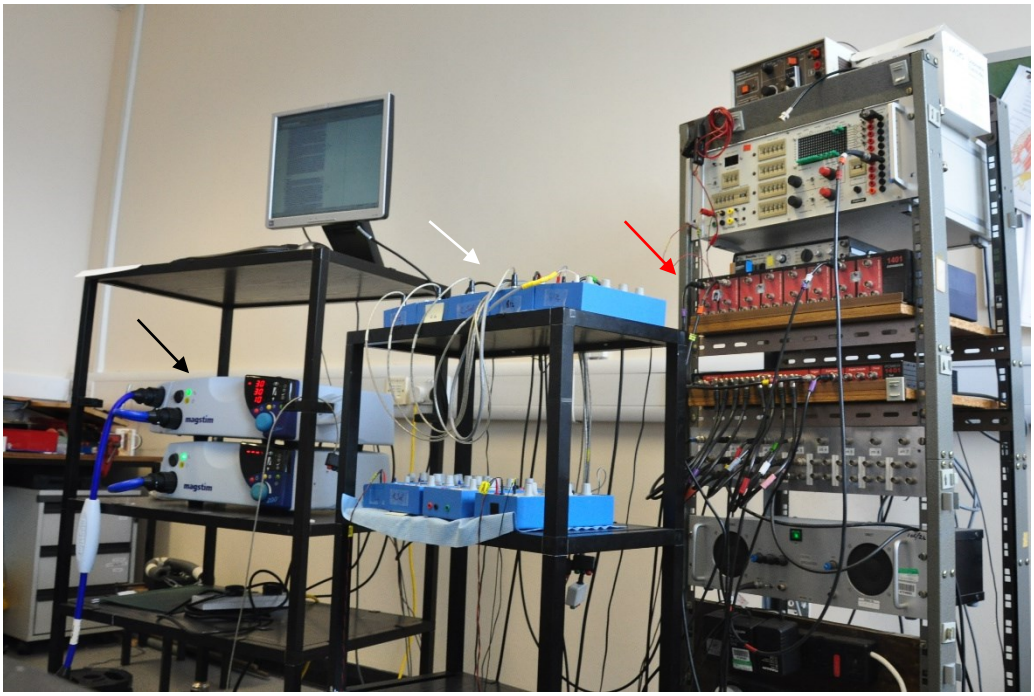

Figure 12: Equipment used in the experiments outlined in this thesis. Under the monitor is the Magstim 200 and Bistim (black arrow), with the ISO Dam amplifiers (white arrows) and CED data acquisition device (red arrow)

Coil Selection and Placement

Different stimulating coils can be used to generate MEPs preferentially from different target muscles. In the experiments outlined in this thesis, the circular, double cone and figure-of-8 coils were all used, with the choice of coil determined by which muscles required stimulation and time constraints of the paradigms (see Chapter 1: Transcranial Stimulation: Stimulation Coils for greater detail).

A 90mm diameter circular coil was used to stimulate upper limb muscles. With the coil positioned A-side facing superiorly, the induced current in the brain flows in a clockwise direction, preferentially activating the left hemisphere and the muscles on the right side of the body (103, 104). With the coil positioned B-side, up, the induced current is anti-clockwise, activating the right hemisphere and left-sided muscles.

A 120mm diameter double-cone coil was used to stimulate lower limb muscles (105). It can induce a stronger magnetic field, thus generating MEPs from the lower limb muscles which are represented deeper within the longitudinal fissure of the brain (with respect to the skull and therefore the coil). The double-cone coil was positioned so that the induced current in the brain flowed in the posterior to anterior direction.

Both the circular and double-cone coil are placed directly over the vertex of the head, with the current induced in the motor cortex. It is located at the intersection of two lines, one running from the nasion to inion, and the other from the tragus of one ear to the tragus of the other. This point was marked on the scalp with a washable marker pen to allow for consistent coil placement between trials (see Figure 13).

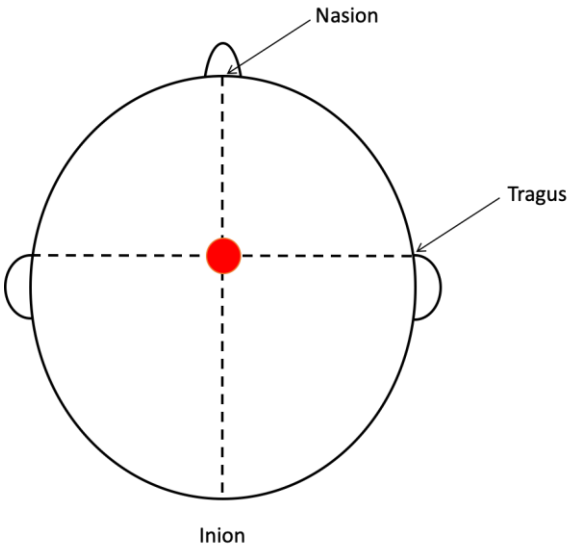

Figure 13: Schematic diagram of view from top of head showing, Nasion-Inion and Inter-tragal lines, with the inter-section point (red dot) indicating the vertex.

The figure-of-8 coil produces a discreet magnetic field under the junction of two circular coils (51). This field allows for targeted stimulation of muscles; however, it lacks depth of penetration and therefore was used to target specific upper limb muscles. To achieve such focused muscle stimulation, the location on the scalp overlying the motor cortical areas representing the muscle of interest was identified. Stimulation of this area, known as the motor hotspot of the muscle, produces the largest MEPs from the desired muscle.

To locate the hotspot, the figure-of-8 coil is held at 45° to the sagittal plane so that the induced current at the junction flows in the posterior to anterior direction. The %MSO was arbitrarily set to 50%. The coil was then moved 1cm at time starting from the vertex, until a point where the MEP peak-to-peak amplitude is greatest. This point is marked on the scalp with a washable pen, which includes the outline of the coil, the junction and an arrow indicating handle direction.

Single pulse TMS

Single pulse TMS was used to calculate the resting motor threshold (RMT) for individual muscles in all experiments, and used in testing for the investigations contained in Chapters 4-6.

Once the hotspot was identified at 50% MSO, the intensity was reduced in steps of 1-5%. The RMT is the lowest stimulation intensity that produces MEPs of ≥50μV in amplitude (106) in at least 50% of stimulus presentations. The stimulation intensity subsequently used in the experiments was 1.2 x RMT; at this supra-threshold intensity, responses fall on the steepest part of the stimulus response curve, so both increases and decreases in responses are more likely to be detected (107).

For the investigations contained in Chapter 6 and 7, the hotspot for the left and right abductor policis brevis (APB) was found using the process above. However, for the remaining studies, the time constraints of the paradigm meant individual muscle hotspots and RMTs could not be identified, thus the cone or circular coil was employed. A 'combined' resting motor threshold (cRMT) was found for the upper and lower limb muscles for each participant, with the circular and cone coils, respectively. This was the lowest stimulation intensity that produced discernable MEPs ≥50μV in amplitude (106) in at least three out of six successive stimuli in either all the upper or all the lower limb muscles; 1.2 x cRMT for the muscle with the highest threshold was therefore used in these investigations.

Paired pulse TMS

The paired-pulse paradigms SICI, SICF, LICI and ICF were employed in the investigations detailed in Chapter 7 – Exploration of mechanisms causing changes in cortical excitability in the motor cortex ipsilateral to an INB. TMS was applied to M1 at the hotspot for each APB muscle (identified as described above) using two Magstim 200^2 stimulators (Magstim Company Ltd., Whitland, Dyfed, UK), connected by the Bistim module. They were connected to a single figure-of-8 coil (The Magstim Company Ltd., Dyfed, UK).

Both left and right APB (LAPB and RAPB, respectively) received single-pulse TMS at 1.2 x RMT, referred to as the "test alone" (TA) state. RAPB also received four further paired stimulations, paired-pulse TMS paradigms or states. Six TA and six of each paired pulse paradigms were delivered at each epoch. The paired-pulse states were randomised by the Signal v5.12 software (CED, UK). The first stimulus in the paired pulse stimulus is referred to as the conditioning stimulus (CS), the second

stimulus as referred to as the test stimulus (TS) and they are separated by a designated time internal, known as the interstimulus interval (ISI); the paired pulse states are summarised in Table 3 (108).

Table 3: Experiment 1 single and paired pulse TMS paradigms. SICI = short interval intracortical inhibition, SICF = short interval intracortical facilitation, LICI = long interval intracortical inhibition, ICF = intracortical facilitation

State	Muscle	Conditioning stimulus (CS) (proportion of RMT)	Test stimulus (TS) (proportion of RMT)	Interstimulus intensity (ISI) (ms)
Test alone (TA)	LAPB, RAPB	-	1.2	-
SICI	RAPB	0.7	1.2	2.0
SICF	RAPB	1.2	0.9	1.5
LICI	RAPB	1.2	1.2	100
ICF	RAPB	0.7	1.2	15

Interhemispheric Inhibition

Two Magstim 200^2 stimulators (Magstim Company Ltd., Whitland, Dyfed, UK) were used to stimulate M1 at the hotspot for each corresponding ABP muscle. A figure-of-8 coil with an 8cm outer wing diameter (The Magstim Company Ltd., Dyfed, UK) provided TMS to the left M1. A second smaller coil of 7cm outer wing diameter delivered TMS to the right M1; it was chosen to allow accurate simultaneous placement of coils to the participants head without the coils clashing.

Using an intensity of 1.2 x RMT, test-alone MEPs were generated for LAPB. During the IHI testing, a CS at 1.15 x RMT was delivered to the right M1, before the test stimulus, at an intensity sufficient to generate RAPB MEPs of approximately 1mV, was delivered to the left M1. The ISI between the CS and TS was 10ms. The optimal intensities and ISI used in the experiment were determined following pilot testing and in keeping with previous work (76, 109).

Electromyography Acquisition

Bipolar arrangement of electrodes was used for recording of surface electromyography (EMG). Pairs of disposable self-adhesive Ag/AgCl electrodes (25mm diameter, 1041PTS, Henleys Medical Supplies Ltd., UK) were applied to the skin overlying the belly of the muscles of interest, after skin preparation with 70% isopropyl alcohol wipes, at a distance of 20mm between the centre of the electrodes (110). A reference electrode was placed over a nearby bony prominence, such as the olecranon process or medial malleolus.

Participants were positioned comfortably either supine or reclined to 45°, as per individual protocols. They were instructed to completely relax all muscles during stimulation to prevent facilitation, which increases MEP amplitude (111).

Following TMS of the motor cortex, the raw EMG signals were band-pass filtered (-3dB between 10 Hz–1 kHz) and differentially amplified (x1000) (Iso-DAM-X, World Precision Instruments, Welwyn Garden City, UK; these were soon decommissioned and replaced by a Digitimer D360 (Digitimer, Welwyn Garden City, UK) and then sampled (at 2 kHz) using an analogue to digital converter (Power 1401 data acquisition device, Cambridge Electronic Design [CED], UK). Signal software v5.12 (CED, UK) was used to record to disk for later data processing. Sweeps (frames) of between 0.5 and 0.75s were obtained with a pre-stimulus time of 200ms recorded to measure background activity prior to the stimulus to ensure muscles were appropriately relaxed.

Analysis of TMS induced MEPs

At each testing epoch during the study protocols, 6 single pulse TMS trials and, where required, 6 of each paired pulse paradigm and IHI, were delivered to M1, and then averaged for analysis of the MEP using Signal software (CED, UK). The unrectified EMG was then analysed using pre-configured scripts. Where TMS was delivered when muscles were not relaxed, a script was used to remove these frames prior to averaging.

Amplitude and Latency

In all studies, the peak-to-peak amplitude of the MEP was measured. Using pre-written scripts in Signal, cursors were manually placed at the start and end of the MEP and the peak-to-peak amplitude, in millivolts (mV) was measured. For studies in Chapter 4, the latency was also analysed; this is the time taken, in milliseconds (msec), from delivery of the TMS pulse to the first discernable deflection of the EMG recording indicating an MEP.

Variability and the Coefficient of Variation (CV)

In Chapter 4: An investigation into the variability of MEPs in patients with peripheral vascular disease, the variability both within a time point and across the whole experiment, was measured. The variability between measurements was determined by the coefficient of variation (CV):

CV = standard deviation/mean.

Data groups with a lower CV are considered to have lower variability; where CV = 1, the standard deviation must equal the mean hence there is greater variability.

Variability within a time point

To determine the extent to which individual MEPs varied between the 6 single-pulse trials at a given epoch, the amplitude of each single-pulse MEP was measured, and the CV of these 6 MEPs was calculated. The CV of the amplitude was calculated for each time point and muscle.

Variability across the whole experiment

To determine the variability of the muscles across the duration of the experiment in Chapter 4, the amplitude of the digitised averaged MEP generated from the 6 single pulse trials, referred to as the averaged MEP (avMEP), at each of the experimental epochs was measured, and then used to calculate the CV. This was performed for all muscles and every participant to calculate the mean CV of the avMEP amplitude; comparison of the different upper and lower limb muscles was then made.

Paired Pulse Paradigms and IHI

As described above, an MEP was generated from a single pulse test stimulus (test alone, TA) within the same epoch as an MEP generated by a paired-pulse or IHI paradigm.

SICI, SICF and ICF were calculated as the ratio (expressed as a percentage) of the averaged peak-to-peak amplitude of the conditioned MEP /unconditioned MEP (i.e. TA MEP) at the same time point.

LICI was calculated as the ratio of the amplitude of the MEP induced by a suprathreshold (1.2 x RMT) stimulus preceded 100ms earlier by a stimulus at the same intensity; it is also expressed as a percentage.

To calculate IHI, a ratio (expressed as a percentage) was calculated between the amplitudes of the averaged MEPs generated by a test stimulus to one hemisphere preceded (10ms) by a conditioning stimulus to the opposite hemisphere and MEPs generated by a test stimulus to one hemisphere only.

Further details of the stimulation patterns for each study protocol and additional analysis pertinent to it, are described within the methods section of the relevant chapter.

Somatosensory Evoked potentials

Somatosensory Evoked potentials were recorded and analysed for the study contained in Chapter 5: Cortical excitability induced by a novel low-pressure INB. See Chapter 5: Somatosensory evoked potentials for greater detail of methodology.

Quantitative Sensory Testing

Quantitative sensory testing was performed for the study contained in Chapter 6 – An investigation of deafferentation induced by ischaemic nerve block. See Chapter 6: Quantitative Sensory Testing (QST) for greater detail of methodology.

Functional Assessments

Near Infrared Light Spectroscopy

NIRS measurements were taken with a Somanetics INVOS™ 5100C Cerebral/Somatic oximeter (Covidien, MA, USA) (see Figure 14)

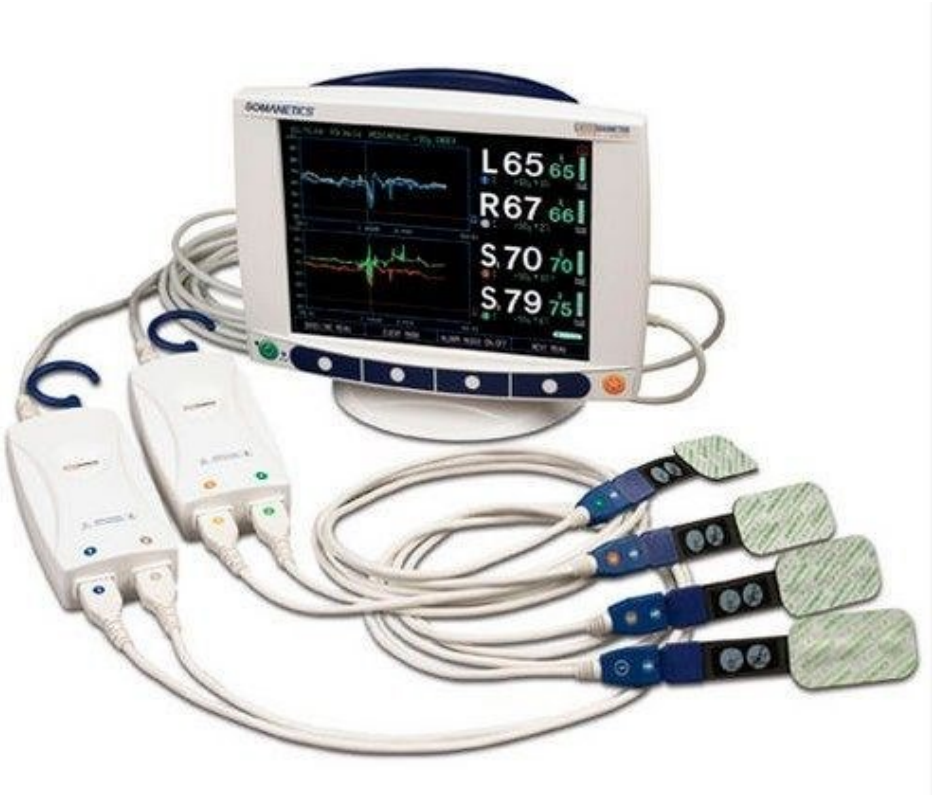

Figure 14: Somanetics INVOS™ 5100C Cerebral/Somatic Oximeter with 4 channels and 4 corresponding optodes attached.

Two self-adhesive optodes (Adult SomaSensor, Covidien, MA, USA) were placed on the medial calf, distal to the ischaemic nerve block (INB) tourniquet on the right leg and proximal to the cathode of the electrical stimulator, and the lateral aspect of the sole of the right foot (see Figure 15 for the experimental setup of the study described in Chapter 5: Cortical excitability induced by a novel low-pressure INB). Additional clinical adhesive tape was used to prevent any movement of the optodes.

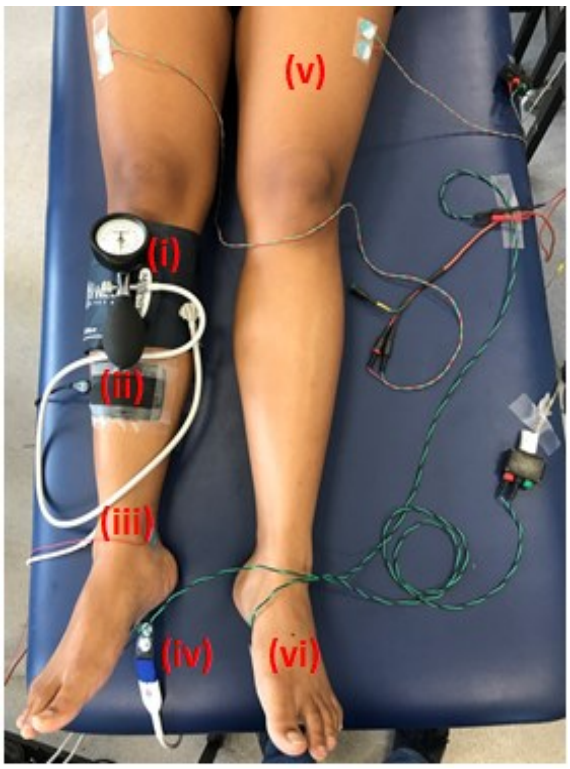

Figure 15: Overview of the experimental set-up for the study described in Chapter 5; (i) Sphygmomanometer cuff providing ischaemic block (ii) SSEP anode (iii) Cathode placed over tibial nerve (iv) NIRS optode placed on sole of foot (gastrocnemius optode not visible) (v) Electrodes placed bilaterally on Vastus Lateralis muscle belly (vi) Electrodes placed bilaterally on Abductor Hallucis muscle belly. The ground electrode was placed on the elbow (not visible).

Measurements were taken at a sampling rate of 0.2Hz. Values were recorded manually before being transferred to Microsoft Excel (Microsoft, USA); back up data was stored within the NIRS monitor and could be extracted as a comma separated values file if required using proprietary software. NIRS values are expressed as raw values of percentage oxygenated haemoglobin present in the underlying tissue (the regional oxygenation, rO_2).

Clinical Assessments

Patient Disease Severity Questionnaire (VascuQol)

Patients completed the King's College Hospital's Vascular Quality of Life Questionnaire (VascuQol) (see Appendix 5 – VascuQol Questionnaire) before participating in the experiment conducted in Chapter

4: An investigation into the variability of MEPs in patients with peripheral vascular disease. This questionnaire is discussed in greater detail in the relevant methods section of the chapter.

Pain Scores

Participant were asked to assess the pain in the ischaemic limb (hand or foot, depending on the study protocol), rather than under the cuff, using a numerical rating scale from 0 to 10 (where 0 is no pain, 10 is maximal pain) (112, 113). This was performed at measurement epochs e.g. when TMS was performed, before, during and after the INB.

Scores are expressed as raw values without normalisation.

Experimental Intervention

Ischaemic Nerve Block

In a semi-recumbent position, participants had their blood pressure measured at rest using an automated blood pressure device (Spot Vital Signs 420 Model 4600-0E 7, WelchAllyn, UK; see Figure 16A) attached to a blood pressure cuff (FlexiPort Reusable Blood Pressure Cuff, WelchAllyn, UK, see Figure 16B). Measurements were taken from the lower leg or upper arm, depending on if the paradigm used a calf or forearm INB, respectively. Three baseline measurements were taken, with the first omitted in case of artificial elevation due to participant anxiety (114).

A manual sphygmomanometer (DS54 Durashock Thumbscrew Blood Pressure Gauge, WelchAllyn, UK; see Figure 16B) was then attached to the same cuff placed around the right upper arm or calf, and inflated to 20-30mmHg above the previously measured systolic pressure, whilst a Doppler probe (Mini Dopplex Doppler, version D900, Huntleigh Healthcare, Cardiff, UK; see Figure 16C) was placed over the right radial or dorsalis pedis pulse, respectively. The loss of the Doppler signal at this inflation pressure was used to confirm occlusion of arterial flow, and thus the pressure was adequate to result

in limb ischaemia. Pilot testing with NIRS and TMS had confirmed this inflation pressure was adequate to result in a sustained reduction in tissue saturation and loss of distal MEPs.

The manual sphygmomanometer cuff was employed as the tourniquet for the INB. During the INB studies, it was inflated to individualised pressures, as described above, and maintained by intermittent inflation as the pressure reduced (an inherent safety feature of the sphygmomanometer is the slow release of the cuff pressure, to prevent inadvertent irreversible tissue ischaemia; periodic manual inflation was therefore required with Doppler signal continuously monitored to confirm occlusion). The cuff was left in situ for baseline measurements and after termination of the INB.

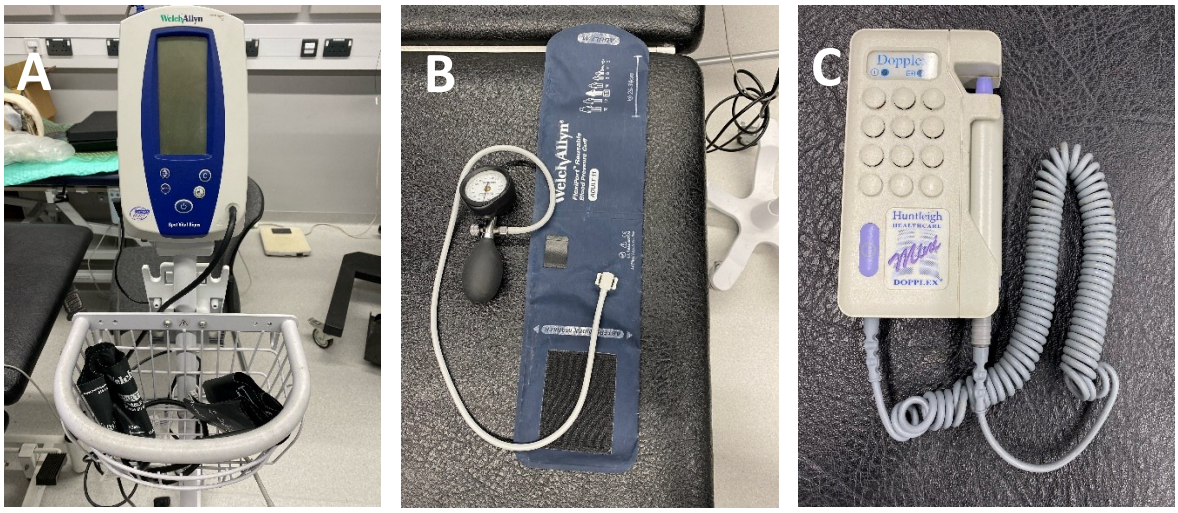

Figure 16: A: Automated sphygmomanometer, B) Manual sphygmomanometer and blood pressure cuff, C) Arterial doppler

Statistical Analysis

Data entry and initial analysis was performed using Microsoft Excel 2016, (Microsoft, USA). Statistical analysis was performed in Sigmaplot version 12.5 (Systat Software, Point Richmond, USA).

All data are given as mean ± standard error of the mean (SEM), unless stated otherwise. Data in figures are means with error bars representing SEM. Data figures were produced in using Sigmaplot v12.5 software.

Background EMG and MEP parameters (amplitudes, latencies) were compared between sides, over time and between muscles (as relevant), using repeated measures ANOVA tests, with post-hoc

multiple comparisons where relevant. A P value of <0.05 is considered significant and adjusted where appropriate for multiple comparisons.

Specific data analysis and statistical tests performed for each experiment are described in the methods sections of the corresponding chapter.

Chapter 3: Survey of Intra-operative Neuromonitoring during TAAA Surgery in the United Kingdom and Ireland

Introduction

At present, remedial interventions to prevent and treat SCI during TAAA surgery are usually employed prophylactically in high-risk patients or intra-operatively when there is clinical suspicion of a SCI. Much work has been undertaken to predict those at risk of developing SCI (18, 115). Similarly, considerable evidence exists for the effectiveness of remedial interventions, both as prophylactic measures and for treating SCI (33). As discussed in Chapter 1: Management of SCI following TAAA repair, interventions to prevent and treat SCI aim to promote blood flow to the spinal cord; deliberately increasing the driving pressure (i.e. mean arterial pressure, MAP) of the arterial blood, whilst simultaneously reducing cerebrospinal fluid pressure with a spinal drain, encourages flow across the spinal cord capillary beds (115). Both techniques are the pillars of spinal cord ischaemia protocols. What is less clear however, is the role of spinal cord monitoring during TAAA repair surgery.

Intra-operative neuromonitoring (IONM) is commonplace in neurosurgical theatres and is employed in surgeries such as scoliosis correction and spinal cord tumour resection to guide surgery (116-119). SSEP and MEPs are the two modalities used; a reduction in somatosensory evoked potentials (SSEPs) or motor evoked potentials (MEPs) amplitude by 50% or increased latency usually suggests impending SCI and prompts corrective measures (120, 121). It has also been shown IONM in TAAA can guide interventions in a targeted way (24), but despite evidence to show its usefulness, it is not actually known how many vascular surgical centres performing TAAA corrective surgery in the United Kingdom (UK) employ IONM of the spinal cord. As such, interventions are rarely guided objectively– and they are not benign risk-free undertakings (122).

Aim

This survey looks to establish the role IONM currently plays in preventing and treating patients with spinal cord ischaemia undergoing TAAA repair in the UK. In doing so, the need for IONM during such high-risk surgery can be identified.

Methods

An online survey (SurveyMonkey Inc, California, USA) was emailed to the Vascular Anaesthetic Society of Great Britain and Ireland (VASGBI) membership through the society's emailing list. The survey consisted of 10 questions (see Table 4), a mixture of multiple-choice questions, multichoice checkboxes and free text entries. The questions were devised to explore the individual views of vascular anaesthetists regarding the uptake of IONM during TAAA surgery, its role in SCI management and possible barriers to its use.

Responses were collected between April and July 2018.

Answers were anonymised and only one response per email was allowed. Partially complete surveys were permitted for analysis. Multiple respondents from the same centre were permitted to capture as many responses as possible. Any conflicting answers from the same centre are discussed further below.

UK Research Ethics Committee and NHS Health Research Authority regulatory permission was not required; this was a survey of practice with no identifiable participant data or interventions that could pose a risk to participant health.

1.	Which hospital performing vascular surgery do you work at? Free text answer
2.	Does your hospital perform thoraco-abdominal aortic aneurysm surgery (open or endovascular)? Yes or no
3.	Have you personally cared for or know of a case of paralysis/paraplegia following TAAA surgery? Yes or No
4.	Does your hospital utilise IONM of the spinal cord during TAAA surgery? Yes or No
5.	If not, why do you not use intra-operative neuromonitoring? (select all that apply) Did not know spinal cord monitoring was available Cost of equipment Cost of staff

	Do not think it is beneficial/will not change outcome Do not know why we don't use it Other
6.	Where it is used, what modality of intra-operative neuromonitoring do you employ? Somatosensory evoked potentials (SSEPs) Motor evoked potentials (MEPs) Both Not applicable (do not use neuromonitoring)
7.	Who performs the intra-operative neuromonitoring? Dedicated neurophysiologist Dedicated anaesthetist Anaesthetist assigned to list Not applicable (do not use neuromonitoring)
8.	What interventions does your trust use to prevent or manage spinal cord ischaemia? CSF (spinal) drain Permissive hypertension Altered surgical approach Cardiopulmonary bypass Surgical shunts Hypothermia Pharmacological e.g. mannitol, N-acetyl-cysteine
9.	Do you have a protocol in place to manage spinal cord ischaemia? Yes or No
10.	Does this protocol incorporate neuromonitoring? Yes, No, Not applicable (no protocol/do not use neuromonitoring)

Table 4: Survey questions. TAAA = thoracoabdominal aortic aneurysm, CSF = cerebrospinal fluid, IONM = intra-operative neuromonitoring

Data Analysis

Responses were generated on the Survey Monkey (California, USA) online platform and then recorded in Microsoft Excel 2016 (Microsoft, USA) for further analysis.

Graphs were created in Sigmaplot v12.5 (Systat Software, UK).

Results

67 responses were received in total.

Questions 1 and 2

The responses reflected 35 unique vascular surgical centres (question 1).

Of these, 27 performed TAAA surgery relevant to this survey (question 2). The results were cross-checked with data from the National Vascular Registry to confirm those 27 performed TAAA surgery (123).

The answers from these 27 centres resulted in 59 respondents (88%). Where a question refers to individual experience or perception (questions 3 and 5), results out of 59 responses are shown; if a question refers to a centre experience (questions 4, 6-10), results as a proportion of 27 are given.

Question 3

49% (29) of the anaesthetists answering this survey had personally cared for patient diagnosed with paraplegia or paralysis following TAAA surgery.

Question 4

Only 4 out of the 27 (15%) unique vascular surgical centres performing TAAA had IONM of the spinal cord available to use.

Question 5

23 centres did not use IONM and the reasons for this were multi-factorial (see Figure 17). Although multiple selections could be made, results produced 59 replies. Table 5 provides the free text answers given in response to this question.

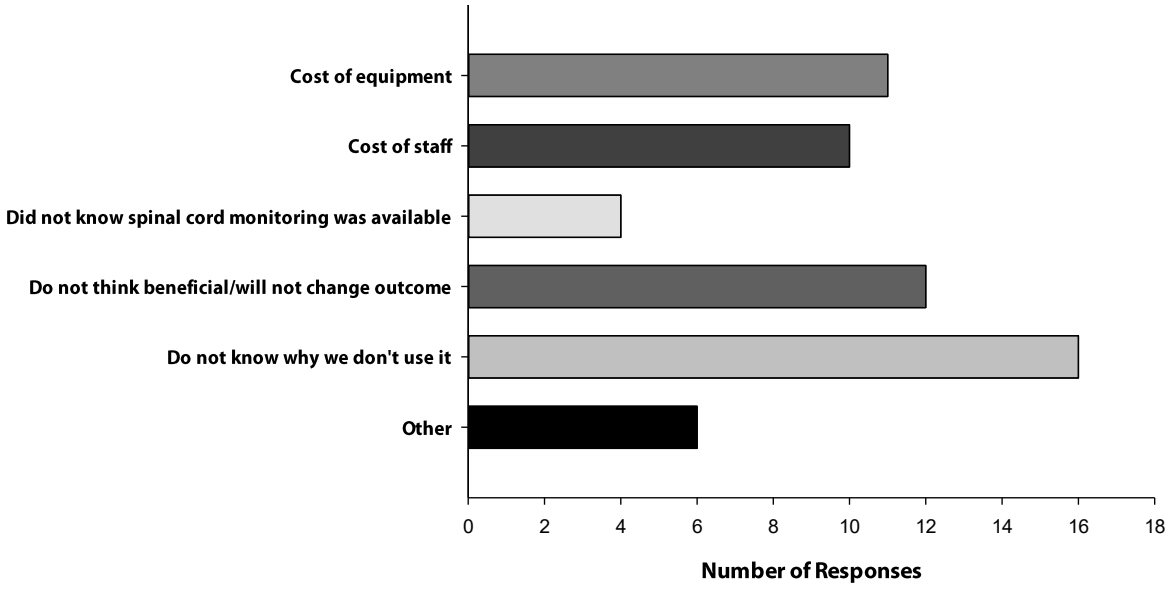

Figure 17: Answers to Question 5 – Where IONM was not used, what do you think the reasons are? Answers given as total number of responses.

Added complexity
I use it in spinal surgery (scoliosis correction) but the staff and equipment are not available for us in vascular
Not available
Our MDT had not identified or defined specific cases that would benefit- it is available
I do the endovascular list and we do not use it due to surgical preference on the endovascular lists
No availability of service, practicalities as our surgery is endovascular.
Equipment and neurophysiologist to monitor not available
I only have experience of TEVAR. Not sure about open - cardiac do those. We use it for spine surgery.

Table 5: Question 5 free text comments, "If not, why do you not use intra-operative neuromonitoring?"

Questions 6 and 7

MEPs and SSEPs were both used in the 4 centres utilising IONM.

One of these centres had 2 different respondents, where 1 claimed to use both modalities whilst another reported that they used only MEPs.

All monitoring was performed by a dedicated neurophysiologist.

Question 8

Figure 18 shows which interventions were performed to prevent or manage cases of spinal cord ischaemia. As multiple interventions were performed and there were some conflicting responses from the same centre, results are expressed as a proportion of 59 respondents; the total number of responses was 136. Fifty-three (90%) anaesthetists used a spinal drain, 28 (47%) employed permissive hypertension and 23 (39%) said the surgical technique was changed to accommodate patients at high risk of SCI.

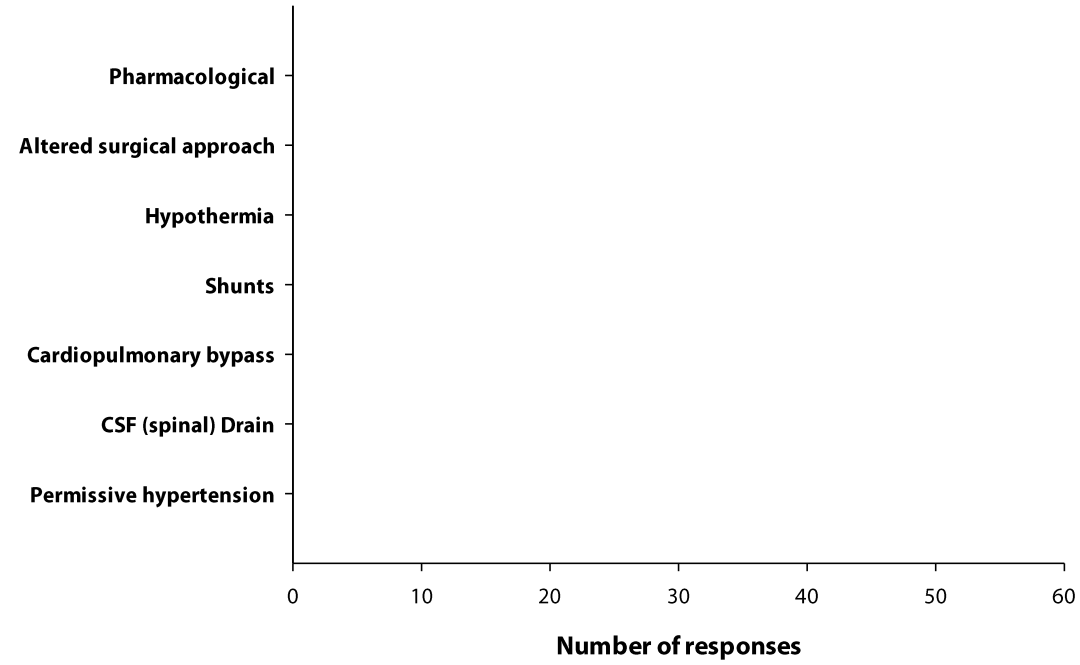

Figure 18: Question 8 –Which interventions were performed to prevent or manage cases of spinal cord ischaemia. Answers as a % of 59 respondents.

Free text comments to this question are provided in Table 6.

Table 6: Question 8 free text comments: What interventions does your Trust use to prevent or manage spinal cord ischaemia?

Staged procedures performed routinely. Post-op CSF drains in symptomatic patients failing to improve with conservative measures.
We have used shunts in two patients, CSF monitoring in one
Only do TEVAR, drain in high-risk cases highlighted at MDT
CSF drain performed once
Where indicated by clinical assessment

Question 9

41 (69%) respondents said they had a formal SCI protocol in place. Further analysis of centres showed 14 (52%) had a SCI protocol, 7 (26%) did not, whereas 6 centres had conflicting responses.

Question 10

Four vascular surgery centres (15%) performing TAAA repair had spinal cord monitoring as part of their post-operative protocol; these however were not the same institutions that utilised IONM (question 4).

Discussion

This is the first survey in the United Kingdom and Ireland showing the ischaemic SCI monitoring practices of 27 vascular centres performing TAAA surgery.

Almost half the anaesthetists had cared for a patient who had developed paraplegia or paralysis following a TAAA repair. The question was designed to reflect the participant experience of SCI, rather than the incidence; based on this survey, SCI is widespread and likely to occur within a vascular anaesthetist's career. Despite this however, only 15% (4/27) of vascular surgery units in the UK have IONM available for TAAA surgery. Broadly, the justification for this was the cost of the equipment and staff, the belief IONM is not beneficial, especially in endovascular surgeries and therefore unlikely to change the outcome, and a lack of awareness of availability. It was also surprising to find that 26-48% of centres did not have any SCI protocol, or anaesthetists were not aware of one. It would be of interest to know how our UK and Ireland survey results compare internationally, however no such data appears to exist.

In the following I shall address the above issues and discuss the potential implications of using prophylactic interventions without IONM or a formal protocol, and as such advocate for widespread use of IONM for all TAAA surgeries.

Cost of Intra-operative Neuromonitoring

The direct costs to the health service of SCIs arise from prolonged inpatient clinical care, management of complications and rehabilitation. There is also the possible loss of the individual's productivity to society from unemployment. Therefore, the health-economic burden is potentially huge. Little research exists specifically regarding ischaemic SCI following TAAA repair, however for all SCIs causing paraplegia, an estimation by the National Spinal Cord Injury Statistical Centre (Birmingham, Alabama, USA) is

approximately US $560 000 in the first year and US $74 000 per year thereafter; lifetime costs vary between US $1.6-2.5 million per patient (124).

The cost of IONM is approximately US $1535 per spinal surgery (e.g. scoliosis correction) with a 52.4% SCI prevention rate. The cost saving benefit exists for all spinal surgeries where there is a SCI rate of 0.3% or greater (125). Given the rate of SCI can be as high as 22% following TAAA repair (9), the initial outlay would be offset by the longer-term savings.

Specific data for ischaemic SCI related costs are not available, but analysis of cost-effectiveness is likely to be similar if not more favourable for the vulnerable vascular patient.

IONM is not beneficial and unlikely to change outcome

Intra-operative monitors can only detect early injury, seen more frequently with open repairs. As one example of many in the literature, of 117 open type I and II TAAA repairs, 17 patients lost MEPs following aortic clamping. Remedial measures returned MEPs in all but 3 patients, who had a persistent neurological injury (126). Three out of the 4 centres which routinely used IOMN performed the greatest number of open TAAA in the UK, all in excess of 20 cases per year (in 2017) according to the National Vascular Registry (NVR). Of the centres that do not utilise IOMN, 7 performed five or more open procedures. It seems pertinent to suggest that any centre performing a high volume (5 or more) open TAAA repairs per year should routinely employ IONM (according to our collaborating consultant vascular surgeon) and should be able to justify it as a clinically necessary, cost-effective tool.

In all centres, most surgeries were performed using the endovascular approach. 18 centres performed 20 or more TEVARs during the NRV collection period 2015-2017 (123). Very few centres employed IOMN; comments stated centres do not use IONM as they exclusively perform TEVARs and that since they are quicker and associated with late injury, there is little value (18). Indeed, late SCI is more likely to occur following a TEVAR, but there is increasing evidence early SCI is seen with endovascular repairs (9, 28, 121). In one study for example, 31 out of 49 patients undergoing TEVAR had a 75% or greater

reduction in MEPs and SSEPs intra-operatively. They recovered in 30 patients following protocolised interventions or when distal blood flow was resumed in response to the MEP changes. One patient however had a persistent loss of evoked potential with subsequent paralysis (37). With evidence to show potential detection of early SCI, the endovascular approach should not preclude the use of IONM. With regards to late SCI, it is true that current spinal cord monitoring is inadequate. However, as discussed below, solutions for this problem may exist and form part of the work undertaken in this PhD.

A major concern with IONM has been the potential for false positives and negatives, which may explain why some respondents felt IONM will not alter outcome. However, a systematic review of 19 studies where open repairs were performed concluded MEP neuromonitoring had 89.1% sensitivity and 99.3% specificity for detecting post-operative paraplegia, and therefore advocated its use (120).

Awareness of IONM

16 of the 59 anaesthetists did not know IONM was available for TAAA repair, despite neuromonitoring being a well-established tool for other spinal cord at-risk surgeries (127, 128). It has been used in vascular surgery for decades (33) and it is therefore surprising that such major surgery would be undertaken without an evidence-based approach, an explanation for which remains unclear.

Interventions designed to prevent or treat SCI

The commonest interventions for SCI are increasing the mean arterial pressure or reducing the cerebrospinal fluid (CSF) pressure using a spinal drain. In unmonitored cases, the interventions are based upon clinical suspicion and risk calculation. However, these interventions are not without significant risk themselves.

CSF drainage is an intervention that has been widely researched with a clear reduction in the relative risk of SCI (34). However, CSF drainage is associated with many complications, from the benign but

debilitating low intracranial pressure headache, to the severe cerebrovascular haemorrhage and nervous system infections (115). In a study of 648 patients undergoing open TAAA repair, 486 had a spinal drain inserted; of these, 14 had an intracerebral bleed, 3 had a bleed with cerebral atrophy and a further 2 patients died from a subdural haematoma (129). In a retrospective study of 130 patients undergoing TEVAR, although 7 developed a late SCI, 2 of which had a permanent injury, of the 71 patients with a prophylactic CSF drain in place, the same number of patients developed neurological deficits from an intrathecal haematoma and bacterial meningitis (121).

Many studies advocate the use of a staged TAAA repair (130) as did 39% of respondents (i.e. from a single open procedure, to a two-stage open or hybrid procedure) to reduce the rates of SCI. There are compelling improvements in short and long-term survival and SCI rate by performing two less extensive surgeries compared to one major procedure; however any potential benefits are offset by the longer total procedural and anaesthetic time and in-patient stay of two procedures (130, 131). If IONM use could decrease SCI rates where used with a one-stage surgery, the risks associated with two procedures could be ameliorated.

IONM could also be used to guide patient-specific blood pressure targets (elevating to a pressure until MEPs return), to site a spinal drain only where blood pressure management alone is insufficient, rather than prophylactically for all, and guide the early removal of a drain in patients not awake on the intensive care unit (115, 132). By increasing confidence that an SCI has not occurred intra-operatively, it may shift the preference to performing a single curative surgery.

Increasing accessibility of IONM in TAAA surgery

As this survey has demonstrated, there is need for spinal cord monitoring, intra-operatively and especially post-operatively, to detect SCI. Although the evidence in the literature makes it apparent that SCI rates can be reduced and interventions can be tailored, the inherent issues with electrical stimulation-based techniques limit its use to the anaesthetised patient and therefore usually to detect early SCI.

Transcranial magnetic stimulation (TMS) of the brain and paraspinal muscle near infrared spectroscopy (NIRS) could allow for a more accessible monitoring modality. Unlike TES, endogenous currents are generated within the motor cortex, circumventing the application of high voltage current to the patient's motor cortex, resulting in near pain-free brain stimulation and MEP generation. It therefore avoids the need for sedation or anaesthesia (121) and can be employed before (to have baseline readings), during (for intra-operative monitoring of early SCI) and after (to detect late SCI seen in TEVARs) surgery. TMS has been previously used successfully during surgery, such as scoliosis correction for example, although the evidence is limited (116). TES requires a dedicated neurophysiologist to setup, which can be time consuming, making it impractical for emergency cases. A dedicated neurophysiologist is also required for interpretation (121). TMS however is comparatively simple and as such could be used and monitored by the anaesthetist, in a similar manner to the way bi-spectral index is a simplified form of electroencephalography employed by an anaesthetist to monitor depth of anaesthesia (133).

NIRS is another emerging technology that has the potential to monitor the cord for ischaemic injury. As discussed in the introduction, animal and human studies have shown an intimate relationship between the paraspinal muscles and intraspinal vasculature. Furthermore, paraspinal muscle desaturation as measured by NIRS has correlated with radicular artery ligation and attenuation of MEP with post-operative neurological deficits (96, 98, 99). The simplicity of setup and use could make paraspinal NIRS a vital adjunct to MEPs or potentially beneficial even in its own right.

Where these technologies could really make a significant clinical difference is in the detection of late SCI following TEVARs. It is likely given the potential benefits of endovascular over open procedures and with an aging population, more endovascular or hybrid procedures are, and will continue to be, performed (130, 134). Being painless, non-invasive and not requiring anaesthesia means TMS and NIRS could be used on the ICU in the sedated patient where clinical assessment is not possible.

Limitations

It is acknowledged that surveys can be prone to selection bias. However, as the focus of the survey was to obtain individual perceptions regarding IONM for the purposes of this PhD, the survey was directed at all members of the VASGBI to mitigate for this. A further limitation is the response rate of approximately 15% of the VASGBI membership and many vascular anaesthetists may not be members of the VASGBI. We believe the results with all anaesthetists involved in vascular surgery are likely to be similar, as the 27 centres include large and small hospitals, teaching and non-teaching centres and have representation from across the country. In the UK and Ireland, there are 76 centres which perform all forms of aortic aneurysm surgery, hence 27 centres which purport to perform TAAA surgery in this survey, correlate with the National Vascular Registry (135). It should also be noted that the results are representative of practice in United Kingdom and Ireland only, and cannot be extrapolated to vascular surgery and anaesthesia internationally where there will be variations in practice.

Conclusions & Recommendations

This survey has demonstrated intra-operative neuromonitoring during TAAA surgery is only employed by centres performing a high volume of open procedures across the United Kingdom. Although many centres perform a high volume of endovascular surgeries, the potential benefit to those patients of IOMN is not offered. It has identified cost and a belief that it is not helpful at changing the outcome of SCI, especially for TEVARs, as potential reasons why IONM is not employed. I believe I have made the argument for IONM as a vital component in the management of TAAA patients where it is cost-effective in the long term and has it been demonstrated in many studies to be useful in detecting SCI.

I have presented the case for emerging technologies that could counter some of the issues identified with current IONM modalities. In the following chapters of this PhD, I shall conduct a series of studies which will further explore their use and address the nuances of using them specifically during TAAA surgery

Chapter 4: An investigation into the variability of MEPs in patients with peripheral vascular disease

Introduction

Transcranial magnetic stimulation as a tool to interrogate the function of the corticospinal tract has considerable evidence as a both a research and clinical tool. It can be used to evaluate neurological and neuromuscular pathologies, such as stroke and sciatica for example (136, 137). TMS has been employed pre-operatively to assess baseline motor tract function in those undergoing non-vascular spinal surgery with a risk of SCI, such as scoliosis correction (138) and has successfully correlated intra-operative MEP changes with post-operative monoplegia (139).

Despite its use as a diagnostic and therapeutic tool for a number of decades (116),(140), there is no evidence for its use as a neurophysiological tool in patients undergoing TAAA surgery to detect early and late SCI. As mentioned in the introduction, TAAA patients commonly have two aetiologies. The smaller cohort are young patients with connective tissue disease, but the vast majority of are older, with vascular pathology secondary to cardiovascular risk factors such as high blood pressure, high cholesterol and smoking (17, 141). As such, they invariably have concomitant peripheral vascular disease (PVD) in addition to an aneurysm, where the arteries of the limbs (usually the legs) are atherosclerotic, leading to occlusion and subsequently chronic limb ischaemia. This can in turn lead to damage of the limb tissues, including nerves and muscles.

MEP characteristics have been extensively studied in healthy young and older adults (142). The MEPs of older patients with known PVD however, have not previously been studied. Understanding changes that occur because of chronic ischaemia is not only beneficial for IONM in general, but potentially for any research or clinical situation where MEPs need to be characterised in the older patient with cardiovascular risk factors. With an aging population and increasing rates of obesity and cardiovascular disease (143), the co-incidence of PVD will also inevitably increase.

TMS studies which investigate MEP characteristics often explore the variability of MEPs, in addition to simply latency and amplitude alone. Previous reliability studies have explored inter- and intra-user reliability of TMS trials i.e. the variability within a set of stimuli by the same user at different times (often days apart) and different users in the same session. They have also explored the effects on reliability from changes in coil position and removal the first MEP generated (144-147). There is little evidence however, of the effect of time alone; the variability and reliability of the TMS-induced MEPs generated over a time-frame similar to that when the spinal cord could be injured has not been investigated.

Aim

The aim of this study was to determine the characteristics (amplitude, latency and coefficient of variation) of TMS-induced MEPs from upper and lower limb muscles in healthy volunteers over a 1 hour period; this is similar to the duration of intensive monitoring during surgery when the spinal cord would be most at risk. The second aim was to collect PVD patients MEP data with this established protocol.

In the context of potential intra-operative neuromonitoring, a variety of upper and lower limb muscles were investigated for their reliability over 1 hour, to determine which would be best employed as the control and monitor muscle, respectively. In addition, the two groups would reflect a similar demographic and co-morbid background as the two cohorts of patients typically undergoing a TAAA repair, the younger connective tissue disease patients (e.g. Marfan's disease) or the older degenerative disease patients (e.g. PVD). To my knowledge, the characteristics of MEPs of patients with PVD has not been studied and would provide essential baseline data for future neurophysiological study of older patients with cardiovascular disease, with possible clinical applications such as the development of an intra-operative neuromonitor.

Methods

Participants

Participant were recruited as per the criteria in Chapter 2: General Methods – Participant Recruitment.

20 healthy volunteers (8 females, 12 males) with a mean (±SD) age of 28 (±7.6) years and 20 patients with confirmed PVD (3 females, 17 males), mean (±SD) age of 63.2 (±11.5) years took part in the study.

Patient Disease Severity Questionnaire (VascuQol)

All patients completed the King's College Hospital's Vascular Quality of Life Questionnaire (VascuQol) questionnaire; this is a clinically validated questionnaire designed to determine disease severity in PVD patients. It utilises 25 questions which explore the debilitating effects of PVD on the physical and emotional well-being of PVD patients (148) (see Appendix 5 – VascuQol Questionnaire)

Each question is graded from 0 to 7, with 0 indicating PVD affects a particular aspect of a patient's life all of the time (i.e. no function or ability to a perform task) and 7 indicating none of the time (i.e. normal function or ability) A mean score of 4.5 or less is strongly associated with symptomatic intermittent claudication (muscle pain secondary to intermittent ischaemia experienced on exercise) whilst a score of 3.1 or less indicates critical limb ischaemia requiring surgical intervention (149).

Surface EMG

All participants were instructed to lie supine on a physiotherapy table. Pairs of disposable Ag/AgCl electrodes (25mm diameter, 1041PTS, Henleys Medical Supplies Ltd., UK) were applied to the skin overlying the muscles of interest after skin preparation with alcohol. For full details, see Chapter 2: Electromyography Acquisition.

Healthy volunteers had electrodes placed over the belly of five muscles of the upper and lower limbs of the left side of the body (brachioradialis (BR), abductor pollicis brevis (APB), vastus lateralis (VL),

tibialis anterior (TA) and abductor hallucis (AH)). These limb muscles were chosen as they are supplied by different major peripheral nerves. To ensure both lower limbs were included to account for potential differences in disease severity between the left and right leg, patients had electrodes placed over left and right VL, TA and AH, as well as left BR and left APB (see Figure 19). A ground electrode was placed over the olecranon process of the ulna.

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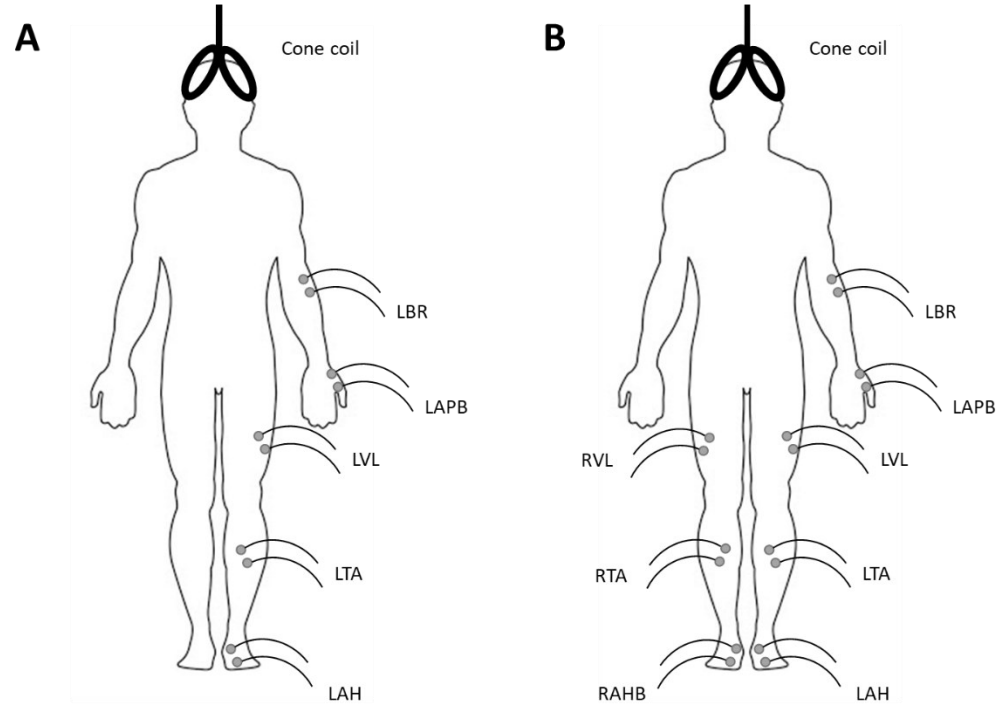


Figure 19: Experimental set up. Electrode position for healthy participants (A) and PVD patients (B)., LBR = left brachioradialis, LABP = left abductor pollicis brevis, , LVL and RVL = left and right vastus lateralis, , LTA and RTA = left and right tibialis anterior, LAH and RAH = left and right abductor hallucis. The cone coil is shown, although both cone and circular coils were used.

Transcranial Magnetic Stimulation

TMS was delivered using two separate Magstim 200² magnetic stimulators (Magstim, Dyfed, UK), each connected to a different coil. A circular coil (90mm outer diameter) was used to stimulate upper limb muscles and an angled double-cone coil (wing outer diameter 120mm), was used to stimulate lower limb muscles. Both the circular and double-cone coil were placed over the vertex of the head overlying the motor cortex, which was marked on the scalp with a washable marker pen to allow for consistent coil placement between trials (Figure 12). A 'combined' resting motor threshold (cRMT) was found for

the upper and lower limb muscles for each participant. This was the lowest stimulation intensity that produced discernable MEPs ≥50μV in amplitude (106) in at least three out of six successive stimuli in either all upper or all lower limb muscles, with the circular and double-cone coils, respectively. Supra-maximal intensities of 120% of the 'combined' RMT for the muscle with the highest threshold were used. For more general details of TMS setup, see Chapter 2: General Methods - Transcranial Magnetic Stimulation.

Experimental Protocol

Each session consisted of six single pulses of TMS from both coils, separately, every 10 minutes over the course of 60 minutes (seven sets of twelve stimulations from baseline (0 minutes) to 60 minutes). Participants were instructed to completely relax all muscles during stimulation to prevent facilitation, which dramatically increases MEP amplitude (111) and to better replicate surgical conditions. Pre-stimulation EMG amplitude was measured to ensure adequate muscle relaxation.

Data analysis

Patient Disease Severity Questionnaire (VascuQol)

The scores from 25 questions were averaged to produce a mean value from 0 to 7 for each participant, which were then averaged to produce a group mean score. Microsoft Excel 2016 (Microsoft, USA) was used to perform calculations and data are presented as mean ± SEM.

Motor Evoked Potentials

Signal Software v5.12 (CED, UK) was used for data analysis by visual positioning of cursors at the start and finish of each MEP.

Peak-to-peak amplitude and latency were subsequently measured.

The variability both within a time point and across the whole experiment was measured in both participant cohorts. The variability between measurements was determined by the coefficient of variation (CV); CV = standard deviation/mean.

Data groups with a lower CV are considered to have lower variability; where CV = 1, the standard deviation is equal to the mean and is evidence of greater variability.

Variability within a time point

To determine the extent to which individual MEPs varied between the 6 stimuli at a given testing epoch (i.e. at 0 min, 10 mins etc), the amplitude of each MEP was measured, and the CV of the 6 MEPs was calculated. The CV of the amplitude was calculated for each time point and muscle.

Variability across the whole experiment

The averaged MEP (avMEP) is a digital averaging process performed by Signal Software, where the 6 MEPs at a given trial are amalgamated to produce a single MEP trace; from this the amplitude and latency can be measured.

To determine the variability of the muscles across the duration of the experiment, the amplitude of the avMEP at each of the 10-minute time points was measured and the CV calculated. This was carried out for each muscle and each participant to calculate the mean CV of the avMEP amplitude; comparison of the different upper and lower limb muscles was then made.

Statistical analysis

Statistical analyses were carried out using Sigmaplot v12 (Systat Software, USA). Data were tested for normality (Shapiro-Wilk) and appropriate parametric or non-parametric tests were used (ANOVA, Friedman on ranks, or t-tests). If a main effect was found, multiple comparison procedures (Holm-Sidak) were performed to isolate the differences.

AvMEP amplitudes, CVs of the avMEP amplitudes and AvMEP latencies were collapsed over the hour and (where appropriate) compared between left and right sides in patients using paired t-tests. As there were no differences between sides, data were averaged between sides and compared between patients and healthy participants using unpaired t-tests. Data collected unilaterally were compared between patients and healthy participants using unpaired t-tests.

To examine changes in variability of MEP amplitudes within a time point over the hour protocol, CVs were compared over time and between left and right sides (for the lower limb muscles) using two-way repeated measures ANOVA (factors TIME and SIDE). Since no effect was seen for either time or side, data from left and right sides were averaged. CVs were assessed for differences over time and between patients and controls using two-way repeated measures ANOVA, with post-hoc multiple comparisons where necessary.

The level of statistical significance was set at $p < 0.05$, or appropriate for multiple comparisons. Data are presented as mean ± SEM in the text and figures.

Results

VascuQol Score in PVD Group

The group mean VascuQol score was 4.3±1.2, representing symptomatic peripheral vascular disease not requiring surgical intervention (as per the main inclusion criteria for the PVD group).

MEP Amplitude

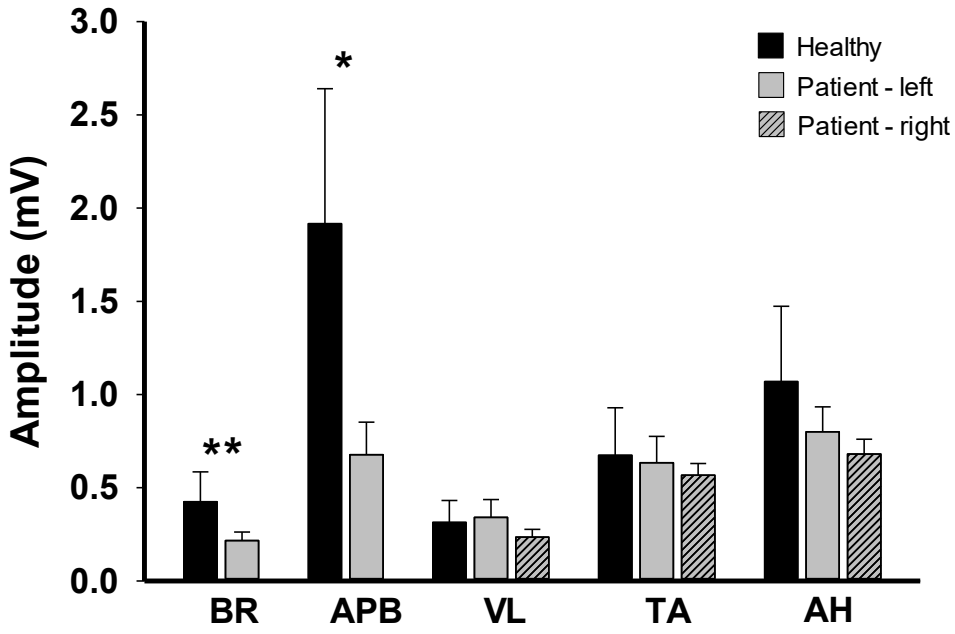


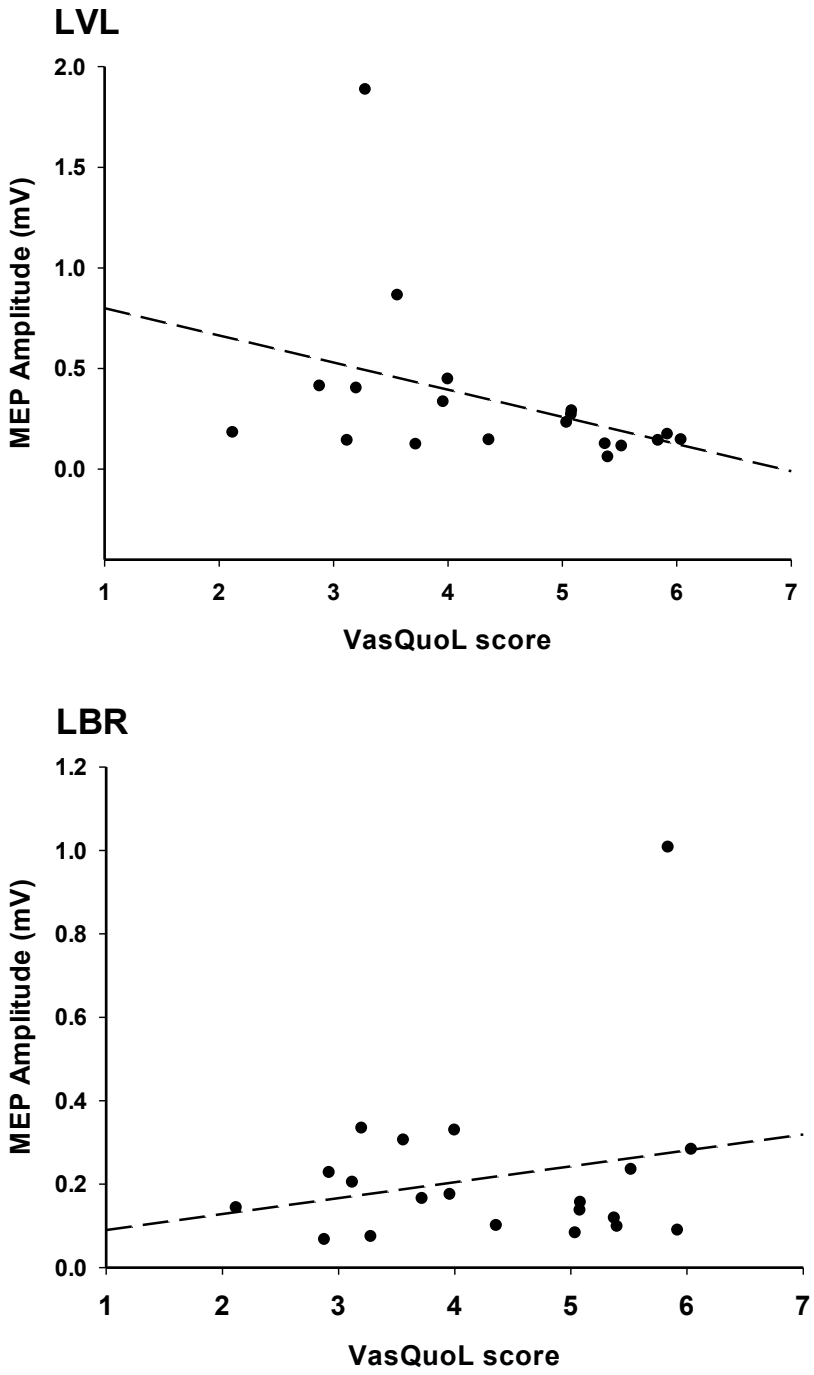
*Figure 20: Amplitude of healthy participant and patient muscles MEPs. Data expressed as mean ±SEM. BR = brachioradialis, APB = abductor pollicis brevis, VL = vastus lateralis, TA = tibialis anterior, AH = abductor hallucis. * denotes significant difference between healthy and patient APB muscles (P <0.05); ** denotes significant difference between healthy and patient BR muscles (P <0.01).*

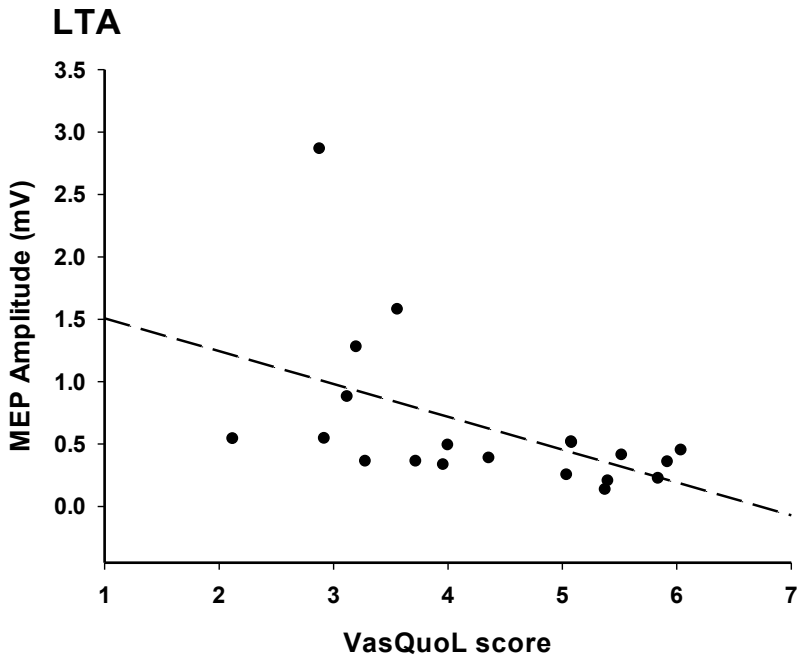
There were no differences in avMEP amplitudes between left and right legs in patients (VL, left 0.34±0.10mV vs right 0.23±0.04mV, P=0.29; TA, left 0.63±0.14mV vs right 0.57±0.06mV, P=0.54; AH, left 0.80±0.13mV vs right 0.68±0.08mV, P=0.22). When collapsed across sides, there were no differences between the MEP amplitudes in the lower limb muscles of patients [P] and healthy [H] participants (VL [P]:0.29±0.06mV vs [H] 0.33±0.06mV, P=0.61; TA [P] 0.60±0.10mV vs [H] 0.67±0.08mV, P=0.60; AH [P] 0.74±0.10mV vs [H] 1.00±0.16mV, P=0.18). The amplitudes of MEPs were smaller in patients than in healthy participants in BR ([P] 0.22±0.05mV vs [H] 0.43±0.04mV, P<0.0001) and APB ([P] 0.68±0.17mV vs [H] 1.87±0.46mV, P=0.02) (see Figure 20).

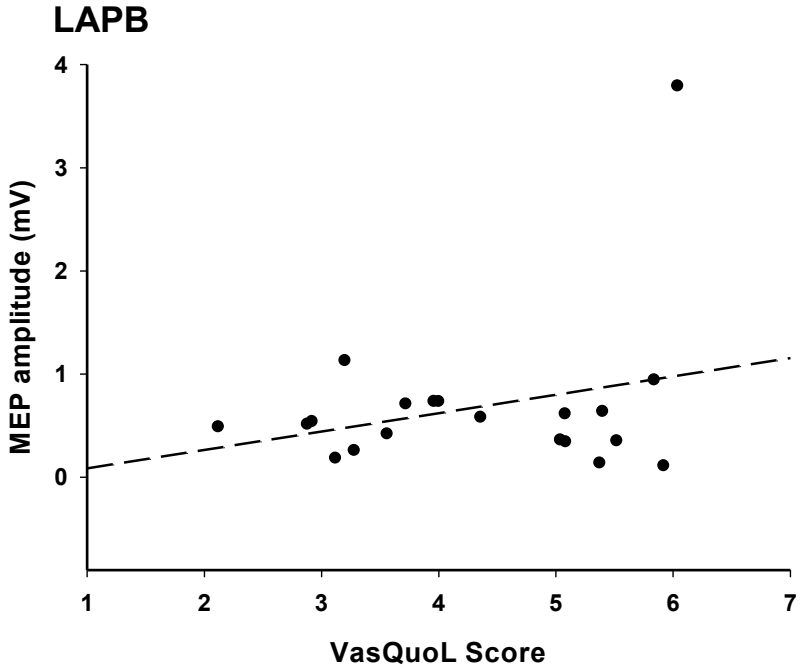
Correlations

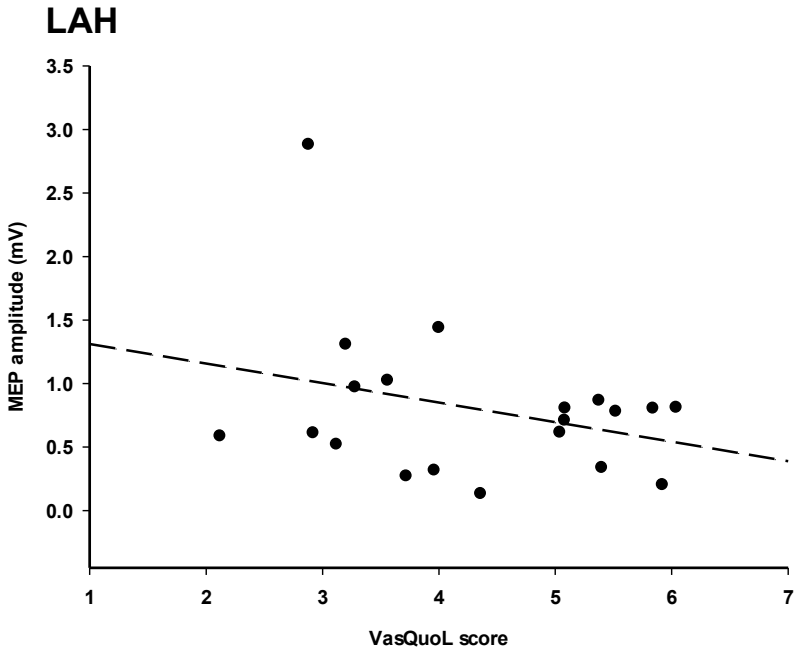
MEP amplitude and VascuQol score

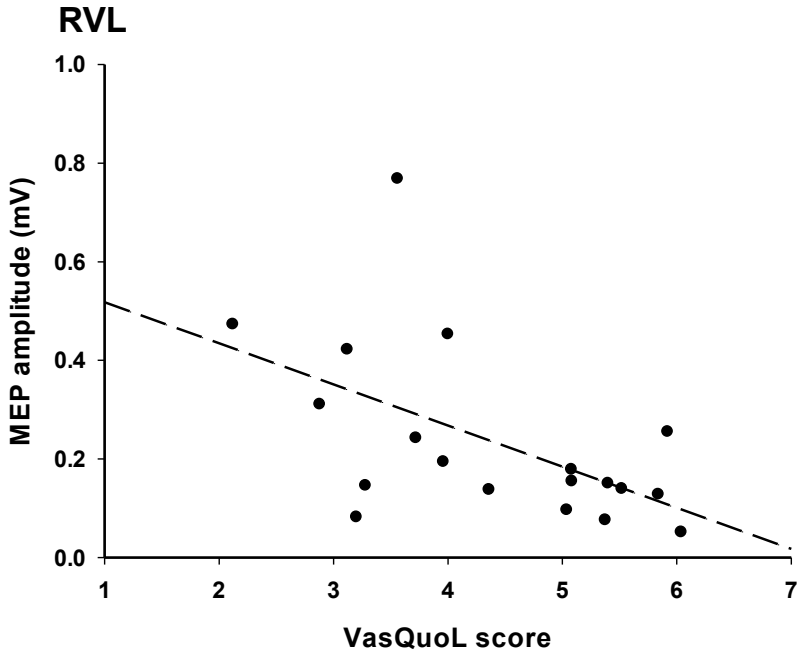
A series of correlations between the individual patient VascuQol scores and the mean avMEP amplitude for each muscle were performed. No correlation was observed for any muscles measured in the patient group; the mean *r* value was -0.21 (range -0.54 to 0.27) (Figure 21 and Table 7).




LTA
MEP Amplitude (mV)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
VasQuoL score


LAPB
MEP amplitude (mV)
4
3
2
1
0
1
2
3
4
5
6
7
VasQuoL Score


LAH
MEP amplitude (mV)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
VasQuoL score


RVL
MEP amplitude (mV)
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
7
VasQuoL score

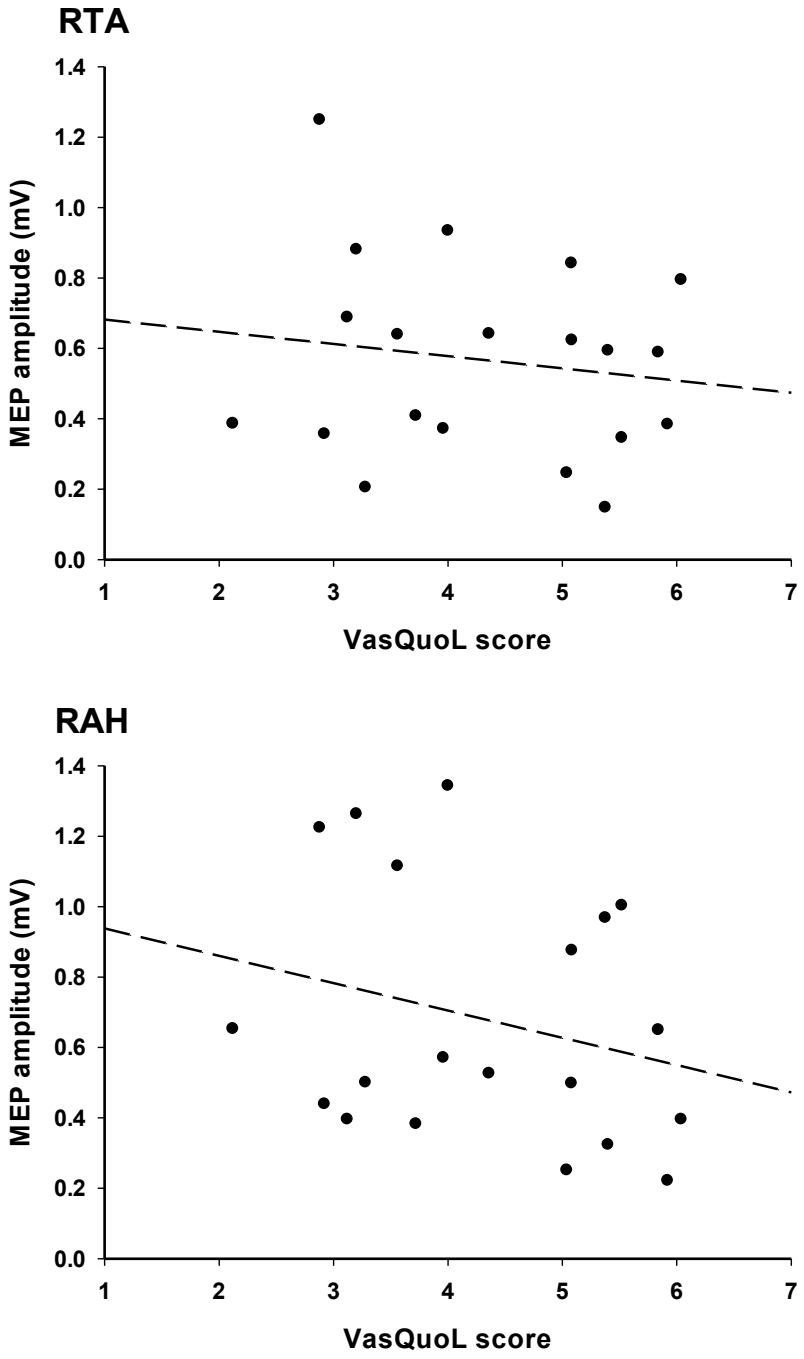


Figure 21: Correlations between individual patient MEP amplitudes and Vascuuol score for each muscle. LBR = left brachioradialis,LAPB = left abductor pollicis brevis, LVL = left vastus lateralis, LTA = left tibialis anterior, LAH = left abductor hallucis, RVL = right vastus lateralis, RTA = right tibialis anterior, RAH = right abductor hallucis.

Muscle	*r*
LBR	0.049
LAPB	0.075
LVL	0.146

LTA	0.246
LAH	0.094
RVL	0.296
RTA	0.022
RAH	0.067

Table 7: Correlation coefficients (r) for MEP amplitudes vs VascuQol score for each muscle.

Mean CV of avMEP and mean avMEP amplitude

There was no correlation between the CV of the avMEP and the mean avMEP amplitude of each muscle (r=0.023).

CV of avMEP amplitude

There were no differences in CV avMEP amplitudes between left and right legs in patients (VL, left 0.35±0.03 vs right 0.41±0.03, P=0.16; TA, left 0.32±0.03 vs right 0.30±0.03, P=0.77; AH, left 0.29±0.03 vs right 0.29±0.02, P=0.97). When collapsed across sides, there were no differences between the CV of avMEP amplitudes in the lower limb muscles of patients [P] and healthy [H] participants (VL [P]: 0.38±0.02 vs [H] 0.34±0.02, P=0.25; TA [P] 0.32±0.03 vs [H] 0.32±0.03, P=0.97; AH [P] 0.29±0.02 vs [H] 0.30±0.04, P=0.94). In the upper limb muscles, there were no differences in BR between patients and healthy participants ([P]: 0.27±0.02 vs [H] 0.31±0.02, P=0.22), but CVs were larger in patients than in healthy participants in APB ([P]: 0.56±0.04 vs [H] 0.38±0.04, P<0.01) (Figure 22).

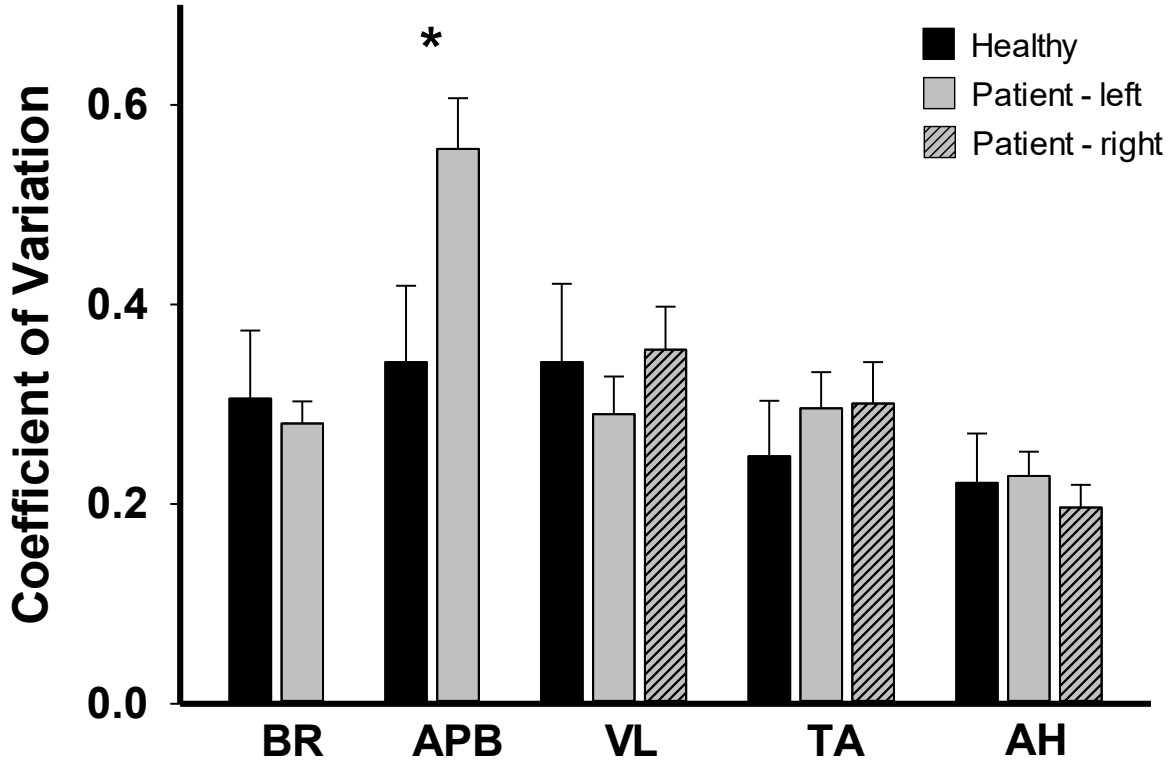
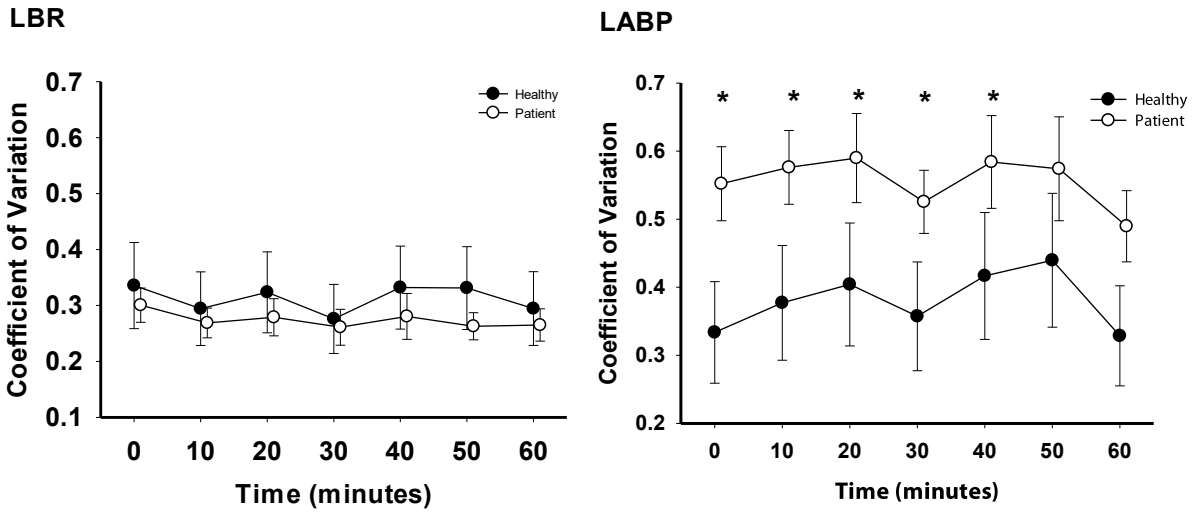


*Figure 22: Coefficient of variation for patient and healthy participant muscles. Data expressed as mean ± SEM. BR = brachioradialis, APB = abductor pollicis brevis, VL = vastus lateralis, TA = tibialis anterior, AH = abductor hallucis. * denotes a significant difference between patient and healthy participant APB muscles (P<0.05)*

Mean CV of MEP amplitude at each time point



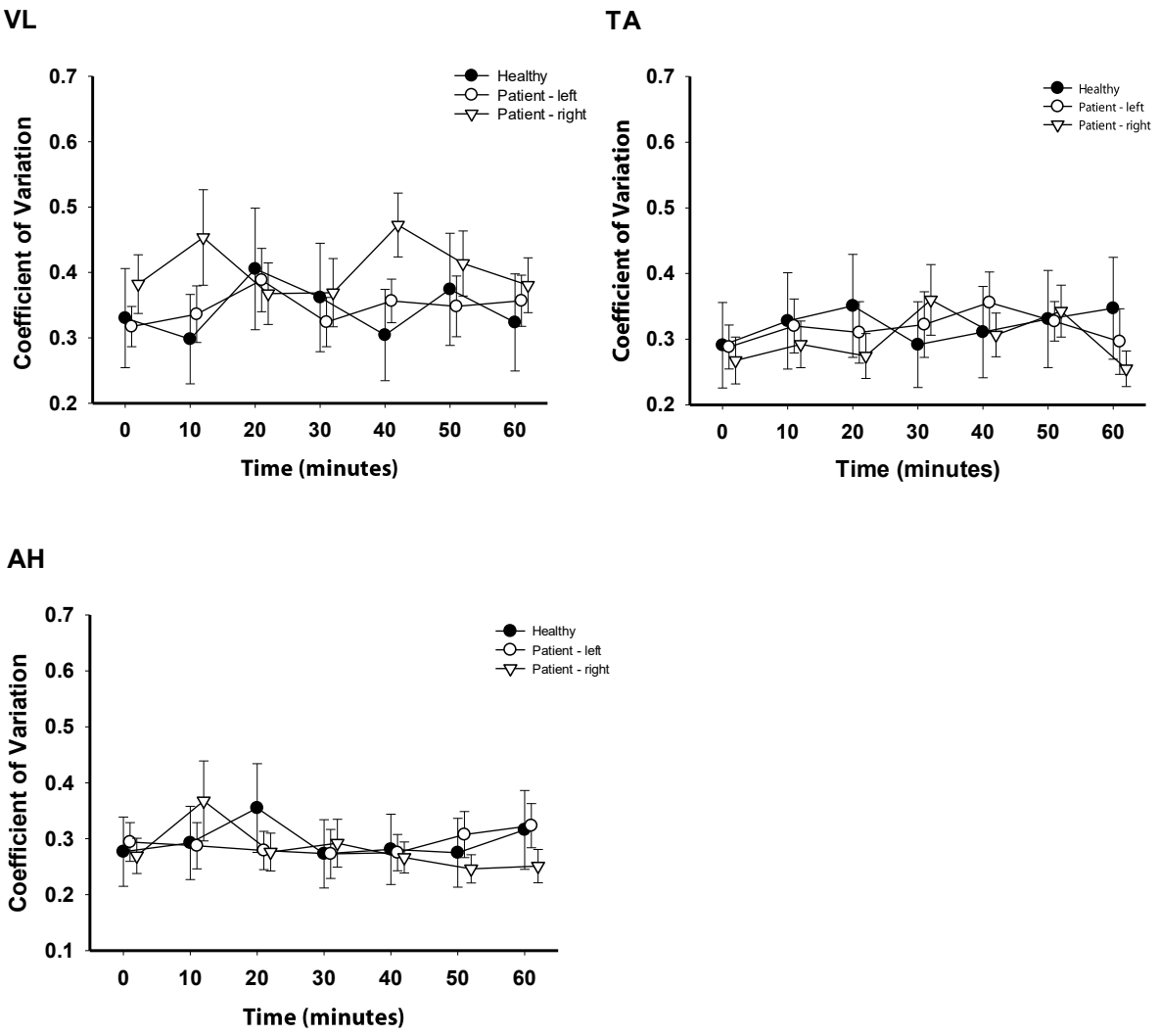


*Figure 23: Coefficient of variation of MEP amplitude at each time point for each muscle. LBR = left brachioradialis, LAPB = left abductor pollicis brevis, VL = vastus lateralis, TA = tibialis anterior, AH = abductor hallucis. For graphs for VL, TA and AH, left and right refers to side of body. * denotes a significant difference between healthy participant and patients' LAPB muscles (P<0.05)*

In the upper limb muscles, ANOVA revealed no effect of time or group (patients or controls) on CV of MEP amplitudes in BR ((time) F=0.59, P=0.74, (group) F=1.69, P=0.20) (see Figure 23A). In APB, there was no effect of time (F=1.50, P=0.18), but a main effect of group (F=9.32, P<0.01). Post-hoc comparisons (Holm-Sidak) revealed differences (P<0.05) in CV of APB at 0, 10, 20, 30, and 40 mins (see Figure 23B).

ANOVA revealed no effect of time or muscle side on CV of MEP amplitudes in the lower limbs of patients (VL, (time) F=0.73, P=0.63, (side) F=3.11, P=0.09; TA, (time) F=1.63, P=0.15, (side) F=0.32, P=0.58; AH, (time) F=0.83, P=0.55, (side) F=0.24, P=0.63). When collapsed across side, there were no

main effects of group (patients or controls) or time on CV of MEP amplitudes (VL, (time) F=0.77, P=0.60, (group) F=0.99, P=0.33; TA, (time) F=0.69, P=0.66, (group) F=0.08, P=0.77; AH, (time) F=0.96, P=0.46, (group) F=0.04, P=0.85) (see Figure 23C-E).

avMEP Latency

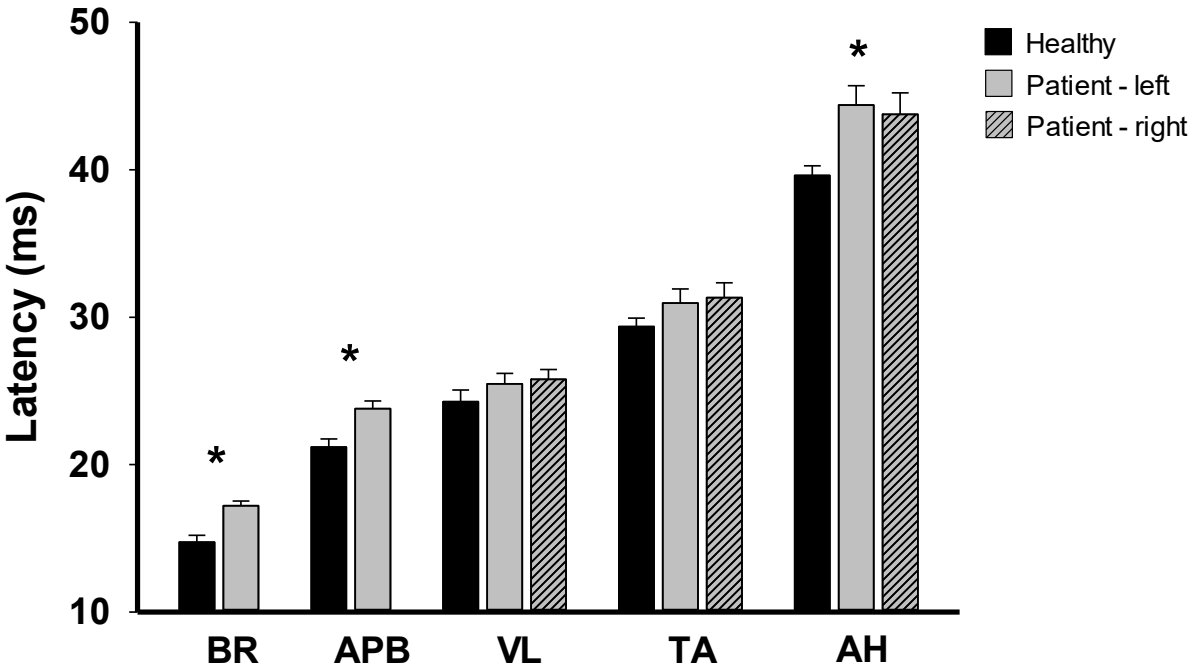


*Figure 25: Latencies of healthy participant and patient muscles MEPs. BR = brachioradialis, APB = abductor pollicis brevis, VL = vastus lateralis, TA = tibialis anterior, AH = abductor hallucis brevis. Data expressed as mean ± SEM. * denotes a significant difference between patient and healthy participant muscles (P<0.05).*

There were no differences in avMEP latencies between left and right legs in patients (VL, left 25.47±0.71ms vs right 25.58±0.68ms, P=0.56; TA, left 30.96±0.96ms vs right 31.71±0.82ms, P=0.20; AH, left 44.39±1.31ms vs right 43.76±1.45ms, P=0.27). When collapsed across sides, there were no differences between the avMEP latencies in the lower limb muscles of patients [P] and healthy [H] participants for VL [P]: 25.61±0.65 vs [H] 24.27±0.79ms, P=0.20 or TA [P] 31.33±0.85ms vs [H] 29.37±0.57ms, P=0.06), but latencies in AH were longer in patients than in healthy participants ([P] 44.08±1.36 vs [H] 39.62±0.65, P<0.01). In the upper limb muscles, MEP latencies in were longer in

patients than in healthy participants (BR [P]: 17.21±0.31ms vs [H] 14.84±0.46ms, P<0.001; APB [P]: 23.79±0.52ms vs [H] 21.27±0.55ms, P<0.01) (see Figure 25).

The latency of avMEPs showed minimal overall variation across all muscles and there was no significant difference between the CVs of avMEP latencies for each of the muscles for both healthy volunteers and patients.

Discussion

The results demonstrate that MEP characteristics are similar between healthy volunteers and PVD patients. There is a small decrease in amplitudes and a small increase in latencies. They have similar variability within a set of TMS stimuli and over time. These changes are likely to be age-related differences between the groups rather than due to pathology, which will be discussed below.

MEP Amplitude and Latency

The results of this study demonstrate that the patient group had smaller MEP amplitudes for the upper limb muscles. MEP latencies were generally longer and significantly different in 3 out of 8 muscles. There were no differences in amplitudes or latencies between the left and right legs. The values for APB, TA and AH amplitude and latency in patients are consistent with those in the literature attributable to the effects of aging (150, 151). These studies commonly compare healthy younger participants with matched older participants and found at both rest and during contraction, MEP amplitudes were smaller in older participants and latencies longer. In addition, no correlation was found between the disease severity score and avMEP amplitude, suggesting negligible impact from moderate PVD on MEP characteristics beyond those expected by aging alone.

The protocol was designed to include patients who had a stable disease with intermittent claudication (symptomatic leg muscle pain on exercise), which was not severe enough to require surgery; the mean VascuQol score of 4.3 reflects this. These patients have an arterial blood supply to their lower limbs

which is reduced but adequate, provided no stress is placed on them, such as prolonged or uphill walking. In this situation, there is a state of intermittent ischaemia. Based on our results, this reduced supply and intermittent compromise does not seem to result in any deterioration in nerve function. To the best of my knowledge this has not been investigated before and provides valuable baseline data for older patients with known cardiovascular morbidity (with or without diagnosed PVD as the risk factors are the same) undergoing neurophysiological investigations, undergoing surgery where IONM is employed, or if participating in research.

MEP amplitude is a key factor to take into consideration when choosing a muscle for use as a monitor during spinal or TAAA surgery. If a muscle has very small MEP amplitudes, difficulties may arise in detecting any decrease in amplitude. Furthermore, anaesthetic agents significantly reduce MEP amplitude and prolong latency, due to their depressive effects on the brain and at the synapse between the axons of the corticospinal tract and the lower motor neurons of the anterior horn (139, 152). The results of this study show for both healthy volunteers and patients, APB and AH produced the largest mean amplitudes of the upper and lower limb muscles, respectively. All the investigated muscles had CVs between approximately 0.3-0.4 (except for APB in patients which was 0.6). Therefore, despite APB having the largest discernible MEPs of any muscle studied, it was the most variable, potentially making it less suitable for use in a monitor of spinal cord function, even as a control muscle above the level of any likely cord injury.

The potential effect of amplitude on the variability of MEPs within a muscle and its coefficient of variation was also considered. A previous study has demonstrated muscles with larger MEP amplitudes, have smaller coefficients of variation (153). The design of the protocol meant there was a disparity between the percentage motor thresholds for each muscle as a single 'combined' test intensity per coil was used. A stimulus intensity of 1.2 x MT of the muscle with the highest threshold was used; therefore, the test intensity would be greater for the remaining muscles. Previous work has shown greater stimulation intensities result in MEPs with not only larger amplitudes but also less variation in amplitudes, likely through increased motor unit activation (107, 153, 154). A correlation between the mean CV of avMEP and the mean amplitude of avMEP for each muscle returned an *r*

value of 0.023, demonstrating there was no correlation between the size of an MEP and its variability in the protocol. Even after removing the data for MEP amplitudes of APB (having such a comparatively large amplitude it appears as an outlier), the correlation coefficient still approached zero.

Reliability

Many neurophysiological studies have previously investigated the variability of TMS-induced MEPs (142, 146, 147, 154-156), both with regard to the extent of the variation and the factors potentially causing it. No previous study to our knowledge has compared the variability of different muscles over a longer period of time, nor in PVD patients with a view to using TMS-induced MEPs for IONM during major aortic surgery.

As can be seen from the results it is clear that there is variability in TMS-induced MEP characteristics between stimuli at each epoch and over time. My interest lay in the extent of this variation between muscles, with the belief that the muscle with the least natural variation would be the most suitable for use as an intra-operative monitor of spinal cord function.

During surgeries where the spinal cord is vulnerable, there will be certain surgical interventions or time points where there is a high risk of SCI. At these crucial timepoints, there will be a need for repeated trains of stimuli to continually assess spinal cord integrity, hence the need to assess variability within trains at a given time point (CV of mean MEP). Determining the variability of TMS-induced MEPs over time is also necessary particularly where frequent but intermittent monitoring is required for a longer duration of time, say to check the IONM system remains operational, hence the need to assess variability over an extended period (CV of avMEP).

TMS-induced MEPs have previously been shown to be variable (153) and this is greater with TMS than with TES, since motor cortical activation occurs trans-synaptically (147, 157). TES generates MEPs through direct cortical activation and produces more consistent results (49) and it is for this reason the neuromonitoring guidelines usually recommend TES be used during surgery (158). However, as

discussed above, there are some practical limitations to its use. TES is painful due to the insertion of scalp screw electrodes and the application of large direct current to the scalp. This necessitates anaesthesia in all cases, limiting its use to the intra-operative setting. In TAAA surgery however, SCI can occur post-operatively also, which currently cannot be diagnosed with TES in the awake or lightly sedated patient. In such a scenario, TMS which is painless, could assist in the diagnosis of late SCI.

Our results have shown that the coefficients of variation for the mean avMEP amplitude for the measured muscles are 0.26-0.55 and 0.22-0.48 in patients and healthy participants, respectively. The mean CV of the avMEP gives an indication of the variation of all the MEPs generated over the course of the one-hour protocol. This is especially true for BR and AH, which had the lowest CVs of the avMEP for upper and lower limb muscles in both groups of participants. These results are in keeping with previous studies in the literature which have shown TMS-related measurements are reliable with good intra and inter-investigator (146) and test-retest (156) reliabilities in healthy young and old participants (142, 153, 159). As demonstrated by the amplitude and latency results, given there were no significant differences between healthy and PVD patient CV values, the normative MEP variability data in the literature where young healthy participants are included appears applicable to patients with cardiovascular disease.

ANOVA testing of the mean CV values over time for each muscle showed no statistical significance for either cohort. The CV values within each of the 10-minute epoch, however, were generally greater than the CV of the avMEP over the hour. The least variable muscles were again BR and AH. These CV values demonstrate a low variation if these muscles were used as part of an intra-operative monitor to interrogate the spinal cord at a specific time point.

Some of the variability in MEPs can also be attributed to methodology (48, 57, 58), such as changes in stimulus intensity or number of stimuli delivered (142), level of muscle contraction at time of stimulation (160, 161), data analysis procedures (such as removal of initial MEP amplitudes when averaging (154)) and technical factors relating to coil position (46, 162). In this study, trains of 6 stimuli were delivered for calculation of the mean CV of the MEP and repeated across the hour to generate

the mean CV of the avMEP and found the variability of all muscles comparable to previously published work (145, 147, 153).

With regard to use of TMS as a monitor during surgery, a concern is the suppressive effects of anaesthesia and adjuvant analgesia on corticospinal excitability (163, 164). This can be overcome with an appropriate anaesthetic to facilitate TMS induced MEPs (165) (see Chapter 8: TMS and NIRS in Theatre). Although in this scenario MEPs may be smaller than in an unanaesthetised participant, the reduced corticospinal excitability may result in reduced variability in MEPs during surgery. Furthermore, the ease of this method of monitoring permits its use in the post-operative period.

Limitations

The use of young healthy volunteers as the controls, rather than healthy age-matched participants, may be viewed as a limitation in this study. However, the atherosclerotic process causing PVD increases with age, with age being an independent risk factor for arterial disease irrespective of health status (166). Thus, it would be difficult to find an older person who is truly disease-free. To confirm this would also require more advanced functional assessments and imaging with blood flow studies of the arterial system (167), which would have been practically prohibitive for this study. As already mentioned, data exist for healthy older adults and the data is compared to the literature in the discussion above.

The stimulus intensities used in the experiments are unlikely to be 120% of the RMT for each muscle. The intensities would only be 120% for the muscle with the highest motor threshold and a varying degree more of the other muscles. Although it is possible to use a specific coil to target each muscle in turn and thus determine the motor threshold for each muscle, this would have been impractical for 8 muscle in the confines of a time critical protocol, thus a “combined motor threshold” was employed.

The healthy and patient group data were collected on different muscles. The healthy volunteer data came from a paradigm performed before study on PVD patients, and it was felt necessary to include

both lower limbs in the patient group. This allowed us to compare the symptomatic leg to the asymptomatic leg (if unilateral symptomology) in case a difference in neurophysiological characteristics was present.

The TMS-induced MEPs were not normalised to M-max, the maximum motor (M) wave that can be generated with electrical stimulation of the peripheral nerve supplying the muscle of interest. However, this was not solely a study investigating variability, but also a protocol development study and that many measurements could not be obtained during the measurement time intervals. Also, were TMS to be used as an IONM, any changes to intra-operative TMS-induced MEPs, that could be indicative of a spinal cord injury, would be judged relative to baseline TMS MEPs. Thus, there would no need to advocate the use of TES to generate M-waves for normalisation purposes.

Conclusions

TMS-induced MEPs in young, healthy participants and older patients with peripheral vascular disease were found to have similar characteristics. The results suggest there are no additional effects of compromised limb blood flow causing intermittent ischaemia seen in this cohort of patients on muscle neurophysiology beyond those expected by aging. As such, normative MEP data in the literature from older healthy participants can be applied to patients with cardiovascular morbidity and peripheral vascular disease who are participating in neurophysiological studies or undergoing surgery where motor spinal cord integrity is monitored.

Chapter 5: Cortical excitability changes induced by a novel low-pressure INB

Introduction

It is well established the brain is plastic with changes occurring following injury such as a stroke or limb amputation (168). These alterations enable the brain to compensate and subsequently augment the function of remaining limbs secondary to an injury. Experimentally, this can be replicated with an ischaemic nerve block (INB), using a tourniquet, where an improvement in functional tasks is observed (169) or with transcranial magnetic stimulation (TMS) where motor evoked potentials (MEPs) increase (87).

Distal to an INB, the occluded muscles become ischaemic and it has been shown that MEPs diminish and eventually disappear; in the non-occluded muscles however, MEPs have been observed to increase in proximal (63, 80) and homologous contralateral limb muscles (59, 87). This reflected increases in corticospinal excitability; no increases in MEP amplitude were observed with spinal or peripheral nerve stimulation. Deafferentation of the distal muscles, analogous to an amputation or nerve injury, is believed to initiate immediate-onset changes within the motor cortex, through a process of disinhibition and exposure of latent excitatory interneurons. These increases in excitability are seen only when TMS is employed, due to transsynaptic activation of cortical motoneurons, and not when sub-cortical or spinal structures are stimulated (87).

Most studies on motor cortical excitability changes following an INB have been performed using the upper limb. There is evidence to show changes occur in the muscles proximal to the INB and in homologous contralateral muscles, but it is unclear if these changes extend to distant muscles (59, 87). In addition, the commonly used model of an INB is where a circumferential tourniquet is inflated to pressures of 220-250mmHg, which will result in nerve compression, as well as ischaemia. Therefore, the results observed previously could be attributable to the effects of ischaemia, nerve compression

and also pain, which can have variable effects on motor cortical excitability, albeit with a preponderance to reduce corticomotor excitability (170).

During TAAA surgery, arterial clamps are used. These are placed on the aorta and iliac arteries to allow aneurysm sac resection without fatal blood loss. The consequence of this is profound and prolonged lower limb ischaemia, effectively producing an INB. Unlike the models used in the aforementioned studies, there is no circumferential limb compression with a tourniquet, but a direct arterial compression, and with the patient being anaesthetised, likely much less pain experienced by the patient. Thus, in attempting to develop a TMS-based intra-operative monitor to detect SCI in TAAA surgery, an INB model more closely resembling the ischaemia seen during this surgery was needed.

Aim

The aims of this study were to:

1. Evaluate the effectiveness of a novel, low pressure INB of the lower limb using a combination of near-infrared spectroscopy distal to the tourniquet to confirm ischaemia, and SSEPs to demonstrate deafferentation.
2. To use this novel, lower limb low-pressure INB to investigate changes in evoked potentials both ipsilateral and contralateral to the block, but also in upper limb muscles distant to the INB site.

The low-pressure is likely to produce less pain and nerve compression, compared to the traditional INB, thus minimising the effects of these on cortical excitability, and more closely resembling ischaemia induced intra-operatively. Therefore, it may allow for the effects of a surgical ischaemia on such a widespread distribution of muscles, those making up the monitored and controlled muscles in a TMS-based model of SCI detection, to be investigated.

Methods

Participants

Participants were recruited as per the criteria in Chapter 2: Participant Recruitment.

Thirty-two participants took part in this study; 19 for experiment 1 (mean(±SD) 21.7± 1.9 years, range 18-34 years, 3 female) and 13 for experiment 2 (mean age 23.7 ± 3.1 years, range 28-47 years, 2 female).

Pre-experiment Vascular Assessments

Blood pressure and subsequently the experimental INB pressure (systolic blood pressure + 20-30mmHg) was determined as per Chapter 2: Ischaemic Nerve Block.

Surface EMG

All participants were instructed to lie supine on a physiotherapy table. Pairs of disposable Ag/AgCl electrodes were applied on the skin overlying vastus lateralis and abductor hallucis bilaterally, parallel to the muscle fibre orientation (Experiment 1; see Figure 26a) or on both brachioradialis and abductor pollicis brevis muscles (Experiment 2; Figure 26b) (110). A ground electrode was placed on the right lateral malleolus or olecranon process, for experiment 1 or 2, respectively.

For greater detail, see Chapter 2: Electromyography Acquisition.

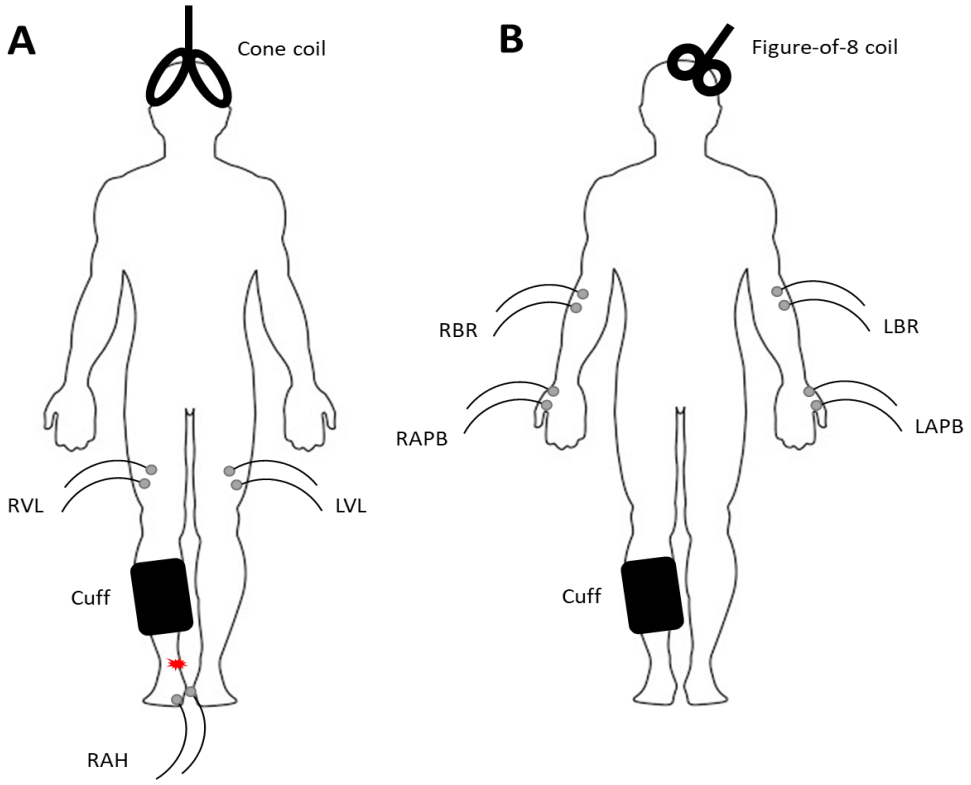


Figure 26: Experimental set up. Electrode position for Experiment 1 (A) and Experiment 2 (B) are shown. LVL and RVL = left and right vastus lateralis, RAH = right abductor hallucis, LBR and RBR = left and right brachioradialis, and LABP and RAPB = left and right abductor pollicis brevis. Red burst indicates tibial nerve stimulation.

Motor Evoked Potential (MEPs)

TMS was applied to the motor cortex using a Magstim 200[2] stimulator (The Magstim Company Ltd., Dyfed, UK); see Chapter 2: Transcranial Magnetic Stimulation.

Various stimulating coils were trialed to establish which type to use (e.g. double cone and figure-of-eight coils). The double cone coil was selected for producing the most reliable MEPs in lower limb muscles, and was positioned over the vertex so that the induced current in the brain flowed in the posterior-to-anterior direction (Experiment 1). The figure-of- eight coil was selected for upper limb muscle MEP generation, which was directed 45° to the midline such that the induced current flowed in a posterior-to-anterior direction (Experiment 2). The coil was placed at the corresponding hotspot for each upper limb muscle.

MEPs were analysed as the average of 6 for each muscle and the peak-to-peak amplitude was measured.

See Chapter 2: Electromyography Acquisition and Chapter 2: Analysis of TMS induced MEPs for greater detail of MEPs sampling and then analysis.

MEPs are expressed as a percentage of baseline for further analysis, where baseline is the average of pre-occlusion amplitudes and expressed as 100%.

Somatosensory Evoked Potentials (SSEPs)

Nerve stimulation

Somatosensory evoked potentials (SSEPs) were generated by electrical stimulation of the tibial nerve, located 1-2cm posteriorly to the medial malleolus of the ankle. The correct location was confirmed by stimulation induced flexion of the hallux. A constant current stimulator (DS7A, Digitimer, UK) was used to deliver 400 stimuli (pulse width of 0.2ms) at 4Hz at each testing epoch, via a cathode, driven by a digital programmer and pulse generator (Digitimer D4030, Digitimer, Welwyn Garden City, UK); the anode was placed approximately 3cm proximal to the cathode over the tibia (171). The output current was increased from 0mA in increments of 0.5mA and set to the lowest value that produced a non-painful flexion of the toes.

Electroencephalogram

The vertex was marked as described above to locate the positions of EEG electrodes. Two Ag/AgCl EEG electrodes (UniMed Electrode Supplies, Farnham, UK) were placed on the scalp at the CP_Z and F_Z position, as per the International Federation of Clinical Neurophysiology 10-20 system (172) (see Figure 27). The sites were first cleaned with 70% isopropyl alcohol wipes, and the scalp gently prepared with abrasive paste to reduced impedance, before adhering the electrodes using electroconductive paste (Ten20, Weaver and Company, USA). Impedance was measured to ensure sufficient signal quality (<5kΩ).

Pilot testing performed before the experimental testing had confirmed the EEG electrodes did not interfere with the TMS stimuli; they did not move and there was no disruption in functional integrity.

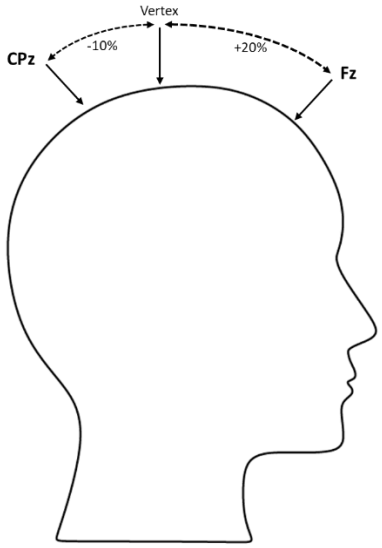


Figure 27: Schematic diagram showing the location of the CPz and Fz positions for EEG electrode placement

SSEP Analysis

Recorded signals were amplified (x1000; Iso-DAM-X, World Precision Instruments, Welwyn Garden City, UK) and band-pass filtered (10-3000Hz). The signals were sampled at 2kHz using an analogue to digital converter (Power 1401 data acquisition device, Cambridge Electronic Design [CED], UK). Signal v5.12 software (CED, UK) was used to record the samples to disk, where an average SSEP trace from the 400 traces was generated at each epoch. Following the application of a 50Hz notch filter to reduce electrical noise, the P37-N45 peak-to-peak amplitude was measured (see Figure 34) (173). The amplitudes are expressed as a percentage of baseline for further analysis, where baseline is the average of pre-experimental intervention amplitudes and expressed as 100%.

Near-infrared Spectroscopy (NIRS)

The optode for the near infrared spectroscopy regional oxygenation measurements (rO_2) was placed distal to the INB on the medial right calf muscle; see Chapter 2: Near Infrared Light Spectroscopy for

a detailed description of the setup. NIRS values are expressed as raw percentages without normalisation.

An image of the experiment is shown in Figure 15.

Occlusion and pain

A pressure of 'calf systolic blood pressure +20-30mmHg' was maintained manually throughout the 30-minute occlusion period of the paradigm. Each participant was asked to assess the pain in the right foot specifically, rather than under the cuff, using a numerical rating scale from 0 to 10 (where 0 is no pain, 10 is maximal pain) (112, 113). This was performed at 5-minute intervals before, during and after the occlusion period. Scores are expressed as raw values without normalisation.

Experimental Protocols

Experiment 1 – Investigating the effects of a low-pressure INB

With participants fully relaxed, a combined resting motor threshold (cRMT) was first determined with the cone coil. This is the stimulator output required to produce MEPs of greater than 50 µV in 3 out of 6 consecutive stimulations (106) in all 4 lower limb muscles; the test intensity used was 120% of this cRMT. A cRMT was used as it was not possible to isolate each muscle consistently within the time constraints of the protocol, therefore the experimental MT is in fact the MT of the lower limb muscle with the highest threshold.

TMS was delivered at 5-minute intervals to the vertex (Figure 28). Each testing point consisted of 6 TMS stimuli. Baseline MEPs were first established over 10 minutes (0, 5 and 10 minutes), following which the cuff was inflated for 30 minutes (from 15 to 45 minutes, with a 5-minute set-up interval between baseline and inflation). MEPs were then recorded immediately after the cuff was deflated (at minute 46), then every minute for 5 minutes (47 to 51 minutes) and finally two further sets 10 and

15 minutes after cuff deflation (at 55 and 60 minutes). The cuff remined in situ to prevent changes in cutaneous afferent input (174, 175).

SSEPs were generated every 5 minutes over the course of the protocol. With the cathode over the tibial nerve, the test current was determined by incrementally increasing the stimulator output from 0mA in 0.5mA steps until a non-painful discernible flexion of the toes was seen. 400 pulses were delivered at each time point, as described above.

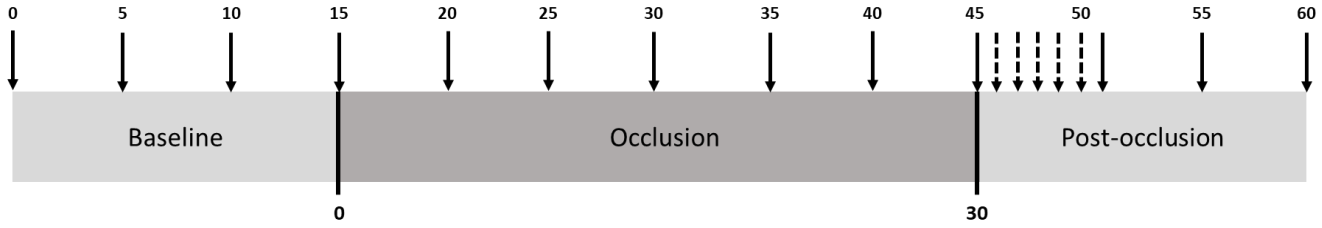


Figure 28: Experiment 1 protocol. Black arrows indicate where MEPs and SSEPs were sampled. The dashed lines indicate where only MEPs were recorded. Numbers along the top represent protocol time in minutes, numbers on the bottom indicate start and end of occlusion.

Experiment 2 – Investigating the spread of cortical excitability following an INB

With participants fully relaxed, individual muscle motor threshold (MT) was determined through identification of the 'hotspot' using the figure-of-8 coil. The 'hotspot' is the area over the motor cortex which consistently produces the largest MEP amplitude at a given stimulator intensity for a chosen muscle. Participants were asked to wear a swimming cap, so each hotspot could be clearly marked on to it. The motor threshold and test intensity for each upper limb muscle was then determined as above.

TMS was delivered at 5-minute intervals with each testing point consisting of 6 TMS stimuli. Baseline MEPs were recorded from left and right APB and BR at 0, 5 and 10 minutes, following which the cuff was inflated for 30 minutes and MEPs measured every 5 minutes. MEPs were then recorded immediately after the cuff was deflated (minute 46), then at 50, 55 and 60 minutes (Figure 29). Distal

AH MEPs were not recorded as the effects of this INB were confirmed in experiment 1. The cuff remained in situ throughout.

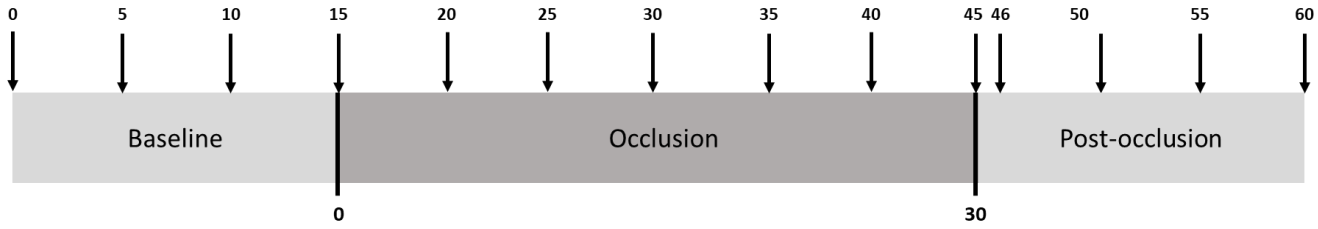


Figure 29: Experiment 2 protocol. Black arrows indicated where MEPs were recorded. Numbers along the top represent protocol time in minutes, numbers on the bottom indicate start and end of occlusion.

Data analysis

Data were taken from Signal software, exported to Microsoft Excel and analysed using SigmaPlot (Systat Software, San Jose, CA). In line with previous research using INB (80, 87), data were grouped into time periods and a mean MEP for that period was used for analysis.

For experiment 1: baseline (BL) (0, 5, 10 minutes), early occlusion (EO) (15, 20, 25, 30, 35 minutes), late occlusion (LO) (40, 45, 46 minutes), early post-occlusion (EPO) (47, 48, 49, 50 minutes) and late post-occlusion (LPO) (51, 55, 60 minutes). For experiment 2: baseline (0, 5, 10 minutes), early occlusion (15, 20, 25, 30, 35 minutes), late occlusion (40, 45, 46 minutes), early post-occlusion (50 minutes) and late post-occlusion (55 and 60 minutes). Data were tested for normality using the Shapiro-Wilk test. Initial one-way repeated measures analysis of variance (RM ANOVA) with post-hoc Holm-Sidak tests on right AH MEPs showed a significant reduction in MEP amplitude from baseline at 40, 45 and 46 minutes, confirming presence of a successful INB which persisted for a minute following cuff deflation. Thus, data from 46 minutes was included in the 'late occlusion' time period and averaged for comparison to the other time period averages. Further one-way RM ANOVA (with Holm-Sidak post-hoc tests) was performed on SSEP and NIRS data with significance set at $p < 0.05$.

MEPs at 45 minutes in those with low pain scores (1-5) were compared to MEPs at 45 minutes in those with high pain scores (6-10) using unpaired t-tests.

Statistical significance was set at $p < 0.05$. Data are presented as means ± SD in the text and as means ± SEM in the figures.

Results

Experiment 1

Motor evoked potentials

The data from 19 participants were analysed. Figure 30 shows representative MEP traces from a single participant, highlighting their disappearance in the right AH and corresponding increases in the ipsilateral vastus lateralis and both contralateral muscles.

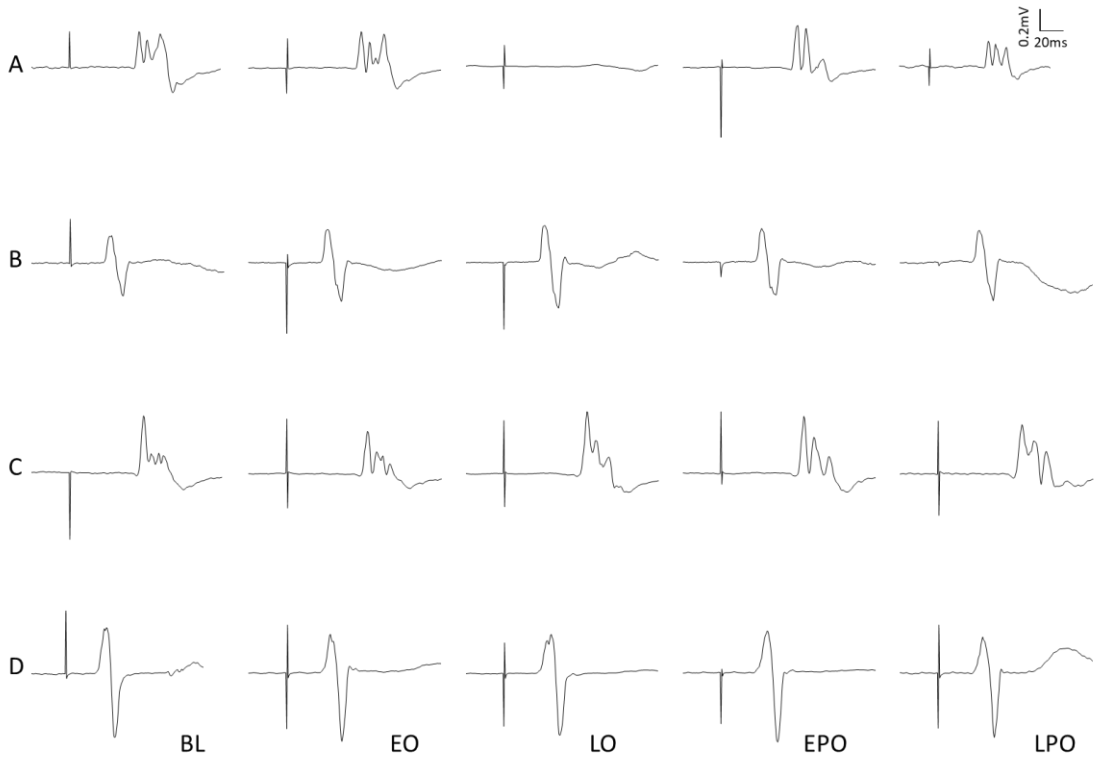


Figure 30: Single participant representative MEPs (averaged) from A) right abductor hallucis brevis, B) right vastus lateralis, C) left abductor hallucis and D) left vastus lateralis at baseline (BL), early occlusion (EO), late occlusion (LO), early post-occlusion (EPO) and late post-occlusion (LPO). The first vertical deflections indicate time of TMS. Note the disappearance of the MEP in trace A during LO and the increase in amplitude seen in traces B-D.

Figure 31 shows the change in mean MEP amplitudes over the course of the experiment. There was a significant reduction in ipsilateral RAH mean amplitude from baseline seen in the LO period (40, 45 and 46 minutes), falling to a mean of 6.5% of baseline (P<0.001). The MEP rapidly recovered following removal of the INB.

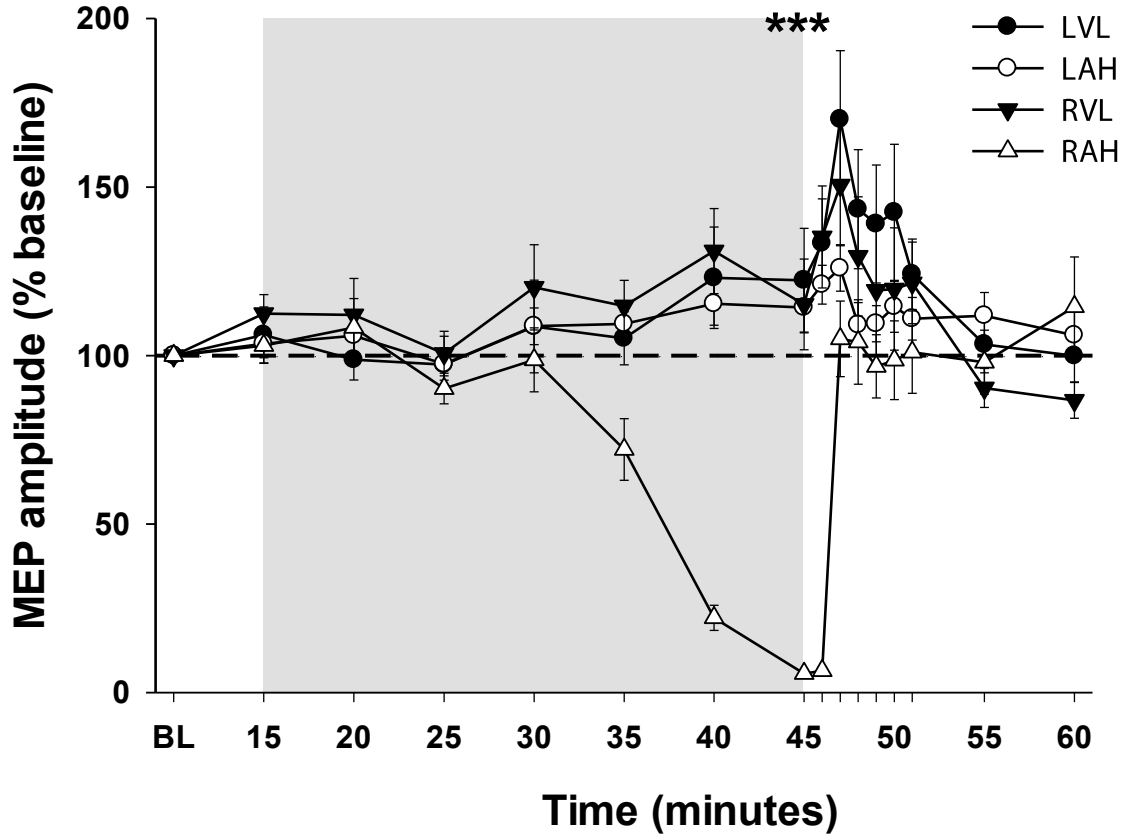


*Figure 31: Mean (±SEM) MEP amplitudes as a percentage of baseline (BL) in left vastus lateralis (LVL), left abductor hallucis (LAH), right vastus lateralis (RVL) and right abductor hallucis (RAH), during experiment 1. The shaded box represents the occlusion period. *** denotes a significant reduction in RAH amplitude compared to BL during LO period (40, 45 and 46 minutes) (P<0.001).*

Data were grouped into time periods for statistical analysis as described in the methods. During LO, LAH and LVL (contralateral, non-occluded) amplitudes increase significantly compared to baseline (P<0.05; see Figure 32). A significant increase in LAH, LVL and RVL (proximal to INB, non-occluded) was also seen during EPO (P<0.05; see Figure 32).

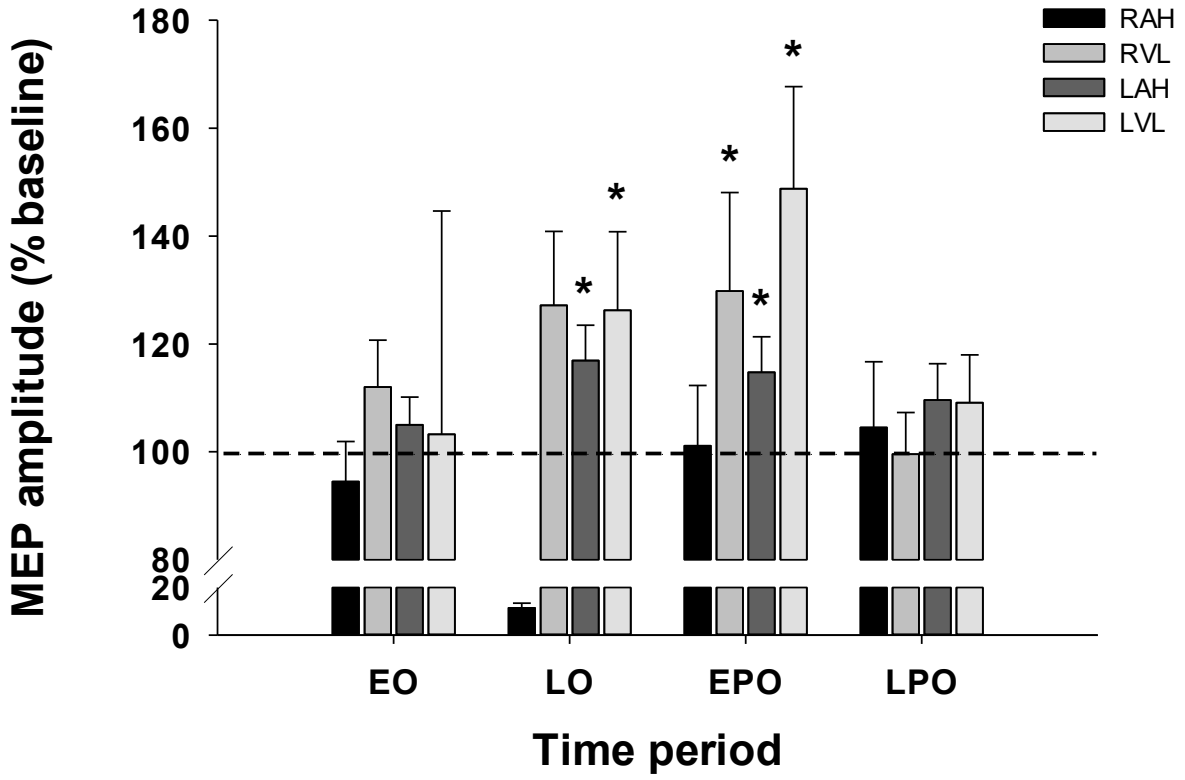


*Figure 32: Mean (±SEM) MEP amplitudes as percentage of baseline (BL) for left vastus lateralis (LVL), left abductor hallucis (LAH), right vastus lateralis (RVL) and right abductor hallucis (RAH), during early occlusion (EO), late occlusion (LO), early post-occlusion (EPO) and late post-occlusion (LPO) time periods. * denotes a significant increase compared to baseline for that muscle, P<0.05).*

Pain

Pain scores increased during the occlusion, peaking at a mean of 5.3 at 45 minutes (P<0.01; see Figure 33).

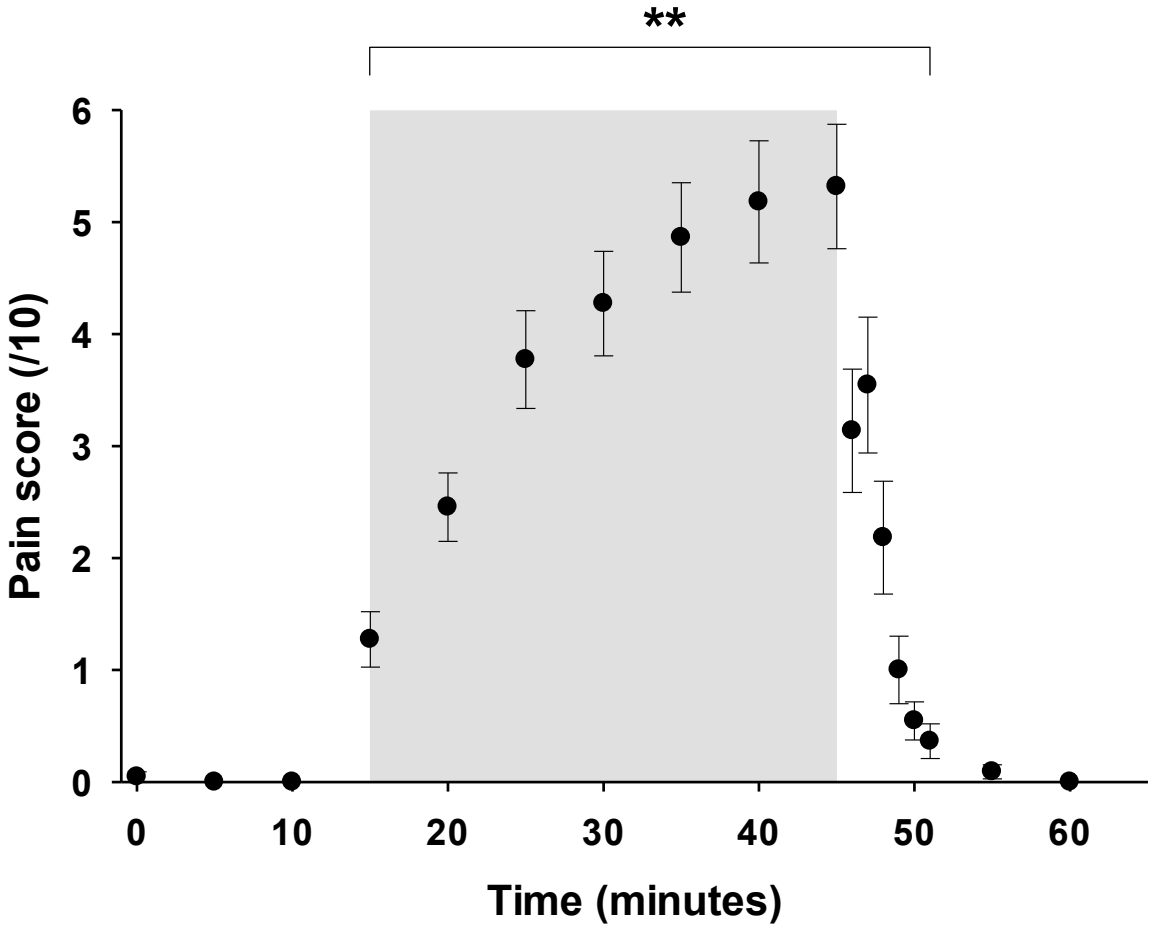


*Figure 33: Mean (±SEM) pain scores (out of 10) during experiment 1. Shaded area represents duration of INB. ** denotes significantly different from baseline of pain scores from 15 to 51 minutes, as indicated by the bracket (P<0.01).*

Further analysis attempted to classify individuals based on those who experienced high or low pain. Higher pain scores (score of 6 to 10) at 45 minutes appear to be associated with greater increases in MEP amplitude from baseline compared to those with lower pain scores (P<0.05; Figure 34).

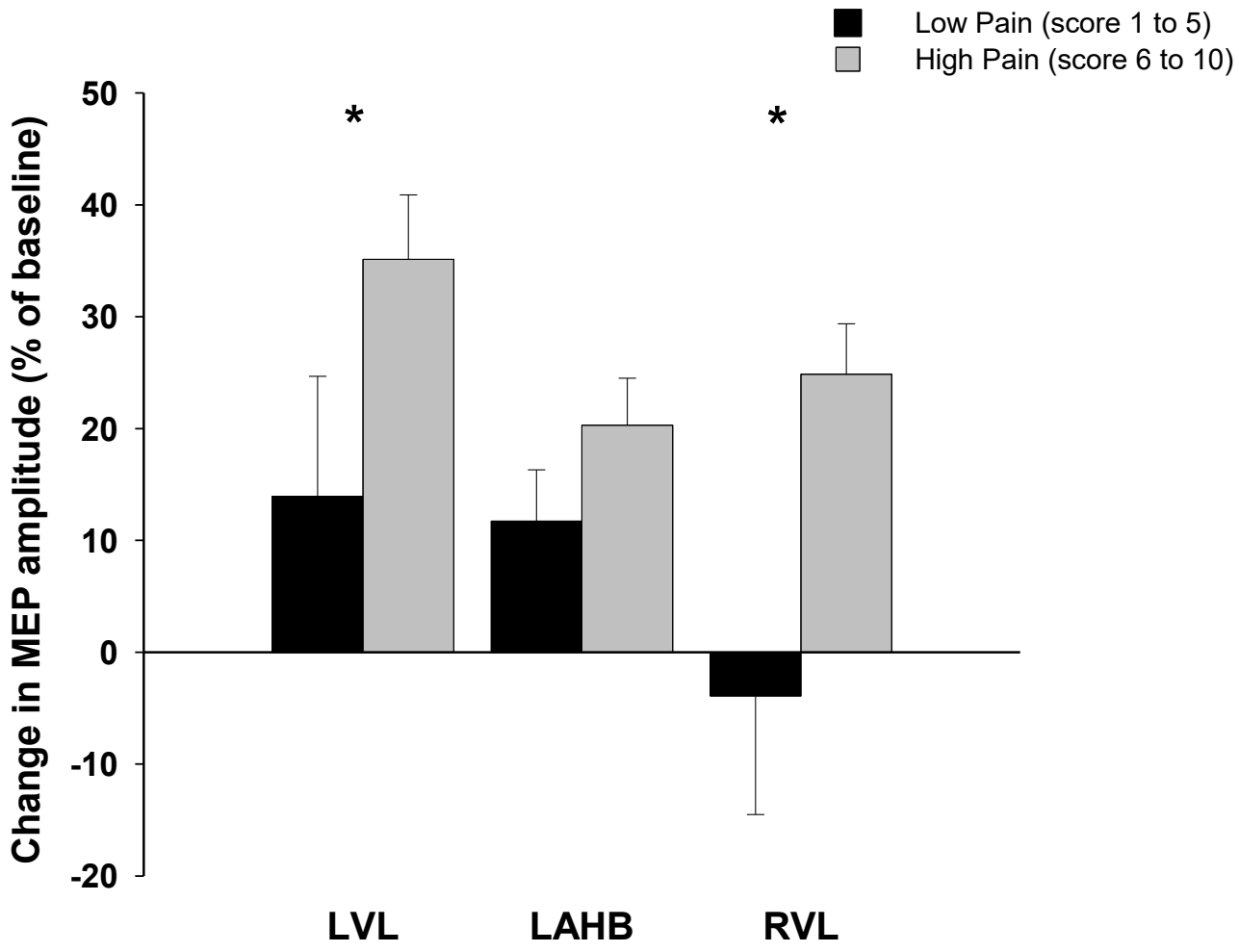


*Figure 34: Mean (±SEM) MEP amplitudes at the end of occlusion (45 minutes) as a percentage of baseline for left vastus lateralis (LVL), left abductor hallucis (LAH) and right vastus lateralis (RVL), grouped according to average low pain (1 to 5) (black bars) or high pain (6 to 10) (grey bars). * denotes a significant difference between low and high pain (P<0.05).*

Somatosensory evoked potentials

Data from 19 participants were analysed. The representative traces in Figure 35 illustrates a reduction but not complete disappearance of SSEPs during late occlusion (SSEP trace from 45 minutes shown as LO).

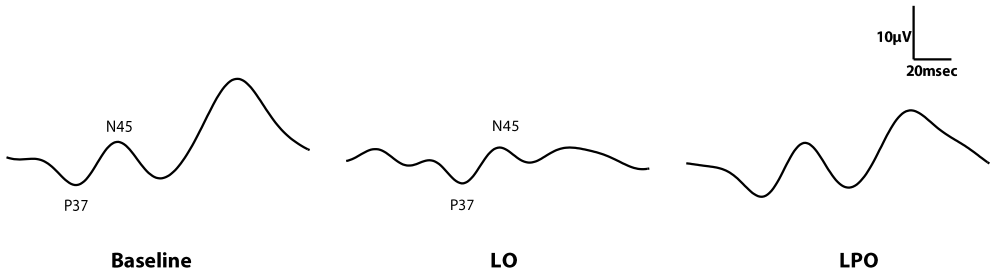


Figure 35: Single participant data for averaged SSEP amplitudes following tibial nerve stimulation at baseline, during late occlusion (LO) and late post occlusion (LPO). The traces begin at the time of nerve stimulation.

There was an initial non-significant increase in mean peak-to-peak amplitude of 23.4% (± 10.7%) followed by a significant decrease to 36.6% (± 8.9%) of baseline by the end of the occlusion period (P<0.01; see Figure 36). This was followed by recovery back to baseline levels at 50 minutes. Note that no measurements were taken at 46-49 minutes, due to the time constraints of consecutive TMS trains.

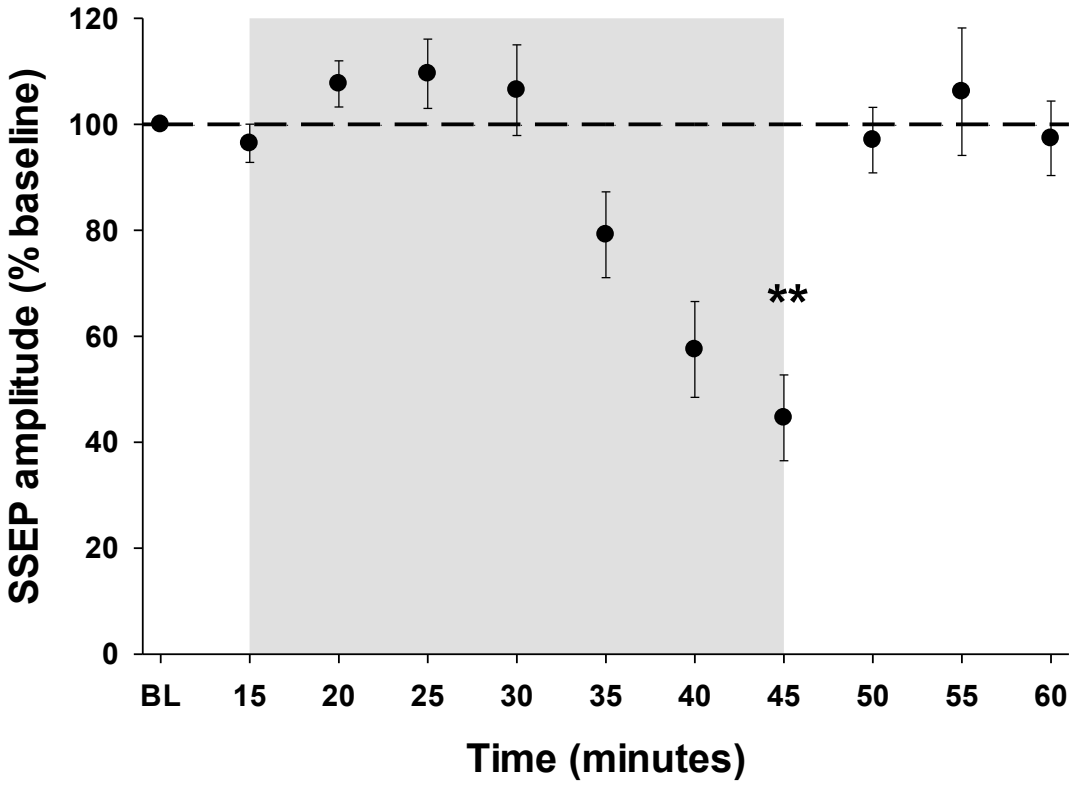


*Figure 36: Mean (±SEM) SSEP P37-N45 peak-to-peak amplitudes as percentage of baseline, following right tibial nerve stimulation. ** denotes significance at 45 minutes compared to baseline (P<0.01).*

Near infrared spectroscopy

Figure 37 shows the average pooled nominal values of regional oxygen tissue saturation (rO_2) as measured by near-infrared spectroscopy from the distal right calf muscle. Calf rO_2 started at a mean of 70% and progressively decreased throughout the duration of the INB ($P<0.01$) , reaching a minimum of 20% at 30 minutes of ischaemia (45 mins on figure 37). It should be noted that rO_2 was significantly lower during and immediately following ischaemia, until 51 minutes, due to the time taken for adequate reperfusion after cuff release. The rO_2 was subsequently significantly higher from 52.5 to 57.5 minutes due to limb hyperperfusion secondary to vasodilation from the metabolites generated within the ischaemic limb.

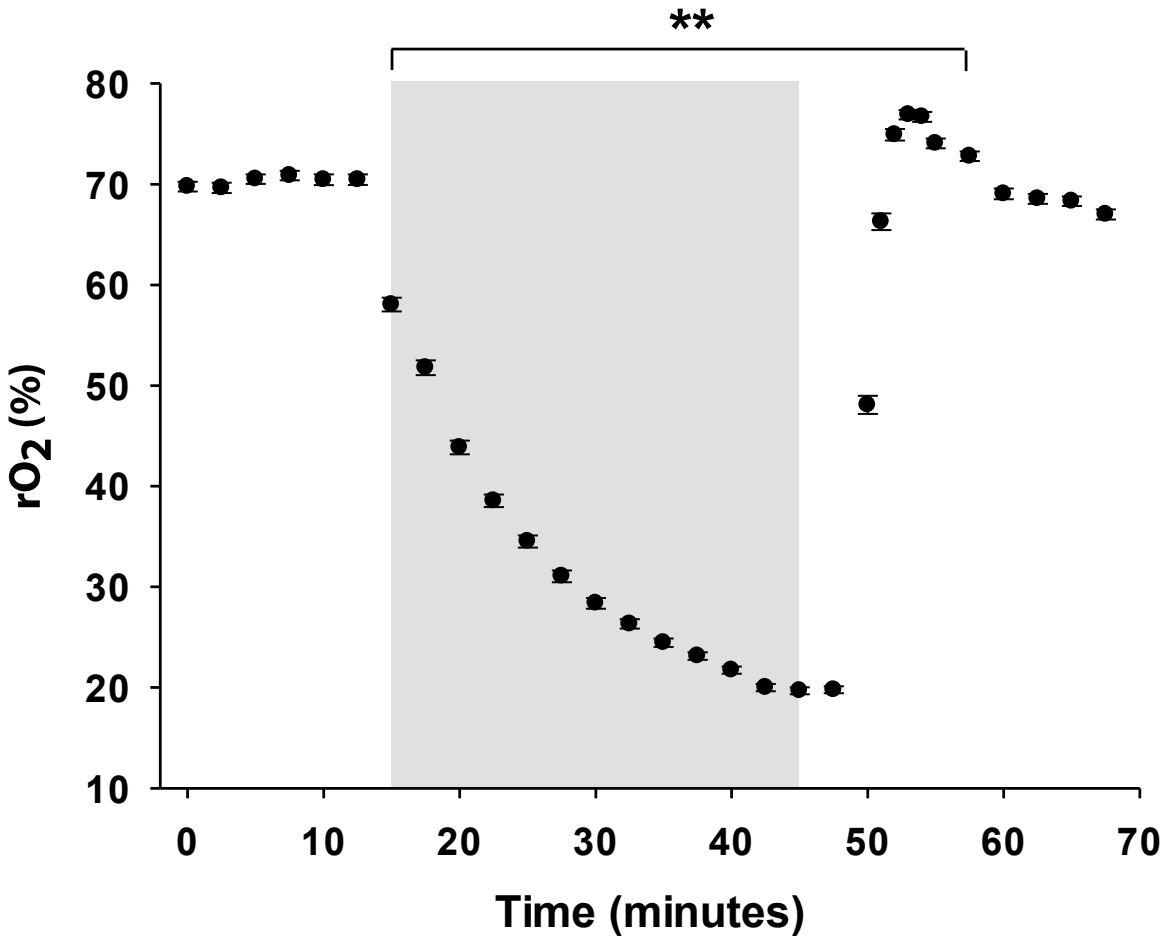


*Figure 37: Mean (±SEM) rSO_2 near infrared spectroscopy for the right calf. Shaded area represents time of INB. ** denotes significant difference compared to mean baseline values, between 15 and 57.5 minutes ($P<0.01$)*

Effectiveness of Low-Pressure Ischaemic Nerve Block

MEPs from the right AH, SSEPs and rO_2 were measured in this experiment to assess the effectiveness of a low-pressure model for an ischaemic nerve block of the lower limb. Figure 38 demonstrates the temporal relationship between these measures. Both MEPs and SSEPs degenerate at this lower pressure, and there is progressive reduction in calf rO_2. Thus, deafferentation and deefferentation occur in the presence of progressive tissue ischaemia, confirming successful ischaemic nerve block.

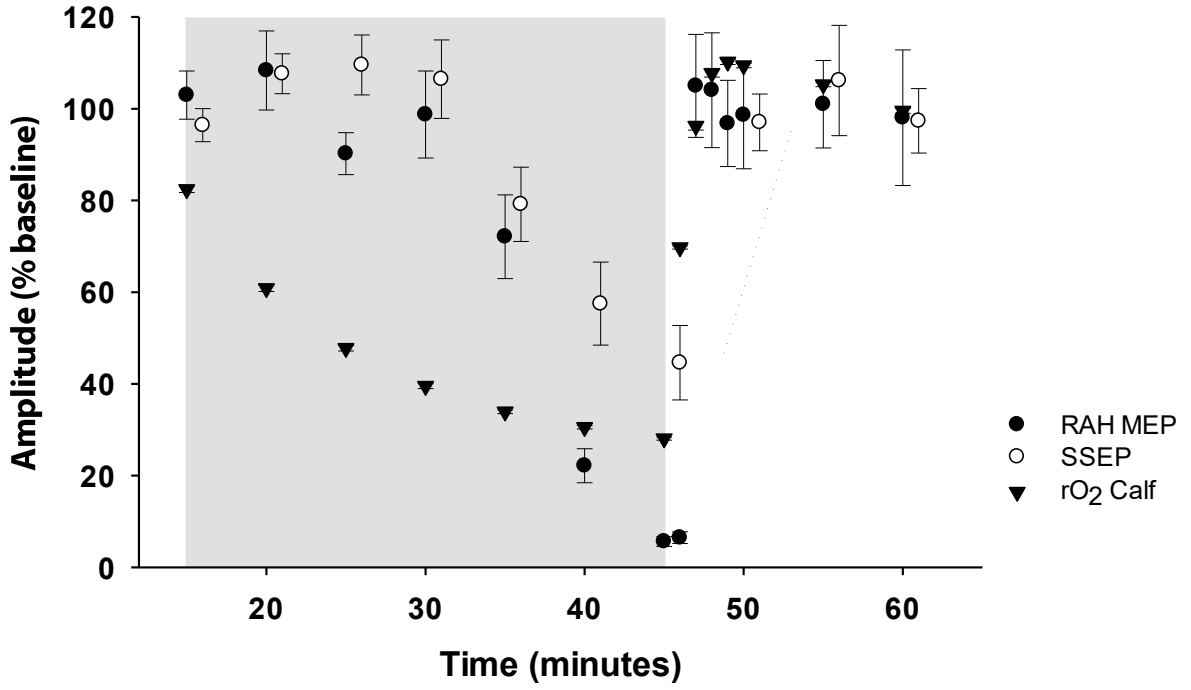


Figure 38: Temporal relationship of right abductor hallucis brevis (RAH) MEPs, right tibial nerve SSEPs and calf muscle regional oxygenation (rO_2) for the assessment of the effectiveness of the low-pressure ischaemic nerve block model. Values are normalised to baseline for each modality and plotted against time. SSEP data adjusted by +1minute for graphical clarity. Data are presented as mean (±SEM). Grey shaded area represents time of INB.

Experiment 2

This protocol was performed after confirming adequate distal motor blockade using the low-pressure INB in experiment 1. As a result, measurement of right AH (occluded) MEPs was not performed. Data represented is the mean data from 13 participants.

Figure 39 shows the change in mean MEP amplitudes in the four muscles over the course of the experiment.

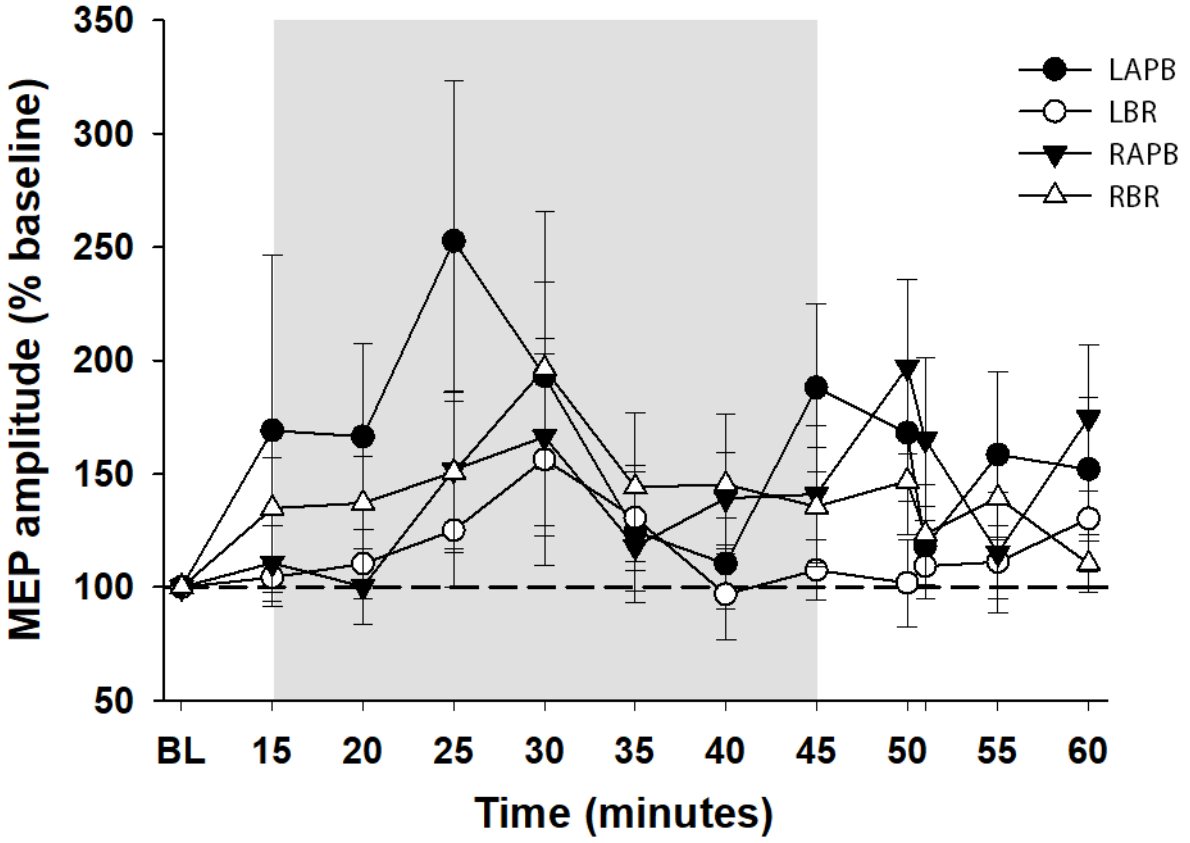


Figure 39: Mean (±SEM) MEP amplitudes as percentage of baseline (BL) for left abductor pollicis brevis (LABP), left brachioradialis (LBR), right abductor pollicis brevis (RABP) and right brachioradialis (RBR), during experiment 2. Grey shaded area represents time of INB.

When data are collapsed into the time periods, there was a significant increase in left APB amplitude during EO and LO, of 181.2% and 155.5%, respectively, compared to baseline ($P<0.05$). No significant changes were observed for LBR, RAPB and RBR (see Figure 40).

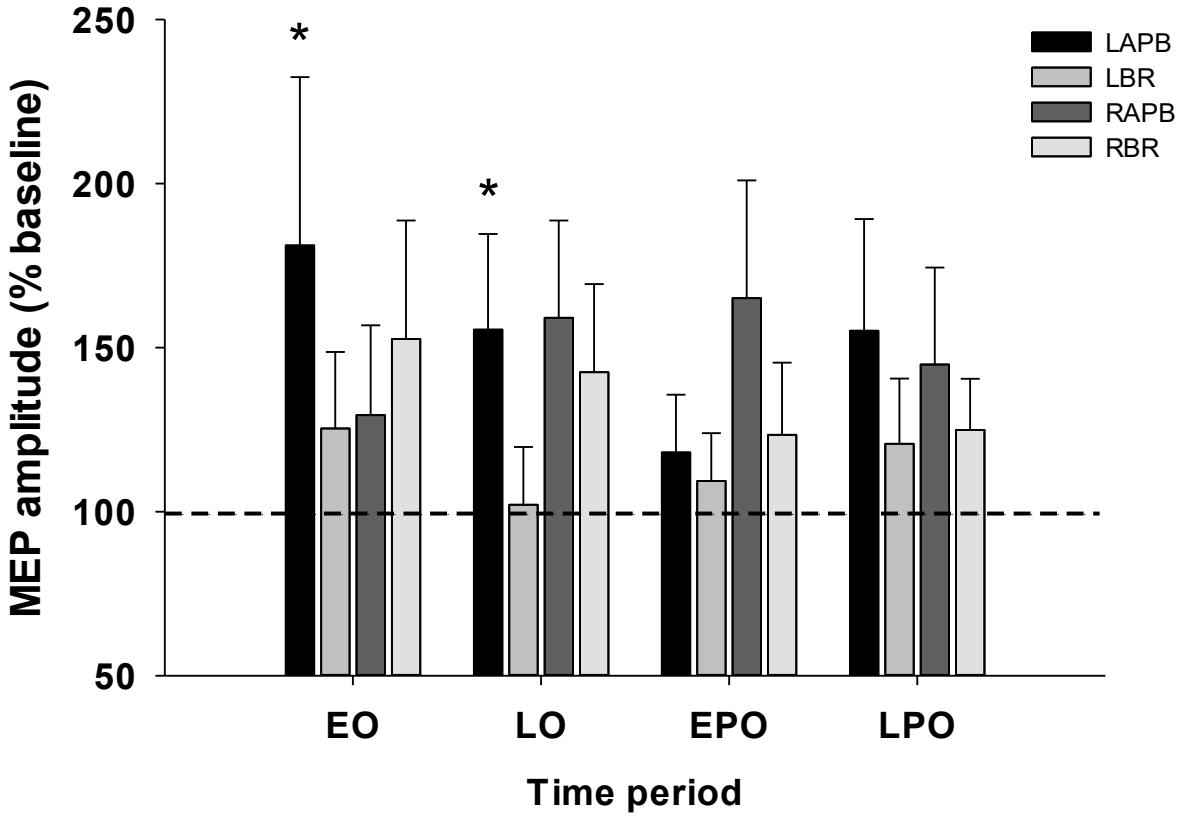


*Figure 40: Mean (±SEM) MEP amplitude as percentage of baseline for left abductor pollicis brevis (LABP), left brachioradialis (LBR), right abductor pollicis brevis (RABP) and right brachioradialis (RBR), during early occlusion (EO), late occlusion (LO), early post-occlusion (EPO) and late post-occlusion time (LPO) time periods. * denotes a significant increase in LAPB compared to baseline during the EO and LO period (P<0.05).*

Discussion

The results of this experiment have shown that a low-pressure model for an ischaemic nerve block is successful at producing distal deafferentation and deefferentation. When applied to the lower limbs, this INB can initiate changes in cortical excitability which in turn lead to an increase in motor evoked potentials from proximal and contralateral lower limb muscles, and distant upper limb muscles. These changes appear to be initiated without complete loss of afferent input. There was a trend for higher pain being associated with greater increases in MEPs of proximal and contralateral muscles. The neurophysiological mechanisms and the implications of this for a TMS-based IONM will be discussed further.

Low-Pressure Ischaemic Nerve Block

Previous studies employing an ischaemic nerve block have used inflation pressures in excess of 200mmHg (63, 80, 87, 176). It is likely that at such pressures, acute nerve compression occurs, which can impede nerve conduction and reduce compound muscle action potential amplitudes (177), but also cause significant pain. The traditional model described in the literature is indeed effective, as their main end point of nerve conduction blockade is achieved. A recent study employed an INB model where inflation pressures equivalent to mean arterial pressure were used. Although this used lower pressures than our model, and achieved ipsilateral deafferentation and deefferentation, contralateral MEP changes were not seen (178). This modified INB was piloted on the legs, but it did not provide an adequate motor block, whilst the standard pressures (200-250mmHg) with the traditional model induced intolerable levels of pain. Therefore, a revised model with minimal inflation pressures but capable of producing an adequate motor blockade was developed.

The results have shown that tissue oxygenation (rO_2) reduced distal to the site of an INB. Use of near infrared spectroscopy is becoming more widespread in the medical field, as it offers a reliable non-invasive measure of tissue oxygen saturation. It is particularly validated in cardiac surgery to measure brain frontal lobe oxygenation, where improved oxygenation has been associated with reduced patient morbidity (89, 179). Visually we could see that the low-pressure INB was causing tissue ischaemia, and NIRS provided objective confirmation. Baseline rO_2 was approximately 70% in our study and reflects a combination of the non-pulsatile arterial and venous component of blood present in tissue (90) and falls to 15-20% at peak occlusion. This is in keeping with previous studies where peripheral limb hypoperfusion in critical limb ischaemia is observed (94, 180).

MEPs and SSEPs were seen to diminish in the late occlusion period. Many studies exploring cortical excitability using a high-pressure INB frequently omit distal MEP measurements, since it was known from previous work in the literature that the traditional INB would be successful. Since a novel lower-pressure lower limb INB was employed, distal MEPs were measured to confirm successful INB. The temporal relationship between right AH MEP, tibial nerve SSEP and calf rO_2, which all decreased

together, provides further evidence that a low-pressure model can cause motor and sensory nerve blockade.

The purpose of developing a low-pressure INB was to better experimentally model the ischaemia seen surgically during a TAAA repair. When performed as an open procedure, an arterial clamp is used intra-operatively, or an intra-arterial occlusive balloon is inflated within an artery, such as an iliac artery. This subsequently produces limb ischaemia, but without any external nerve compression and the associated pain, and therefore an INB unlike that seen with traditional models. Reproducing this surgical INB in the laboratory setting is not possible, hence a model minimising the pain and compression from a tourniquet was developed to reduce their impact on neurophysiological measures. The low-pressure INB could then be employed in further healthy volunteer experiments to interrogate the neurophysiological consequences of ischaemia and deafferentation. As discussed above, the trans-synaptic activation of TMS-induced MEPs makes them susceptible to changes in cortical excitability, which in turn is influenced by deafferentation. Therefore, before TMS can be used as an IONM of SCI in TAAA surgery where limb ischaemia is observed routinely, further laboratory testing is required.

Cortical Excitability

The results of this study support and extend findings in the current literature. Previous work focusing on the upper limb has found the MEPs of muscles proximal to an ischaemic nerve block increase but MEPs in homologous contralateral limb muscles have been shown to either increase (87) or remain unchanged (181). In the limited work where the lower limb has been studied (176), this excitability is seen in ipsilateral proximal thigh muscles and homologous contralateral thigh muscles; it did not extend to the contralateral foot muscle. The data has demonstrated that following successful low-pressure INB of the leg, proximal and contralateral thigh and contralateral foot muscle MEPs increase. However, the excitability changes appear to extend to different body part representations and is not topographically specific as seen in previous studies (87). The left APB MEPs increased significantly

during early and late occlusion, with the remaining arm muscles displaying a trend towards increasing. These results are consistent with a general increase in motor cortical excitability (59, 80).

Increased cortical excitability is believed to arise secondary to the loss of afferent input; this deafferentation consequently leads to a reduction in inhibition within the cortex (181) The mechanisms are thought to be cortical in origin, since responses from stimulation at the level of the brain stem (87), spinal cord (182) or of the peripheral nerves (80) following ischaemic deafferentation are not altered. The rapid timescale over which the changes occur also implies alterations to existing neuronal circuits must occur, rather than a structural change in cell populations, which would occur after two or more days (183). The disinhibition of the cortex is believed to be mediated by loss of GABAergic input, based upon previous imaging, pharmacological and paired-pulse TMS studies (87, 184, 185). The mechanisms underlying interhemispheric inhibition however, along with the role of other intracortical interactions, are not fully understood (186-188). Further, it has been shown that cortical plasticity is mediated by increased glutaminergic transmission, new axonal connections and long-term potentiation in regions where GABAergic inhibition is reduced in more chronic deafferentation, such as those seen in amputees (189).

If the stimulus initiating the increase in motor cortical excitability is the loss of afferent input, or deafferentation, then it should follow that a loss of SSEPs preceeds an increase in MEP amplitude. However, the data from this experiment do not support this hypothesis: increases in MEP amplitudes occurred in the late occlusion period without loss of SSEPs – SSEP amplitude decreases to only 36.6% of baseline at peak occlusion. The data therefore suggest partial deafferentation is adequate as the stimulus.

SSEPs provide information about afferent transmission from the periphery via the dorsal column-lemiscal system to the sensory cortex following stimulation of a peripheral nerve, with its many components reflecting signal propagation through sequential structures of the sensory pathway (190-192). They do not provide information about individual sensory modalities directly since all sensory fibres in a mixed nerve are activated by the supramaximal electrical nerve stimulus (193). The P37, N45 and P60 peaks are cortical in origin as evident by their long latency (193). The P37-N45 peak-to-peak amplitude of the SSEP waveform measured in the current study, is a commonly measured parameter in clinical electrophysiological studies (171); short latency components are less consistently reproduced (191). Ischaemia differentially affects nerves, with large, myelinated fibres being more susceptible to its effects than smaller unmyelinated fibres (194, 195). Given the reporting of a "dull ache" in the distal ischaemic leg by the participants alongside increasing pain scores (one would expect the scores to fall once a partial or full INB is achieved), this would support the idea of preservation of C-fibre function. It has been shown that during ischaemia of a peripheral limb, short latency components are lost first, with long latency components mostly preserved. (196). This occurs over 24-30 minutes, a similar timescale to the current study (196, 197). Therefore ischaemia-sensitive myelinated afferent fibres lose their function due to ischaemia, evidenced by the loss of short latency components of the SSEPs. The short latency components were not measured with the EEG montage employed in this study. However, the near complete loss of MEPs indicates the function of large, myelinated motor fibres was lost, and therefore one can assume myelinated afferent fibres would be similarly affected. The SSEP data show a reduction, rather than a complete loss, of the P37-N45 amplitude over 30 minutes of ischaemia. This is in keeping with using a lower pressure INB, as data in the literature where previously a complete loss of the SSEP waveform has been reported, was following the traditional higher pressure INB (197-200). There is likely to be direct nerve compression (177), confounded by greater pain. These results suggest a loss of function of larger, myelinated A fibres, with relative preservation of smaller fibres (Aδ and C) is adequate to unmask latent excitatory pathways and initiate cortical excitability changes in non-occluded muscles; via a partial rather than

complete deafferentation. Therefore, selective block of fibre types may reveal the contributions to changes in cortical excitability.

It has also been proposed that there is a role for 'deefferentation' in inducing changes in cortical excitability alongside changes in afferent input (81, 181). Rat models where a motor facial nerve transection was performed, have shown expansion of forelimb and eye-eyelid motor territories into adjacent vibrissae representations; a change occurring rapidly over hours (201, 202) but persisting for months (203). Similarly, human studies in patients with idiopathic peripheral facial nerve palsy, a similarly pure motor lesion, have shown that deefferentation alone leads to considerable cortical remapping in sensorimotor cortices (204). This INB paradigm was not designed to, nor able to, inhibit the motor or sensory system in isolation but did demonstrate that deefferentation and deafferentation were present, suggesting the close interplay between the sensory and motor systems may play a role in cortical excitability.

Effect of pain

The participants in this study experienced increasing pain as the occlusion progressed. This was both distal to the tourniquet, as well as under the cuff itself, despite minimising the occlusion pressure used.

The effects of pain on the motor system during an INB remain unclear. There is conflicting evidence to suggest that pain can both inhibit and enhance motor excitability (170, 205-212). MEPs from muscles proximal to the induction of pain with hypertonic saline for example, have been shown to both increase and decrease in amplitude, changes which are cortically mediated (213, 214). The literature is similarly conflicted with regards to changes in cortical circuitry which mediate changes in excitability in response to pain. Immediately following a noxious stimulus, there is an increase in short interval intracortical inhibition (SICI) and a decrease in intracortical facilitation (ICF), manifesting as reduced MEP amplitudes (215). It has been suggested following a pain stimulus the somatosensory cortex inhibits the motor cortex bilaterally, possibly in an effort to limit movement of a painful body

part and limit further damage (209, 216). Over time however, there is a reversal of this inhibition and SICI is reduced, whilst ICF is increased, perhaps reflecting an attempt to improve motor function in the long-term and promote regaining of lost function (217, 218). What the literature does agree upon however, is that there is a complex interplay between the duration of pain, type of muscle studied with regards to its function (for example, extensor versus flexor) and the mechanism of pain induction (219).

The experimental paradigms showed a greater experience of pain was associated with greater increases in MEP amplitudes compared to less participant pain. This may be a protective mechanism, whereby the increased perception of pain leads to increased cortical excitability to proximal muscles, to facilitate a greater flexion response and therefore withdrawal of the limb (220, 221). In keeping with these results, others have found that pain augments the excitability to proximal muscles seen following an INB of the forearm (222). The increases in excitability observed in the contralateral muscles may have also been influenced by the pain, as it has been demonstrated that pain can reduce interhemispheric inhibition in experimental pain models (218).

Limitations

Whilst the likely interference of pain in these data has been discussed, previous research has utilised far higher tourniquet pressures (80). It was attempted in this nerve block model to maintain ischaemia with as low a tourniquet pressure as possible to minimise pain. However, participants still reported varying levels of pain and our results suggest this had an impact on the changes in cortical excitability.

A single test intensity for TMS was used throughout the experiment, being 1.2 x MT for the muscle with the highest threshold. Hence, for 3 out of 4 muscles, this test intensity would have been greater than of MT. In order to generate MEPs and SSEPs in the narrow 5-minute interval for measurements,

it was not possible to use muscle-specific test intensities. The cone coil is also more reliable at generating lower limb MEPs, especially where a single intensity is used. Single intensities have been used previously in experiments of this nature (87).

Conclusion

This study has shown that corticospinal excitability to muscles ipsilateral, contralateral and distant to a novel low-pressure nerve block increased, to varying degrees, following 30 minutes of lower limb ischaemia. The incomplete loss of SSEPs with concurrent increases in MEP amplitude, suggest that complete deafferentation is unlikely to be required for the changes in cortical excitability to be evoked. It is plausible that selective deafferentation of larger myelinated fibres in the occluded limb is sufficient to initiate this. Pain likely influenced the amplitude of the MEPs despite the use of a lower pressure INB which was designed to minimise pain from direct nerve compression.

In the development of intra-operative neuromonitor of SCI, where TMS is employed to interrogate the function of the spinal cord, it is essential to elucidate the changes in cortical excitability induced by progressive limb ischaemia. To detect a possible injury, how baseline MEP characteristics evolve secondary to alterations in cortical excitability need to be determined first. This study has modelled the potential changes that could be seen and has furthered the development of such a monitor.

Chapter 6: An investigation of deafferentation induced by ischaemic nerve block

Introduction

It is well established in the literature and in the previous investigation (see chapter 5) that short term and rapid changes in corticospinal excitability can be initiated using an ischaemic nerve block (INB) (80, 87). The deafferentation by the INB is believed to initiate changes within the motor cortices which augment the neurophysiological and functional characteristics of muscles both proximal and contralateral to the INB (223).

The intracortical mechanisms responsible for the increased excitability are believed to include disinhibition of $GABA_A$-mediated interneurons, with deafferentation of peripheral sensory nerves and subsequent reduced sensory input being responsible for initiating the process (87). As discussed in the previous chapter, some sensory function, as measured by the persistence of somatosensory evoked potential (SSEPs), remained during a 30-minute INB, whilst proximal and contralateral muscle motor evoked potentials (MEPs) increased. It would seem therefore that only partial loss of sensory function was adequate for cortical excitability to occur (224).

SSEPs can be generated following the stimulation of a mixed peripheral nerve and allow assessment of sensory function from the periphery to the sensory cortex, with the peaks and troughs reflecting different constituent parts of the pathway. They are not however, able to provide assessment of different nerve types and sensory modalities (225). Quantitative sensory testing (QST) allows interrogation of the function of individual nerve fibre types, and therefore specific somatosensory modalities, using a battery of non-invasive tests (226). It is a standardized method for objectively assessing somatosensory thresholds and can identify increased or decreased sensory function in clinical pain states or experimentally in human surrogate pain models (227). Therefore, QST may be

able to provide further mechanistic insight into the INB-mediated deafferentation thought to increase cortical excitability.

Aim

The aim of this study is to explore the contribution of specific fibre types to the development of INB-induced cortical plasticity using a truncated QST paradigm.

Methods

Participants

Participant were recruited as per described in Chapter 2: Participant Recruitment.

16 healthy adult participants (male: female 9:7; mean (±SD) age 21.8 (±0.3) years) were recruited.

Pre-experiment blood pressure assessment

Blood pressure and subsequently the experimental INB pressure (systolic blood pressure + 20-30mmHg) was determined as per Chapter 2: Ischaemic Nerve Block.

Surface EMG

All participants were instructed to recline at 45° on a physiotherapy table. Pairs of disposable Ag/AgCl electrodes applied on the skin overlying the brachioradialis and abductor pollicis brevis muscles bilaterally, parallel to the muscle fibre orientation (see Figure 41). A ground electrode was placed over the left olecranon process.

For greater detail, see Chapter 2: Electromyography Acquisition.

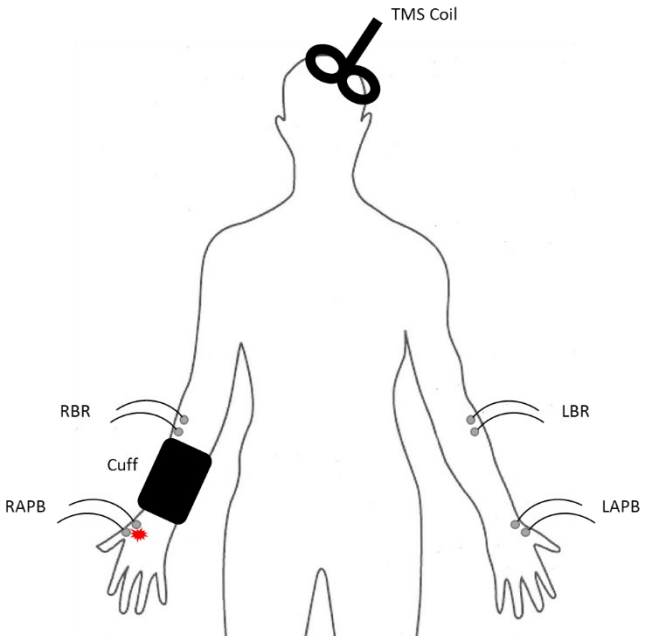


Figure 41: Experiment set. TMS Coil used was the 'figure-of-8' coil. RBR = right brachioradialis, RAPB = right abductor pollicis brevis, LBR = left brachioradialis, LAPB = left abductor pollicis brevis. The red star on the right hand represents the area where sensory testing was performed.

Motor evoked potential

The hotspot for the 4 muscles was determined and TMS was applied at 1.2 x RMT to the motor cortex at the hotspot for each corresponding muscle using a Magstim 200^2 stimulator connected to a figure-of-8 coil (The Magstim Company Ltd., Dyfed, UK); for greater methodological detail, see Chapter 2: Transcranial Magnetic Stimulation.

MEPs were analysed as the average of 6 for each muscle at each time point, and the peak-to-peak amplitude was measured. For further details, see Chapter 2: Electromyography Acquisition and Chapter 2: Analysis of TMS induced MEPs, for greater detail of MEP sampling and then analysis.

MEPs are expressed as a percentage of baseline for further analysis, where baseline is the average of pre-occlusion amplitudes and expressed as 100%.

Quantitative Sensory Testing (QST)

A truncated QST paradigm was used in this investigation. Due to the short time interval to complete each set of measurements (see Experimental Protocol below), tests were selected to interrogate distinct nerve fibre types, from small unmyelinated fibres to large, myelinated fibres. Dynamic sensation (light touch), punctate sensation (pin prick) and heat pain detection thresholds were tested in this experiment, corresponding to the function of Aβ, Aδ and C fibres respectively (228). Each test was performed after the TMS trials at each testing epoch.

Participants were seated comfortably in a quiet room, to avoid distraction.

Dynamic sensation was measured by applying a standardised brush (200-400mN per stroke; Somedic, Sweden)(226). The brush stroke was applied over an area of approximately $4cm^2$ to the proximal, palmar thenar area of the right hand (corresponding to dermatome C6), adjacent to the EMG electrodes for TMS measurements.

Punctate sensation was measured using a 512mN weighted pin prick stimulator (MRC Systems GmbH, Germany). Both were tested 5 times over same area as above and were preceded by a single stimulus on the contralateral hand for reference.

Heat pain threshold (HPT) was tested using a thermal sensory testing device (TSA 2001-II, MEDOC, Israel). The HPT is the point at which the perception of temperature changes from ''warm'' or ''hot'' to an additional impression of a ''burning'' or ''stinging''. The thermode was positioned on the C6 skin area lateral to the electrodes as above. The temperature of the thermode increased from 32°C at a rate of 1.5°C/sec, to maximum of 50.6°C; the participant indicated when the sensation changed to painful at which point the ramping up of the temperature was terminated by pressing a mouse button. Five HPTs were measured at each time point.

Pain score

Participants were asked to rate the level of pain they felt, from 0-10, directly under the BP cuff at each time point (see Chapter 2: Pain scores).

Experiment Protocol

With participants were reclined on a physiotherapy couch, pre-stimulus EMG amplitude was measured to ensure adequate relaxation. There were 8 testing time points throughout the protocol (see Figure 42). At each time point, 6 TMS stimuli were delivered to the 4 pre-marked scalp locations corresponding to the muscle hotspots, at rest. This was followed by the QST paradigm as described above. For dynamic and pinprick sensations, one stimulus was also delivered to the participant's contralateral hand (non-ischemic hand) as a reference for normal. Symmetry of sensation between the hands was determined before starting. Following each stimulus to the right (occluded) hand, the participant was asked to give a score on a scale of 0 – 10 (a score of 10 indicated no change in sensation and the same as the control hand; 0 indicated a complete loss of sensation to the stimulus). Where the participant experienced an exaggerated response to brush stroke or pinprick, a score of 10+ was recorded. Heat pain threshold was measured after tactile and pinprick was performed and recorded using the Medoc software as described above.

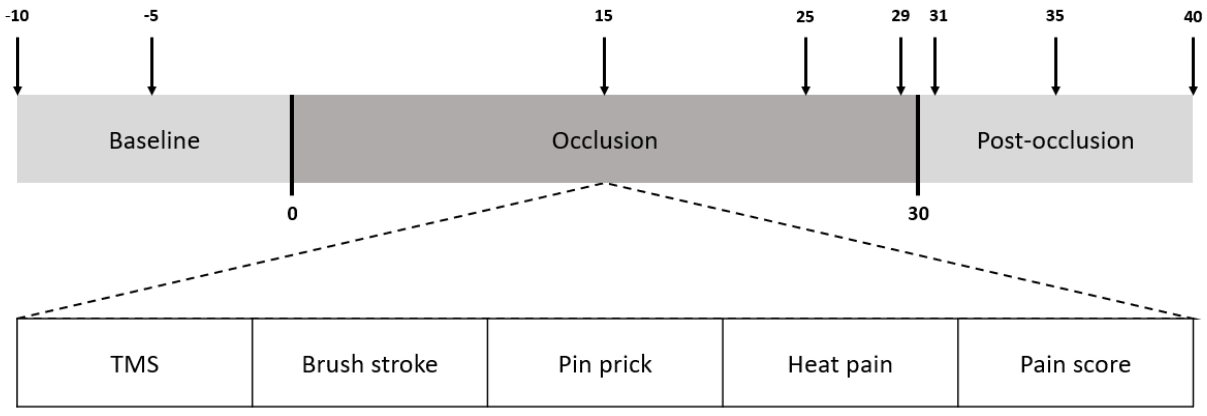


Figure 42: Experimental protocol diagram. Shaded box (top) showing the 3 phases of the experiment (baseline, occlusion and post-occlusion) with the black arrows showing the time (in minutes) at which testing occurred, with respect to the occlusion period. The white box (bottom) shows which tests were performed at each time point

Two baseline sets of tests (-10 and -5 minutes) were obtained. The cuff was then inflated for 30 minutes at the pre-determined pressure for each participant. 3 sets of measurements were carried out during the occlusion (at 15, 25 and 29 minutes). After deflation, a further 3 sets of post-occlusion measurements were performed at 1, 5 and 10 minutes after the cuff was released (discussed further as timepoints 31, 35 and 40 minutes).

Data Analysis

Each set of 6 stimuli were averaged to produce one MEP waveform in Signal v5.12 software (CED, UK) and the peak-to-peak amplitude was measured at each time point for each muscle.

A mean baseline (BL) MEP amplitude was calculated by averaging the 2 measurements from pre-occlusion (-10 and -5 minutes). All subsequent MEPs were normalised to the mean baseline value. MEPs for all muscles were grouped into three time periods: mid-occlusion (MO) is the MEPs recorded at 15 minutes; late occlusion (LO) is an average of MEPs at 29 and 31 minutes (there was no statistical difference between these; (P=0.608)) and post-occlusion (PO) is an average of MEPs at 35 and 40 minutes (also no difference; (P=0.585)).

A mean was calculated from 3 stimuli for dynamic and punctate sensation (the first 2 readings are not included, as is standard procedure) and from 5 heat pain thresholds at each measurement timepoint. The heat pain thresholds were also normalised to the mean baseline. Sensory testing scores were also grouped into time periods. BL scores are an average of -10 and -5 minutes and MO scores are measurements taken at 15 minutes only, as with MEPs. Light touch and pinprick pain scores were significantly lower at 29 minutes compared to 31 minutes ($P<0.05$), however. Therefore, LO for sensory testing includes mean scores from only 29 minutes as reperfusion of the hand at 31 minutes was thought to have a significant effect on sensation; 31minute scores are therefore omitted. PO

scores are an average of 35 and 40 minutes as they were not statistically different for light touch (P=0.912) or pinprick (P=0.600).

Microsoft Excel (Microsoft, USA) was used for data storage and processing. All data are presented as the mean ± SEM.

Statistical analysis

Data were tested for normality using the Shapiro-Wilk statistical test. Repeated measures ANOVA or Friedman tests with appropriate post-hoc tests were used to test for differences.

To enable correlation analysis, the punctate sensation scores where the participant experienced exaggerated pain were arbitrarily converted to a score of 12.

Statistical significance was set at $P<0.05$ and adjusted for multiple comparison testing as appropriate with Bonferroni's correction.

Results

Normalised MEP Amplitudes

Figure 43 shows the normalised RAPB MEP amplitudes (distal to INB) decreased as the occlusion progressed. This was significant at 29 and 31 minutes ($P<0.01$).

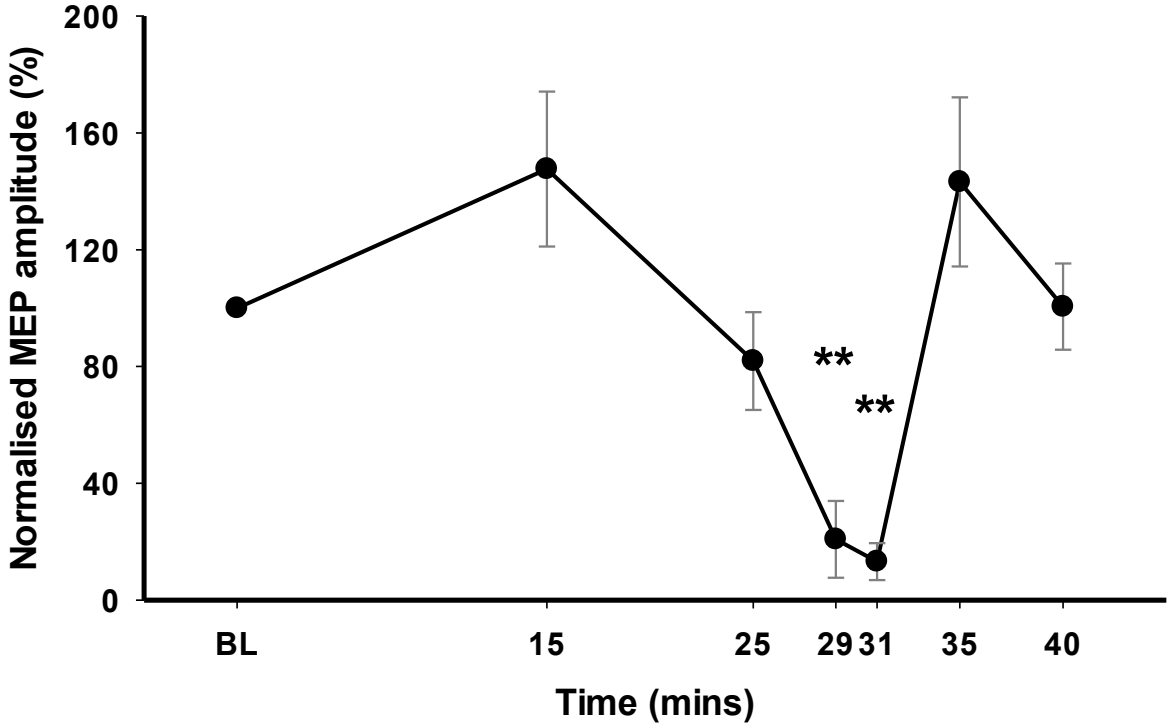


*Figure 43: Normalised RAPB MEP amplitude. Shaded area represents the occlusion period. Data expressed as mean ± SEM. ** denotes significance at 29 and 31 minutes compared to baseline (BL) (P<0.01).*

When data were grouped into the different time periods, the decrease at LO was significant (P=0.002) (see Figure 44).

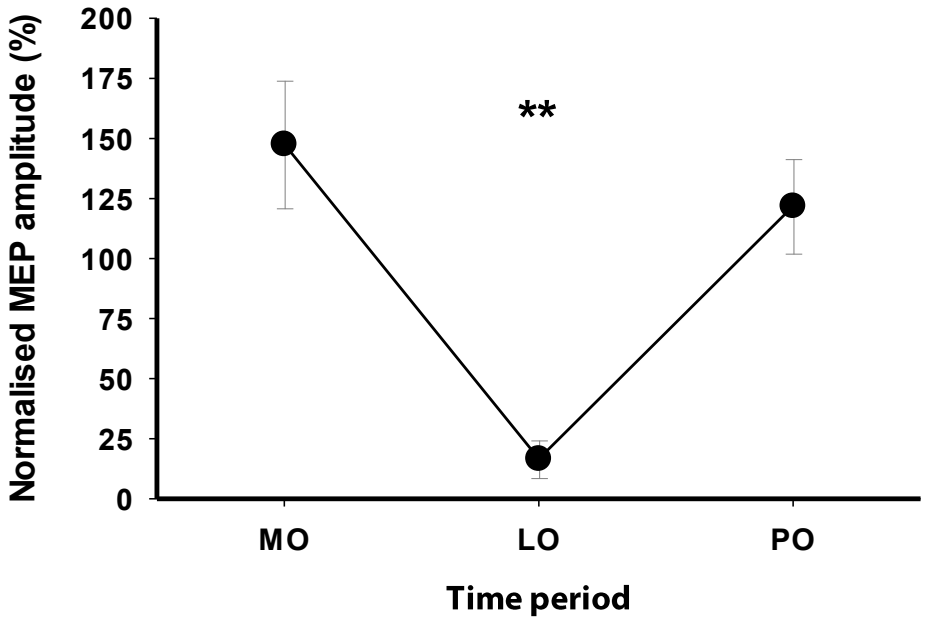
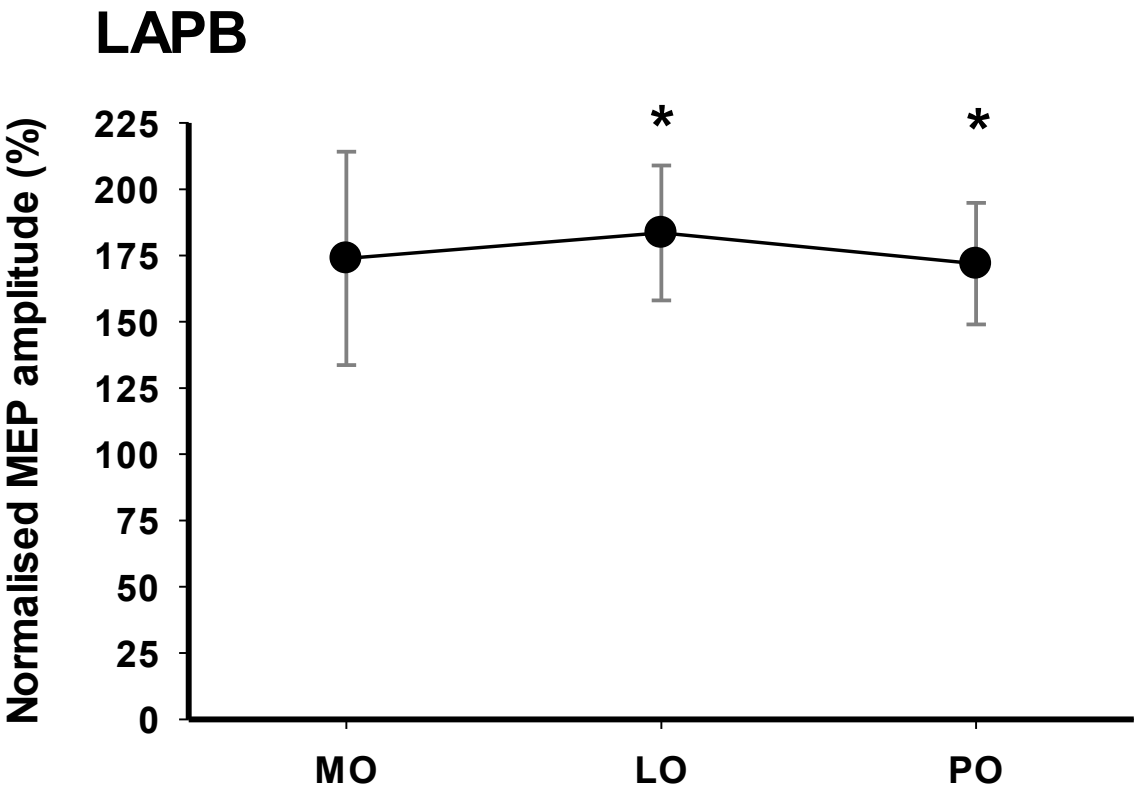
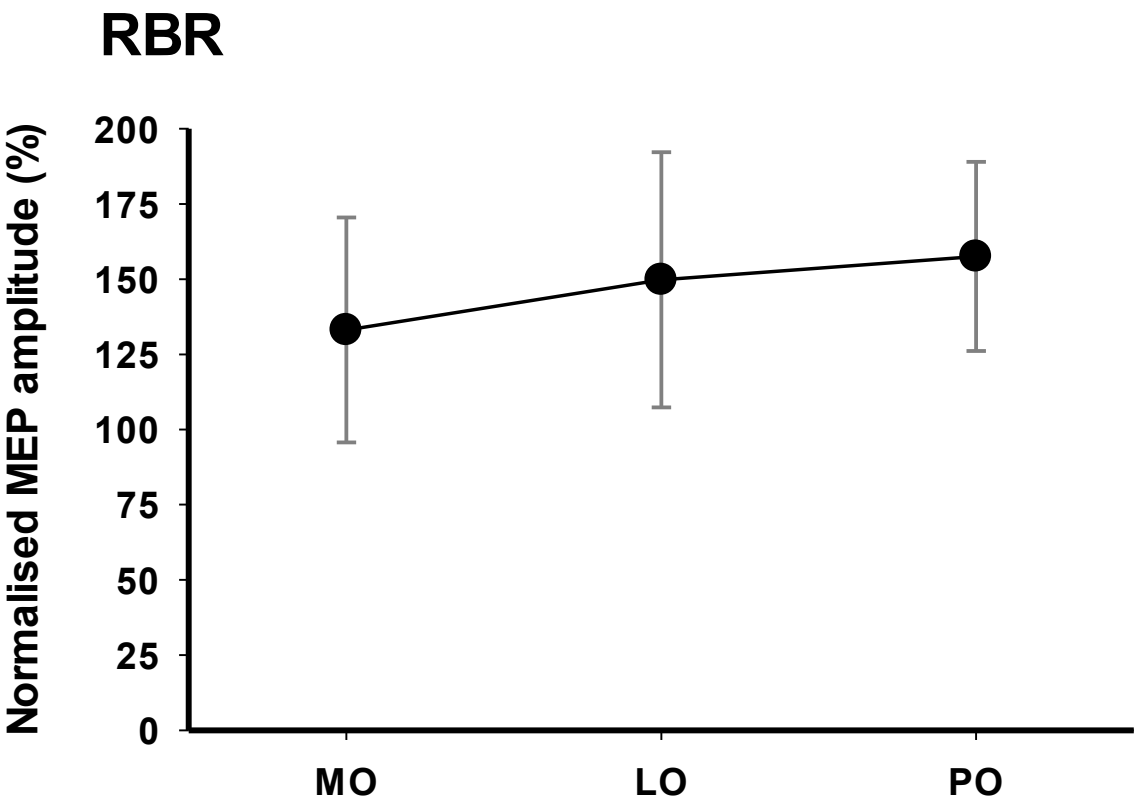


*Figure 44: Normalised RAPB MEP amplitudes grouped into time periods. MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Date expressed as mean ± SEM. ** denotes a significant reduction compared to baseline (P < 0.01).*

Normalised LAPB MEP amplitudes were significantly different from BL (P=0.006). Post-hoc testing reveal MEPs were higher at LO and PO than BL (P<0.05). MEP amplitudes were on average 153% and 154% of baseline for RBR and LBR, respectively, but were not significantly different from baseline (P>0.05) (see Figure 45A-C).




RBR
Normalised MEP amplitude (%)
200
175
150
125
100
75
50
25
0
MO
LO
PO

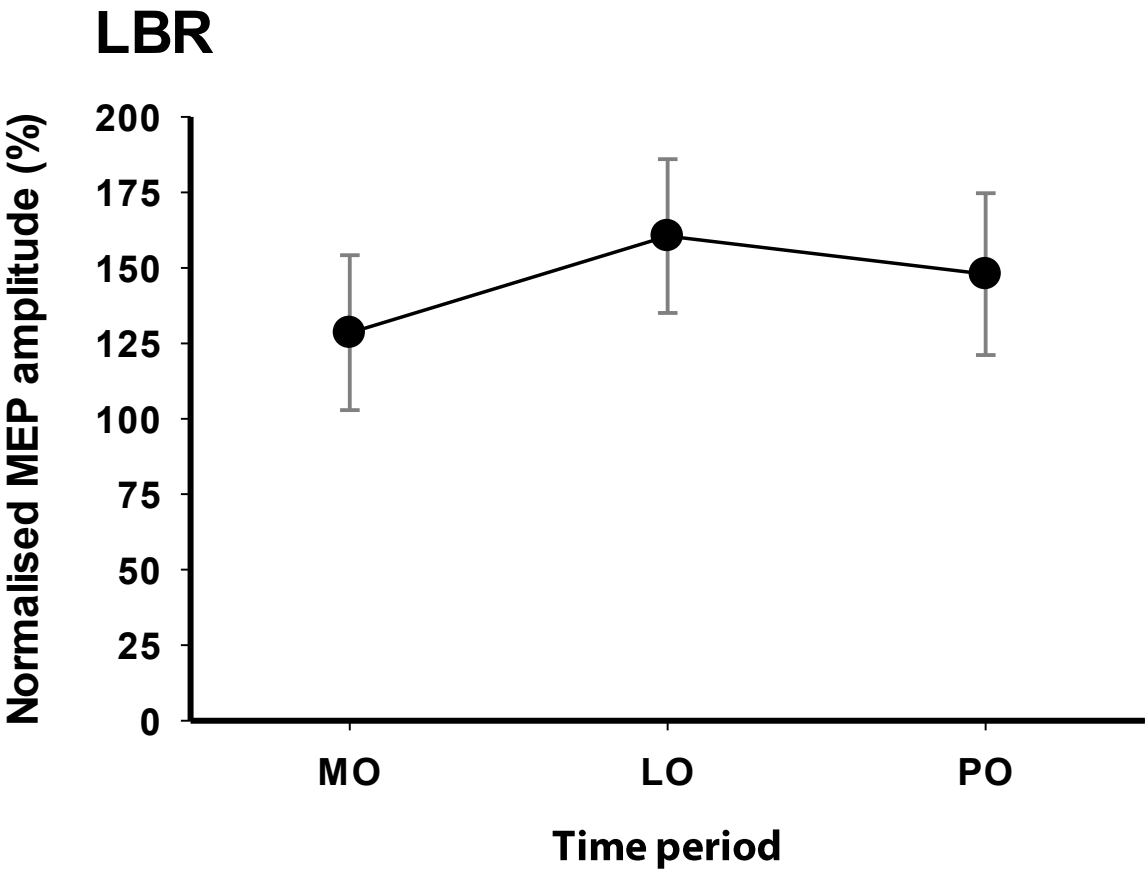

LBR
Normalised MEP amplitude (%)
200
175
150
125
100
75
50
25
0
MO
LO
PO
Time period

*Figure 45: Normalised mean MEP amplitudes for lleft abductor pollicis brevis (LAPB),right brachioradialis (RBR) and left brachioradialis (LBR) muscles. MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM; * denotes a significant difference compared to baseline (P<0.05).*

Pain scores

Pain scores (under the INB forearm cuff) are shown in Figure 46 for 15 participants; data from 1 participant was not recorded, in error. Pain scores were significantly higher at MO and LO (P<0.05) and returned to baseline values at PO.

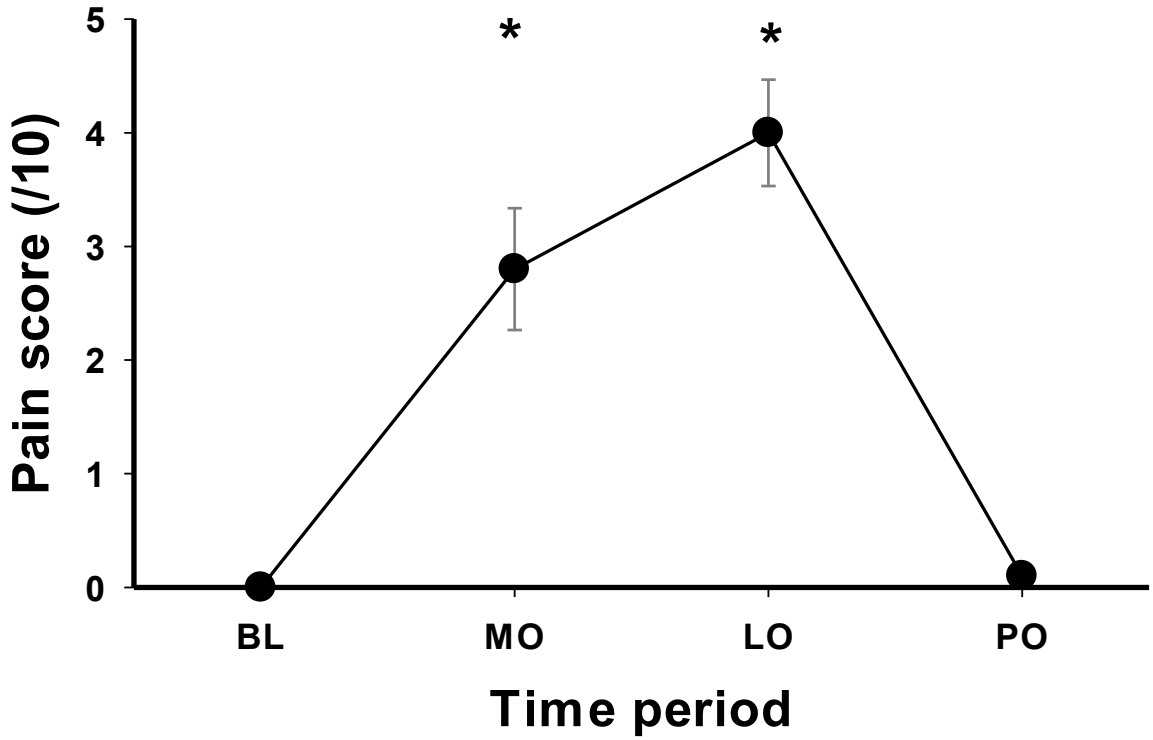


*Figure 46: Pain under the cuff. Scores range from 0 – 10, where 10 is most pain experienced and 0 is no pain (baseline). BL = baseline, MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM, * denotes a significant difference compared to baseline (P<0.05). Note: some error bars too small to be visible.*

Quantitative Sensory Testing

Dynamic Sensation

There was a decrease in dynamic sensation (brush stroke) distal to the INB when compared to baseline during MO and LO ($P<0.05$) (see Figure 47).

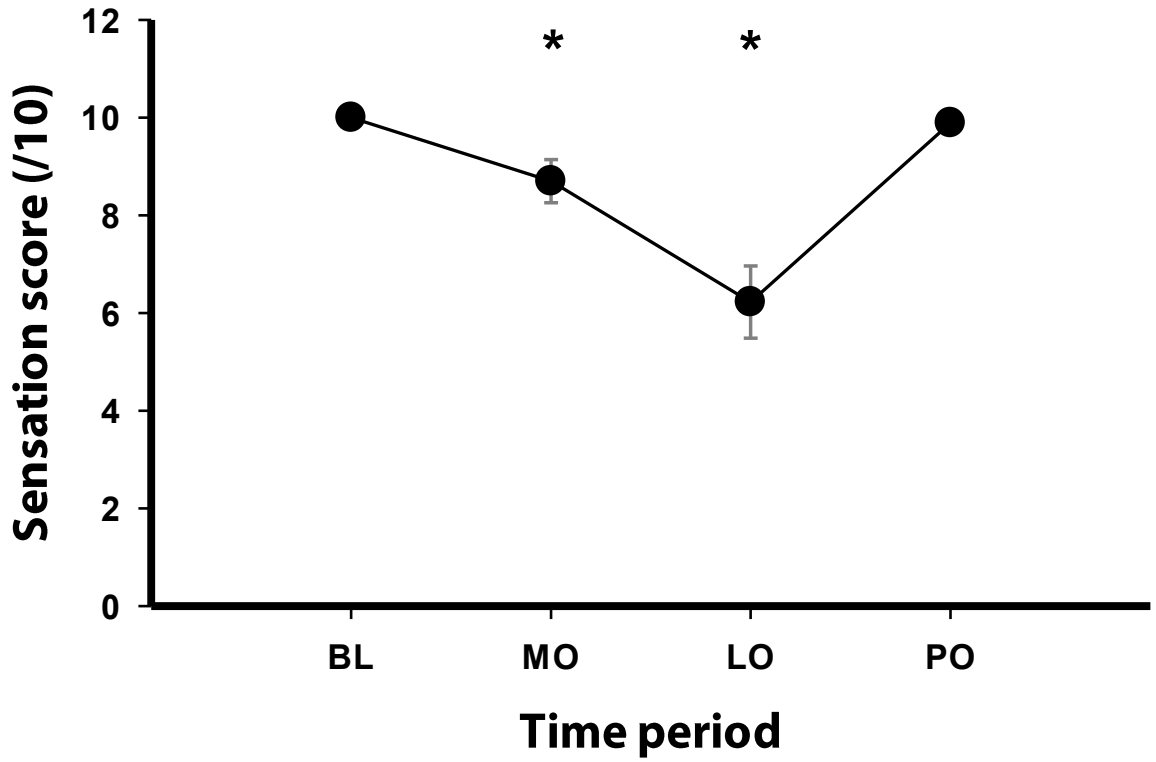


*Figure 47: Dynamic sensation as compared to the contralateral side. Score range from 0 – 10, when 10 is dynamic sensation pre-occlusion. BL = baseline, MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM. * denotes a significant difference compared to baseline (P<0.05). Note: some error bars too small to be visible.*

Heat Pain Threshold

Figure 48 shows the mean normalised heat pain threshold, which did not significantly change throughout the experiment ($P=0.16$). The raw mean values ranged from 44.9°C to 47.1°C.

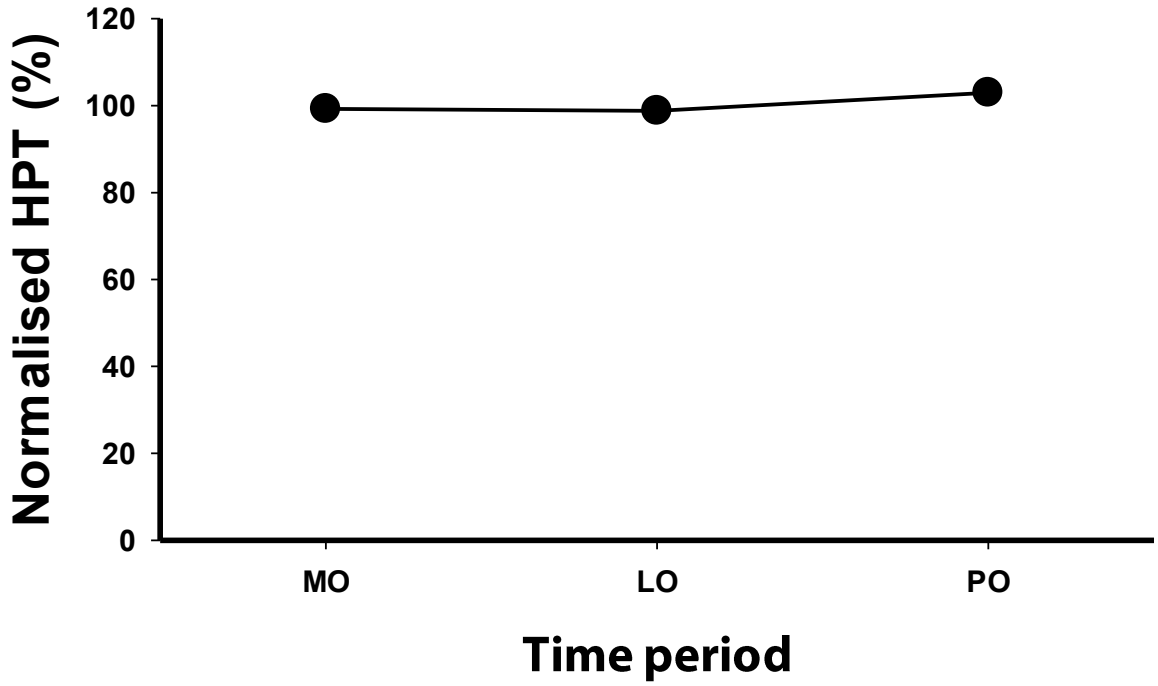


Figure 48: Normalised heat pain thresholds (HPT) compared to the contralateral side (n = 16). BL = baseline, MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM. Note: error bars too small to be visible.

Punctate Sensation

The mean punctate sensation score when compared to baseline for participants who had a reduction in sensation (a 'hyposensitive' response; see discussion below) (n=10) is shown in Figure 49. Sensation scores during the late occlusion period were significantly lower compared to BL ($P<0.001$).

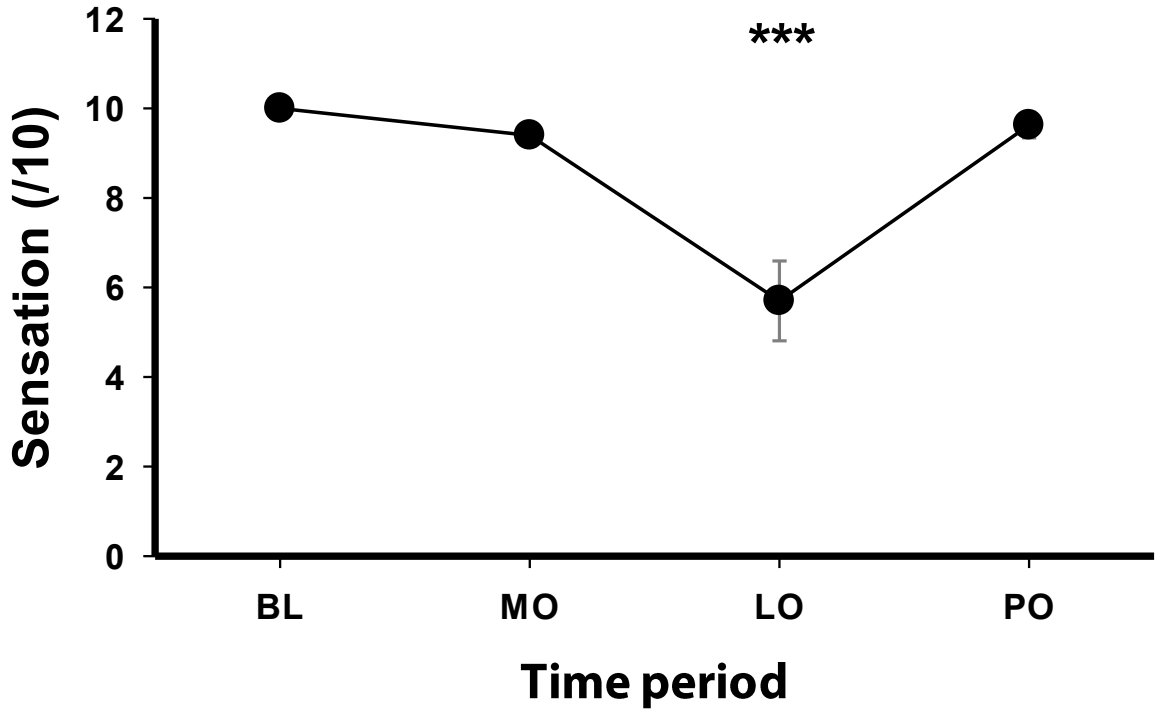


*Figure 49: Punctate sensation as compared to the contralateral side, in 10 participants who experienced a reduction in sensation (n = 10). Score range from 0 – 10, where 10 is sensation pre-occlusion. BL = baseline, MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM; *** denotes a significant difference compared to baseline (P<0.001). Note: some error bars too small to be visible.*

6 participants had an increased punctate sensation score (a 'hypersensitive' response). Since the scoring scale was 0 to 10, with 10 being normal as compared to baseline and the contralateral control side, a score of 10+ was given. MEP changes in LAPB, RBR and LBR after grouping the data according to the punctate sensation (hyper- or hyposensitive) were then analysed

Hypersensitive vs Hyposensitive Response to Punctate Sensation

The hyper-(n=6) and hyposensitive (n=10) groups had similar demographic profiles, with a 1:1 gender ratio; groups were not significantly different in age (mean age±SD; hypersensitive 22.5yrs±1.4yrs vs hyposensitive 21.4yrs±1.1yrs, p=0.130), mean cuff pressures (151.7 mmHg and 149 mmHg, respectively, P=0.754) and mean circumferential force exerted on the arm (611.8 N and 599.9 N respectively, P=0.870).

Sensory Differences

There were no sensation differences in the other QST modalities between the hyper- and hyposensitive groups across all time points; dynamic sensation (P=0.486) and heat pain threshold (P=0.747). Pain under the cuff did not differ significantly at any time point between the two groups (P=0.724). There was no correlation between cuff pain score and the punctate sensation at the time of maximal cuff pain (LO; R = 0.11).

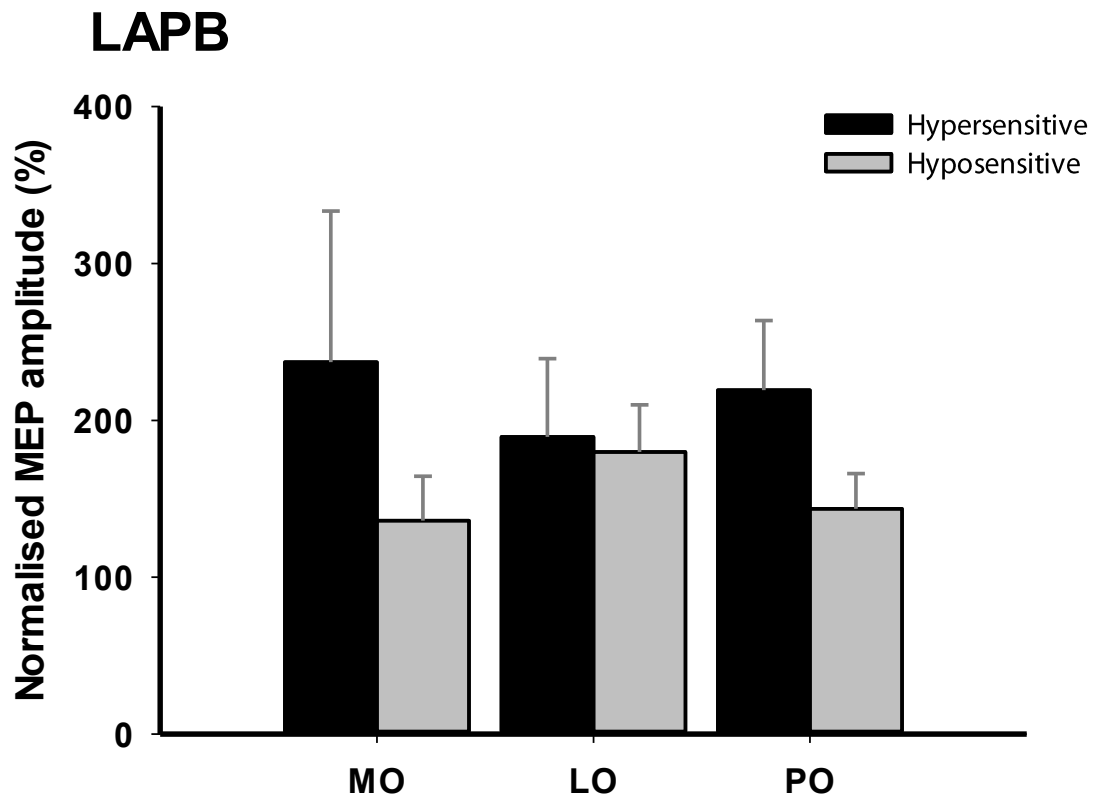
MEP Amplitude

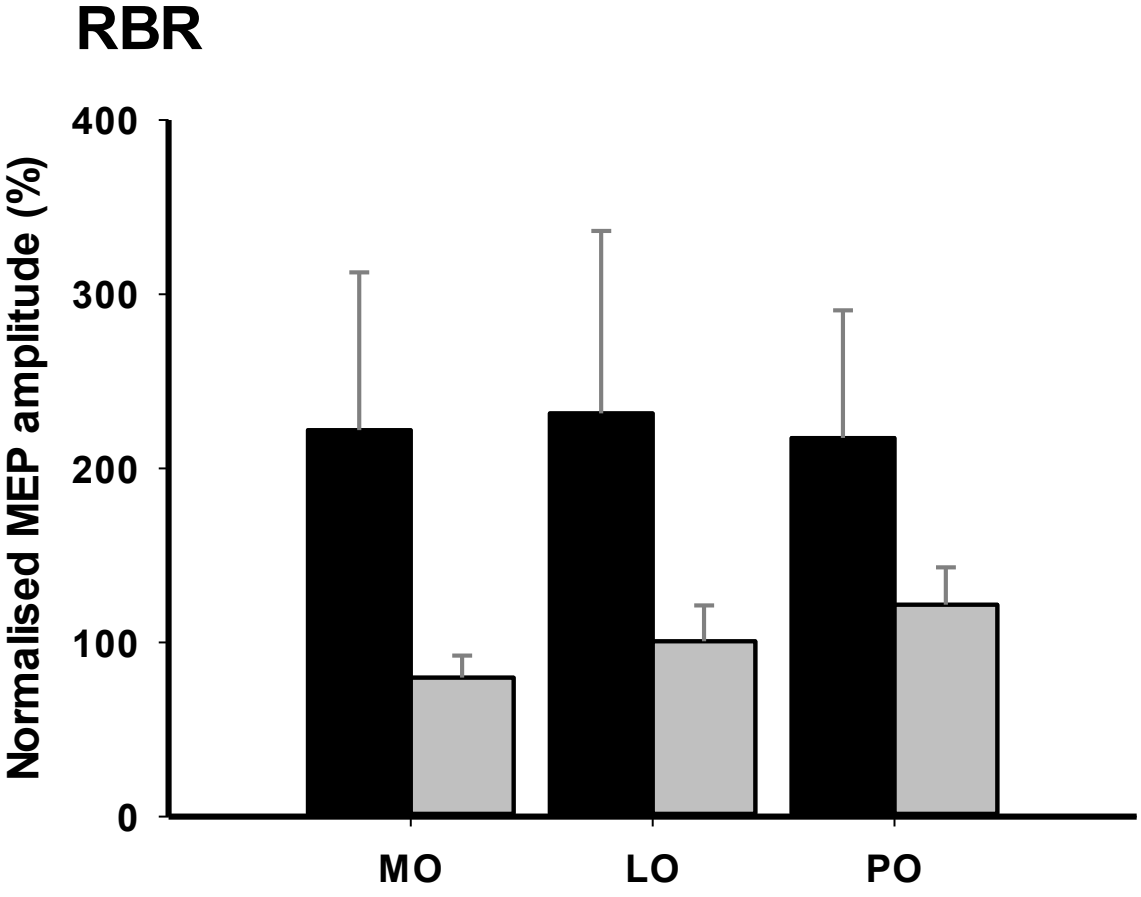
Separate two-way RM ANOVA for each muscle with factors time (BL, MO, LO and PO) and group (hypo- and hypersensitive) were performed.

For LAPB there was an effect of time (P=0.048) but there was no effect of group (P=0.826) (see Figure 50). Multiple comparison tests could not identify differences between the time points.

For RBR there was no effect of time (p=0.443) or group (P=0.794) on MEP amplitude (see Figure 50)

For LBR there was no effect of group (P=0.787) on MEP amplitude, but there was an effect of time (p=0.037) and post-hoc analysis revealed there was a difference between MO and LO (p=0.028) (see Figure 50).


LAPB
Hypersensitive
Hyposensitive
Normalised MEP amplitude (%)
400
300
200
100
0
MO
LO
PO


RBR
Normalised MEP amplitude (%)
400
300
200
100
0
MO
LO
PO

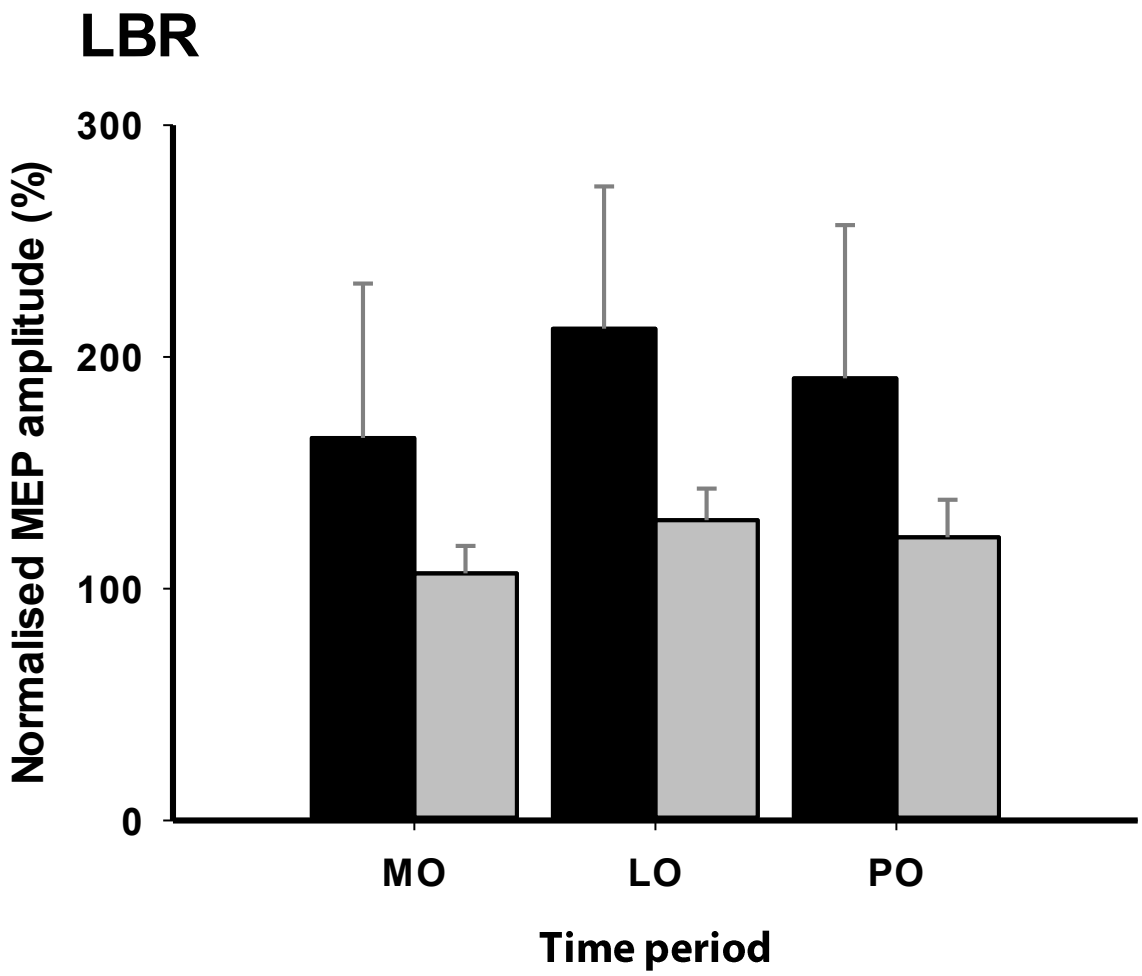


Figure 50: Normalised MEP amplitudes for hypersensitive (black bars) and hyposensitive (grey bars) responders shown for each muscle. (LAPB = left abductor pollicis brevis, RBR = right bracioradialis, LBR = left brachioradialis). MO = mid-occlusion, LO = late occlusion, PO = post-occlusion. Data expressed as mean ± SEM.

Discussion

An expected decrease in MEP amplitude of the occluded muscle was observed following a low-pressure upper limb INB. This was accompanied by a significant increase in MEP amplitude in the contralateral homologous arm muscle, indicating an increase in corticospinal excitability. QST demonstrated dynamic sensation and punctate sensation significantly decreased during the INB whereas HPT remained unchanged. A sub-group of participants had an unexpected increase in the perception of punctate sensation during the occlusion period and our results indicate these participants may have greater mean MEP amplitudes. The findings suggest that there is a differential effect of INB on somatosensory function with a loss of A-fibre function and preservation of C-fibre

function, which initiates changes in corticospinal excitability. In addition, the maintenance of C-fibre function following INB could be a contributing factor to the differential responses to punctate sensation and subsequent possible differences in sensorimotor plasticity.

Changes in corticospinal excitability

The results of this study are in keeping with those in the literature (229-232). MEPs in the proximal and contralateral muscles showed a tendency to increase, the latter significantly.

As discussed in chapter 5, increased cortical excitability arises secondary to deafferentation and the unmasking of latent cortical excitatory interneurons, via disinhibition of the cortex mediated by the loss of GABAergic input (87, 185). The rapid timescale over which the changes occur also implies alterations to existing neuronal circuitry, rather than structural changes in cell populations (183).

To be an initiating event, deafferentation should precede changes in motor cortical excitability. As the data from chapter 5 demonstrated, an increase in MEP amplitudes late into an INB was observed without complete loss of somatosensory evoked potentials. It was postulated that selective preservation of unmyelinated afferent nerve fibres, presumably ischaemia-insensitive nerve fibres, could account for these findings. The unchanged heat pain threshold in this experiment substantiates this and demonstrates preserved C-fibre function, whilst the reduction in mean dynamic sensation and punctate sensation responses therefore indicates the deafferentation mediated by Aβ and Aδ fibres is required to induce changes in cortical excitability.

Sensory Changes

Dynamic sensation and punctate sensation reduced during the occlusion, indicating a reduction in myelinated Aβ and Aδ fibre function. Previous work has demonstrated that myelinated fibre function begins to diminish after 12 minutes of compressive and non-compressive ischaemia of the rabbit femoral nerve. The same study also demonstrated that Aδ function is more sensitive to ischaemia than Aβ fibres (233). The later finding was not replicated in our study as activity in Aβ fibres appeared to

diminish significantly before that in Aδ fibres at MO. It is possible that direct nerve compression by the cuff could be a confounding factor as larger myelinated fibres are more sensitive to compression than smaller ones (233), which would fit with loss of the larger Aβ fibres earlier than the smaller Aδ fibres.

Preservation of C-fibre function was observed, as evidenced by there being no change in HPT throughout occlusion. This finding is in keeping with the literature, where direct compression of a nerve for over 30 minutes resulted in preservation of heat pain sensation (234). Unmyelinated fibres appear to require less ATP than myelinated fibres due to their lack of Schwann cells, and as such they are able to tolerate ischaemia for longer (235). Preserved C-fibre function would therefore account for the persistence of the ischaemic pain and the pain under the cuff which many of our participants experienced (236, 237).

Differing Sensory Responses

Unexpectedly, a number of participants reported an increased response to punctate pain compared to baseline and their contralateral control hand. Sub-group analysis found this cohort of 'hypersensitive' also appeared to have larger MEPs in the non-occluded muscles, albeit these findings were not significant (with respect to group). It should be noted that this was an unexpected finding, hence the differences in MEP amplitude between the hyper- and hyposensitive groups may not be significant as the paradigm was underpowered. Post-hoc power analysis showed a power of only 20% and to power the investigation adequately (80%) would require a sample size in excess of 16 in each of the hypo- and hypersensitive groups.

These results seem to suggest that hypersensitivity to punctate sensation is associated wi th augmentation of motor cortical excitability increases following an INB. The mechanism for this is speculative; it is possible the preserved C-fibre function coupled with the ongoing pain following INB may have contributed to the hypersensitivity to punctate pain observed in 6 participants (see below).

An important consideration to bear in mind is that this study was not designed to explore and therefore quantify the level of hypersensitivity experienced by some participants; this was an

unexpected finding as previously mentioned. Had it been, the use of a scoring system with a greater range or one designed for neuropathic pain would for example have improved the study design and the interrogation of the hypersensitive responses (238).

Effects of Pain and Hypersensitivity

Pain is a complex pathopsychological state with a myriad causes, manifestations and individual responses to treatments. The effect of pain on the motor system is similarly complex and can vary depending on a number of variables (type of stimulus, duration of pain, its location). Although there is some conflicting evidence in the literature, most TMS studies have shown that acute experimental pain has an inhibitory effect on corticospinal excitability, with TES studies showing the effect is attributed to changes in the motor cortex (205, 212). This inhibitory effect has also been demonstrated to reduce motor cortical excitability and suppress the ability to learn new motor tasks (239). TMS experiments exploring the effects of pain on the motor cortex often use injection of hypertonic saline directly into muscles or the application of capsaicin to the skin, which activate Aδ and C-fibre nociceptive afferents (240, 241). The ischaemic pain seen with an INB most likely activates at least these nociceptive fibres, and does so continuously. The preservation of HPTs in this study suggests ongoing C-fibre function, activation of which causes hyperalgesia in some participants, which in turn causes an increase in MEP amplitude in muscles contralateral to the ischaemic pain.

These models of acute experimental pain are distinct from the pain observed with neuropathic or chronic pain. In this investigation, sensory nerve fibre interrogation was performed in the context of ischaemia, which can mimic a neuropathic pain state. This commonly presents with sensory loss, thermal hyperalgesia or mechanical hyperalgesia (242), such as that exhibited by the “hypersensitive” responders, which have been attributed to changes in peripheral and central nociceptive pathways (243). It is thought that the extra territorial spread of punctate (pin prick) hypersensitivity is a result of central sensitisation; it is therefore possible that in the current study there was a subgroup who have developed additional plasticity in the spinal cord as a result of the ongoing C-fibre activation following an INB.

TMS studies exploring the effects of chronic or neuropathic pain on corticospinal excitability are less consistent than those with acute pain. As an example of many, one study showed reduced short interval intracortical inhibition and increased I-wave facilitation in the hemisphere contralateral to the affected limb of patients with complex regional pain syndrome type 1 (a chronic pain disease state), suggesting chronic pain causes increases in cortical excitability (244). In contrast, patients with chronic leg pain from sciatica had higher mean thresholds for MEPS and the cortical silent period compared to healthy control participants, implying decreased excitability (137). This ambiguity has been repeatedly demonstrated also in studies with phantom limb pain; one study showed increased TMS-induced MEPs from the stump muscles relative to the contralateral muscles in patients with phantom limb pain compared to those without (245), whereas another study showed pharmacologically reducing cortical excitability did not correlate with a reduction in phantom limb pain (246). The explanation of these differences is unclear, but may be due to the heterogeneity in the sensorimotor deficit experienced by patients with a clinical pain state as opposed to the acute experimental pain state (247).

Potential Mechanism for Hypersensitive Response

The continuous unmyelinated C- fibre activation throughout occlusion may have led to sensitisation at the level of the spinal cord. As reported, Aδ fibres progressively lose function during ischaemia (236, 248), whereas their response appears to be exaggerated in the “hypersensitive” participants. Despite this, correlations performed between cuff related pain scores and changes in punctate sensation did not demonstrate a relationship.

Under normal conditions, nociceptors activate second order neurons by releasing glutamate, which predominantly binds to AMPA receptors. In cases of high pain states, a barrage of nociceptive activity and release of glutamate leads to predominantly NMDA receptor activation. NMDA activation in the second order neuron leads to increased perception of pain (249). In this investigation, the INB may have induced this effect in some participants via preserved unmyelinated axonal activation by the cuff. This may have in turn sensitised the residual Aδ afferent signaling in the spinal cord leading to a

hypersensitive response to punctate sensation sensation. The differences found between participants may have been due to differences in susceptibility to this central sensitisation mechanism (243). Central sensitisation is a process whereby the synaptic efficacy and membrane excitability of sensory fibres in the spinal cord increases, which then causes amplification of pain (250). This is influenced by the descending inhibitory pathway (251). The effectiveness of descending inhibitory control is variable within a human population due to genetic and environmental factors (252). The extent to which differences in response to the same stimulus (e.g. INB) may cause differential effects on corticospinal excitability remains to be explored.

Conclusion

The results of this study add to our understanding of the mechanisms underlying INB-mediated changes in corticospinal excitability and further expose the complex influence of pain on these pathways. Although selective deafferentation by an INB of Aβ and Aδ fibres appears to be sufficient to increase corticospinal excitability, intact C-fibre function most likely permits on-going pain perception; it is possible to speculate that this preserved C-fibre then augments Aδ function in susceptible individuals to promote changes in excitability via neuropathic pain mechanism. The data from this study adds to understanding of the complex interplay between mechanisms underlying changes in corticospinal excitability and pain.

Chapter 7: Exploration of mechanisms causing changes in cortical excitability in the motor cortex ipsilateral to an INB

Introduction

As has been seen in previous chapters, an INB is capable to inducing changes in cortical excitability, likely via deafferentation to the contralateral hemisphere. This increased cortical excitability has also been documented in the literature, and can manifest with increased MEP amplitude in muscles proximal to (59, 63, 80), and contralateral to (223) the INB. Much work has been undertaken to explore the mechanism responsible for the changes in the deafferented motor cortex, with alterations in intracortical facilitatory and inhibitory neuronal networks being implicated (60, 73, 253). However, to my knowledge, little evidence exists to explain the changes that occur within the ipsilateral, non-deafferented hemisphere.

The MEP amplitude is a measure of the strength of synaptic transmission within the motor cortex (59), which is modifiable by the activity of intracortical and interhemispheric circuits. These inhibitory (SICI, LICI) and facilitatory (SICF, ICF) intracortical pathways represent distinct neuronal networks (60), whilst interhemispheric inhibition is the transcallosal transfer on inhibitory effects from one M1 to the contralateral M1, likely via augmentation of local circuits (109); all can be probed with TMS paradigms. See Chapter 2: Paired Pulse Paradigms and IHI for greater detail.

Aim

The aim of this study was to use an INB of the forearm to investigate the mechanisms responsible for the increase in cortical excitability to the contralateral APB muscles. Paired pulse TMS paradigms were employed to interrogate the activity of intracortical pathways within, and interhemispheric pathways

to, the non-deafferented M1 ipsilateral to the INB, in order to gain a better understanding of the mechanisms which underlie these effects.

Methods

This investigation comprised 2 separate experiments, designed to determine if intracortical and inter-hemispheric mechanisms underlie the changes in motor cortical excitability seen following an INB:

- Experiment 1: Intracortical inhibition and facilitation (SICI, SICF, LICI and ICF)
- Experiment 2: interhemispheric inhibition (IHI)

Participants

Participant were recruited as per described in Chapter 2: Participant Recruitment.

Thirty-one healthy adult participants were recruited; 17 participants (male: female 9:8; mean (±SD) age 21.9 (±1.6) years) for Experiment 1 and 14 participants (male: female 9:5; mean (±SD) age 29.4 (±7.6) years) for experiment 2.

Pre-experiment blood pressure assessment

Resting blood pressure and subsequently the experimental INB pressure (systolic blood pressure + 20-30mmHg) was determined, as per Chapter 2: Ischaemic Nerve Block.

Surface EMG

All participants were instructed to recline at 45° on a physiotherapy table, with arms resting semi-pronated on their lap. Pairs of disposable Ag/AgCl electrodes were applied to the skin overlying the abductor pollicis brevis muscles bilaterally, parallel to the muscle fibre orientation (see Figure 51) for

both experiments. A ground electrode was placed on the left olecranon process. Pre-stimulation EMG was recorded before TMS stimulation to ensure adequate relaxation was present.

For greater detail on EMG recording, see Chapter 2: Electromyography Acquisition.

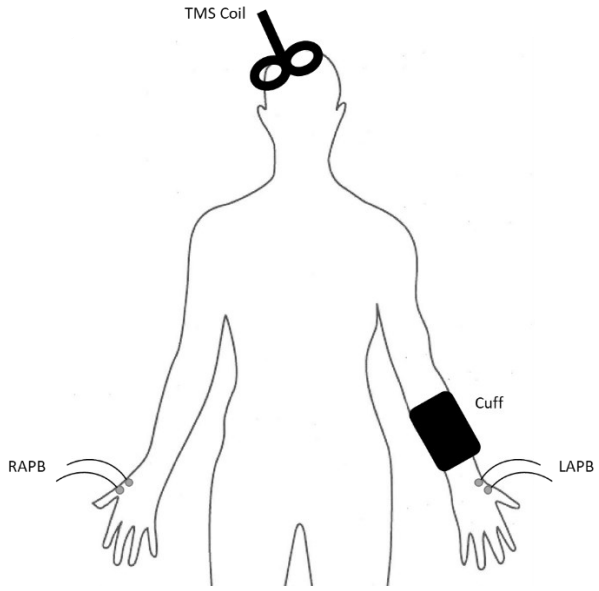


Figure 51: Experimental setup. TMS Coil used was the 'figure-of-8' coil. RAPB = right abductor pollicis brevis, LAPB = left abductor pollicis brevis. A single coil is shown in the diagram, as would be employed in experiment 1. For experiment 2, two coils placed on both ABP hotspots would be seen.

Motor evoked potential

Experiment 1

TMS was applied to the motor cortex at the hotspot for each corresponding muscle using two Magstim 200^2 stimulators (Magstim Company Ltd., Whitland, Dyfed, UK), placed in the Bistim mode. They were connected to a single figure-of-8 coil (The Magstim Company Ltd., Dyfed, UK); see Chapter: Transcranial Magnetic Stimulation.

In this experiment, MEPs were generated in LAPB (occluded muscle) and RAPB (non-occluded muscle) using single pulses of TMS at 1.2xRMT, referred to as the "test alone" (TA) state. Further pairs of stimuli were delivered over the left motor cortex (for RAPB) to explore intracortical inhibition (SICI, LICI) and facilitation (ICF, SICF). Paired-pulse states were randomised by the Signal v5.12 software (CED, UK).

The conditioning stimulus (CS), test stimulus (TS) and interstimulus interval (ISI) for each set of paired pulses are summarised in Table 3 above, with greater methodological detail provided in Chapter 2: Paired Pulse Paradigms.

Experiment 2

Two Magstim 200^2 stimulators (Magstim Company Ltd., Whitland, Dyfed, UK) were used to stimulate the motor cortex at the hotspot for each corresponding ABP muscle. A figure-of-8 coil with an 8cm wing diameter (The Magstim Company Ltd., Dyfed, UK) provided TMS to the left M1. A second smaller coil of 7cm wing diameter delivered TMS to the right M1; it was chosen to allow accurate simultaneous placement of coils to the participants head without the coils clashing.

Using an intensity of 1.2 x RMT, test-alone MEPs were generated for LABP to confirm INB success. During the IHI testing, a conditioning stimulus (CS) at 1.15 x RMT was delivered to the right M1, before the test stimulus (TS), at an intensity sufficient to generate MEPs in RABP of approximately 1mV, was delivered to the left M1. The interstimulus interval (ISI) between the CS and TS was 10ms. The optimal intensities and ISI used in the experiment were determined following pilot testing and in keeping with previous work (76, 109).

Experiment Protocols

Experiment 1

There were 7 testing time points throughout the protocol (see Figure 52). At every time point, 6 TMS trials for each state were performed; 6 single stimuli to each motor cortex for the test alone states and 6 pairs of stimuli to the left motor cortex for the paired-pulse paradigms. TA and paired-pulse stimuli were delivered in random order.

Two baseline sets of tests (-10 and -5 minutes) were obtained. The cuff was then inflated for 30 minutes at the pre-determined pressure for each participant. 3 sets of measurements were carried

out during the occlusion (at 10, 25 and 29 minutes). After deflation, a further 2 sets of post-occlusion measurements were performed at 35 and 40 minutes (5 and 10 minutes after the cuff was released). The intervals between measurement epochs were influenced by the time taken to perform all single and paired-pulse TMS paradigms.

Experiment 2

There were 9 testing time points throughout this protocol. At each time point, 6 single TMS stimuli were delivered to each of the motor cortices for both TA states, and pairs of 6 stimuli were delivered to both cortices for IHI testing (see Figure 52). TA and IHI stimuli were delivered in random order.

Two baseline sets of tests (-10 and -5 minutes) were obtained. The cuff was then inflated for 30 minutes at the pre-determined pressure for each participant. 4 sets of measurements were carried out during the occlusion (at 10, 20, 25 and 29 minutes). After deflation, a further 3 sets of post-occlusion measurements were performed at 31, 35 and 40 minutes (1, 5 and 10 minutes after the cuff was released. Unlike experiment 1, there was sufficient time between measurement epochs at 29 and 31 minutes to perform all measurements.

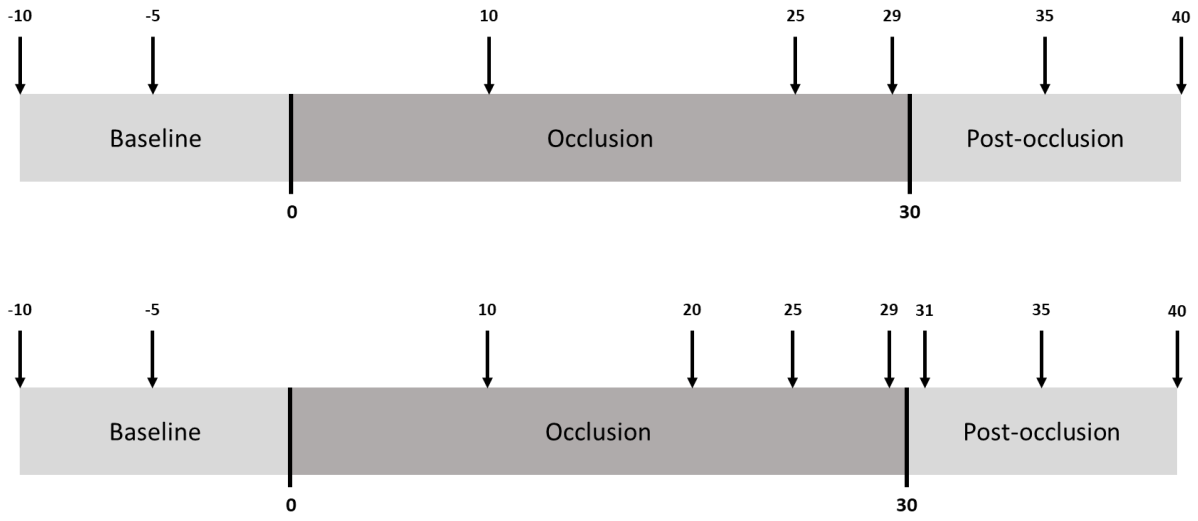


Figure 52: Protocol diagrams for experiment 1 (top) and experiment 2 (bottom). Shaded box showing the 3 phases of the experiment (baseline, occlusion and post-occlusion) with the black arrows showing the time (in minutes) at which testing occurred, with respect to the occlusion period.

Data Analysis

For both experiments, the set of 6 trials at a measuring epoch for each state, were digitally averaged to produce a single MEP waveform in Signal v5.12 software (CED, UK).

See Chapter 2: Electromyography Acquisition and Chapter 2:Analysis of TMS induced MEPs for greater MEPs sampling and then analysis detail.

Microsoft Excel (Microsoft, USA) was used for data storage and processing. All data are presented as the mean ± SEM.

Experiment 1

For further analysis, a mean baseline (BL) MEP amplitude for each state and muscle was calculated by averaging the 2 pre-occlusion measurements at -10 and -5 minutes; similarly measurements from 35 and 40 minutes were collapsed to generate a single post-occlusion (PO) average MEP amplitude. For the purposes of discussion, measurements from 10 minutes are referred to as early occlusion (EO), and from 29 minutes as late occlusion (LO). MEP amplitudes from EO, LO and PO for each state and muscle are presented as normalised values, relative to their respective baseline.

For LAPB and RAPB test alone pulses, the peak-to-peak MEP amplitudes were measured. The 6 MEPs at each time point were averaged to generate a single MEP amplitude for each participant, before being averaged for analysis.

SICI, SICF and ICF were calculated as the ratio of the averaged peak-to-peak amplitudes between the MEPs generated from the paired-pulse paradigm (conditioned MEP) and the averaged MEPs resulting from the TA stimulus (test MEP) at the same time point; they are expressed as a percentage (also referred to cMEP/tMEP). LICI was calculated as the ratio of the MEP amplitude generated from a suprathreshold stimulus preceded (100ms) by a suprathreshold stimulus; it is expressed as a percentage. The values for SICI, SICF, ICF and LICI were collapsed into BL, EO, LO and PO time periods and then averaged across subjects for further analysis.

Experiment 2

As above, data were grouped where appropriate for further analysis. A mean baseline (-5 and -10min measurements), late occlusion (LO; 25 and 29 minutes) and post-occlusion (PO; 35 and 40min measurements) MEP amplitude for each state and muscle was calculated. Data from 10 minutes is referred to as early occlusion (EO). MEP amplitudes from BL, EO, LO and PO for each state and muscle are presented as the normalised values, relative to their respective baseline.

For LAPB and RAPB test alone pulses, the averaged peak-to-peak MEP amplitudes were measured, before being averaged across subjects for analysis.

To calculate IHI, the amplitude of the MEP generated by the IHI state, the averaged conditioned MEP (cMEP), was measured and then expressed as a percentage of the amplitude of the test alone averaged MEP for RABP (tMEP). The values for IHI were collapsed into BL, EO, LO and PO time periods and then averaged across subjects for further analysis.

Statistical analysis

Data were tested for normality using the Shapiro-Wilk normality tests. Changes in MEP amplitudes to single pulse TMS and parameters of intracortical inhibition and facilitation and interhemispheric inhibition were examined using one-way repeated measures ANOVA (or Friedman test) followed by *post-hoc* analysis, where appropriate. Where ANOVA showed no significant differences over time, Pearson correlations were performed for non-parametric data and Spearman correlations for parametric data to examine any trends of changes over time.

Significance was set at $P<0.05$ or appropriate for multiple comparisons.

Results

Experiment 1

Data presented are from 13 participants; 4 subjects could not complete the protocols due to the TMS coil overheating.

MEP amplitudes (single pulse)

There was a reduction in MEP amplitudes in the occluded LABP muscle during the occlusion, reaching 43.1% ± 13.4 of baseline at LO (P<0.05), indicating successful INB. The non-occluded RAPB muscle MEP amplitude had a trend towards an increase but this was not significant (p=0.07); see Figure 53 and Figure 54.

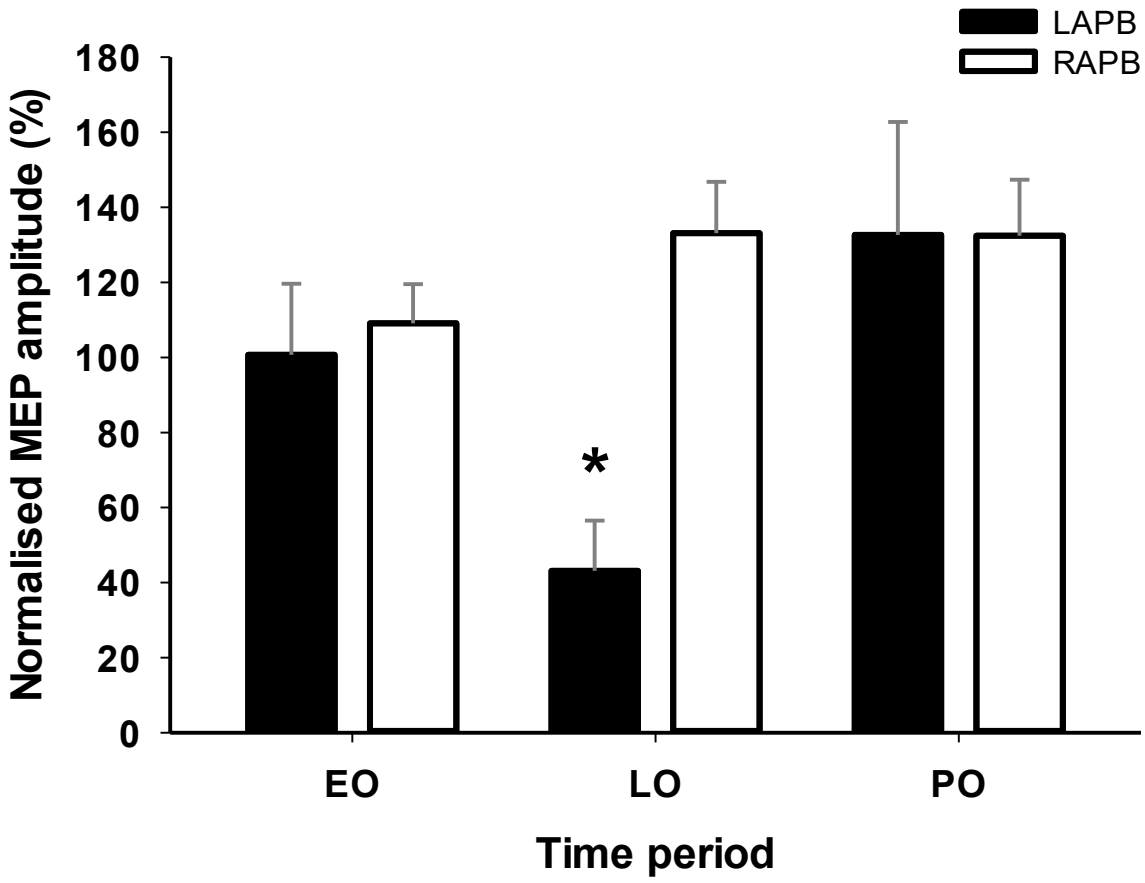


*Figure 53: Normalised MEP amplitudes for test alone states. Black bars represent MEP amplitudes from the occluded left abductor pollicis brevis (LAPB) muscle and the white bars represent the non-occluded right abductor pollicis brevis (RAPB). EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values are mean ± SEM. *denotes P<0.05 with respect to baseline.*

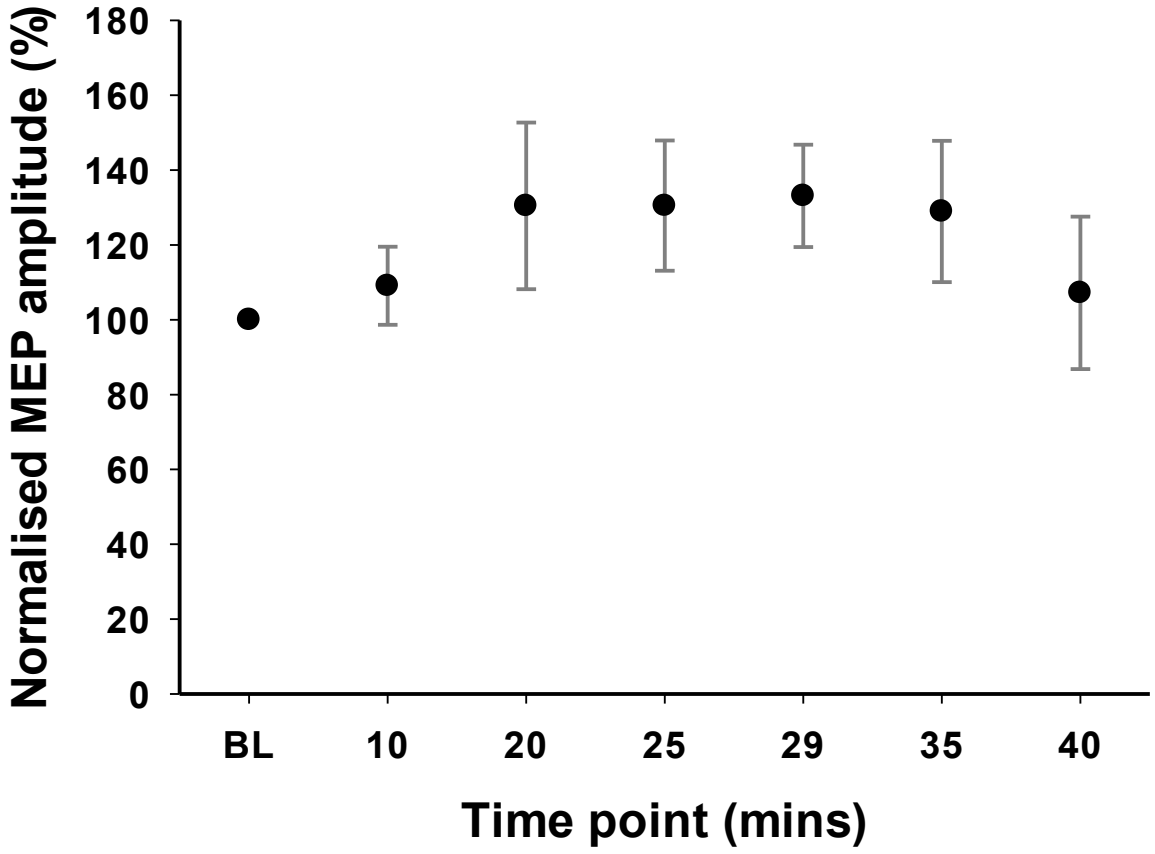


Figure 54: Normalised RAPB MEP amplitudes over time for Experiment 1. BL = baseline. Data represented as mean ±SEM

When raw RAPB MEP amplitudes were plotted against time, a strong positive correlation of $r^2 = 0.94$, (p=0.018) was observed; see Figure 55.

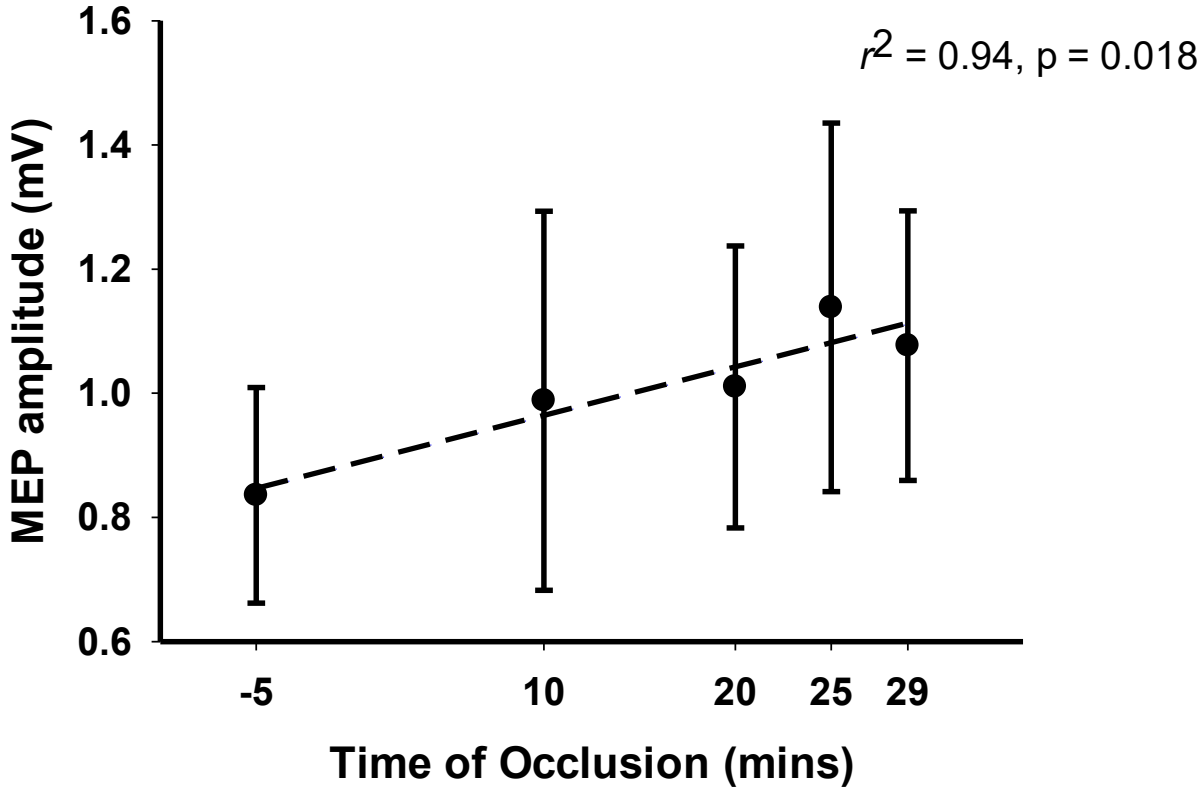


Figure 55: Graph showing correlation between raw MEP amplitudes for RAPB test-alone state and time. Data represented as mean ± SEM.

MEP amplitudes (paired-pulse)

Figure 56 shows the baseline levels of inhibition and facilitation induced by the four paired-pulse paradigms. There was a significant decrease in the conditioned MEP amplitude during SICI and LICI paradigms (as shown by values less than 100% of unconditioned MEP amplitude) and a significant increase during SICF and ICF paradigms (as shown by values greater than 100% of unconditioned MEP amplitude). These data demonstrate the success of the paired pulse paradigm parameters.

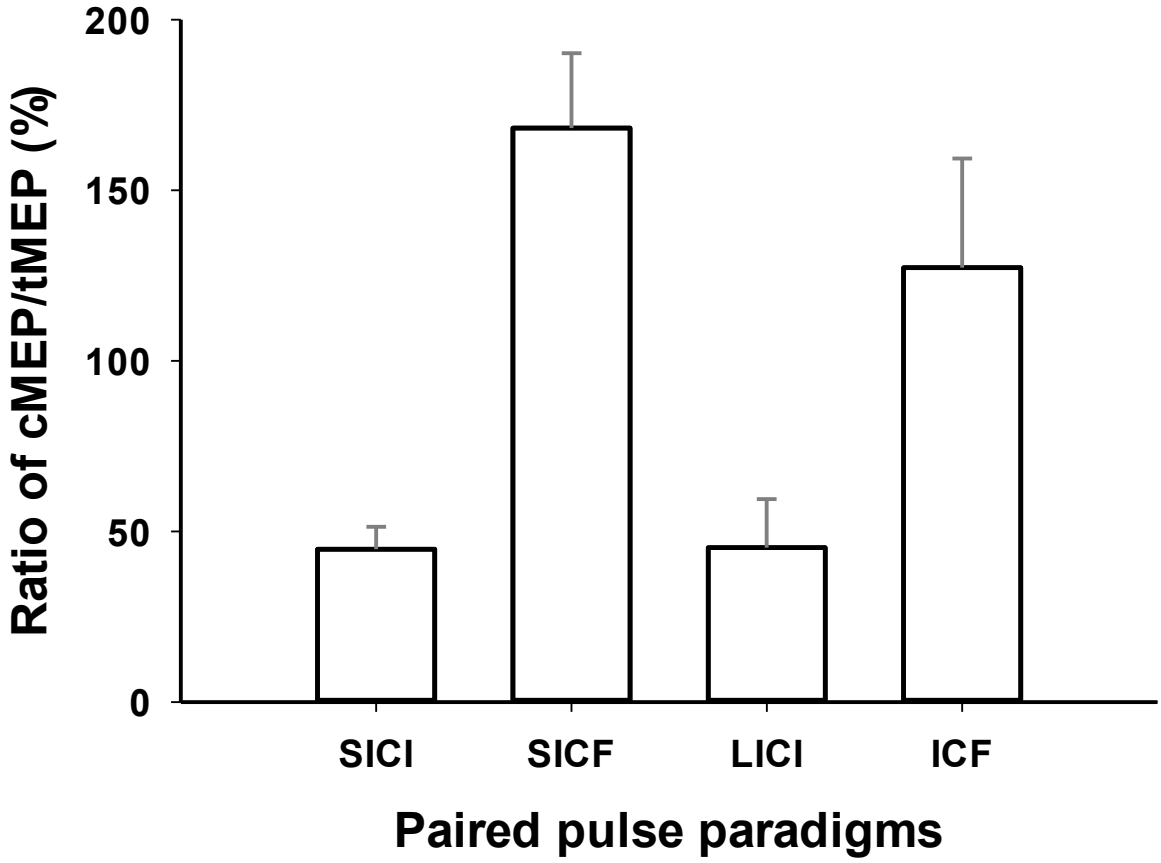
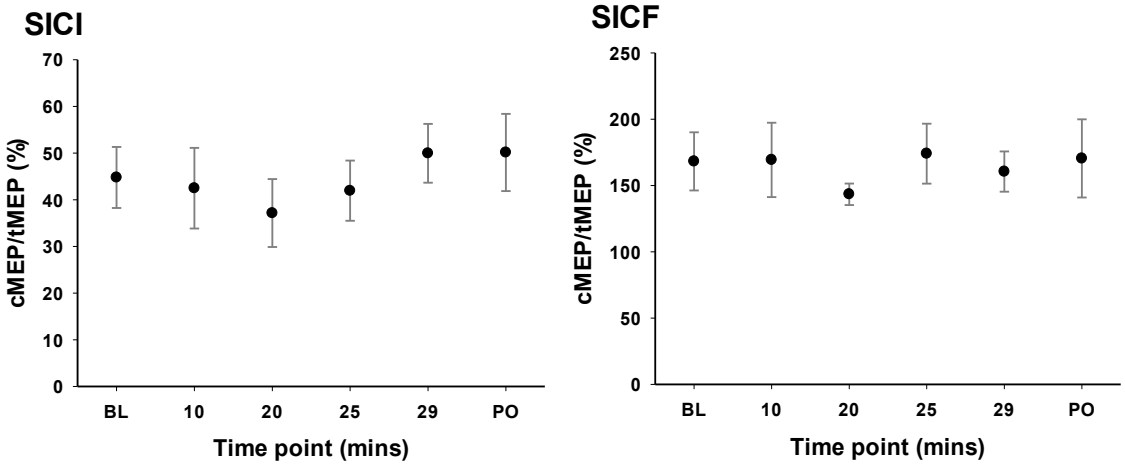


Figure 56: Ratio of cMEP/tMEP for each of the paired-pulse paradigms at baseline. SICI = short interval intracortical inhibition, SICI = short interval intracortical facilitation, LICI = long-interval intracortical inhibition, ICF = intracortical facilitation. Data represented as mean ± SEM.

There were no significant changes in SICI, SICF, ICF or LICI over time (see Figure 57). Further, there were no significant correlations between SICI, SICF, ICF or LICI and time.



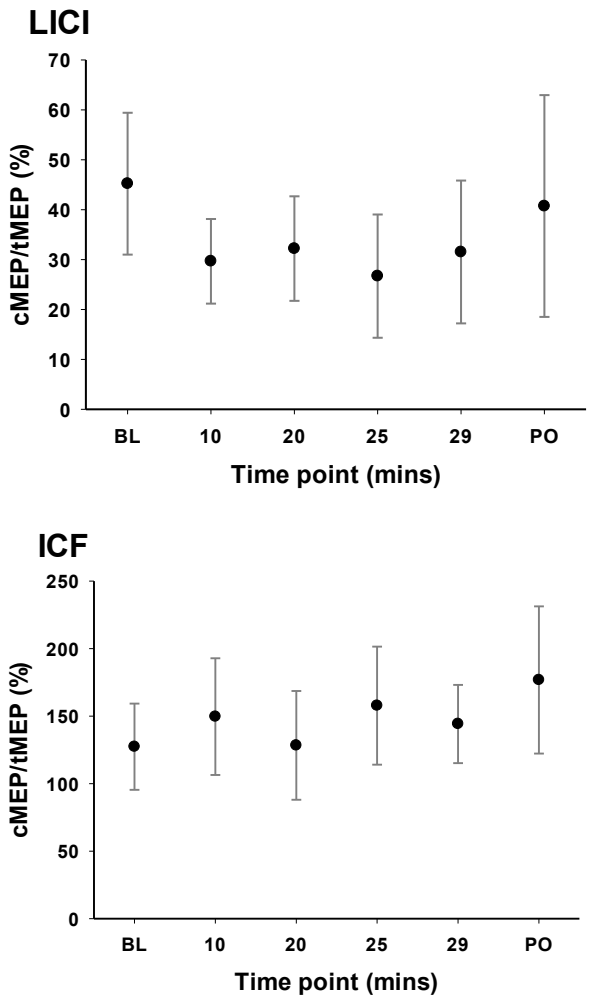


Figure 57: Conditioned MEP/test MEP amplitude showing SICI, SICF, LICI and ICF over the course of the experiment. BL = baseline, PO = post-occlusion. Data shown as mean ± SEM.

Experiment 2

MEP amplitudes (single pulse)

There was a significant reduction in MEP amplitudes in the occluded LABP following 30mins of INB, dropping to 28.3% (±6.8%) of baseline ($P<0.05$)(see Figure 58).

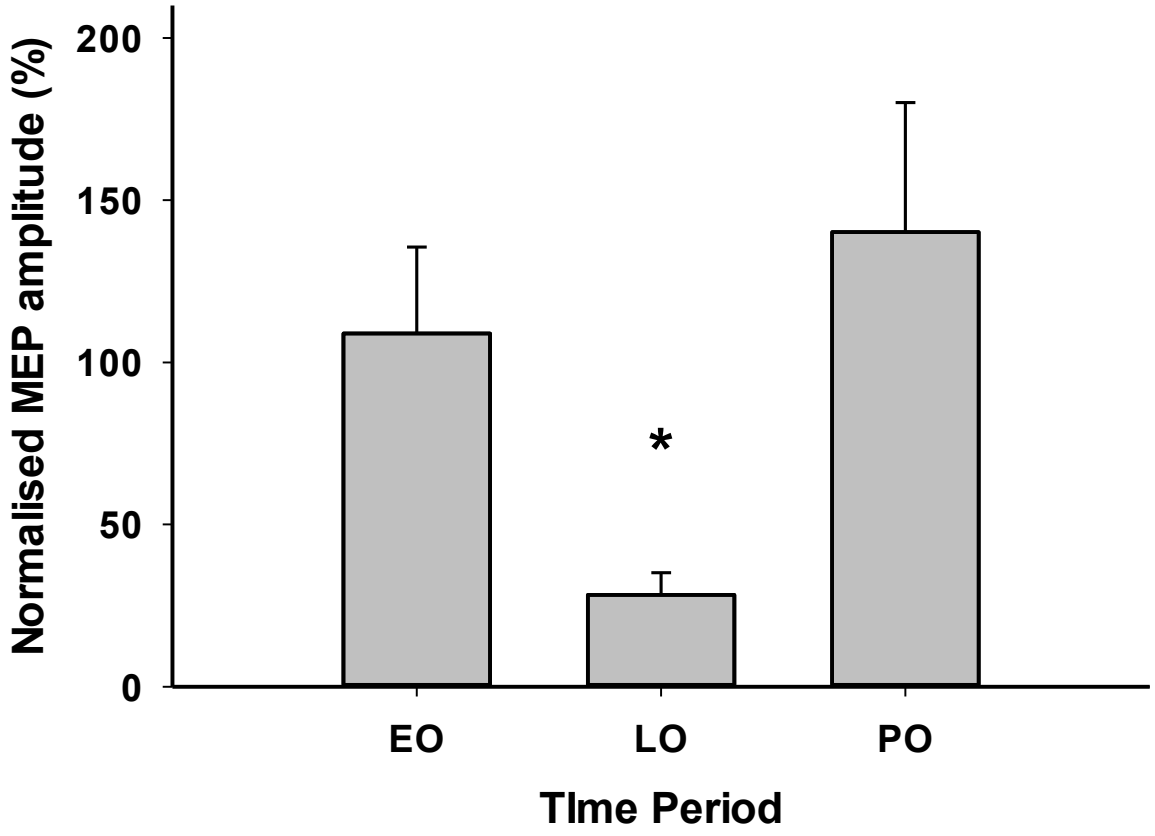


*Figure 58: Normalised MEP amplitudes for the LABP test alone state during experiment 2. BL = baseline, EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values represented as mean ± SEM. *denotes P<0.05 with respect to baseline.*

Figure 59 shows normalised MEP amplitudes from the non-occluded RAPB muscles in response to single pulse TMS. At both LO and PO, a significant increase in the mean RAPB MEP amplitude was seen, indicating an increase in cortical excitability in the contralateral limb to the INB.

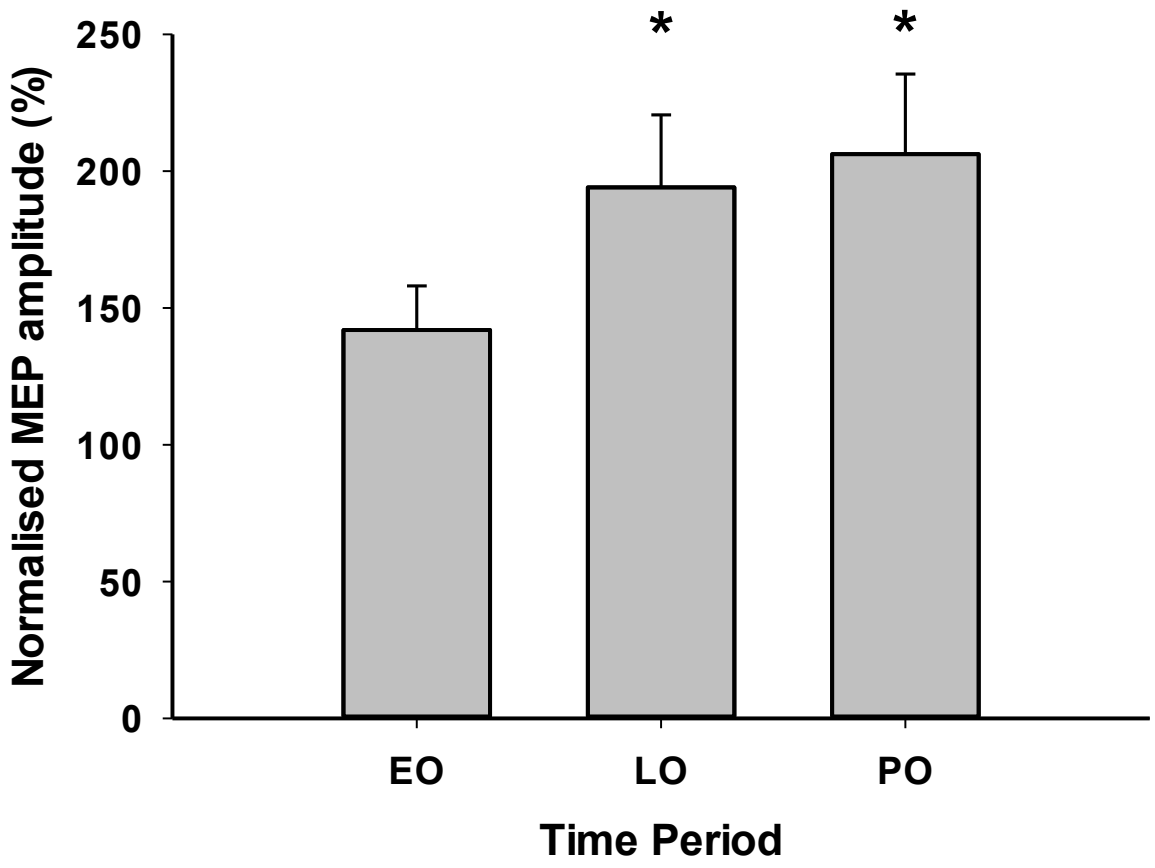


*Figure 59: Normalised MEP amplitudes in RABP during experiment 2. BL = baseline, EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values represented as mean ± SEM. *denotes P<0.05 with respect to baseline.*

As can be seen in Figure 60, the MEP amplitude in RAPB amplitude was lower than the desired 1mV, despite determining the intensity required to reach this during hotspot testing for each participant. However, previous work in the literature has shown IHI does not change over the range of 0.2 to 1mV for the single pulse MEP amplitude (109).

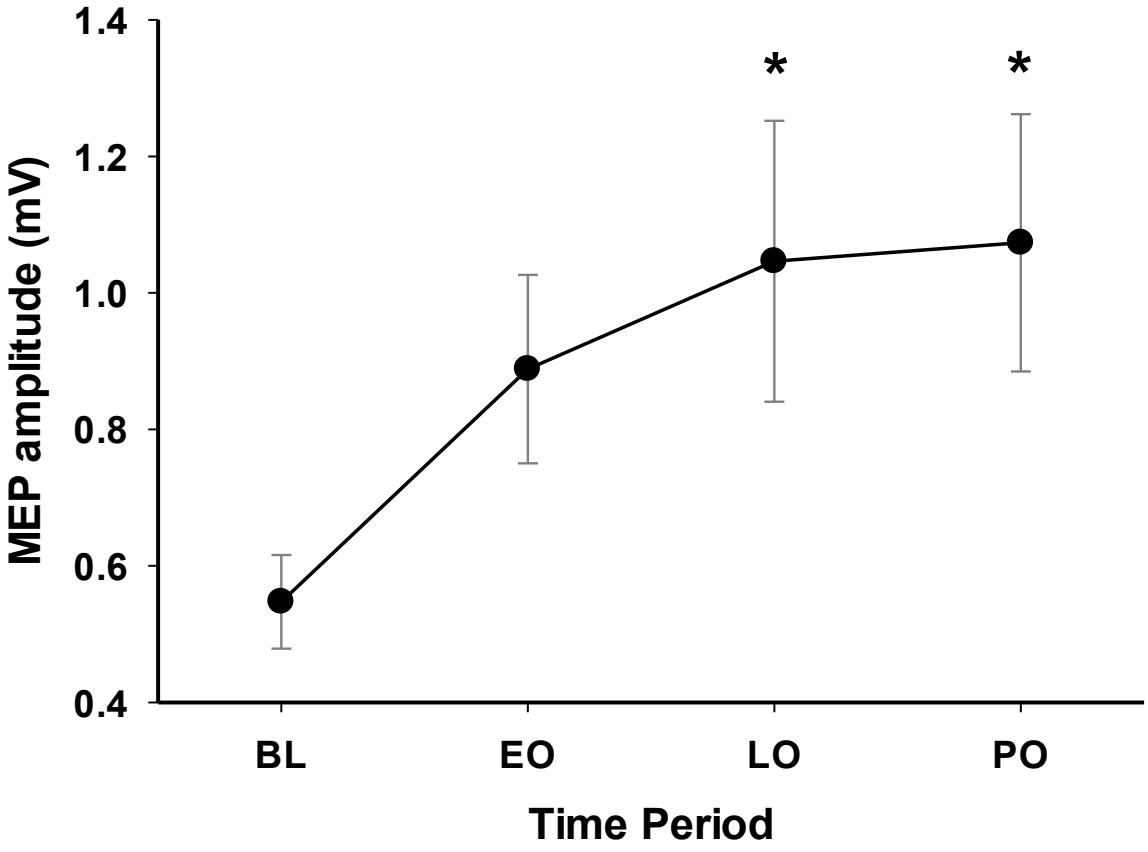


*Figure 60: Raw MEP amplitudes in RABP during experiment 2. BL = baseline, EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values represented as mean ± SEM. *denotes P<0.05 with respect to baseline.*

Interhemispheric Inhibition paradigm

During 30 minutes of INB, there was a significant decrease in IHI. At LO, the cMEP/tMEP was 151.7±23.9% when compared to baseline (p=0.037) (see Figure 61). As is convention, an increase in the cMEP/tMEP during an IHI paradigm indicates a decrease in the amount of IHI present.

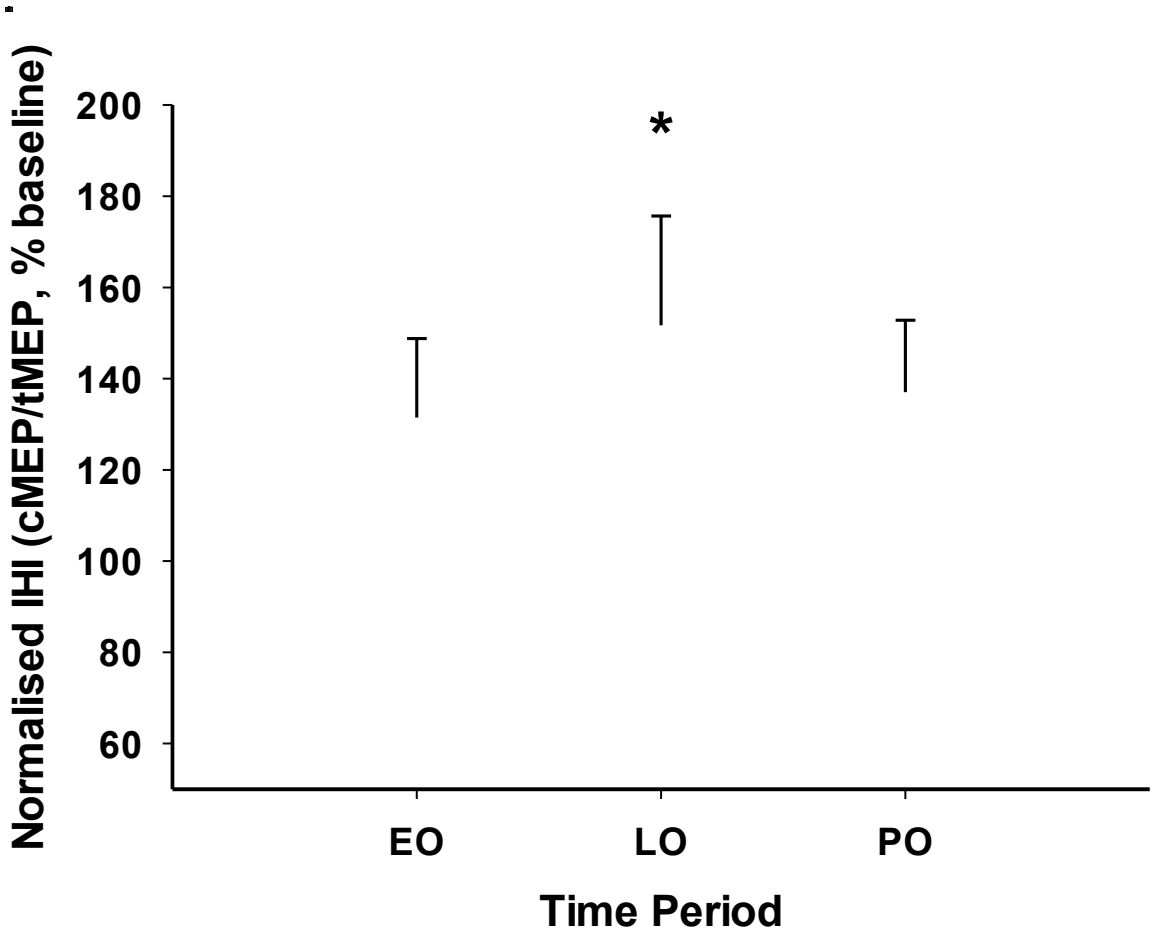


*Figure 61: Extent of interhemispheric inhibition, normalised to baseline, present during Experiment 2. EO = early occlusion, LO = late occlusion and PO = post-occlusion. Values represented as mean ± SEM. *denotes a significant change with respect to baseline, P<0.05*

A correlation performed between the RAPB MEP amplitude and the corresponding IHI value at both -10min and -5min baseline, showed IHI did not change over the range of MEP amplitudes obtained and data were therefore used for further analysis of IHI changes during INB (see Figure 62).

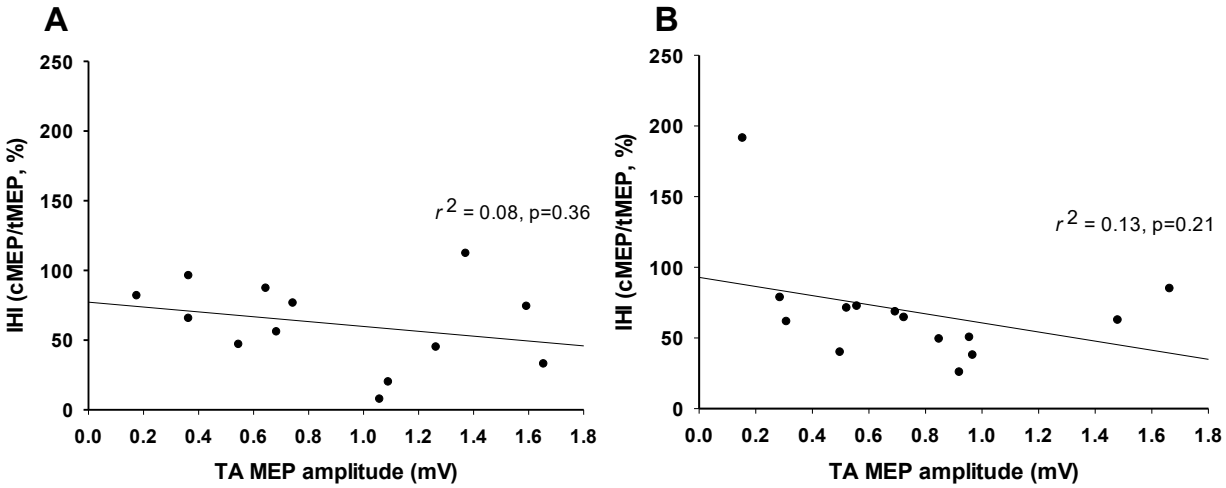


Figure 62: Graphs showing the correlation between RABP MEP amplitudes against the amount of IHI present at (A) -5mins and (B) -10mins baseline. As shown by the r^2 values, there is no correlation present over the range of RAPB MEP amplitudes generated in experiment 2.

Discussion

In this investigation, an ischemic nerve block was performed on the left forearm of healthy participants, whilst MEPs were recorded from the ipsilateral and contralateral abductor pollicis brevis muscles. The results of this study demonstrate a successful INB was performed, as evidenced by a significant reduction in mean LABP MEP amplitude following 30 minutes of occlusion in both experiments. A significant positive correlation was observed between the non-occluded RAPB MEP amplitude and the duration of occlusion in experiment 1, whilst a significant increase in RABP MEP amplitudes was seen during LO and PO in experiment 2, indicating an increase in corticospinal excitability. Whilst interrogation of the intrahemispheric pathways using the paired pulse paradigms SICI, SICF, LICI and ICF, did not demonstrate any changes, interhemispheric inhibition decreased, suggesting it plays a role in the increased motor cortical excitability. In the following, I shall review the literature and discuss the evidence that supports or refutes these findings.

As has been discussed in previous chapters, the increase in motor cortical excitability to proximal upper limb muscles following an INB has been attributed to deafferentation mediated disinhibition;

loss of sensory input to the motor cortex causing reduced levels of inhibition and thus the exposure of latent excitatory circuits resulting in increased excitability (254) (see Chapter 1: Mechanisms of Cortical Plasticity). This would explain the increased excitability to muscles proximal to an INB (80, 87); reduced sensory input would occur in the same hemisphere that shows an increase in motor cortical excitability. However, this would not be true for muscles contralateral to the deafferentation, where an increase in MEP amplitude is also observed, and which would require transcallosal communication (87).

A previous investigation explored the consequences of an INB performed at the wrist. MEPs were recorded from proximal forearm flexors, whilst SICI, SICF and LICI (with an ISI of 80ms and 150ms) was interrogated (73). Unexpectedly, the authors found only an increase in LICI with an ISI of 150ms. This would suggest a greater degree of inhibition occurs following an INB, in a cortex exhibiting increased excitability (as evidenced by the increased in MEP amplitude from proximal muscles). The authors concluded an intracortical pathway other than that being investigated, or a subgroup of neurones which could be explored with different TMS paradigms could be responsible (73). The data presented in this chapter interrogated the non-deafferented M1, to see if any changes occurred in the muscles on the opposite side to the INB and, if so, could changes in inhibitory, facilitatory intracortical neural networks explain these changes. The present findings of the lack of change in intracortical inhibitory or facilitatory circuits may not be so unexpected given the lack of change seen in the intracortical circuits of a deafferented hemisphere (73).

In another study, the influence of the projections from the non-deafferented M1 to the deafferented hemisphere driving the muscles proximal to an INB was explored. Bicep brachii (BB) MEPs were recorded under conditions with repetitive TMS (rTMS) to the contralateral deafferented hemisphere and ipsilateral non-deafferented hemisphere, with and without an INB, or with just an INB alone. Measures of excitability included MEP amplitudes, SICI and ICF. INB alone resulted in an increase in proximal BB amplitude, but no change in SICI and ICF to those muscles. However, rTMS to the contralateral hemisphere augmented this excitability with a reduction in SICI and an increase in ICF; ipsilateral rTMS however, prevented the increase induced by INB alone (63). Other studies are in

support of this finding, and it can be assumed therefore the transcallosal connection between two homonymous motor representations is predominantly inhibitory (76). As will be discussed below, the transcallosal communication has a major inhibitory role and the data from this study would be in keeping with this.

The increase in the RAPB MEPs contralateral to a left forearm INB would suggest an increase in the motor cortical output from the hemisphere where the sensory input remains intact i.e. non-deafferented. This would therefore mean the deafferented hemisphere must be influencing the non-deafferented hemisphere such that its excitability has increased. Therefore, a reduction in interhemispheric inhibition to this hemisphere as observed in the data, would be in keeping with this mechanistically and the most likely cause for this change (87).

Interhemispheric inhibition is one of the three inhibitory cortico-cortical processes. Here, a stimulus to one motor cortex reduces the size of the MEP generated following a stimulus to the opposite hemisphere (76, 255). IHI pathways influence control during unimanual and bimanual tasks by connecting homologous regions of the primary motor cortex via the corpus collosum (transcallosal pathways) (109, 256). In its simplest form, it limits the movement of one hand whilst the other performs a task,helping to fine-tune motor control; writing is an example of such unilateral activity where mirror activity is inhibited in the non-writing hand (257). It also promotes interlimb transfer of motor skills, whereby motor skills learnt by one hemisphere can promote the execution of the same task, in terms of accuracy and speed, by the untrained hand; there is a direct correlation with the amount of IHI reduction and the acquisition of the motor skill (258). An imbalance has been observed in neurological pathology, such as following a stroke; it has been demonstrated that there is an increase in IHI from the non-lesioned hemisphere to the lesioned hemisphere, thereby limiting movement initiation, skilled movements and functional recovery (259).

In keeping with other inhibitory pathways, GABA is the neurotransmitter responsible for IHI. Two forms of IHI, short and long, have been proposed, acting at $GABA_A$ and $GABA_B$ receptors, respectively,

and occurring at ISIs of 6 and 50ms, respectively (260). However, GABAergic neurons are not thought to cross through the corpus callosum to mediate IHI directly (261). IHI neurones are likely excitatory transcallosal neurons that augment the function of local GABAergic neurons, thereby increasing inhibition in the contralateral motor cortex. (109). This local inhibitory interneuron network is also most likely responsible for LICI and they likely share $GABA_B$ mediated transmission as it is a common pathway (108, 262). Studies agree that IHI has a cortical origin, given its close association to LICI and no inhibition of the H-reflex during IHI paradigms or following electrical stimulation (76, 263).

The results of the present study have shown a significant reduction in IHI from the deafferented hemisphere, with an associated significant increase in cortical excitability (as evidence by the increase in MEP amplitude in the contralateral, non-occluded APB). This has been observed in a previous study, where an increase in FDI MEP amplitude contralateral to the INB was associated with a reduction in IHI to the ipsilateral M1 (87). As has been discussed above, previous research has shown deafferentation results in the exposure of latent excitatory pathways, via disinhibition, which causes an increase in cortical excitability of the deafferented hemisphere, in turn increasing the MEP amplitude of muscles proximal to an INB. For MEPs to increase in muscles contralateral to an INB with an associated decrease in IHI, the function of the excitatory IHI neurones must diminish, thereby decreasing their promotion of inhibitory GABAergic interneurons in the non-deafferented hemisphere and increasing cortical excitability. In the study above (87), Lorazepam, which enhances GABA activity at $GABA_A$-receptors, prevented the increase in contralateral FDI MEP amplitude, implicating GABAergic transmission and thus the release of excitation within the non-deafferented hemisphere, in keeping with disinhibition of the deafferented M1 and the possible mechanism, laid out above. Thus, the deafferentation from the INB leads not only to inhibition of inhibitory neurones in the contralateral hemisphere, but the inhibition of the excitatory neurons mediating IHI.

Pain is inevitable with all INBs and in keeping with the investigations of previous chapters and in the literature, participants experienced similar levels of pain in this study. Unilateral pain can have effects

on motor cortical plasticity in both hemispheres. This investigation was not designed to explore the effects of pain on changes in cortical excitability, however it is likely to have a confounding influence and hence warrants some discussion.

Many studies have shown pain can influence cortical excitability, both increasing and decreasing it. Many factors influence these changes, such as the type of painful stimulus, its duration, and the location of the pain. One study demonstrated heat pain caused by laser cutaneous stimulation increased MEPs, with changes restricted to only the painful muscle (264). Hypertonic saline however, injected into the first dorsal interosseous (FDI) has been shown to cause a decrease in MEP amplitudes in the FDI muscle, as well as the nearby abductor digiti minimi. SICI following this noxious stimulus was increased, whilst ICF was decreased both during and after pain, suggesting differential effects on inhibitory and facilitatory interneurons within an affected motor cortex (215). Cutaneous pain secondary to topical capsaicin in contrast to the previous study, resulted in reduced SICI in the contralateral M1 region with no corresponding change in ICF (265). In the acute pain setting, this decrease in cortical excitability is thought to be a protective mechanism, whereby pain limits muscle activity and movement, although as may be apparent from the literature, the changes in cortical excitability do not always support this. This is supported by patient studies, where reduced inhibition has been demonstrated in the contralateral M1 of unilateral hand pain sufferers and those with isolated peripheral nerve lesions; SICI in the ipsilateral “pain-free” hemisphere however, was equivalent to age-matched healthy control (266, 267).

The aforementioned studies have shown alterations in the cortical circuits assessed using paired pulse paradigms in the contralateral hemisphere following different acute and chronic painful stimuli. The results in the present chapter show no change however, in levels of intracortical facilitation or inhibition. The difference in this investigation is of course, that the increase in MEP amplitude occurs in the APB contralateral to the painful hand undergoing an INB, with an increase in excitability in the ipsilateral “pain-free” M1. Therefore, the influence of pain on interhemipsheric pathways may offer mechanistic insights.

There is evidence in the literature that unilateral limb pain can cause bilateral changes in muscle function and neurophysiology. Changes in motor function such as alterations in speed of movements and reactions times, in the both the ipsilateral (affected) and contralateral (unaffected) limb of patients with unilateral lateral epicondylitis (268) have been observed. In addition, the subjective experience of pain itself is sensed in the unaffected limb (269). This shares similarities with INB paradigm. These bilateral phenomena, where changes in sensorimotor function are seen in a disease-free limb, implicate transcallosal transfer of effects between hemispheres. Given the role of IHI may play in over-exerting its influence from the contralesional to ipsilesional M1 and impairing motor recovery following chronic stroke (270), much work has explored a potentially similar role of IHI in mediating bilateral pain and sensorimotor changes in unilateral pain states. It should be noted however, in all the aforementioned studies, the pain states described are different to the pain caused by an INB.

In one study, IHI was investigated by injecting nerve growth factor into an upper limb extensor muscle over several days to induce persistent musculoskeletal pain. That study found IHI decreased from the contralateral M1 (i.e. the M1 representing the painful muscle) to the unaffected ipsilateral M1. In addition to this, an associated increased sensitivity to mechanical stimuli was observed in the "non-painful" limb (271). The same research group found acute pain instigated by hypertonic saline injected into a hand muscle also caused IHI to decrease from the affected M1 region of the hand muscle to the homologous M1 region of the unaffected hemisphere. These changes occurred at 30minutes after the painful stimulus. There was no pain in the unaffected hand, but there were reduced pressure pain thresholds relative to baseline, with no such change seen in distant control leg muscles. Despite the decrease in IHI, motor cortical excitability of the ipsilateral hemisphere did not change, as evidence by no change in contralateral hand muscle MEP amplitudes (272). In the present study, IHI from the deafferented M1 also reduced to the hemisphere ipsilateral to an INB, in keeping with this pain study above. Thus, it could be the experience of pain, rather than deafferentation, that could initiate a change in cortical excitability via IHI to the muscles contralateral to an INB. Unlike the acute pain study

mentioned above however, contralateral hand muscles MEPs did increase, suggesting an increase in cortical excitability of the unaffected (non-deafferented) hemisphere.

The reasons for this discrepancy may lie in the different mechanisms and durations of pain. The acute pain from the hypertonic saline lasted 9 minutes (272), compared to 30 minutes from an INB. The nature of ischaemic pain from an INB is very different to the pain modelled by a single noxious or mechanical stimulus. As discussed in Chapter 5: Cortical excitability induced by a novel low-pressure INB, ischaemic pain is multi-factorial, being a combination of nociceptive and inflammatory pain, exacerbated by autonomic involvement and microvascular injury (273). It is mostly likely mediated through group III, IV afferent fibres. It differs greatly from localised and comparatively simple mechano-thermal pain models above, which can preferentially activate single afferent nerve types (241). Therefore, despite some evidence in the literature suggesting interhemispheric effects of pain, the complex nature of ischaemic pain, coupled with the complex effects of pain on the sensorimotor neural networks, the influence of pain on data from the present study are difficult to interpret. Indeed, the paradigm was not designed to explore the effects of pain.

A clinical situation where a cerebral hemisphere has been deafferented is stroke. Stroke patients can present with a sensory deficit in up to 80% of cases alongside motor deficits (274), similar to the sequelae of an INB. Also, TMS and functional imaging studies of stroke patients have shown increased cortical excitability in the non-lesioned (unaffected) motor cortex (275, 276), in keeping with changes seen with a unilateral limb pain. Hence, stroke patients could offer some insight into contralateral changes in cortical excitability. In acute stroke, which is reflective of an INB, decreased SICI is observed in the non-lesioned M1 ipsilateral to a paretic hand alongside the increased excitability. These changes, however, correlate with the motor recovery of the paretic side and thus functional output of the lesioned M1, implicating transcallosal effects (277). Although decreased SICI was not seen in the present study, a similar increase in cortical excitability to the contralateral APB was observed. The functional consequence of this increase was not investigated, but it may well have increased in response to the INB and warrants further investigation.

Another potential explanation for the results of this study could be the interactions between the different cortical circuits probed by the paired pulse paradigms. Both interhemispheric and intracortical neural networks can influence the function of each other.

All four intracortical paradigms were performed together at each epoch, but were not measured at precisely the same time; they were performed with approximately 8 seconds between each stimulus. Hence, there is potential for contamination of one state by the measurement of the other. SICI for example is capable of increasing SICF, counterintuitively, via disinhibition. SICI interneurons inhibit a group of $GABA_A$-mediated inhibitory interneurons which normally suppress I-wave generation, causing facilitation. This is particularly true at ISIs of 2-3msec used in this investigation (278). ICF can suppress SICF mediated by these late I-waves, by promoting inhibitory interneuron function (279), which could explain why no net change in facilitation is measured.

Despite these potential interactions, it was demonstrated in this study that baseline measurements before cuff inflation for each paradigm produced the expected change in conditioned MEP amplitude. This would suggest any potential contamination between the intracortical paradigms to be minimal, and the lack of change from baseline during the course of the INB, to be true. However, this does not take into account for any interhemispheric influence.

One study explored the interactions between IHI with SICI and LICI. The authors found significantly less SICI in the presence of IHI compared to SICI in the absence of IHI, and those participants with stronger IHI also had weaker SICI, suggests it is the IHI neurones suppressing the SICI pathways (109). A similar inverse relationship is observed between LICI and SICI (72) and a further similarity is that as the test stimulus intensity increases, IHI and LICI reduce but SICI increases (72, 76).

IHI and LICI are reduced in the presence of each other (109). One explanation offered is that a "saturation effect" may occur (108). IHI is believed to be mediated via the same neural networks responsible for LICI; they both require suprathreshold conditioning stimuli and both inhibit SICI for example. IHI neurones are most likely excitatory and promote the inhibitory LICI interneurons in the

opposite hemisphere (261). Sharing the same effective neural networks therefore means when one inhibitory process has already activated the low threshold neurones that cause inhibition, there are few neurones left for the second process to cause any more inhibition. Also, LICI and IHI, are mediated by $GABA_B$ receptors. These are usually found pre-synaptically, the activation of which can cause autoinhibition (280), thus result in LICI and IHI inhibiting each other.

With regards to facilitatory mechanisms, IHI is not known to have any effect on ICF (108); as mentioned above, IHI is mediated by inhibitory LICI circuits. Interestingly however, IHI can indirectly increase SICF. When SICI and IHI are examined together, the inhibitory effect of IHI on SICI results in the unmasking of SICF with the paradigm probing SICI, thus causing an increase in MEP amplitude after the test stimulus (109)

In the present study, IHI decreased following 30 minutes of INB, but the intracortical pathways remained unchanged. Since it is known IHI can suppress SICI, it is possible there may have been less SICI present towards the end of the occlusion but the reduction in IHI may have increased SICI back to baseline levels. LICI too may have fallen in tandem with IHI and contributed to increasing SICI. IHI has no effect on ICF and if SICI is not reduced there is no unmasking of SICF circuits. The net result therefore is no change in intracortical circuits.

It is evident there are complex interactions between IHI and the intracortical pathways in the non-deafferented M1 ipsilateral to the INB. As a result, we may not have observed any changes in these mechanisms potentially responsible for the increase in cortical excitability to the contralateral APB. Unfortunately, with our current setup, it was not possible to measure intracortical and interhemispheric circuits simultaneously or in isolation to investigate this further.

In addition to the interactions between the intracortical circuits, TS intensity can also influence measurement of intracortical inhibition and facilitation. SICI, ICF and SICF are demonstrable with a test intensity capable of producing a 0.5mV MEP (281). As the test pulse increases, SICI also increases but a reduction in SICF and ICF is seen. This effect is seen between 0.2 and 1mV, with no further effect up to 4mV (279). For this reason, the desired TS amplitude for this study was 1mV, and a mean of 0.89

± 0.13mV was achieved across EO, LO and PO, which would produce adequate SICI, ICF and SICF without an impact on SICI and ICF.

Conclusion

The results of this investigation have shown that a unilateral INB can result in an increase in the corticospinal excitability in the ipsilateral motor cortex not deafferented. This effect is mediated by a decrease in interhemispheric inhibition from the deafferented M1, with no apparent change in the intracortical circuits of the non-deafferented M1.

Since unilateral and bilateral lower limb ischaemia occurs during TAAA surgery, the results described here are relevant to the development IONM of SCI, given the widespread changes in corticospinal excitability which occur in muscles opposite to the INB. Therefore, a greater understanding of the mechanisms underlying these changes will allow accurate interpretation of changes in intraoperative MEPs in the development of spinal cord function monitor.

Chapter 8: TMS and NIRS in Theatre

Introduction

Chapter 1: Introduction and Background and Chapter 3: Survey of Intra-operative Neuromonitoring during TAAA Surgery in the United Kingdom and Ireland, have identified a greater need for intraoperative neuromonitoring (IONM) of the spinal cord during TAAA surgery. With their ease of use and non-invasive qualities, transcranial magnetic stimulation (TMS) and near infrared spectroscopy (NIRS) have been proposed to address this issue. TMS testing so far has shown it to be reliable over a prolonged period of time, such as would be required during surgery, with MEP characteristics in the peripheral vascular disease patient cohort in keeping with heathy volunteers and age-matched control data in the literature. The potential influence of an INB, that will occur iatrogenically during a TAAA repair, on intraoperative TMS-induced MEPs secondary to changes in cortical excitability, has also been investigated and possible mechanisms discussed.

The culmination of the investigations in the previous chapters was to subsequently introduce TMS MEPs into the theatre setting, alongside paraspinal muscle oximetry using NIRS. There were a number of aims that I hoped to achieve. Firstly, an anaesthetic regimen suitable to allow MEP generation needed to be determined and the potential effects of different agents on cortical excitability explored; secondly, the effect of limb ischaemia on cortical excitability and on MEP characteristics under surgical conditions; and thirdly, utilising both TMS and NIRS together intraoperatively and postoperatively, seeing how parameters measured by these methodologies fluctuate with changes in patient physiology, and ultimately, to detect early and late SCI, respectively.

Due to unforeseen circumstances out of my control, this study did not proceed as planned. I encountered many significant obstacles to patient recruitment, which will be discussed in greater detail in the limitations section below. As a result, unfortunately the clinical investigations fell short of what was proposed from the outset; I was only able to perform a small number of TMS and NIRS studies on patients in the operating theatre. Although they are incomplete investigations, it is possible

to make some tentative conclusions regarding the use of these technologies in the surgical setting, and they can provide a framework for future investigations in this area of clinical medicine research.

Experiment 1: TMS in Orthopaedic Surgery

Vascular patients undergoing elective procedures were frequently postponed due to hospital bed shortages, hence recruitment was severely impacted. It was subsequently decided to recruit orthopaedic surgical patients undergoing lower limb procedures where a tourniquet was used, thus resulting in a lower limb ischaemia analogous to that by an arterial clamp. As demonstrated in Chapter 4: An investigation into the variability of MEPs in patients with peripheral vascular disease, the MEP characteristics of PVD patients are comparable to healthy controls, with any differences attributable to age rather than disease. Thus, the inclusion of this relatively well group of patients, who were undergoing day case procedures (i.e. no hospital beds required as the patients would be discharged home the same day), circumvented the problems seen with vascular patient recruitment.

2 patients undergoing arthroscopic knee surgery (keyhole surgery of the knee to usually remove damaged cartilage) underwent upper and lower limb TMS. The patients were fit and healthy, with meniscal injuries. MEPs were recorded from muscles of the upper limb (ABP) and lower limb (TA and/or AH) from the non-operative side, prior to the induction of anaesthesia (baseline), immediately after induction (post-induction), then every 5 minutes after the inflation of the lower limb tourniquet, and on release of the tourniquet. The tourniquet employed was a dedicated pneumatic tourniquet [AT4 Surgical Tourniquet System, Avanthe Health Solutions, USA], with a cuff placed around the mid-thigh and inflated to 300mmHg.

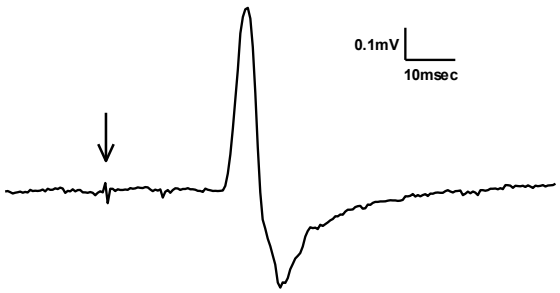
Patient A

56yr old male with degenerative cartilage of the right knee underwent an arthroscopic washout. Induction of anaesthesia was with intravenous fentanyl (an opiate drug) and propofol (liquid

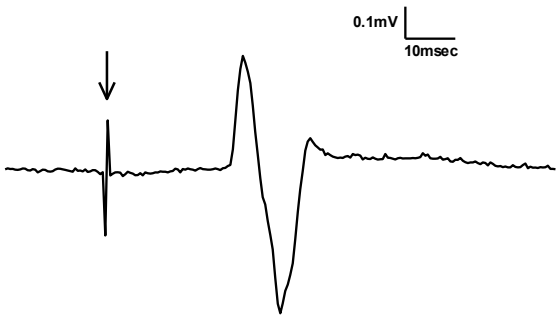
anaesthetic agent), and anaesthesia was maintained using the inhalation volatile anaesthetic gas (or vapour) sevoflurane, carried by an oxygen-air mixture. During the procedure, additional analgesia was provided by boluses of morphine, paracetamol and ibuprofen.

MEPs from LABP, RAPB and LAH were measured at the timepoints described above. RAH could not be recorded from as the foot was part of the surgical field and could not be contaminated with the research electrodes. As can be seen by the representative MEPs from Patient A (see Figure 63), upper and lower limb MEPs were recordable prior to the induction of anaesthesia. Immediately after however, they were no longer present throughout the surgery from any muscles (see Figure 64) and returned only after the anaesthetic agent had been washed out from the patient (i.e. the amount of gas present in the expired air was near zero).

Patient A - Baseline LAPB



Patient A - Baseline RAPB



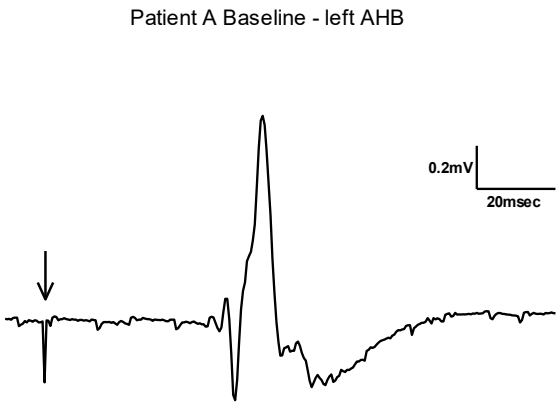
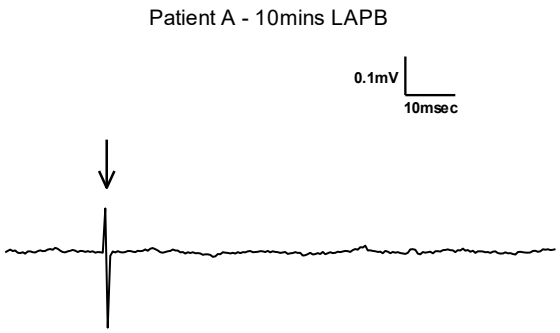
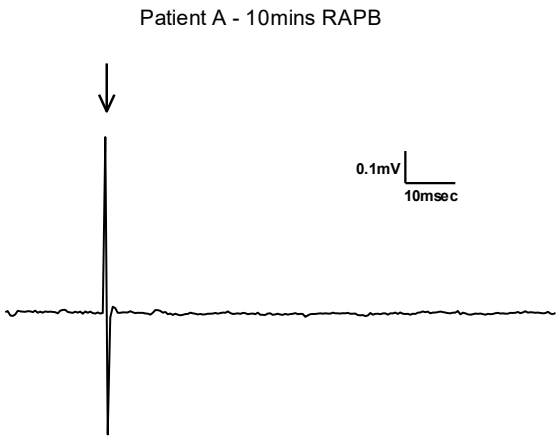


Figure 63: Representative MEPs at baseline from patient A's left abductor pollicis brevis (LAPB), right abductor pollicis brevis (RAPB) and left abductor hallucis (LAH) (top to bottom). Black arrow indicates time of TMS stimulus.





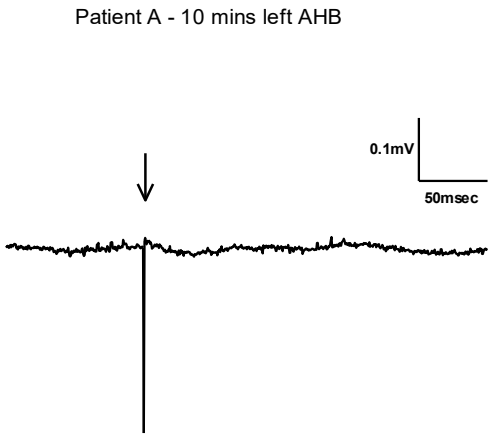


Figure 64: Representative MEPs post-induction from patient A's left abductor pollicis brevis (LAPB), right abductor pollicis brevis (RAPB) and left abductor hallucis (LAH) (top to bottom). Black arrow indicates time of TMS stimulus.

Patient B

24yr old male undergoing a right knee arthroscopy to repair a meniscal tear. Induction and maintenance of anaesthesia was provided by titrated infusions of remifentanil (a short-acting, potent opiate) and propofol, delivered by programmable syringe drivers; a technique known as total intravenous anaesthesia (TIVA). An oxygen-air mixture was used to ventilate the patient without the need for a volatile anaesthetic gas. Analgesia was provided using morphine boluses, diclofenac (a non-steroidal anti-inflammatory drug) and paracetamol.

MEPs were recorded from APB bilaterally and left TA and left AH; the left AH recording was lost on transfer to the operating table, likely due to disconnection, it was not possible to remedy this. Figure 65 shows representative baseline recordings prior to anaesthesia. As can be seen in Figure 66, MEPs were still present post-induction following TMS stimulation, albeit smaller and requiring higher stimulus intensity (100% MSO).

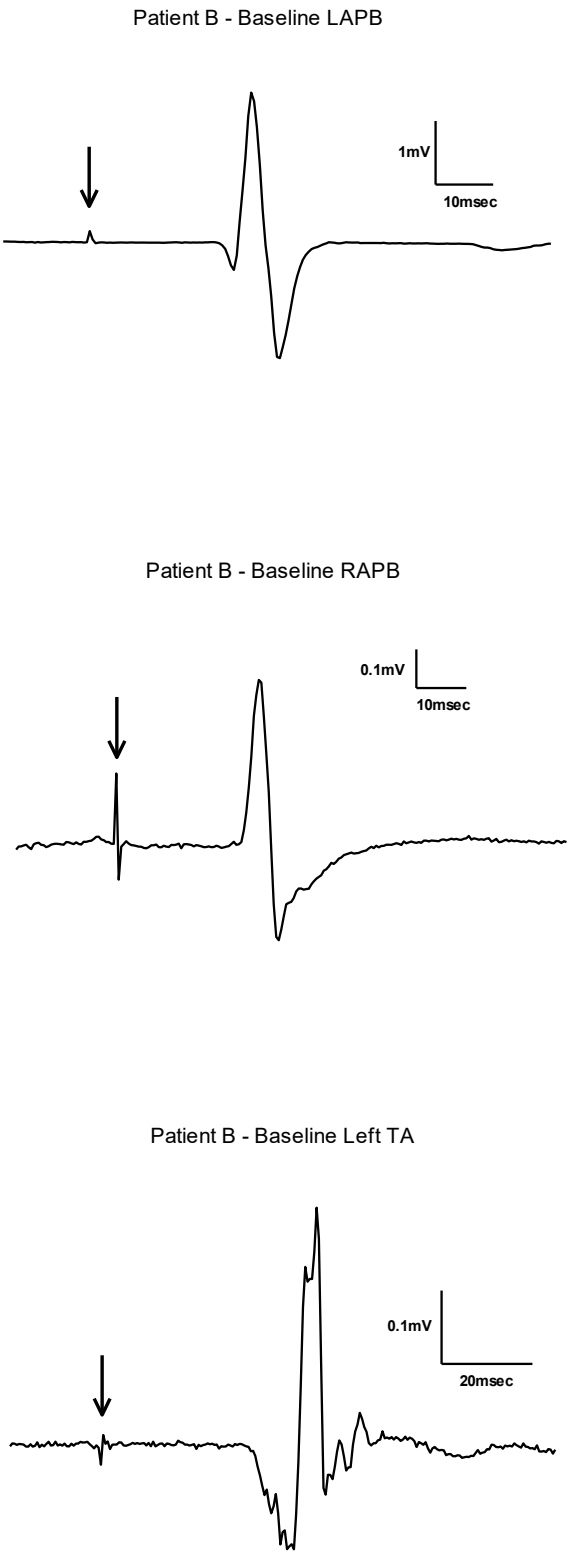


Figure 65: Representative MEPs at baseline from patient B's left abductor pollicis brevis (LAPB), right abductor pollicis brevis (RAPB) and left tibialis anterior (LTA) (top to bottom). Black arrow indicates time of TMS stimulus.

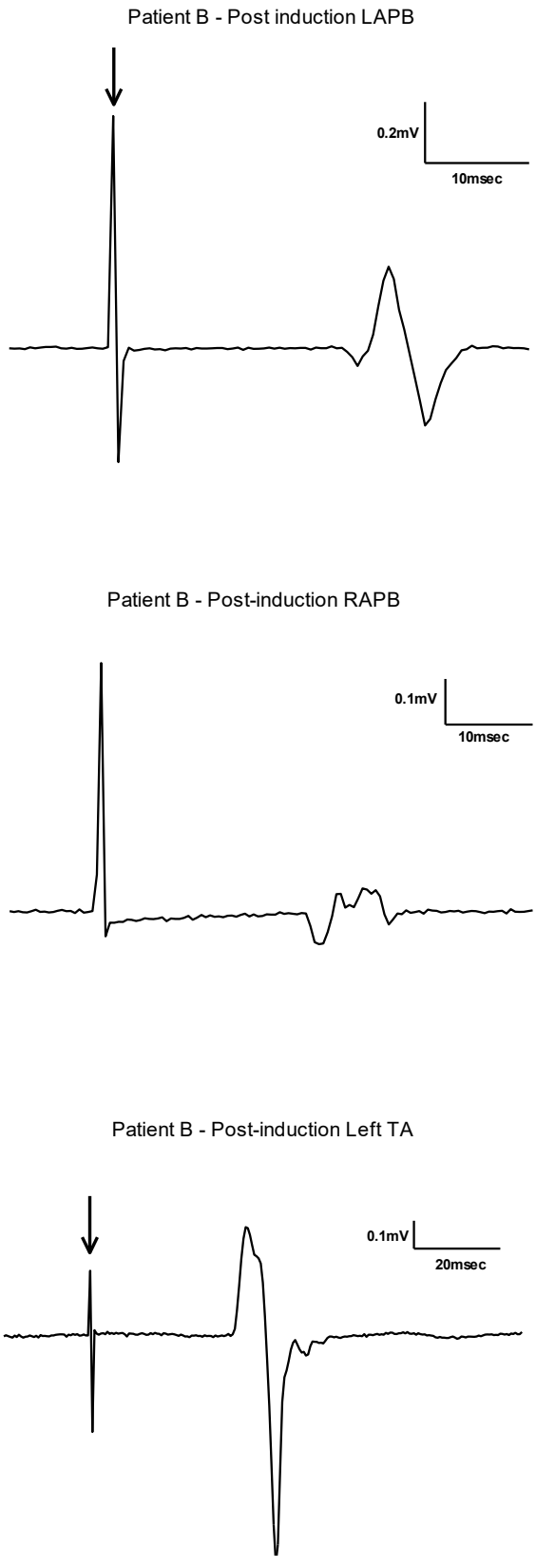


Figure 66: Representative post-induction MEPs from patient B's left abductor pollicis brevis (LAPB), right abductor pollicis brevis (RAPB) and left tibialis anterior (LTA) (top to bottom). Black arrow indicates time of TMS stimulus.

Following 30mins of surgical tourniquet inflation applied to the right thigh, APB and left TA MEPs remained present but were smaller than those recorded immediately post-induction. Unlike in previous Chapter 5-7, where an INB was performed in the laboratory setting, contralateral and distant MEPs did not exhibit an increase in amplitude (see Figure 67).

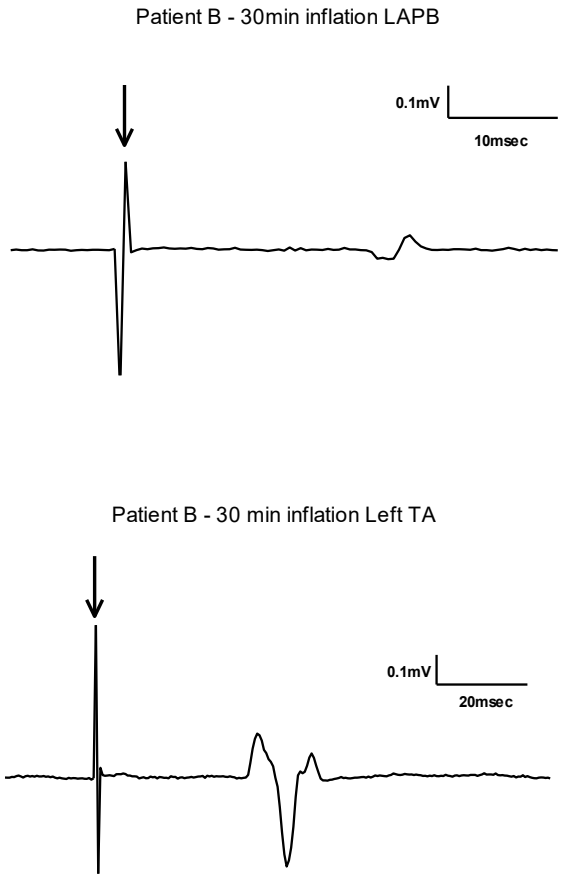


Figure 67: Representative MEPs from patient B's B's left abductor pollicis brevis (LAPB) and left tibialis anterior (LTA) (top to bottom) following 30mins of tourniquet inflation. MEPs from the RAPB were no longer measurable due to interference. Black arrow indicates time of TMS stimulus.

Discussion

Much work has been undertaken to explore the complex mechanisms underlying how anaesthetic agents produce their effects. Drugs within the same class appear to have heterogenous effects, both

at a molecular level and clinically (282), whilst the same effect by different drugs appear to be generated by different mechanisms (283). Albeit a simplification for the purposes of this discussion, the following briefly summarises how volatile anaesthetic agents and propofol produce anaesthesia, how this explains the results of the 2 case studies above and the potential impact this may have on utilising TMS as an intraoperative monitor of spinal cord function.

As shown by the 2 example case studies above, TMS induced MEPs can be generated under anaesthesia. However, this is only possible under certain conditions. Both patients underwent near identical procedures, with respect to surgeon, surgical technique, and duration. They also received the same intra-operative medications; analgesia consisting of paracetamol, the non-steroidal anti-inflammatory drug diclofenac, the opiate morphine, as well as the anti-emetic ondansetron. The only difference was the choice of anaesthetic maintenance agents. Patient A, who lost their MEPs after induction of anaesthesia, was induced by propofol and the opiate fentanyl, then maintained with the volatile agent Seveflurane. Patient B was induced and maintained with an infusion of propofol and the opiate remifentanil.

Volatile anaesthetic agents or "gases" in regular use are halogenated methyl ethyl ethers, differing chiefly in the number of fluoride molecules present in their molecular structure. This in turn changes the lipid and water solubility properties and thus alters properties such as speed of onset and offset of anaesthesia. Anaesthetic gases act by promoting inhibitory $GABA_A$ and glycine transmission whilst reducing excitatory nicotinic acetylcholine (nACh) receptor activation, both the neuronal and muscle sub-types, thus suppressing neuronal transmission (284). This has a global suppressive effect on most brain areas, particularly in the thalamus and midbrain reticular formation (285), resulting in the desired hypnosis with some analgesia. Muscle relaxation and hence immobility, is produced through depression of spinal neuronal transmission and to a lesser degree at the motor endplate, which ultimately prevents muscle contraction and the generation of MEPs (286). This effect is profound; the usual percentage concentration of sevoflurane to produce adequate anaesthesia is 1.6%, whilst MEPs can be significantly attenuated at concentrations of only 0.25% (165).

Propofol's anaesthetic effects are believed to be mediated through effects on a wider range of receptor types. Like volatile agents, propofol promotes glycine and $GABA_A$ transmission within the brain. Unlike the gases however, it has a much less potent inhibitory action on nACh-receptors. In addition, a relatively low potency suppressive effect on excitatory AMPA and NMDA receptors is also observed (see (284)). It too produces the anaesthetic triad of hypnosis, analgesia and muscle relaxation, but it is primarily the first effect that is seen, with the latter being observed at higher propofol doses (284, 287, 288). Studies have demonstrated that compared to volatile agents, propofol has less of an effect on spinal transmission (289). Thus, it is possible to titrate propofol to produce hypnosis whilst maintaining spinal motor neurone function, allowing MEPs to be generated by both TES and TMS.

The data from the 2 patient case studies above are in keeping with the literature. It is well established that volatile anaesthetic agents significantly depress or abolish TMS induced MEPs in animal and human participants; nitrous oxide, the non-halogenated methyl ethyl ether, being an exception (290, 291). This occurs to a lesser degree with TES induced MEPs; the suppressive effects of these gases can be overcome by increasing the TES stimulation intensity, frequency and the number of stimuli per trial being employed (292). This is not the case with propofol, however, where MEPs can be more easily generated with both modes of motor cortical stimulation.

It has been demonstrated in primates lightly anaesthetised by a volatile anaesthetic, both the directly and indirectly (trans-synaptically) activated corticospinal volleys were required to activate the alpha motor neurones and record MEPs where magnetic stimulation was used (293). As the concentration of the anaesthetic gas is increased, I-waves are suppressed and only attenuated directly activated motor corticospinal volleys remain (294, 295). As discussed in

Chapter 1: Introduction and Background, TES and MEP generate MEPs via different mechanisms. At high intensities, TMS, like TES, can directly and indirectly activate corticospinal neurons (296). However, even at high stimulator intensities, TMS cannot produce the same degree of direct activation of TES. Sevoflurane, used with patient A, also preferentially suppresses I-waves (297) and given TMS primarily produces MEPs via trans-synaptic activation of corticospinal neurons, this would explain the loss of MEPs observed in patient A. He was anaesthetised sufficiently deeply to allow surgery to proceed, and therefore to a degree where I wave generation would be abolished.

Where propofol is used as the sole anaesthetic agent, or supplemented by an opioid, TES- and TMS-induced MEPs are more readily produced. As discussed above, propofol has comparatively less suppression of alpha motor neurones at the level of the spinal grey matter; MEPs are seen with TES and TMS but have smaller amplitudes compared to pre-anaesthetic baseline MEPs. This suppression at the spinal level is a dose-dependent effect (298). Compared to volatile anaesthetics, I wave activity is reduced significantly less with propofol. After seveoflurane anaesthesia, I wave activity is reduced by 70% of baseline values, whereas it is only reduced by 39% with propofol (297). This property, coupled with the rapid metabolism of propofol, makes it ideally suited to being increased to the desired end-point of anaesthesia, whilst allowing MEP generation with TMS that is not seen with volatile agents. In keeping with other studies, it was possible to obtain smaller amplitude MEPs, compared to pre-induction MEPs, with patient B undergoing total intravenous propofol anaesthesia(298, 299). The post-induction MEPs could be used as the new baseline with which intra-operative MEPs could be compared.

Improving anaesthetic practices to increase MEP acquisition

MEPs have been recorded under other non-volatile agent anaesthesia. However, more common anaesthetic practices usually limit these now to being adjuncts to supplement propofol, permitting its more judicious use and minimising suppressive effects.

A commonly used class of drugs is opiates, which provide analgesia with some hypnotic effects at higher doses. They reduce ascending transmission to the sensory cortex and depress cortical excitation but have minimal effects at the dorsal horn and on spinal transmission (300). Opiates therefore reduce the amount of volatile or propofol required, whilst maintaining adequate depth of anaesthesia, allowing production of MEPs with larger amplitudes compared to the use of anaesthetic agents alone. As opiates are not true anaesthetic agents, they are seldom used for surgery alone. Infusion at high doses, well above that used clinically, has been shown to reduce MEP amplitudes (298). Ketamine, an NMDA receptor agonist, is used as both an anaesthetic drug and analgesic, particular in the management of chronic pain. Unlike the commonly used anaesthetic agents, it has predominantly excitatory effects and produces a 'dissociative' anaesthesia – it does not produce hypnosis, but a complete disengagement with one's surroundings. At low doses supplementing opiates or propofol, this excitatory effect extents to the cortex and spinal cord, and studies have shown small doses of ketamine can augment MEP amplitudes. Profound psychedelic effects, however, limits its use as a sole agent at higher doses (301). Etomidate is another atypical anaesthetic. It appears to disinhibit subcortical structures, which in turn increases excitability of motor system (302). This makes it ideally suited for use with TMS, as minimal suppressive effects are observed (303). It can be used as an infusion on its own or to supplement volatile agents and propofol. However, due to profound adrenocortical suppression, the risk secondary hypotension, electrolyte disturbances and possible increased infection risk that can occur as a result, needs to be balanced with the need spinal cord monitoring. Unlike other inhalational anaesthetic agents, nitrous oxide is significantly less suppressive. Where used in low concentrations up to 50% as an adjunct to other agents, minimal additional reduction in MEP amplitude is seen(304). In keeping with all agents, higher concentrations of nitrous oxide can cause suppression (305); rarely in current practice is this nitrous oxide used in isolation or at such high concentration however.

Although these effects are beneficial to MEP generation under anaesthesia, they are adjuncts rather than sole agents. They act to improve the anaesthetic, such as provide analgesia, or to limit the amount of anaesthetic administered, thereby reducing side-effects like cardiac suppression. As such,

they are unlikely to be used alone. Certainly, for major surgery such as TAAA repair, which is extremely painful and stimulating, deep anaesthesia is required. Common practice in TAAA repair for example, is to use a titrated propofol infusion with a potent opiate infusion. This provides an adequate balance whereby an appropriate anaesthetic is delivered, but MEPs can still be monitored.

Improving methodology to improve MEP acquisition

Patient A and B both underwent single-pulse TMS. Multi-pulse TMS, which is where 4-6 pulses are delivered at varying frequencies using a dedicated multi-pulse magnetic stimulator, could increase the yield in future studies. This creates temporal summation of excitatory post-synaptic potentials at the ventral horn cells, which increases the likelihood of reaching the threshold for alpha motor neurone firing. Thus, multi-pulse stimulation can overcome the suppressive effects of anaesthetic agents on the motor neurone (306). Single pulse TMS induced MEPs have a smaller amplitude and show considerably more variability (307), whereas increasing the number of stimuli to 4 pulses has been shown to double the MEP amplitude (298). Hence, where TMS has been studied under anaesthesia in the literature, multi-pulse TMS is usually employed (298, 308). For the same reasons, where TES is employed under anaesthesia, multi-pulse stimulation patterns are used (292, 309).

The impact of physiological factors on MEPs also needs to be considered. Hypothermia has been shown in animal models to increase the stimulation threshold and latency of MEPs (310). Interestingly, amplitudes initially increase, but then decrease with progressive hypothermia to 25°C (311). Although in most cases this temperature is not reached, there are some surgeries such as TAAA repair, where deep circulatory arrest is utilised, and such temperatures are achieved. In these circumstances, one needs to be aware of the effects on MEPs if IONM is being used. Hypotension can also reduce MEP amplitudes, secondary to reduced profusion to the spine. Provided the mean arterial pressure is maintained greater than 60mmHg however, this effect is minimal (163). As will be discussed below, pressures lower than this can result in a reduction in MEP amplitudes. In and of itself, hypoxia does not alter MEP characteristics, unless inhaled concentrations are very low (312), where all normal

cellular function, including maintaining electrical potentials and ion gradients, is likely to cease. This would be exceptionally unlikely in the theatre setting, with an airway obstruction preventing ventilation of the patient being the only possible scenario. Ischaemia of the motor pathways, from the cerebral cortex to the muscle, however, due to hypoxia and hypotension or secondary to direct blood flow cessation, can abolish MEPs (313-316).

Anaesthetics and Excitability

As discussed above, anaesthetics modulate inhibition within the brain, in the most part via enhancement of $GABA_A$-receptor mediated transmission. Increases in cortical excitability appear to be due to disinhibitory pathways, where a reduction in GABA transmission is observed (see Chapter 7 – Exploration of mechanisms causing changes in cortical excitability in the motor cortex ipsilateral to an INB, for an in depth discussion). It would follow therefore, that under anaesthesia, no increase in cortical excitability would be observed and more likely a reduction would be seen. A rodent TMS study showed a dose-dependent reduction in MEP amplitude and increase in LICI, when the animals received low and high dose propofol infusions (317). Other studies have shown propofol causes a widespread reduction in cholinergic (318) and glutamatergic (319) transmission, as well inhibiting the function of sodium, potassium and calcium channels throughout the nervous system (320, 321). Thus, in isolation, anaesthetics cause a reduction in cortical excitability.

Patient B, who had measurable MEPs under propofol TIVA, did not demonstrate an increase in MEPs in muscles contralateral or distant to a 30-minute INB; non-anaesthetised healthy volunteer studies in the literature and within this thesis show this would prompt an increase. Of course, patient B did not undergo an INB in isolation. Therefore, patient B results coupled to the data in the literature, would suggest that under anaesthesia, an increase in cortical excitability following limb ischaemia with an INB would seem unlikely. To my knowledge however, there is no formal investigation of the net effects on cortical excitability of an INB, which increases excitation, under general anaesthesia, which reduces excitation. In addition, the duration of an intra-operative INB during TAAA repair would be 60

minutes approximately, which might also increase the likelihood overcoming the tonic suppression of anaesthesia.

This certainly warrants further investigation and a conclusion on the impact of anaesthesia whilst performing an INB cannot be reached from analysis of the data from one patient. Future studies should focus on the effects of additional anaesthetic drugs, such as ketamine, on cortical excitability whilst an INB performed and the impact of a longer INB. As discussed above, stimulation patterns affect MEP amplitude and their impact on excitability under anaesthesia should be explored.

Limitations and TMS in Theatre

The data of the patients are limited in their interpretation as only 2 investigations were performed under anaesthesia. This is due to a number of unforeseen circumstances which severely restricted recruitment. The initial plan had been to perform TMS on patients undergoing lower limb arterial or abdominal aortic aneurysms procedures. This however was hindered by last-minute cancellation of patients' surgery who had consented to participate in the research; cancellations were often due to a shortage of appropriate post-operative inpatient beds. Often when these patients were rebooked for surgery, I was not informed or had clinical commitments elsewhere. Thus, to increase recruitment, orthopaedic patients having knee or lower leg surgery were added to the criteria. Although not the same cohort of surgical patient, lower limb ischaemia was performed with a pneumatic tourniquet, mimicking that seen in vascular surgical patients. This started to yield success and 2 patients were quickly recruited. However, then the Covid-19 pandemic occurred, and all non-urgent surgical cases were postponed, non-essential research stopped, and clinical staff (including myself) were redeployed to support the health service. Many attempts have been made to re-establish the research project, however patient reluctance, hospital restrictions on non-essential individuals within the hospital and theatres, as well as my personal increased clinical demand, has prevented further patient study.

Despite this, some useful scientific data has been obtained and is discussed above. Just as importantly, much needed experience has been gained as to the feasibility of performing TMS in the theatre

setting. For one, the time taken to perform baseline and intra-operative measures has been determined, which can better inform the clinicians about how much extra time the study will add to their case. In addition, a number of practicalities were resolved. These include, but are not limited to, where it is best to situate the Magstim stimulator so as to not obstruct staff, doors or the patient bed; where to run the electrode wires so they are not a nuisance to the clinical setup; and even how to secure electrodes to prevent displacement during surgery when they are inaccessible. All of these factors will allow for smoother and more efficient running of future theatre investigations.

Experiment 2: NIRS in TAAA Surgery

As outlined in Chapter 1: Introduction and Background, the potential of paraspinal muscle rO_2 measured using near-infrared spectroscopy (NIRS) for detecting ischaemic spinal cord injury was discussed. Animal and patient studies (albeit with very low participant numbers) had shown paraspinal muscle desaturation secondary to deliberate intercostal segmental artery ligation or intraoperative events correlated with TES MEP amplitude decreases or clinical paralysis (96, 99).

The first phase of the study involved measuring paraspinal rO_2 in conjunction with routine intraoperative TES MEPs in patients deemed at high risk of SCI. The aim of this investigation was to build upon this evidence in the literature and to gain experience of performing the measurements. The second phase was to then introduce TMS MEP measurements alongside the current gold standard TES MEPS. This would be done intraoperatively, having learnt how to generate them during surgery and their behaviour during limb ischaemia, using data obtained from Experiment 1 (see above). The NIRS would also then be continued in the post-operative period for those patients undergoing TEVARs at risk of late spinal cord ischaemia; if a desaturation (a reduction in rO_2 reflecting a reduction in blood supply) were to be observed then TMS MEPs would be measured to determine if an injury had occurred.

Unfortunately, due to the difficulties discussed above with patient recruitment and study interruptions, much of this study was not possible. The data presented is from the 4 patients that were recruited, and their findings are presented and discussed below.

Patient Demographics

The data presented for experiment 2 are from 4 patients (see Table 8).

Patient	Age (yrs)	Sex	Pathology	AAA extent	Surgery	Outcome
1	27	M	Marfan's	2	Open	Paraparesis - recovered
2	47	M	Marfan's	2	Hybrid	No SCI
3	45	F	Marfan's	2	Hybrid	Died on ICU
4	66	M	Previous aortic dissection secondary to hypertension	2	Open	No SCI

Table 8: Demographic data of patients enrolled in experiment 2. M = male, F = female, Open = open TAAA repair. Hybrid = open and endovascular TAAA repair, ICU = intensive care unit.

Setup and intra-operative protocol

NIRS

NIRS optodes (Adult SomaSensor, Covidien, MA, USA) were placed vertically over the left and right paraspinal muscles at the vertebral level L4/5 ("lumbar" level) and T3/4 ("thoracic" level) (98) and connected to a cerebral oximetry monitor (Somanetics INVOS^{TM} 5100C Cerebral/Somatic oximeter; Covidien, MA, USA (see Figure 14)). Recordings were started 10 minutes pre-induction of anaesthesia to establish baseline values and continued until the end of surgery. During transfer of the patient from the anaesthetic room to the theatre table, the optodes were temporarily disconnected whilst the patient was settled for surgery.

The NIRS monitor has an inbuilt sample rate of 5 seconds, and automatically stores the recordings on an in-built hard drive. This was later extracted to Microsoft Excel for analysis.

Blood pressure

The blood pressure was manually recorded on to an experimental sheet. The values were obtained from an invasive arterial blood pressure line inserted in the radial artery, by the anaesthetic team responsible for the patients' care.

Transcranial electrical stimulation MEPs

TES MEPs were recorded by a commercial neurophysiologist employed specifically for these high-risk cases. As a result, some details of the stimulation technique and equipment are unknown.

Once anaesthetised, invasive, stimulating scalp electrodes were screwed into the scalp overlying the motor cortex of each patient. Recording needle electrodes were inserted bilaterally into abductor pollicis brevis, flexor carpi ulnaris, vastus lateralis, tibialis anterior and abductor hallucis muscles.

Stimulation patterns and intensities were determined by the neurophysiologist independent of the research and clinical teams. MEP data, such as amplitudes, were provided to the research team for further analysis in Microsoft Excel.

Anaesthesia

The conduct of anaesthesia was determined by the clinical teams. All patients received total intravenous anaesthesia using a combination of propofol and the opiate remifentanil. A small dose of the muscle relaxant rocuronium was administered to facilitate intubation of the trachea, but its effects had worn off by the time MEPs were being recorded. Patients also received small, regular doses of ketamine for analgesia. All patients had a spinal drain inserted to measure cerebrospinal fluid (CSF) pressure.

Surgery

All patients required an open procedure; 2 procedures were combined in part with endovascular surgery to aortic branch vessels. This consisted of a large incision from left axilla to the lower abdomen, exposing the chest and abdominal contests. All patients also went on to left heart bypass; this is where the left atrium is cannulated, and the blood pumped with a centrifugal device to the iliac

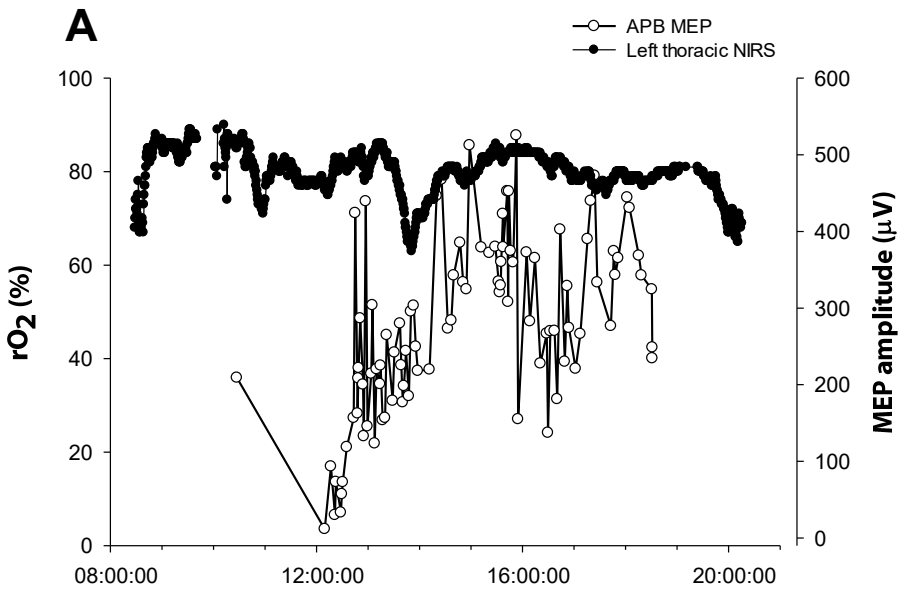
or femoral artery. This allows oxygenated blood to perfuse the pelvis and lower limbs, whilst 'bypassing' the aorta and allowing the surgeons to dissect the aneurysm. As a result, there is limited flow in the mesenteric and radicular circulations, increasing the risk of SCI.

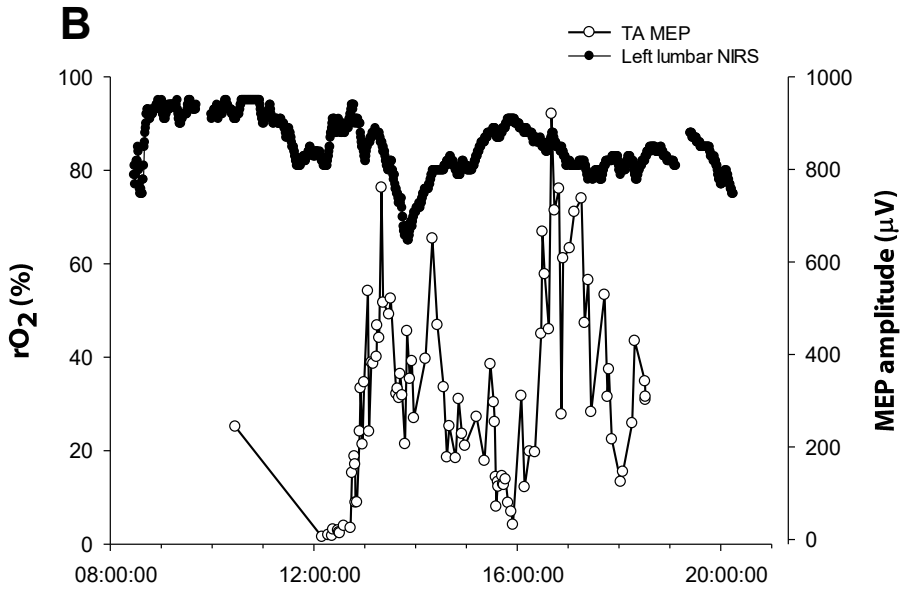
Results

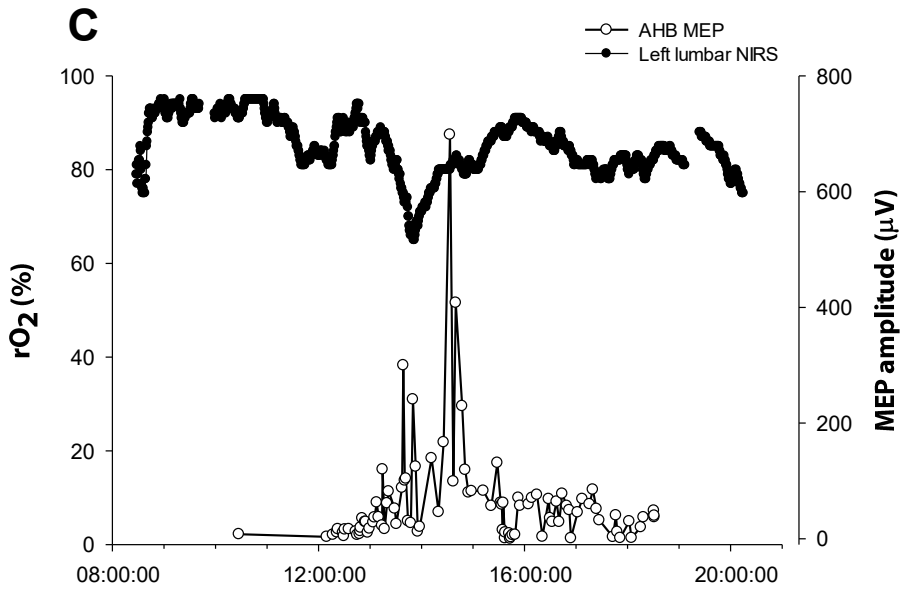
The results below are from these 4 patients. Where data show an event of particular interest, it has been highlighted here for later discussion.

Patient 1

The results below show the MEP amplitude and the rO_2 values over time (see Figure 68). Upper limb muscle MEPs have been plotted with thoracic rO_2 and lower limb muscle MEPs with lumbar rO_2, with their corresponding laterality. Data from right TA is not included here due to loss of EMG..




B
TA MEP
Left lumbar NIRS
100
80
60
40
20
0
1000
800
600
400
200
0
rO2 (%)
MEP amplitude (μV)
08:00:00
12:00:00
16:00:00
20:00:00


C
AHB MEP
Left lumbar NIRS
100
80
60
40
20
0
800
600
400
200
0
rO2 (%)
MEP amplitude (μV)
08:00:00
12:00:00
16:00:00
20:00:00

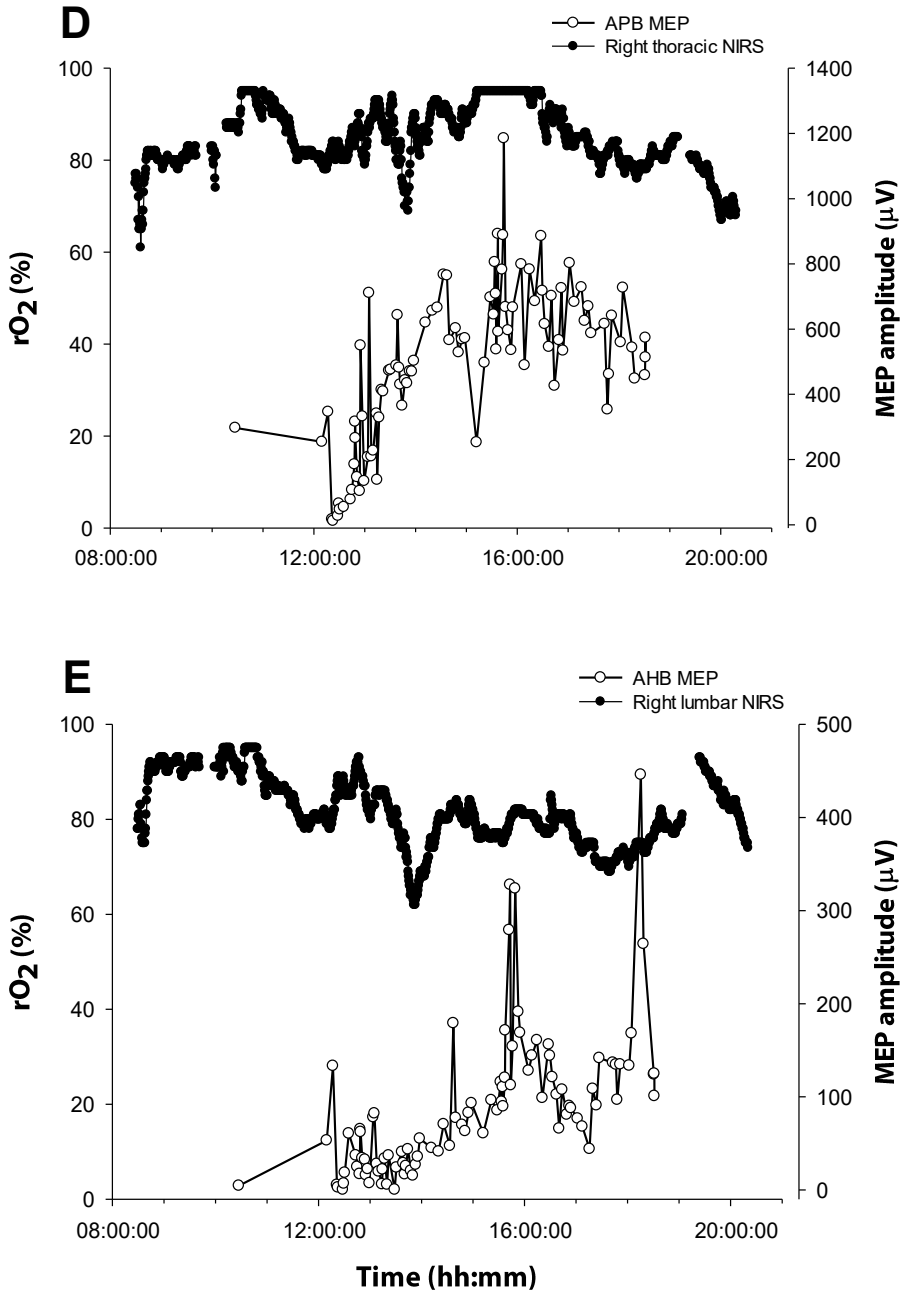


Figure 68: Graphs displaying the MEP amplitude (μV) and paraspinal rO_2 (%) against time of surgery (hh:mm) for A) left APB, B) left TA, C) left AH, D) right APB and E) right AH for Patient 1. Upper limb muscles plotted with thoracic rO_2 and lower limb muscles with lumbar rO_2.

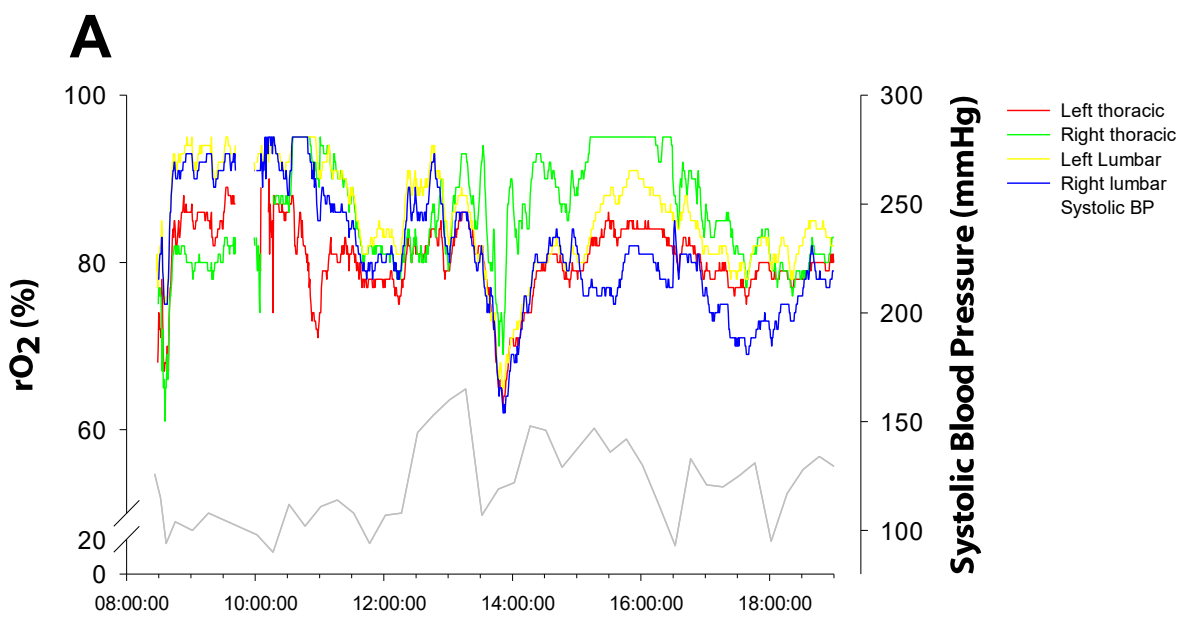
The starting rO_2 for all locations is between 60-70%. Soon after induction of aneasthesia, the values increase to 85-90%, reflecting increased delivery of oxygen during anaesthesia, as is routine practice, thus increased oxygen delivery to the paraspinal muscles. MEP recording is initiated 90-120mins after NIRS, reflecting the time taken to insert intravenous lines and invasive monitoring required for safe surgery; once these are established, the neurophysiologist is permitted to insert their electrodes and

start monitoring. In general (except for in Figure 68E), baseline MEPs amplitudes are higher than those recorded subsequently. Before surgery commences, although the patient 'asleep', they are not heavily (or 'deeply') anaesthetised, as there is no external stimulation e.g. pain from surgical incision. When surgery starts, the level of anaesthesia is increased to mitigate for increased afferent stimulation, which in turn suppresses MEP amplitude. Following this, rO_2 and MEP amplitude changes reflect alterations in physiology, anaesthetic depth and may also reflect changes in stimulation parameters of TES, determined by the phases of surgery. MEP recording stops 90-120 minutes before the completion of surgery, whilst NIRS remains in place, as the neurophysiologist terminates stimulation once the risk to the cord ceases i.e. surgery is over except for closes the muscle and skin layers. This initial and final process is true for all patients discussed in this section of the chapter. At 19:30 approximately, the sensors are temporarily disconnected as rhe patient's position is altered, as can be seen by the loss of rO_2 in all graphs in Figure 66Figure 68.

Figure 69A shows the rO_2 values from all four sensors plotted against systolic blood pressure (SBP) across the duration of surgery. As can be seen, rO_2 values correlate well with SBP values and in general, changing congruently. Figure 69B is a snapshot of 2hours which particularly demonstrates this close relationship. The global reduction in all NIRS values at this particular point in surgery reflects a period of sudden blood loss. The blood pressure is reasonably maintained by vasopressor medcation, but the comparative lack of blood (and hence haemoglobin) causes reduced rO_2. Once the blood is replaced, the rO_2 values increase, as does the SBP. MEPs remain present (see Figure 68) as rO_2 remain within 20% of baseline. In Figure 69C there is a divergence of this relationship, where all values gradually fall but especially the right lumbar rO_2, which is lower than the others, having previously being the same or higher (see Figure 69B) but eventually recovers over hours. This reduction is about 20% of baseline with no obvious associated cause.

On waking in the ICU some days later, patient 1 had mild weakness of right lower limb. On retrospective review of intra-operative MEPs, there is no MEP loss; both left and right lower limb MEPs are present albeit smaller than the upper limb MEPs, and no different compared to baseline. As mentioned above, a reduction in right lumbar rO_2 was observed, but at the time was not considered

indicative of an injury given MEPS remained present. Over the coming weeks, the patient made a full recovery of their motor function and was discharged well.



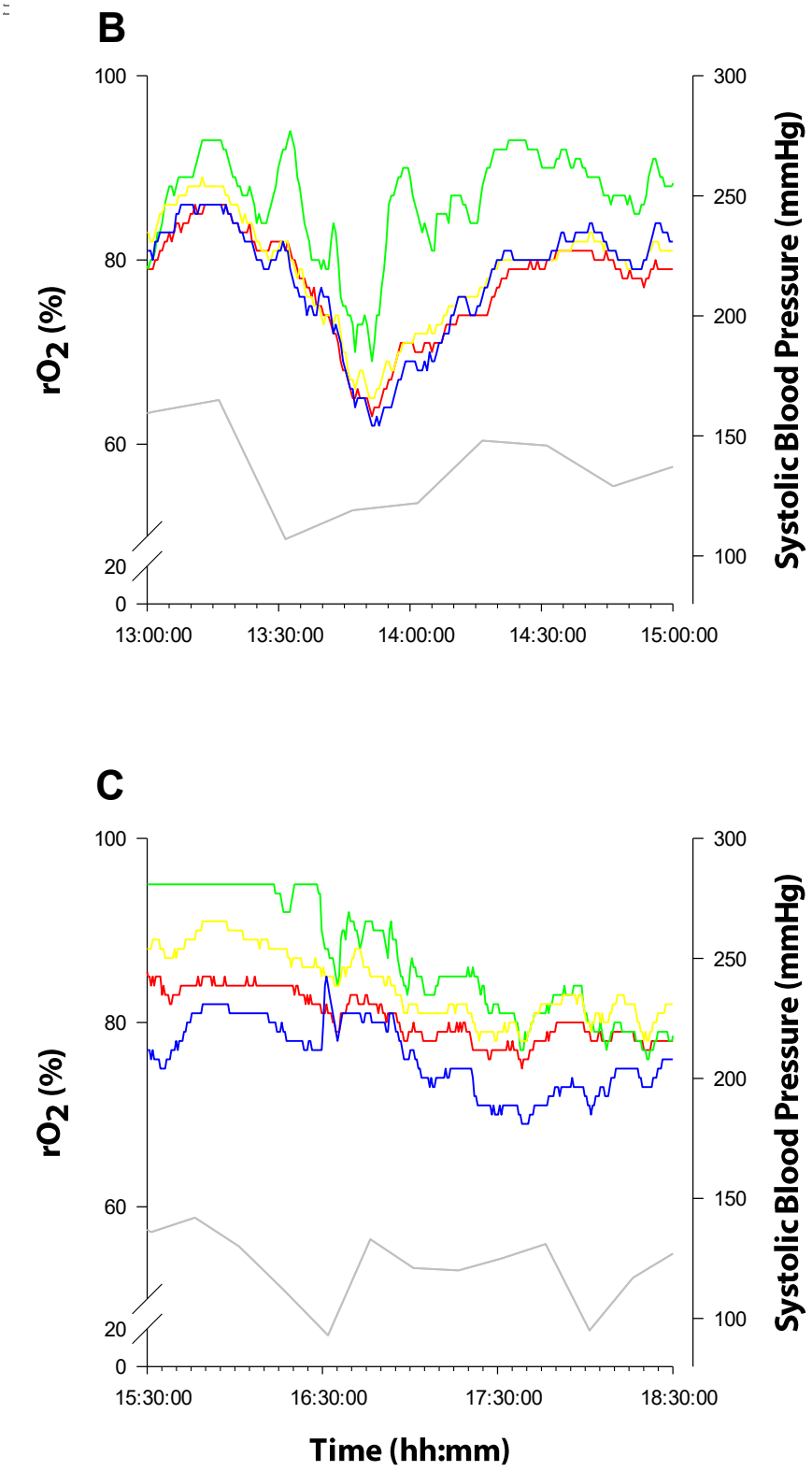
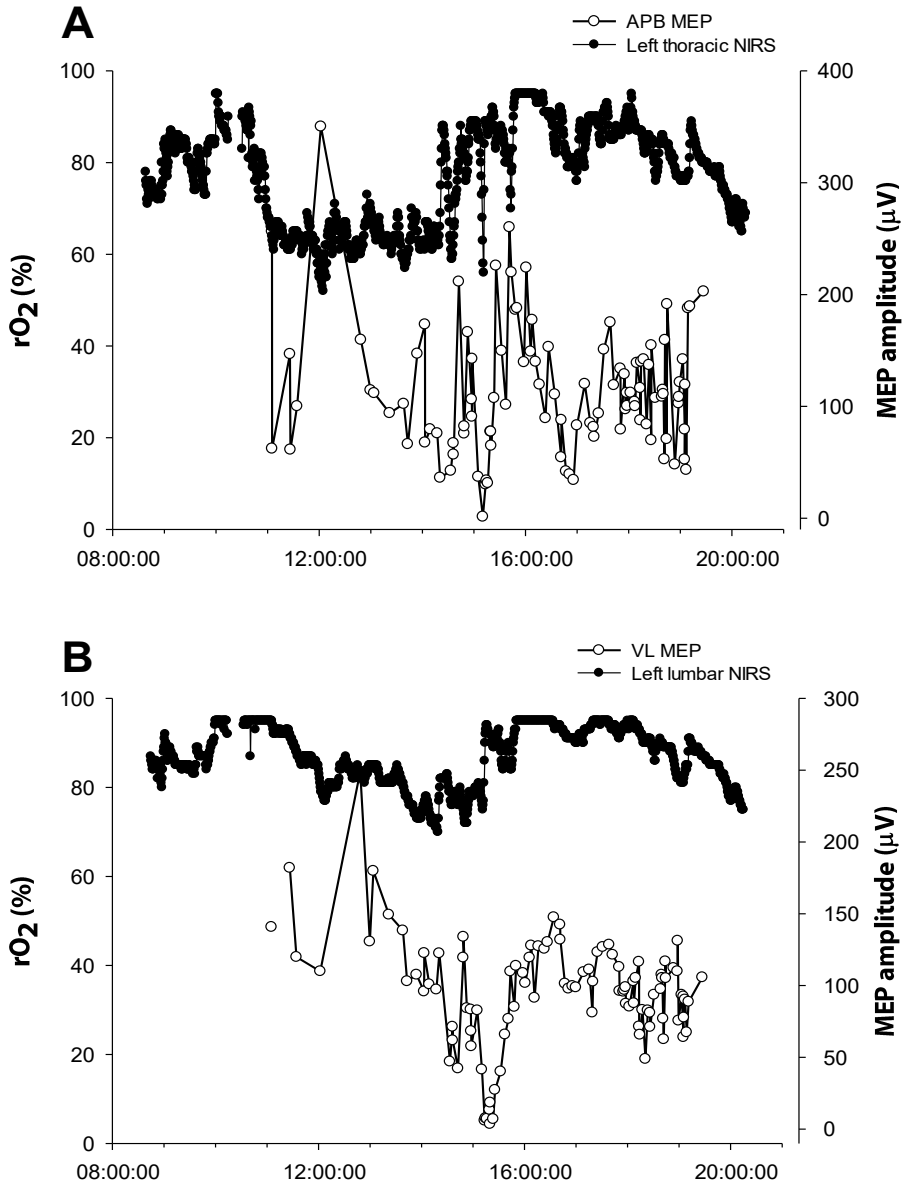


Figure 69: Systolic blood pressure (mmHg) and rO_2 (%) from the 4 optodes plotted against time (hh:mm) for patient 1; A) whole surgery, B) from 13:00 to 15:00 showing close relationship between variables, and C) from 15:30 to 18:30 where a fall is seen, particularly in right lumbar rO_2 (blue line).

Patient 2

Figure 70 and Figure 71 shows the left and right sided MEP and rO_2, respectively, plotted for the duration of surgery for patient 2. As described with Patient 1 above, early initial and final periods of monitoring show a similar recording pattern. During the surgery, there is a gradual reduction in rO_2 , with a nadir at approximately 15:00. This is associated with a a gradual reduction in all MEPs, which are also at their lowest at approximately 15:00. . Despite the reduction in rO_2, the values remain within 20% of pre-anaesthetic baseline values, except interestingly for bilateral thoracic rO_2. This reduced oxygenation to the upper part of the paraspinal muscles and upper body most likely reflect the change in perfusion dynamics secondary to the left heart bypass, which increases flow to the gut, pelvis and lower limbs. With lumbar rO_2 remaining within 20% of baseline, and all associated MEPs remaining measurable, a SCI is not likely to be present. Another interesting observation is the right sided thoracic and lumbar rO_2 values are higher than their paired left sided values; right sided values in fact reach a maximum (90%) and remain there for some period of time (see Figure 72). This is due to the patient being turned slightly on to their right side to assist surgical access, which results in increased blood flow due to gravity to the dependent right side, thus increasing rO_2.

Widespread changes in MEPs such as these, with tandem reduction in are usually due to the patient being hypotensive and subsequent hypoperfusion to the limb nerves and muscles. As can be seen in Figure 72, patient 2 is persistently hypotensive, which is reflected in the rO_2 values. When the SBP is increased (alongside a reduction in anaesthetic depth, on suggestion of the neurophysiologist), both the MEPs and paraspinal rO_2 recover.


A
APB MEP
Left thoracic NIRS
100
80
60
40
20
0
400
300
200
100
0
rO2 (%)
MEP amplitude (μV)
08:00:00
12:00:00
16:00:00
20:00:00
B
VL MEP
Left lumbar NIRS
100
80
60
40
20
0
300
250
200
150
100
50
0
rO2 (%)
MEP amplitude (μV)
08:00:00
12:00:00
16:00:00
20:00:00

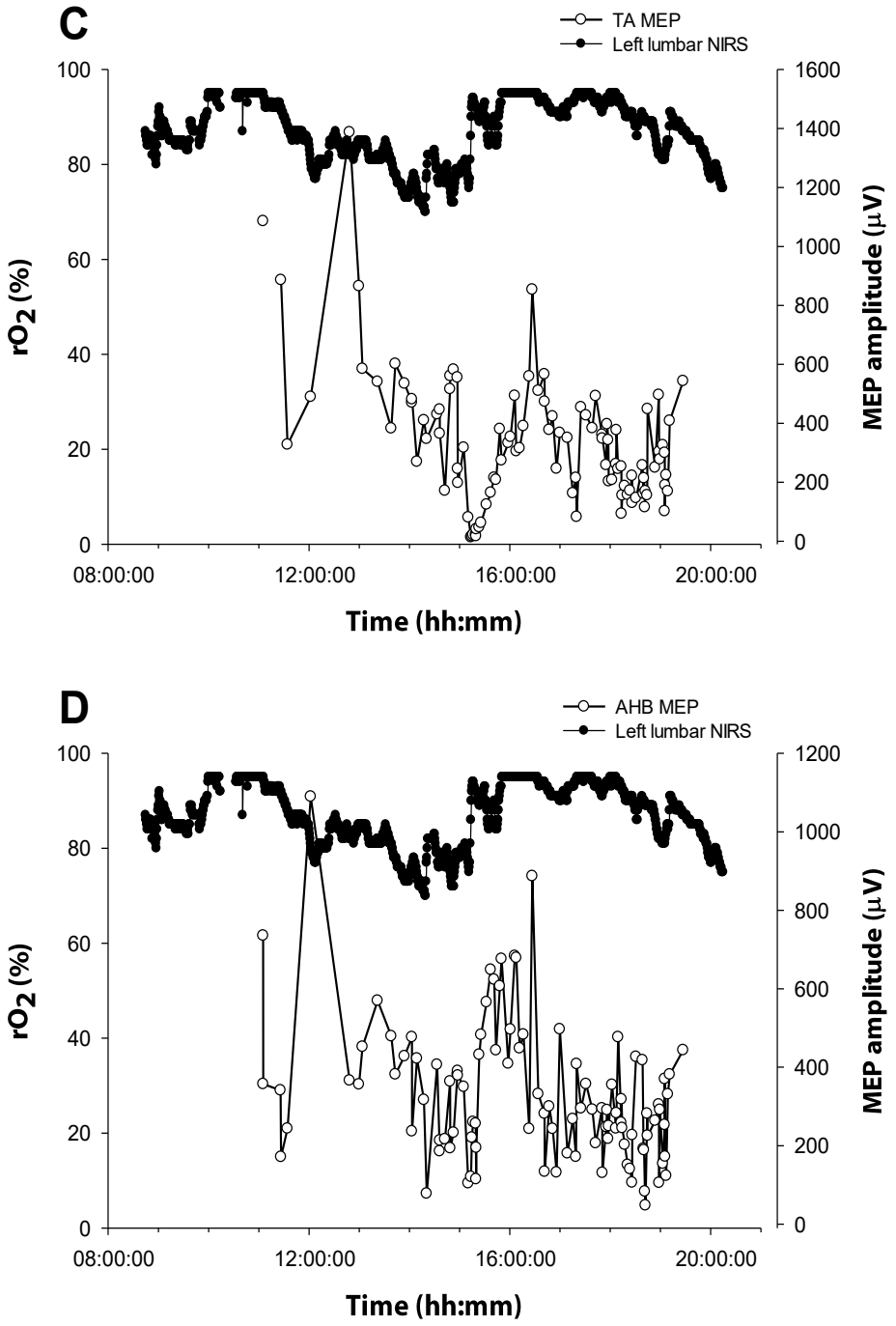
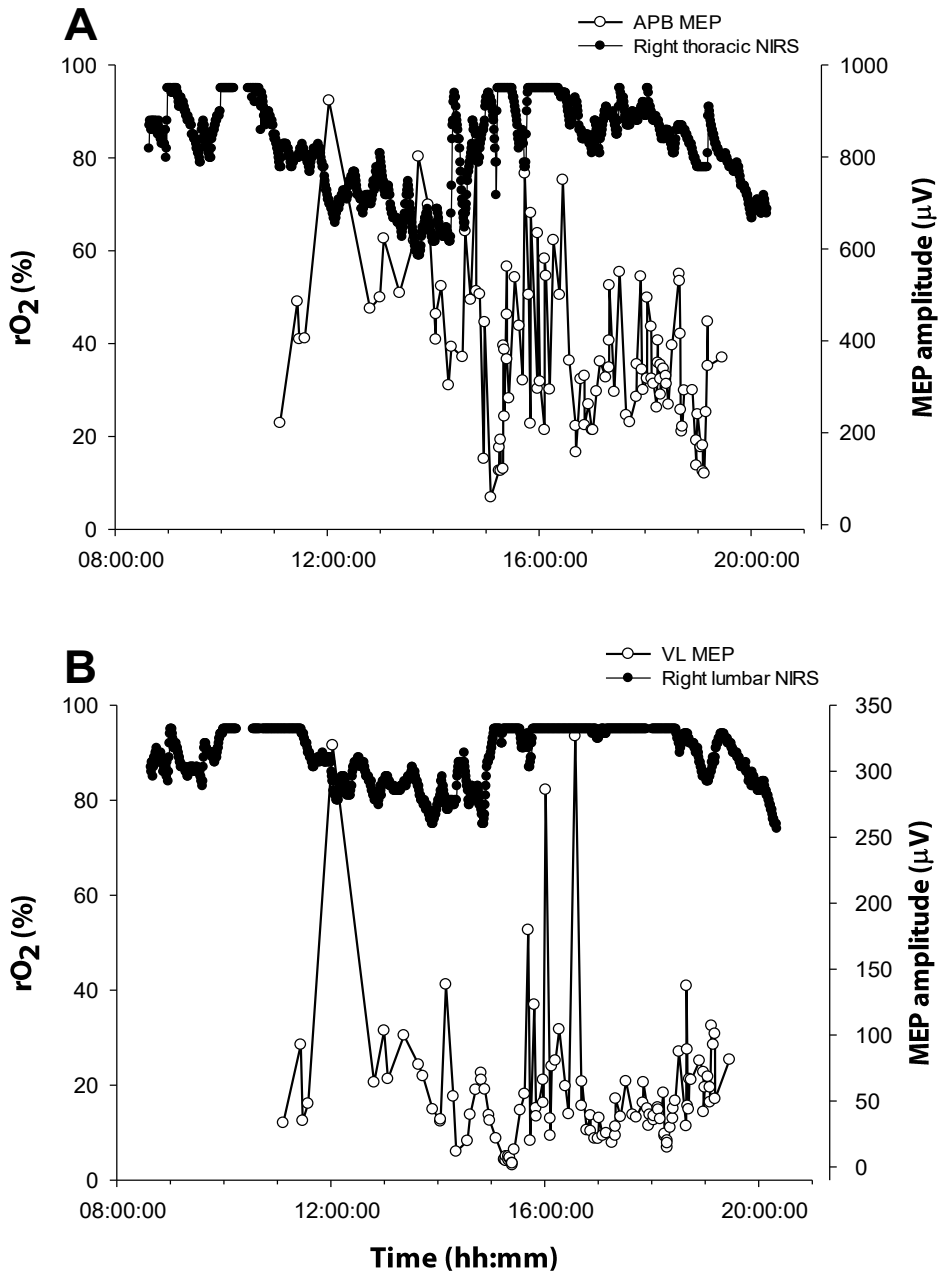


Figure 70: MEP amplitude (μV) and rO_2 (%) across time (hh:mm) for left A) ABP, B) VL, C) TA and D) AH muscles for patient 2.


A
APB MEP
Right thoracic NIRS
100
80
60
40
20
0
1000
800
600
400
200
0
rO2 (%)
MEP amplitude (μV)
08:00:00
12:00:00
16:00:00
20:00:00
B
VL MEP
Right lumbar NIRS
350
300
250
200
150
100
50
Time (hh:mm)

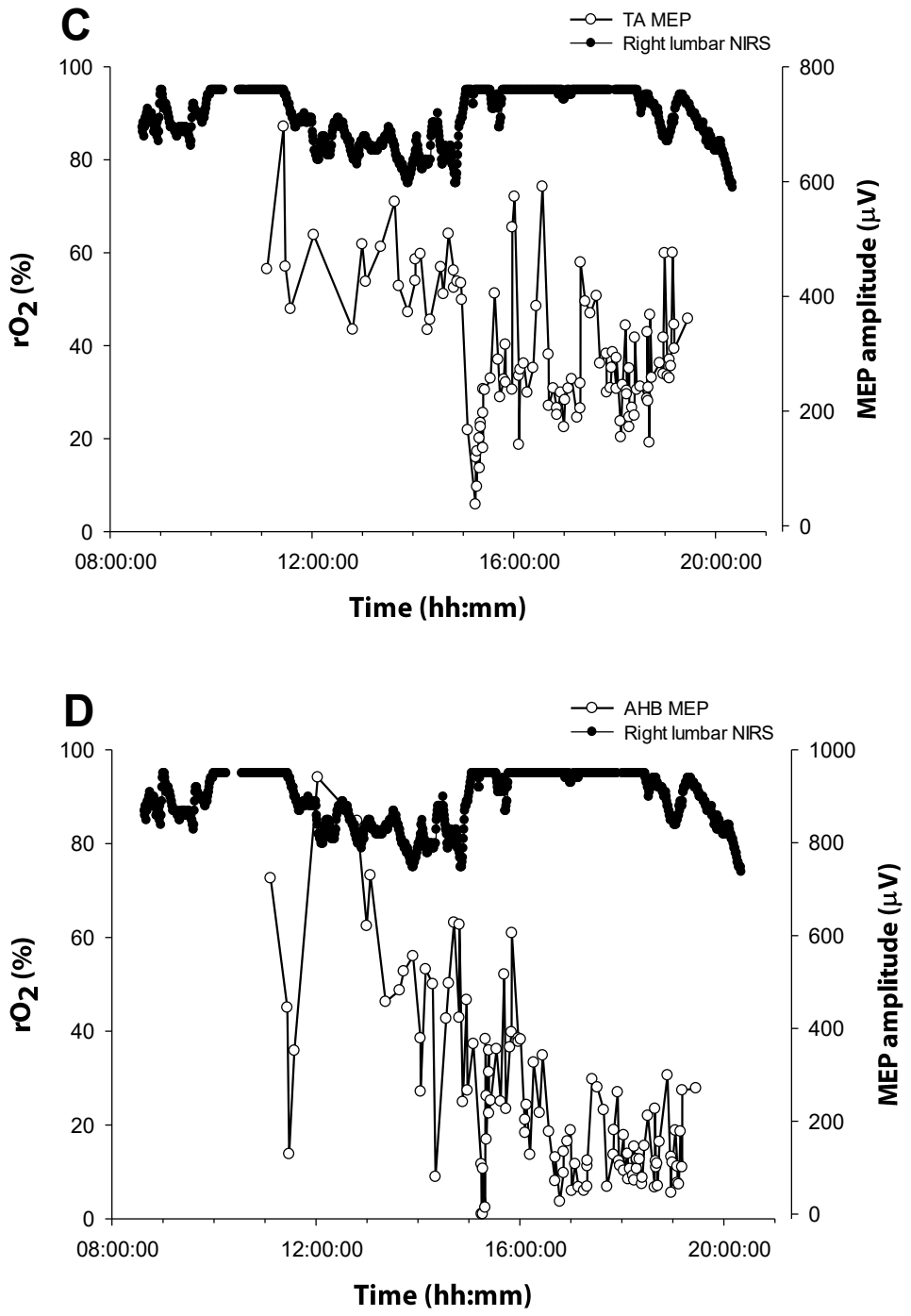


Figure 71: MEP amplitude (μV) and rO_2 (%) across time (hh:mm) for right A) ABP, B) VL, C) TA and D) AH muscles for patient 2.

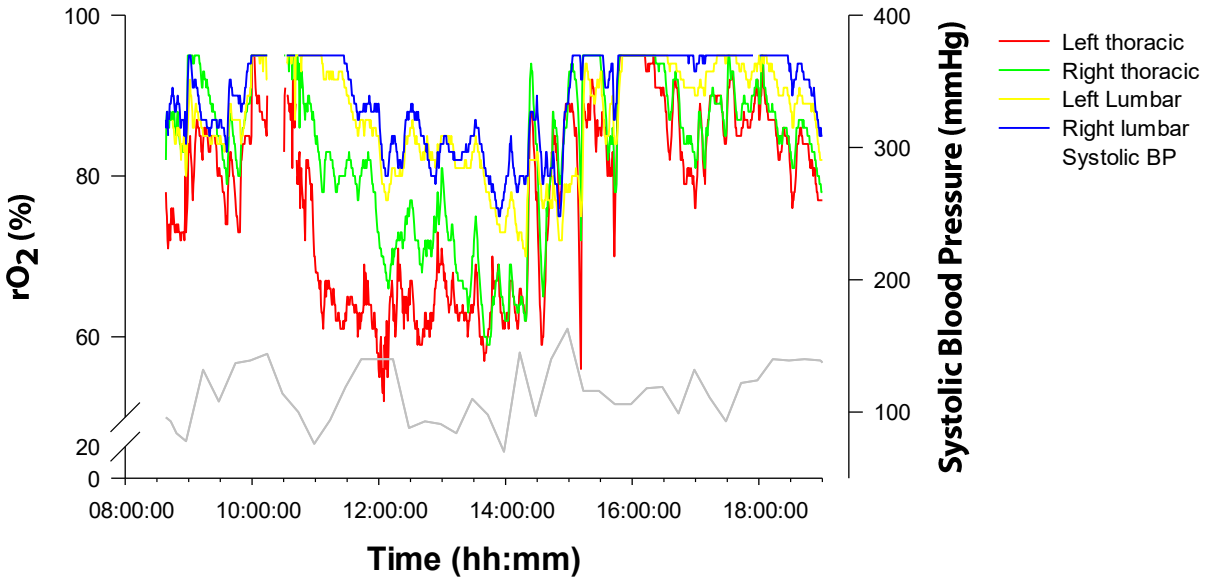


Figure 72: Graph showing the paraspinal rO_2 (%) from 4 optodes and the systolic blood pressure (mmHg) plotted against time (hh:mm) for patient 2.

Patient 3

The results of patient 3 are shown below. At approximately 14:30, the patient had a profound hypotension and peri-cardiac arrest (see Figure 73). This is evident in the data where a precipitous and dramatic fall in SBP is associated with a reduction in all rO_2 values, especially bilateral thoracic values. At this point the patient was still on left heart bypass, hence the lower body circulation was assisted to a degree by the cardiopulmonary bypass circuit and hence the lumbar values are greater.

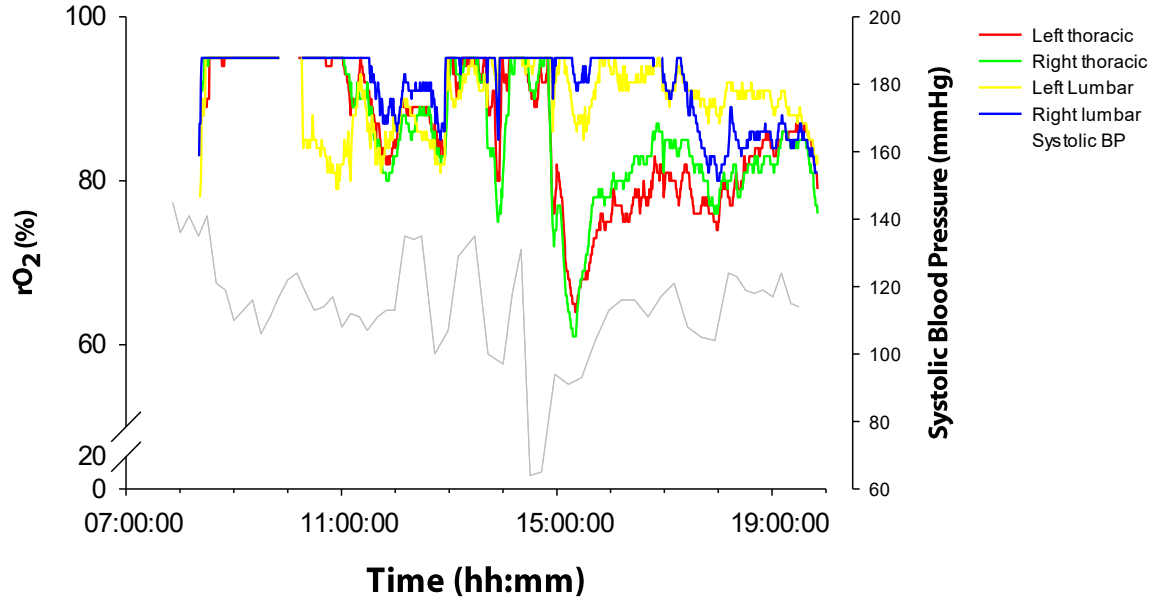
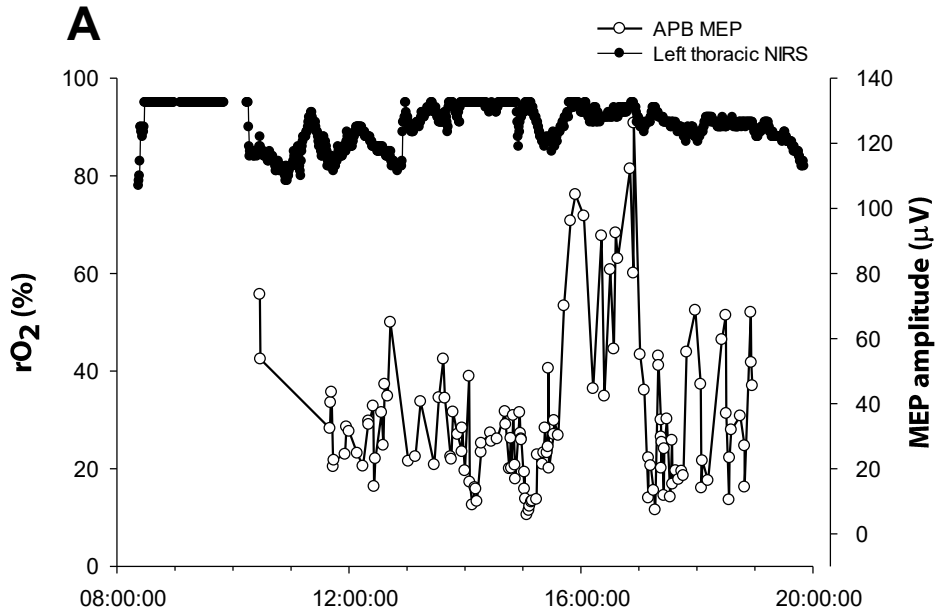
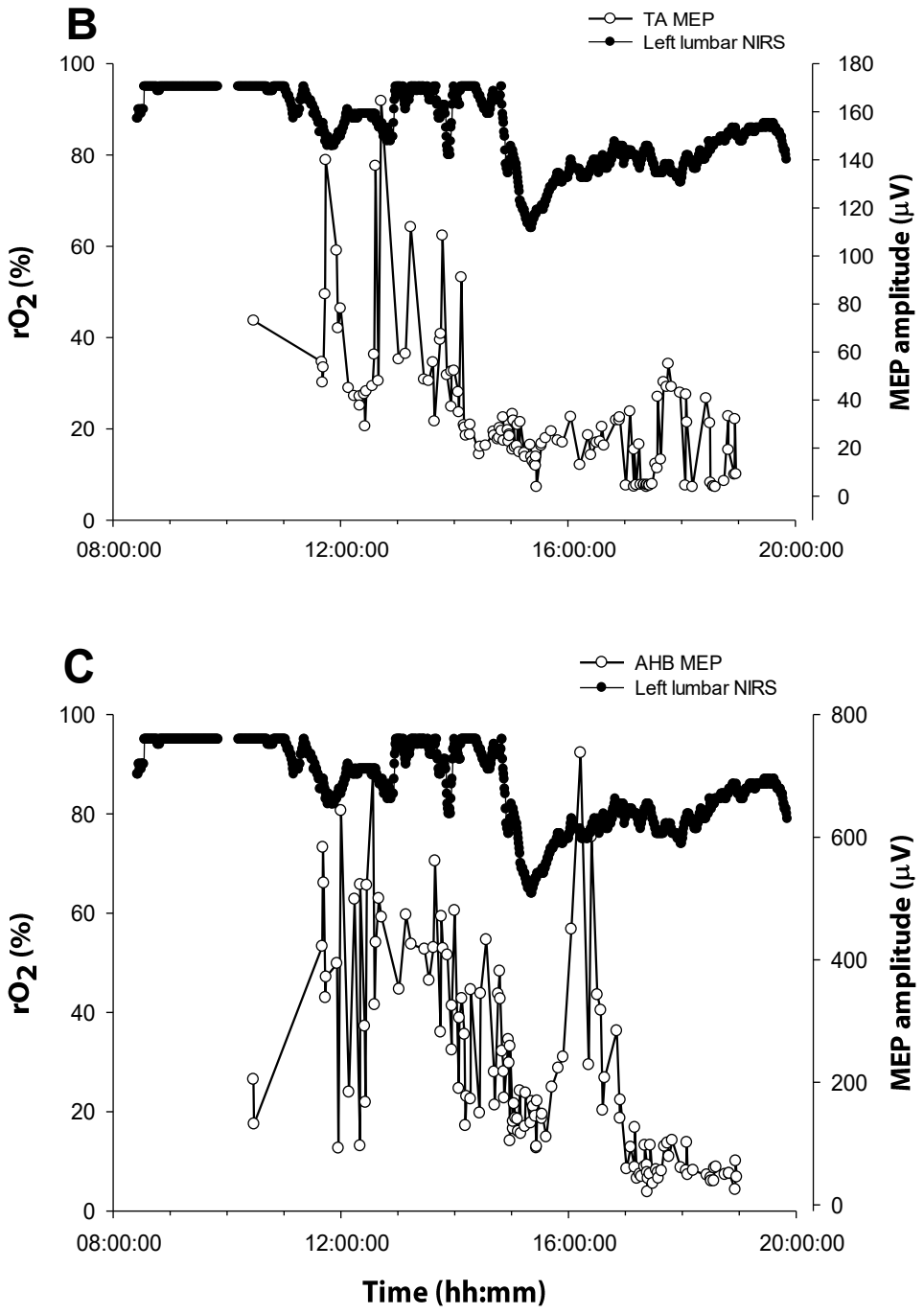


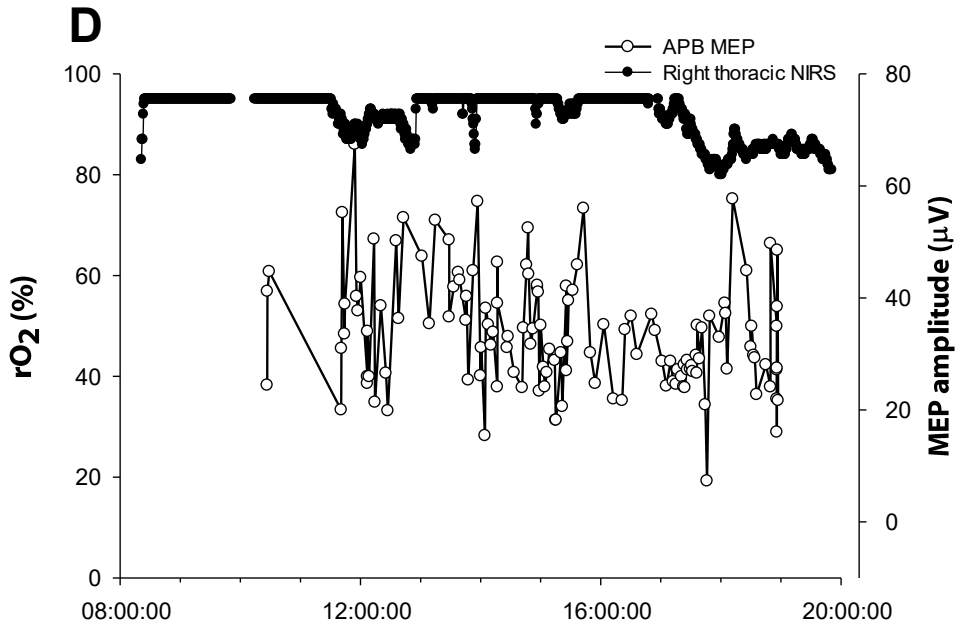
Figure 73: Graph showing the paraspinal rO_2 (%) from 4 optodes and the systolic blood pressure (mmHg) plotted against time (hh:mm) for patient 3.

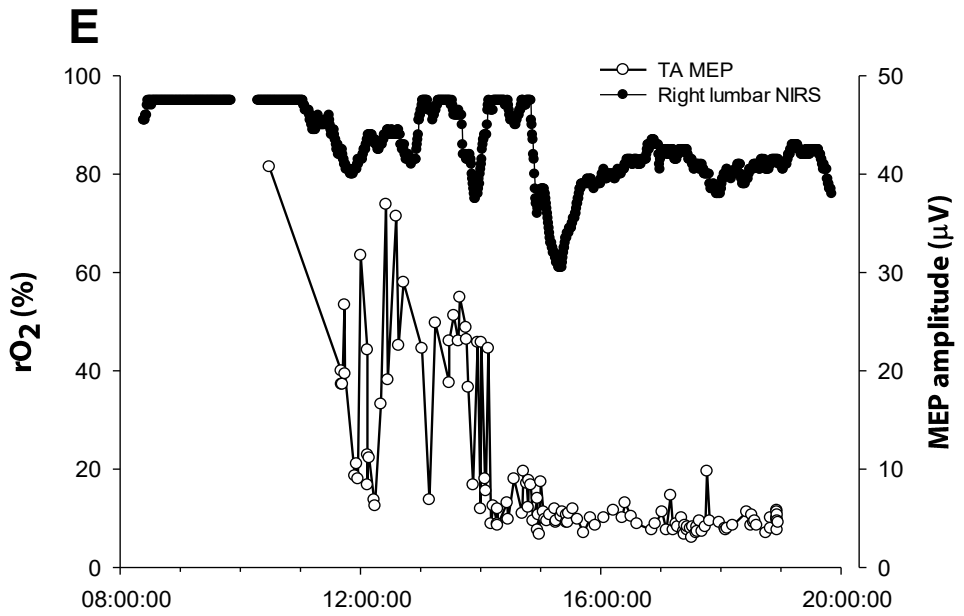
This event is associated with a reduction in MEPs of the lower limbs, which occurs soon after the profound hypotension (see Figure 74). The APB muscle MEPs are preserved (see Figure 74A and D), however there is a dramatic fall in lower limb MEPs bilaterally (see Figure 74B, C, E and F). Despite an increase in stimulation intensity and frequency, which temporarily regenerates left AH MEPs (see Figure 74C), these too also diminish.

This pattern is most indicative of a SCI secondary to the hypotension and subsequent ischaemia. Although this patient survived surgery and made it to the ICU, they unfortunately died 2 weeks later from multi-organ failure due to huge systemic inflammatory response from surgery. The clinical team were unable to assess lower limb motor function to confirm this suspicion.




B
TA MEP
Left lumbar NIRS
rO2 (%)
MEP amplitude (μV)
100
80
60
40
20
0
180
160
140
120
100
80
60
40
20
0
08:00:00
12:00:00
16:00:00
20:00:00
C
AHB MEP
Left lumbar NIRS
rO2 (%)
MEP amplitude (μV)
800
600
400
200
0
Time (hh:mm)


D
APB MEP
Right thoracic NIRS
rO2 (%)
MEP amplitude (μV)
100
80
60
40
20
0
80
60
40
20
0
08:00:00
12:00:00
16:00:00
20:00:00


E
TA MEP
Right lumbar NIRS
rO2 (%)
MEP amplitude (μV)
100
80
60
40
20
0
50
40
30
20
10
0
08:00:00
12:00:00
16:00:00
20:00:00

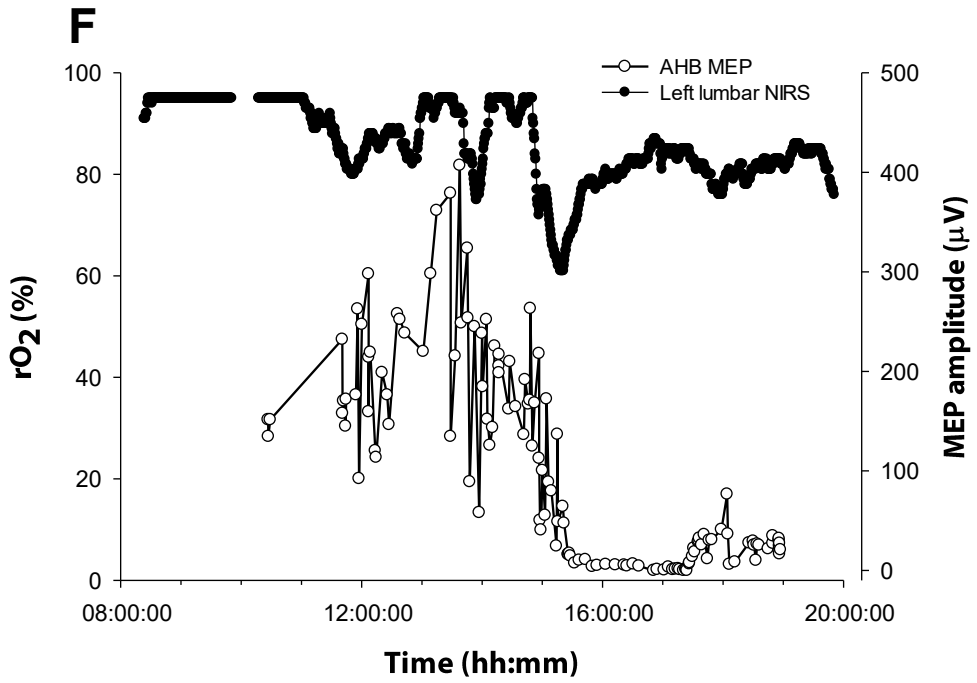


Figure 74: MEP amplitude (μV) and rO_2 (%) across time (hh:mm) for left A) ABP, B) TA and C) AH and right D) ABP, E) TA and F) AH muscles for patient 3.

Patient 4

The results from patient 4 are shown in Figure 75 and Figure 76. This patient had an uncomplicated surgery, although there was clinical concern regarding the possibility of an SCI. At approximately 16:00, there was an expected fall in SBP as the patient comes off left heart bypass; this was associated with a reduction in rO_2 values, with lumbar values decreasing more than thoracic values (see Figure 75). In Figure 76, this reduction in lumbar rO_2 values occurred alongside a reduction in left and right TA and AH MEP amplitudes (see Figure 76B, C, E and F). Thoracic rO_2 values, however, did not decrease more than 20% of baseline and there was less profound decrease in bilateral ABP MEP amplitudes (see Figure 76A and D). Once the blood pressure was deliberately increased to greater than baseline (permissive hypertension) (see Figure 75), so the lumbar rO_2 rapidly increased and the lower limb MEPs also increase. Post-operatively, the patient's blood pressure was maintained at a supra-normal level and the spinal drain was used to reduced CSF pressure and aid spinal cord perfusion.

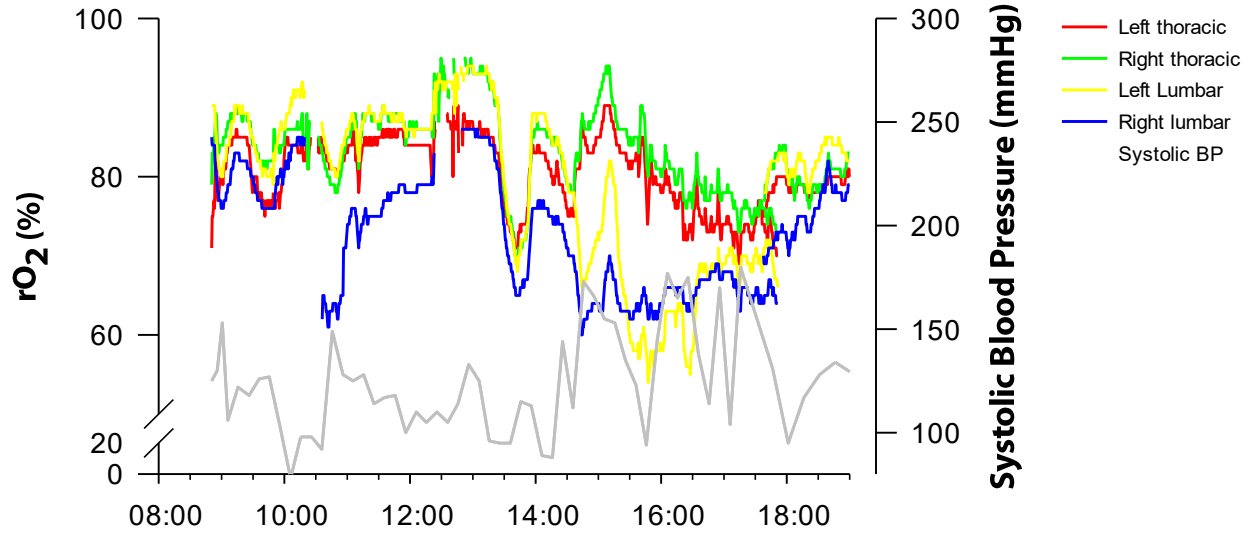
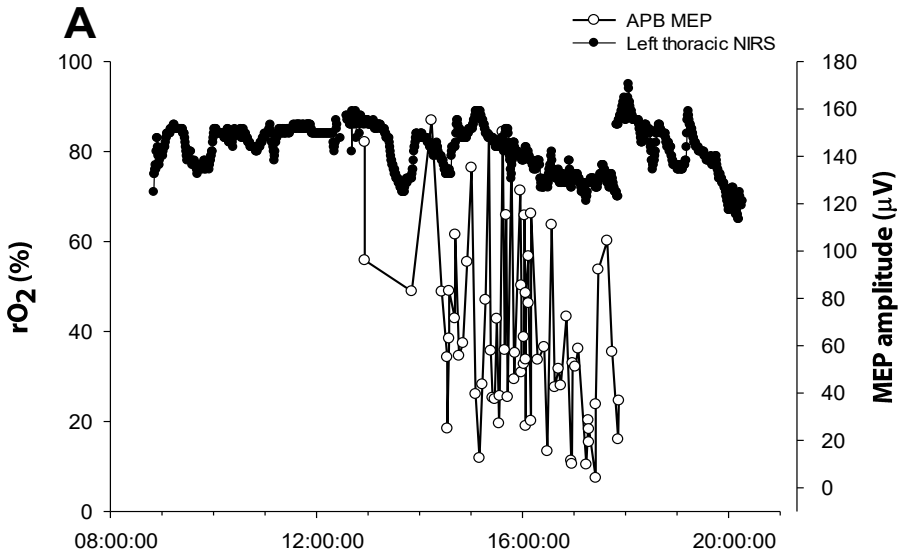
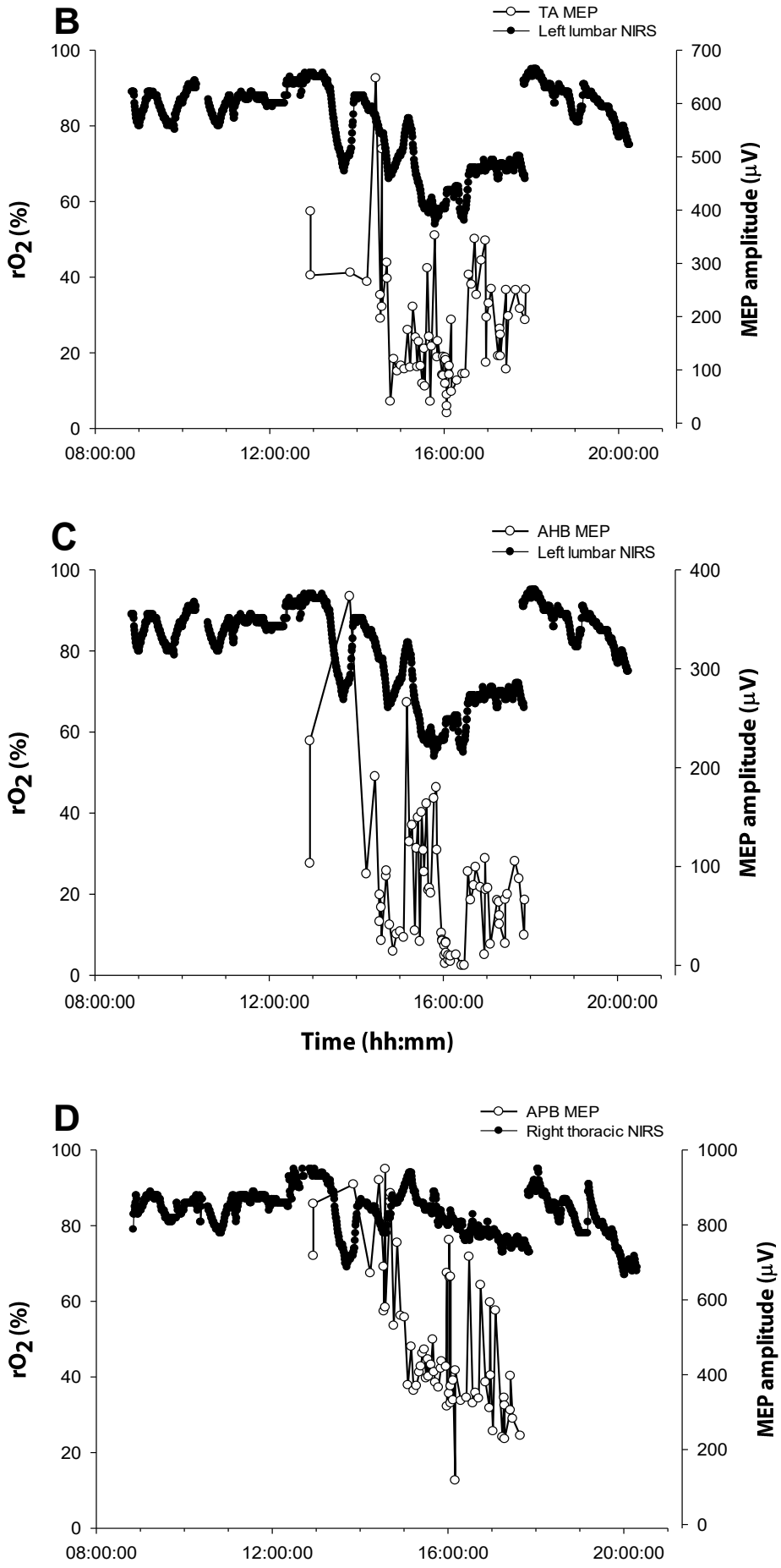


Figure 75: Graph showing the paraspinal rO_2 (%) from 4 optodes and the systolic blood pressure (mmHg) plotted against time (hh:mm) for patient 4.




B
TA MEP
Left lumbar NIRS
rO2 (%)
MEP amplitude (μV)
C
AHB MEP
Left lumbar NIRS
rO2 (%)
MEP amplitude (μV)
Time (hh:mm)
D
APB MEP
Right thoracic NIRS
rO2 (%)
MEP amplitude (μV)
08:00:00
12:00:00
16:00:00
20:00:00

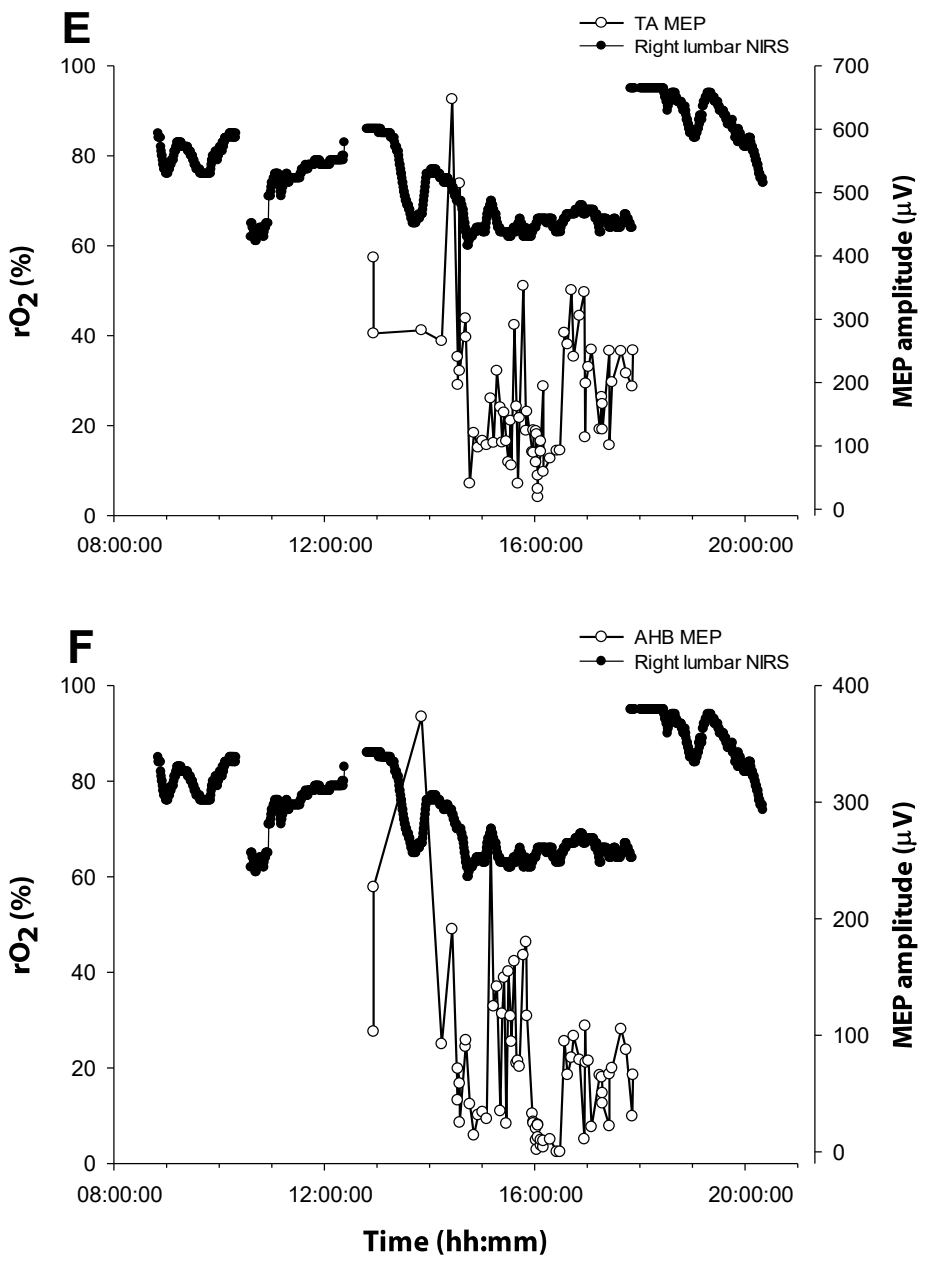


Figure 76: MEP amplitude (μV) and rO_2 (%) across time (hh:mm) for left A) ABP, B) TA and C) AHB and right D) ABP, E) TA and F) AHB muscles for patient 4.

Discussion

Although there is limited patient data in experiment 2 of this study, the potential value of NIRS for detecting SCI is evident. Despite only 4 participants recruited, a number of important clinical scenarios were encountered, which will be discussed further

Blood pressure changes and end organ perfusion

NIRS is commonly employed as a monitor of cerebral perfusion in clinical practice (322). Much clinical data in the literature is from patients undergoing cardiac surgery, where maintenance of cerebral saturations within 20% of baseline has shown reduced post-operative cognitive decline and shorter hospital stay (323). In carotid endarterectomy surgery, where a plaque within a stenosed carotid artery is removed to reduce the risk of stroke, the use of cerebral oximetry allows indirect monitoring of the brain's blood supply whilst the artery is temporarily occluded. This prevents the use of additional invasive monitors and more complex surgical or anaesthetic techniques, reducing risk to the patient (324, 325). Increasingly, evidence exists now for the use of NIRS in detecting limb ischaemia, especially post-operatively to determine the success of limb re-vascularistion surgery (180, 326).

The rO_2 is the algorithmically calculated percentage of oxygen present in the organ of interest (90). All the clinical uses outlined above, therefore, employ NIRS as a surrogate measure of end organ perfusion i.e. how much oxygen carrying blood gets to the target organ of interest. In the case of SCI, the paraspinal muscles here are the end-organs of interest, they themselves being representative of the intra-spinal circulation (327). Perfusion is determined by a number of factors, chiefly the driving pressure of blood to the tissue, the systolic blood pressure. As such, alterations in blood pressure will impact the rO_2 of all tissues, as was seen in the patients in this study, and highlighted particularly with patient 1's results. There is a short time lag of minutes between changes in SBP and rO_2, reflecting the time taken for oxygen extraction and utilisation by the tissues.

Although based on the limited data set of 4 patients above, this time lag is similar to the time taken for MEPs to change with blood pressure changes, seen most clearly with patient 4. This patient had a fall in SBP following recommencement of their native circulation. This is followed by a fall in rO_2 at both thoracic and lumbar levels, secondary to reduced paraspinal muscle perfusion. There is also a corresponding decrease in upper and lower limb MEPs bilaterally, especially evident in the lower limb MEPs. The lumbar rO_2 decreased more than thoracic, and thus lower limb MEPs appear to decrease

more than upper limb MEPs. Given that changes to SBP are mirrored by paraspinal rO_2, which is believed to indicate intra-spinal oxygen saturations, and MEPs are a measure of the spinal cord integrity, it would follow therefore that paraspinal rO_2 as measured by NIRS could also be a measure of spinal cord function.

It should be noted here however, that NIRS can potentially only detect spinal cord injury secondary to ischaemia, thus having a potential role in TAAA surgery. It would not diagnose a spinal cord injury due to spinal cord transection, from trauma for example, or following malignancy. In this case, the blood supply to the cord at the level of the injury may be compromised, but most likely would remain intact. Certainly, the blood supply to the paraspinal muscles would remain unchanged.

A further consideration worth mentioning is whether the global reduction in MEPs reflects peripheral nerve and muscles ischaemia, rather than spinal cord perfusion. Although this is a possibility, it can be seen from patient 3 and 4's MEP data, the rapidity with which they decrease. As has been demonstrated throughout the studies of this thesis (see Chapters 5, 6 and 7) and with INB studies in the literature (80, 87), ischaemia induced MEP loss takes approximately 30minutes to occur. Therefore, the MEPs decreases occurring over minutes most likely reflect compromise of spinal cord integrity.

Spinal cord ischaemia

The current incidence of spinal cord injury following a TAAA is between 11-22% (8). With a sample of 4 patients, it was unlikely one would occur, however one patient experienced temporary paraplegia, one had neurophysiological evidence of injury, and a third was at considerable risk of SCI and required mitigating interventions (see subsection below Guiding SCI management). It is likely that this is reflective of the high risk these patients inherently faced of acquiring a SCI, hence why their MEPs were measured. It is common for no monitoring to occur due to the limited financial resources of the surgical institution, with a neurophysiologist only requested when the risk is considered substantial.

Patient 1 had clinical limb weakness of the right leg on waking in the ICU 2 days after completion of surgery. The weakness was incomplete, with some leg function still present, which resolved over the coming weeks. There was no indication during the surgery from the MEPs that there was a right sided SCI and retrospective review of the MEP data produces a similar conclusion.

As shown in the results section, the only finding of interest is that the right lumber rO_2 decreases to a lower value than the others. The right lumber rO_2 becomes the lowest value having been the highest near the start of surgery, falling from 86% to 70%. This change could be when the SCI occurred. But it may be the case that this observation is only considered relevant with hindsight; there is no neurophysiological evidence to confirm this. In a study with 20 patients undergoing TAAA correction with paraspinal oximetry alone, the 3 patients experiencing SCI had a persistent reduction of bilateral lumbar rO_2 of 25% from baseline for 15minutes (98). Patient 1 had a reduction of 19% from baseline, but did so for considerably longer, 40minutes approximately. Thus, it could be that it is not just an absolute desaturation that is clinically significant, but also the time spent being ischaemic i.e. a function of the degree of desaturation and time. Further work would be required to explore this and no conclusion can be made from one patient.

The leg weakness experienced by patient 1 may also not represent a SCI at all. The weakness from a SCI is usually bilateral following TAAA repair (10). The unilateral nature may represent a lumbar or sacral injury, possibly at the root or even lumbar plexus given hip flexion weakness, which could be secondary to the surgical damage and patient positioning (328). No post-operative neurophysiological tests were performed however to confirm or refute this.

Patient 3 had the most convincing evidence for SCI. There was profound reduction in SBP secondary to near cardiac arrest which persisted for approximately 30 mins. The thoracic rO_2 remained stable due the assistance of left heart bypass, which maintains output to the proximal aortic arch and therefore the upper body and limbs. The lumbar rO_2 bilaterally decreases from 90% to 64%, a reduction approaching 30%, associated with a sudden precipitous fall in MEPs from TA and AH bilaterally. An increase in intensity and frequency of stimulation temporarily recovered left AH MEPs

but they were not sustained. Despite measures to restore spinal cord perfusion (discussed below), the MEPs remained very small compared to before this event.

The lumbar rO_2 desaturation, coupled with the sudden reduction lower limb MEPs due to a fall in SBP and hypoperfusion to the lower body, contrasts with the preservation of thoracic rO_2 and APB MEPs as a result of continued blood flow from the bypass circuit. This presents a unique scenario and data set which in the same patient demonstrate the potential ability of NIRS to detect ischaemic SCI.

It was not possible to clinically confirm however if an ischaemic SCI had occurred. CT imaging after surgery showed the patient had experienced a stroke and developed bowel ischaemia, all end organ perfusion deficit events. These findings alongside the intra-operative NIRS and MEP data make a spinal cord injury very likely. The patient did not recover and died on ICU.

Guiding SCI management

Patient 4 experienced a lumbar desaturation from 90% to 57% at its lowest. This too was associated with lower limb MEP reduction. Thoracic O_2 and ABP MEPs remained stable however, and there was no significant fall in SBP; preceding the desaturation it had in fact been higher than normal. The patient had however come off left heart bypass and flow through the aorta and new graft commenced. In resecting the aneurysm, it was likely important segmental arteries supplying the spinal cord which branched off from the resected aorta, were lost. The bypass circuit maintained cord perfusion artificially which now the surgically altered vasculature could not maintain, resulting in impending SCI. Thus, to prevent this, a number of remedial measures were instigated.

It was not possible for surgical correction, as the aneurysmal sac had been resected, although the use of shunts is possible. Therefore, the anaesthetic team augmented the blood pressure and raised it to supra-normal level, whilst simultaneous opening the CSF (spinal) drain and reducing CSF pressure. This effectively forces more blood flow via the remaining vessels, including the collateral circulation in the paraspinal muscles, thus increasing cord perfusion. As can be seen in patient 4 results, the SBP is increased to over 175mmHg; this is associated with an increased in all rO_2 values (they in fact

overshoot to well above baseline) and a restoration of TA and AH MEPs. This augmentation of spinal cord perfusion was then maintained on the ICU whilst the spinal cord arterial supply reorganises.

The data from patient 4 provides clear evidence of the potential use of NIRS and paraspinal oximetry in guiding SCI treatment interventions. Given the correlation between lower limb MEP amplitude and lumbar rO_2 levels, paraspinal rO_2 could in addition to, or instead of invasive MEPs, guide CSF pressure or SBP targets in treating ischaemic SCI.

Future perspectives and clinical use

The data from only 4 patients has demonstrated the exciting potential of paraspinal NIRS for detecting ischaemia SCI following TAAA repair. In the cases above, it has been able to track haemodynamic changes, detect a probable SCI and how it could be used to guide interventions for treating a SCI. However, a number of considerations remain to be explored. It must also be remembered that a much larger sample is required before using NIRS clinically on a routine basis.

As mentioned, paraspinal rO_2 is a marker of intra-spinal perfusion, and in itself does not detect a SCI has occurred. This can only be confirmed using primary endpoints of cord function i.e. MEPs and/or SSEPs. Further work would need to explore to what degree of desaturation, and for how long, would be associated with a SCI. Would a shorter, more profound desaturation be equivalent to a persistent but less severe desaturation? This would require large scale human observational studies, as randomised controlled trials would be unethical. Animal models have been utilised before, using successive ligation of segmental arteries, but they do not account for potential changes in the collateral circulation secondary to a chronic aneurysm (96, 327). Confirmation of the injury until this can be determined will still require neurophysiological measures.

Currently, the commonest intra-operative neurophysiological monitor is the use of MEPs, which have been more sensitive than SSEPs for detecting a SCI and paralysis (38, 121). As seen with the patients above, and in small sample patient studies, paraspinal rO_2 correlates well MEP changes, suggestive of

injury (99). Only animal models to date have confirmed the association with a clinically confirmed SCI (96, 327). The proposed study intended to include 40 patients, which had the intention to confirm a desaturation with an SCI, but due to recruitment problems discussed above, this study remains to be completed.

Patient 4 exemplifies very clearly the potential role for NIRS in managing a SCI and its role post-operatively on the ICU. Promoting cord perfusion by manipulating the SBP and CSF pressure resulted in an increase in rO_2 and MEPs; this then allows for physiological target setting for ongoing care on the ICU. However, once the patient leaves theatre, confirmation that these targets remain adequate can no longer be confirmed with MEPs. Monitoring with TES MEPs is confined to theatre, the reasons for which are discussed in Chapter 3: Survey of Intra-operative Neuromonitoring during TAAA Surgery in the United Kingdom and Ireland; TES is not practical on the sedated patient. Also discussed in that chapter are the potential harms caused by the remedial measures and the risk of undertaking these on clinical suspicion alone. If a desaturation level associated with an SCI can be clearly defined, then it could be continued on the ICU, circumventing concerns with unguided management. However, it is likely that with NIRS only providing a surrogate marker of an ischaemic SCI, neurophysiological confirmation of an injury before embarking on greater interventions, radiological imaging or even further surgery would be required.

This is where the role for transcranial magnetic stimulation could be especially invaluable. Data from experiment 1 has shown TMS can be performed in theatre provided considerations are made to the choice of anaesthetic. All patients in experiment 2 had TIVA without muscle relaxant to generate TES MEPs, the same anaesthetic needed for intra-operative TMS MEPs. Therefore, future studies would need to trial TMS in theatre, most likely alongside the current gold-standard TES MEPs until they can be confirmed to be equivalent. They would also need to be used with NIRS to correlate rO_2 levels with TMS MEPs and a SCI. However, the particular opportunity for TMS is for it to be used on the ICU, where TES is not possible, for guiding SCI management and to detect late SCI. As discussed in

Chapter 1: Introduction and Background, late SCI is increasingly prevalent as more endovascular TAAA repairs (TEVARs) are performed, manifesting 24hrs after surgery (121). With most patients being sedated and not clinically examined until fully awake several days later, the window to mitigate againts late SCI has often passed. TMS could be utilised on the ICU to routinely probe the integrity of the spinal cord. As the patients would be sedated (as opposed to anaesthetised) with propofol and an opiate, TMS MEPs could be generated with ease; this level of sedation would be insufficient for the pain caused by TES however. TMS could be performed hourly alongside the documentation of standard physiological variables, with the frequency of TMS trials increased if and when paraspinal rO_2 shows a desaturation suggestive of an injury. With prompt SCI detection, time-critical remedial measures could be initiated immediately, providing the best opportunity to prevent injury. This potential role for TMS could completely revolutionise the management of late SCI and warrants future study.

Conclusion

Despite the patient studies being severely impacted by the pandemic and intense pressures on the National Health Service, the limited patient data has provided valuable insights. I have demonstrated that TMS MEPs can be generated under the appropriate anaesthetic regimen and that the potential effects of cortical excitability following an INB on distant muscles may be negligible. In addition, practical lessons on how to perform TMS in theatre were learnt, making future patient study more streamlined. Data from the TAAA patients supports the limited evidence in the literature of the potential benefit of using non-invasive NIRS of the paraspinal musculature to detect an ischaemic SCI. When correlated with TES MEPs, paraspinal rO_2 follows trends suggestive of hypoperfusion and injury, and increasing the patient size sample in further studies would likely diagnose a sadly inevitable SCI. Future work should also focus on utilising TMS alongside NIRS in the post-operative phase in particular, where their uniquely non-invasive and simple-to-perform properties make them ideally suited to detect, and then treat, late SCI.

Chapter 9: Conclusions

The aim of this thesis was two-fold; to investigate the role of TMS and NIRS as potential monitors to detect spinal cord ischaemia seen during TAAA repair, and to further characterise and explain the effects of limb ischaemia on cortical excitability that could influence TMS induced MEPs used in this monitor.

SCI following TAAA is a life-changing, and potentially life ending, complication with a significant health-economic burden. With modern endovascular repair techniques, this pathology is no longer limited to the operating theatre and can occur even in the post-operative recovery phase (see Chapter 1: Introduction and Background). There is a need therefore for spinal cord monitoring that is available both whilst the patient is anaesthetised and awake, and which is more readily accessible.

A survey of current monitoring and management procedures to detect and treat SCI following TAAA repair in the UK and Ireland, showed that it is a widespread problem; anaesthetists participating in TAAA surgery will encounter this devastating complication (see Chapter 3: Survey of Intra-operative Neuromonitoring during TAAA Surgery in the United Kingdom and Ireland). Despite this, intraoperative neuromonitoring in its current guise has limited use and availability and rarely features in the management protocols for many vascular surgery specialist centres. Prohibitive costs of IONM were cited as a barrier to routine use, as well as a perception of low clinical benefit, issues which could be overcome with the novel monitoring techniques contained in this thesis.

Transcranial magnetic stimulation is used predominantly in neurophysiological research. It is non-invasive, near painless way to interrogate the neuronal pathways of the brain and corticospinal tract. Its use as a clinical diagnostic tool and intraoperative neuromonitor, however, is limited. In Chapter 4: An investigation into the variability of MEPs in patients with peripheral vascular disease, healthy participants and patients with peripheral vascular disease, who contribute to the majority of TAAA pathology, underwent single pulse TMS over a one-hour time period at rest; this was to simulate the time of intense monitoring during surgery when the spinal cord would be most at risk. Patients and

healthy controls had no difference in their MEP characteristics and variability beyond which could be explained by age, suggesting PVD itself (and therefore the chronically reduced blood flow to the nerves) had no influence of on muscle neurophysiology. In addition, potential muscles that could be used as control and monitor muscles were identified based on those most consistent after 1 hour of testing.

A feature of TAAA surgery is the need to clamp arteries to perform surgery in a bloodless field and prevent major haemorrhage. In doing so, the limbs are rendered ischaemic, resulting in an ischaemic nerve block. It is well known in the literature that an INB can cause an increase in cortical excitability, particularly affecting muscles proximal to it. This was further explored with a novel low-pressure INB (see Chapter 5: Cortical excitability induced by a novel low-pressure INB). After validating its effectiveness, this INB was employed on the lower limb to demonstrate that the increase in cortical excitability extended to not only the proximal muscles, but also contralateral and distant arm muscles. In addition, SSEP analysis from the ischaemic leg showed only a partial deafferentation was required to initiate these changes. How MEPs are influenced following an INB is necessary to characterise if TMS is to be utilised intraoperatively during TAAA surgery.

Following on from these findings, an investigation into the partial deafferentation was undertaken. Using a truncated quantitative sensory testing regimen, the function of each afferent nerve fibre type was assessed (see Chapter 6 – An investigation of deafferentation induced by ischaemic nerve block). The INB resulted in cortical excitability in keeping with previous investigations, with QST showing preservation of C-fibre function. Thus, it would seem selective deafferentation of A-fibres was responsible to initiating an increase in cortical excitability. An unexpected finding was an exaggerated response to punctate pain experienced by some participants which was associated with a greater increase in MEP amplitude, possibly indicating a subtype of participant who experiences a greater increase in cortical excitability secondary to pain.

In Chapter 7 – Exploration of mechanisms causing changes in cortical excitability in the motor cortex ipsilateral to an INB, scientific inquiries were performed to explain the mechanisms responsible for

the increase in MEP amplitudes in muscles contralateral to an INB. Explanations for proximal muscle increases can be found in the literature with little to explain changes in cortical excitability resulting in contralateral changes. Interrogation of the intrahemispheric pathways (SICI, SICF, LICI and ICF) using the paired pulse paradigms, controlling the contralateral APB did not demonstrate any changes.

In the final experimental chapter of this thesis, Chapter 8: TMS and NIRS in Theatre, preliminary testing of both TMS and near infrared spectroscopy is undertaken. Limited by the coronavirus pandemic, a series of case studies was performed. TMS was capable of producing MEPs under the appropriate anaesthetic conditions. However, unlike experimental conditions, no effect of limb ischaemia on cortical excitability was discernible; interpretation of course is speculative given the sample size of one. NIRS monitoring of paraspinal muscle oxygenation was utilised on patients undergoing TAAA repair alongside traditional MEPs monitoring with transcranial electrical stimulation. The data from these cases demonstrated NIRS values change alongside changes in blood pressure and also in conjunction with MEP changes. Where a patient has paraparesis, retrospective analysis of rO_2 showed a desaturation which could have been responsible. A further patient who lost their MEPs likely due to a SCI, also had a corresponding profound desaturation of lumbar rO_2. The potential of NIRS to guide remedial measures was also seen in a third patient. Despite the small sample size, the potential for TMS and NIRS at detecting an SCI intra-operatively and guiding post-operative management is evident, and presents an exciting avenue for future research.

To summarise the main findings in this thesis, TMS and NIRS could be used intraoperatively to monitor for SCI. Experimental testing in the laboratory has shown TMS induced MEPs in patients likely to develop a TAAA are in keeping with those in the literature for age-matched controls. The simulated limb ischaemia encountered intra-operatively during TAAA repair has the potential to increase cortical excitability and thus augment MEPs across all limbs, and these changes need to be accounted for by a TMS-based monitor of SCI. Although only limited data were acquired, exploration of different anaesthesic regimens demonstrated the potential for TMS to be utilised in the theatres, although this will require further testing. Scientific exploration into the increase in motor cortical excitability found only partial deafferentation is required to initiate changes, with perseveration of C-fibre function,

whilst intercortical rather than intracortical mechanisms are likely to sustain the increase. NIRS has the potential to compliment any neuromonitor of spinal cord function given its tandem changes with MEP fluctuations. Further intraoperative and postoperative testing of TMS and NIRS is required to fully realise their potential as safe, non-invasive and affordable monitors of spinal cord function.

Appendix 1: classification of TAAAs

Aortic aneurysms are classified based on their location. The main categories are:

- Thoracic
- Thoraco-abdominal
- Abdominal

TAAA are classified originally according to the Crawford Classification System, which has been modified since (329). The system is based on the extent of aortic involvement (see Table 9).

Type	Description
Type 1	Aneursym of the descending thoracic aorta above the sixth intercostal space, extending to include the origins of the celiac axis and superior mesenteric arteries. Does not extend distal to origin of renal arteries (supra-renal).
Type 2	Aneurysm arises above the sixth intercostal space, extending beyond the origins of the renal arteries to include the infra-renal aortic segment. May involve the ascending thoracic aorta, hence entire aorta can be involved.
Type 3	Aneurysm of the distal half of the descending thoracic aorta below the sixth intercostal space, to the aortic bifurcation.
Type 4	Aneurysm of the entire abdominal aorta from the diaphragm to origin of the iliac arteries.
Type 5	Distal half of the descending thoracic aorta below the sixth intercostal space to the origin of the renal arteries.

Table 9: Modified Crawford Classification of TAAA

TAAA comprise only 10% of all aneurysms involving the thoracic aorta, which have a separate classification system, also based on location.

Aneurysms can be also be classified by their morphological appearance. Fusiform aneurysms are more common, describing a symmetrical dilation of an artery wall. These occur as a result of atherosclerotic disease of the artery. Saccular aneurysms are usually asymmetrical dilatations of one side of the vessel wall and occur due to arterial ulceration or infection.

The purpose of classification is to guide management and prognosticate outcomes. Repair of Crawford type 2 TAAA for example, has the highest risk of spinal cord ischaemic injury and hence paralysis (38).

Appendix 2: Methods to reduce SCI

Overview

Methods to reduce the incidence of SCI can be categorised as follows (30):

Early Detection	Intra-operative SSEPs/MEPs (early SCI) Focused clinical examination Radiological (unclear pathology i.e. SCI vs epidural haematoma)
Augment spinal cord perfusion pressure	Increase mean arterial pressure Reduced CSF pressure (or CVP) Re-implant critical arteries Revascualrise critical arteries Distal aortic perfusion/left heart bypass
Increase ischaemic tolerance	Hypothermia Pharmacological interventions

Maintaining spinal cord perfusion:

Spinal cord perfusion is determined by the following principle:

Spinal perfusion pressure = MAP – CVP (or CSF pressure)[330]

MAP is mean arterial pressure, CVP is central venous pressure and CSF is cerebrospinal fluid.

Mean arterial pressure

Spinal cord perfusion is not simply determined by the driving mean arterial pressure. Flow in any tube, including blood vessels and vascular beds is across a pressure gradient, from high to low. Thus, to

maintain perfusion to an injured spinal cord which may have increased intrathecal pressures due to oedema, and therefore where the high CSF pressure causes compression of the relatively lower pressure spinal veins, a high mean arterial pressure and a low intrathecal (cerebrospinal spinal fluid) pressure is employed.

The data for these recommendations is level IIa and III at best i.e. expert opinion (331) and animal studies (332). A study from 2008 where 10 patients out of a series of 858 developed late SCI (within 48hrs but normal SSEPs intra-operatively) found the absolute MAP value in patients with the SCI was the same as those without. However, relative to their pre-surgery baseline, the relative MAPs were closer to 80% of baseline. Thus, MAPs ideally should be tailored to each patient (333). Despite this, most centres use will advocate a supranormal MAP target (MAP >90mmHg) which most likely is to provide safer and consistent care amongst clinician and nursing staff (331). This is achieved through the use of intravenous fluid administration and vasopressor drugs, the choice of which makes little difference.

CSF Pressure

Low CSF (or CVP pressure) of <10mmHg is achieved using a "spinal drain." This drain removes CSF fluid and subsequently results on reducing CSF pressure. It is commonly employed in all surgeries where many segmental arteries are likely to be sacrificed, where neuromonitoing has identified patients with or at risk of SCI and those who have a neurological deficit on examination (16). A pressure greater than 10mmHg in the post-operative period has also been observed in late SCI (334). The American College of Cardiology/American Heart Association guidelines advocate spinal drains in all open TAAA repairs and in TEVARs where many risk factors for SCI are present (158). Maintenance of a spinal cord perfusion pressure of >80mmHg is continued for up to 48hrs and can initiated post-operatively where late SCI develops.

A series of 142 TEVARs found no benefit of prophylactic spinal drains, but were associated with a 6% incidence of severe harm from their placement (cranial hypotension syndrome requiring

rehabilitation)(17). Another study found higher mean CVP pressures (which will be seen in the epidural veins thus be greater than CSF pressure) early in the post-operative period was seen in patients with late SCI, whereas lower mean CVP pressures were seen in those without late SCI.

Currently, most centres would place a CSF drain prophylactically where the predicted risk of SCI is high, especially for open repairs where early injury is seen. However, owing to the uncertainty of benefit in endovascular repair from emerging evidence, there may be a shift to inserting them once signs of a SCI are seen.

Hypothermia

Limited studies have explored the role of hypothermia. It is employed sporadically by few centres performing open TAAA repair (132). Mild hypothermia of 32-35°C often occurs by not warming the patient intra-operatively; moderate hypothermia of 28-32°C may be neuroprotective, but limited evidence exists.

Surgical techniques

Current

Surgical techniques have evolved in recent times to minimise the risk of SCI. Most commonly, surgeons attempt to identity and then preserve or re-implant important segmental arteries. Such techniques include:

- Delineation of intercostal and lumbar arteries for ligation and serial ligation (16)
    - Prevents reversal of blood flow within the anterior spinal network and thus reduces steal phenomenon.
    - Before the aneurysm is opened, segmental arteries that are connected to the aneurysm are detached slowly, whilst neuromonitoring is used to determine their importance to spinal perfusion

- This slowly reduces input to the anterior spinal artery

- Re-implantation of intercostal arteries
    - Before segmental arteries are permanently tied off, they are temporarily occluded
    - If there is a change in the neuromonitoring, then this suggest that artery is vital to spinal cord perfusion and hence is re-attached.
    - An alternative is to test flow through this artery by returning blood from the bypass circuit back to the segmental artery to see if MEPs return
    - The reason that not all arteries are simply re-anastomosed are that this can significantly prolong surgical time, exposing the high rick patients to unnecessary anaesthesia, but also every anastomoses has the risk of re-bleeding, which would be catastrophic (335).

Emerging

Newer surgical techniques attempt to precondition the spine to potential ischaemia. By reducing the blood flow, a partial ischaemic state is created, thus promoting vasoactive mediators and the formation of collateral arteries to supply the spinal cord.

Staging

Where a long graft is required to cover a large aneurysm (>30cm), performing the repair (usually endovascular) in two stages is advocated to reduce the risk of SCI (36). As mentioned earlier, it possibly helps collateralisation to occur and diversion of blood to the intramedullary arteries.

Temporary Aneurysm Sac Perfusion

Here, a staged TEVAR procedure is performed. In the first procedure, the graft used has a small branch (an endobranch) which eventually will be used to feed a visceral artery. In the meantime however, it is left disconnected and therefore allows blood to flow into the original aneurysm sac. During the

second stage performed up to 3 months later, a further stent connects the endobranch to the desired visceral. This technique has shown significant promise in reducing SCI, likely through improved collateralisation a discussed above (336).

Appendix 3 – Sample Patient information sheet and consent form

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Transcranial Magnetic Stimulation (TMS) in Vascular Disease

Can TMS be used as a novel intra-operative monitor of spinal cord function?

A Preliminary Study

Patient Information Sheet – Orthopaedic

Thank you for taking time to read this information sheet. We would like to invite you to join a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

We want to make clear that the complication outlined below is **not** seen following orthopaedic (bone) surgery of the lower limbs. Your potential inclusion in this study is because a technique used during your surgery is very similar to that employed during vascular surgery, and the effects on your body as a result of this is where the interest of this study lies. In addition, your age and past medical history is similar to, but not the same as, those undergoing vascular surgery.

To re-iterate, *you are not at risk* of the below complication from the surgery you are having.

What is the purpose of the study?

Spinal cord injury following surgery to the main blood vessel in the body, the thoracic aorta, represents the most feared and significant complication. Even with the advent of minimally-invasive techniques, paralysis can affect up to 13% of patients. This is a life-changing complication.

Monitoring of these types of responses is not routinely carried out during surgery and when it is, it is undertaken using painful electrical stimulation of the brain with needles positioned in the scalp. The electric currents used are large and cause significant discomfort, hence is done after patients have been anaesthetised. Magnetic stimulation does not require high electrical currents or needles (so is non-invasive) and is painless. It has the potential to be used in the awake patient after surgery too.

This study will establish the responses of a number of different muscles to magnetic brain stimulation in patients undergoing surgery of lower limb blood vessels (*not you*) and those undergoing surgery of the lower limb bones, patients such as you. Although these surgeries are very different, a tourniquet is used in both, to improve the surgeon's view. Thus, the data from your surgery is similar to that of patients undergoing surgery of the blood vessels (vascular surgery).

The ultimate aim is to use this painless and non-invasive method to monitor blood flow to the spinal cord during complex surgery of the thoracic aorta. This project on patients, could pave the way for routine monitoring during vascular surgery which would ultimately lead to a reduced likelihood of patients having a potentially catastrophic spinal cord injury and being left paralysed.

Why have I been invited?

We are asking you to think about joining this study because you are a patient undergoing orthopaedic surgery of the lower limb where a tourniquet will be used, under the care of the orthopaedic team at Imperial College Healthcare NHS Trust.

Who are we looking for?

You can take part in the study and will be allocated to group B (*as highlighted below*) if you:

- Group A
    - Experience symptomatic lower limb claudication pain (cramping in lower leg), not currently suitable for surgery.
    - Are under regular review by the Vascular Surgery Department at Imperial College NHS Trust for peripheral vascular disease (the build-up of fatty deposits in the arteries (blood vessels) that restrict blood supply in the lower legs) and are not currently suitable for surgery.
- **Group B**
    - Have peripheral vascular disease of the lower limbs, requiring surgery of the arterial system of the lower limbs
    - Are awaiting surgery of abdominal aorta e.g. endovascular aneurysm repair
    - **Are undergoing orthopaedic surgery of the lower leg requiring the use of an external pneumatic tourniquet e.g. knee replacement**

You cannot take part in this study if any of the following applies to you:

- Age < 18yrs
- Past medical history of:
    - Diabetes Mellitus (High blood sugar level) with secondary complications e.g. eye disease (retinopathy) or nerve injury (peripheral neuropathy)
    - Neurological or psychiatric disease, including but not limited to: epilepsy, undiagnosed/non-specific seizures, intracranial mass or space occupying lesion, deep brain stimulator, aneurysm clips
    - Use of anti-epileptics or anti-convulsant medication or any medication thought to reduce seizure threshold
    - Cochlear implants (Surgically implanted device that aids hearing)
    - Having previously being denied a magnetic resonance imaging (MRI) scan (type of scan using magnetic fields and radio waves to produce detailed images of the body)
- Confirmed or possibility of pregnancy
- Open wounds or skin lesions, including wet gangrene (dry gangrene permitted)
- Inability to speak and understand English fluently
- Surgical procedure being performed without a general anaesthetic (i.e. neuraxial anaesthetic block)

- Patients undergoing thoracic or thoraco-abdominal aneurysm repair

Do I have to take part?

No. It is entirely up to you. If you would like to take part, you will be asked to sign a consent form. Even after you have signed this consent form and agree to join the study, you are free to withdraw from the study at any time with no impact on the healthcare you are provided. If you decide to withdraw from the study, data collected up to the point of withdrawal will be retained in the study.

What will happen to me if I take part?

You will be assigned to group B of the study.

All measurements and data will be collected on the day of your surgery at St Mary's Hospital (Praed Street, London) or Charing Cross Hospital (Fulham Palace Road, London).

We will record your age, sex, ethnicity, height and weight as well as any other medical conditions you have (co-morbidities). Clinical data regarding your disease pathology and severity and functional limitation will also be recorded using a questionnaire (VascuQol).

Before you are anaesthetised, small, non-painful adhesive electrodes (similar to ECG 'dots') will be attached to various muscles on your arms and legs. These will be connected to an amplifier and computer which will measure muscle activity. Each muscle will be stimulated 6-12 times. A specifically designed magnet will be held on your head and when activated, will cause muscles to contract. A series of measurements will be made from each limb.

Measurements (muscle activity) will be taken immediately before the start of the anaesthetic and will take approximately 30 minutes. Taking part in the study may delay the administration of the anaesthetic for your surgery whilst the adhesive electrodes are attached and initial measurements carried out, but the delay will be short and not affect your care in any way. They will then be taken intermittently during the surgery whilst you remain under anaesthesia and will continue for a short

period after the conclusion of surgery, including in the recovery area. The Co-Investigators named on this study who have an NHS contract will be responsible for taking your measurements in theatre.

What are the side effects or risks of any of the methods received when taking part?

There are no long-lasting risks of taking part in this study. The assessment techniques are safe and non-invasive and there are minimal risks from having these tests performed under strict safety guidelines, which include stringent exclusion criteria. TMS involves holding a magnetic coil over the head, similar in shape to a table-tennis bat. When this is activated, it results in a tapping sensation, which may feel unusual to some participants. All tests will be performed within your limits of tolerance. You may feel a mild discomfort during the removal of the sticky electrodes from the skin, this will be minimised by skin preparation before the experiment.

What is the drug or procedure that is being tested?

There are no drugs being tested in this study and no new treatments.

What are the possible benefits of taking part?

There are no direct benefits to you for taking part.

However, this research may help develop a new form of routine monitoring during aortic surgery which would ultimately lead to a reduced likelihood of patients developing a spinal cord injury.

Will I be paid be take part in the study?

We are not able to pay you for your time to take part in this study. However, we can reimburse your travel expenses to and from the MSK Laboratory, Charing Cross Hospital Campus.

What if new information becomes available?

Any new information derived through this study will be made available to all of the subjects should they request this.

What if something goes wrong?

Imperial College London holds insurance policies, which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Dr. Paul Strutton; p.strutton@imperial.ac.uk/Tel: 020 3313 8837). The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

For independent advice about participating within a research study, please contact the patient advice and liaison service (PALS) based at Charing Cross Hospital, Fulham Place Road, London W6 8RF (Tel: 020 3313 0088).

Will my taking part in this study be kept confidential?

Any information you give us will be kept confidential. If the study is published in a book or scientific journal, no individual will be identified in any way. All personal identifiable information will be kept in case we to contact participants and will be stored on secure laboratory server or a non-network computer that meets the college criteria for storing personal data. Once assessments have been completed, the contact details will be deleted. The data will be held in accordance with the Data

Protection Act, which means that we keep it safely and cannot reveal it to other people, without your permission. Only the research team will have access to the research data.

All data will be anonymised at point of consent. Your data will be stored using a unique identification number to ensure patient confidentiality. The data will be stored and analysed at the coordinating site, Charing Cross Hospital (Imperial College London) by the research team. The data will be stored with Imperial College London for duration of 10 years following completion of the study.

What will happen to the results of the research study?

The results of the study will be analysed by the research team and presented at physiology, anaesthesia and other health care conferences and published in scientific journals. No individual subject will be identified in any report or presentation arising from the research.

Who is organising and funding the research?

The study will be run by a research team based at Imperial College London and funded **by the Association of Anaesthetists of Great Britain and Ireland**, Imperial College Healthcare NHS Trust and Imperial College London.

Who has reviewed the study?

This study has been approved by the Central London Research Ethics Committees and Health Research Authority (HRA).

Contact for further information about this study.

For further information please contact:

Dr Paul Strutton

Imperial College London

Charing Cross Campus, London W6 8RF

Tel: 020 3313 8837

Email: p.strutton@imperial.ac.uk

Thank you for considering taking part in this study. Please contact one of the investigators if you have any further questions.

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Co-Investigators: Dr Pav Sarai/Dr Nathalie Courtois/Mr. Colin Bicknell

Study Identification Number:

CONSENT FORM - Orthopaedic

Transcranial Magnetic Stimulation (TMS) in Vascular Surgery

Can TMS be used as a novel intra-operative monitor of spinal cord function during vascular surgery?

A Preliminary Study

		Please initial box
1.	I confirm that I have read and understand the information sheet "Version 1.3 Orthopaedic", dated 21/01/2019 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2.	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, and without my medical care or legal rights being affected. If you decide to withdraw from the study, data collected up to the point of withdrawal will be retained in the study.	☐
3.	I agree to have my personal data stored confidentially on a secure Imperial College London laboratory server or a non-networked computer, for the purposes of contacting you only (compliant with Data Protection Act). Personal data will be deleted after the completion of the study but this consent form will be stored for 10 years with Imperial College London including measurements taken as part of this study. I understand that published data will be anonymised.	☐
4.	I understand that sections of my research notes and data may be accessed by responsible persons from the research team, from Imperial College London and/or from regulatory authorities where it is relevant to the research and in order to check that it is being conducted correctly.	☐
5.	I agree to take part in the above study.	☐

_____	_____	_____
Full name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

Appendix 4 – TMS Safety Questionnaire

Safety Screening Questionnaire for Transcranial Magnetic Stimulation (TMS)

Please click to put a check mark in the relevant box if completing electronically

1. Do you have epilepsy or have you ever had a convulsion or a seizure? ☐Yes ☐No
2. Have you ever had a fainting spell or syncope? ☐Yes ☐No

   If yes, please describe on which occasion(s)?

3. Have you ever had a head trauma that was diagnosed as a concussion or was associated with loss of consciousness? ☐Yes ☐No
4. Do you have any hearing problems or ringing in your ears? ☐Yes ☐No
5. Do you have cochlear implants? ☐Yes ☐No
6. Are you pregnant or is there any chance that you might be? ☐Yes ☐No
7. Do you have metal in the brain, skull or elsewhere in your body (e.g., splinters, fragments, clips, etc.)?

   If so, specify the type of metal. ☐Yes ☐No
8. Do you have an implanted neurostimulator (e.g., DBS, epidural/subdural, VNS)? ☐Yes ☐No
9. Do you have a cardiac pacemaker or intracardiac lines? ☐Yes ☐No
10. Do you have a medication infusion device? ☐Yes ☐No
11. Are you taking any medications? (please list below) ☐Yes ☐No
12. Did you ever undergo TMS in the past? ☐Yes ☐No

    If yes, were there any problems? ☐Yes ☐No
13. Did you ever undergo MRI in the past? ☐Yes ☐No

    If so, were there any problems? ☐Yes ☐No

Please list any medications you are taking

Safety Screening questionnaire for TMS.docx 29/11/2013

Rossi et al. (2011). Screening questionnaire before TMS: an update. Clinical Neurophysiology 122:1686.

Groppa et al. (2012) A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee Clinical Neurophysiology. 123 5: 858-882

Appendix 5 – VascuQol Questionnaire

VascuQol Questionnaire

(To be completed by the patient at follow-up. Please complete text in BLOCK CAPITALS, tick the appropriate box.)

Date of Completion: ☐☐/☐☐/☐☐ (dd/mm/yy)

Full Name: _____

Date of Birth: ☐☐/☐☐/☐☐ (dd/mm/yy)

Hospital Name: _____

Completed at: 3 months ☐ 6 months ☐ 12 months ☐

2 years ☐ 3 years ☐ 4 years ☐

Instructions: These questions ask you how you have been affected by poor circulation to your legs over the last two weeks.

You will be asked about the symptoms you have had, the way that your activities have been affected and how you have been feeling.

Please read each bit of the answer and then tick the one that applies best to you.

If you are unsure about how to answer a question, please give the best answer you can.

There is no right or wrong answer.

Please answer every question. Thank you.

1. In the last two weeks **I have had pain in the leg (or foot) when walking** ……

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

2. In the last two weeks **I have been worried that I might injure my leg** ……

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

3. In the last two weeks **cold feet have given me** …….

(tick one)

1. A very great deal of discomfort or distress ☐ 1
2. A great deal of discomfort or distress ☐ 2
3. A good deal of discomfort or distress ☐ 3
4. A moderate amount of discomfort or distress ☐ 4

5. Some discomfort or distress ☐ 5
6. Very little discomfort or distress ☐ 6
7. No discomfort or distress ☐ 7

4. In the last two weeks, because of the poor circulation to my legs, **my ability to take exercise or to play any sports has been** …..

(tick one)

1. Totally limited, couldn't exercise at all ☐ 1
2. Extremely limited ☐ 2
3. Very limited ☐ 3
4. Moderately limited ☐ 4
5. A little limited ☐ 5
6. Only very slightly limited ☐ 6
7. Not at all limited ☐ 7

5. In the last two weeks **my legs have felt tired or weak** …..

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

6. In the last two weeks, because of the poor circulation to my legs, **I have been restricted in spending time with my friends or relatives** …..

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

7. In the last two weeks **I have had pain in the foot (or leg) after going to bed at night** …..

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

8. In the last two weeks **pins and needles or numbness in my leg (or foot)** have caused me ….

(tick one)

1. A very great deal of discomfort or distress ☐ 1
2. A great deal of discomfort or distress ☐ 2
3. A good deal of discomfort or distress ☐ 3
4. A moderate amount of discomfort or distress ☐ 4
5. Some discomfort or distress ☐ 5
6. Very little discomfort or distress ☐ 6
7. No discomfort or distress ☐ 7

9. In the last two weeks **the distance I can <u>walk has improved</u>** …..

(tick one)

1. Not at all (tick this if distance is unchanged or has decreased) ☐ 1
2. A little ☐ 2
3. Somewhat ☐ 3
4. Moderately ☐ 4
5. A good deal ☐ 5
6. A great deal ☐ 6
7. A very great deal ☐ 7

10. In the last two weeks, because of the poor circulation to my legs, **my ability to walk has been**

(tick one)

1. Totally limited, couldn't walk at all ☐ 1
2. Extremely limited ☐ 2
3. Very limited ☐ 3
4. Moderately limited ☐ 4
5. A little limited ☐ 5
6. Only very slightly limited ☐ 6
7. Not at all limited ☐ 7

11. In the last two weeks **being (or becoming) housebound has been a concern of mine** …..

(tick one)

1. A very great deal ☐ 1
2. A great deal ☐ 2
3. A good deal ☐ 3
4. A moderate amount ☐ 4
5. Some discomfort ☐ 5
6. A little ☐ 6
7. Not at all ☐ 7

12. In the last two weeks **I have been concerned about having poor circulation to my legs** …..

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

13. In the last two weeks **I have had pain in the foot (or leg) when I am at rest** …..

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

14. In the last two weeks, because of the poor circulation to my legs**, my ability to climb stairs has been** …..

(tick one)

1. Totally limited, couldn't walk at all ☐ 1
2. Extremely limited ☐ 2
3. Very limited ☐ 3
4. Moderately limited ☐ 4
5. A little limited ☐ 5
6. Only very slightly limited ☐ 6
7. Not at all limited ☐ 7

15. In the last two weeks, because of the poor circulation to my legs**, my ability to take part in social activities has been** …..

(tick one)

1. Totally limited, couldn't socialise at all ☐ 1
2. Extremely limited ☐ 2
3. Very limited ☐ 3
4. Moderately limited ☐ 4
5. A little limited ☐ 5
6. Only very slightly limited ☐ 6
7. Not at all limited ☐ 7

16. In the last two weeks, because of the poor circulation to my legs**, my ability to perform routine household work has been** …..

(tick one)

1. Totally limited, couldn't perform housework at all ☐ 1
2. Extremely limited ☐ 2
3. Very limited ☐ 3
4. Moderately limited ☐ 4
5. A little limited ☐ 5
6. Only very slightly limited ☐ 6
7. Not at all limited ☐ 7

17. In the last two weeks **ulcers in the leg (or foot) have given me pain or distress** …..

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

18. Because of poor circulation to my legs, **the overall range of activities that I would have liked to do in the last two weeks has been** …..

(tick one)

1. Severely limited – most activities not done ☐ 1
2. Very limited ☐ 2
3. Moderately limited – several activities not done ☐ 3
4. Slightly limited ☐ 4
5. Very slightly limited – very few activities not done ☐ 5
6. Hardly limited at all ☐ 6
7. Not limited at all – have done all the activities that I wanted to ☐ 7

19. In the last two weeks **the poor circulation to the legs have made me feel frustrated** …..

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

20. In the last two weeks **when I do get pain in my leg (or foot) it has given me** ….

(tick one)

1. A very great deal of discomfort or distress ☐ 1
2. A great deal of discomfort or distress ☐ 2
3. A good deal of discomfort or distress ☐ 3
4. A moderate amount of discomfort or distress ☐ 4
5. Some discomfort or distress ☐ 5
6. Very little discomfort or distress ☐ 6
7. No discomfort or distress ☐ 7

21. In the last two weeks **I have felt guilty about relying on friends or relatives** …..

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

22. In the last two weeks, because of the poor circulation to my legs**, my ability to go shopping or carry bags has been** …..

(tick one)

1. Totally limited, couldn't go shopping at all ☐ 1
2. Extremely limited ☐ 2
3. Very limited ☐ 3
4. Moderately limited ☐ 4
5. A little limited ☐ 5
6. Only very slightly limited ☐ 6
7. Not at all limited ☐ 7

23. In the last two weeks **I have worried I might be in danger of losing a part of my leg or foot**

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6

7. None of the time ☐ 7

24. In the last two weeks **the distance I can walk <u>has become less</u>** …..

1. A very great deal ☐ 1
2. A great deal ☐ 2
3. A good deal ☐ 3
4. Moderately ☐ 4
5. Somewhat ☐ 5
6. A little ☐ 6
7. Not at all – tick if distance is unchanged or has increased ☐ 7

25. In the last two weeks **I have been depressed about the poor circulation to my legs** …..

(tick one)

1. All of the time ☐ 1
2. Most of the time ☐ 2
3. A good bit of the time ☐ 3
4. Some of the time ☐ 4
5. A little of the time ☐ 5
6. Hardly any of the time ☐ 6
7. None of the time ☐ 7

Thank you for completing this questionnaire

Appendix 6 – Papers & Abstarcts

Papers

1. Gimzewska, M., Berthelot, M., **Sarai, P.**, Geoghegan, L., Onida, S., Strutton, P., Shalhoub, J., Davies, A. H. (2022). Evaluation of a novel wireless near infrared spectroscopy (NIRS) device in the detection of tourniquet induced ischaemia. BMJ Innovations. http://dx.doi.org/10.1136/bmjinnov-2021-000752
2. **Sarai, P.**, Bradshaw, J. M., Milwood Hargrave, J. F. S., Rohani-Shukla, C., Chatterjee, N., Strutton, P.H. (2022). Cortical plasticity induced by a novel low-pressure ischemic nerve block of the lower limb. (in preparation for resubmission).
3. **Sarai, P.**, Luff, C., Rohani-Shukla, C., Strutton, P.H. (2022). Characteristics of motor evoked potentials in patients with peripheral vascular disease. (in preparation for resubmission).
4. **Sarai, P.**, Perring, R., Courtois, N., Bicknell, C., Strutton, P.H. (2022). Availability of intra-operative spinal cord monitoring for thoraco-abdominal aortic aneurysm repair: a survey of vascular anaesthetists in the United Kingdom and Ireland. (in preparation for resubmission).

Abstracts

1. Cai, W., Hepworth Lloyd, F. G., **Sarai, P.**, Hughes, S., Strutton, P.H. (2019). An investigation into the differential effects on somatosensory functions during deafferentation. Abstracts of the Society for Neuroscience. 49.
2. Naulls, S., **Sarai, P.**, Strutton, P.H. (2018). Changes in cortical excitability during ischaemic nerve block. Abstracts of the Society for Neuroscience. 48.
3. Constantinescu, M., **Sarai, P.**, Strutton, P.H. (2018). Mechanisms underlying changes in cortical excitability following ischaemic nerve block of the upper limb. Abstracts of the Society for Neuroscience. 48.
4. Watson, A. M., **Sarai, P.**, Strutton, P.H. (2018). Use of ischaemic nerve block to assess the potential of transcranial magnetic stimulation to monitor spinal cord perfusion. Abstracts of the Society for Neuroscience. 48.
5. **Sarai, P.**, Bicknell, C., Courtois, N., Strutton, P.H. (2018). Can Transcranial Magnetic Stimulation be used to detect potential spinal cord injury following thoracic endovascular aortic repair (TEVAR)? A preliminary study to characterise baseline motor evoked potentials in vascular disease patients. European Journal of Vascular and Endovascular Surgery. https://doi.org/10.1016/j.ejvs.2018.08.025.
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7. Luff, C., **Sarai, P.**, Rohani-Shukla, C., Strutton, P.H. (2017). Could transcranial magnetic stimulation reliably be used as an intra-operative monitor of spinal cord function during thoraco-abdominal aortic aneurysm repair surgery? A pilot study in healthy volunteers. VASGBI annual meeting, London.

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