Neuroplasticity measured via brain stimulation in healthy and depressed subjects

Author:
Player, Michael

Publication Date:
2013

DOI:
https://doi.org/10.26190/unsworks/16561

NEUROPLASTICITY MEASURED VIA BRAIN STIMULATION IN HEALTHY AND DEPRESSED SUBJECTS

MICHAEL J. PLAYER

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

SCHOOL OF PSYCHIATRY, FACULTY OF MEDICINE
THE UNIVERSITY OF NEW SOUTH WALES, SYDNEY AUSTRALIA

October 2013

Thesis/Dissertation Sheet

THE UNIVERSITY OF NEW SOUTH WALES
Thesis/Dissertation Sheet

Surname or Family name: PLAYER

First name: MICHAEL **Other name/s: John**

Abbreviation for degree as given in the University calendar:

School: PSYCHIATRY **Faculty: MEDICINE**

Title: Neuroplasticity measured via brain stimulation in healthy and depressed subjects

Major Depressive Disorder (MDD) is a debilitating and pervasive illness with a lifetime prevalence of between 10-15% of the world's population. The prevailing hypothesis of depression is the stress neurotrophic hypothesis, and is characterised by excessive levels of stress and glucocorticoids. Excessive stress and glucocorticoids result in detrimental changes to the structure and functioning of the brain, including effects upon neuroplasticity. Neuroplasticity allows the brain to differentially respond to stimuli, and adapt to changes in the environment. Impaired neuroplasticity is linked to a number of symptoms in depression.

The thesis aims were to find a means to objectively test neuroplasticity in subjects suffering MDD, and to compare neuroplasticity with matched controls. A secondary aim was to discover if neuroplasticity changed with treatment for depression. To achieve these aims, three separate experiments were carried out.

The aim of the first study was to find a conditioning protocol that induced robust and consistent increases in motor cortical excitability, thus providing a means of measuring neuroplasticity, in healthy subjects. The selected conditioning protocol would be used for measurement of neuroplasticity in healthy and depressed populations in two subsequent studies.

Using the paired associative stimulation (PAS) protocol selected from study 1, the aim for study 2 was to compare neuroplasticity in depressed subjects with that of age and gender matched controls. By measuring motor cortical plasticity before and after PAS conditioning, this study provided one of the first objective demonstrations of impaired neuroplasticity in individuals with MDD that is not confounded by subject effort or motivation.

In study 3, PAS-induced neuroplasticity was measured twice in the same subjects. The first measure was taken while subjects were depressed, the second, after a treatment course of transcranial direct current stimulation. This study showed a significant improvement in neuroplasticity and mood state after treatment, though change in mood did not correlate with change in neuroplasticity.

This research supports a hypothesis of impaired neuroplasticity in depression. Thesis findings provide evidence of improved neuroplasticity and depressive symptoms after treatment, and thus provide important information about the pathophysiology and treatment of MDD.

COPYRIGHT STATEMENT

Michael Player
11 October 2013

ORIGINALITY STATEMENT

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at UNSW or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.

Michael Player
11 October 2013

AUTHENTICITY STATEMENT

I certify that the Library deposit digital copy is a direct equivalent of the final officially approved version of my thesis. No emendation of content has occurred and if there are any minor variations in formatting, they are the result of the conversion to digital format.

Michael Player
11 October 2013

ACKNOWLEDGEMENTS

I'd like to thank my supervisors, Professors Colleen K. Loo, Janet L. Taylor and Perminder Sachdev for supporting me in these particularly optimistic endeavours. Janet, it is not possible to express how grateful I am for your role throughout this thesis. What you provided was so much more than anyone could imagine of a co-supervisor. I am in awe of your dignified presence, your selfless sharing, and your honest and supportive nature. Thank you for the time you gave to me.

I wholeheartedly thank my primary supervisor Colleen for giving me the opportunity to work on a fascinating study that has kept my passion and interest burning from the beginning. I have a true admiration for you and the way you go about your work. It was a privilege to see your innovative influence and drive over a wide range of research projects. I am forever grateful for the opportunity to work in your lab. To the Brain Stimulation team, thank you for the positive work environment and the companionship offered. In particular, thank you to Dr Angelo Alonzo for your patience, support and sagely statistical advice. I am especially grateful to Dr Donel Martin, Dr Bronwyn Hegarty and also Dr Kathryn Fletcher for the clear thinking and the time you made available for me and my many questions. Sincere thanks and gratitude goes to Kerrie-Anne Ho and Dr Veronica Galvez for the good company, friendship and cheerfulness you offered.

Thank you also to Scientia Professor Perminder Sachdev for the tremendous advice and support I received from you and your team at the Centre for Healthy Brain Aging (CHEBA). I never went without anything I needed.

I have been blessed to work on some very important studies with researchers of the calibre of Scientia Professor Phil Mitchell, and Scientia Professor Cyndi Weickert. What a pleasure and true honour it was to work with you both.

Finally, thank you to my family. Your love, support and understanding made this journey much easier. To my mother and father, just knowing you were there as support meant a great deal more than I can express. Thank you to my brothers and sisters and nieces, I am sorry for all the missed celebrations over the last four years. However, the time spent away will ensure that I value every one of these in the coming years.

PUBLICATIONS ARISING FROM THESIS

STUDY 1

Player MJ, Taylor JL, Alonzo A, Loo CK (2012) Paired associative stimulation increases motor cortex excitability more effectively than theta-burst stimulation. Clinical Neurophysiology 123:2220-2226. **(Chapter Two)**

STUDY 2

Player MJ, Taylor JL, Weickert CS, Alonzo A, Sachdev P, Martin D, Mitchell PB, Loo CK (2013) Neuroplasticity in depressed individuals compared with healthy controls. Neuropsychopharmacology 38:2101-2108. **(Chapter Three)**

STUDY 3

Player MJ, Taylor JL, Weickert CS, Alonzo A, Sachdev P, Martin D, Mitchell PB, Loo CK Increase in neuroplasticity after a treatment course of transcranial direct current stimulation for depression (*in review).* **(Chapter Four)**

Abstract

Major Depressive Disorder (MDD) is a debilitating and pervasive illness with a lifetime prevalence of between 10-15% of the world's population. The prevailing hypothesis of depression is the stress neurotrophic hypothesis, and is characterised by excessive levels of stress and glucocorticoids. Excessive stress and glucocorticoids result in detrimental changes to the structure and functioning of the brain, including effects upon neuroplasticity. Neuroplasticity allows the brain to differentially respond to stimuli, and adapt to changes in the environment. Impaired neuroplasticity is linked to a number of symptoms in depression.

The thesis aims were to find a means to objectively test neuroplasticity in subjects suffering MDD, and to compare neuroplasticity with matched controls. A secondary aim was to discover if neuroplasticity changed with treatment for depression. To achieve these aims, three separate experiments were carried out.

The aim of the first study was to find a conditioning protocol that induced robust and consistent increases in motor cortical excitability, thus providing a means of measuring neuroplasticity, in healthy subjects. The selected conditioning protocol would be used for measurement of neuroplasticity in healthy and depressed populations in two subsequent studies.

Using the paired associative stimulation (PAS) protocol selected from study 1, the aim for study 2 was to compare neuroplasticity in depressed subjects with that of age and gender matched controls. By measuring motor cortical plasticity before and after PAS conditioning, this study provided one of the first objective demonstrations of impaired neuroplasticity in individuals with MDD that is not confounded by subject effort or motivation.

In study 3, PAS-induced neuroplasticity was measured twice in the same subjects. The first measure was taken while subjects were depressed, the second, after a treatment course of transcranial direct current stimulation. This study showed a significant improvement in neuroplasticity and mood state after treatment, though change in mood did not correlate with change in neuroplasticity.

This research supports a hypothesis of impaired neuroplasticity in depression. Thesis findings provide evidence of improved neuroplasticity and depressive symptoms after treatment, and thus provide important information about the pathophysiology and treatment of MDD.

Table of Contents

LIST OF TABLES

LIST of FIGURES

Chapter 1: Introduction

Synapses in motion tend to stay in motion. Synapses at rest tend to stay at rest.

– Richard Powers, *Galatea 2.2*

1. Overview

Major Depressive Disorder (MDD) is a debilitating disease affecting near 300 million people worldwide. MDD is a growing disorder in the global burden of disease, and makes up a major cost in economic terms, and years lived with a disability (YLD) (Vos et al., 2013). Remission with currently available treatments occurs in only about 50% of those with MDD after two steps of treatment with medication and psychological therapy (Star*D, Rush et al., 2009). Low remission and recovery rates reflect the poorly understood aetiology of MDD, highlighting the need for more effective treatments (Warden et al., 2007). Clearer understanding of the biological changes that underlie depression would assist in the development of more novel and efficacious treatment options.

In recent years, it has become increasingly clear that the brain is not a static structure, as once believed. Instead, neural connections and circuits have been shown to undergo structural and functional changes throughout the lifespan. This 'neuroplasticity' allows the brain to differentially respond to stimuli and adapt to changes in the environment. Neuroplasticity comprises a number of mechanisms essential for normal brain development and function.

It has been proposed that dysfunctional neuroplasticity may contribute to the aetiology and progression of neuropsychiatric disorders, including MDD (Krystal et al.,

2009). This thesis will present background information and new clinical evidence testing the hypothesis that impairment of neuroplasticity is associated with the aetiology and continuation of depressive symptomology. While a great deal of research exists on both MDD and neuroplasticity independently, this thesis will address areas relevant to the intersection of these two domains.

This introductory chapter provides an overview of the major themes that underlie this thesis: neuroplasticity (section 1.1), MDD (section 1.2), and the techniques used to measure neuroplasticity in health and depression (section 1.3), with the chapter culminating in thesis hypotheses (section 1.4). Chapters 2-4 detail experiments undertaken to test the hypothesis of impaired neuroplasticity in MDD. Chapter 5 provides a summary of novel findings derived from these experiments, and offers a conclusion to the thesis proposition.

1.1 Neuroplasticity

Neuroplasticity refers to the capability of nerve cells to alter structure and function in response to changes in environmental stimuli or experience (Cohen et al., 1998, Rapoport and Gogtay, 2008). 'Neuroplasticity,' 'synaptic plasticity,' and 'plasticity' are terms sometimes used interchangeably, however some distinctions may be made. '*Synaptic plasticity*' refers to cellular processes that affect variability in the strength of neurotransmission between cells, such as change in neurotransmitter release, and change in the number and strength of existing synaptic connections. '*Neuroplasticity*' and '*plasticity*' are more general terms of change, inclusive of a wider range of cellular mechanisms, and will be used in this thesis interchangeably. Neuroplasticity and plasticity encompass the mechanisms of synaptic plasticity, but also a broader range of mechanisms that underlie changes in neuronal structure and function.

These changes include: shifts in intracellular signalling and gene regulation, nerve cell pruning coupled with the loss of connections and dendritic complexity, formation of new synaptic connections, and even neurogenesis (Zarate Jr et al., 2010). Neuroplasticity is crucial to allow functional adaptation of neural systems to different requirements. Failure in this capacity limits an organism's fitness for survival, since some brain structures or circuits may be unable to maintain homeostasis in response to change, and thus become vulnerable to disease (Calabrese et al., 2009).

There are many forms of excitatory and inhibitory neuroplasticity that take place in normal neuronal activities. In this Introduction however, the focus is primarily on three forms of Long-Term Potentiation (LTP: LTP1, LTP2, and LTP3), as they provide a highly relevant means of testing neuroplasticity *in vivo*. Cellular mechanisms and processes that are related to the decrease in synaptic strength, known as Long-Term Depression (LTD), are also discussed. LTP and LTD are the two basic mechanisms for experience-dependent modification of synaptic strength (Buonomano and Merzenich, 1998). While the decrease in synaptic strength via LTD is equally important to an organism's wellbeing as LTP, discussion will mainly focus on the latter. This is because the mechanisms that underlie LTP are critical to the experimental investigations in this thesis. These investigations use facilitatory brain stimulation protocols to induce LTP-like changes in cortical excitability, thus providing a means of measuring neuroplasticity in human subjects. In addition, dysfunctional LTP is thought responsible for a number of cognitive and behavioural symptoms of depression (Pittenger and Duman, 2008).

1.1.1 Mechanisms of Neuroplasticity – Long-Term Potentiation and Long-Term Depression

Multiple forms of plasticity have been discovered at virtually all synapses in organisms that range from very simple invertebrates right through to mammals (Zucker and Regehr, 2002, Malenka and Bear, 2004). Broadly speaking, the two basic mechanisms underlying experience-dependent modification of synaptic strength are LTP and LTD (Buonomano and Merzenich, 1998). LTP increases synaptic strength between neighbouring cells that are activated synchronously. Conversely, LTD reduces synaptic strength and thus the connectivity between adjacent cells (Cooke and Bliss, 2006). Through alteration of synaptic strength between neurons, LTP and LTD provide the cellular mechanisms thought to underpin learning, memory and adaptation in the central nervous system (Malenka and Nicoll, 1999, Martin et al., 2000, Whitlock et al., 2006, Sweatt, 2008).

LTP consists of a complex range of incompletely understood mechanisms. Different forms of LTP are found in different structures in the brain, and even within the same structure (Malenka and Bear, 2004, Raymond, 2007) to optimise flexibility in stimulus response and neural adaptation. Research into LTP has primarily focused on the hippocampus, but also the prefrontal cortex (PFC), where preclinical and human studies have improved understanding of how LTP affects organism function (Pittenger and Duman, 2008). For example, mechanisms of LTP are relied upon in cognitive control tasks shown in the PFC, the region primarily responsible for executive function (Otani, 2002, Otani et al., 2003, Rocher et al., 2004, Liston et al., 2006, Walsh et al., 2009). Mechanisms of LTP are also an important component of hippocampus-dependent memory formation (Sweatt, 2008). Despite the many known forms of LTP, discussion in this thesis will be limited to postsynaptic LTP, on which most research has

been focused. Postsynaptic LTP is the same mode of synaptic change hypothesised to occur after the brain stimulation protocols used in this thesis (Di Lazzaro et al., 2008c, Di Lazzaro et al., 2009).

1.1.2 Stages of Long-Term Potentiation

Postsynaptic LTP is not a unitary construct, and can be distinguished by time duration, with longevity ranging from momentary changes right through to changes that last for hours, days and even longer (Citri and Malenka, 2008). Variation exists between laboratories in the terminology used, and the delineation between phases of LTP in models of plasticity (Sweatt, 2008). For this thesis, a multiple phase model of LTP is used, distinguishing short lasting LTP which may last for up to 2 hours, intermediate LTP which may last up to 8 hours, and long-lasting LTP which may last for days, weeks or longer (Raymond, 2007, Reymann and Frey, 2007). Following the nomenclature adopted by Raymond et al. (2007), these phases will be referred to as LTP1, LTP2, and LTP3 respectively.

Stability and longevity of LTP is enhanced by cellular processes which supplant those inducing the simpler, more transient plasticity (Frey et al., 2001, Sweatt, 2008). For example, longer lasting plasticity may require a different combination or concentration of ions and neurotransmitters, initiation of additional cell signalling cascades, extra receptor trafficking, and protein synthesis (Reymann and Frey, 2007, Sweatt, 2008). A brief description of these LTP phases and mechanistic difference is provided below. While this thesis is not intended for molecular biologists, an understanding of the different phases of LTP provides an important reference point for comprehension of LTP-like changes that are hypothesised to occur as a result of the different brain stimulation protocols used in this thesis.

1.1.2.1 LTP1

LTP1 is a brief, temporary change in synaptic efficacy that has often been referred to as Early-LTP. LTP1 can be induced by weak stimulus protocols (Raymond, 2007). Enhanced presynaptic transmission induces greater calcium influx into the postsynaptic cell through ionotropic glutamate receptor activation. In general, glutamate released from presynaptic neurons binds to alpha-amino-3-hydroxy-5-methylisoxazole propionate (AMPA) and N-methyl-D-aspartate (NMDA) receptors. Binding of glutamate to AMPA receptors opens their channels to sodium and potassium ions lowering the resting membrane potential of the postsynaptic neuron. Coincident postsynaptic membrane depolarisation then induces NMDA receptor activation which facilitates calcium influx, and initiates postsynaptic LTP cascades (Citri and Malenka, 2008). Postsynaptic calcium levels must first reach a critical threshold which will then initiate an internal signalling cascade, with cyclic adenosine monophosphate (cAMP) activating protein kinases such as calcium-calmodulin-dependent kinase II (CaMKII). CaMKII is considered the primary mediator of short lasting LTP through the phosphorylation of pre-existing synaptic proteins (Raymond, 2007). Thus, CaMKII activity leads to an increase in the conductance of AMPA receptors as well as insertion of additional AMPA receptors into the postsynaptic membrane (Citri and Malenka, 2008). This activity results in LTP1, a form of synaptic plasticity characterised by fast initial onset that decays over a period of a few hours. LTP1 is independent of protein synthesis or gene transcription (Raymond, 2007) (see Figure 1.1). LTP1, confusingly, may have some mechanistic overlap with Short-Term Potentiation (STP). STP is acknowledged as the first stage of LTP, and lasts about 30 minutes. Induction of STP is independent of protein kinase activity, however, not a great deal more is known about STP mechanisms (Sweatt, 2008).

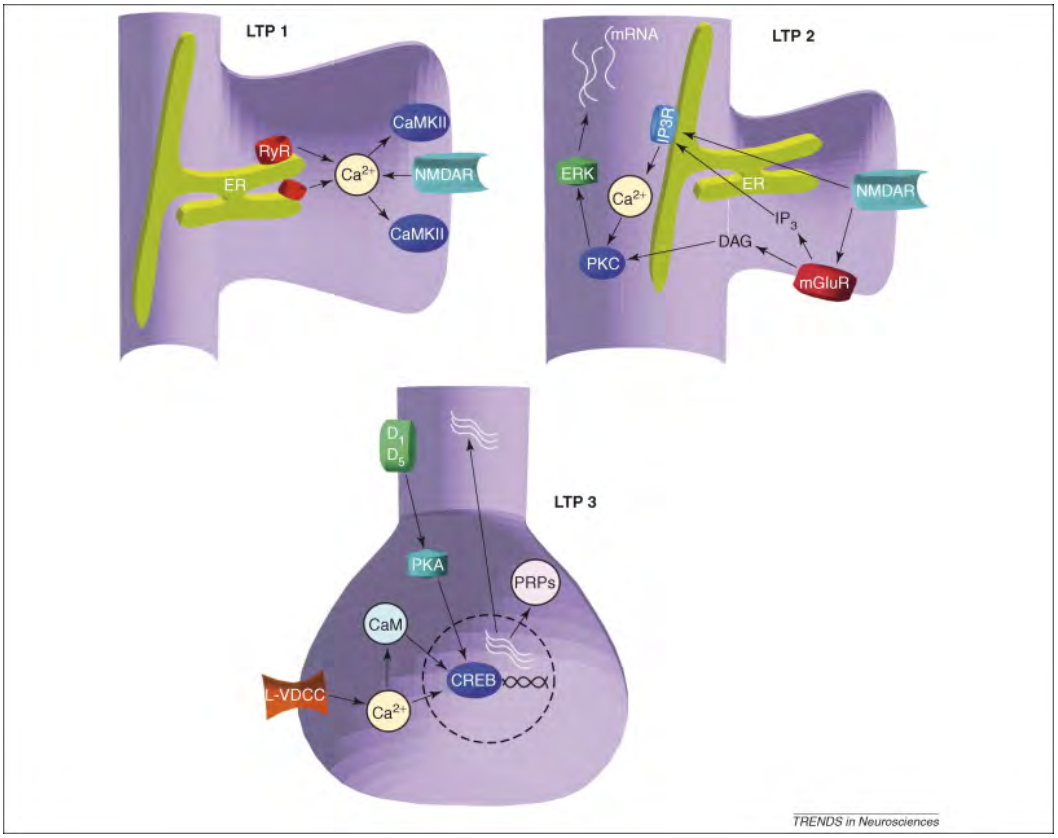


Figure 1.1 Summary of the hypothesised mechanisms underlying LTP1, LTP2, LTP3.

Each later phase of LTP extends upon the more transient mechanisms of the LTP phase previous. LTP1 relies on protein-kinase activity in dendrites improving glutamatergic receptor function leading to potentiation which lasts up to a few hours. LTP2 is an intermediate phase of potentiation which also relies on protein kinase activity (for example, PKC) to induce synthesis of proteins. The LTP3 phase depends upon gene transcription to further sustain changes to synaptic strength and synapse morphology. CaM, calcium–calmodulin complex; CaMKII, calcium-calmodulin-dependent kinase II; Ca2+, calcium; CREB, cyclic AMP responsive element binding protein; D1, dopamine receptor 1, D5, dopamine receptor 5; DAG, diacylglycerol; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; IP3, inositol (1,4,5)-triphosphate; IP3R, inositol (1,4,5)-trisphosphate receptors, L-VDCC, ligand voltage-dependent calcium channels; mGluR, metabotropic glutamate receptors; mRNA, messenger ribonucleic acid; NMDAR, N-methyl-D-aspartate receptors; PKC, protein kinase C; PKA, protein kinase A; PRP, prion protein; RYR, ryanodine receptors. Figure from (Raymond, 2007).

1.1.2.2 LTP2

LTP of intermediate duration (LTP2) requires a greater degree of presynaptic cell activation than observed in LTP1. This may be achieved by longer, or stronger tetanic stimulation. Sufficient neuronal tetanisation generates higher levels of postsynaptic calcium, through parallel activation of NMDA, and metabotropic glutamate (mGlu) receptors (Raymond, 2007). This increase in postsynaptic calcium concentration facilitates longer lasting LTP through the synthesis of proteins, which stabilises synaptic strength and differentiates LTP2 from LTP1. Protein synthesis occurs via a number of protein kinase pathways (Citri and Malenka, 2008), using mRNA already existing in dendrites (Otani et al., 1989). Protein synthesis (independent of gene transcription) alters the strength of a synapse, and even its morphology, and has been shown to last for up to 8 hours after cell tetanisation (Reymann and Frey, 2007).

1.1.2.3 LTP3

LTP in its most durable form (LTP3) can be induced by even greater intensity of stimulatory inputs than in LTP2. For example, higher frequency, or higher intensity tetanic stimulation enables greater calcium influx into the postsynaptic cell, in contrast to the more moderate stimulation linked with LTP2 (Raymond, 2007). This greater postsynaptic calcium concentration facilitates additional cell processes that sustain changes in plasticity. For example, a distinguishing feature of LTP3 is reliance on gene transcription. Gene transcription occurs in the cell soma and facilitates the synthesis of proteins in addition to those produced in LTP2 (Raymond, 2007). Gene transcription is initiated through the activation of the cyclic AMP responsive element binding protein (CREB) pathway. This pathway is mediated by higher postsynaptic calcium concentrations (Raymond, 2007). However CREB activity is also influenced by other neuromodulatory inputs. For example, dopaminergic signaling triggers additional

protein kinase pathways and regulates transcription activity (Reymann and Frey, 2007). New mRNA transcripts are either translated into plasticity-related proteins in the soma or transported to the dendrites, where cell proteins are synthesised akin to that in LTP2. Proteins encoded by newly transcribed mRNA are responsible for the resilient nature of this longer lasting LTP. LTP3 improves synaptic efficacy above that of transient LTP, and if the signal is adequate, induces and maintains long-lasting structural changes at the synapse.

1.1.3 Role of Neuroplasticity in Mental Health

On the whole, neuroplasticity is seen as a beneficial process essential for optimal neuronal development and functioning, while the absence of plasticity is seen as detrimental to the maintenance of good health. However, there is some evidence that excessive neuroplasticity may be pathological, contributing to neurological disorders such as dystonia (Quartarone and Pisani, 2011), or more subtle functional impairments characteristic of psychiatric illness (Krystal et al., 2009). An example of a functional impairment is the exaggerated fear reaction found in the amygdala in response to fear-provoking stimuli (Vouimba et al., 2006), but also neutral stimuli (Citri and Malenka, 2008), which is often found in anxiety disorders. Therefore, while neuroplasticity is critical to an organism's survival, optimal functioning requires an appropriate level in each brain region.

1.2 Neuroplasticity in Major Depressive Disorder

The following section discusses changes in brain structure and function in depression, and the prevailing hypotheses for this disorder. MDD, and animal models of depression are often characterised by greater levels of stress and glucocorticoids

(Sheline, 2003, Willner, 2005). Excessive stress and glucocorticoids affect LTP and LTD expression resulting in dysfunctional neuroplastic changes, which in turn are hypothesised to underlie a number of the symptoms in depression. Structural and functional brain abnormalities in MDD are hypothesised to result from dysfunctional neuroplastic changes, with such abnormalities also observed in animal models of depression. This thesis will focus primarily on structural abnormalities in the hippocampus, frontal cortices, amygdala, and the major depression circuits, where evidence is most available. Functional abnormalities are also discussed. Specifically, the focus will be on cognitive and behavioural symptoms that likely result from impaired neuroplasticity. The state versus trait related features of structural and functional abnormalities are also considered, highlighting whether neuroplasticity impairments and associated symptoms are state-dependent (i.e. only present during a major depressive episode) and thus able to be reversed with treatment. Understanding of depressive symptomotology may therefore assist in addressing the overarching questions of this thesis – is neuroplasticity impaired in MDD, and does it improve when people are no longer depressed?

1.2.1 Major Depressive Disorder

MDD is a pervasive illness currently affecting over 4% of the population worldwide (Vos et al., 2013), with a lifetime prevalence of 10-15% (Bromet et al., 2011). The World Health Organisation (WHO) estimates MDD to be the fourth leading contributor to the global burden of disease, with predictions that the disorder will become the largest cause of disease burden by 2030 (Mathers et al., 2008). The predicted growth in disease prevalence and economic cost to the community position MDD as an important illness for treatment investigation. At present, the most common treatments for depression include antidepressant medication, psychotherapy such as

Cognitive Behavioural Therapy, and physical treatments such as Electroconvulsive Therapy (ECT).

Diagnoses of MDD are made with reference to diagnostic tools such as the Diagnostic and Statistical Manual of Mental Disorders (DSM-V; American Psychiatric Association, 2013). The DSM-V criteria capture distinctive physical, cognitive and behavioural symptoms of MDD affecting mood, thought patterns, and psychomotor disturbances.

1.2.1.1 Aetiology of MDD

Since the 1960s, the 'monoamine hypothesis' of depression has been a dominant theory regarding MDD aetiology. This hypothesis indicates that the pathophysiology of depression involves abnormalities in monoamine neurotransmission – specifically, a reduction in serotonergic, noradrenergic and/or dopaminergic activity. The majority of antidepressant medications target the monoamine system, increasing serotonin, norepinephrine and/or dopamine availability and improving monoaminergic receptor functioning. The upregulation of one or more of these neurotransmitters has been shown to improve intracellular signaling and restore functioning within neuronal systems, resulting in measurable improvement in depressive symptoms (Vaidya and Duman, 2001, Kharade et al., 2010).

Monoamine-focused interventions have not, however, provided a comprehensive treatment solution for all depressed individuals. The inadequate remission rate after antidepressant treatment (Rush et al., 2009), high relapse rates, residual symptomology, and the existence of antidepressants that are not primarily monoaminergic in action (for example, ketamine), provide clear evidence that impaired monoaminergic transmission is not the sole cause of depressive symptoms (Paul and Skolnick, 2003). Evidence also

shows that mood improvements following antidepressant treatment generally only occur after a number of weeks of administration, despite rapid enhancement in monoaminergic transmission. This delay in symptom improvement may indicate that monoaminergic deficits might be secondary to upstream abnormalities (Manji et al., 2001).

In light of the inadequacy of the monoamine hypothesis to fully account for depression aetiology and treatment, a number of other theories posit alternative explanations for the illness. These alternative hypotheses include:

- The 'network hypothesis' posits that depressive symptoms result from abnormalities in interneuronal communication within brain networks, impairing the transfer and storage of information necessary for maintaining good health (Castrén, 2005).
- The 'inflammation hypothesis', holds that environmental stressors result in increased levels of cytokines. Abnormal cytokine levels impair neuronal communication and neuroplasticity, which is thought to be responsible for depressive symptoms (Hayley et al., 2005).
- The 'neurotoxic hypothesis' posits that structural and functional plasticity abnormalities caused by depression make neurons vulnerable to insults (Sapolsky et al., 1986, Lupien et al., 2009). Recurrent depressive episodes enhance the deleterious effect upon neurons inducing cumulative maladaptive changes to brain structure and functionality (Fossati et al., 2004).
- The 'neuroplasticity hypothesis' of depression – which is a main tenet of this thesis, is that the inability of an organism to adapt its neural structure and function to changing internal and external cues contributes to depressive symptoms (Nissen et al., 2010). This theory is supported by evidence of an

inability to make appropriate adaptive responses to environmental stimuli (Duman et al., 1999). Examples of dysfunctional experience-dependent plasticity are hypothesised to include impaired learning and memory, and inappropriate or exaggerated response to stress or other aversive stimuli (Pittenger and Duman, 2008).

There is persuasive experimental evidence for each of the aforementioned hypotheses in the aetiology of depression. However, none of these hypotheses comprehensively explain the aetiology of the disease, and all its symptoms. Furthermore, these depression hypotheses have considerable overlap and are not mutually exclusive. Therefore, it is possible that an individual's experience of MDD results from a combination of the above aetiologies. In addition, MDD may not be a single illness and thus, may have different aetiologies in different individuals. Diverse aetiologies would be expected to lead to variable cortical effects and to induce diverse symptoms which may have different time courses, and complicate treatment approaches. Nonetheless, research to date has favoured a 'stress neurotrophic' hypothesis as the theory which currently best accounts for MDD aetiology, symptomology and the antidepressant response. This hypothesis posits that lower levels of neurotrophins, such as brain derived neurotrophic factor (BDNF) result from downregulated regional neuronal activity found in MDD. Lower neurotrophin levels may induce neuronal atrophy (Duman et al., 2000), and impair both neuroplasticity and functionality in specific depression-related regions that lead to depressive symptoms (Duman and Monteggia, 2006). However, it has been shown that neurotrophin depletion might just increase the risk of MDD but not be sufficient in and of itself to cause depressive symptoms (Duman et al., 2007). Research has shown that there is an important environmental interaction – exposure to stress, which may induce depressive phenotype (Duman and Voleti, 2012).

The ‘stress neurotrophic’ hypothesis is therefore considered to provide the most comprehensive theory for the aetiology of the disease. However, the neuroplasticity hypothesis provides the most appropriate explanation for many cognitive and behavioural symptoms in depression, and the experimental investigations that are discussed in this thesis. While understanding of MDD is complicated by the multiple mechanisms and time courses of MDD symptoms and their response to treatment, it is hypothesised that stress and neurotrophic changes cause changes to neuroplastic mechanisms that induce specific symptoms while an individual is depressed. The ‘stress neurotrophic’ hypothesis is now considered in more detail.

1.2.1.2 Stress Neurotrophic Hypothesis of MDD

The environmental influence of stress makes the stress neurotrophic hypothesis of MDD a more comprehensive account of depression pathophysiology. The stress neurotrophic hypothesis posits that the brain reacts to acute and chronic stressors through activation of the hypothalamic-pituitary-adrenal (HPA) axis, which stimulates adrenal release of glucocorticoids. Glucocorticoids are steroid hormones, which are essential for normal, healthy brain maturation. However, a chronically overactive HPA axis may produce repeated episodes of hypercortisolemia (Sheline, 2003, Burke et al., 2005). Excessive levels of glucocorticoids can have a detrimental effect on synaptic connectivity, brain development and brain functioning, and may induce neuronal atrophy (Sapolsky et al., 2000, see section 1.2.3-1.2.5). Atrophy and other changes detrimental to neuronal functioning have been directly measured in animal models, in which chronic stress is used to induce depression.

1.2.2 Animal Models of Depression

Animal models of depression have been developed to aid in understanding the aetiology of MDD and its sequelae. These models primarily include acute and chronic

stress paradigms designed to activate the HPA axis, and induce analogous neurochemical and behavioural changes to those observed in MDD patients (Nestler et al., 2002, Tannenbaum et al., 2002). Animals subjected to chronic stress paradigms characteristically exhibit elevated glucocorticoid levels, increases in anxiety and depression-linked behaviours, impairment in cognitive functions, and regionally specific neuronal atrophy (Willner, 2005, Autry and Monteggia, 2012). Models in which animals receive direct administration of glucocorticoids demonstrate similar cognitive impairments and neuronal atrophy. Thus animal models may be useful in studying MDD.

Animal models have also highlighted potential mechanisms that may underlie the chronicity of MDD. These models show that stress results in the accumulation of glucocorticoids in glucocorticoid sensitive neurons. Glucocorticoid accumulation, in conjunction with altered expression and impaired functioning of the glucocorticoid receptors in the hypothalamus may cause a breakdown in the negative feedback loop that ordinarily halts further glucocorticoid release from the HPA axis (Nestler et al., 2002) (see Figure 1.2). In addition to impaired functioning of the hypothalamus, there is a loss of inhibitory control over HPA activity through damage to neurons in the hippocampus (Sapolsky et al., 1984, Sheline et al., 1996, Nestler et al., 2002) and frontal cortices (Diorio et al., 1993, Lupien et al., 2009). Animal models predict that in depression, common environmental stressors may invoke an exaggerated stress response through excessive circulating glucocorticoids (Sheline, 2003). Dysregulated levels of glucocorticoids could be responsible for the generation of a number of the symptoms of depression (see section 1.2.3-1.2.5). Increases in depressive symptomology may be induced by changes to cellular metabolic capacity, synaptic connectivity, glutamatergic excitotoxicity and ultimately, neuronal atrophy (Pittenger

and Duman, 2008). Therefore, stress-induced mechanisms are implicated in structural and functional changes in hippocampal and frontal regions found in depression (Sapolsky et al., 2000, Duman and Monteggia, 2006, Goosens and Sapolsky, 2007).

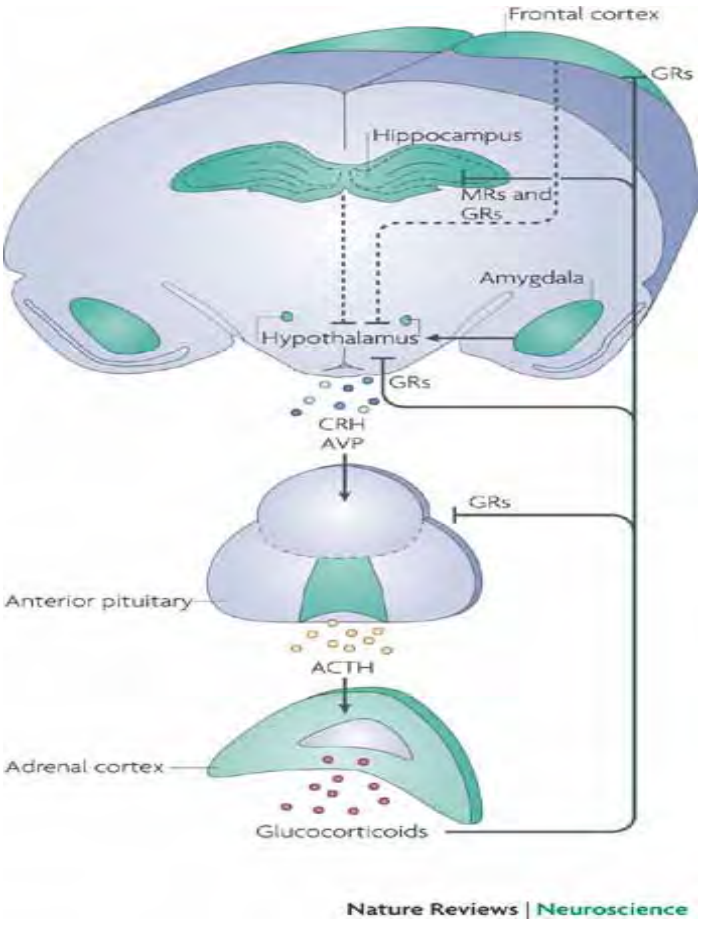


Figure 1.2 Hypothesised HPA axis response to stressor, response shown with negative feedback loop

When a threat is detected a stress response is initiated that crucially involves the HPA axis. Corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are released from the hypothalamus triggering the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland. This leads to the production and release of glucocorticoids from the adrenal cortex. The stress response is determined by the ability of glucocorticoids to further regulate the ACTH and CRH release by binding to the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). Once the HPA axis has been activated and the stressor has subsided, feedback loops are triggered at various levels of the system, i.e. from the adrenal gland to the hypothalamus, and other brain regions such as the hippocampus and the frontal cortex in order to shut down the HPA axis and to return to a set homeostatic point. Damage to neurons in the hippocampus and PFC may impair the feedback loop and thus fail to halt glucocorticoid release. Figure from (Lupien et al., 2009).

1.2.3 Stress and Glucocorticoid Induced Structural and Functional Changes

It is hypothesised that depression, excessive stress or glucocorticoid administration cause structural and functional changes in the brain. These changes can be measured to assist in disease detection and treatment. Structural brain changes can be assessed *in vivo* via magnetic resonance imaging (MRI). Functional MRI (fMRI) and positron emission tomography (PET) are used to determine the functional integrity of the brain. These two functional imaging techniques allow assessment of neuronal connectivity and regional activity levels via measurement of cerebral blood flow (CBF) and glucose metabolism.

1.2.3.1 Hippocampus

The hippocampus is a key structure for consolidating emotionally relevant information into memory. It is well established that hippocampal function is negatively affected by exposure to excessive levels of stress and glucocorticoids (Carlson et al., 2006). However, inconsistent evidence for the functioning of the hippocampus has been shown in depression (Savitz and Drevets, 2009). In a review of depression-related changes in activity levels, Drevets reported non-significant differences in glucose metabolism and CBF between depressed subjects and controls, signifying no difference in metabolic activity (Drevets and Furey, 2009). In contrast, some research has reported an increase in hippocampal activity compared to controls (Videbech et al., 2001), while other research points to regional decreases in activity (Sapolsky et al., 1984). For example, Sapolsky and colleagues (1984) reported that the hippocampal projection to the hypothalamus becomes hypoactive in MDD, resulting in the ineffective control of HPA axis activity, and to an exaggerated stress response. There are a number of possible explanations for reports of inconsistency in hippocampal activity in depression. One interpretation is that some inputs and outputs of the hippocampus may either be

hyperactive or hypoactive, with no resultant overall change in activity. Other possible explanations for inconsistencies include changes in hippocampal volume, which affect the relative measure of glucose metabolism and CBF (Drevets and Furey, 2009). Another possibility is variation in the imaging techniques between the studies, which may not have consistently delineated hippocampal regions (Videbech et al., 2001).

Despite inconsistencies in reported activity levels, studies investigating structural changes in depression and models of depression have generally found reductions in hippocampal volume (Sapolsky et al., 2000, Campbell et al., 2004). Animal models have consistently shown that chronic stress or the chronic administration of glucocorticoids cause dendritic atrophy in hippocampal neurons (Magariños and McEwen, 1995) leading to regional volume reductions (Sapolsky et al., 2000, Goosens and Sapolsky, 2007). Similarly, in human studies, hypercortisolemia and glutamatergic excitotoxicity result from HPA axis overactivity in the context of stress (Sheline et al., 1996), and are associated with the retraction of dendrites and neuronal atrophy in hippocampal pyramidal cells (McEwen, 1999, McEwen and Magarinos, 2001). Lower hippocampal volumes are found using imaging data in those with a history of depression (Sheline et al., 1996, Shah et al., 1998, Bremner et al., 2000, Bell-McGinty et al., 2002, MacQueen et al., 2003, Campbell et al., 2004). This finding has been confirmed in post-mortem studies (Stockmeier et al., 2004). Reductions in hippocampal volume are more pronounced in those who have suffered multiple depressive episodes (Kessing, 1998, MacQueen et al., 2003), and may correlate with illness duration (Sheline et al., 1996, Sheline et al., 1999), or disease severity (Vakili et al., 2000). Overall, an association between chronic stress, glucocorticoids and reduced hippocampal volume (Lupien et al., 2007), has been consistently demonstrated in

depression (Campbell et al., 2004, Stockmeier et al., 2004, Goosens and Sapolsky, 2007).

1.2.3.2 Prefrontal Cortex

The PFC is generally considered to be the centre of executive function. In the PFC, stress and glucocorticoids have the similar effect of reducing activity and volume as in the hippocampus, with these effects also linked to the pathophysiology of depression (Carlson et al., 2006). Overall, in most PFC regions, there are decreases in volume and activity, however some structures within the PFC have shown increases in volume and activity (Drevets et al., 1992, Drevets, 1998, Drevets et al., 2008, Savitz and Drevets, 2009). This variability in PFC volume and activity in depression has been supported by investigations of stress and glucocorticoid exposure in animal models (Liston et al., 2006, Pittenger and Duman, 2008).

Animal models of depression have shown dendritic retraction and reduction in dendritic spine numbers in the PFC generally following chronic stress (Izquierdo et al., 2006). Administration of glucocorticoids in animal models has similarly resulted in reduced volume in prefrontal cortical regions (Cerqueira et al., 2005). In humans, overall, the PFC is hypoactive in MDD, shown through reduced CBF and glucose metabolism (Drevets, 2000). PET studies have shown reduced CBF in the dorsomedial PFC (Drevets et al., 1999), dorsolateral PFC (Bench et al., 1995), and the dorsal anterior cingulate (ACC) (Drevets et al., 1999, Goethals et al., 2005). In addition, a study in healthy individuals showed that after induction of sadness, subjects had a similar pattern of decreases in prefrontal CBF as those suffering a depressive episode (Mayberg, 1997). Furthermore, human neuropathological studies have found decreases in frontal lobe volume of depressed subjects compared to controls (Coffey et al., 1993). These changes are characterised by regional reductions in neuronal and glial size and density

(Rajkowska et al., 1999). Evidence supporting the relationship between regional decreases in volume and MDD is shown by a negative correlation found between dorsolateral PFC grey matter volume and depression severity (Chen et al., 2007). Overall these findings suggest that stress, sadness and depression generally lower neuronal activity in the PFC (Manji et al., 2001). This lower neuronal activity in conjunction with regional atrophy and impaired functionality led to the hypothesis of hypofrontality in patients with MDD (Goethals et al., 2005, Pittenger and Duman, 2008).

1.2.3.3 Amygdala

The crucial function of the amygdala is determination of the emotional valence of perceptual experience, and the interpretation of emotionally laden memories and social cues (Carlson et al., 2006). The effects of excessive stress or glucocorticoids on the amygdala remain unclear. Some evidence suggests that exposure to excessive stress or glucocorticoids has opposite effects in the amygdala to those in the hippocampus and the PFC. Thus, in contrast to generally reduced activity and volume observed in hippocampus and PFC, studies have shown an increase in activity, dendritic complexity, and volume of the amygdala in response to excessive stress or glucocorticoids (Mitra et al., 2005, Mitra and Sapolsky, 2008, Pittenger and Duman, 2008, Lupien et al., 2009). Furthermore, increased resting levels of CBF and glucose metabolism in the amygdala have been shown to correlate positively with the intensity of negative mood (Drevets et al., 1992, Abercrombie et al., 1998, Drevets et al., 2002, Hamilton and Gotlib, 2008). However evidence of increased amygdala volume in MDD is not entirely consistent, with a recent meta-analysis finding decreases in volume compared to controls (Sacher et al., 2012). This inconsistency might be explained by inter-subject differences in antidepressant treatment history. One other recent meta-analysis into amygdala volume

in depression found decreases in unmedicated patients compared to controls, but comparative increases in patients taking antidepressants (Hamilton et al., 2008).

1.2.4 Stress and Glucocorticoid Induced Changes to Neuroplasticity and Substrates of Neuroplasticity in MDD

The evidence shown above (see section 1.2.3) for changes in structure and activity in fronto-limbic regions induced by stress and glucocorticoids provides support for the stress neurotrophic hypothesis of depression. In addition to these effects observed at the structural level, stress and glucocorticoids may change the efficacy of critical molecular and cellular processes inducing functional changes. For example, neuroplasticity allows the organism to change structure and function with experience, and provides a life-sustaining mechanism known to be affected by stress and glucocorticoids (Masi and Brovedani, 2011, Schoenfeld and Gould, 2012). For instance, one form of neuroplasticity, neurogenesis, is reduced in the hippocampus in preclinical models by stress (Gould et al., 1997, Czéh et al., 2001, Koo et al., 2010) and glucocorticoid administration (Wong and Herbert, 2006, Lucassen et al., 2010). Stress and glucocorticoids are hypothesised to affect other crucial forms of neuroplasticity, shown through regionally-specific changes in LTP and LTD expression. The following discussion of how the stress neurotrophic hypothesis may explain changes in neuroplasticity in MDD will centre on how stress and glucocorticoids specifically affect synaptic plasticity. In particular, how stress and glucocorticoids affect the requisite substrates of synaptic plasticity such as BDNF, glial cells, glutamate, and the location and functioning of ionotropic receptors.

1.2.4.1 BDNF

BDNF and other neurotrophins have been shown to play a key role in neuronal growth, survival and differentiation (Mamounas et al., 1995, Manji et al., 2003, Brunoni

et al., 2008). BDNF levels are reduced by exposure to stress (Smith et al., 1995) or extended periods of glucocorticoid administration (Jacobsen and Mørk, 2006). BDNF expression appears to be related to regional variations in brain volume and activity in MDD (Autry and Monteggia, 2012) (see section 1.2.3). For example, decreased levels of BDNF have been found in the hippocampus and in the PFC of depressed subjects (Castrén et al., 2007, Dwivedi, 2009). Evidence is presently inconclusive as to whether BDNF levels correlate with volume change in the amygdala in depression (Autry and Monteggia, 2012). However, in animal models of depression, there is enhanced BDNF expression in the amygdala in response to stress (Yu and Chen, 2011). Thus overall, BDNF changes resulting from stress and depression are consistent with the hypothesis that BDNF expression is likely related to MDD pathophysiology (Autry and Monteggia, 2012). This hypothesis is supported by a number of meta-analyses which show relationships between BDNF expression and clinical abnormalities in MDD (Sen et al., 2008). However, not all evidence is supportive of the involvement of BDNF in pathophysiology of depression, for review see (Groves, 2007).

BDNF signalling contributes to both functional and structural plasticity (Egan et al., 2003, Autry and Monteggia, 2012). BDNF is hypothesised to play a role in structural changes, through for example, the enlargement of dendritic spines (Carvalho et al., 2008, Yoshii and Constantine-Paton, 2010). In addition, BDNF has acute effects on mechanisms of synaptic plasticity through facilitation of the release of neurotransmitters glutamate and gamma-aminobutyric acid (GABA), which underlie excitatory and inhibitory potentials respectively (Goggi et al., 2002). BDNF can strengthen excitatory transmission by enhancing inward currents through AMPA receptors (Manji et al., 2003). In contrast, lower BDNF levels induced by genetic or pharmacological manipulations weaken synaptic efficacy, evidenced by comparatively

impaired LTP, and decreased learning and memory in preclinical models (Lu et al., 2008).

BDNF has also been associated with neuroplasticity essential for symptom improvement after administration of antidepressants (Duman and Aghajanian, 2012). For example, the normalisation of synaptic transmission after antidepressant treatment relies on the action of BDNF (Vetencourt et al., 2008, Duman and Voleti, 2012). In stress models of depression, antidepressant-induced behavioural improvements that rely on neuroplastic mechanisms are blocked in mice with BDNF deletion, and mice genetically modified to have lower BDNF expression (Chen et al., 2006, Castrén and Rantamäki, 2010, Duman and Voleti, 2012). Therefore, evidence supports the proposal that stress-induced reductions in BDNF levels may crucially impair neuroplasticity. Further, antidepressant treatments rely on BDNF to improve neuroplasticity and, as shown in animal models, neuroplasticity is crucial to improvement in the depressive syndrome (Castrén, 2005, Son et al., 2012).

Of particular relevance to the potential of BDNF to enhance neuroplasticity, a functional non-synonymous single nucleotide polymorphism resulting in the substitution of amino acid valine by methionine has been identified in codon 66 (Val66Met, rs6265) of the BDNF gene (Egan et al., 2003). Val homozygotes are thought to exhibit greater synaptic plasticity through more efficient packaging and secretion of BDNF than Met carriers. In humans, this polymorphism has been associated with a change in neuroplastic response after exposure to a variety of plasticity-inducing stimuli, such as brain stimulation protocols (Cheeran et al., 2008) and antidepressant treatments (Duman and Aghajanian, 2012). It is hypothesised that Met carriers have an increased risk of abnormal neurobiological change compared to Val homozygotes. For example, Met carriers are reported to have comparative regional

volume loss in the hippocampus (Pezawas et al., 2004), and this extends to the PFC in the case of individuals who suffered childhood stress (Gerritsen et al., 2012). Met carriers may also have an increased risk of psychiatric disorders. For example, Verhagen and colleagues reported that males carrying the Met polymorphism have an increased risk of depression (Verhagen et al., 2010), and Gatt and colleagues found that Met carriers were more likely to develop depression as a result of childhood stress (Gatt et al., 2009). However, the overall evidence for an association of BDNF Val/Met polymorphism with disease risk remains inconclusive (Calabrese et al., 2009), with two meta-analyses failing to show an overall relationship between Val66Met polymorphism and depressive disorders (Gratacòs et al., 2007, Verhagen et al., 2010).

1.2.4.2 Glial Cells

The function of glial cells is to maintain metabolic and ionic homeostasis within neurons. In animal models, there is reduced glial cell production after chronic stress (Banasr et al., 2007) and glucocorticoid exposure (Alonso, 2000). Similarly in depression, glial cell density corresponds with established depression-related changes in metabolic activity and volume (see section 1.2.3). For example, reductions in glial cell density have been reported in frontal regions (Rajkowska and Miguel-Hidalgo, 2007), and specifically in the ACC (Öngür et al., 1998), dorsolateral PFC (Rajkowska et al., 1999, Cotter et al., 2001) and orbital cortex (Rajkowska et al., 1999). In contrast, there is an apparent increase of glial density in the hippocampus of depressed individuals (Stockmeier et al., 2004). However, it has been suggested that this finding may simply reflect the more substantial reduction in hippocampal neuronal volume (Rajkowska and Miguel-Hidalgo, 2007).

Reduction in the number and density of glial cells provides another mechanism by which neuroplasticity may be impaired in MDD (Manji et al., 2003, Rajkowska and

Miguel-Hidalgo, 2007). Glial cells have a clear role in both the synthesis and clearance of neurotransmitters, particularly glutamate (Rajkowska and Miguel-Hidalgo, 2007). A reduction in glial cell functioning may contribute to glutamatergic dysfunction in MDD (Ritchie et al., 2004, Sanacora et al., 2004). This dysfunction may consist of an imbalance between synaptic and extrasynaptic concentrations of glutamate. Synaptic glutamatergic neurotransmission is positive for cell maintenance, cell survival and LTP. In contrast, excessive extrasynaptic glutamatergic concentrations bias regional synaptic plasticity toward LTD (Papouin et al., 2012). Excessive extrasynaptic glutamate levels may also reduce BDNF expression, impair cellular maintenance, and increase cell vulnerability to excitotoxicity and volume loss (Pittenger et al., 2007). Overall, these findings suggest that glial cells may be crucially involved in regulating neuroplasticity through the uptake of glutamate, and changes in glial cell functioning are therefore relevant to the pathogenesis of depression.

1.2.4.3 Excessive Extrasynaptic Ionotropic Receptor Activation

Stress and glucocorticoids are thought to modulate the regionally specific expression of ionotropic receptors within the cortico-limbic region (Yuen et al., 2008, Caudal et al., 2010, Marsden, 2011). High levels of stress and glucocorticoids induce excessive levels of synaptic and extrasynaptic glutamate (Marsden, 2011). Higher levels of extrasynaptic glutamate result in the activation of a greater number of extrasynaptic NMDA and non-NMDA (AMPA and kainate) receptors. This greater glutamatergic receptor activation is particularly evident in stress susceptible regions such as the hippocampus, and PFC (Moghaddam, 1993, Moghaddam et al., 1994, Lowy et al., 1995, Ritchie et al., 2004). A disproportionate activation of extrasynaptic glutamatergic receptors results in excessive increases in intracellular calcium influx. Excessive intracellular calcium levels lead to dysfunction in multiple cellular processes, impairing

cell maintenance and stability (Marsden, 2011). For example, excessive intracellular calcium has been shown to reduce LTP, generate oxygen free radicals, induce excitotoxicity, and may even cause cell death (Armanini et al., 1990, Takahashi et al., 2002, Manji et al., 2003). Thus greater extrasynaptic ionotropic receptor activation induced by excessive glutamate may reduce LTP and cell resilience, which corresponds with regional activity and volume reductions found in depression (Marsden, 2011).

1.2.4.4 Synaptic Plasticity: LTP and LTD

Stress and glucocorticoid exposure modulate levels of LTP via an inverse U-shaped curve (Goosens and Sapolsky, 2007). Higher levels of acute (Foy et al., 1987) and chronic (Pavlides et al., 2002) stressors reduce LTP, but have been shown to facilitate LTD in preclinical studies (Artola et al., 2006, Yang et al., 2006). Similarly, the effect of glucocorticoid administration on synaptic plasticity mimics that of stress, with low levels amplifying LTP, and higher levels reducing LTP but increasing LTD (Pittenger and Duman, 2008). This modulation of synaptic plasticity after exposure to stress and glucocorticoid administration has been shown in the hippocampus and PFC, with the opposing pattern of increasing levels of LTP found in the amygdala (Marsden, 2011). A hypothesis for the change in the directional bias for synaptic plasticity is the reduction in glutamate uptake due to glial cell dysfunction as described above (see section 1.2.4.2). Thus stress and glucocorticoids directly affect the expression of synaptic plasticity in a regionally specific manner, with reduction in LTP found in areas of lower activity and volume in depression.

1.2.5 Cognitive Abnormalities in MDD Due to Excessive Stress and Glucocorticoid Exposure

LTP has been shown to be crucial for learning and memory formation (Bliss and Collingridge, 1993, Sweatt, 2008). Excessive levels of stress and glucocorticoids

change functioning in the substrates of synaptic plasticity leading to regionally-specific changes in LTP induction (see section 1.2.4). In depression, impairment of LTP induction in the hippocampus and PFC has been linked to profound functional changes in learning, memory and executive function (Pittenger and Duman, 2008, Savitz and Drevets, 2009). Therefore, in addition to providing an explanation for the structural changes in depression (see section 1.2.3), the stress neurotrophic hypothesis also presents an explanation for some of the cognitive and behavioural symptoms of depression (Nestler et al., 2002). Evidence for impaired neuroplasticity inducing changes in cognitive abilities is considered below.

1.2.5.1 Learning, Memory and Executive Function

Within the hippocampus, an inverted U curve relationship exists between levels of stress and glucocorticoids and cognitive abilities such as learning and memory. Mild levels of stress and glucocorticoids enhance learning and memory (Luine et al., 1996, Lupien and McEwen, 1997), while chronic or severe stressors, as well as excessive levels of glucocorticoids have detrimental effects on learning and memory (Luine et al., 1994, Lupien and McEwen, 1997, Sapolsky, 2003). In depression, studies have reliably shown comparatively impaired declarative memory (Burt et al., 1995, Zakzanis et al., 1998, Nissen et al., 2010), in addition to poor episodic (Sweeney et al., 2000), recollection (MacQueen et al., 2003) and working memory (Landrø et al., 2001). Thus evidence for impaired memory is consistent with the impairment of hippocampal-dependent LTP in depression, and corresponds with downstream functional consequences of the stress neurotrophic hypothesis (see section 1.2.4).

Excessive stress and glucocorticoids also reduce executive function (Wellman, 2001, Egeland et al., 2005, Pittenger and Duman, 2008). Executive function particularly relies on the structural and functional integrity of the PFC (Barense et al., 2002, Liston

et al., 2006, Walsh et al., 2009). In animals, attentional set-shifting tasks have been used to measure executive function (Barense et al., 2002, Dalley et al., 2004). Task performance is significantly reduced in rats subject to chronic stress compared to controls. This impairment was shown to correspond with a 20% decrease in dendritic arbours in the mPFC, linking impaired structure with impaired function (Liston et al., 2006). Impaired executive function has also been found in subjects with depression (Ottowitz et al., 2002, Paelecke-Habermann et al., 2005, Wagner et al., 2012). For example, comparatively poorer performance has been consistently reported in studies using the Wisconsin Card Sorting Test (WCST), one of the most widely used tests of executive function (Merriam et al., 1999, McClintock et al., 2010). This cognitive test assesses performance in a number of executive function domains. Impaired WCST performance in depression is considered to reflect an inability to adequately cope with changing stimuli, and a tendency to persevere with decisions, which demonstrates a lack of flexibility in thinking. However, poor performance might also reflect a failure to learn and follow test rules, or a failure to maintain the required task set in working memory, or even slowed processing potential (Grant et al., 2001). Mechanisms of plasticity are thus implicated in performing each of the cognitive skills sets required in WCST performance. Research has shown a correlation between those with greater severity (Merriam et al., 1999, McClintock et al., 2010) or length of depressive episodes (Grant et al., 2001) and worse performance on the WCST, suggesting it may be a core feature of depression. Impaired performance on the WCST and attentional set-shifting deficits are therefore consistent with findings of impaired plasticity in the PFC of depressed subjects and in preclinical models.

1.2.6 Relationship Between Structural and Functional Changes in Depression: Hippocampus, PFC and the Amygdala

In addition to structural and functional abnormalities (see sections 1.2.3-1.2.5) found independently in depression, and animal models of depression, there is some evidence of a relationship between these structural and functional changes (Frodl et al., 2006, Liston et al., 2006, McClintock et al., 2010). For example, a relationship has been shown between cognitive performance and hippocampal volumes in depression (Frodl et al., 2006), and in animal models (Issa et al., 1990). A corresponding relationship is hypothesised between cognitive performance and the dendritic structure of the PFC (Miracle et al., 2006). In addition, the volume of ventromedial PFC correlated with greater memory of extinction learning (Milad et al., 2005), showing the relationship between structure and function existing in healthy humans.

Therefore, the evidence above (see sections 1.2.3-1.2.6) supports a hypothesised relationship between stress-induced cognitive and behavioural changes, and structural abnormalities in depression. A higher propensity for LTD and lower propensity for LTP (see section 1.2.4.4) may be accompanied by dendrite retraction, shown, for example in the hippocampus (Wang et al., 2007), which is consistent with lower hippocampal volumes in depression (Stockmeier et al., 2004). Physiologically, a regional bias toward LTD may underlie reduced ability of the hippocampus and PFC to respond to demands of changing stimuli, resulting in reduced working memory and learning ability (Baune et al., 2010, Nissen et al., 2010). These findings therefore implicate dysfunctional mechanisms of neuroplasticity in depression (Frodl et al., 2006, Pittenger and Duman, 2008), likely through changes to plasticity mechanisms as explained by the stress neurotrophic hypothesis (Duman and Monteggia, 2006). At the level of the organism, impaired neuroplasticity likely makes it difficult to adapt appropriately, or to see

possibilities or solutions to challenging life events. This inability to shift mindset feeds back to increase the level of stress, and feelings of hopelessness characteristic in depression, and preclinical models of depression (Marsden, 2011).

In contrast to hippocampal and PFC regions, the amygdala exhibits increased neuroplasticity in MDD, and after stress paradigms in animals (Pittenger and Duman, 2008). The amygdala is responsible for organising the autonomic, neuroendocrine, and behavioural manifestations of emotional response. Stress results in increased glutamate expression which induces greater synaptic NMDA receptor activation in the amygdala, and a thus regional bias for LTP over LTD (Tsvetkov et al., 2004, Marsden, 2011). This increased plasticity is evident in enhanced fear learning in depression and stress models (Conrad et al., 1999, Nissen et al., 2010). Increases in amygdala volume and activity (Lupien et al., 2009) are therefore also thought to be related to functional changes in depression and models of depression (Drevets, 2003). Another example of the structure and function relationship is shown through control of amygdala function. The hippocampus and the dorsolateral PFC are hypothesised to exert top-down control over the amygdala. Depression-related dysfunction in these controlling regions results in chronic over-activity of the amygdala (Lupien et al., 2009). This chronic overactivity in turn increases negative emotions (Davidson et al., 2002), and an enhanced reactivity to stress through defensive behaviours, intensified anxiety and a state of hypervigilence (Davidson et al., 2002, Carlson et al., 2006). Thus structural and functional changes in depression may induce chronic amygdala overactivity, and a greater propensity for excitatory neuroplastic change in this region.

Therefore, the evidence above shows reductions in LTP and regional volume and a tendency for increased LTD in the PFC and hippocampus. This contrasts with enhanced activity, LTP, and dendritic complexity in the amygdala (Marsden, 2011).

Functional abnormalities, such as maladaptive cognitive and behavioural changes may therefore be related either to structural changes or to dysfunctional plasticity mechanisms in depression. However, it is presently difficult to measure directly, dysfunctional neuroplasticity in-vivo. Conclusions of impaired neuroplasticity so far have been primarily based upon indirect measures of plasticity such as learning and memory. These measures rely on factors other than just plasticity in neural circuits. Indeed, MDD is associated with profound deficits in attention and many believe that the attentional deficits are primary too much of the effects on learning and memory. Therefore, neuropsychological measures are limited in their usefulness for evaluation of neuroplasticity mechanisms *in vivo*.

1.2.7 Direct Evidence of Impaired Neuroplasticity in MDD

In addition to the indirect evidence above (see section 1.2.5), there is a very small amount of direct evidence of dysfunctional neuroplasticity in depressed subjects. This direct evidence provides experimental support for the premise that impaired neuroplasticity may be a feature of depression. One of these studies used changes in the amplitude of visual evoked potentials (VEPs) to evaluate synaptic plasticity *in vivo* (Normann et al., 2007). Plasticity in the visual cortex is proposed to share common mechanisms with LTP shown in brain slices. For example, changes in VEPs in response to repeated visual stimuli are reliant on NMDA receptor activation (Normann et al., 2007). Researchers have reported significant increases in the VEPs after a train of patterned visual stimuli. However, increases in VEP amplitude were significantly smaller in depressed subjects compared to age and gender matched healthy controls. This finding suggests plasticity is impaired in the visual cortex in depression (Normann et al., 2007). This evidence is limited to visual cortex, and therefore only offers partial support for the impaired neuroplasticity hypothesis.

Studies investigating plasticity in corticospinal pathways have also found evidence of differences in adaptive potential between subjects with MDD and controls. Decreased facilitation of motor response (motor evoked potentials, MEPs) to TMS was found following isometric exercise in depressed subjects compared to controls (Samii et al., 1996, Shajahan et al., 1999a). However it is unclear whether the immediate post-exercise facilitation of MEPs in fact represents a type of neuroplasticity. Furthermore, differences between healthy and depressed subjects in motor response are not always apparent (Loo et al., 2008). Another study investigated MEPs after application of repetitive TMS (rTMS), which is recognised as inducing neuroplastic changes at a cortical level (Grunhaus et al., 2003). While the study found increases in MEP amplitude after rTMS, there was no difference between depressed subjects and matched controls (Grunhaus et al., 2003). However, in this study, the rTMS was not delivered directly to the motor area, where excitability was tested by eliciting MEPs, but was delivered over the dorsolateral PFC. Therefore, the potential for plastic changes in the motor cortex has not yet been properly tested in patients with depression.

1.2.8 Reversal of Cognitive and Behavioural Impairments with Treatment for MDD

Evidence of enhanced learning, memory and executive functioning in humans, and improved responses to stress paradigms in animals have been found after a variety of treatments for depression (Vythilingam et al., 2004, Fregni et al., 2006, Boggio et al., 2007a, Bhagya et al., 2011, Wagner et al., 2012). These findings suggest at least some symptoms in depression are state rather than trait characteristics and therefore can be targeted for improvement (Pittenger and Duman, 2008). A discussion of the treatment options and outcomes for depression is given below.

1.2.8.1 Neuroplasticity Improvement with Antidepressant Treatment

Antidepressant medications are frequently used as the first line of treatment in episodes of depression (Berton and Nestler, 2006). Many of these drugs increase synaptic levels of serotonin, norepinephrine, and dopamine by blocking the reuptake or breakdown of these monoamines. Increased neurotransmission resulting from antidepressant treatment results in activity-dependent improvements in cellular and neural network functioning (Castrén, 2005). One way cellular functioning is improved is through the activity-dependent release of neurotrophic factors such as BDNF, which is shown after antidepressant treatment (Duman et al., 1999, Sen et al., 2008). For activity-dependent changes in BDNF to occur, a presynaptic neuron must sufficiently stimulate a postsynaptic neuron in order to signal the production and release of the neurotrophic factor (Castrén, 2005). Antidepressant improvement of neurotransmission and also neuroplasticity is shown via regulation of presynaptic glutamate release, and through the normalisation of AMPA and NMDA receptor activity, and the cyclic AMP response element binding protein (CREB) pathway (Carlson et al., 2006). Therefore, greater synaptic connectivity through improved neurotransmission is hypothesised to underlie activity-dependent improvement in cellular processes such as neuroplasticity and neurogenesis (Duman et al., 2000, Son et al., 2012). In addition, antidepressants have been shown to reinstate the balance of glial cells (Elsayed et al., 2012), reduce the HPA axis response to stress (Delbende et al., 1993), and assist in the normalisation of neurochemistry (Czéh et al., 2001). These activity-dependent processes are integral to the improvement in mood, learning, memory and executive functioning (Egan et al., 2003, Castrén et al., 2007, Kuczewski et al., 2010).

Similarly, some of the known sequelae of depression, such as regional decreases in volume and neural activity (Sheline, 2003) are reversed after antidepressant treatment (Czéh et al., 2001, Kennedy et al., 2001, Seminowicz et al., 2004). Experimental evidence of impaired neuroplasticity as shown above (see section 1.2.7) may also be reversed after antidepressant treatment. The lack of MEP facilitation found after isometric exercise in depression, was shown to normalise in subjects taking psychotropic medication, and who then achieved remission (Shajahan et al., 1999b). Furthermore, Norman et al. (2007) administered antidepressants (SSRIs) to a subset of healthy controls, which resulted in a significant increase in VEP amplitude compared to baseline levels. Thus, in healthy controls, a direct measure of neuroplasticity improved after antidepressant administration. This evidence underlines the importance of activity-dependent changes in synaptic plasticity induced by antidepressant treatment, which may lead to symptom improvement in depression (Son et al., 2012).

1.2.8.2 Neurobiological Improvements with Other Treatments for Depression

A number of brain stimulation treatments have been shown to be effective in treating depression. These treatments include electroconvulsive therapy (ECT) (Geddes et al., 2003), rTMS (Schutter, 2009, Slotema et al., 2010), and transcranial direct current stimulation (tDCS) (Fregni et al., 2006, Boggio et al., 2008, Kalu et al., 2012, Loo et al., 2012, Brunoni et al., 2013). Changes in neurobiology identified after these treatments include increases in hippocampal volume (Nordanskog et al., 2010), BDNF levels (Nibuya et al., 1995, Zanardini et al., 2006, Fritsch et al., 2010), and neurogenesis (Malberg et al., 2000).

TDCS, which is used in Chapter 4 of this thesis, is a mild form of brain stimulation that is gaining considerable experimental support for the treatment of MDD

(Loo et al., 2012, Brunoni et al., 2013). The application of a weak electrical current through electrodes placed upon the scalp has been shown to be safe and convenient for use in humans. TDCS changes cortical excitability and may induce activity-dependent modulation of brain circuits (Liebetanz et al., 2002). Repetitive application of tDCS has demonstrated neuroplastic effects in healthy individuals (Galvez et al., 2013) and also improvements in cognitive function in healthy and depressed subjects (Kuo and Nitsche, 2012, Oliveira et al., 2013). Structural changes have been shown following tDCS, with improvements in functional connectivity and motor function after a stroke (Hummel and Cohen, 2005). These features of the stimulation and its hypothesised mode of cortical effects make tDCS a useful option for the treatment of the symptoms of depression.

The evidence outlined above suggests that structural and functional abnormalities induced by excessive stress, glucocorticoids, and depression may be improved by antidepressant treatment. The time course of response to treatments for depression are varied. This may reflect improvement or normalisation of dysfunctional brain processes responsible for the generation of depressive symptoms (see section 1.2.1.1), including the improvement of different neuroplastic processes. For example, the antidepressant response to ketamine infusions can be as short as a few hours, while response to ECT ranges from hours to days, and from days to weeks for tDCS and antidepressant medications. However, antidepressant treatment is believed to lead to activity-dependent changes in cellular functioning resulting in the improvement of neuroplasticity (Castrén, 2005, Son et al., 2012). Improvements in neuroplasticity are believed to contribute to improvements in cognitive and behavioural functions, enhancing learning, memory, and executive functioning (Pittenger and Duman, 2008). Activity-dependent improvement in cellular functioning that occurs in neural regions

associated with emotion regulation is thought to underlie improvement in mood after antidepressant treatment (Krystal et al., 2009). So improvement in depressive symptoms may be reliant upon the treatment approach and its unique influence on underlying brain processes, and particularly neuroplastic processes – the focus of thesis investigations. It is likely that some antidepressant treatments are more effective at improving neuroplastic processes and could explain the differences in the type, time course, and stability of symptom reduction.

1.2.9 Summary

The aforementioned evidence (see section 1.2) provides considerable support for the stress neurotrophic hypothesis of depression. The evidence indicates that exposure to excessive levels of stress and glucocorticoids induce structural and functional neurological changes that correspond with those in depression. Stress is one of the major precipitating factors in MDD (Caspi et al., 2003), and is also a strong modulator of synaptic plasticity (Goosens and Sapolsky, 2007). Stress therefore provides a potential link between the features of neuroplasticity and brain circuitry models of MDD (Carlson et al., 2006). As mentioned above (see section 1.2.1.1), heterogeneity in MDD symptomology and its longevity complicates the understanding of the relationship between the factors - stress, neuroplasticity and MDD. This understanding is confounded by the multiple cortical mechanisms and time courses of these factors. Nonetheless, treatment for depression has been shown to induce structural changes in the brain and improve neurocognitive function (Normann et al., 2007, Son et al., 2012). Activity-dependent improvement in the substrates of neuroplasticity is found after antidepressant treatment. Improvement in neuroplasticity is believed to contribute to

particular depressive symptom reduction (Vythilingam et al., 2004, Castrén et al., 2007, Son et al., 2012).

Dysfunctional neuroplasticity is therefore hypothesised in the aetiology of some of the cognitive, behavioural and physiological symptoms of depression (Pittenger and Duman, 2008). However, at this time, aside from impairment inferred from these secondary measures, there is limited direct evidence that neuroplasticity is impaired in those suffering MDD (Normann et al., 2007). The difficulty in relying on cognitive and behavioural measures as indicators of plasticity in neural circuits is that they are indirect measures, which rely on other factors, such as subject effort and motivation, known to be impaired in depression (Austin et al., 2001, Scheurich et al., 2008). At present, evidence of abnormalities in direct measures of neuroplasticity in depression is sparse, inconclusive and sometimes contradictory (Grunhaus et al., 2003, Loo et al., 2008). Therefore in order to provide meaningful support for the hypothesis that dysfunctional neuroplasticity is a core feature of depression, it is necessary to demonstrate impairment in a study using a direct measure, which is independent of subject confounds. The next section discusses means to further test neuroplasticity directly using non-invasive brain stimulation techniques in the motor cortex.

1.3 *In vivo* Measurement of Neuroplasticity

This section focuses on specific experimental methods that allow the measurement of neuroplasticity in humans. For decades, a variety of methods have been used to induce and measure synaptic plasticity in preclinical studies. However, the applicability of these methods for *in vivo* investigations in humans is limited. The adaptation of methods that allow analogous measures of plasticity in humans is

discussed here. In addition, the validity of using brain stimulation protocols over the motor cortex to measure neuroplasticity in depression is evaluated. Although there are many different brain stimulation protocols presently available, this section focuses on those shown to be the most consistent and reliable in inducing and measuring discrete changes in human motor cortical excitability.

1.3.1 Methods for Measuring Neuroplasticity in Humans

As discussed in section 1.2, neuroplasticity is essential for many brain functions including learning and memory. These neurocognitive functions are often used as surrogate measures of neuroplasticity within subjects. However, learning and memory are complex behaviours. They involve multiple brain areas and mechanisms (Sweatt, 2008, Rajji et al., 2011), and thus cannot provide a pure measure of neuroplasticity. Furthermore, they rely on subject motivation and effort which are often impaired in depressed subjects (Austin et al., 2001, Scheurich et al., 2008), introducing additional confounds for the use of learning and memory tests as measures of neuroplasticity in depression.

In principle, an objective test requires both a means of inducing neuroplastic changes and a means of measuring these changes in the brain, independent of subject effort (though subject cooperation may still be required). The requirement for tests to be independent of subject effort essentially implies some form of external stimulus to induce lasting changes in excitability, plus an evoked potential by which changes in excitability can be measured. The possible combinations could include: visual stimuli and visual evoked potentials (VEPs) (Normann et al., 2007), somatosensory stimuli and sensory evoked potentials (SEPs) (Kriváneková et al., 2011), auditory stimuli and auditory evoked potentials (AEPs) (Purdy et al., 2001), or motor stimuli and motor

evoked potentials (MEPs) (Huang et al., 2009). Each of these combinations has the potential to provide direct measurements of the plasticity of different regions of the cortex, independent of subject effort.

Of these potential methodologies, visual stimulation and VEPs have been used (see section 1.2.7) to demonstrate plasticity changes in healthy and clinical populations (Normann et al., 2007). A limitation of using VEPs is that compared to MEP investigations, there is a lack of evidence of reproducibility of this methodology in humans, particularly in a psychiatric population. Sensory stimulation combined with TMS has been shown to induce measurable changes in SEP amplitude (Wolters et al., 2005). However these changes are weak and inconsistent between studies (Litvak et al., 2007, Bliem et al., 2008, Murakami et al., 2008, Kriváneková et al., 2011). In comparison to the above methodologies, motor cortical stimulation and collection of MEPs has been widely used and allows for the relatively consistent, rapid induction and measurement of cortical change (Ilić and Ziemann, 2005).

1.3.1.1 Motor Stimulus and Motor Evoked Potentials

Within this thesis, the use of a motor stimulus and measurement of MEPs was selected for the *in vivo* testing of neuroplasticity in humans. A large body of evidence supports this combination as a sensitive measure of the brain's ability to change (Di Lazzaro et al., 2008c, Djuric et al., 2010). A variety of methods can be used to stimulate the motor cortex to induce plasticity, including repetitive TMS (rTMS), Paired Associative Stimulation (PAS), Theta Burst Stimulation (TBS) and transcranial Direct Current Stimulation (tDCS) (Nitsche and Paulus, 2000, Classen et al., 2004, Huang et al., 2005, Di Lazzaro et al., 2008c, Galvez et al., 2013). Motor cortical stimulation protocols are well defined, convenient to implement, and equally well received in

healthy and clinical populations (Frantseva et al., 2008). Within the range of motor cortical stimulation protocols, many require only very short stimulation times to induce significant, long-lasting cortical change (Huang et al., 2005, Ilić and Ziemann, 2005). Motor stimulation protocols are relatively painless and can be performed while the subject is conscious, making them practical for use in a clinical population. The levels of stimulus input and evoked output can also be readily quantified by objective measures, making the measurement of motor cortical plasticity a suitable measure of neuroplasticity in humans.

1.3.1.2 Motor Cortical Abnormalities in MDD

In addition to the procedural advantages of motor cortical stimulation protocols, a number of characteristics of MDD also support the use of this particular method for the determination of plasticity within this thesis. New methodologies for measuring motor abnormalities have confirmed findings of psychomotor disturbances (Lohr et al., 2013) which have been widely reported in MDD (Sobin and Sackeim, 1997, Loo et al., 2008, Buyukdura et al., 2011). There is evidence of altered motor cortical excitation and inhibition in depression that has come from TMS studies. For example, there is consistent evidence of comparatively reduced motor cortical excitability after isometric exercise in depression (Samii et al., 1996, Shajahan et al., 1999a, Reid et al., 2002). Studies in the motor cortex have also shown reduced measures of cortical inhibition (Fitzgerald et al., 2004, Bajbouj et al., 2006, Lefaucheur et al., 2008, Levinson et al., 2010, Radhu et al., 2013).

1.3.1.3 Motor Cortical Facilitation

Brain stimulation protocols can be used to induce and measure either the facilitation or inhibition of cortical excitability. For the studies in this thesis, facilitatory

brain stimulation protocols were chosen, since facilitation of motor cortical excitability is thought to rely on LTP mechanisms (Stefan et al., 2000, Huang et al., 2005, Lang et al., 2005, Lefaucheur et al., 2008, Nitsche et al., 2008). The neurocognitive functions of learning, memory and executive function, which are often impaired in depression, are also hypothesised to rely on LTP (see section 1.2.5.1). At present there is evidence to indicate impaired LTP in the hippocampus and the PFC (see section 1.2.4.4), the centres for these neurocognitive functions – and also the visual cortex (Normann et al., 2007). Thus, dysfunctional LTP may be widespread in the brain, and could be an important marker in the pathophysiology of depression. It is therefore important to ascertain if deficiencies of LTP also exist in the motor cortex.

In summary, neuroplasticity assessed in the motor cortex using brain stimulation protocols offers a direct physiological measure of plasticity. This measure is appropriate in light of hypothesised changes in the neurophysiology of MDD and the hypothesised mechanisms underlying some of the symptoms of depression. Measures of motor cortical plasticity also overcome the potential confounds which exist in neuropsychological tests such as subjective rating and analysis, subject education levels, practice effects or motivational factors.

1.3.2 Stimulation of the Motor Cortex using Transcranial Magnetic Stimulation

Motor cortical stimulation is used to both induce and measure excitability changes in the motor cortex. Changes in motor cortical excitability, which are achieved through the use of brain stimulation protocols such as TMS, are often used as measures of neuroplasticity.

In TMS, an electrical charge is stored in capacitors and discharged periodically through a conducting coil, producing a time-varying electrical field. When the coil is

held over the scalp, this electrical field produces a short-lasting magnetic field that induces a current in a secondary circuit, such as neurons, lying in close proximity. The magnetic field passes through into the cortex unimpeded and can depolarise neurons beneath the coil. By avoiding direct activation of scalp afferent nerves and muscles, TMS overcomes the pain associated with transcranial electrical stimulation (Hallett, 2000).

Understanding of how TMS affects the brain has been improved through direct electrophysiological recordings taken from electrodes implanted in the cervical epidural space of patients to relieve chronic pain (Di Lazzaro et al., 1998, Di Lazzaro et al., 2004, Di Lazzaro et al., 2005, Di Lazzaro et al., 2008a). Low intensity TMS initially elicits descending corticospinal volleys that are labelled indirect-waves (I-waves). I-waves are believed to result from the indirect activation of pyramidal tract neurons via synaptic inputs onto pyramidal neurons. Increasing TMS intensity induces additional I-waves and can evoke a direct-wave (D-wave) at cell membrane threshold. A D-wave represents a descending volley in pyramidal tract axons which results from the direct activation of corticospinal neurons (Di Lazzaro et al., 1998, Rothwell, 2003, Di Lazzaro et al., 2008c). A D-wave is distinguished by the shorter latency of the descending volley compared to I-waves (Di Lazzaro et al., 2004).

1.3.2.1 TMS – Assessing Motor Cortical Excitability

TMS-evoked MEPs in a target muscle can be used as a measure of motor cortical excitability. Magnetic stimulation that is sufficient to induce pyramidal cell firing results in multiple action potentials travelling down corticospinal axons (Rothwell, 1991) to where they terminate onto motoneurons in the ventral horn of the spinal cord. These descending volleys generate excitatory postsynaptic potentials in

motoneurons that innervate muscles of the limbs and trunk. Temporal and spatial summation of descending volleys sufficient to generate action potentials in motoneurons in turn generate MEPs that can be measured in a target muscle via surface electromyography (Sale, 2009). MEPs therefore represent the output from the motor cortex and its connecting pathways. Thus, MEP amplitude can be influenced by motor cortical output cell excitability and the excitability of other cells in the motor pathway, in addition to the strength of synapses between cells in this pathway. Despite subcortical influences, changes in motor cortical excitability are generally reflected by changes in MEP amplitude. Throughout this thesis, changes in the amplitude of MEPs are interpreted as changes in 'motor cortical excitability', with the understood caveat that subcortical effects cannot always be excluded.

TMS over the motor cortex is comparatively convenient and safe in almost all subjects (Wassermann, 1998, Ilić and Ziemann, 2005, Thirugnanasambandam et al., 2011). The amplitude of the MEP can be used as a measure of excitability in neuroplasticity measurements. MEP amplitude following the application of TMS is influenced by the features of the stimulation and characteristics of the neurons activated. The pattern of neurons activated by brain stimulation protocols depends on neuronal size, shape and orientation. MEP size will also be directly influenced by stimulation parameters including frequency, intensity, duration of stimulus train, and the total number of stimuli (Classen and Ziemann, 2003). Testing of corticospinal excitability is usually conducted at a frequency of around 0.1 Hz. This low TMS frequency is favoured as it is considered below the stimulus frequency which could induce changes in motor cortical excitability during the measurement process itself (Chen et al., 1997). The stimulus waveform produced by the magnetic stimulator, the type of TMS coil, and

its placement relative to the scalp also affect MEP amplitude (Rothwell, 2003), as discussed below.

1.3.2.2 Coil Shape and Placement

As mentioned, measures of MEP amplitude crucially depend upon the shape and the relative placement of the TMS conducting coil (Rothwell, 2003, Cárdenas-Morales et al., 2010). The type of conducting coil selected for brain stimulation experiments is usually the commonly available circular coil or a figure-of-eight shaped coil. The shape of the coil influences the strength and site of the electrical current induced in the brain (Di Lazzaro et al., 2008c) (see Figure 1.3). Figure-of-eight shaped coils provide greater spatial acuity than circular coils due to a superior ability to focus the magnetic field (Ueno et al., 1988). When the coil is held flat against the scalp surface with handle of the coil pointing posteolaterally, an electrical current is generated within the brain, which runs in the posterolateral to anteromedial direction (Di Lazzaro et al., 2008c). This current direction is optimal for activating descending pyramidal neurons, whilst also activating interneurons that modulate pyramidal neuron firing, thus optimising evoked MEPs (Amassian and Deletis, 1999).

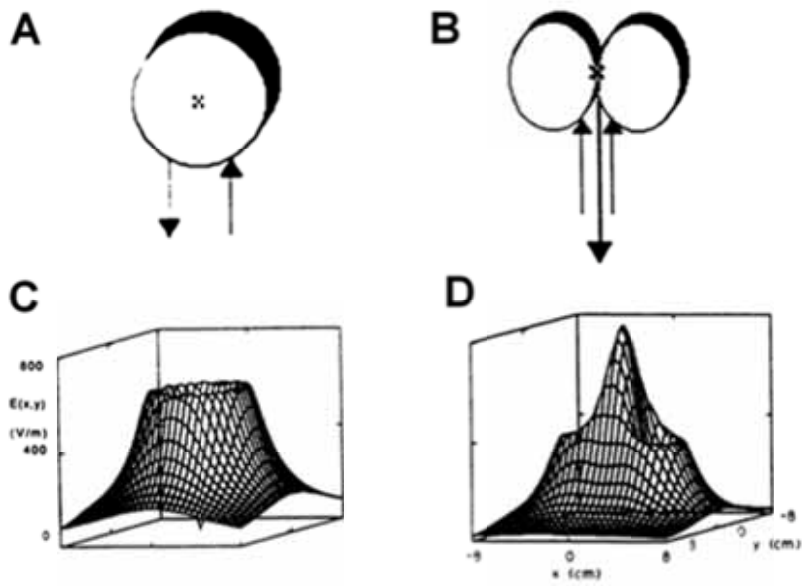


Figure 1.3 Round and figure-of-eight conducting coils with models of induced electrical fields in the cortex

Two coils are shown with different shapes (A, circular coil and B, figure-of-eight coil), with models of their induced electrical fields in the cortex. Coil B possesses a more focused magnetic field resulting in a greater spatial acuity than the circular coil. Figure from (Hallett, 2007).

1.3.2.3 Monophasic and Biphasic Waveforms

Various magnetic stimulators are capable of producing monophasic or biphasic waveforms, which induce different patterns of neuronal stimulation in the brain. The monophasic waveform induces a current in only one direction (for example, posterior to anterior), which is opposite to the current direction within the coil (Di Lazzaro et al., 2004). The monophasic waveform is thought to activate a uniform neuronal population, inducing larger summated changes in synaptic strength, and thus potentially larger influences on excitability (Arai et al., 2007). Biphasic stimulation induces a bi-directional current in the brain and may activate differently orientated neurons. The biphasic waveform might also activate neurons at variable latencies, potentially leading to phase cancellations among neurons (Arai et al., 2005, Arai et al., 2007). This pattern

of neuronal activation may therefore result in changes in excitability that could offset each other. Thus the particular characteristics of the stimulus waveform likely result in different accumulated measures of excitability (Taylor and Loo, 2007).

In order to measure the excitability of corticospinal circuits, single pulse (monophasic) TMS provides the most appropriate investigatory tool due to the increased summating effects on a relatively uniform population of neurons (Arai et al., 2007).

1.3.2.4 The Resting Motor Threshold and Active Motor Threshold

In assessing motor cortical excitability, an optimal spot for eliciting MEPs in a target muscle is identified, and marked on the scalp overlying the motor cortex. This spot is used as the point of stimulation, and used to measure a subject's motor threshold (MT). Finding the MT is essential for determining the appropriate TMS intensity to measure corticospinal excitability in plasticity experiments. MT is defined as the lowest stimulation intensity necessary to evoke an MEP of a specified size, usually 50-100 microvolts, in a discrete number of TMS pulses (Rossini et al., 1994). Two slightly different MTs are used in brain stimulation paradigms. Resting MT (RMT) is taken while a subject's target muscle is at rest, while active MT (AMT) is measured while the target muscle is contracting weakly. The AMT results in comparatively lower stimulus intensity as voluntary activity increases both cortical and motoneuronal excitability. AMT is most commonly used to determine stimulus intensity in theta-burst stimulation (TBS) protocols (see section 1.3.5).

1.3.3 Inducing Facilitatory Neuroplastic Change in Motor Cortex

Transient changes in an individual's motor cortical excitability provide a measure of neuroplasticity (Ridding and Uy, 2003, Ilić and Ziemann, 2005, Huang et al., 2009). Motor cortical excitability can be altered through implementation of conditioning stimulation. Conditioning stimulation protocols act to either increase or decrease corticospinal excitability depending upon the stimulation parameters. Excitability changes measured through differences in MT or mean MEP amplitude before and after the conditioning stimulation provides a measure of cortical responsiveness to the stimulation, and thus a measure of plasticity in these circuits (Stefan et al., 2000, Huang et al., 2005).

Researchers have used a range of brain stimulation protocols including rTMS, PAS, and TBS as conditioning stimulation methods in the measurement of plasticity *in vivo*. Each of these brain stimulation protocols have been found to induce changes in the human motor cortex that are analogous to aspects of plasticity seen in preclinical studies. For example, high frequency rTMS stimulation increases cortical excitability, and low frequency rTMS stimulation decreases cortical excitability. These changes are analogous to *in vitro* LTP and LTD induced by high and low frequency tetanus respectively (Cooke and Bliss, 2006). Stimulation paradigms with paired inputs such as PAS are thought to mimic spike-timing dependent plasticity also shown in preclinical experiments (Dan and Poo, 2004). TBS is hypothesised to mimic natural cortical rhythms that occur during voluntary motor processes (Cárdenas-Morales et al., 2010).

PAS and TBS were selected for use in this thesis as the most suitable for testing neuroplasticity in humans. Their selection was based upon prior experimental evidence of each protocol inducing sizeable excitability changes in healthy populations (Di

Lazzaro et al., 2011). These protocols have been widely reported to consistently induce changes in cortical excitability that outlast the period of exposure (Huang et al., 2005, Rosenkranz and Rothwell, 2006, Ilić et al., 2009, Di Lazzaro et al., 2011). Changes in cortical excitability after PAS and TBS are believed to reflect LTP- and LTD-like changes in synaptic efficacy (Stefan et al., 2002, Huang et al., 2005, Hallett, 2007). The characteristics of the stimulation and experimental support for their selection are discussed below.

1.3.4 Paired Associative Stimulation (PAS)

PAS involves the repetitive pairing of electrical nerve stimulation, which provides input to motor cortical neurons via the thalamic and somatosensory cortex, with single pulse TMS over the contralateral motor cortex, which also activates motor cortical neurons, both directly and through other interneuronal inputs (Stefan et al., 2000). The temporal pattern of stimulation is designed to generate the arrival of both inputs into the motor cortex at approximately the same time, inducing spike-timing dependent plasticity (Hebb, 1949, Wolters et al., 2003).

The resultant change in cortical excitability after PAS depends on the ordering of presynaptic and postsynaptic cell spiking. LTP is induced if the afferent sensory pulse from the peripheral nerve stimulation reaches the motor cortex immediately before the TMS activation of motor cortical cells. In contrast, LTD results if the TMS stimulation of the motor cortex precedes the arrival of the afferent impulse. Thus the interstimulus interval (ISI) in the PAS protocol is crucial in determining the induction of LTP or LTD, and the corresponding increase or decrease in synaptic strength (see Figure 1.4). Protocols in which the TMS pulse is delivered 25ms after peripheral stimulation (PAS25) result in LTP, and have been shown to generate increases in

cortical excitability lasting at least 60 minutes in humans (Stefan et al., 2000). If the ISI is reduced to 10ms (PAS10), however, the order of stimulus arrival is reversed, resulting in LTD, and a decrease in cortical excitability of up to 90 minutes (Wolters et al., 2003).

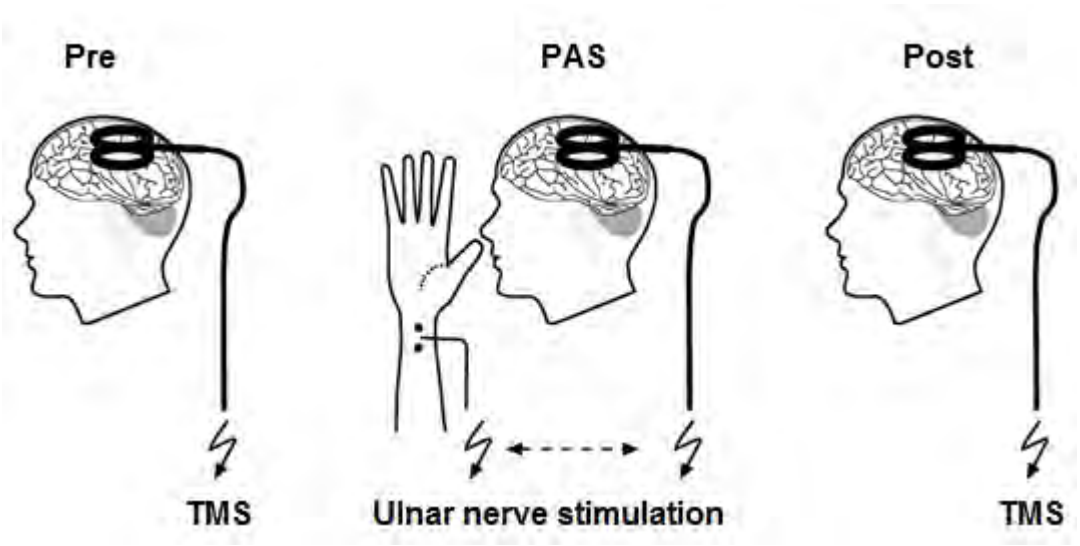


Figure 1.4 Experimental design of PAS conditioning study measuring motor-evoked potentials (MEPs)

TMS evoked potentials are recorded before and after PAS conditioning, with the difference between these measures held to be a measure of motor cortical plasticity resulting from the conditioning. Figure adapted from (Cooke and Bliss, 2006).

Importantly, there are multiple points within sensory-motor cortical pathways at which PAS may affect excitability. However, recordings from electrodes surgically implanted in the cervical epidural space support the hypothesis that PAS-induced changes in MEPs, result from changes that take place in the motor cortex itself. Analysis of TMS evoked corticospinal volleys after the PAS conditioning show that PAS changes the amplitude of I-waves, particularly later I-waves, which are considered to be the result of cortico-cortical connections (Di Lazzaro et al., 2009). The

hypothesised site of cortical change makes PAS a suitable choice as a motor cortical conditioning protocol.

1.3.4.1 Does PAS use Similar Mechanisms to Postsynaptic NMDA-Dependent LTP?

The evidence above (see section 1.3.4) supports the contention that PAS conditioning induces changes in motor cortical excitability. In addition to knowing where the changes are taking place, it is also necessary to demonstrate that these changes form appropriate models for testing neuroplasticity. Evidence presented below suggests that changes induced by PAS exhibit characteristics that correspond with, or rely on the same mechanisms as those that underlie synaptic plasticity measured *in vitro*.

Strong support for the use of PAS as a means to measure neuroplasticity comes from evidence that post-PAS excitability changes outlast the period of conditioning (Stefan et al., 2000). This strengthens the hypothesis that excitability changes are occurring at the cellular level. Changes in cortical excitability – either increases or decreases in MEP amplitude – have been shown to last for up to 60 and 90 minutes after facilitatory and inhibitory PAS protocols respectively (Stefan et al., 2000, Wolters et al., 2003). Similarly, EEG signals show alterations in brain activity that remain after PAS conditioning (Litvak et al., 2007). Overall these PAS effects are consistent with the concept of neuroplasticity, that is, they result in a change in structure or function that outlasts the period of exposure (Di Lazzaro et al., 2010).

Preclinical studies have shown that the number of stimuli, and the pattern of stimulus presentation alter synaptic strength. For example, *in vitro* LTP and LTD are alternatively induced depending upon the temporal ordering of presynaptic and postsynaptic spiking, with the longevity of synaptic effects related to the number of

stimuli (Dan and Poo, 2004). Similarly, the *in vivo* effects of PAS are reliant on the number and the presentation of stimuli, which determine the direction and durability of changes in cortical excitability. PAS studies have shown that the longevity of change in cortical excitability is increased through longer periods of stimulation (Nitsche et al., 2007). In addition, altering the ISI from 25ms to 10ms changes the cortical effects from facilitation to inhibition (Wolters et al., 2005). Thus, the number of stimuli, and the temporal spacing between stimuli have a direct influence on MEP amplitude – our measure of cortical excitability – and the durability of this change (Ziemann et al., 2004). These *in vivo* neuroplasticity findings thus correspond with preclinical studies of measures of synaptic plasticity.

Further support for the use of brain stimulation protocols, including PAS, in inducing and measuring neuroplasticity *in vivo* comes from evidence that the stimulation interacts with other inputs at the synapse known to change synaptic strength. The influential Bienenstock-Cooper-Munro model of bidirectional synaptic plasticity states that the amount of LTP or LTD that can be induced depends on the previous history of plasticity at the synapse (Bienenstock et al., 1982). This importantly maintains synaptic strength and neuronal firing within a physiological range that preserves homeostasis at the synapse (Abraham and Bear, 1996, Abraham, 2008). This homeostatic effect means the threshold for LTP will increase if postsynaptic neuronal activity was previously high, making it harder to induce LTP, but easier to induce LTD at the same synapse. In contrast, the threshold for LTD will increase if prior synaptic activity were low, making it harder to induce LTD, but easier to induce LTP (Ziemann and Siebner, 2008).

A change in the potential for further synaptic plasticity has been shown experimentally through combining two facilitatory PAS protocols. Müller and

colleagues found that when a facilitatory PAS protocol was primed with the same facilitatory PAS protocol, there was an extended decrease in MEP amplitude (Müller et al., 2007). This suggests that the response to the second excitatory PAS input was affected by the excitable state of motor cortical synapses induced by the first PAS protocol. This interaction is in line with the theory of metaplasticity – the plasticity of synaptic plasticity (Abraham and Bear, 1996). Metaplastic interactions have also been shown between PAS and other inputs, for example, repetitive contraction of the target muscle (Jung and Ziemann, 2009). This interaction may result in changes in the direction of synaptic strength other than expected from presentation of the second protocol input alone. This demonstration that PAS interacts with motor learning, which relies on LTP (Rioult-Pedotti et al., 2000, Ziemann et al., 2004), suggests at least some shared cortical mechanisms (Jung and Ziemann, 2009) and is consistent with PAS inducing LTP-like changes at the cortex.

Further evidence that the effects of PAS correspond with processes of synaptic plasticity is provided by pharmacological challenge studies. PAS-induced change in excitability is shown to rely on at least some of the mechanisms essential for changes in *in vitro* synaptic strength. For example, Stefan et al. (2002) showed that a NMDA antagonist, dextromethorphan, blocked changes in MEP amplitude after PAS. PAS-induced plasticity is also impaired at times of high circulating cortisol levels (Sale et al., 2008). High levels of cortisol have been shown to be detrimental to LTP (see section 1.2.4.4). This pharmacological evidence provides further support for the assertion that PAS relies on LTP-like mechanisms for inducing changes in excitability in the cortex.

Genetic investigations provide a final piece of support for the premise that PAS induces LTP-like changes in the motor cortex. For example, studies show that subjects with the Val/Val polymorphism of the BDNF gene respond with increased MEP

amplitude following facilitatory PAS conditioning (Cheeran et al., 2008). BDNF secretion has been shown to be important for changes in synaptic plasticity (Egan et al., 2003) and the Val/Val polymorphism is linked to improved BDNF release. In contrast, abnormalities of BDNF release in Met carriers are thought to lead to impaired synaptic plasticity, and thus less change in cortical excitability after PAS. The findings of Cheeran et al. (2008) that Val/Val subjects have a greater response to PAS than Met carriers, provides another mechanistic association between synaptic plasticity and this form of brain stimulation.

1.3.5 Theta-Burst Stimulation (TBS)

TBS is a novel variation of rTMS. TBS appears to induce motor cortical excitability changes more efficiently than conventional rTMS (Huang et al., 2007, Oberman et al., 2011). This comparative efficiency is shown by the lower number of pulses, stimulation times, and stimulation intensities required to induce changes in cortical excitability (Zafar et al., 2008). TBS is based upon a stimulatory protocol comprising a burst of three pulses given at 50Hz, repeated at 5Hz. This stimulatory pattern is modelled upon the coupling of gamma and theta rhythms that naturally occur during cognitive processing in humans (Canolty et al., 2006, Cárdenas-Morales et al., 2010). The stimulus intensity most commonly used in TBS is 80-90% of AMT, which is considerably lower than the stimulus intensity used in conventional TMS paradigms. The reduced stimulus intensity compensates for the much higher pulse frequency than in conventional TMS, and along with shorter stimulation time, lowers the risk of seizure (Cárdenas-Morales, 2010). In order to stimulate at the high frequencies required, a biphasic magnetic stimulator must be used. The design of biphasic magnetic stimulators allows the utilisation of a proportion of the return charge following stimulus discharge, which facilitates ongoing rapid pulse discharge. Thus the biphasic stimulus used in TBS

protocols provides an efficient and effective way to stimulate discrete regions of the cortex.

The duration and pattern of TBS applications can induce substantially different effects on corticospinal excitability. For example, forty seconds of continuous TBS (cTBS) has been shown to reduce cortical excitability for periods up to 60 minutes (Huang et al., 2005), while the same number of stimuli, 600, delivered in an intermittent pattern (iTBS), by means of a 2 second train of TBS repeated every 10 seconds (190 seconds), will generally increase MEPs for at least 15 minutes (Huang et al., 2005). Direct recordings from electrodes placed into the cervical epidural space of patients suggest that TBS-induced changes, like those of PAS, occur in the motor cortex. TMS-evoked corticospinal volleys following both cTBS and iTBS, show change in I-wave excitability, which is considered to reflect change of the strength of cortico-cortical inputs in the motor cortex (Di Lazzaro et al., 2005, Di Lazzaro et al., 2008a) (see Figure 1.5).

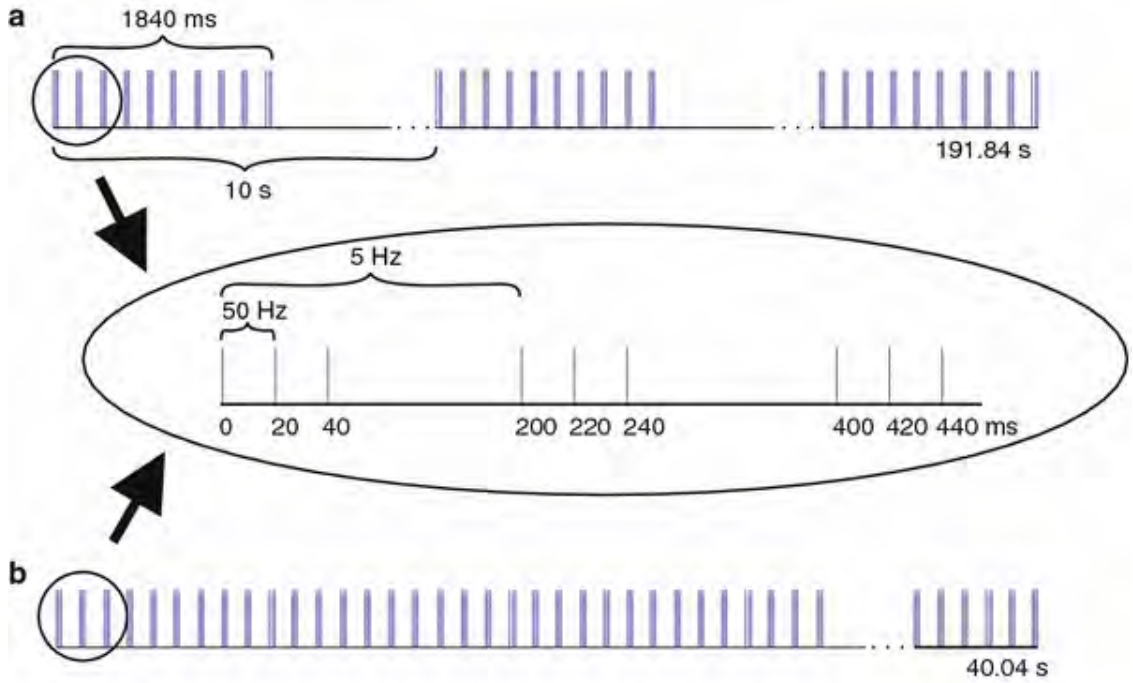


Figure 1.5 Two modalities of theta burst stimulation (TBS)

The pattern consists of 3 pulses delivered at 50Hz, every 200ms (5Hz). **a.** Shows intermittent (iTBS), 2 seconds of TBS are given every 10 seconds, with 600 pulses delivered over 190 seconds. **b.** Shows continuous (cTBS), 200 bursts (600 pulses) are given uninterrupted over 40 seconds. Figure from (Cárdenas-Morales et al., 2010).

1.3.5.1 Does TBS use Similar Mechanisms to Postsynaptic NMDA-Dependent LTP?

Similar to the PAS protocol (see 1.3.4.1), TBS is thought to rely on NMDA-dependent LTP or LTD for changes in cortical excitability. Changes in cortical excitability outlast the period of TBS conditioning (Huang et al., 2005, Hubl et al., 2008, Zafar et al., 2008). The number of stimuli and the pattern of stimuli alter synaptic strength analogous to *in vitro* studies measuring LTP and LTD (Huang et al., 2005, Gentner et al., 2008). TBS also interacts with other synaptic inputs known to change the direction and duration of synaptic strength, thus providing examples of homeostatic plasticity and metaplasticity (Huang et al., 2008, Iezzi et al., 2008). The aftereffects of

TBS rely on NMDA receptor activation, known to be crucial for MEP amplitude change (Huang et al., 2007). Genetic investigations have also shown that the BDNF polymorphism is important for changes in cortical excitability after TBS (Cheeran et al., 2008). These findings provide support for the premise that TBS-induced changes in excitability correspond with LTP- and LTD-like synaptic changes *in vitro*.

1.3.6 Are PAS and TBS Induced Changes Really Postsynaptic NMDA-Dependent LTP and/or LTD?

Research clearly indicates that cortical changes induced by PAS and TBS share several critical features with LTP- and LTD-like changes in synaptic strength (see sections 1.3.4.1 and 1.3.5.1). However, this does not provide definitive proof that they are the same process. Thus while there is persuasive evidence supporting PAS and TBS to provide good models of neuroplasticity, this must be balanced by some crucial caveats. For example, cortical changes after PAS and TBS, along with *in vitro* measures of synaptic plasticity are very broad physical concepts each influenced by a range of factors, many of which are not yet fully understood. This therefore complicates the finding of a direct link between them (Hoogendam et al., 2010). The measures of LTP and LTD *in vitro* may appear very different from the measures resulting from brain stimulation. In LTP and LTD studies, stimulation and measurement is especially focal. Tiny electrodes are placed on a single presynaptic and postsynaptic neuron, and the changes in the excitatory postsynaptic potentials are used to measure change in synaptic plasticity. However, in brain stimulation studies, the outcome measure is the amplitude of an MEP in a target muscle stimulated by multiple neurons that are at least two synapses away. This is therefore a much more imprecise measure of synaptic plasticity (Hoogendam et al., 2010). In addition, changes in excitability after brain stimulation protocols have been shown to induce structural and functional connectivity changes far

from the site of stimulation (Bestmann et al., 2003), and are thus above the level of the single synapse measured *in vitro* (Hoogendam et al., 2010). Therefore, when PAS and TBS alter MEP amplitude reflecting a change in motor cortical output, this may include a change in synaptic strength, which may represent the induction of LTP or LTD as seen in preclinical studies. However, this does not offer causal evidence that they invoke, or are limited to the same mechanisms. The only way to provide causal proof would be to conduct invasive recordings of changes in synaptic transmission in conscious humans, which is not immediately foreseeable (Hoogendam et al., 2010).

1.3.7 Priming

Based on the theory of homeostatic metaplasticity, priming a facilitatory brain stimulation protocol with an inhibitory protocol is hypothesised to induce a greater increase in MEP amplitude than a single protocol (Todd et al., 2009). Changes in cortical excitability after both TBS and PAS have been shown to be increased by priming (Müller et al., 2007, Todd et al., 2009). Thus, the addition of priming to TBS and PAS protocols may result in MEP amplitude changes of a larger magnitude, or possibly more consistent between-subject changes, both of which would be useful when the stimulation protocol is applied to detect any differences in neuroplasticity between depressed and healthy subjects.

1.4 Summary and Thesis Overview

As discussed in the Introduction, LTP and LTD are considered to be two basic mechanisms for experience-dependent modification of synaptic strength. Changes in synaptic strength are essential for crucial everyday functions, and impairment of plasticity may be maladaptive. For example, impairment in LTP is thought to be responsible for a number of cognitive and behavioural symptoms of depression.

Measures of LTP induced by brain stimulation protocols *in vivo* provide a functional means by which impairment may be experimentally measured. There is much support for the use of brain stimulation as an objective measure of neuroplasticity *in vivo,* allowing comparisons between healthy and clinical populations. Such comparisons may shed light on the aetiology, and hence optimal treatment, of depressive symptoms.

1.4.1 Thesis Hypotheses

The primary hypothesis of this thesis is that individuals with MDD have impaired neuroplasticity, as demonstrated in functioning of the motor cortex. This impairment in neuroplasticity is expected to be widespread in the cortex, including the motor cortex, where one form of neuroplasticity may be readily measured. Although in this thesis, a single construct is used to examine neuroplasticity, neuroplasticity is a complex phenomenon which has multiple expressions (for example - LTP, LTD, neurogenesis, altered cortical excitability) which may not be uniformly related or expressions of the same neuroplastic process. It is assumed that MEPs, which are the response to magnetic stimulation of the motor cortex, measured using EMG in peripheral muscles, provide a useful surrogate measure for neuroplasticity (Classen et al., 2004). It is hypothesised that depressed subjects will show impaired MEP response to a conditioning brain stimulation protocol compared to the response of healthy controls. This hypothesis is supported by consistent findings in MDD of impairments in cognitive and behavioural measures that rely on neuroplastic mechanisms (Landrø et al., 2001, Porter et al., 2003, Naismith et al., 2006, Normann et al., 2007, Nissen et al., 2010). Previous research using motor cortical plasticity protocols provide a framework for testing neuroplasticity in psychiatric disorders (Daskalakis et al., 2008, Frantseva et al., 2008), while limited direct evidence of impaired neuroplasticity in depression provides a mandate for this investigation.

The secondary hypothesis is that motor cortical plasticity improves after treatment for depression. This hypothesis is supported by evidence of plasticity improvements after antidepressant administration, shown directly through increased VEPs in healthy subjects (Normann et al., 2007), and indirectly through improved learning and memory after antidepressant treatment (Vythilingam et al., 2004, Wagner et al., 2012).

Additional hypotheses were that there would be correlations between separate measures of neuroplasticity taken from the same subject, and there would be relationships found between measures of neuroplasticity and neuroplasticity mediating factors, such as BDNF.

1.4.2 Significance of Research

This thesis undertakes the first systematic investigation of motor cortical plasticity in MDD. Studies objectively test motor cortical plasticity in individuals with MDD, and compare it with matched healthy controls. Studies are also designed to discover if this measure of neuroplasticity changed following treatment for depression. Direct investigations of neuroplasticity, and its possible links to symptoms of depression are significantly under-researched in MDD. As very few studies to date have directly examined neuroplasticity changes in MDD, the current thesis seeks to address this gap in the literature. Results are likely to lead to a better understanding of the disorder's aetiology and provide guidance for treatment.

1.4.3 Overview of Studies

The studies proposed in this thesis utilise a number of brain stimulation protocols designed to induce and measure cortical excitability changes in healthy, and

depressed subjects. It is proposed that LTP and LTD, the two basic mechanisms for experience-dependent modification of synaptic strength, are integral to these changes (Bliss and Collingridge, 1993, Malenka and Bear, 2004). The brain stimulation protocols chosen provide robust and relatively consistent changes in cortical excitability, which have been shown to outlast the period of exposure (Stefan et al., 2000, Huang et al., 2005), making them well suited for the study hypotheses.

This thesis comprises three studies. Study 1 (Chapter 2) aimed to identify a brain stimulation protocol which reliably induced excitatory motor cortical change that could subsequently be used in a clinical population. The study compared the effects of two commonly used brain stimulation protocols (PAS25 and iTBS), shown to facilitate motor cortical excitability. The effects of PAS25 and iTBS were additionally evaluated when they were immediately preceded by 40 seconds of cTBS, which results in reduced motor cortical excitability. The theory of homeostatic metaplasticity predicts that after a reduction in cortical excitability, a greater level of LTP might result from an excitatory stimulation protocol (Todd et al., 2009). It was also hypothesised that priming with cTBS might reduce the variability of the response to the excitatory protocol across subjects by standardising the preceding history of cortical activity. Finally, in order to determine the functional significance of neuroplasticity as measured by changes in MEP amplitude, motor learning was assessed using a computerised version of the rotor pursuit task.

Study 1 results showed that the single facilitatory PAS protocol induced the greatest change in motor cortical excitability measured by increased MEP amplitude. Therefore in Study 2, PAS was used to test the motor cortical plasticity of depressed subjects, and contrasted with healthy controls. A cross-sectional analysis compared

change in motor cortex excitability (MEP amplitude) after PAS, motor learning (assessed with the rotor pursuit task), BDNF serum levels and BDNF (Val66Met) genotype. Changes in MEP amplitude were compared with motor learning, BDNF genotype and BDNF serum levels. Subject mood (Montgomery-Äsberg Depression Rating Scale, MADRS) (Montgomery and Äsberg, 1979) was also measured to assess the relationship between neuroplasticity and mood state.

Indirect evidence from studies of neurocognitive function and direct evidence from Study 2 supports a hypothesis of impaired neuroplasticity in MDD. Performance on neurocognitive measures improves with effective treatment for depression (Vythilingam et al., 2004, Wagner et al., 2012). In a longitudinal analysis, Study 3 used the PAS protocol to test the neuroplasticity of depressed subjects before and after a treatment course of tDCS. Mood and BDNF serum levels were also assessed. There is emerging evidence that tDCS is effective for the improvement of depressive symptoms such as mood and cognition. Thus, enhanced measures of mood, improved neuroplasticity (increased MEP amplitude after PAS) and increased serum BDNF were expected post-tDCS compared to the pre-tDCS time point.

The three studies are presented in chapters 2, 3 and 4 in the format of journal articles. Studies 1 and 2 are published in *Clinical Neurophysiology* and *Neuropsychopharmacology* respectively. Study 3 has been submitted for consideration for publication and is currently under review.

Chapter 2: Paired Associative Stimulation Increases Motor Cortex Excitability More Effectively than Theta-burst Stimulation

2.1 Abstract

The objective was to examine the effects of theta burst stimulation (TBS) and paired associative stimulation (PAS) on excitability in the human motor cortex. Sixteen healthy young subjects received intermittent TBS (iTBS) or PAS to the primary motor cortex on two testing occasions, at least a week apart. Ten of the subjects also received iTBS or PAS after conditioning with continuous TBS on two other occasions. Cortical excitability was assessed with single TMS pulses to the motor cortex. Motor evoked potentials (MEPs) were measured from the first dorsal interosseus (FDI) muscle before TBS or PAS stimulation, and every 10 mins for 60 mins after stimulation. Changes in excitability were compared against the potential for motor learning, assessed with the rotor pursuit task. After the PAS protocol, MEP amplitudes were significantly increased. This increase was greater than after intermittent TBS, which did not change MEPs significantly. Conditioning with continuous TBS showed no significant effect. Subjects' responses were not correlated across protocols and were not correlated with rotor pursuit learning. In conclusion, PAS was the only protocol which induced significant increases in MEP amplitude. PAS demonstrated robust induction of excitatory cortical change. This makes it a suitable protocol for testing plasticity in healthy and patient groups.

2.2 Introduction

Neuroplasticity refers to the ability of nerve cells to modify their structure and function. This includes all possible modes of neuronal reorganisation, including neurogenesis, alterations in dendritic complexity, synaptogenesis, and changes in the strength of existing synaptic connections (Duffau, 2006). These changes can occur in response to activity, injury, insult, or other environmental factors, with the changes outlasting the period of exposure. Plasticity is therefore inherent within the human nervous system and provides the mechanisms for development and learning. Recently, there has been increasing recognition that neuroplasticity may be impaired in neurological and psychiatric disorders (Duman et al., 1999, Johnston, 2004). Thus the development of methods for assessing neuroplasticity *in vivo* is potentially useful for investigating changes in plasticity in disease states and evaluating the effects of treatment.

Neuroplasticity may be tested in people through the use of non-invasive brain stimulation protocols which induce transient changes in cortical excitability. Two protocols that have been reported to induce significant increases in cortical excitability are paired associative stimulation (PAS) and intermittent theta burst stimulation (iTBS) (Stefan et al., 2000, Huang et al., 2005, Di Lazzaro et al., 2011). In an excitatory form of PAS (PAS25), peripheral stimulation of afferent sensory nerves is given approximately 25 milliseconds prior to transcranial magnetic stimulation (TMS) to the motor cortex, so that afferent stimuli arrive at the sensory motor cortex just prior to TMS activation of motor cortical pathways. TBS involves bursts of 3 cortical stimuli at 50Hz, repeated at 200ms intervals (5 Hz). In iTBS, trains of TBS are separated by rest intervals, e.g., typically 2-s trains with 8-s inter-train intervals. Both PAS25 and iTBS increase cortical excitability by facilitating later indirect (I) waves and both forms of

facilitation are at least partially dependent on N-methyl-D-aspartate (NMDA) receptors (Huang et al., 2007, Teo et al., 2007). However, PAS is based upon the Hebbian concept of spike-timing-dependent plasticity in which the precise timing of paired presynaptic and postsynaptic changes in membrane potential are critical (Stefan et al., 2000), while TBS protocols mimic the coupling between gamma (50 Hz) and theta (5 Hz) oscillations in the cortex (Cárdenas-Morales et al., 2010).

Prior research has shown substantial inter-individual as well as intra-individual variability in responses to brain stimulation protocols (Bolognini et al., 2009). Studies have found variations in post–stimulation motor evoked potentials (MEPs) to be influenced by subject age (Müller-Dahlhaus et al., 2008, Todd et al., 2010), attention (Stefan et al., 2004, Conte et al., 2007), exercise status (Cirillo et al., 2009) genetic variations (Cheeran et al., 2008) and the time of day of testing (Sale et al., 2007, 2008). The prior and current state of cortical excitability (Ziemann et al., 2004, Nitsche et al., 2007) and priming with another brain stimulation protocol (Potter-Nerger et al., 2009) will also influence the outcome of subsequent stimulation protocols - a process known as metaplasticity (Müller et al., 2007).

This study aimed to examine the suitability of the iTBS and PAS25 protocols as tests for evaluating neuroplasticity. To have clinical utility in distinguishing health from disease, a test should reliably induce significant changes in the majority of healthy subjects to allow comparison with patients. Ideally, the degree of inter-individual variation in response among healthy subjects would be small, and test outcomes would correlate with measures of functional relevance. Protocols of short duration will also offer practical advantages.

To minimise the influence of confounding factors on the outcomes of different stimulation protocols, subjects were restricted to healthy young adults and all testing was done in the afternoon, when neuroplasticity has been found to be greatest (Sale et al., 2007, 2008). In addition, the combination of neuroplasticity protocols with a prior priming stimulation has been shown to increase the magnitude of induced changes (Iyer et al., 2003, Lang et al., 2004). In particular, priming stimulation with contrary effects to the subsequent main stimulation protocol has been shown to increase the effects of the main stimulation, due to the principle of homeostatic metaplasticity (Todd et al., 2009). Hence, this study also explored whether combining PAS25 and iTBS, which facilitate cortical excitability, with prior (priming) stimulation that reduces cortical excitability (continuous TBS; cTBS), would increase the magnitude of induced changes and reduce the variability of results. Finally, to determine the functional significance of "plasticity" as measured by changes in MEP amplitudes, motor learning was assessed using the rotor pursuit task.

2.3 Methods

Subjects

Sixteen (nine male) healthy adults who were right handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971) participated after giving written, informed consent. The study was approved by the research ethics committee of the University of New South Wales. Subjects were screened to ensure the absence of any illicit drug use or excessive alcohol use, major medical illness, musculoskeletal, psychological or neurological disorder, or electronic implants.

Research Plan

Ten subjects visited the laboratory on four occasions. After completing the rotor pursuit task, they undertook PAS25, iTBS, cTBS-PAS25 and cTBS-iTBS in a within-subjects crossover design. From the 24 different sequences of protocols possible, 10 were selected that would provide an equal number of protocol comparisons if subjects dropped out after the first 2 sessions. Subjects were randomly allocated to one of these 10 sequences. Each protocol was tested in a separate afternoon experimental session separated by at least a week to avoid carry-over effects. Two protocols (PAS25, iTBS) were tested in a further six subjects. These subjects first completed the rotor pursuit task and then completed the stimulation protocols in a pseudo-randomised within-subjects crossover design. Three subjects started with PAS25 and three with iTBS. Motor evoked potentials (MEPs) were assessed before and after each stimulation protocol.

Materials and Equipment

For each of the four protocols, electromyographic activity (EMG) was recorded through (Ag/AgCl) surface electrodes placed over the belly and tendon of the right first dorsal interosseous (FDI) muscle. EMG signals were amplified (x1000) using a 1902 amplifier (Cambridge Electronics Design, Cambridge, UK), band-pass filtered (16-1000Hz) and sampled (2000Hz). EMG signals were digitised using an A/D converter (1401, version 4.02, Cambridge Electronics Design, Cambridge, UK).

MEPs were elicited by single-pulse TMS delivered by a Magstim 200 stimulator (Magstim Co., Whitland, UK) using a figure-of-eight coil with 7cm diameter wings. TBS protocols were delivered with a similar figure-of-eight coil but using a Magstim

Rapid stimulator (Magstim Co., Whitland, UK). In the PAS sessions, single-pulse TMS (Magstim 200 stimulator) was combined with electrical stimuli which were delivered to the ulnar nerve through a bipolar electrode using square-wave pulses of 200μs from a constant-current stimulator (DS7 stimulator, Digitimer Co. Ltd., Hertfordshire, UK). A computerised version of the rotor pursuit task (Life Sciences Associates, Inc., Bayport, N.Y.) was used.

Motor Learning Task

Subjects were seated in front of a computer screen (38x30cm) that featured a circular track with a round red target that orbited at discretely controlled speeds. Subjects were required to manoeuvre a pointed arrow cursor with a computer mouse in order to keep it within the moving target for the duration of the trial. Each trial, regardless of the speed, lasted for 30s. Each subject completed a pre-baseline block, a baseline block and five test blocks, each consisting of 5x30s trials. At the end of each trial, subjects were given feedback of their 'time on target', which was the dependent variable of interest and was measured as a percentage of the trial time. The baseline and testing session speed was based upon performance in the pre-baseline block and designed to have subjects perform with around 25% time on target.

Brain Stimulation Experimental Procedures

The two main stimulation protocols were PAS25 and iTBS. In two further sessions, these protocols were preceded by an inhibitory continuous TBS conditioning

(cTBS-PAS25, cTBS-iTBS). The interval between the cTBS and subsequent PAS25 or iTBS was ~2 mins.

During all brain stimulation protocols, subjects were seated in a chair with both hands lying comfortably on a pillow in their lap. EMG was recorded from the right FDI.

At the beginning of each testing session, the optimal site for eliciting MEPs in the contralateral FDI muscle was established empirically. The TMS coil was placed tangentially over the left motor cortex with the handle pointing postero-laterally. A black soft-tipped pen was used to mark the optimal spot, which was used for subsequent testing in the session. Resting motor threshold was defined as the minimum stimulus intensity required to evoke a motor response of at least 50 µV in the relaxed FDI in five of ten consecutive trials. TMS stimulus intensity was selected to evoke an MEP of around 1mV in the relaxed FDI, but did not exceed 130% of the resting motor threshold (Stefan et al., 2000, Stefan et al., 2002, Wolters et al., 2003).

For the sessions in which theta-burst stimulation was given, the optimal spot for eliciting a MEP with the (biphasic) Magstim Rapid Stimulator was also determined at the start of the session and marked on the scalp with a different coloured pen. Active motor threshold was determined while subjects maintained a contraction 10-20% of maximum effort using visual feedback of EMG. Active motor threshold was defined as the minimum stimulus intensity to evoke a motor response of at least 200µV in the target muscle in five of ten consecutive trials (Huang et al., 2005). In the experimental sequence, active motor threshold was determined after resting motor threshold and was completed 3-5 mins before collection of the first baseline block of MEPs. Stimulus intensity for TBS was set at 80% active motor threshold. TBS comprised bursts of 3 pulses at 50 Hz given every 200 ms (5 Hz). In the facilitatory iTBS protocol, 2-s bursts

of TBS were spaced by 8-s intervals delivering 600 stimuli over 190-s (Huang et al., 2005). The inhibitory cTBS protocol comprised an uninterrupted train of 600 TBS pulses over 40 seconds.

For the PAS25 protocol, single-pulse TMS to the left motor cortex was combined with right ulnar nerve stimulation. Two electrodes were placed over the ulnar nerve ~3cm and 5cm proximal to the wrist and lateral to the flexor carpi ulnaris tendon. Perceptual threshold was measured as the lowest intensity at which sensation radiated into the hand. PAS25 comprised 200 pairs of stimuli (TMS and ulnar nerve) given at 0.25Hz over ~13 min (Ziemann et al., 2004). In each pair, ulnar nerve stimulation (300% of perceptual threshold) preceded the TMS pulse (130% RMT) by 25ms. As it has been shown previously that subject attention to the area of stimulation increases cortical response (Stefan et al., 2004), ring electrodes were placed upon the right index finger and subjects were asked to count the number of electrical stimuli randomly delivered to the finger during each PAS session.

In each session, MEPs were tested before and after a conditioning stimulation protocol. Each testing block consisted of 20 MEPs at 0.1Hz.Two blocks of MEPs were recorded at baseline. A block of MEPs was recorded immediately after the stimulation protocol and then every ten minutes for one hour (see Figure 2.1).

During all stimulation protocols, subject EMG was continuously visually monitored to ensure adequate muscle relaxation.

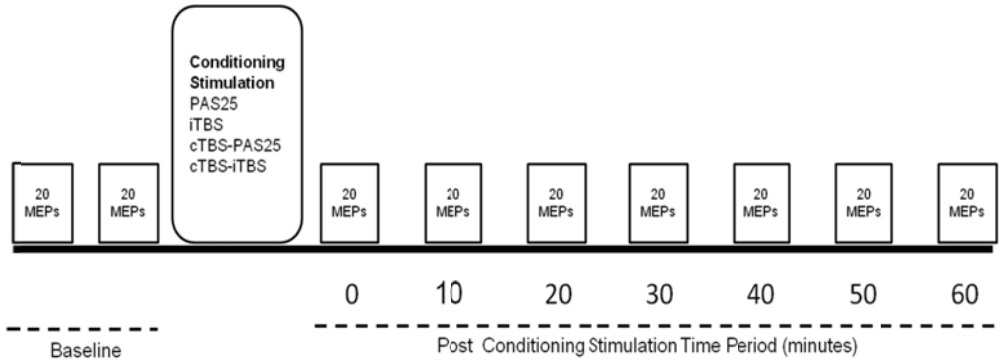


Figure 2.1 Chapter 2 experimental design

Experimental design showing the procedure for each of the four brain stimulation testing sessions. Key: PAS25, paired associative stimulation with 25 ms interstimulus interval; iTBS, intermittent theta-burst stimulation; cTBS, continuous theta-burst stimulation.

Data Analysis

MEP amplitudes were measured peak-to-peak in each individual frame. Any frames with voluntary EMG activity preceding the stimulus were excluded from analysis. Amplitudes for each block of 20 MEPs were averaged. A mean baseline value was calculated from the two baseline blocks. Repeated measures analyses of variance (RM ANOVA) were carried out to statistically compare mean raw baseline MEP amplitudes across protocols. The mean MEP values for subsequent blocks were normalised to the baseline value before further analysis.

Changes in cortical excitability

Normalised MEP amplitudes were analysed in 16 subjects by a 2 x 8 RM ANOVA with the two factors being Protocol (PAS and iTBS) and Time (baseline, 0mins, 10mins, 20mins, 30mins, 40mins, 50mins, 60mins) with a planned contrast investigating a linear trend over the Time factor. Planned contrasts comparing all post-

stimulation time points to baseline were conducted for PAS and iTBS separately with Bonferroni correction used for multiple comparisons (significance level $p<0.007$).

The effect of cTBS conditioning was assessed by first averaging the normalised post stimulation blocks of MEPs into one "post-stimulation" MEP value. These were assessed in a further 2 x 2 RM ANOVA with the two factors of Conditioning (cTBS, no cTBS) and Protocol (PAS25, iTBS) for 10 subjects. Associations between subjects' responses across protocols were sought by means of Pearson's correlations. Comparison between PAS25 and iTBS was for 16 subjects. Comparisons involving cTBS-iTBS and cTBS-PAS25 included 10 subjects.

To determine the number of subjects in whom MEP amplitudes were significantly increased, a one-way ANOVA was calculated for all pre- and post-stimulation MEPs for each subject in each protocol. Positive responders were defined as subjects who had a significant positive increase in post-stimulation amplitudes compared to baseline. Negative responders showed a significant decrease in post-stimulation MEPs. Analysis of variance on RANKS was carried out for non-parametric data.

Motor learning and correlates of neuroplasticity

The outcome measure for the motor learning task was the subject's time on target, which was collected for the five testing blocks and one baseline block. The mean time on target for each block was normalised to baseline. The mean of testing blocks 5 minus 1 is considered a measure of learning (Test Block Learning) and was examined for correlations with the mean, normalised, post-stimulation MEP amplitudes for each of the four protocols in 16 subjects (PAS25, iTBS) and 10 subjects (cTBS-iTBS, cTBS-PAS25). However, it was noted during the task that some subject learning took place

within the initial baseline block. Thus a further analysis using Pearson's correlation examined for associations between Baseline Learning (last trial minus the first trial of the baseline block) and the mean, post-stimulation MEP amplitudes of each of the four protocols.

2.4 Results

MEP amplitudes at baseline were similar between test days for the 16 subjects who completed PAS25 and iTBS only, (PAS25, 1.00±0.48 mV; iTBS, 0.96±0.64 mV; [$F(1,15)=0.033$, $p=0.859$)]. Similarly, there were no differences between test days for the four stimulation protocols (PAS25, 1.03±0.62 mV; iTBS, 0.95±0.56 mV; cTBS-PAS, 0.96±0.45 mV and cTBS-iTBS, 0.87±0.47 mV); [$F(3,27)=0.383$, $p=0.766$)].

Changes in cortical excitability

Comparison of normalised MEP amplitudes before and after PAS25 and iTBS found significant main effects of Time [linear trend $F(1,15) = 5.989$, $p=0.027$] and Protocol [$F(1,15) = 5.624$, $p=0.032$] as well as a significant Time*Protocol interaction [$F(1,15) = 4.967$, $p=0.042$]. Planned contrasts showed significant increases in MEP amplitudes after PAS25 at time points 30, 40 and 60mins ($p<0.007$) and at trend level at 50mins ($p=0.013$; See Figure 2.2a). No significant changes were seen after iTBS ($p>0.007$).

When the averaged post-stimulus MEP amplitudes were compared across all four protocols (10 subjects), there was a trend level main effect for MEPs after the PAS25 protocols to be larger than after the iTBS protocols [$F(1,9) = 3.972$, $p=0.077$] but no main effect of cTBS Conditioning [$F(1,9) = 0.368$, $p=0.559$], and no Protocol*Conditioning interaction [$F(1,9) = 0.059$, $p=0.813$; See Figure 2.2b].

In the correlational analysis of post-stimulation MEP amplitudes, there was no significant association between any of the four protocols (R=-0.4 to 0.5, p=0.137 to 0.913; See Figure 2.3).

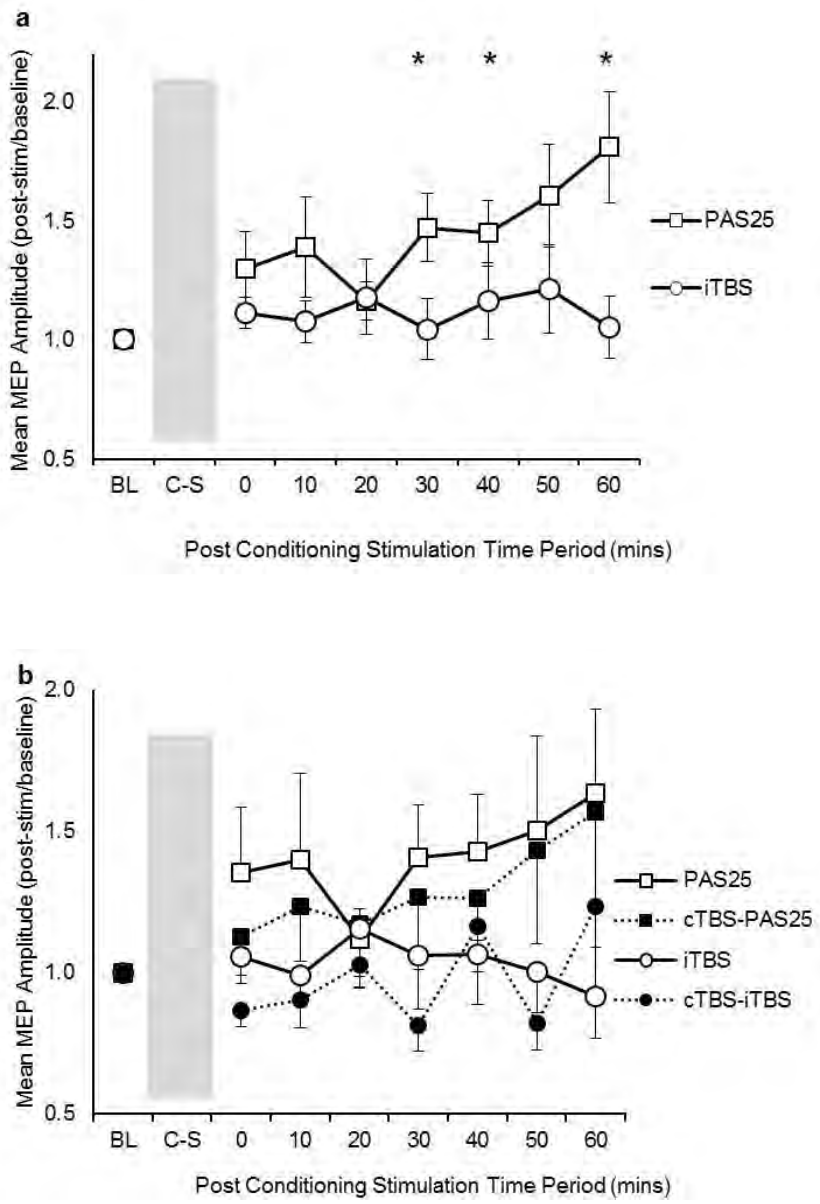


Figure 2.2 Time course of mean MEP amplitudes after conditioning protocols

a. Time course of mean MEP amplitudes after the PAS25 and iTBS stimulation protocols in 16 subjects. **b**. Time course of mean MEP amplitudes after each of the four conditioning stimulation protocols in 10 subjects. MEP amplitudes are normalised to baseline values. Mean and standard errors are shown, (*) indicates significant change from baseline. Key: BL, baseline; C-S, conditioning stimulation.

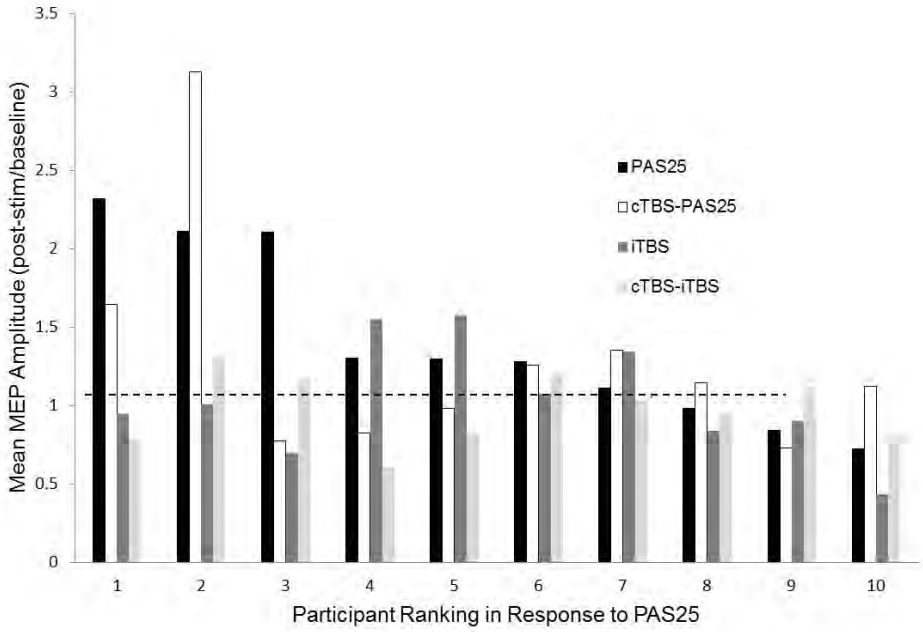


Figure 2.3 Comparison of individual mean MEP amplitudes after each conditioning stimulation protocol

Mean MEP amplitudes after each of the four conditioning stimulation protocols are shown for the individual subjects who completed all protocols. Subjects are ranked from greatest to least response to the PAS25 protocol. MEPs shown are the mean post-stimulation values normalised to baseline.

The number of positive and negative responders in each protocol was 12 and 3 (PAS25), 10 and 4(iTBS), 5 and 3 (cTBS-PAS25), and 3 and 4 (cTBS-iTBS). The remaining subjects did not have a significant change in MEP amplitudes from baseline levels.

Motor learning and correlates of neuroplasticity

There were no significant correlations between Test Block Learning and the mean post-stimulation MEP amplitudes for any of the four protocols (see Figure 2.4). However, a positive correlation was found at trend level between Baseline Learning and mean normalised, post-stimulation MEP amplitudes for PAS25 (R=0.448, p =0.082).

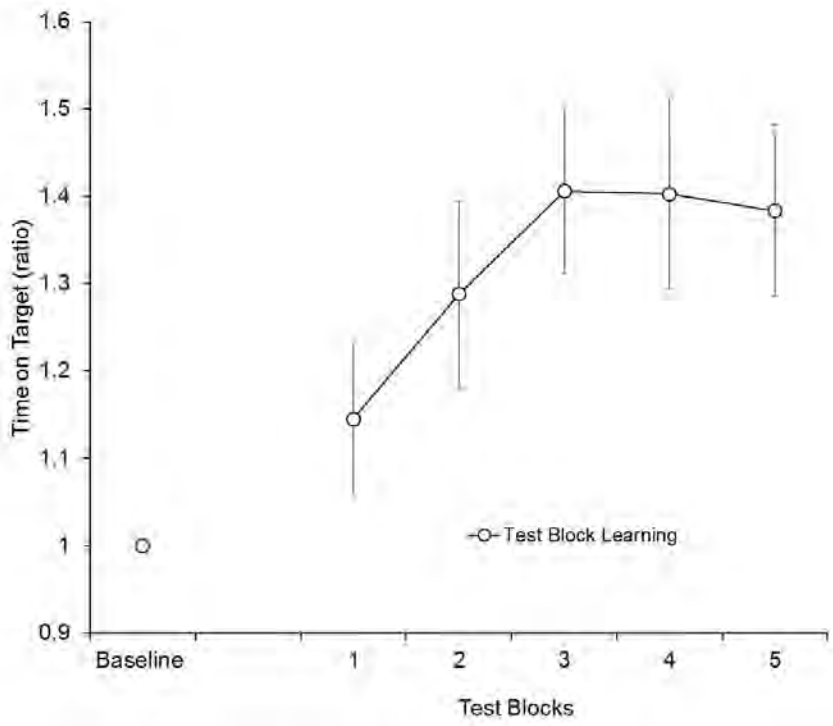


Figure 2.4 Group normalised mean motor learning performance over five testing blocks

Group data (means ± SEM) for 16 subjects for performance on the rotor pursuit task. Time on target during each test block is shown normalised to mean value of the baseline block.

2.5 Discussion

This is the first comparison of iTBS and PAS25, given alone and after preconditioning with cTBS, in the same subjects. Of the four protocols tested, PAS25 was found to be the most effective protocol in inducing increases in MEP amplitudes, both in terms of mean group response and in the number of subjects showing a significant increase.

Results show changes in MEP amplitude of over 80% after PAS25, with an ongoing upward trend 60 minutes after stimulation. These changes are of comparable or greater magnitude to those found in other studies using PAS25 (Stefan et al., 2000,Ziemann et al., 2004, Frantseva et al., 2008, Di Lazzaro et al., 2011). Thus the

results of our study support earlier suggestions that the PAS25 protocol is more robust than other non-invasive brain stimulation protocols in inducing changes in cortical excitability (Rosenkranz and Rothwell, 2006, Ilić et al., 2009)

Unlike some earlier studies (Huang et al., 2005, Iezzi et al., 2008), iTBS did not lead to significant increases in cortical excitability in this study. Two points may be relevant here. First, variability between subjects is common in response to brain stimulation protocols including iTBS and PAS (e.g. (Fratello et al., 2006, Di Lazzaro et al., 2008a, Todd et al., 2009). Here, more than half of subjects (10 of 16) responded to iTBS with increased post-stimulation MEP amplitudes, but the remaining subjects had significant decreases or no significant change. Hence, there was no significant change for the group. After PAS25, facilitation occurred in 75% of subjects (12 of 16). As detailed in the Introduction, many factors such as time of day, stimulation history, attention and previous activity can influence responses. Despite our attempt to standardise a number of these factors, inter-individual variability remained. Second, we measured MEP amplitudes every 10 minutes for 60 minutes. While strong effects of iTBS have been shown at 20-30 minutes (Huang et al., 2005, Teo et al., 2007), they may peak much earlier (Di Lazzaro et al., 2008a, Zafar et al., 2008, Di Lazzaro et al., 2011) and a brief peak may have been missed in our protocol. In contrast, PAS-induced changes can last at least 60 minutes after stimulation (Stefan et al., 2000, Delvendahl et al., 2010). When all time points were considered, PAS showed a significant increase in MEP amplitudes from 30mins post-stimulation onwards, while the maximal increase after iTBS was just over 20% and was not significantly different from baseline (see Figure 2.2).

PAS25 and iTBS

Both PAS25 and iTBS are thought to involve changes in neurotransmission at the cortical level (Paulus et al., 2008). It is postulated that the changes reflect long-term potentiation (LTP). Consistent with this, there is evidence that NMDA receptors are important for the effects of both PAS (Stefan et al., 2002) and TBS (Huang et al., 2007). However the two protocols attempt to produce LTP in different ways. In TBS, bursts of high-frequency stimuli at low intensity are designed to activate cortical neurones which provide synaptic inputs to motor cortical output cells without overtly activating them (Stefan et al., 2000, Huang et al., 2005). In PAS25, a sensory volley is timed to arrive at the motor cortex (via the thalamus and somatosensory cortex) milliseconds before TMS is delivered at an intensity which activates motor cortical output cells both directly and through input from other cortical neurones (Stefan et al., 2000).

The use of suprathreshold stimuli in PAS25 compared to the subthreshold stimuli in TBS may have influenced the success of PAS25 in increasing MEP size. In practical terms, application of suprathreshold stimuli is more secure. Observation of a MEP during PAS confirms the appropriate site and intensity of TMS and report from the subject confirms the peripheral stimulation. In contrast, with stimuli delivered at the low intensity of 80% active motor threshold, there is no EMG or other response so there is no overt confirmation that any neurones are activated when TBS is applied. More theoretically, the intensity of stimulation may affect calcium influx and hence the induction of plasticity. The induction of NMDA-receptor-dependent synaptic plasticity relies on the level of calcium influx into the postsynaptic cell (Gamboa et al., 2010). High intracellular calcium leads to the cascade of events which result in LTP whereas a weaker, moderate calcium influx leads to long-term depression (LTD) (Lisman, 2001, Wankerl et al., 2010). Furthermore, an intermediate level, referred to as “no man’s

land," may result in no plasticity at all (Cho et al., 2001, Lisman, 2001). For TBS, each subthreshold stimulus will have a small effect on the postsynaptic cell. Sufficient intracellular calcium to initiate LTP will need to be accumulated by the repeated inputs in a burst and may be marginal. In contrast, depolarisation that leads to action potential generation produces large increases in intracellular calcium. Thus for PAS, intracellular calcium levels are not likely to be a problem. Rather, the timing of the sensory input compared to TMS is critical.

Another possible explanation for divergence in MEP amplitudes after PAS25 and iTBS is that subjects were instructed to attend to the site of stimulation in PAS but not in TBS as is common practice. As attention has been shown to increase induced effects (Stefan et al., 2004), it could also provide for the greater change in MEP amplitudes observed after PAS25.

Our results differed from earlier research comparing iTBS and PAS25 within subjects. Di Lazzarro et al. (2011) found greater changes in MEP amplitudes after iTBS than PAS25. This may be due to methodological differences in the protocols used and in the study design overall. Di Lazzaro et al. (2011) in their comparison of six separate stimulation protocols used PAS with 90 pairs at 0.05 Hz over 30 minutes, whereas our protocol had 200 pulses at 0.25 Hz lasting around 13 minutes. Furthermore, Di Lazzaro et al. (2011) used a complex experimental design, in which resting and active thresholds, and intracortical inhibition and facilitation were tested in addition to MEP amplitudes. This additional stimulation and muscle contraction may have influenced subsequent measures of MEP amplitudes (Kujirai et al., 2006, Huang et al., 2008). Di Lazzaro et al. (2011) found increases in MEP amplitudes after iTBS that were only marginally greater than after PAS25, and only at 5 minutes post-stimulation.

Priming with cTBS

Priming with cTBS did not improve the effectiveness of either PAS25 or iTBS in increasing MEP amplitude post stimulation. It had been expected that priming with cTBS followed by an excitatory stimulation protocol would create greater cortical facilitation through the principle of metaplasticity (Iyer et al., 2003). Metaplasticity operates on the cellular level and refers to changes in the susceptibility of synapses to the induction of plasticity (Abraham and Bear, 1996). Homeostatic metaplasticity, for which there is considerable supporting evidence (Ziemann and Siebner, 2008), proposes that the threshold for LTP or LTD induction is flexible and dependent on the recent history of postsynaptic activity: high activity increases the LTP threshold and decreases LTD threshold whereas low activity has the opposite effects (Ridding and Ziemann, 2010). For the human motor cortex, a number of studies have reported induction of increased cortical facilitation following priming with a stimulation protocol designed to reduce excitability (Müller et al., 2007, Potter-Nerger et al., 2009). One possible explanation for the absence of enhanced facilitation with the double protocols in the current study is that the inhibitory and facilitatory stimulation protocols used may have their main effect on different sets of neurones. Epidural recording has shown that cTBS mainly reduces the size of I_1, which suggests that its action is on the synapses between the interneurones responsible for the first I-wave and the corticospinal neurones (Di Lazzaro et al., 2005). In contrast, iTBS and PAS both appear to influence neural networks affecting later I-wave transmission (Di Lazzaro et al., 2008a, Di Lazzaro et al., 2009). The Bienenstock-Cooper-Munro (BCM) theory proposes heterosynaptic expression of synaptic plasticity. That is, activity-induced changes in synaptic thresholds will affect the subsequent activity of all synapses onto the postsynaptic cell (Bienenstock et al., 1982, Abraham et al., 2001). However, if cTBS or iTBS and PAS25

do not all act at synapses onto the same neurones, metaplastic interactions would be limited. A counter to this argument, however, is that a reversal of one of the current protocols (iTBS prior to cTBS) was effective in enhancing the cTBS-induced depression of MEPs (Todd et al., 2009).

Another possibility is that the time interval between the two types of stimulation in the double protocols was not optimal for enhancing facilitatory effects of the second stimulation. Huang et al., (2010) demonstrated that the time gap between two theta-burst protocols in the human motor cortex determined the effectiveness of the interaction. They found MEP changes after cTBS and iTBS were abolished by 150 pulses of iTBS and cTBS respectively, but only when given 1 minute after and not 10 minutes after original stimulation. We observed that MEP amplitudes actually tended to be lower after the double protocols. This may have been due to lasting inhibitory effects from the priming cTBS protocol. Earlier studies have suggested that cTBS has significant effects on MEP amplitude after 7mins (Di Lazzaro et al., 2005), with cortical effects still consistently found 20-30 mins after stimulation (Huang et al., 2005, Zafar et al., 2008).

Intra-individual comparison of plasticity measures

The consistency of MEP changes for each individual across the different stimulation protocols is also of interest. Li Voti et al. (2011) demonstrated within the same subjects a correlation between MEP amplitude changes brought about by 5 Hz rTMS and iTBS which induce short-term potentiation (STP) and LTP respectively, and which typically induce increases in MEP amplitude (Huang et al., 2005, Quartarone et al., 2009, Li Voti et al., 2011). However, most studies comparing different brain stimulation protocols within individuals in the motor cortex have not commented upon

the reliability of cortical effects between protocols (Rosenkranz and Rothwell, 2006, Zafar et al., 2008, Di Lazzaro et al., 2011). As shown in Figure 2.3, there was no systematic consistency between measures of plasticity across the protocols. For each subject, changes in MEP amplitudes induced by one protocol did not reliably predict changes in MEP amplitudes after the other three protocols. As discussed above, a possible explanation for this finding is that the stimulation protocols exert effects through different mechanisms. Alternatively, the lack of consistency between results could have arisen from variability attributable to different occasions of testing. Fratello et al. (2006) found that results differed between two sessions of PAS testing, even in the same individuals. Nonetheless, there is no evidence to support the use of multiple testing protocols as a strategy to derive a single, more reliable measure of plasticity instead of using a single protocol such as PAS25

Motor Learning

Learning across the main blocks of the rotor pursuit task did not correlate with outcomes of any of the stimulation protocols, which were examined as potential tests of plasticity. In contrast, Frantseva et al. (2008) found a significant correlation with PAS25 induced plasticity and motor learning in patients with schizophrenia. This might be explained by the exact method used to measure motor learning. For example, Frantseva et al. (2008) had patients trace a moving object with a hand-held stylus with results precisely recorded by a photoelectric device. The computerised version of the rotor pursuit task used in the current study might not be sensitive enough to distinguish differences in a healthy cohort.

Conclusion

PAS25 was the only stimulation protocol which induced significant changes in motor cortical excitability, and was the protocol which resulted in significant changes in the greatest number of subjects. Priming with cTBS with approximately two minutes lapse between protocols did not increase the excitatory effects of PAS25 and iTBS stimulation as had been expected. Response to one type of stimulation was not related to response to the other stimulation protocols, suggesting the involvement of different mechanisms. While cortical excitation has been consistently shown after iTBS, results of this study show that PAS25 is a more robust protocol for assessing facilitatory neuroplasticity and is likely to be suitable for comparing plasticity between control and patient groups.

Chapter 3: Neuroplasticity in Depressed Individuals Compared to Healthy Controls

3.1 Abstract

Several lines of evidence suggest that neuroplasticity is impaired in depression. This study aimed to compare neuroplasticity in 23 subjects with DSM-IV Major Depressive Episode and 23 age and gender matched healthy controls using an objective test that is independent of subject effort and motivation. Neuroplasticity was assessed in the motor cortex using a brain stimulation paradigm known as paired associative stimulation (PAS), which induces transient changes in motor cortical function. Motor cortical excitability was assessed before and after PAS using single pulse transcranial magnetic stimulation (TMS) to induce motor evoked potentials (MEPs) in a hand muscle. After PAS, MEP amplitudes significantly increased in healthy controls compared to depressed subjects (p=.002). The functional significance of motor cortical changes was assessed using a motor learning task – a computerised version of the rotor pursuit task. Healthy controls also performed better on motor learning (p=.02). BDNF blood levels and genotype were assayed to determine any relationship with motor cortical plasticity. However, PAS results did not correlate with motor learning, nor appear to be related to BDNF measures. The significance of these findings is that it provides one of the first direct demonstrations of reduced neuroplasticity in depressed subjects, using an objective test.

3.2 Introduction

The neurobiology of major depressive disorder (MDD) includes evidence for altered cortical activity (Savitz and Drevets, 2009) shrinkage of neurons and glial cells, loss of dendritic complexity in the hippocampus and prefrontal cortex (PFC) (Kanner, 2004, Carlson et al., 2006), and lower levels of brain derived neurotrophic factor (BDNF) (Sen et al., 2008). Reduction of regional volume in these affected brain areas has been confirmed in post-mortem studies (Rajkowska et al., 1999, Stockmeier et al., 2004) and shown to be greater in those with multiple episodes, longer illness duration (MacQueen et al., 2003) and increased symptom severity (Kumar et al., 1998). The pathophysiological processes which lead to these neurotrophic changes are also thought to be responsible for impaired cellular resilience and loss of neuroplasticity (Pittenger and Duman, 2008).

For example, depression is often accompanied by impairment of learning and memory processes (Landrø et al., 2001, Porter et al., 2003) which are known to require neural adaptation (Malenka and Bear, 2004). Though a finding of impaired neuroplasticity in depression may be inferred from these observations, it is also possible that reduced motivation and effort may account for these deficits. This study therefore aimed to objectively assess neuroplasticity in depressed subjects compared to age and gender matched controls, through the use of a non-invasive brain stimulation protocol which induces temporary neuroplastic changes independent of subject effort. A range of brain stimulation protocols have shown the capacity to induce short-lasting motor cortical plasticity (Ziemann and Siebner, 2008). In a prior study we compared two stimulation protocols for which robust effects have been described – paired associative stimulation (PAS) and theta burst stimulation (TBS), and found that more consistent changes in motor cortical excitability were induced by PAS (Chapter 2). PAS-induced

increases in cortical excitability are considered to be at least partially dependent on associative long-term potentiation (LTP) (Stefan et al., 2002), which is modulated by BDNF through both presynaptic and postsynaptic mechanisms (Yoshii and Constantine-Paton, 2010), known to be crucial for neuroplasticity. These mechanisms are believed to underlie motor learning and memory formation (Letzkus et al., 2007, Sweatt, 2008), hence providing an appropriate model for testing neuroplasticity. The PAS protocol has previously been used to demonstrate reduced synaptic change in schizophrenia (Frantseva et al., 2008), but has hitherto not been tested in depressed patients. To determine whether neuroplasticity as measured by the PAS protocol was related to functional ability in learning a motor task, subjects were also tested with a computerised version of the rotor pursuit task. A second, exploratory aspect of the study was to test if BDNF Val66Met genotype and BDNF serum levels may relate to neuroplasticity.

3.3 Methods and Materials

Subjects

The sample consisted of 23 depressed subjects (10 male, 13 female) who met DSM-IV criteria for a Major Depressive Episode (20 with MDD, 3 with bipolar disorder), assessed using the Structured Clinical Interview for DSM Disorders IV (SCID), and 23 healthy controls, matched for gender and age (within 2 years). All were right handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971) and participated after providing written, informed consent. The study was approved by the Human Research Ethics Committee of the University of New South Wales in accordance with the Helsinki Declaration.

Current depression severity was assessed using the Montgomery-Äsberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979). Criteria for inclusion were a MADRS of ≥ 20 for MDD subjects, and < 6 for healthy controls

(Bandelow et al., 2006). In addition, depressed subjects were required to have no change in doses of psychotropic medication for at least 4 weeks prior to the study (6 weeks if newly initiated medication). Length of current depressive episode, treatment resistance (number of failed adequate trials of antidepressant medications), and current psychotropic medication were assessed in depressed subjects. Healthy controls had no prior history of depression, and were not on psychotropic medications. Other exclusion criteria were illicit drug use, alcohol abuse, musculoskeletal or neurological disorder, and electronic implants.

Research Plan

During the same experimental day, subjects underwent blood collection for BDNF genotyping and serum measurements (n=36) [or had DNA collected by buccal swab if blood tests were refused (n=9), or declined all DNA testing (n=1)], were tested with the rotor pursuit test (to assess motor learning) and ~ 45 minutes later completed the PAS protocol (at noon).

BDNF Genotyping and Serum Levels

Serum BDNF levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA) using the PromegaE_{max} kit (G7610) according to manufacturers instructions. Subject BDNF serum levels were excluded if greater than 2 standard deviations from group means (n=1, healthy control; n=1, depressed).

Genomic DNA was isolated from blood or buccal swab samples using a PUREGENE DNA purification kit (QIAGEN). The BDNF SNP rs6265 (Val/Met) polymorphism was assayed by TaqMan Allele discrimination assay (Cat# C_11592758_10, Life Technologies Inc.) analyzed with Sequence Detection Software (SDS) version 2.3 (ABI, Life Technologies, Inc.).

Motor Learning Task

A computerised version of the rotor pursuit task (Life Sciences Associates, Inc, NY) was used. Subjects sat in front of a computer screen that featured a round red target orbiting a circular track at discrete speeds. Subjects moved a computer mouse to keep a cursor within the moving target for the trial duration (30s per trial). Subjects completed a pre-baseline block, baseline block and 5 test blocks, each consisting of 5 trials. After each trial, feedback was given of 'time on target' (TOT), the dependent variable of interest, measured as % of trial time. The testing session speed was set to have subjects perform at 25% TOT, based on performance in the pre-baseline block (Schwartz et al., 1996).

Paired Associative Stimulation (PAS)

During the brain stimulation protocol, subjects sat with both hands on a pillow. Electromyographic activity (EMG) was recorded through (Ag/AgCl) surface electrodes over the right first dorsal interosseous (FDI) muscle. EMG was amplified (x1000), filtered (16-1000Hz) and digitised (2000Hz) (Cambridge Electronics Design, UK). Motor evoked potentials (MEPs) were elicited by TMS (Magstim 200 stimulator, Magstim Co, UK) using a 70mm figure-of-eight coil oriented with the handle postero-lateral.

Initially, the optimal site for eliciting MEPs in right FDI was established and marked on the scalp. Resting motor threshold (RMT) was defined as the minimum stimulus intensity to evoke MEPs of ≥50 μV in relaxed FDI in 5 of 10 consecutive trials. TMS intensity for evoking test MEPs was selected to elicit a 1mV response. If this intensity exceeded 130% RMT then 130% RMT was used (Table 3.1).

The PAS25 protocol was used as previously described (Chapter 2). Briefly, TMS (130% RMT) to the left motor cortex was combined with electrical stimuli (200µs duration, 300% perceptual threshold, DS7 stimulator, Digitimer Co. Ltd, UK) to the right ulnar nerve proximal to the wrist. Two hundred pairs of stimuli (TMS and ulnar nerve) were given at 0.25Hz over ~13 min. In each pair, ulnar nerve stimulation preceded TMS by 25ms. Electrical stimuli were delivered occasionally to the right index finger during PAS. Subjects counted the stimuli and reported the number at session end to ensure sensory attention to the hand (Stefan et al., 2004).

Test MEPs were recorded before and after PAS. Each testing block consisted of 20 MEPs at 0.1 Hz. Two blocks of MEPs were recorded at baseline. A block of MEPs was recorded immediately after PAS and then every 10 minutes for 1 hour (see Figure 3.1). During all stimulation, EMG from the FDI muscle was monitored to ensure muscle relaxation.

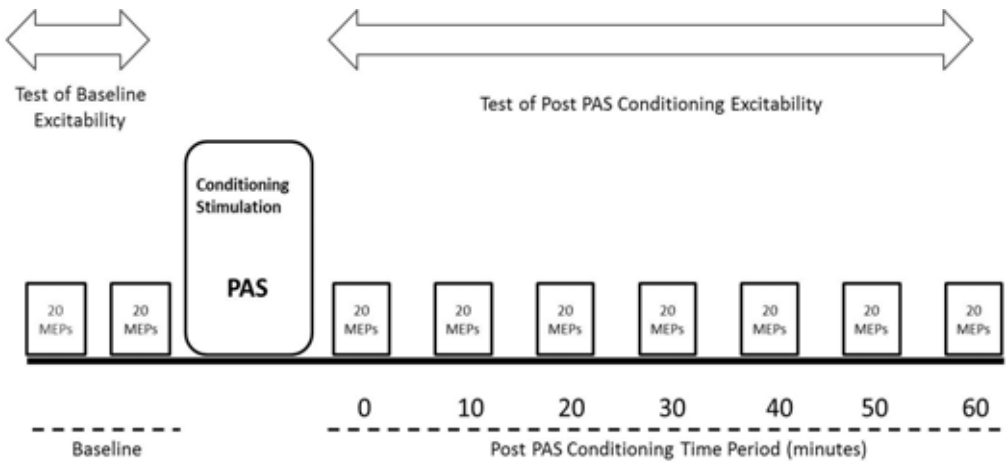


Figure 3.1 Chapter 3 experimental design

Experimental design. Motor evoked potentials (MEPs) were elicited to measure motor cortex excitability before and after a period of conditioning stimulation, which comprised 13 min of repeated paired brain and peripheral nerve stimulation (PAS, paired associative stimulation).

Data Analysis

Independent sample t-tests compared demographic and other experimental data between diagnostic groups: depressed (DEP) and healthy controls (HC). MEP amplitudes were measured peak-to-peak. MEPs with preceding voluntary EMG were excluded from analysis. Amplitudes for each block of 20 MEPs were averaged. A mean baseline value was calculated from the 2 baseline blocks. The mean MEP values for subsequent blocks were normalised by dividing by this baseline value. For each subject, an overall (normalised) post-PAS mean MEP amplitude (averaged across all post stimulation time points) was then calculated as the primary outcome of interest.

Changes in cortical excitability

To examine overall change in cortical excitability after PAS, a 2 x 2 mixed ANOVA was conducted with Group as the between-subjects factor (DEP or HC) and Time (Baseline, Post-PAS) as the repeated measures factor. To then examine the time course of change in cortical excitability after PAS, a mixed 2 x 8 ANOVA was conducted with Group (DEP or HC) as the between-subjects factor, and Time (Baseline, 0mins, 10mins, 20mins, 30mins, 40mins, 50mins, 60mins) as the repeated measures factor. Planned polynomial contrasts tested for changes across Time, while significant interactions were interpreted with tests of simple effects. As there are potential neurobiological differences between bipolar and unipolar depressed subjects, we conducted a further 2 x 2 mixed ANOVA comparing change in cortical excitability after PAS with Group as the between-subjects factor (unipolar depressed or matched controls) and Time (Baseline, Post-PAS) as the repeated measures factor.

Depression severity and episode duration

To determine whether severity and duration of depressive symptoms had an impact upon neuroplasticity, correlations between normalised mean post-PAS MEP amplitude, MADRS score, and duration of the current depressive episode were assessed in the depressed cohort.

Motor learning

The outcome measure for the motor learning task was the subject's TOT, which was collected for the five test blocks and one baseline block. The mean TOT values for test blocks were normalised to the baseline value by dividing TOT for each test block by the TOT in the baseline block. The mean of all five test blocks is considered a measure of motor learning (Rajji et al., 2011). A 2 x 2 mixed ANOVA was conducted with Group as the between-subjects factor (DEP or HC) and Trial Block (baseline, test) as the repeated measures factor. Simple effects were conducted after any significant interactions to interpret the changes.

Effects of disease state and BDNF genotype on neuroplasticity

BDNF genotype data were successfully obtained for 42 of 45 subjects tested. To examine whether the relationship between neuroplasticity (as measured by Post-PAS MEPs and motor learning) and disease state was influenced by an individual's BDNF genotype, we conducted a 2 x 2 ANOVA in subjects with genotype data (n=42), with Group (DEP, HC) and Genotype (Val/Val, Met carrier) as between-group factors, and mean post-PAS MEP amplitude as the outcome measure. In the event of a significant interaction, simple effects were conducted to interpret the differences.

Correlates of neuroplasticity

Correlational analyses were then performed to analyze the relationship between mean post-PAS MEP amplitude, and performance on the motor learning task, and with serum BDNF levels for the whole cohort, and for each diagnostic Group (HC, DEP) separately. This was to determine whether neuroplasticity after PAS correlated with a measure of functional significance (motor learning), and was related to BDNF levels, a known facilitator of cellular change. Pearson's correlation was used for these analyses.

3.4 Results

Between group (DEP, HC) comparison of demographic factors and baseline experimental measures demonstrated no significant difference apart from the expected greater MADRS scores (lower mood) in the depressed cohort (Table 3.1). In the depressed group, duration of the current episode was 27.9 ± 20.9 months, mean number of failed antidepressants was 2.61 ± 2.5 (current episode), and 4.17 ± 3.1 (lifetime). Seventeen subjects in the depressed group were on psychotropic medications: selective serotonin reuptake inhibitors (n=7), atypical antipsychotics (n=6), serotonin-noradrenaline reuptake inhibitors (n=5), lithium (n=4), anticonvulsants (n=2), tricyclic antidepressants (n=2), agomelatine (n=2), moclobemide (n=1). During the PAS protocol, depressed patients and healthy controls gave matching attention to the hand, as shown by detection of similar numbers of the stimuli delivered to the index finger at random times (Table 3.1).

Table 3.1 Subject Demographic and Experimental Characteristics.

		Group		
		Depressed	**Healthy Controls**	***t*** ***p***
Age (years)		38.0 (12.8)	38.5 (13.1)	t=-0.14, p=0.88
MADRS score		30.7 (3.4)	0.4 (0.7)	t=41.53, p<0.001*
Resting Motor Threshold (% stimulator output)		42.2 (7.9)	44.5 (9.9)	t=-0.85, p=0.40
Ulnar Nerve Stimulus Threshold (mA)		39.3 (8.5)	39.4 (7.7)	t=-0.05, p=0.96
Testing Stimulus Intensity (% stimulator output)		49.3 (10.0)	53.3 (13.1)	t=0.15, p=0.26
Mean Number of Random Sensory Stimuli Detected (measure of attention during PAS)		37.6 (7.1)	37.4 (5.1)	t=0.94, p=0.91
Motor Learning	Task Speed (rpm)	24.1 (2.2)	24.6 (1.8)	t=0.18, p=0.40
	Time on Target (learning- ratio)	1.4 (0.2)	1.7 (0.5)	t=-2.48, p=0.02*
BDNF Genotype	VAL/VAL	13/23	14/23	
	MET carrier	8/23	7/23	
	Undetermined	2/23	2/23	
	BDNF serum level (ng/mL)	19.4 (7.0)	17.9 (5.9)	t=0.66, p=0.51

BDNF, Brain-Derived Neurotrophic Factor; mA, milliampere; MADRS, Montgomery-Äsberg Depression Rating Scale; ng/mL, nanograms per millilitre; PAS, Paired Associative Stimulation; rpm, revolutions per minute.

Learning (test Time on Target/baseline Time on Target) is the mean test block Time on Target divided by mean baseline Time on Target.

*P<0.05.

Changes in cortical excitability

The 2 x 2 ANOVA showed a main effect of Group [$F(1, 44) = 11.244$, p=0.002], no significant effect of Time [$F(1, 44) = 3.997$, p=0.052] and a significant Group x Time interaction [$F(1, 44) = 11.244$, p=0.002]. Tests of simple effects found a

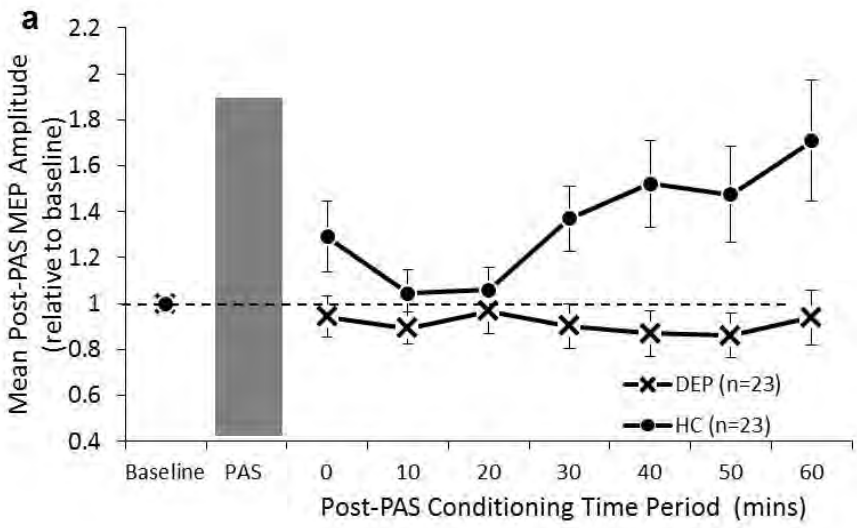
significant increase in the amplitude of MEPs after PAS for the HC group [$F(1, 44) = 14.32$, $p < 0.001$], while there was no change for the DEP group [$F(1, 44) = 0.92$, $p = 0.344$]. The 2 x 2 ANOVA comparing only subjects with unipolar depression and matched controls also showed a significant difference main effect of Group [$F(1, 38) = 7.748$, $p=0.008$], no significant effect of Time [$F(1, 38) = 3.654$, $p=0.063$] and a significant Group x Time interaction [$F(1, 38) = 7.748$, $p=0.008$]. MEP amplitude was significantly increased after PAS for the HC group [$F(1, 38) = 11.02$, $p = 0.002$] but not changed for the unipolar DEP group [$F(1, 38) = 0.38$, $p = 0.541$].

The 2 x 8 ANOVA showed a main effect of Group [$F(1, 44) = 11.244$, $p=0.002$], no significant effect of Time [$F(1, 44) = 3.640$, $p=0.063$] and a significant Group x Time interaction [$F(1, 44) = 6.089$, $p=0.018$; Figure 3.2a]. Simple effects analysis showed a linear increase in MEP amplitude in the healthy controls [$F(1, 44) = 5.44$, $p<0.001$], but no change in the depressed cohort [$F(1, 44) = 0.19$, $p=.987$].

As 17 of 23 subjects in the depressed cohort were on psychotropic medications (16 on antidepressant medications), a further one-way ANOVA compared mean post-PAS MEP amplitude between depressed subjects on medication (DEP-meds; 17), depressed subjects not on medication (DEP-no-meds; 6; all unipolar depression), and healthy controls (HC; 23). This ANOVA demonstrated a main effect of Group [$F(2, 43) = 6.446$, $p=0.004$]. Bonferroni-adjusted pair-wise analyses showed significant differences in means between healthy controls (1.35 ± 0.52) and medicated subjects (0.98 ± 0.39, $p = 0.01$), and healthy controls and unmedicated subjects (0.72 ± 0.09, $p = 0.035$), but no difference between the two depressed groups ($p = 0.675$; Figure 3.2b).

Depression severity and episode duration

In the depressed cohort, there was an inverse correlation at trend level between mean post-stimulation MEP amplitude and MADRS score (r=-0.364, *p*=0.088), but no association between mean post-PAS MEP amplitude and current episode duration (r=0.299, *p*=0.166).



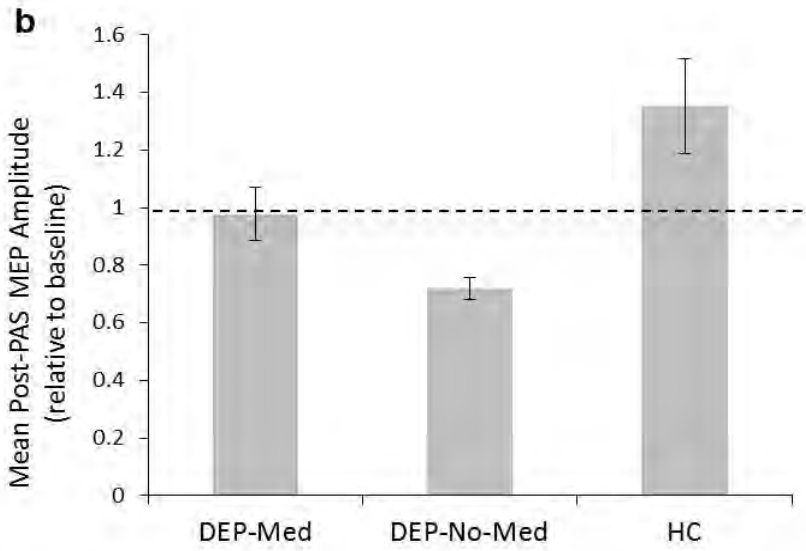


Figure 3.2 Time course comparison of post-PAS mean MEP amplitude for depressed and healthy controls, and group result compared across medication status

a. Group data (mean±SEM) showing the time course of changes in the amplitude of motor evoked potentials (MEPs) after conditioning stimulation (PAS) in depressed subjects (crosses) and healthy controls (filled circles). MEP amplitudes were normalised to baseline values for each subject. **b.** Mean normalised MEP amplitudes after PAS conditioning stimulation in depressed subjects on medication (DEP-med; n=17), depressed subjects without medication (DEP-no-med; n=6), and healthy controls (HC; n=23). The seven post-PAS time points shown in panel **(a)** are collapsed into single group values. Means and standard errors are shown.

Motor learning

A 2 x 2 ANOVA compared motor learning between groups. Two missing values (one DEP and one HC) were replaced with the Expectation Maximisation (Schafer and Olsen, 1998) function of the Statistical Package for the Social Sciences (SPSS) for Windows, Version 20.0 (IBM Software Group, IL, USA). Motor learning was comparatively impaired in the depressed cohort shown by a significant main effect of Group [$F(1, 44) = 5.582$, $p=0.023$], Time [$F(1, 44) = 85.583$, $p<0.001$] and Group x Time interaction [$F(1, 44) = 5.582$, $p=0.023$]. Simple effects analysis showed significant increases in motor learning in both healthy controls [$F(1, 44) = 67.44$, $p<.001$] and the depressed cohort [$F(1, 44) = 23.73$, $p<0.001$; Figure 3.3].

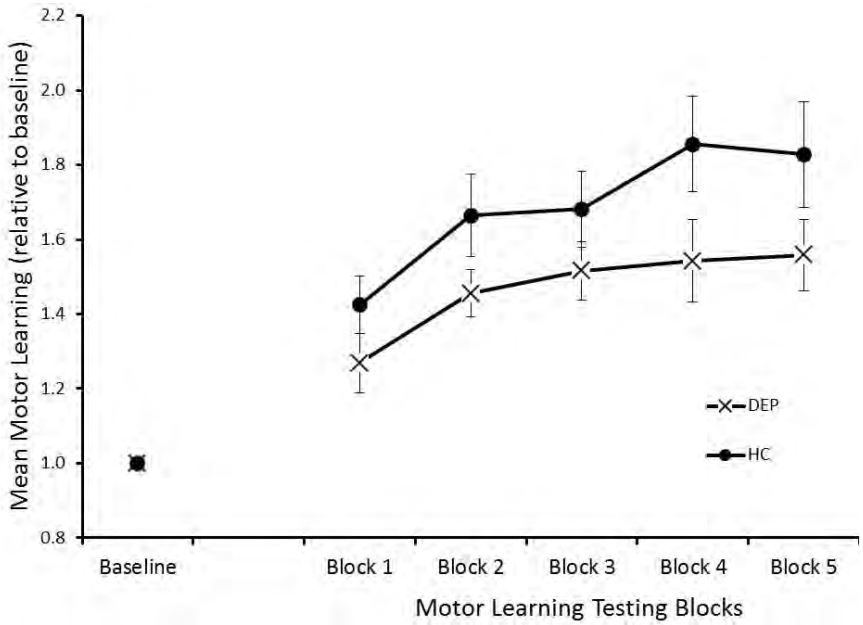


Figure 3.3 Comparison of normalised mean motor learning performance between healthy and depressed subjects

Comparison of time on target on the rotor pursuit task in the depressed cohort (DEP, crosses) and healthy controls (HC, filled circles). Time on target in each test block was normalised to the baseline block. Mean and standard errors are shown. Increasing time on target indicates motor learning.

Effects of disease state and BDNF genotype on neuroplasticity

BDNF genotype was obtained in 42 subjects (21 DEP, 21 HC). Neither the distribution of BDNF genotypes nor the BDNF serum levels differed between the groups. Furthermore, there was no difference in BDNF serum levels when subjects were divided by genotype (Val/Val vs. Met carrier, 18.4 ± 6.3 vs. 18.4 ± 7.9 ng/ml respectively). For the 2 x 2 ANOVA (factors Group, Genotype) with mean post-PAS MEP amplitude as the dependent variable, there was a main effect of Group with greater post-PAS MEP amplitude in healthy controls than in the depressed patients [$F(1, 38) = 8.295$, $p=0.006$], no significant effect of Genotype [$F(1, 38) = 3.718$, $p=0.061$], (though trend for higher mean post-PAS MEP amplitude in Met allele carriers) and no Group x Genotype interaction [$F(1, 38) = 0.178$, $p=0.676$].

Correlates of neuroplasticity

Mean post-PAS MEP amplitude did not correlate with motor learning (r=0.075, $p=0.674$, n=46), or BDNF serum levels (r=-0.007, p=0.967, n=34). However, when investigating each diagnostic group separately, there was a trend level correlation between mean post-PAS MEP amplitude and BDNF serum levels in the depressed group (r=0.410, $p=0.091$, n=18).

3.5 Discussion

This study clearly demonstrated a significant deficit of neuroplasticity in depressed subjects compared with age and gender matched healthy controls. The finding is significant as one of the first objective demonstrations of impaired neuroplasticity in depression. In this study a physiological measure of plasticity not confounded by factors such as subjective rating and analysis, subject education levels,

practice effects or motivational factors was used, conferring clear advantages compared with cognitive or other behavioural tests previously used to assess plasticity in depression (Landrø et al., 2001, Porter et al., 2003).

In the brain stimulation protocol used, ulnar nerve stimuli were repeatedly delivered 25 ms before TMS so that each pair of stimuli arrived at the cortex at nearly the same time. Such PAS protocols result in prolonged facilitation of the excitability of the motor cortex as measured by the amplitude of MEPs elicited by TMS (Stefan et al., 2004, Chapter 2). Changes in cortical excitability after facilitatory PAS are thought to reflect associative LTP (Stefan et al., 2002). Thus, increases in synaptic strength are considered to underlie the increases in MEP amplitude (Thickbroom et al., 2006). While there is limited and indirect knowledge of the processes underlying associative LTP in humans, it is believed to involve glutamate signaling, and postsynaptic cell depolarisation through N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methylisoxazole propionate (AMPA) receptor activation (Pittenger and Duman, 2008) which are each enhanced by BDNF (Carlson et al., 2006). Glutamate acts synergistically with BDNF and tyrosine-related kinase B (TrkB) signaling to increase NMDA activity and AMPA receptor expression (Yoshii and Constantine-Paton, 2010) which both result in greater intracellular calcium influx. The level of postsynaptic calcium influx determines the induction of synaptic LTP, or its opposite, long-term depression (LTD). Fast increases in intracellular calcium inflows lead to LTP of the synapse (Wankerl et al., 2010). In our current study, MEP amplitudes were increased after PAS only in healthy controls, suggesting that the mechanisms underlying LTP induction were impaired in the motor cortex of those with depression.

This novel finding of no change in MEP amplitude after a stimulation protocol known to produce facilitatory motor cortical changes supports earlier reports of

impaired neuroplasticity in depression. Dysfunctional neuroplasticity has been implicated in depression-related cognitive and behavioural changes such as impaired learning, memory and inability to respond appropriately to stress or aversive stimuli (Pittenger and Duman, 2008). However, only one prior study has examined neuroplasticity in depression using an objective neurological test (Normann et al., 2007). This study used changes in visual evoked potentials (VEPs) in response to repeated visual stimuli as a means of examining changes in synaptic plasticity. Such plasticity is proposed to share common features with LTP shown in brain slices, including reliance on NMDA receptor activation. Researchers reported significant increases in VEPs in subjects after a train of patterned visual stimuli. However, increases in VEP amplitude were significantly smaller in depressed subjects compared to healthy controls (Normann et al., 2007). Impairment of synaptic plasticity in depression has also been proposed to occur in other telencephalic regions such as the hippocampus. This is evidenced by impairment in declarative memory consolidation (Porter et al., 2003). Together, these deficits in motor cortex, sensory cortex and hippocampus suggest a deficiency in facilitatory synaptic plasticity that is widespread, and not merely in limbic and frontal regions traditionally considered central to depression.

Various mechanisms may underlie alterations in synaptic plasticity in depression. For example, the activation of synaptically localised NMDA receptors stimulates LTP, whereas excessive glutamatergic activation of extrasynaptic NMDA receptors instead may induce LTD (Papouin et al., 2012). In the current study, MEPs were reduced by over 20% after PAS in approximately half of the depressed cohort, which may indicate an increased tendency for LTD in some depressed subjects. Reduction in LTP can occur through dysregulated glutamate at the synapse as a result of

a number of factors. For example, glial cells not only provide metabolic support to sustain the neuron, but also affect synaptic plasticity through a crucial role in the clearance and reuptake of neurotransmitters such as glutamate (Citri and Malenka, 2008). Glial cell numbers and functioning are negatively affected by chronic stress (Rajkowska et al., 1999, Pittenger and Duman, 2008), which is commonly associated with depression. Furthermore, stress also impairs LTP and facilitates LTD through increased glucocorticoid exposure, shown in animal models of depression. These alterations in synaptic plasticity may differentially affect specific brain regions, with associated functional implications (Pittenger and Duman, 2008).

Additionally, changes in cortical plasticity require balanced excitatory and inhibitory neuronal activity (Yazaki-Sugiyama et al., 2009). Thus, weak inhibition, as may be found in depression, can prevent cortical plasticity (Fagiolini et al., 2004). In depression, *in-vivo* magnetic resonance spectroscopy studies have found reduced cortical gamma-aminobutyric acid (GABA) concentrations (Sanacora et al., 1999, Price et al., 2009) with deficits in GABA receptor-mediated inhibition (Levinson et al., 2010). Further, there is evidence that the rate-limiting enzyme for GABA synthesis (GAD67) is reduced in the cortex of individuals with bipolar disorder (Thompson et al., 2009) and hippocampus of depressed individuals (Thompson Ray et al., 2011). While robust changes in cortical GAD67 mRNA are not typically found in the PFC of individuals with depression (Thompson et al., 2009, Sibille et al., 2011), significant decreases in somatostatin suggest that interneuron deficits may be especially prominent in a subset of interneurons shown to directly contribute to cortical plasticity (Lazarus and Huang, 2011). Since inhibitory interneuron deficits are shared among those with depression, bipolar disorder and schizophrenia (Daskalakis et al., 2002, Hashimoto et al., 2008,

Thompson et al., 2009, Fung et al., 2010, Thompson Ray et al., 2011), cortical plasticity deficits would also be expected to be found in multiple forms of psychopathology.

Learning is thought to rely on neuroplastic processes. A motor learning task was included in this study as a functional test of neuroplasticity. While both healthy and depressed subjects showed significant improvement in the rotor pursuit task, motor learning was comparatively impaired in depressed subjects. The lack of correlation between motor learning and PAS test results may be considered surprising as motor learning is considered at least partially reliant on LTP in the primary motor cortex (Ziemann et al., 2004). However, very few studies have found a significant relationship between motor learning outcomes and results of brain stimulation tests of motor cortex plasticity. Thus, the impact of altered plasticity, as measured by MEP changes, on functional motor performance is unclear. There are several possible explanations for the lack of relationship. There may be only partial overlap between the cortical processes moderated by brain stimulation and those involved in motor learning protocols (Li Voti et al., 2011). For example, performance of the rotor pursuit task used in this study also involves visual and subcortical striatal systems (Rajji et al., 2011). Functional motor learning tasks also rely on subject motivation and effort, as well as strategy and planning specific to the task. Thus poorer learning in the depressed subjects may have reflected impairment of these higher order functions, whereas the PAS protocol directly assessed motor and sensory cortical function independent of subject effort.

BDNF mediates changes in synaptic plasticity, and can further influence structural changes that can be found within minutes of LTP-inducing stimulation of synapses in rat brain slices (Tanaka et al., 2008). While decreased BDNF serum levels are reported in MDD, we found no differences in BDNF serum levels in depressed subjects compared to healthy controls. However, 16 of the depressed subjects were

taking antidepressant medications which have been shown to increase serum concentrations of BDNF (Sen et al., 2008). Similarly, no relationship was seen between synaptic plasticity (as assessed by PAS) and serum BDNF levels in the whole study sample. In terms of BDNF genotype, we did not confirm a prior report that Val/Val homozygotes demonstrated greater MEP amplitude after PAS (Cheeran et al., 2008).

There are three important limitations to this study. First, the majority of depressed subjects were on antidepressant medications. This is important because there is some experimental evidence that antidepressant medications may affect neuroplasticity, though these studies found they tended to enhance rather than reduce plasticity (Rocher et al., 2004, Normann et al., 2007). Further analysis of medicated and non-medicated depressed subjects found no difference in MEP changes after PAS between these groups, though both differed significantly from healthy controls. However, as the number of subjects not on psychotropic medications was small, this study could not resolve the degree to which the presence of medications may have influenced results. Inspection of the results suggests that the presence of antidepressant medications increased the variability in neuroplasticity measured in depressed subjects (see Figure 3.2b) which may have confounded exploration of secondary outcomes, i.e. the relationship between neuroplasticity, motor learning and BDNF levels.

A second limitation is that neuroplasticity was measured only once in the depressed cohort and this was while they were symptomatic. A second measurement during remission would enable us to evaluate if impaired neuroplasticity is a state or trait phenomenon and may assist in elucidating the underlying mechanisms. The suggestion that impaired neuroplasticity is a state phenomenon is supported by improvements in indirect measures of neuroplasticity, such as learning and memory,

while patients are in remission (Gallagher et al., 2007) and also after antidepressant treatment in humans and in animal models of depression (Pittenger and Duman, 2008).

A third limitation is that neuroplasticity was assessed in the motor cortex, which is not considered the primary site of cerebral dysfunction in depression. This study used the PAS protocol as it provides an accessible physiological measure of plasticity that is not confounded by factors such as subject motivation, or prior learning and experience. Moreover, it supports other studies which have found abnormalities in motor cortical function in depressed subjects (Shajahan et al., 1999a, Loo et al., 2008, Levinson et al., 2010). It is possible that motor cortical abnormalities may reflect global pathophysiological disturbances in depression.

Conclusion

In conclusion, this is one of the first studies to demonstrate reduced neuroplasticity in depression, using a test of experimentally induced plasticity. Findings also suggested that motor learning is impaired.

Chapter 4: Increase in Neuroplasticity after a Treatment Course of Transcranial Direct Current Stimulation for Depression

4.1 Abstract

Several lines of evidence suggest that neuroplasticity is impaired in depression and improves with effective treatment. This study aimed to measure neuroplasticity in depressed subjects before and after a course of anodal transcranial direct current stimulation (tDCS), given as treatment for depression. Neuroplasticity was assessed in the motor cortex using paired associative stimulation (PAS), which induces short term neuroplastic changes. The relationships between PAS results, mood state, brain-derived neurotrophic factor (BDNF) serum levels and BDNF genotype were examined.

In 18 depressed subjects, neuroplasticity (PAS-induced change) was increased after a course of tDCS ($t(17) = -2.651$, $p=0.017$). Treatment with tDCS also led to significant mood improvement, but unexpectedly, this did not correlate with improved neuroplasticity. Serum BDNF levels did not change after tDCS, or correlate with change in neuroplasticity achieved after tDCS. BDNF genotype did not predict mood or neuroplasticity results. This study demonstrated improvement of neuroplasticity after effective treatment for depression using a test (PAS) which is independent of subject effort.

4.2 Introduction

Major depressive disorder (MDD) is a highly disabling illness affecting near 300 million people worldwide (Vos et al., 2013). While there is incomplete understanding of the mechanisms underlying MDD, neurobiological changes are considered fundamental to depression symptomology (Duman, 2009).

It has been hypothesised that some symptoms of depression such as poor learning and memory may be the result of impaired neuroplasticity (Pittenger and Duman, 2008). Neuroplasticity refers to adaptive changes in neuronal structure and function with experience. Two critical physiological processes underlying neuroplasticity are long-term potentiation (LTP) and long-term depression (LTD). LTP and LTD rely on molecular and cellular mechanisms to increase and decrease, respectively, the strength of synaptic connectivity (Roy et al., 2007). These changes affect neural function and thus, are fundamental to healthy cognitive and behavioural performance (Letzkus et al., 2007, Sweatt, 2008). Further, recovery of neuroplastic mechanisms may be responsible for improvements in neurocognitive function after depression treatments (Vythilingam et al., 2004, Bhagya et al., 2011, Wagner et al., 2012). The depressed state is also accompanied by lower levels of neurotrophins such as brain derived neurotrophic factor (BDNF), which normalise with treatment and recovery (Brunoni et al., 2008). Neurotrophins provide neurons with trophic support, and have been implicated in both the development and resolution of depression (Duman and Monteggia, 2006). BDNF is linked to neuroplasticity through its effects on LTP (Egan et al., 2003, Fritsch et al., 2010). The above lines of evidence suggest that neuroplasticity is impaired in the depressed state, and normalises with treatment for depression.

Until recently, the evidence for impaired neuroplasticity in depression has been mostly indirect, i.e. inferred from impaired learning and memory (Merriam et al., 1999, Landrø et al., 2001, Naismith et al., 2006). However, two studies have provided direct evidence of dysfunctional neuroplastic mechanisms in depressed cohorts. First, reduced visual evoked potentials after repetitive visual stimuli were demonstrated in patients with MDD (Normann et al., 2007). Second, our group demonstrated that individuals with depression showed a significantly reduced response to a facilitatory brain stimulation protocol – paired associative stimulation (PAS) (Chapter 3). In healthy controls, the output of the motor cortex to external stimuli is enhanced after a brief period of conditioning stimulation with PAS (Stefan et al., 2000; Chapter 2). This enhancement was significantly reduced in depressed subjects (Chapter 3). The degree of neural potentiation after PAS can be assessed through changes in the size of motor responses (motor evoked potentials, MEPs) induced in a hand muscle by single pulse transcranial magnetic stimulation (TMS) to the motor cortex. Increases in the size of MEPs after PAS are thought to rely on LTP of synapses in the sensorimotor cortex (Stefan et al., 2000, Pellicciari et al., 2009). Thus, the ability of the motor cortex to respond to PAS may be a useful marker of cortical neuroplasticity in depression.

Recently, transcranial direct current stimulation (tDCS) – a non-invasive form of brain stimulation – has been demonstrated to have therapeutic effects in alleviating depression (Kalu et al., 2012, Loo et al., 2012, Brunoni et al., 2013). TDCS has also led to demonstrated neuroplastic benefits, enhancing cognitive functioning in healthy and depressed individuals and in some individuals with schizophrenia (Nitsche et al., 2008, Vercammen et al., 2011, Javadi and Walsh, 2012, Kuo and Nitsche, 2012, Demirtas-Tatlidede et al., 2013, Oliveira et al., 2013). Additionally, tDCS-induced improvements in motor function have been reported following a stroke (Boggio et al., 2007b), through

either up-regulating excitability in the lesioned motor cortex or down-regulating excitability in the contralateral region (Fregni and Pascual-Leone, 2007). Therapeutic applications of tDCS are based on the principle that repeated sessions induce cumulative and lasting changes in neuronal function (Boggio et al., 2007b, Reis et al., 2009, Alonzo et al., 2012, Galvez et al., 2013). Studies suggest these changes are mediated through both membrane and synaptic mechanisms (Liebetanz et al., 2002, Nitsche et al., 2003, Arul-Anandam and Loo, 2009).

Presently it is unknown whether neuroplasticity is related to mood state in subjects suffering depression or is reflective of ongoing trait dysfunction. Apart from evidence of enhanced learning and memory, and increase in BDNF levels, after successful treatment of depression, there has been no objective demonstration of improvement in neuroplasticity with resolution of depression. This study aimed to assess neuroplasticity in depressed subjects before and after a treatment course of tDCS. Depression severity (rated using the Montgomery-Äsberg Depression Rating Scale, MADRS) (Montgomery and Asberg, 1979), neuroplasticity (measured with the PAS protocol) and BDNF levels were measured before and after tDCS treatment. We hypothesised that improvement in mood after tDCS treatment would be accompanied by increases in neuroplasticity and serum BDNF. BDNF genotype (Val66Met polymorphism) was also analysed as it has been shown to influence BDNF secretion and neuroplastic potential (Egan et al., 2003).

4.3 Methods

Subjects

The sample consisted of 18 depressed subjects (11 male, 7 female) who received tDCS treatment in one of several clinical trials. All subjects met DSM-IV criteria for a Major Depressive Episode (17 with MDD, 1 with Bipolar Disorder II), assessed using the Structured Clinical Interview for DSM Disorders IV (SCID) (First et al., 2002). Subjects were right handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971) and participated after providing written, informed consent. The study was approved by the Human Research Ethics Committee of the University of New South Wales in accordance with the Helsinki Declaration.

Current depression severity was assessed using the MADRS. Criteria for inclusion was a MADRS score of 20 or greater, and absence of illicit drug use, alcohol abuse, musculoskeletal or neurological disorder, and electronic implants.

Research Plan

Mood ratings, neuroplasticity assessments, blood sampling, and a test of motor learning, were performed at baseline (Time 1: T1) and after tDCS treatment (Time 2: T2; See Figure 4.1). Six subjects received a course of sham tDCS prior to active tDCS and were also tested before (Time 0, T0) and after (T1) sham tDCS (see Figure 4.1). On each testing occasion (T1, T2), blood was taken in the morning for BDNF serum levels and genotyping (n=14), the subjects then completed a motor learning task, and underwent the PAS protocol (about midday). If blood tests were refused, DNA was collected (with consent) by buccal swab (n=3) or spit tube (n=1). As nine subjects were included from an earlier study which assessed neuroplasticity (prior to tDCS treatment) using a computerised version of the rotor pursuit task prior to the PAS protocol

(Chapter 3), all additional subjects in the present study performed the same rotor pursuit task before each PAS testing session to maintain consistency. However due to likely practice effects from repeat testing with the rotor pursuit task, these results were not analysed. The above tests were done 1-3 days prior to the first tDCS session and 1-3 days after the final tDCS session (Figure 4.1). Pre-treatment neuroplasticity data (PAS, BDNF genotype and serum levels) from nine subjects (Chapter 3), and mood results for one subject (from the trial of Loo et al., 2012) have been previously reported.

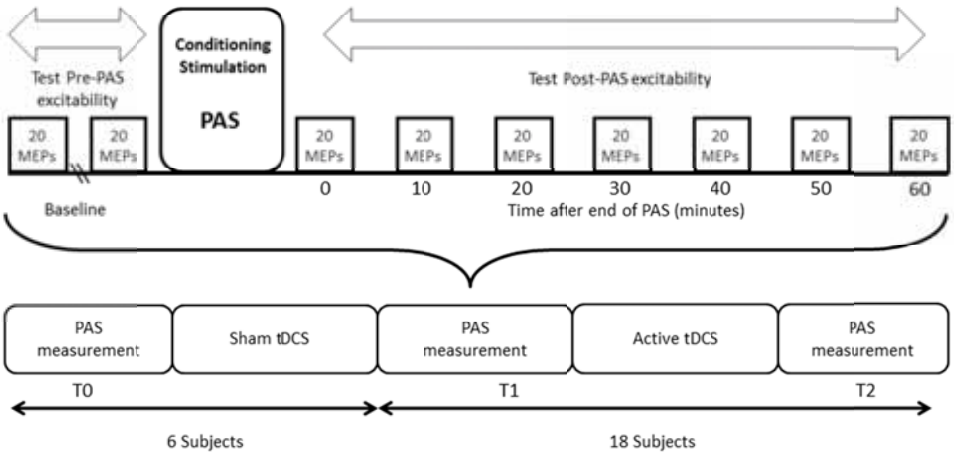


Figure 4.1 Chapter 4 experimental design

PAS sessions were conducted before and after a treatment course of transcranial direct current stimulation (tDCS). During PAS, motor evoked potentials (MEPs) were collected to measure motor cortex excitability before and after the period of conditioning stimulation with PAS.

Paired Associative Stimulation (PAS)

During the brain stimulation protocol, subjects were seated with both hands lying comfortably on a pillow in their lap. Electromyographic activity (EMG) was recorded through (Ag/AgCl) surface electrodes placed over the belly and tendon of the right first dorsal interosseous (FDI) muscle. EMG signals were amplified (x1000) using a 1902 amplifier (Cambridge Electronics Design, Cambridge, UK), filtered (16-1000Hz) and sampled at 2000Hz (1401, version 4.02, Cambridge Electronics Design, Cambridge, UK).

Motor evoked potentials (MEPs) were elicited by single-pulse TMS (Magstim 200 stimulator, Magstim Co., Whitland, UK) using a figure-of-eight coil with 7cm diameter wings which was placed tangentially over the left motor cortex with the handle pointing postero-laterally. The optimal site for eliciting MEPs in the right FDI muscle was established empirically at the beginning of each testing session and marked on the scalp. Resting motor threshold (RMT) was identified as the minimum stimulus intensity required to evoke a motor response of at least 50 μV in the relaxed FDI in five of ten consecutive trials. The intensity of TMS for evoking test MEPs was selected to elicit a response of around 1mV in the relaxed FDI, but did not exceed 130% RMT.

The PAS protocol was used as previously described (Chapter 2). Briefly, single-pulse TMS (at 130% RMT) to the left motor cortex was combined with electrical stimuli (200μs duration, 300% perceptual threshold) which were delivered to the right ulnar nerve proximal to the wrist using a constant-current stimulator (DS7 stimulator, Digitimer Co. Ltd., Hertfordshire, UK). Two hundred pairs of stimuli (TMS and ulnar nerve) were given at 0.25Hz over ~13 min. In each pair, ulnar nerve stimulation preceded the TMS pulse by 25ms (Ziemann et al., 2004). To ensure attention to the

hand, subjects counted occasional electrical stimuli delivered to the digital nerves of the right index finger at random intervals during the PAS protocol.

Test MEPs were recorded before and after PAS. Each testing block consisted of 20 MEPs at 0.1 Hz. Two blocks of MEPs were recorded at baseline. A block of MEPs was recorded immediately after the PAS protocol and then every ten minutes for one hour (see Figure 4.1). During all stimulation, subject EMG was monitored to ensure adequate muscle relaxation.

Transcranial Direct Current Stimulation

Subjects received a course of anodal tDCS in one of several clinical trials, under open-label conditions (N=10) or under double-blind, sham-controlled conditions (n=8). TDCS treatments were given on consecutive weekdays with an Eldith DC-stimulator (NeuroConn GmbH, Germany) (15 subjects), or a Soterix DC-stimulator (Soterix Medical Inc. 160 Convent Ave New York, NY, 10031) (3 subjects). The number of tDCS sessions received ranged from 13-21 (*M*: 19.1, *SD*: 2.2), depending on the trial protocol and subject compliance. All subjects received anodal tDCS to the left prefrontal cortex [PFC-specifically-F3 (international 10/20 EEG system)]. Eleven subjects received bifrontal tDCS and five subjects received fronto-extracephalic tDCS, with electrodes placed as previously described (Martin et al., 2011). The other two subjects received tDCS with a 5x7 cm anode at pF1 (international 10/20 EEG system) and a 10x10 cm cathode centred over the occipital lobe (POz) or the cerebellum (Oz). TDCS was given at 2-2.5 milliamperes (mA) for 20-30 minutes.

Mood Assessment

Subject mood was assessed using the MADRS (Montgomery and Asberg, 1979), administered by a trained rater at T1 and T2 (n=18). Raters were aware that subjects received active tDCS during open label phases, but were blinded to treatment assignment during the sham-controlled study periods.

BDNF Genotyping and Serum Levels

Serum samples were collected from subjects between 10am and 11am and allowed to clot at room temperature in Serum-Separator tubes for half an hour (hr), before being centrifuged (2000g, 5 minutes; mins), aliquoted and stored at -80°C. Serum BDNF levels were measured by sandwich enzyme-linked immunosorbent assay using the Promega E_{max} kit (G7610). The plates were first coated with monoclonal BDNF antibody t [1:1000 dilution in carbonate coating buffer (pH 9.7) and incubated overnight at +4°C]. The plate was washed with Tris buffered saline (TBST, pH 7.6), and the nonspecific binding was blocked in Block & Sample Buffer (BSB) at room temperature (RT) for 1 hr. Samples were vortexed thoroughly upon thawing and spun down to remove particulates. Serum underwent a 2-step dilution and was used at 1:250. Samples were all randomised and coded and processed in duplicate within the same plate. The plate was incubated with anti-human BDNF polyclonal Ab (1:500 dilution) for an additional 2 hrs at RT while shaking. This was followed by anti-IgY HRP conjugate (1:200 dilution) for 1 hr at RT with shaking. TMB One Solution was then

added to each well for 10 min with 1M hydrochloric acid to stop the reaction. Color intensity indicating BDNF protein level was detected by a plate reader at 450nm absorbance (Fluostar Optima, BMG Labtech).

Genomic DNA was isolated from 8mL whole blood collected in EDTA tubes using a PUREGENE DNA purification kit (QIAGEN). The BDNF SNP rs6265 (val/met) polymorphism was assayed by TaqMan Allele discrimination assay (Cat # C_11592758_10, Life Technologies Inc.) analyzed with Sequence Detection Software (SDS) version 2.3 (ABI, Life Technologies, Inc.).

Data Analysis

Cortical excitability

Peak-to-peak amplitudes of individual MEPs were measured. Any MEPs with voluntary EMG activity in the 50 ms preceding the stimulus were excluded from analysis. Amplitudes for each block of 20 MEPs were averaged. For each subject, for each session, a mean baseline value was calculated from the two baseline blocks. The mean MEP amplitudes for subsequent blocks were normalised to this baseline value. Mean MEP values from all post-PAS blocks were then averaged to derive a single overall, normalised, mean post-PAS MEP amplitude for each session. PAS testing parameters at the T1 and T2 testing occasions were compared using paired-sample t-tests (Table 4.1).

To examine overall change in the effect of PAS on cortical excitability, a paired sample t-test was conducted to determine changes between normalised (overall) mean post-PAS MEP amplitude at the T1 (PAS_T1), and the T2 (PAS_T2) time points.

Mood assessment

Subject mood was recorded by clinician MADRS rating. To examine overall change in mood, a paired sample t-test was used to determine differences in MADRS scores between the T1 (MADRS_T1), and T2 (MADRS_T2) time points.

BDNF serum levels

In addition, BDNF serum levels were collected at T1 (BDNF_T1), and T2 (BDNF_T2) in 14 subjects, which allowed determination of any differences in BDNF serum levels scores between the T1 (BDNF_T1), and T2 (BDNF_T2) time points via a paired sample t-test.

Correlations

Correlational analyses were performed to analyse relationships between the percentage change in PAS-induced plasticity from T1 to T2 with the percentage change in MADRS, and serum BDNF over the same time period, the number of tDCS sessions, medication status (i.e. psychotropic vs no psychotropic meds), and BDNF genotype (Val/Val vs Met carriers). Pearson's correlation was used for these analyses. In addition, effect sizes (Cohen's *d)* were used for the comparison with binomial variables.

4.4 Results

Comparison between testing occasions demonstrated no significant changes in experimental test parameters, including resting motor threshold, between T0 and T1, and between T1 and T2. Comparison of experimental characteristics for the PAS_T1 and PAS_T2 with 18 subjects is shown in Table 4.1 Thirteen subjects were receiving psychotropic medications, with doses unchanged for at least 4 weeks prior to, and throughout, the study period. All thirteen were on antidepressants: SNRI (5), SSRI (1),

tricyclics (4), agomelatine (2), mirtazapine (1), tranylcypromine (1). One subject was on combined amitriptyline and agomelatine. Five were also on lithium for augmentation and four were on small adjunctive doses of atypical antipsychotics.

Table 4.1 Subject Experimental Characteristics For PAS Sessions With 18 Subjects.

	Testing Time Point		
	T1	**T2**	***t*** ***p***
Resting Motor Threshold (% stimulator output)	45.7 (6.6)	46.4 (8.5)	t=-0.92, p=0.37
TMS Test Stimulus Intensity (% stimulator output)	53.3 (8.4)	53.4 (9.2)	t=-0.92, p=0.84
Ulnar Nerve Stimulus Threshold (mA)	38.1 (7.7)	40.7 (10.4)	t=-1.96, p=0.07
Mean Number of Random Sensory Stimuli Detected (measure of attention during PAS)	38.6 (3.1)	38.8 (2.5)	t=-0.31, p=0.76

Key: mA, milliampere; PAS, Paired Associative Stimulation.

Cortical excitability

After sham tDCS, there was no increase in the effect of PAS on MEP amplitude. PAS_T1 (M: .983, S.D: .322) was not significantly different to PAS_T0 (M: .907, S.D: .258) [t(5) = -0.489, p=0.646] (see figure 4.2)

After treatment with tDCS, there was an increase in the effect of PAS on MEP amplitude. A paired sample t-test demonstrated that PAS_T2 was significantly greater than PAS_T1 [t(17) = -2.651, p=0.017; See Figure 4.2]. At T2, thirteen subjects showed an increase in MEP amplitude above baseline in response to PAS whereas eight subjects showed such an increase at T1.

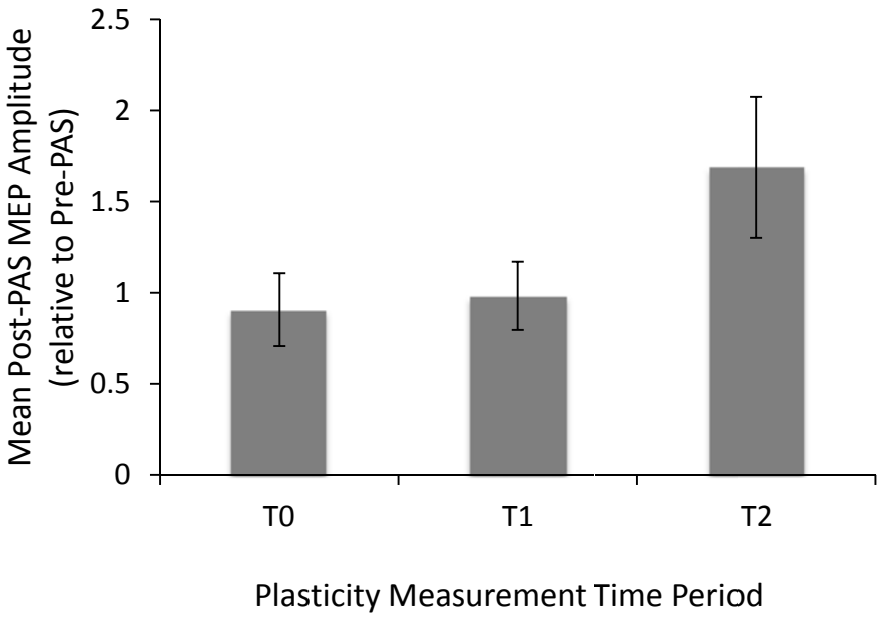


Figure 4.2 Time course comparison of post-PAS mean MEP amplitudes in six subjects before and after sham and active tDCS treatment.

Overall mean normalised post-PAS MEP amplitudes after conditioning stimulation in six depressed subjects shown before sham tDCS (T0), after sham tDCS (T1) and after active tDCS (T2). The 7 post-PAS time points collected are collapsed into a single value. Means and standard errors are shown.

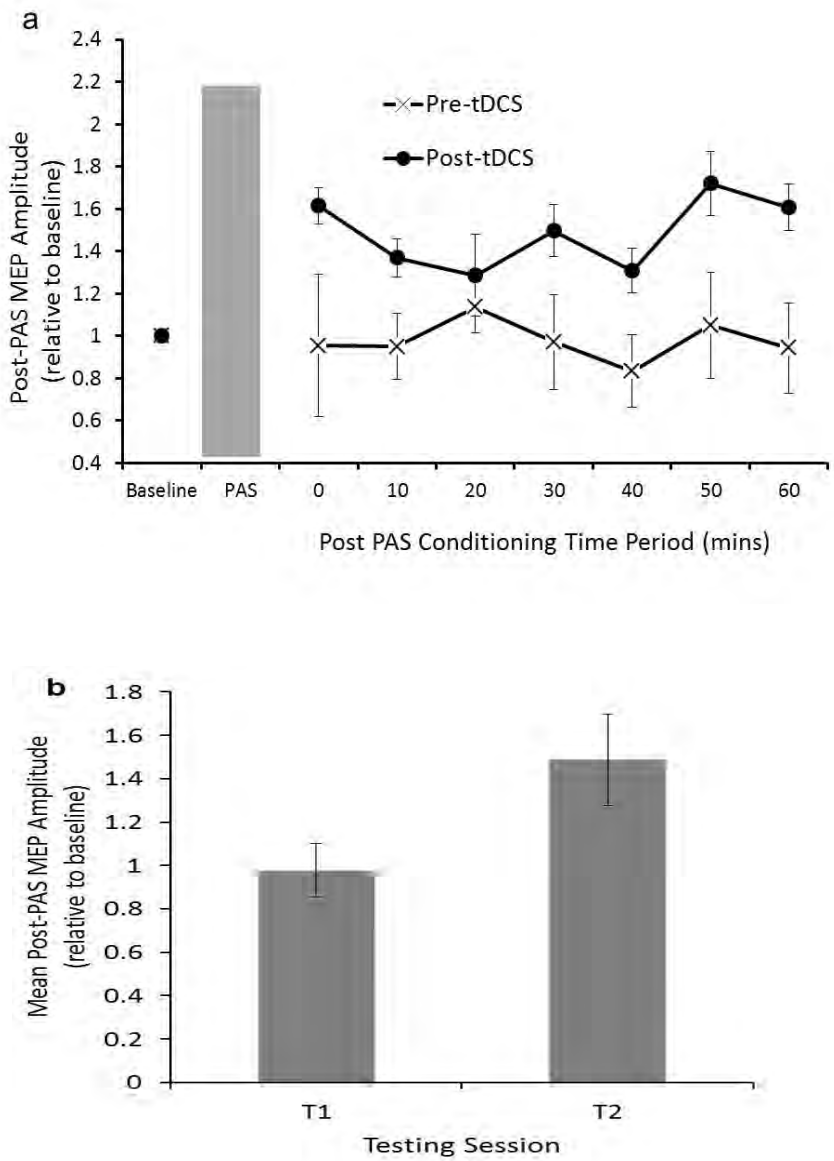


Figure 4.3 Time course comparison of post-PAS mean MEP amplitudes before and after tDCS treatment, and single mean pre and post tDCS group comparison

a. Group data (mean ± SEM) showing the time course of changes in the amplitude of motor evoked potentials (MEPs) after conditioning stimulation (PAS) in depressed subjects before tDCS treatment (cross) and after tDCS treatment (filled circle). MEP amplitudes were normalised to baseline values for each subject. **b.** Overall mean normalised post-PAS MEP amplitudes after conditioning stimulation in depressed subjects before tDCS (T1) and after tDCS (T2). The 7 post-PAS time points shown in **(a)** are collapsed into a single value. Means and standard errors are shown.

Mood assessment

A paired sample t-test also showed a significant decrease in MADRS scores, reflecting mood improvement after tDCS; MADRS_T2 (*M*: 21.00, *SD*: 8.83) vs MADRS_T1 (*M*: 29.78, *SD*: 4.71), $t(17) = 4.366$, $p < 0.001$].

BDNF Serum Levels and Genotype

A paired samples t-test showed no change in BDNF serum levels between BDNF_T1 (*M*: 13.73, *SD*: 7.00) and BDNF_T2 (*M*: 13.52, *SD*: 7.64), $t(13) = 0.094$, $p = 0.926$. BDNF genotype data were successfully obtained for 16 of 18 subjects, with 11 Val homozygotes and 5 Met carriers in the sample tested.

Correlations

Percentage change in plasticity between T1 and T2, did not correlate with percentage changes in MADRS (r=-0.385, p=0.115, n=18), or percentage change in serum BDNF levels (r=-0.235, p=0.419, n=14) over the same period; nor was there a significant relationship with medication status (r=0.128, p=0.614, n=18; Cohen's *d=0.298*), or BDNF genotype (r=0.180, p=0.504, n=16; Cohen's *d=0.330*). However, a relationship at trend level was found between the change in plasticity and the number of tDCS sessions, (r=0.454, p=0.058, n=18) (see Figure 4.3).

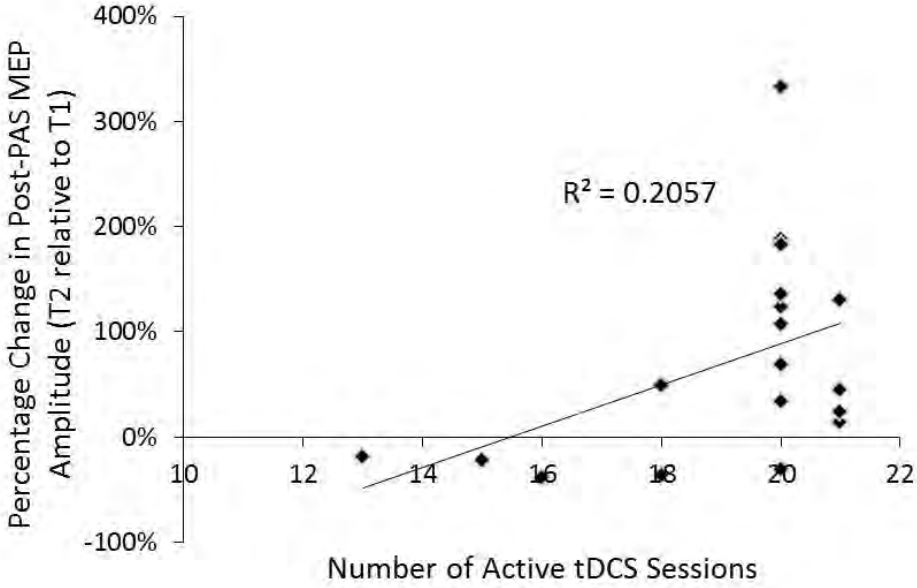


Figure 4.4 Comparison of the percentage change mean post-PAS MEP amplitude from pre tDCS to after tDCS with number of tDCS sessions in depressed subjects

Comparison of the percentage change in overall mean normalised post-PAS MEP amplitudes from pre tDCS (T1) to after tDCS (T2) with the number of tDCS sessions in depressed subjects.

Five of 18 subjects met the criteria for clinical response which was a 50% or greater reduction in MADRS score. After active tDCS, these 5 responders showed a mean normalised PAS response (M: 1.232, SD: 0.51). There were 13 non-responders showed a mean normalised PAS response (M: 1.59, SD: 0.77). There was no significant difference between these groups. Three of the five responders had an increase in their normalised PAS response. Additionally, there was 1 remitter (MADRS score <10) – mean normalised PAS response (M: 0.79) and 17 non-remitters – mean normalised PAS response (M: 1.52, SD: 0.714).

4.5 Discussion

This is the first study to demonstrate a significant improvement in cortical plasticity following a treatment course of tDCS in depressed subjects, using an objective test that is independent of subject effort. Treatment with tDCS also led to improvement in mood, though contrary to expectations, there was no obvious relationship between improvement in depression scores and increase in our measure of cortical neuroplasticity following treatment.

In this study, the ability of PAS to potentiate a motor response was used to test the neuroplasticity of subjects. This technique pairs electrical nerve stimulation and TMS so that these inputs interact at the cortical level. PAS can either facilitate or depress motor cortical excitability depending on the precise timing of stimuli. In the form of PAS used here, the sensory volley from nerve stimulation arrives at the motor cortex almost simultaneously with the TMS pulse and this is known to be facilitatory (Stefan et al., 2000; Chapter 2). That is, this form of PAS increases MEP amplitude, which is believed to reflect associative LTP (Roy et al., 2007). The process of LTP relies on activation of NMDA receptors to increase postsynaptic calcium levels (Sweatt, 2008) and induce activity-dependent cellular mechanisms that increase synaptic efficacy (Pittenger and Duman, 2008). Hence, LTP increases synaptic strength and results in enhanced motor cortical responses to single-pulse TMS (Roy et al., 2007). Thus, the increase in MEPs after PAS provides a measure of neuroplasticity (Stefan et al., 2000, Stefan et al., 2002).

In prior studies, we found that the PAS protocol used here reliably enhanced MEP amplitude for groups of healthy subjects, but not for subjects with depression. Over two thirds of healthy individuals showed enhanced MEP amplitude after PAS

(12/16, Chapter 2; 16/23, Chapter 3) in contrast to nine of twenty-three depressed subjects (Chapter 3), suggesting that neuroplastic processes were impaired in the depressed state in the majority of subjects. Similarly, in the current study, prior to tDCS, only eight of eighteen depressed subjects showed an increase in MEP amplitude after PAS, and there was no significant increase in motor cortical excitability overall for the group. Importantly, after a course of tDCS, the majority (thirteen of eighteen) of depressed subjects now showed increases in MEP amplitude after PAS. This proportion of positive response to PAS in depressed individuals treated with tDCS is comparable to that previously found in healthy subjects. Further, repeat testing after tDCS treatment now showed a significant increase in MEP amplitudes after PAS for the group as a whole. As the PAS sessions were separated by approximately a month, it is unlikely that the change in response to PAS resulted from subjects undergoing two PAS sessions. This assertion is supported by the absence of change in plasticity after four weeks of sham stimulation in six subjects. Thus, our findings suggest that neuroplasticity was improved in the motor cortex of depressed subjects after a course of anodal tDCS.

There are multiple mechanisms by which a treatment course of tDCS may potentially have influenced the change in neuroplasticity. Some of these are discussed below, including direct effects of tDCS on the motor cortex, changes in BDNF and remission from depression.

Like PAS, a single session of tDCS applied over the motor cortex induces changes in MEPs that outlast the period of stimulation (Nitsche and Paulus, 2001). This direct neuroplastic effect of tDCS occurs even though intracerebral current densities produced by tDCS are sub-threshold for direct activation of cortical neurons (Tehovnik, 1996, Wagner et al., 2007, Fritsch et al., 2010). Pharmacological challenge studies suggest that tDCS transiently alters membrane potential by altering ion channel

permeability (Liebetanz et al., 2002, Bolognini et al., 2009). Through altering neuronal membrane potentials, tDCS increases spontaneous neuronal firing rates (Bindman et al., 1962, Liebetanz et al., 2002), and may lead to greater synaptic efficacy through enhanced pairing of pre and postsynaptic firing (Fritsch et al., 2010). Coincident pre and postsynaptic neuronal depolarisation can induce LTP. For tDCS, like PAS, this process is believed to be crucially dependent on activation of NMDA receptors. NMDA receptor blockade prevents the neuroplastic effects of tDCS (Liebetanz et al., 2002). There is also preliminary evidence from neuroimaging studies (Rae et al., 2009) of altered glutamatergic transmission after tDCS. For a more detailed review of tDCS mechanisms, see (Arul-Anandam and Loo, 2009).

PAS and tDCS share some common mechanisms and have been shown to interact in the short term when both are applied over the motor area. The neuroplastic effects of PAS were enhanced when immediately preceded by motor cortical tDCS. This resulted in an increase in MEP amplitude which was greater than after PAS alone and lasted for approximately 90 minutes (Nitsche et al., 2007). In the present study, PAS testing was done 1-3 days before the first tDCS session (PAS_T1), and again 1-3 days after the last tDCS session (PAS_T2), so it is unlikely that the change in the effect of PAS represents a short-term metaplastic interaction between the PAS and tDCS protocols. However, daily application of tDCS can have cumulative effects. When tDCS was applied over the motor cortex for 5 consecutive days in healthy subjects, the baseline excitability of the cortex and the neuroplastic effect of tDCS were both increased (Alonzo et al., 2012, Galvez et al., 2013). Repeated daily application of tDCS also facilitated improvements in motor function in stroke patients (Boggio et al., 2007b). Therefore, the relatively large number of tDCS sessions given in this study may account for the robust changes in neuroplasticity demonstrated. The trend level

(p=0.058) association between the number of tDCS sessions and change in neuroplasticity from T1 to T2 is therefore of interest, though this finding failed to reach significance and should be regarded as tentative.

An important consequential question is how anodal tDCS centred over the PFC might induce changes in the MEP response to PAS, which probes the function of the sensorimotor cortex. One possible explanation is that tDCS led to improvement in depression, which was associated with an overall improvement in neuroplasticity. The lack of significant correlation between change in mood scores and change in PAS outcomes does not support this explanation, though it is possible that the small sample and the variable sensitivities of the scales used contributed to our inability to demonstrate such a relationship. Another possibility is that, though anodal tDCS was centred on the frontal cortex, tDCS leads to relatively diffuse brain stimulation, and a significant level of stimulation may have occurred directly at the motor cortex, as suggested by computer modeling studies of the effects of tDCS (Sadleir et al., 2010). Thus, tDCS may have directly induced neuroplastic effects at the motor cortex, independent of its antidepressant effects. However, in other studies that have applied tDCS over the motor cortex, direct effects on motor cortical function were suggested by a change in resting motor threshold or baseline excitability over the course of repeated tDCS sessions (Alonzo et al., 2012, Galvez et al., 2013). In contrast, in this study there was no change in resting motor threshold between the two PAS sessions. Thus, it is unclear whether the improvement in neuroplasticity tested in the motor cortex was secondary to improvement in mood, or an independent effect of tDCS.

This study also investigated the role of BDNF serum levels and genotype in neuroplasticity in depression. BDNF is an important mediator of neuroplasticity (Duman and Monteggia, 2006, Carvalho et al., 2008), with a recent study finding that

tDCS-induced neuroplastic changes were accompanied by, and appeared to depend on, secretion of BDNF, as these changes did not occur in BDNF knockout animals (Fritsch et al., 2010). However, in the current study, mean serum BDNF levels did not increase after a course of repeated daily tDCS and there was no relationship between BDNF levels and changes in measured neuroplasticity. A recent meta-analysis of BDNF levels measured before and after antidepressant treatment (of various modalities) found that serum BDNF levels tended to increase after a course of antidepressant medication, but not after a course of brain stimulation treatment (e.g. electroconvulsive therapy, repetitive TMS) (Brunoni et al., 2008). It was hypothesised that this was due to treatment with antidepressant medications prior to, as well as during, the brain stimulation treatments, such that BDNF levels were already raised prior to the brain stimulation and had limited potential for further increases. This may also be a possible explanation for our results, given that thirteen of eighteen subjects were on antidepressant medications prior to, and during tDCS treatment. BDNF genotype has been shown previously to differentially influence neuroplasticity, for example, memory, in healthy subjects. Val homozygotes are thought to have greater synaptic change through superior packaging and increased secretion of BDNF (Egan et al., 2003). In the current small sample, we could not identify a BDNF genotype related change in BDNF serum levels or neuroplasticity (mean post-PAS MEP amplitudes) after a course of tDCS, suggesting that genotype effects on these measures may not be robust.

Consistent with the growing literature on the use of tDCS to treat depression – including two recent large randomised, sham-controlled trials, and a meta-analysis of change in mean depression scores after tDCS (Loo et al., 2008, Kalu et al., 2012, Brunoni et al., 2013) – significant improvement in mood was demonstrated in our study after treatment with tDCS. The results of the current study, however, suggest that

restoration of neuroplasticity may not be the sole mechanism responsible for antidepressant effects, as no relationship was found between antidepressant response and change in neuroplasticity.

The lack of relationship between improvement in neuroplasticity and mood is akin to previous reports of tDCS-induced neurocognitive enhancement, which also did not correlate with mood improvement in depressed subjects who received a treatment course of tDCS (Fregni et al., 2006, Boggio et al., 2007a, Loo et al., 2012). It is possible that these processes rely on different mechanisms and occur over different time courses. TDCS-induced neurocognitive effects (Boggio et al., 2007a), and improved motor functioning in stroke patients (Hummel and Cohen, 2005), have been shown to occur immediately, while mood improvement is only evident after repeated stimulation session (Loo et al., 2012, Brunoni et al., 2013). In this respect, tDCS is similar to antidepressant medications, which enhance neurocognitive function independently of improvements in mood. For example, one study found a neurocognitive improvement in memory performance after three months of selective serotonin re-uptake inhibitor (SSRI) treatment that did not correlate with subjects' significant mood improvement (Vythilingam et al., 2004). An animal study similarly showed – after 14 days of SSRI treatment – improved learning on the radial arm maze, and increased levels of hippocampal LTP, which were independent of an enhanced behavioural response to the forced swim test, a measure reflecting improved mood (Bhagya et al., 2011).

This study measured the neuroplasticity of a depressed cohort who underwent treatment with tDCS. It is unknown whether a similar neuroplastic change would occur in depressed patients who improved with other forms of antidepressant treatment – an interesting question for further investigation.

There were a number of limitations to this investigation. This was a preliminary study in a relatively small sample of eighteen depressed subjects, who received anodal prefrontal tDCS in a range of clinical trials. Thirteen of the eighteen subjects were on antidepressant medications, which are known to influence BDNF levels (Wong et al., 2010), and neuroplasticity (Rocher et al., 2004, Normann et al., 2007). For each subject, medication had been unchanged for at least 4 weeks prior to study entry and was kept constant over the study period, and was therefore unlikely to account for the increase in neuroplasticity demonstrated by PAS testing. However, the presence of antidepressant medication and the small sample size meant the study could not definitively resolve the relationship between neuroplasticity, mood and BDNF. In addition, this study used the PAS protocol, which we have shown to be robust and reliable in inducing cortical change at a group level (Chapter 2). Similarly, robust group effects have been demonstrated in a group of individuals tested across two separate PAS sessions, though reliability is less at an individual test-retest level (Fratello et al., 2006). The lack of increase in our plasticity measure after four weeks of sham tDCS suggests that the finding of increased plasticity after active tDCS was not a spurious finding.

Conclusion

This is the first study to demonstrate an increase in neuroplasticity following treatment of depression, using a test of plasticity that is independent of subject effort and motivation. The results support the hypothesis that depression is associated with impaired neuroplasticity, and that this can improve after effective treatment.

Chapter 5: Summary and Concluding Remarks

In recent years, research has been drawn to the investigation of the brain's ability to change. It has been hypothesised that plasticity of the brain is impaired in depression, and that this impairment may contribute to some of the symptoms associated with the disorder (Pittenger and Duman, 2008). However, this theory has been based on findings in preclinical models and results from cognitive and behavioural tests, which may have been confounded by suboptimal motivation and effort in depressed subjects. The main aims of this thesis were to objectively test neuroplasticity in individuals with MDD, to compare it to matched healthy controls, and to discover if this measure of neuroplasticity changed following treatment for depression. It is hoped that investigation of neuroplasticity, and its linkages to depressive symptoms may contribute to a better understanding of the disorder's aetiology and provide guidance for treatment.

Three separate studies were carried out. The first study investigated which of a range of brain stimulation protocols reliably induced the greatest change in cortical excitability in normal subjects, in order to provide the methodology for the subsequent studies (Chapter 2). The second study made a cross-sectional comparison of neuroplasticity in subjects with MDD against age and gender-matched controls (Chapter 3). Finally, a longitudinal investigation of neuroplasticity in subjects diagnosed with MDD was performed, before and after tDCS treatment for their depression (Chapter 4). Study results are summarised below and the significance of the findings discussed.

5.1 Study Findings

In the first study, neuroplasticity was tested in healthy young subjects on four separate occasions using the conditioning protocols PAS, iTBS, cTBS-PAS and cTBS-iTBS. Priming with cTBS was used in an attempt to induce greater changes in motor cortical excitability than single protocols through mechanisms of homeostatic metaplasticity (Todd et al., 2009). PAS proved to be the most robust of the four protocols in terms of inducing the greatest increase in excitability in the greatest proportion of subjects. PAS was also the only protocol to induce a significant mean group change in excitability. MEP amplitude increases of up to 80% relative to baseline were shown after PAS, with an upward trend continuing at the 60 minute point. PAS was therefore selected as the most suitable conditioning protocol for use in subsequent experiments (Rosenkranz and Rothwell, 2006, Ilić et al., 2009).

Direct comparisons between conditioning protocols in the same subjects have been rare (Di Lazzaro et al., 2011; Chapter 2). However, the greater comparative reliability of PAS to induce change in motor cortical excitability found in Study 1 is supported by recent evidence. Hamada and colleagues found in a study of 52 subjects tested with facilitatory and inhibitory TBS that only 25% of subjects had changes in excitability in the expected direction for both types of stimulation, and there were no overall mean group changes (Hamada et al., 2013), see also (Vernet et al., 2013). Similarly, the results of Study 1 demonstrated considerable variability in the response to TBS. Ten of sixteen subjects had significant increases in excitability after the facilitatory iTBS, however variability from the remaining subjects led to a finding of no overall group change. Variability in individual response extends to other brain stimulation protocols, including PAS (Fratello et al., 2006, Müller-Dahlhaus et al., 2008, Bolognini et al., 2009, Ridding and Ziemann, 2010). Possible explanations for the

comparatively greater reliability of PAS relate to characteristics of the stimuli and subject attentional factors in each protocol. In PAS, suprathreshold pairing of sensory and motor stimuli may induce superior changes in synaptic strength over that of subthreshold motor stimulation only, due to greater postsynaptic calcium levels (see section 2.5). In addition, during PAS, subjects are instructed to attend to the target finger, which increases PAS-induced effects (Stefan et al., 2004). There was no matching instruction for attention in the TBS protocols, although there is evidence that attention to the target hand significantly increases cortical excitability following rTMS protocols (Conte et al., 2007). Thus subject attention may have been a factor in the relative effects of the protocols used in this study. Understanding of the factors that cause protocol variability remains incomplete, and remains a caveat for conclusions of neuroplasticity drawn from brain stimulation paradigms.

Against expectations, priming PAS and iTBS with cTBS did not result in greater increases in cortical excitability than single protocols. It is possible that the particular inhibitory (cTBS) and facilitatory (iTBS and PAS) stimulation protocols used in this study affected different neurons, thus limiting the potential for metaplastic interactions (Di Lazzaro et al., 2005, Di Lazzaro et al., 2008c). Alternatively it may be that the timing between the two protocols may not have been optimal, with this also limiting any metaplastic interaction (Huang et al., 2010).

Results from Study 1 showed that subjects' MEP responses did not correlate across the four plasticity-inducing protocols. This finding suggests that each protocol might recruit different cortical neurons or circuits to effect change in cortical excitability, and thus may reflect heterogeneity of neuroplastic processes even within the motor cortex. However it may be that this within-subject variability is simply due to

inconsistencies of testing motor cortical plasticity on different occasions (Fratello et al., 2006).

Study 2 used the PAS protocol selected from Study 1 to compare neuroplasticity in depressed subjects with that of age and gender matched, non-depressed controls. In healthy controls but not in depressed subjects, PAS conditioning resulted in significantly increased mean MEP amplitude. Furthermore, motor learning (a second measure of plasticity) was impaired in the depressed cohort compared to healthy controls. Together, these results provide evidence that neuroplasticity is impaired in depression, supporting the primary hypothesis of the thesis.

Subjects with a lower mood-state tended to have lower post-conditioning MEP amplitude. Although this difference did not reach statistical significance, the tendency is consistent with expectations that if impaired plasticity is a feature in depression, then more depressed subjects would possess greater impairment.

This study provided one of the first investigations of experimentally induced neuroplasticity in individuals diagnosed with MDD. Importantly, the methodology used in this study ensured that the findings were not confounded by subject effort or motivation – known to be impaired in depression, or subjective analysis, which may be present in cognitive or behavioural measures of plasticity.

In Study 3, the relationship between neuroplasticity and mood state was further explored in a longitudinal manner, with neuroplasticity being measured on two separate occasions using a within-subject design. Neuroplasticity was measured using the PAS protocol in subjects while they were depressed, and again after treatment for depression with transcranial Direct Current Stimulation (tDCS).

As expected, tDCS led to a significant mean group improvement in mood state (Kalu et al., 2012, Loo et al., 2012, Brunoni et al., 2013). In support of the hypothesis that neuroplasticity would improve with antidepressant treatment, the mean group MEP response to PAS conditioning was greater after a course of tDCS, than the pre-treatment MEP response. This mean group improvement was matched with an increase in the number of subjects who responded (from 8/18 to 13/18) with positive increases in post-PAS MEPs. It should be noted however, that the change in mood did not correlate with change in the PAS response, indicating that tDCS may have directly induced neuroplastic effects at the motor cortex, which were independent of its antidepressant effects. A trend level association was found between the number of tDCS sessions and change in neuroplasticity. This suggests a cumulative effect of treatment dose, which is consistent with earlier findings of tDCS applications (Alonzo et al., 2012, Galvez et al., 2013).

Overall this study demonstrated that neuroplasticity appeared similar to that of healthy controls after an effective treatment for depression. This improvement in neuroplasticity was reflected in the ratio of responders to the PAS protocol. The ratio of PAS responders in Study 3 was similar to the ratio of healthy control responders in Study 2. However, as a number of the Study 3 findings failed to reach significance, further research is required to substantiate any link between tDCS and neuroplastic effects, or the possible relationship between mood state and neuroplasticity.

It should also be noted that, surprisingly, motor cortical plasticity assessed by changes in MEP amplitude after PAS in Study 1 and Study 2 showed no correlation with respective motor learning results assessed by the rotor pursuit task. This may be due to there being only partial overlap between cortical mechanisms involved in these two processes (Li Voti et al., 2011). Alternatively, the lack of a relationship may be due

to PAS and rotor pursuit learning being measures with limited psychometric reliability. For example, PAS may be sufficiently reliable to show group effects (changes in means) but insufficiently reliable to show correlations with other imperfect measures. The absence of a correlation limits functional inferences that can be made about changes in PAS-induced motor cortical plasticity.

Whilst the main objective of this thesis was to investigate neuroplastic changes in depression and with antidepressant treatment, an additional aim was to evaluate relationships between neuroplasticity, and neuroplasticity mediating factors, such as BDNF.

Unlike a number of studies that evaluated BDNF, we did not find that BDNF serum levels and BDNF genotype polymorphisms consistently influenced plasticity (Figurov et al., 1996, Egan et al., 2003, Cheeran et al., 2008). PAS-induced plasticity was positively related to BDNF serum levels in the depressed cohort of Study 2, supporting the hypothesised relationship between BDNF levels and neuroplasticity (Carlson et al., 2006, Tanaka et al., 2008, Fritsch et al., 2010, Yoshii and Constantine-Paton, 2010). However, the same relationship was not observed in healthy subjects. Furthermore, no association between change in BDNF serum levels and change in neuroplasticity was seen in the Study 3 cohort. This inconsistency between BDNF levels and neuroplasticity in the depressed cohorts, may be due to sample size, and therefore limits the inferences we can draw from thesis results.

In addition, BDNF genotype did not predict neuroplastic change following brain stimulation, a result inconsistent with that of Cheeran et al. (2008). Further, in contrast to previous research, no differences were found in serum BDNF levels across genotype (Egan et al., 2003, Teixeira et al., 2010), nor was there a change in serum BDNF levels

after tDCS (Fritsch et al., 2010). The reason for the difference between the current results and previous findings may again be related to sample size or the proportion of subjects with each BDNF polymorphism in the studies. Thus, while these findings highlight potential paths for investigation, the small sample size limits the conclusions that can be drawn for the influence of BDNF genotype and BDNF serum levels on neuroplasticity.

Treatment with tDCS may have induced an increase in activity-dependent neuronal plasticity, analogous to that hypothesised to occur following administration of antidepressant medications (Castrén, 2005). An increase in activity-dependent plasticity after antidepressants is hypothesised to occur through the enhanced neurotransmission, neural network functioning, and levels of neurotrophic factors which all support experience-dependent synaptic change (Duman et al., 2000, Castrén, 2005, Elsayed et al., 2012, Son et al., 2012). An increase in BDNF levels would therefore be predicted to follow treatment with brain stimulation (Nibuya et al., 1995, Zanardini et al., 2006, Brunoni et al., 2008, Fritsch et al., 2010), and correspond with improvement in activity-dependent plasticity, hypothesised to underlie improved mood and motor cortical plasticity.

However, in Study 3, no increase in serum BDNF levels after tDCS was observed. One explanation for this finding is that rather than a change in serum BDNF levels, there may have been a change in the composition of BDNF, which might support this change in plasticity. Specifically, serum BDNF levels are composed of ‘Mature BDNF’ (mBDNF), and its precursor peptide ‘Pro BDNF’ (pBDNF) which are complicit in LTP and LTD respectively (Yoshii and Constantine-Paton, 2010). It is possible that greater neuronal activity resulting from tDCS stimulation may have induced activity-dependent proteolytic cleaving of pBDNF to the more trophic mBDNF (Pang et al.,

2004). This cleaving process may have facilitated increased neuroplasticity while leaving BDNF serum levels unchanged. Differentiation between these forms of BDNF requires 'Western Blot' analyses (Nagappan et al., 2009), which were beyond the scope of this study. However this hypothesis is speculative, and anticipated increases in serum BDNF levels that correspond with improved neuroplasticity might become evident with a larger sample tested.

A larger sample would also assist in understanding the relationship between tDCS, BDNF, neuroplasticity and mood, which is not clear from Study 3, and is complicated by the concurrent treatment of many of the subjects with antidepressant medications. The mechanisms by which tDCS exerts neuroplastic effects are incompletely understood. Nonetheless, it is promising that a treatment course of tDCS led to an improvement in neuroplasticity as measured by excitability changes after PAS, and also to improved mood. In this small sample however, the two changes in outcome measures were not correlated. Thus it is unclear whether the improvement in mood accounted for the improvement in neuroplasticity or vice versa. It is however possible that improved mood and motor cortical excitability may independently result from a return toward normal levels of activity-dependent neuronal plasticity in all brain regions.

5.2 Study Assumptions and Limitations

This series of studies involved a number of assumptions and limitations that should be considered. Firstly, the change in post-PAS motor cortical excitability was used as a measure of neuroplasticity. A pure measure of synaptic plasticity is not possible to observe in humans after brain stimulation without invasive cell recording

procedures. Therefore, the change in MEP amplitude after PAS conditioning has been used as a surrogate measure of neuroplasticity (Classen et al., 2004).

This study assessed neuroplasticity in the motor cortex, as it provides an objective, convenient and generally painless way to evaluate neuroplasticity *in vivo*. However, the motor cortex is not considered the primary site of cerebral dysfunction in depression. It would be advantageous to measure neuroplasticity in regions that regulate emotion, given this is more salient in depression. Despite this limitation, results from the motor cortex correspond with evidence in depression of impaired plasticity from other tests, in other regions in the brain (Landrø et al., 2001, Porter et al., 2003, Naismith et al., 2006, Normann et al., 2007, Nissen et al., 2010). Thus, the finding of impaired motor cortical plasticity is of substantial interest as it may indicate that impairment of neuroplasticity is widespread in the brain of depressed subjects.

In Study 3, the proportion of depressed subjects treated with tDCS who then responded positively to PAS was similar to that found in healthy subjects. However, it has been assumed that tDCS is an effective treatment for depression. Though the efficacy of tDCS in treating depression is supported by several sham-controlled clinical trials (Kalu et al., 2012, Loo et al., 2012, Brunoni et al., 2013), this treatment is still considered experimental. A tDCS treatment group was chosen because it was a population that was readily available. Study methodology would be improved by the addition of a sham tDCS comparison group. Also, it is unknown whether a similar change in motor cortical plasticity would occur in depressed subjects who achieved symptom reduction under other forms of treatment, such as antidepressant medications. Such research would provide greater insight into the relationship between depressive symptoms and mechanisms of neuroplasticity.

Finally, in the three studies, excitability measures have been taken of the corticospinal pathway and discussion of synaptic changes has primarily focused on excitatory (glutamatergic) interneuronal mechanisms. However, activity of glutamatergic neurons is modulated by inhibitory interneurons that use the major neurotransmitter GABA (Zhang, 2006). Therefore, the measures of motor cortical excitability in these studies are influenced by inhibitory (GABAergic) circuits in the motor cortex. The excitability of GABAergic circuits can be assessed through measures of the cortical silent period (Chen et al., 1999) and intracortical inhibition (Kujirai et al., 1993). For example, measurement of the short-interval intracortical inhibition (SICI) is achieved using paired pulse TMS paradigms (Kujirai et al., 1993, Ziemann et al., 1996, Hanajima et al., 1998, Ilić et al., 2002). There has been evidence of comparative GABAergic deficits in depression with a recent meta-analysis finding significantly shortened CSP and decreased SICI in depressed subjects (Radhu et al., 2013). Distinction of a change from healthy controls in excitability of excitatory (glutamate) and inhibitory (GABA) circuits may increase understanding of the neurophysiology of impaired plasticity in depression. Therefore, measures of intracortical inhibition would have provided a useful addition to study findings.

5.3 Future Directions

Study findings raise several important questions for further research. First, improvement in neuroplasticity was observed following treatment with tDCS (Study 3). It would be of great interest to know if there was a similar improvement in neuroplasticity with remission from depression after other forms of antidepressant treatment. Discovery of changes in neuroplasticity following other antidepressant treatments may improve understanding of MDD pathophysiology, and thus provide additional guidance for treatment.

Second, research within this thesis demonstrated impaired plasticity in the motor cortex of subjects with MDD. This novel finding adds to evidence of impaired plasticity in the visual cortex (Normann et al., 2007), and indirect evidence of impaired plasticity in PFC and hippocampus, reflected by learning and memory deficits (Merriam et al., 1999, Landrø et al., 2001, Porter et al., 2003, Naismith et al., 2006). To further support the hypothesis that widespread, impaired neuroplasticity is a marker of depression, it would be useful to test neuroplasticity in other brain regions. Testing of neuroplasticity in other brain regions may be objectively achieved through the measurement of sensory-evoked potentials. These may be, for example, either somatosensory-evoked potentials elicited by tactile or electrical stimulation of a sensory or mixed nerve in the periphery (Pascual-Leone and Torres, 1993, Tamura et al., 2009), or auditory-evoked potentials, which are elicited by a click or tone presented through earphones (Purdy et al., 2001).

Third, the measurement of neuroplasticity in this thesis involved using facilitatory brain stimulation protocols and testing for increases in motor cortical excitability. Increases in motor cortical output are hypothesised to occur through induction of LTP at synapses in the motor cortical pathway (Stefan et al., 2000, Stefan et al., 2002, Huang et al., 2005). However knowledge of how synaptic plasticity is affected in MDD may be improved by testing the motor cortical response to inhibitory brain stimulatory protocols in depressed subjects. Inhibitory protocols are hypothesised to reduce cortical excitability, mainly through LTD mechanisms, at excitatory synapses (Fitzgerald et al., 2006). However, as mentioned above (see section 5.2), the activity of excitatory glutamatergic neurons are modulated by inhibitory GABAergic neurons. The output from stimulated corticospinal neurons is thus dependent on the sum of all excitatory and inhibitory inputs onto output neurons. In healthy subjects, inhibitory protocols such as low-frequency rTMS or PAS10 induce a reduction in post-

conditioning corticospinal excitability (measured via MEPs) to TMS test pulses (Chen et al., 1997, Wolters et al., 2003, Fitzgerald et al., 2006, Di Lazzaro et al., 2008b). An experiment conducted with an inhibitory protocol might assist in the determination of whether subjects with depression have a functional impairment of both facilitatory and inhibitory plasticity, or whether the deficit is only of LTP-like mechanisms. For example, in Study 2, MEPs were reduced by over 20% after PAS in approximately half of the depressed cohort, which might represent an increased tendency for LTD. To clarify this question, a future experiment could test depressed subjects with an inhibitory protocol to identify any bias toward LTD in MDD, in addition to impaired LTP evidenced in this thesis. As seen above (see section 1.2), impaired LTP and enhanced LTD could be related to increased levels of circulating cortisol, considered to result from an overactive HPA axis in depression (see section 1.2.4.4). Evidence of these changes in motor cortical excitability after an inhibitory protocol may increase understanding of the pathophysiology of MDD.

5.4 Concluding Remarks

The prevailing stress neurotrophic hypothesis of depression is that MDD is associated with over-activity in the HPA axis, and this results in excessive levels of glucocorticoids, which may impair neuroplasticity. The research in this thesis is consistent with the stress neurotrophic hypothesis in that it demonstrates impairment of facilitatory neuroplasticity in the motor cortex of depressed subjects. This finding is consistent with current hypotheses that individuals with depression are impaired in their ability to develop new synaptic connections or change existing connections, which may be reflected through impaired learning and memory formation (Landrø et al., 2001,

Porter et al., 2003, Naismith et al., 2006, Nissen et al., 2010). Lowered ability for the brain to change also provides an explanation for a number of other symptoms pervasive in depression such as executive function deficits, anhedonia, and a lessened ability to deal with novelty and change (Pittenger and Duman, 2008, Marsden, 2011). The finding of impaired motor cortical plasticity using brain stimulation protocols suggests that neurocognitive deficits in depression cannot be solely accounted for by lowered effort or motivation. The implication of this finding, in conjunction with previous neurocognitive evidence, is that impaired plasticity might be widespread in the cortex. However, the measure of neuroplasticity used in this thesis improved after treatment with a course of non-invasive brain stimulation, which also improved depressive symptoms, though these two outcomes were not correlated.

The research contained within this thesis significantly adds to the body of knowledge of neuroplasticity in depression, with the results providing guidance for a number of additional avenues for investigation (see section 5.3). This is the first study to use an objective test (independent of subject effort) to both demonstrate impaired neuroplasticity in the depressed state, and improvement of neuroplasticity after antidepressant treatment. This improvement mirrors the improvement in activity-dependent neuroplasticity in cognitive regions suggested through improved learning and memory with recovery from depression. The studies in this thesis significantly contribute to understanding the aetiology and maintenance of symptoms in depression, and offer further insight into their treatment.

Reference List

Abercrombie HC, Schaefer SM, Larson CL, Oakes TR, Lindgren KA, Holden JE, Perlman SB, Turski PA, Krahn DD, Benca RM, Davidson RJ (1998) Metabolic rate in the right amygdala predicts negative affect in depressed patients. NeuroReport 9:3301-3307.

Abraham WC (2008) Metaplasticity: Tuning synapses and networks for plasticity. Nature Reviews Neuroscience 9:387-399.

Abraham WC, Bear MF (1996) Metaplasticity: The plasticity of synaptic plasticity. Trends in Neuroscience 19:126-130.

Abraham WC, Mason-Parker SE, Bear MF, Webb S, Tate WP (2001) Heterosynaptic metaplasticity in the hippocampus in vivo: A BCM-like modifiable threshold for LTP. Proceedings of the National Academy of Sciences of the United States of America 98:10924-10929.

Alonso G (2000) Prolonged corticosterone treatment of adult rats inhibits the proliferation of oligodendrocyte progenitors present throughout white and gray matter regions of the brain. Glia 31:219-231.

Alonzo A, Brassil J, Taylor JL, Martin D, Loo CK (2012) Daily transcranial direct current stimulation (tDCS) leads to greater increases in cortical excitability than second daily transcranial direct current stimulation. Brain Stimulation 5:208-213.

Amassian VE, Deletis V (1999) Relationships between animal and human corticospinal responses. Electroencephalography and Clinical Neurophysiology Supplement 51:79-92.

American Psychiatric Association (2000) Diagnostic and Statistical Manual of Mental Disorders. 4th ed. text revsion (DSM-IV-TR). Washington: American Psychiatric Association.

Arai N, Okabe S, Furubayashi T, Mochizuki H, Iwata NK, Hanajima R, Terao Y, Ugawa Y (2007) Differences in after-effect between monophasic and biphasic high-frequency rTMS of the human motor cortex. Clinical Neurophysiology 118:2227-2233.

Arai N, Okabe S, Furubayashi T, Terao Y, Yuasa K, Ugawa Y (2005) Comparison between short train, monophasic and biphasic repetitive transcranial magnetic stimulation (rTMS) of the human motor cortex. Clinical Neurophysiology 116:605-613.

Armanini MP, Hutchins C, Stein BA, Sapolsky RM (1990) Glucocorticoid endangerment of hippocampal neurons is NMDA-receptor dependent. Brain Research 532:7-12.

Artola A, Von Frijtag JC, Fermont PCJ, Gispen WH, Schrama LH, Kamal A, Spruijt BM (2006) Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. European Journal of Neuroscience 23:261-272.

Arul-Anandam AP, Loo C (2009) Transcranial direct current stimulation: A new tool for the treatment of depression? Journal of Affective Disorders 117:137-145.

Austin MP, Mitchell P, Goodwin GM (2001) Cognitive deficits in depression: Possible implications for functional neuropathology. British Journal of Psychiatry 178:200-206.

Autry AE, Monteggia LM (2012) Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacological Reviews 64:238-258.

Bajbouj M, Lisanby SH, Lang UE, Danker-Hopfe H, Heuser I, Neu P (2006) Evidence for impaired cortical inhibition in patients with unipolar major depression. Biological Psychiatry 59:395-400.

Banasr M, Valentine GW, Li XY, Gourley SL, Taylor JR, Duman RS (2007) Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. Biological Psychiatry 62:496-504.

Bandelow B, Baldwin DS, Dolberg OT, Andersen HF, Stein DJ (2006) What is the threshold for symptomatic response and remission for major depressive disorder, panic disorder, social anxiety disorder, and generalized anxiety disorder? Journal of Clinical Psychiatry 67:1428-1434.

Barense MD, Fox MT, Baxter MG (2002) Aged rats are impaired on an attentional set-shifting task sensitive to medial frontal cortex damage in young rats. Learning and Memory 9:191-201.

Baune BT, Miller R, McAfoose J, Johnson M, Quirk F, Mitchell D (2010) The role of cognitive impairment in general functioning in major depression. Psychiatry Research 176:183-189.

Bell-McGinty S, Butters MA, Meltzer CC, Greer PJ, Reynolds Iii CF, Becker JT (2002) Brain morphometric abnormalities in geriatric depression: Long-term neurobiological effects of illness duration. American Journal of Psychiatry 159:1424-1427.

Bench CJ, Frackowiak RSJ, Dolan RJ (1995) Changes in regional cerebral blood flow on recovery from depression. Psychological Medicine 25:247-261.

Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: Beyond monoamines. Nature Reviews Neuroscience 7:137-151.

Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J (2003) Subthreshold high-frequency TMS of human primary motor cortex modulates interconnected frontal motor areas as detected by interleaved fMRI-TMS. NeuroImage 20:1685-1696.

Bhagya V, Srikumar BN, Raju TR, Rao BS (2011) Chronic escitalopram treatment restores spatial learning, monoamine levels, and hippocampal long-term potentiation in an animal model of depression. Psychopharmacology 214:477-494.

Bienenstock EL, Cooper LN, Munro PW (1982) Theory for the development of neuron selectivity: Orientation specificity and binocular interaction in visual cortex. Journal of Neuroscience 2:32-48.

Bindman LJ, Lippold OCJ, Redfearn JWT (1962) Long-lasting changes in the level of the electrical activity of the cerebral cortex produced by polarizing currents. Nature 196:584-585.

Bliem B, Müller-Dahlhaus JFM, Dinse HR, Ziemann U (2008) Homeostatic metaplasticity in the human somatosensory cortex. Journal of Cognitive Neuroscience 20:1517-1528.

Bliss TVP, Collingridge GL (1993) A synaptic model of memory: Long-term potentiation in the hippocampus. Nature 361:31-39.

Boggio PS, Bermpohl F, Vergara AO, Muniz AL, Nahas FH, Leme PB, Rigonatti SP, Fregni F (2007a) Go-no-go task performance improvement after anodal transcranial DC stimulation of the left dorsolateral prefrontal cortex in major depression. Journal of Affective Disorders 101:91-98.

Boggio PS, Nunes A, Rigonattia SP, Nitsche MA, Pascual-Leone A, Fregni F (2007b) Repeated sessions of noninvasive brain DC stimulation is associated with motor

function improvement in stroke patients. Restorative Neurology and Neuroscience 25:123-129.

Boggio PS, Rigonatti SP, Ribeiro RB, Myczkowski ML, Nitsche MA, Pascual-Leone A, Fregni F (2008) A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. International Journal of Neuropsychopharmacology 11:249-254.

Bolognini N, Pascual-Leone A, Fregni F (2009) Using non-invasive brain stimulation to augment motor training-induced plasticity. Journal of Neuroengineering and Rehabilitation 6:8.

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS (2000) Hippocampal volume reduction in major depression. American Journal of Psychiatry 157:115-117.

Bromet E, Andrade LH, Hwang I, Sampson NA, Alonso J, de Girolamo G, de Graaf R, Demyttenaere K, Hu C, Iwata N, Karam AN, Kaur J, Kostyuchenko S, Lépine J, Levinson D, Matschinger H, Mora MEM, Browne MO, Posada-Villa J, Viana MC, Williams DR, Kessler RC (2011) Cross-national epidemiology of DSM-IV major depressive episode. BMC Medicine 9:90.

Brunoni AR, Lopes M, Fregni F (2008) A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: Implications for the role of neuroplasticity in depression. International Journal of Neuropsychopharmacology 11:1169-1180.

Brunoni AR, Valiengo L, Baccaro A, Zanão TA, De Oliveira JF, Goulart A, Boggio PS, Lotufo PA, Benseñor IM, Fregni F (2013) The sertraline vs electrical current therapy for treating depression clinical study. JAMA Psychiatry 70:383-391.

Buonomano DV, Merzenich MM (1998) Cortical plasticity: From synapses to maps. Annual Review of Neurosciences 21:149-186.

Burke HM, Davis MC, Otte C, Mohr DC (2005) Depression and cortisol responses to psychological stress: A meta-analysis. Psychoneuroendocrinology 30:846-856.

Burt DB, Zembar MJ, Niederehe G (1995) Depression and memory impairment: A meta-analysis of the association, its pattern, and specificity. Psychological Bulletin 117:285-305.

Buyukdura JS, McClintock SM, Croarkin PE (2011) Psychomotor retardation in depression: Biological underpinnings, measurement, and treatment. Progress in Neuro-Psychopharmacology and Biological Psychiatry 35:395-409.

Calabrese F, Molteni R, Racagni G, Riva MA (2009) Neuronal plasticity: A link between stress and mood disorders. Psychoneuroendocrinology 34 Supplement 1:S208-216.

Campbell S, Marriott M, Nahmias C, MacQueen GM (2004) Lower hippocampal volume in patients suffering from depression: A meta-analysis. American Journal of Psychiatry 161:598-607.

Canolty RT, Edwards E, Dalal SS, Soltani M, Nagarajan SS, Kirsch HE, Berger MS, Barbare NM, Knight RT (2006) High gamma power is phase-locked to theta oscillations in human neocortex. Science 313:1626-1628.

Cárdenas-Morales L (2010) Effects of Theta-Burst Transcranial Magnetic Stimulation over the Human Motor Cortex: A Neuroimaging Study. Doctoral thesis from the Hospital for Psychiatry and Psychotherapy, Universität Ulm, Ulm.

Cárdenas-Morales L, Nowak DA, Kammer T, Wolf RC, Schönfeldt-Lecuona C (2010) Mechanisms and applications of theta-burst rTMS on the human motor cortex. Brain Topography 22:294-306.

Carlson PJ, Singh JB, Zarate CA, Jr., Drevets WC, Manji HK (2006) Neural circuitry and neuroplasticity in mood disorders: Insights for novel therapeutic targets. NeuroRx : Journal of the American Society for Experimental NeuroTherapeutics 3:22-41.

Carvalho AL, Caldeira MV, Santos SD, Duarte CB (2008) Role of the brain-derived neurotrophic factor at glutamatergic synapses. British Journal of Pharmacology 153 Supplement 1:S310-324.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science 301:386-389.

Castrén E (2005) Is mood chemistry? Nature Reviews Neuroscience 6:241-246.

Castrén E, Rantamäki T (2010) The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. Developmental Neurobiology 70:289-297.

Castrén E, Võikar V, Rantamäki T (2007) Role of neurotrophic factors in depression. Current Opinion in Pharmacology 7:18-21.

Caudal D, Godsil BP, Mailliet F, Bergerot D, Jay TM (2010) Acute stress induces contrasting changes in AMPA receptor subunit phosphorylation within the prefrontal cortex, amygdala and hippocampus. PLoS ONE 5(12):e15282.

Cerqueira JJ, Pêgo JM, Taipa R, Bessa JM, Almeida OFX, Sousa N (2005) Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. Journal of Neuroscience 25:7792-7800.

Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, Houlden H, Bhatia K, Greenwood R, Rothwell JC (2008) A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. Journal of Physiology 586:5717-5725.

Chen CH, Ridler K, Suckling J, Williams S, Fu CHY, Merlo-Pich E, Bullmore E (2007) Brain imaging correlates of depressive symptom severity and predictors of symptom improvement after antidepressant treatment. Biological Psychiatry 62:407-414.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, Cohen LG (1997) Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 48:1398-1403.

Chen R, Lozano AM, Ashby P (1999) Mechanism of the silent period following transcranial magnetic stimulation: Evidence from epidural recordings. Experimental Brain Research 128:539-542.

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140-143.

Cho K, Aggleton JP, Brown MW, Bashir ZI (2001) An experimental test of the role of postsynaptic calcium levels in determining synaptic strength using perirhinal cortex of rat. Journal of Physiology 532:459-466.

Cirillo J, Lavender AP, Ridding MC, Semmler JG (2009) Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals. Journal of Physiology 587:5831-5842.

Citri A, Malenka RC (2008) Synaptic plasticity: Multiple forms, functions, and mechanisms. Neuropsychopharmacology 33:18-41.

Classen J, Wolters A, Stefan K, Wycislo M, Sandbrink F, Schmidt A, Kunesch E (2004) Paired Associative Stimulation. In: Advances in Clinical Neurophysiology: proeedings of the 27th international Congress of Clinical Neurophysiology, vol. 57 (Hallett, M., ed), pp 563-569 San Francisco: Elsevier, 2004.

Classen J, Ziemann U (2003) Stimulation-Induced Plasticity in Human Motor Cortex. In: Plasticity in the Human Nervous System: Investigations with Transcranial

Magnetic Stimulation (Boniface, S. and Ziemann, U., eds) Cambridge, UK: Cambridge University Press.

Coffey CE, Wilkinson WE, Weiner RD, Parashos IA, Djang WT, Webb MC, Figiel GS, Spritzer CE (1993) Quantitative cerebral anatomy in depression: A controlled magnetic resonance imaging study. Archives of General Psychiatry 50:7-16.

Cohen LG, Ziemann U, Chen R, Classen J, Hallett M, Gerloff C, Butefisch C (1998) Studies of neuroplasticity with transcranial magnetic stimulation. Journal of Clinical Neurophysiology 15:305-324.

Conrad CD, LeDoux JE, Magariños AM, McEwen BS (1999) Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. Behavioral Neuroscience 113:902-913.

Conte A, Gilio F, Iezzi E, Frasca V, Inghilleri M, Berardelli A (2007) Attention influences the excitability of cortical motor areas in healthy humans. Experimental Brain Research 182:109-117.

Cooke SF, Bliss TV (2006) Plasticity in the human central nervous system. Brain 129:1659-1673.

Cotter D, Mackay D, Landau S, Kerwin R, Everall I (2001) Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. Archives of General Psychiatry 58:545-553.

Czéh B, Michaelis T, Watanabe T, Frahm J, De Biurrun G, Van Kampen M, Bartolomucci A, Fuchs E (2001) Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proceedings of the National Academy of Sciences of the United States of America 98:12796-12801.

Dalley JW, Cardinal RN, Robbins TW (2004) Prefrontal executive and cognitive functions in rodents: Neural and neurochemical substrates. Neuroscience and Biobehavioral Reviews 28:771-784.

Dan Y, Poo MM (2004) Spike timing-dependent plasticity of neural circuits. Neuron 44:23-30.

Daskalakis ZJ, Christensen BK, Chen R, Fitzgerald PB, Zipursky RB, Kapur S (2002) Evidence for impaired cortical inhibition in schizophrenia using transcranial magnetic stimulation. Archives of General Psychiatry 59:347-354.

Daskalakis ZJ, Christensen BK, Fitzgerald PB, Chen R (2008) Dysfunctional neural plasticity in patients with schizophrenia. Archives of General Psychiatry 65:378-385.

Davidson RJ, Pizzagalli D, Nitschke JB, Putnam K (2002) Depression: Perspectives from affective neuroscience. Annual Review of Psychology 53:545-574.

Delbende C, Mocaer E, Rettori M, Kamoun A, Vaudry H (1993) Effect of the antidepressant tianeptine on the activity of the hypothalamo-pituitary-adrenal axis. European Psychiatry 8:49s-54s.

Delvendahl I, Jung NH, Mainberger F, Kuhnke NG, Cronjaeger M, Mall V (2010) Occlusion of bidirectional plasticity by preceding low-frequency stimulation in the human motor cortex. Clinical Neurophysiology 121:594-602.

Demirtas-Tatlidede A, Vahabzadeh-Hagh AM, Pascual-Leone A (2013) Can noninvasive brain stimulation enhance cognition in neuropsychiatric disorders? Neuropharmacology 64:566-578.

Di Lazzaro V, Dileone M, Pilato F, Capone F, Musumeci G, Ranieri F, Ricci V, Bria P, Iorio RD, de Waure C, Pasqualetti P, Profice P (2011) Modulation of motor cortex neuronal networks by rTMS: Comparison of local and remote effects of six different protocols of stimulation. Journal of Neurophysiology 105:2150-2156.

Di Lazzaro V, Dileone M, Pilato F, Profice P, Oliviero A, Mazzone P, Insola A, Capone F, Ranieri F, Tonali PA (2009) Associative motor cortex plasticity: Direct evidence in humans. Cerebral Cortex 19:2326-2330.

Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Mazzone P, Insola A, Tonali PA, Rothwell JC (2004) The physiological basis of transcranial motor cortex stimulation in conscious humans. Clinical Neurophysiology 115:255-266.

Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, Mazzone P, Tonali P, Rothwell JC (1998) Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. Electroencephalography and Clinical Neurophysiology - Electromyography and Motor Control 109:397-401.

Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, Insola A, Ranieri F, Meglio M, Tonali PA, Rothwell JC (2008a) The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. Journal of Physiology 586:3871-3879.

Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, Insola A, Ranieri F, Tonali PA, Rothwell JC (2008b) Low-frequency repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. Journal of Physiology 586:4481-4487.

Di Lazzaro V, Pilato F, Saturno E, Oliviero A, Dileone M, Mazzone P, Insola A, Tonali PA, Ranieri F, Huang YZ, Rothwell JC (2005) Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. Journal of Physiology 565:945-950.

Di Lazzaro V, Profice P, Pilato F, Dileone M, Oliviero A, Ziemann U (2010) The effects of motor cortex rTMS on corticospinal descending activity. Clinical Neurophysiology 121:464-473.

Di Lazzaro V, Ziemann U, Lemon RN (2008c) State of the art: Physiology of transcranial motor cortex stimulation. Brain Stimulation 1:345-362.

Diorio D, Viau V, Meaney MJ (1993) The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. Journal of Neuroscience 13:3839-3847.

Djuric S, Djuric V, Zivkovic M, Milosevic V, Jolic M, Stamenovic J, Djordjevic G, Calixto M (2010) Are somatosensory evoked potentials of the tibial nerve the most sensitive test in diagnosing multiple sclerosis? Neurology India 58:537-541.

Drevets WC (1998) Functional neuroimaging studies of depression: The anatomy of melancholia. Annual Review Medicine 49:341-361.

Drevets WC (2000) Neuroimaging studies of mood disorders. Biological Psychiatry 48:813-829.

Drevets WC (2003) Neuroimaging abnormalities in the amygdala in mood disorders. Annals of New York Academy of Sciences vol. 985, pp 420-444.

Drevets WC, Furey ML (2009) Depression and the Brain. Encyclopedia of Neuroscience 3:459-470.

Drevets WC, Gadde K, Krishnan R (1999) Neuroimaging Studies of Depression. In: Neurobiology of Mental Illness (Charney, D. et al., eds), pp 394-418 New York, USA: Oxford University Press.

Drevets WC, Price JL, Bardgett ME, Reich T, Todd RD, Raichle ME (2002) Glucose metabolism in the amygdala in depression: Relationship to diagnostic subtype

and plasma cortisol levels. Pharmacology Biochemistry and Behavior 71:431-447.

Drevets WC, Savitz J, Trimble M (2008) The subgenual anterior cingulate cortex in mood disorders. CNS Spectrums 13:663-681.

Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME (1992) A functional anatomical study of unipolar depression. Journal of Neuroscience 12:3628-3641.

Duffau H (2006) Brain plasticity: From pathophysiological mechanisms to therapeutic applications. Journal of Clinical Neuroscience 13:885-897.

Duman CH, Schlesinger L, Kodama M, Russell DS, Duman RS (2007) A role for MAP Kinase signaling in behavioral models of depression and antidepressant treatment. Biological Psychiatry 61:661-670.

Duman RS (2009) Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: Stress and depression. Dialogues in Clinical Neuroscience 11:239-255.

Duman RS, Aghajanian GK (2012) Synaptic dysfunction in depression: Potential therapeutic targets. Science 338:68-72.

Duman RS, Malberg J, Nakagawa S, D'Sa C (2000) Neuronal plasticity and survival in mood disorders. Biological Psychiatry 48:732-739.

Duman RS, Malberg J, Thome J (1999) Neural plasticity to stress and antidepressant treatment. Biological Psychiatry 46:1181-1191.

Duman RS, Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. Biological Psychiatry 59:1116-1127.

Duman RS, Voleti B (2012) Signaling pathways underlying the pathophysiology and treatment of depression: Novel mechanisms for rapid-acting agents. Trends in Neuroscience 35:47-56.

Dwivedi Y (2009) Brain-derived neurotrophic factor: Role in depression and suicide. Neuropsychiatric Disease and Treatment 5:433-449.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev. E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257-269.

Egeland J, Lund A, Landrø NI, Rund BR, Sundet K, Asbjørnsen A, Mjellem N, Roness A, Stordal KI (2005) Cortisol level predicts executive and memory function in

depression, symptom level predicts psychomotor speed. Acta Pychiatrica Scandinavica 112:434-441.

Elsayed M, Banasr M, Duric V, Fournier NM, Licznerski P, Duman RS (2012) Antidepressant effects of fibroblast growth factor-2 in behavioral and cellular models of depression. Biological Psychiatry 72:258-265.

Fagiolini M, Fritschy JM, Löw K, Möhler H, Rudolph U, Hensch TK (2004) Specific GABAA circuits for visual cortical plasticity. Science 303:1681-1683.

Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B (1996) Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature 381:706-709.

First MB, Spitzer RL, Miriam. G, Williams JBW (2002) Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition With Psychotic Screen (SCID-I/P W/ PSY SCREEN). (Biometrics Research, N. Y. S. P. I., ed) New York, USA.

Fitzgerald PB, Brown TL, Marston NAU, Daskalakis ZJ, De Castella A, Bradshaw JL, Kulkarni J (2004) Motor cortical excitability and clinical response to rTMS in depression. Journal of Affective Disorder 82:71-76.

Fitzgerald PB, Fountain S, Daskalakis ZJ (2006) A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clinical Neurophysiology 117:2584-2596.

Fossati P, Radtchenko A, Boyer P (2004) Neuroplasticity: From MRI to depressive symptoms. European Neuropsychopharmacology 14 Supplement 5:S503-510.

Foy MR, Stanton ME, Levine S, Thompson RF (1987) Behavioral stress impairs long-term potentiation in rodent hippocampus. Behavioral and Neural Biology 48:138-149.

Frantseva MV, Fitzgerald PB, Chen R, Moller B, Daigle M, Daskalakis ZJ (2008) Evidence for impaired long-term potentiation in schizophrenia and its relationship to motor skill learning. Cerebral Cortex 18:990-996.

Fratello F, Veniero D, Curcio G, Ferrara M, Marzano C, Moroni F, Pellicciari MC, Bertini M, Rossini PM, De Gennaro L (2006) Modulation of corticospinal excitability by paired associative stimulation: Reproducibility of effects and intraindividual reliability. Clinical Neurophysiology 117:2667-2674.

Fregni F, Boggio PS, Nitsche MA, Rigonatti SP, Pascual-Leone A (2006) Cognitive effects of repeated sessions of transcranial direct current stimulation in patients with depression. Depression and Anxiety 23:482-484.

Fregni F, Pascual-Leone A (2007) Technology insight: Noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. Nature Clinical Practice Neurology 3:383-393.

Frey S, Bergado-Rosado J, Seidenbecher T, Pape HC, Frey JU (2001) Reinforcement of early long-term potentiation (early-LTP) in dentate gyrus by stimulation of the basolateral amygdala: Heterosynaptic induction mechanisms of late-LTP. Journal of Neuroscience 21:3697-3703.

Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, Lu B (2010) Direct current stimulation promotes BDNF-dependent synaptic plasticity: Potential implications for motor learning. Neuron 66:198-204.

Frodl T, Schaub A, Banac S, Charypar M, Jäger M, Kümmler P, Bottlender R, Zetzsche T, Born C, Leinsinger G, Reiser M, Möller H-Jr, Meisenzahl EM (2006) Reduced hippocampal volume correlates with executive dysfunctioning in major depression. Journal of Psychiatry & Neuroscience 31:316-325.

Fung SJ, Webster MJ, Sivagnanasundaram S, Duncan C, Elashoff M, Weickert CS (2010) Expression of interneuron markers in the dorsolateral prefrontal cortex of the developing human and in schizophrenia. American Journal of Psychiatry 167:1479-1488.

Gallagher P, Robinson L, Gray J, Young A, Porter R (2007) Neurocognitive function following remission in major depressive disorder: Potential objective marker of response? Australian and New Zealand Journal of Psychiatry 41:54-61.

Galvez V, Alonzo A, Martin D, Loo CK (2013) Transcranial direct current stimulation treatment protocols: Should stimulus intensity be constant or incremental over multiple sessions? International Journal of Neuropsychopharmacology 16:13-21.

Gamboa OL, Antal A, Moliadze V, Paulus W (2010) Simply longer is not better: Reversal of theta burst after-effect with prolonged stimulation. Experimental Brain Research 204:181-187.

Gatt JM, Nemeroff CB, Dobson-Stone C, Paul RH, Bryant RA, Schofield PR, Gordon E, Kemp AH, Williams LM (2009) Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. Molecular Psychiatry 14:681-695.

Geddes J, Carney S, Cowen P, Goodwin G, Rogers R, Dearness K, Tomlin A, Eastaugh J, Freemantle N, Lester H, Harvey A, Scott A (2003) Efficacy and safety of electroconvulsive therapy in depressive disorders: A systematic review and meta-analysis. Lancet 361:799-808.

Gentner R, Wankerl K, Reinsberger C, Zeller D, Classen J (2008) Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: Evidence of rapid polarity-reversing metaplasticity. Cerebral Cortex 18:2046-2053.

Gerritsen L, Tendolkar I, Franke B, Vasquez AA, Kooijman S, Buitelaar J, Fernández G, Rijpkema M (2012) BDNF Val66Met genotype modulates the effect of childhood adversity on subgenual anterior cingulate cortex volume in healthy subjects. Molecular Psychiatry 17:597-603.

Goethals I, Audenaert K, Jacobs F, Van De Wiele C, Ham H, Pyck H, Vandierendonck A, Van Heeringen C, Dierckx R (2005) Blunted prefrontal perfusion in depressed patients performing the Tower of London task. Psychiatry Research - Neuroimaging 139:31-40.

Goggi J, Pullar IA, Carney SL, Bradford HF (2002) Modulation of neurotransmitter release induced by brain-derived neurotrophic factor in rat brain striatal slices in vitro. Brain Research 941:34-42.

Goosens KA, Sapolsky RM (2007) Stress and Glucocorticoid Contributions to Normal and Pathological Aging. In: Brain Aging: Models, Methods, and Mechanisms (Riddle, D. R., ed) Boca Raton (FL): CRC Press.

Gould E, McEwen BS, Tanapat P, Galea LAM, Fuchs E (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. Journal of Neuroscience 17:2492-2498.

Grant MM, Thase ME, Sweeney JA (2001) Cognitive disturbance in outpatient depressed younger adults: Evidence of modest impairment. Biological Psychiatry 50:35-43.

Gratacòs M, González JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X (2007) Brain-derived neurotrophic factor Val66Met and psychiatric disorders: Meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. Biological Psychiatry 61:911-922.

Groves JO (2007) Is it time to reassess the BDNF hypothesis of depression? Molecular Psychiatry 12:1079-1088.

Grunhaus L, Polak D, Amiaz R, Dannon PN (2003) Motor-evoked potential amplitudes elicited by transcranial magnetic stimulation do not differentiate between patients and normal controls. International Journal of Neuropsychopharmacology 6:371-378.

Hallett M (2000) Transcranial magnetic stimulation and the human brain. Nature 406:147-150.

Hallett M (2007) Transcranial magnetic stimulation: A primer. Neuron 55:187-199.

Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC (2013) The role of interneuron networks in driving human motor cortical plasticity. Cerebral Cortex 23:1593-1605.

Hamilton JP, Gotlib IH (2008) Neural substrates of increased memory sensitivity for negative stimuli in major depression. Biological Psychiatry 63:1155-1162.

Hamilton JP, Siemer M, Gotlib IH (2008) Amygdala volume in major depressive disorder: A meta-analysis of magnetic resonance imaging studies. Molecular Psychiatry 13:993-1000.

Hanajima R, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, Kanazawa I (1998) Paired-pulse magnetic stimulation of the human motor cortex: Differences among I waves. Journal of Physiology 509:607-618.

Hashimoto T, Arion D, Unger T, Maldonado-Avilés JG, Morris HM, Volk DW, Mirnics K, Lewis DA (2008) Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. Molecular Psychiatry 13:147-161.

Hayley S, Poulter MO, Merali Z, Anisman H (2005) The pathogenesis of clinical depression: Stressor- and cytokine-induced alterations of neuroplasticity. Neuroscience 135:659-678.

Hebb D (1949) The Organization of Behavior: A Neuropsychological Theory. New York, USA: Wiley.

Hoogendam JM, Ramakers GMJ, Di Lazzaro V (2010) Physiology of repetitive transcranial magnetic stimulation of the human brain. Brain Stimulation 3:95-118.

Huang YZ, Chen RS, Rothwell JC, Wen HY (2007) The after-effect of human theta burst stimulation is NMDA receptor dependent. Clinical Neurophysiology 118:1028-1032.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC (2005) Theta burst stimulation of the human motor cortex. Neuron 45:201-206.

Huang YZ, Rothwell JC, Edwards MJ, Chen RS (2008) Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. Cerebral Cortex 18:563-570.

Huang YZ, Rothwell JC, Lu CS, Chuang WL, Lin WY, Chen RS (2010) Reversal of plasticity-like effects in the human motor cortex. Journal of Physiology-London 588:3683-3693.

Huang YZ, Sommer M, Thickbroom G, Hamada M, Pascual-Leonne A, Paulus W, Classen J, Peterchev AV, Zangen A, Ugawa Y (2009) Consensus: New methodologies for brain stimulation. Brain Stimulation 2:2-13.

Hubl D, Nyffeler T, Wurtz P, Chaves S, Pflugshaupt T, Lüthi M, von Wartburg R, Wiest R, Dierks T, Strik WK, Hess CW, Müri RM (2008) Time course of blood oxygenation level-dependent signal response after theta burst transcranial magnetic stimulation of the frontal eye field. Neuroscience 151:921-928.

Hummel FC, Cohen LG (2005) Drivers of brain plasticity. Current Opinion in Neurology 18:667-674.

Iezzi E, Conte A, Suppa A, Agostino R, Dinapoli L, Scontrini A, Berardelli A (2008) Phasic voluntary movements reverse the aftereffects of subsequent theta-burst stimulation in humans. Journal of Neurophysiology 100:2070-2076.

Ilić NV, Sajić J, Mišković M, Krstić J, Milanović S, Vesović-Potić V, Ljubisavljević M, Ilić TV (2009) The efficacy of two protocols for inducing motor cortex plasticity in healthy humans - TMS study. General Physiology and Biophysics 28:228-234.

Ilić TV, Meintzschel F, Cleff U, Ruge D, Kessler KR, Ziemann U (2002) Short-interval paired-pulse inhibition and facilitation of human motor cortex: The dimension of stimulus intensity. Journal of Physiology 545:153-167.

Ilić TV, Ziemann U (2005) Exploring motor cortical plasticity using transcranial magnetic stimulation in humans. Annals of New York Academy of Sciences 1048:175-184.

Issa AM, Rowe W, Gauthier S, Meaney MJ (1990) Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. Journal of Neuroscience 10:3247-3254.

Iyer MB, Schleper N, Wassermann EM (2003) Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. Journal of Neuroscience 23:10867-10872.

Izquierdo A, Wellman CL, Holmes A (2006) Brief uncontrollable stress causes dendritic retraction in infralimbic cortex and resistance to fear extinction in mice. Journal of Neuroscience 26:5733-5738.

Jacobsen JPR, Mørk A (2006) Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. Brain Research 1110:221-225.

Javadi AH, Walsh V (2012) Transcranial direct current stimulation (tDCS) of the left dorsolateral prefrontal cortex modulates declarative memory. Brain Stimulation 5:231-241.

Johnston MV (2004) Clinical disorders of brain plasticity. Brain and Development 26:73-80.

Jung P, Ziemann U (2009) Homeostatic and nonhomeostatic modulation of learning in human motor cortex. Journal of Neuroscience 29:5597-5604.

Kalu UG, Sexton CE, Loo CK, Ebmeier KP (2012) Transcranial direct current stimulation in the treatment of major depression: A meta-analysis. Psychological Medicine 42:1791-1800.

Kanner A (2004) Structural MRI changes of the brain in depression. Clinical EEG and Neuroscience 35:46-52.

Kennedy SH, Evans KR, Krüger S, Mayberg HS, Meyer JH, McCann S, Arifuzzman AI, Houle S, Vaccarino FJ (2001) Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression. American Journal of Psychiatry 158:899-905.

Kessing LV (1998) Cognitive impairment in the euthymic phase of affective disorder. Psychological Medicine 28:1027-1038.

Kharade SM, Gumate DS, Naikwade NS (2010) A review: Hypothesis of depression and role of antidepressant drugs. International Journal of Pharmacy and Pharmaceutical Sciences 2:3-6.

Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman RS (2010) Nuclear factor-κB is a critical mediator of stress-impaired neurogenesis and depressive behavior. Proceedings of the National Academy of Sciences of the United States of America 107:2669-2674.

Kriváneková L, Lu MK, Bliem B, Ziemann U (2011) Modulation of excitability in human primary somatosensory and motor cortex by paired associative stimulation targeting the primary somatosensory cortex. European Journal of Neuroscience 34:1292-1300.

Krystal JH, Tolin DF, Sanacora G, Castner SA, Williams GV, Aikins DE, Hoffman RE, D'Souza DC (2009) Neuroplasticity as a target for the pharmacotherapy of anxiety disorders, mood disorders, and schizophrenia. Drug Discovery Today 14:690-697.

Kuczewski N, Porcher C, Gaiarsa JL (2010) Activity-dependent dendritic secretion of brain-derived neurotrophic factor modulates synaptic plasticity. European Journal of Neuroscience 32:1239-1244.

Kujirai K, Kujirai T, Sinkjaer T, Rothwell JC (2006) Associative plasticity in human motor cortex during voluntary muscle contraction. Journal of Neurophysiology 96:1337-1346.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD (1993) Corticocortical inhibition in human motor cortex. Journal of Physiology 471:501-519.

Kumar A, Jin Z, Bilker W, Udupa J, Gottlieb G (1998) Late-onset minor and major depression: Early evidence for common neuroanatomical substrates detected by using MRI. Proceedings of the National Academy of Sciences of the United States of America 95:7654-7658.

Kuo MF, Nitsche MA (2012) Effects of transcranial electrical stimulation on cognition. Clinical EEG and Neuroscience 43:192-199.

Landrø NI, Stiles TC, Sletvold H (2001) Neuropsychological function in nonpsychotic unipolar major depression. Neuropsychiatry, Neuropsychology, and Behavioral Neurology 14:233-240.

Lang N, Siebner HR, Ernst D, Nitsche MA, Paulus W, Lemon RN, Rothwell JC (2004) Preconditioning with transcranial direct current stimulation sensitizes the motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects. Biological Psychiatry 56:634-639.

Lang N, Siebner HR, Ward NS, Lee L, Nitsche MA, Paulus W, Rothwell JC, Lemon RN, Frackowiak RS (2005) How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? European Journal of Neuroscience 22:495-504.

Lazarus MS, Huang JZ (2011) Distinct maturation profiles of perisomatic and dendritic targeting GABAergic interneurons in the mouse primary visual cortex during the critical period of ocular dominance plasticity. Journal of Neurophysiology 106:775-787.

Lefaucheur JP, Antal A, Ahdab R, de Andrade DC, Fregni F, Khedr EM, Nitsche M, Paulus W (2008) The use of repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) to relieve pain. Brain Stimulation 1:337-344.

Letzkus JJ, Kampa BM, Stuart GJ (2007) Does spike timing-dependent synaptic plasticity underlie memory formation? Clinical and Experimental Pharmacology and Physiology 34:1070-1076.

Levinson AJ, Fitzgerald PB, Favalli G, Blumberger DM, Daigle M, Daskalakis ZJ (2010) Evidence of cortical inhibitory deficits in major depressive disorder. Biological Psychiatry 67:458-464.

Li Voti P, Conte A, Suppa A, Iezzi E, Bologna M, Aniello MS, Defazio G, Rothwell JC, Berardelli A (2011) Correlation between cortical plasticity, motor learning and BDNF genotype in healthy subjects. Experimental Brain Research 212:91-99.

Liebetanz D, Nitsche MA, Tergau F, Paulus W (2002) Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain 125:2238-2247.

Lisman JE (2001) Three Ca2+ levels affect plasticity differently: The LTP zone, the LTD zone and no man's land. Journal of Physiology 532:285.

Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, Morrison JH, McEwen BS (2006) Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. Journal of Neuroscience 26:7870-7874.

Litvak V, Zeller D, Oostenveld R, Maris E, Cohen A, Schramm A, Gentner R, Zaaroor M, Pratt H, Classen J (2007) LTP-like changes induced by paired associative stimulation of the primary somatosensory cortex in humans: Source analysis and associated changes in behaviour. European Journal of Neuroscience 25:2862-2874.

Lohr JB, May T, Caligiuri MP (2013) Quantitative assessment of motor abnormalities in untreated patients with major depressive disorder. Journal Affective Disorder 146:84-90.

Loo CK, Alonzo A, Martin D, Mitchell PB, Galvez V, Sachdev P (2012) Transcranial direct current stimulation for depression: 3-week, randomised, sham-controlled trial. British Journal of Psychiatry 200:52-59.

Loo CK, Sachdev P, Mitchell PB, Gandevia SC, Malhi GS, Todd G, Taylor JL (2008) A study using transcranial magnetic stimulation to investigate motor mechanisms in psychomotor retardation in depression. International Journal of Neuropsychopharmacology 11:935-946.

Lowy MT, Wittenberg L, Yamamoto BK (1995) Effect of acute stress on hippocampal glutamate levels and spectrin proteolysis in young and aged rats. Journal of Neurochemistry 65:268-274.

Lu Y, Christian K, Lu B (2008) BDNF: A key regulator for protein synthesis-dependent LTP and long-term memory? Neurobiology of Learning and Memory 89:312-323.

Lucassen PJ, Meerlo P, Naylor AS, van Dam AM, Dayer AG, Fuchs E, Oomen CA, Czéh B (2010) Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: Implications for depression and antidepressant action. European Neuropsychopharmacology 20:1-17.

Luine V, Martinez C, Villegas M, Magariños AM, McEwen BS (1996) Restraint stress reversibly enhances spatial memory performance. Physiology and Behavior 59:27-32.

Luine V, Villegas M, Martinez C, McEwen BS (1994) Repeated stress causes reversible impairments of spatial memory performance. Brain Research 639:167-170.

Lupien SJ, Evans A, Lord C, Miles J, Pruessner M, Pike B, Pruessner JC (2007) Hippocampal volume is as variable in young as in older adults: Implications for the notion of hippocampal atrophy in humans. NeuroImage 34:479-485.

Lupien SJ, McEwen BS (1997) The acute effects of corticosteroids on cognition: Integration of animal and human model studies. Brain Research Reviews 24:1-27.

Lupien SJ, McEwen BS, Gunnar MR, Heim C (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature Reviews Neuroscience 10:434-445.

MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT, Nahmias C, Young LT (2003) Course of illness, hippocampal function, and hippocampal volume in major depression. Proceedings of the National Academy of Sciences of the United States of America 100:1387-1392.

Magariños AM, McEwen BS (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Involvement of glucocorticoid secretion and excitatory amino acid receptors. Neuroscience 69:89-98.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. Journal of Neuroscience 20:9104-9110.

Malenka RC, Bear MF (2004) LTP and LTD: An embarrassment of riches. Neuron 44:5-21.

Malenka RC, Nicoll RA (1999) Long-term potentiation - A decade of progress? Science 285:1870-1874.

Mamounas LA, Blue ME, Siuciak JA, Altar CA (1995) Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. Journal of Neuroscience 15:7929-7939.

Manji HK, Drevets WC, Charney DS (2001) The cellular neurobiology of depression. Nature Medicine 7:541-547.

Manji HK, Quiroz JA, Sporn J, Payne JL, Denicoff K, A. Gray N, Zarate CA, Charney DS (2003) Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. Biological Psychiatry 53:707-742.

Marsden WN (2011) Stressor-induced NMDAR dysfunction as a unifying hypothesis for the aetiology, pathogenesis and comorbidity of clinical depression. Medical Hypotheses 77:508-528.

Martin DM, Alonzo A, Mitchell PB, Sachdev P, Gálvez V, Loo CK (2011) Fronto-extracephalic transcranial direct current stimulation as a treatment for major depression: An open-label pilot study. Journal of Affective Disorders 134:459-463.

Martin SJ, Grimwood PD, Morris RGM (2000) Synaptic plasticity and memory: An evaluation of the hypothesis. Annual Review of Neuroscience 23:649-711.

Masi G, Brovedani P (2011) The hippocampus, neurotrophic factors and depression: Possible implications for the pharmacotherapy of depression. CNS Drugs 25:913-931.

Mathers C, Boerma T, Ma Fat D (2008) The global burden of disease: 2004 Update. Switzerland: WHO Press.

Mayberg HS (1997) Limbic-cortical dysregulation: A proposed model of depression. Journal of Neuropsychiatry and Clinical Neurosciences 9:471-481.

McClintock SM, Husain MM, Greer TL, Cullum CM (2010) Association between depression severity and neurocognitive function in major depressive disorder: A review and synthesis. Neuropsychology 24:9-34.

McEwen BS (1999) Stress and hippocampal plasticity. Annual Review of Neuroscience 22:105-122.

McEwen BS, Magarinos AM (2001) Stress and hippocampal plasticity: Implications for the pathophysiology of affective disorders. Human Psychopharmacology 16:S7-S19.

Merriam EP, Thase ME, Haas GL, Keshavan MS, Sweeney JA (1999) Prefrontal cortical dysfunction in depression determined by Wisconsin Card Sorting Test performance. American Journal of Psychiatry 156:780-782.

Milad MR, Quinn BT, Pitman RK, Orr SP, Fischl B, Rauch SL (2005) Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. Proceedings of the National Academy of Sciences of the United States of America 102:10706-10711.

Miracle AD, Brace MF, Huyck KD, Singler SA, Wellman CL (2006) Chronic stress impairs recall of extinction of conditioned fear. Neurobiology of Learning and Memory 85:213-218.

Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S (2005) Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. Proceedings of the National Academy of Sciences of the United States of America 102:9371-9376.

Mitra R, Sapolsky RM (2008) Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. Proceedings of the National Academy of Sciences of the United States of America 105:5573-5578.

Moghaddam B (1993) Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: Comparison to hippocampus and basal ganglia. Journal of Neurochemistry 60:1650-1657.

Moghaddam B, Bolinao ML, Stein-Behrens B, Sapolsky R (1994) Glucocorticoids mediate the stress-induced extracellular accumulation of glutamate. Brain Research 655:251-254.

Montgomery SA, Äsberg M (1979) A new depression scale designed to be sensitive to change. British Journal of Psychiatry 134:382-389.

Müller JFM, Orekhov Y, Liu Y, Ziemann U (2007) Homeostatic plasticity in human motor cortex demonstrated by two consecutive sessions of paired associative stimulation. European Journal of Neuroscience 25:3461-3468.

Müller-Dahlhaus JFM, Orekhov Y, Liu Y, Ziemann U (2008) Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Experimental Brain Research 187:467-475.

Murakami T, Sakuma K, Nomura T, Uemura Y, Hashimoto I, Nakashima K (2008) Changes in somatosensory-evoked potentials and high-frequency oscillations after paired-associative stimulation. Experimental Brain Research 184:339-347.

Nagappan G, Zaitsev E, Senatorov Jr VV, Yang J, Hempstead BL, Lu B (2009) Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. Proceedings of the National Academy of Sciences of the United States of America 106:1267-1272.

Naismith SL, Hickie IB, Ward PB, Scott E, Little C (2006) Impaired implicit sequence learning in depression: A probe for frontostriatal dysfunction? Psychological Medicine 36:313-323.

Nestler EJ, Gould E, Manji H (2002) Preclinical models: Status of basic research in depression. Biological Psychiatry 52:503-528.

Nibuya M, Morinobu S, Duman RS (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. Journal of Neuroscience 15:7539-7547.

Nissen C, Holz J, Blechert J, Feige B, Riemann D, Voderholzer U, Normann C (2010) Learning as a model for neural plasticity in major depression. Biological Psychiatry 68:544-552.

Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F, Pascual-Leone A (2008) Transcranial direct current stimulation: State of the art. Brain Stimulation 1:206-223.

Nitsche MA, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, Henning S, Tergau F, Paulus W (2003) Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. Journal of Physiology 553:293-301.

Nitsche MA, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct stimulation. Journal of Physiology 527:633-639.

Nitsche MA, Paulus W (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 57:1899-1901.

Nitsche MA, Roth A, Kuo MF, Fischer AK, Liebetanz D, Lang N, Tergau F, Paulus W (2007) Timing-dependent modulation of associative plasticity by general network excitability in the human motor cortex. Journal of Neuroscience 27:3807-3812.

Nordanskog P, Dahlstrand U, Larsson MR, Larsson EM, Knutsson L, Johanson A (2010) Increase in hippocampal volume after electroconvulsive therapy in patients with depression: A volumetric magnetic resonance imaging study. Journal of ECT 26:62-67.

Normann C, Schmitz D, Furmaier A, Doing C, Bach M (2007) Long-term plasticity of visually evoked potentials in humans is altered in major depression. Biological Psychiatry 62:373-380.

Oberman L, Edwards D, Eldaief M, Pascual-Leone A (2011) Safety of theta burst transcranial magnetic stimulation: A systematic review of the literature. Journal of Clinical Neurophysiology 28:67-74.

Oldfield RC (1971) The assessment and analysis of handedness: The Edinburgh inventory. Neuropsychologia 9:97-113.

Oliveira JF, Zanão TA, Valiengo L, Lotufo PA, Benseñor IM, Fregni F, Brunoni AR (2013) Acute working memory improvement after tDCS in antidepressant-free patients with major depressive disorder. Neuroscience Letters 537:60-64.

Öngür D, Drevets WC, Price JL (1998) Glial reduction in the subgenual prefrontal cortex in mood disorders. Proceedings of the National Academy of Sciences of the United States of America 95:13290-13295.

Otani S (2002) Memory trace in prefrontal cortex: Theory for the cognitive switch. Biological Reviews of the Cambridge Philosophical Society 77:563-577.

Otani S, Daniel H, Roisin MP, Crepel F (2003) Dopaminergic Modulation of Long-term Synaptic Plasticity in Rat Prefrontal Neurons. Cerebral Cortex 13:1251-1256.

Otani S, Marshall CJ, Tate WP, Goddard GV, Abraham WC (1989) Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenger RNA synthesis immediately post-tetanization. Neuroscience 28:519-526.

Ottowitz WE, Dougherty DD, Savage CR (2002) The neural network basis for abnormalities of attention and executive function in major depressive disorder: Implications for application of the medical disease model to psychiatric disorders. Harvard Review of Psychiatry 10:86-99.

Paelecke-Habermann Y, Pohl J, Leplow B (2005) Attention and executive functions in remitted major depression patients. Journal of Affective Disorders 89:125-135.

Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung WH, Hempstead BL, Lu B (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 306:487-491.

Papouin T, Ladepeche L, Ruel J, Sacchi S, Labasque M, Hanini M, Groc L, Pollegioni L, Mothet JP, Oliet SH (2012) Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150:633-646.

Parker G, Hadzi-Pavlovic D, Boyce P (1989) Endogenous depression as a construct: A quantitative analysis of the literature and a study of clinician judgement. Australian and New Zealand Journal of Psychiatry 23:357-368.

Pascual-Leone A, Torres F (1993) Plasticity of the sensorimotor cortex representation of the reading finger in Braille readers. Brain 116:39-52.

Paul IA, Skolnick P (2003) Glutamate and depression: Clinical and preclinical studies. Annals of New york Academy of Sciences 1003:250-272.

Paulus W, Classen J, Cohen LG, Large CH, Di Lazzaro V, Nitsche M, Pascual-Leone A, Rosenow F, Rothwell JC, Ziemann U (2008) State of the art: Pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation. Brain Stimulation 1:151-163.

Pavlides C, Nivón LG, McEwen BS (2002) Effects of chronic stress on hippocampal long-term potentiation. Hippocampus 12:245-257.

Pellicciari MC, Miniussi C, Rossini PM, De Gennaro L (2009) Increased cortical plasticity in the elderly: Changes in the somatosensory cortex after paired associative stimulation. Neuroscience 163:266-276.

Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, Egan MF, Meyer-Lindenberg A, Weinberger DR (2004) The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. Journal of Neuroscience 24:10099-10102.

Pittenger C, Duman RS (2008) Stress, depression, and neuroplasticity: A convergence of mechanisms. Neuropsychopharmacology 33:88-109.

Pittenger C, Sanacora G, Krystal JH (2007) The NMDA receptor as a therapeutic target in major depressive disorder. CNS and Neurological Disorders - Drug Targets 6:101-115.

Porter RJ, Gallagher P, Thompson JM, Young AH (2003) Neurocognitive impairment in drug-free patients with major depressive disorder. British Journal of Psychiatry 182:214-220.

Potter-Nerger M, Fischer S, Mastroeni C, Groppa S, Deuschl G, Volkmann J, Quartarone A, Munchau A, Siebner HR (2009) Inducing homeostatic-like plasticity in human motor cortex through converging corticocortical inputs. Journal of Neurophysiology 102:3180-3190.

Price RB, Shungu DC, Mao X, Nestadt P, Kelly C, Collins KA, Murrough JW, Charney DS, Mathew SJ (2009) Amino acid neurotransmitters assessed by proton magnetic resonance spectroscopy: Relationship to treatment resistance in major depressive disorder. Biological Psychiatry 65:792-800.

Purdy SC, Kelly AS, Thorne PR (2001) Auditory evoked potentials as measures of plasticity in humans. Audiology and Neuro-Otology 6:211-215.

Quartarone A, Classen J, Morgante F, Rosenkranz K, Hallett M (2009) Consensus paper: Use of transcranial magnetic stimulation to probe motor cortex plasticity in dystonia and levodopa-induced dyskinesia. Brain Stimulation 2:108-117.

Quartarone A, Pisani A (2011) Abnormal plasticity in dystonia: Disruption of synaptic homeostasis. Neurobiology of Disease 42:162-170.

Radhu N, de Jesus DR, Ravindran LN, Zanjani A, Fitzgerald PB, Daskalakis ZJ (2013) A meta-analysis of cortical inhibition and excitability using transcranial magnetic stimulation in psychiatric disorders. Clinical Neurophysiology 124:1309-1320.

Rae C, Arul-Anandam P, Moffat K, Loo C (2009) Plugged in and switched on: The effect of acute transcranial direct current stimulation in healthy controls. Proc Int Soc Magn Reson Med 17:3460.

Rajji TK, Liu SK, Frantseva MV, Mulsant BH, Thoma J, Chen R, Fitzgerald PB, Daskalakis ZJ (2011) Exploring the effect of inducing long-term potentiation in the human motor cortex on motor learning. Brain Stimulation 4:137-144.

Rajkowska G, Miguel-Hidalgo JJ (2007) Gliogenesis and glial pathology in depression. CNS and Neurological Disorders - Drug Targets 6:219-233.

Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, Overholser JC, Roth BL, Stockmeier CA (1999) Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biological Psychiatry 45:1085-1098.

Rapoport JL, Gogtay N (2008) Brain neuroplasticity in healthy, hyperactive and psychotic children: Insights from neuroimaging. Neuropsychopharmacology 33:181-197.

Raymond CR (2007) LTP forms 1, 2 and 3: Different mechanisms for the "long" in long-term potentiation. Trends in Neurosciences 30:167-175.

Reid PD, Daniels B, Rybak M, Turnier-Shea Y, Pridmore S (2002) Cortical excitability of psychiatric disorders: Reduced post-exercise facilitation in depression compared to schizophrenia and controls. Australian and New Zealand Journal of Psychiatry 36:669-673.

Reis J, Schambra HM, Cohen LG, Buch ER, Fritsch B, Zarahn E, Celnik PA, Krakauer JW (2009) Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proceedings of the National Academy of Sciences of the United States of America 106:1590-1595.

Reymann KG, Frey JU (2007) The late maintenance of hippocampal LTP: Requirements, phases, 'synaptic tagging', 'late-associativity' and implications. Neuropharmacology 52:24-40.

Ridding MC, Uy J (2003) Changes in motor cortical excitability induced by paired associative stimulation. Clinical Neurophysiology 114:1437-1444.

Ridding MC, Ziemann U (2010) Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. Journal of Physiology 588:2291-2304.

Rioult-Pedotti MS, Friedman D, Donoghue JP (2000) Learning-induced LTP in neocortex. Science 290:533-536.

Ritchie LJ, De Butte M, Pappas BA (2004) Chronic mild stress exacerbates the effects of permanent bilateral common carotid artery occlusion on CA1 neurons. Brain Research 1014:228-235.

Rocher C, Spedding M, Munoz C, Jay TrsM (2004) Acute stress-induced changes in hippocampal/prefrontal circuits in rats: Effects of antidepressants. Cerebral Cortex 14:224-229.

Rosenkranz K, Rothwell JC (2006) Differences between the effects of three plasticity inducing protocols on the organization of the human motor cortex. European Journal of Neuroscience 23:822-829.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lucking CH, Maertens De Noordhout AL, Marsden CD, Murray NMF, Rothwell JC, Swash M, Tomberg C (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: Basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalography and Clinical Neurophysiology 91:79-92.

Rothwell J (2003) Techniques of transcranial magnetic stimulation. In: Plasticity in the Human Nervous System: Investigations with Transcranial Magnetic Stimulation (Boniface, S. and Ziemann, U., eds), pp 26-61. Cambridge, UK: Cambridge University Press.

Rothwell JC (1991) Physiological studies of electric and magnetic stimulation of the human brain. Electroencephalography and Clinical Neurophysiology Supplement 43:29-35.

Roy FD, Norton JA, Gorassini MA (2007) Role of sustained excitability of the leg motor cortex after transcranial magnetic stimulation in associative plasticity. Journal of Neurophysiology 98:657-667.

Rush AJ, Weissenburger JE (1994) Melancholic symptom features and DSM-IV. American Journal of Psychiatry 151:489-498.

Rush JA, Warden D, Wisniewski SR, Fava M, Trivedi MH, Gaynes BN, Nierenberg AA (2009) STAR*D: Revising conventional wisdom. CNS Drugs 23:627-647.

Sacher J, Neumann J, Fünfstück T, Soliman A, Villringer A, Schroeter ML (2012) Mapping the depressed brain: A meta-analysis of structural and functional

alterations in major depressive disorder. Journal of Affective Disorders 140:142-148.

Sadleir RJ, Vannorsdall TD, Schretlen DJ, Gordon B (2010) Transcranial direct current stimulation (tDCS) in a realistic head model. NeuroImage 51:1310-1318.

Sale MV (2009) Factors Influencing the Induction of Neuroplastic Changes in the Human Motor Cortex. Doctoral thesis from School of Molecular and Biomedical Science, University of Adelaide, Adelaide, S.A.

Sale MV, Ridding MC, Nordstrom MA (2007) Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Experimental Brain Research 181:615-626.

Sale MV, Ridding MC, Nordstrom MA (2008) Cortisol inhibits neuroplasticity induction in human motor cortex. Journal of Neuroscience 28:8285-8293.

Samii A, Wassermann EM, Ikoma K, Mercuri B, George MS, O'Fallon A, Dale JK, Straus SE, Hallett M (1996) Decreased postexercise facilitation of motor evoked potentials in patients with chronic fatigue syndrome or depression. Neurology 47:1410-1414.

Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, Krystal JH, Mason GF (2004) Subtype-specific alterations of γ-aminobutyric acid and glutamate in patients with major depression. Archives of General Psychiatry 61:705-713.

Sanacora G, Mason GF, Rothman DL, Behar KL, Hyder F, Petroff OAC, Berman RM, Charney DS, Krystal JH (1999) Reduced cortical γ-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. Archives of General Psychiatry 56:1043-1047.

Sapolsky RM (2003) Stress and Plasticity in the Limbic System. Neurochemical Research 28:1735-1742.

Sapolsky RM, Krey LC, McEwen BS (1984) Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. Proceedings of the National Academy of Sciences of the United States of America 81:6174-6177.

Sapolsky RM, Krey LC, McEwen BS (1986) The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. Endocrine Reviews 7:284-301.

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21:55-89.

Savitz J, Drevets WC (2009) Bipolar and major depressive disorder: Neuroimaging the developmental-degenerative divide. Neuroscience and Biobehavioral Reviews 33:699-771.

Schafer JL, Olsen MK (1998) Multiple imputation for multivariate missing-data problems: A data analyst's perspective. Multivariate Behavioral Research 33:545-571.

Scheurich A, Fellgiebel A, Schermuly I, Bauer S, Wölfges R, Müller MJ (2008) Experimental evidence for a motivational origin of cognitive impairment in major depression. Psychological Medicine 38:237-246.

Schoenfeld TJ, Gould E (2012) Stress, stress hormones, and adult neurogenesis. Experimental Neurology 233:12-21.

Schutter DJLG (2009) Antidepressant efficacy of high-frequency transcranial magnetic stimulation over the left dorsolateral prefrontal cortex in double-blind sham-controlled designs: A meta-analysis. Psychological Medicine 39:65-75.

Schwartz BL, Rosse RB, Veazey C, Deutsch SI (1996) Impaired motor skill learning in schizophrenia: Implications for corticostriatal dysfunction. Biological Psychiatry 39:241-248.

Seminowicz DA, Mayberg HS, McIntosh AR, Goldapple K, Kennedy S, Segal Z, Rafi-Tari S (2004) Limbic-frontal circuitry in major depression: A path modeling metanalysis. NeuroImage 22:409-418.

Sen S, Duman R, Sanacora G (2008) Serum brain-derived neurotrophic factor, depression, and antidepressant medications: Meta-analyses and implications. Biological Psychiatry 64:527-532.

Shah PJ, Ebmeier KP, Glabus MF, Goodwin GM (1998) Cortical grey matter reductions associated with treatment-resistant chronic unipolar depression: Controlled magnetic resonance imaging study. British Journal of Psychiatry 172:527-332.

Shajahan PM, Glabus MF, Gooding PA, Shah PJ, Ebmeier KP (1999a) Reduced cortical excitability in depression. Impaired post-exercise motor facilitation with transcranial magnetic stimulation. The British Journal of Psychiatry 174:449-454.

Shajahan PM, Glabus MF, Jenkins JA, Ebmeier KP (1999b) Postexercise motor evoked potentials in depressed patients, recovered depressed patients, and controls. Neurology 53:644-646.

Sheline YI (2003) Neuroimaging studies of mood disorder effects on the brain. Biological Psychiatry 54:338-352.

Sheline YI, Sanghavi M, Mintun MA, Gado MH (1999) Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. Journal of Neuroscience 19:5034-5043.

Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996) Hippocampal atrophy in recurrent major depression. Proceedings of the National Academy of Sciences of the United States of America 93:3908-3913.

Sibille E, Morris HM, Kota RS, Lewis DA (2011) GABA-related transcripts in the dorsolateral prefrontal cortex in mood disorders. International Journal of Neuropsychopharmacology 14:721-734.

Slotema CW, Blom JD, Hoek HW, Sommer IEC (2010) Should we expand the toolbox of psychiatric treatment methods to include repetitive transcranial magnetic stimulation (rTMS)? A meta-analysis of the efficacy of rTMS in psychiatric disorders. Journal of Clinical Psychiatry 71:873-884.

Smith MA, Makino S, Kvetnansky R, Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. Journal of Neuroscience 15:1768-1777.

Sobin C, Sackeim HA (1997) Psychomotor symptoms of depression. American Journal of Psychiatry 154:4-17.

Son H, Banasr M, Choi M, Chae SY, Licznerski P, Lee B, Voleti B, Li N, Lepack A, Fournier NM, Lee KR, Lee IY, Kim J, Kim JH, Kim YH, Jung SJ, Duman RS (2012) Neuritin produces antidepressant actions and blocks the neuronal and behavioral deficits caused by chronic stress. Proceedings of the National Academy of Sciences of the United States of America 109:11378-11383.

Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J (2002) Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. Journal of Physiology 543:699-708.

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J (2000) Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123:572-584.

Stefan K, Wycislo M, Classen J (2004) Modulation of associative human motor cortical plasticity by attention. Journal of Neurophysiology 92:66-72.

Stockmeier CA, Mahajan GJ, Konick LC, Overholser JC, Jurjus GJ, Meltzer HY, Uylings HB, Friedman L, Rajkowska G (2004) Cellular changes in the postmortem hippocampus in major depression. Biological Psychiatry 56:640-650.

Sweatt JD (2008) Long-term potentiation—A candidate cellular mechanism for information storage in the central nervous system. Molecular Mechanisms of Memory 4:295-326.

Sweeney JA, Kmiec JA, Kupfer DJ (2000) Neuropsychologic impairments in bipolar and unipolar mood disorders on the CANTAB neurocognitive battery. Biological Psychiatry 48:674-684.

Takahashi T, Kimoto T, Tanabe N, Hattori TA, Yasumatsu N, Kawato S (2002) Corticosterone acutely prolonged N-methyl-D-aspartate receptor-mediated Ca2+ elevation in cultured rat hippocampal neurons. Journal of Neurochemistry 83:1441-1451.

Tamura Y, Ueki Y, Lin P, Vorbach S, Mima T, Kakigi R, Hallett M (2009) Disordered plasticity in the primary somatosensory cortex in focal hand dystonia. Brain 132:749-755.

Tanaka J, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GC, Kasai H (2008) Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. Science 319:1683-1687.

Tannenbaum B, Tannenbaum GS, Sudom K, Anisman H (2002) Neurochemical and behavioral alterations elicited by a chronic intermittent stressor regimen: Implications for allostatic load. Brain Research 953:82-92.

Taylor JL, Loo CK (2007) Stimulus waveform influences the efficacy of repetitive transcranial magnetic stimulation. Journal of Affective Disorders 97:271-276.

Tehovnik EJ (1996) Electrical stimulation of neural tissue to evoke behavioral responses. Journal of Neuroscience Methods 65:1-17.

Teixeira AL, Barbosa IG, Diniz BS, Kummer A (2010) Circulating levels of brain-derived neurotrophic factor: Correlation with mood, cognition and motor function. Biomarkers in Medicine 4:871-887.

Teo JTH, Swayne OB, Rothwell JC (2007) Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. Clinical Neurophysiology 118:1649-1651.

Thickbroom GW, Byrnes ML, Edwards DJ, Mastaglia FL (2006) Repetitive paired-pulse TMS at I-wave periodicity markedly increases corticospinal excitability: A new technique for modulating synaptic plasticity. Clinical Neurophysiology 117:61-66.

Thirugnanasambandam N, Grundey J, Adam K, Drees A, Skwirba AC, Lang N, Paulus W, Nitsche MA (2011) Nicotinergic impact on focal and non-focal neuroplasticity induced by non-invasive brain stimulation in non-smoking humans. Neuropsychopharmacology 36:879-886.

Thompson M, Weickert CS, Wyatt E, Webster MJ (2009) Decreased glutamic acid decarboxylase67 mRNA expression in multiple brain areas of patients with schizophrenia and mood disorders. Journal of Psychiatric Research 43:970-977.

Thompson Ray M, Weickert CS, Wyatt E, Webster MJ (2011) Decreased BDNF, trkB-TK+ and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. Journal of Psychiatry & Neuroscience 36:195-203.

Todd G, Flavel S, Ridding M (2009) Priming theta-burst repetitive transcranial magnetic stimulation with low- and high-frequency stimulation. Experimental Brain Research 195:307-315.

Todd G, Kimber TE, Ridding MC, Semmler JG (2010) Reduced motor cortex plasticity following inhibitory rTMS in older adults. Clinical Neurophysiology 121:441-447.

Tsvetkov E, Shin RM, Bolshakov VY (2004) Glutamate uptake determines pathway specificity of long-term potentiation in the neural circuitry of fear conditioning. Neuron 41:139-151.

Ueno S, Tashiro T, Harada K (1988) Localized stimulation of neural tissues in the brain by means of a paired configuration of time-varying magnetic fields. Journal of Applied Physics 64:5862-5864.

Vaidya VA, Duman RS (2001) Depression - Emerging insights from neurobiology. British Medical Bulletin 57:61-79.

Vakili K, Pillay SS, Lafer B, Fava M, Renshaw PF, Bonello-Cintron CM, Yurgelun-Todd DA (2000) Hippocampal volume in primary unipolar major depression: A magnetic resonance imaging study. Biological Psychiatry 47:1087-1090.

Vercammen A, Rushby JA, Loo C, Short B, Weickert CS, Weickert TW (2011) Transcranial direct current stimulation influences probabilistic association learning in schizophrenia. Schizophrenia Research 131:198-205.

Verhagen M, Van Der Meij A, Van Deurzen PM, Janzing J, Arias-Vásquez A, Buitelaar J, Franke B (2010) Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: Effects of gender and ethnicity. Molecular Psychiatry 15:260-271.

Vernet M, Bashir S, Yoo WK, Oberman L, Mizrahi I, Ifert-Miller F, Beck CJ, Pascual-Leone A (2013) Reproducibility of the effects of theta burst stimulation on motor cortical plasticity in healthy participants. Clinical Neurophysiology (in press).

Vetencourt JFM, Sale A, Viegi A, Baroncelli L, De Pasquale R, O'Leary OF, Castrén E, Maffei L (2008) The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science 320:385-388.

Videbech P, Ravnkilde B, Pedersen AR, Egander A, Landbo B, Rasmussen NA, Andersen F, Stødkilde-Jørgensen H, Gjedde A, Rosenberg R (2001) The Danish PET/depression project: PET findings in patients with major depression. Psychological Medicine 31:1147-1158.

Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basáñez MG, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabé E, Bhalla K, Bhandari B, Bikbov B, Bin Abdulhak A, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie

BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, De Leo D, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD (2013) Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2163-2196.

Vouimba RM, Muñoz C, Diamond DM (2006) Differential effects of predator stress and the antidepressant tianeptine on physiological plasticity in the hippocampus and basolateral amygdala. Stress 9:29-40.

Vythilingam M, Vermetten E, Anderson GM, Luckenbaugh D, Anderson ER, Snow J, Staib LH, Charney DS, Bremner JD (2004) Hippocampal volume, memory, and cortisol status in major depressive disorder: Effects of treatment. Biological Psychiatry 56:101-112.

Wagner S, Doering B, Helmreich I, Lieb K, Tadic A (2012) A meta-analysis of executive dysfunctions in unipolar major depressive disorder without psychotic symptoms and their changes during antidepressant treatment. Acta Psychiatrica Scandinavica 125:281-292.

Wagner T, Valero-Cabre A, Pascual-Leone A (2007) Noninvasive human brain stimulation. Annual Review of Biomedical Enginerring 9:527-565.

Walsh ND, Seal ML, Williams SCR, Mehta MA (2009) An investigation of cognitive 'branching' processes in major depression. BMC Psychiatry 9:69.

Wang XB, Yang Y, Zhou Q (2007) Independent expression of synaptic and morphological plasticity associated with long-term depression. Journal of Neuroscience 27:12419-12429.

Wankerl K, Weise D, Gentner R, Rumpf JJ, Classen J (2010) L-type voltage-gated Ca2+ channels: A single molecular switch for long-term potentiation/long-term depression-like plasticity and activity-dependent metaplasticity in humans. Journal of Neuroscience 30:6197-6204.

Warden D, Rush AJ, Trivedi MH, Fava M, Wisniewski SR (2007) The STAR*D project results: A comprehensive review of findings. Current Psychiatry Reports 9:449-459.

Wassermann EM (1998) Risk and safety of repetitive transcranial magnetic stimulation: Report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996.

Electroencephalography and Clinical Neurophysiology - Evoked Potentials 108:1-16.

Wellman CL (2001) Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. Journal of Neurobiology 49:245-253.

Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. Science 313:1093-1097.

Willner P (2005) Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52:90-110.

Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, Benecke R, Classen J (2003) A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. Journal of Neurophysiology 89:2339-2345.

Wolters A, Schmidt A, Schramm A, Zeller D, Naumann M, Kunesch E, Benecke R, Reiners K, Classen J (2005) Timing-dependent plasticity in human primary somatosensory cortex. Journal of Physiology 565:1039-1052.

Wong EYH, Herbert J (2006) Raised circulating corticosterone inhibits neuronal differentiation of progenitor cells in the adult hippocampus. Neuroscience 137:83-92.

Wong J, Hyde TM, Cassano HL, Deep-Soboslay A, Kleinman JE, Weickert CS (2010) Promoter specific alterations of brain-derived neurotrophic factor mRNA in schizophrenia. Neuroscience 169:1071-1084.

Yang J, Han H, Cui M, Wang L, Cao J, Li L, Xu L (2006) Acute behavioural stress facilitates long-term depression in temporoammonic-CA1 pathway. NeuroReport 17:753-757.

Yazaki-Sugiyama Y, Kang S, Cteau H, Fukai T, Hensch TK (2009) Bidirectional plasticity in fast-spiking GABA circuits by visual experience. Nature 462:218-221.

Yoshii A, Constantine-Paton M (2010) Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. Developmental Neurobiology 70:304-322.

Yu H, Chen ZY (2011) The role of BDNF in depression on the basis of its location in the neural circuitry. Acta Pharmacologica Sinica 32:3-11.

Yuen EY, Jiang Q, Chen P, Feng J, Yan Z (2008) Activation of 5-HT2A/C receptors counteracts 5-HT1A regulation of N-methyl-D-aspartate receptor channels in

pyramidal neurons of prefrontal cortex. Journal of Biological Chemistry 283:17194-17204.

Zafar N, Paulus W, Sommer M (2008) Comparative assessment of best conventional with best theta burst repetitive transcranial magnetic stimulation protocols on human motor cortex excitability. Clinical Neurophysiology 119:1393-1399.

Zakzanis KK, Leach L, Kaplan E (1998) On the nature and pattern of neurocognitive function in major depressive disorder. Neuropsychiatry, Neuropsychology and Behavioral Neurology 11:111-119.

Zanardini R, Gazzoli A, Ventriglia M, Perez J, Bignotti S, Maria Rossini P, Gennarelli M, Bocchio-Chiavetto L (2006) Effect of repetitive transcranial magnetic stimulation on serum brain derived neurotrophic factor in drug resistant depressed patients. Journal of Affective Disorders 91:83-86.

Zarate Jr C, MacHado-Vieira R, Henter I, Ibrahim L, Diazgranados N, Salvadore G (2010) Glutamatergic modulators: The future of treating mood disorders. Harvard Review of Psychiatry 18:293-303.

Zhang ZW (2006) Canadian association of neurosciences review: Postnatal development of the mammalian neocortex: Role of activity revisited. Canadian Journal of Neurological Sciences 33:158-169.

Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D (2004) Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. Journal of Neuroscience 24:1666-1672.

Ziemann U, Lönnecker S, Steinhoff BJ, Paulus W (1996) Effects of antiepileptic drugs on motor cortex excitability in humans: A transcranial magnetic stimulation study. Annals of Neurology 40:367-378.

Ziemann U, Siebner HR (2008) Modifying motor learning through gating and homeostatic metaplasticity. Brain Stimulation 1:60-66.

Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Annual Review of Physiology 64:355-405.