Memory Modulation by Offline Consolidation and Transcranial Direct Current Stimulation

A thesis presented for the degree of

Doctor of Philosophy

by

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Declaration

I, Amir Homayoun JAVADI ARJOMAND, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Amir Homayoun JAVADI ARJOMAND

March 2011

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I wish everybody would have a life as fortunate as mine, where you can wake up every morning and say, “I do not want anything more.”[*] I do not want anything more just because of the gifts that I have been given; the confidence and courage my dad gives me; the passion and faith my mum has in me; the love and support that my sister gives me; and the most welcoming cheerful brother-in-law. I love you all deeply and with all my heart. As always, nothing I have achieved could have been done without you. This thesis is an outcome of all the love and support that you gave me. I am ashamed that this is far too little compared to what you gave me.

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[*] Brest, M. (1998) *Meet Joe Black*, USA: Universal Pictures.

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Abstract

Two groups of experiments are discussed in this thesis, (a) procedural memory consolidation during sleep and wakefulness, to study the contribution of emotion in consolidation of procedural skill learning, and (b) memory modulation using electrical brain stimulation, to study the effects of long- and short-duration stimulation of left dorsolateral prefrontal cortex (DLPFC) on verbal episodic memory.

Memory consolidation; The first study showed that participants who were trained in a mirror tracing task with negative emotional stimuli benefited more compared to the participants who were trained with neutral or positive emotional stimuli. The second experiment aimed to investigate the modulatory effect of stimuli with emotional content in a modified serial reaction time task (SRTT). This experiment failed to achieve any main effect of emotional content, retention type, their interaction or their interaction with session number. The only significant effect was found for the session number in which participants showed significantly higher performance in the second session. It is more likely that this outcome is due to the training effects over blocks.

Brain stimulation; The first study showed that 20min anodal stimulation enhanced memory performance while the stimulation was delivered during the encoding phase, 20min cathodal stimulation impaired memory performance for the words that were encoded prior to the stimulation and impaired the recognition performance while it was delivered during the testing phase. The second study was similar to the first experiment with the exception that

stimulation was delivered for 1.6s for each presented word in three different conditions: no stimulation, early-stimulation and late-stimulation. Results showed that early stimulation has significantly stronger effects on the memory performance of the participants compared to no-stimulation and late-stimulation in both anodal and cathodal stimulation types. Results also showed that early anodal stimulation enhanced the memory performance and early cathodal stimulation impaired the memory performance.

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Preface

I began my PhD studies with the goal of studying the contribution of sleep to behaviour and cognition. Within the first couple of months I decided to work on memory consolidation through wakefulness and sleep. I ran experiments with two groups of participants (day and night groups) to study the effect of sleep on memory consolidation. The experiments were as follows in chronological order:

1. Memory consolidation of serial or parallel presentation of paired words; 50 subjects were tested in 4 groups of experimental conditions with different retention type (day/night) and stimuli presentation (serial/parallel), 2 sessions each (training/testing).
2. Memory consolidation of parallel presentation of paired abstract images; 53 subjects were tested in 4 groups of experimental conditions with different retention type (day/night) and stimuli presentation (serial/parallel), 2 sessions each (training/testing).
3. Transitive inference; A follow-up study of Ellenbogen and colleagues (2007) with complex networks; 27 subjects were tested in 2 groups of experimental conditions with different retention type (day/night), 2 sessions each (training/testing).
4. Memory consolidation of semantically related and unrelated word-pairs (Javadi & Walsh, 2009); 66 subjects were tested in 4 groups of experimental conditions with different retention type (day/night) and pair-type (semantically related/unrelated), 2 sessions each (training/testing).

5. Mirror tracing with embedded emotion (Javadi, Walsh, & Lewis, 2011); An experiment in which the contribution of emotion in procedural learning was studied. 99 subjects were tested in 9 groups of experimental conditions with different retention type (day/night/control) and emotional type (positive/negative/neutral), 2 sessions each (training/testing).
6. Serial reaction time task with embedded emotion (Javadi, Yang, Bellesi, Lewis, & Walsh, In preparation); It was a follow-up study of my previous study to study the extendibility of previous findings. 48 subjects were tested in 4 groups of experimental conditions with different retention type (day/night) and stimulus type (negative/neutral), 2 sessions each (training/testing) (Javadi, Yang, Bellesi, Lewis, & Walsh, 2010); In a separate study I used standard serial reaction time task with no emotional content with the aim of better understanding the results of the previous experiment. 30 subjects were tested in 2 groups of experimental conditions with different retention type (day/night), 2 sessions each (training/testing).

The first 3 experiments did not show clear robust effects. The 4th experiment was presented as a poster in the 23rd annual meeting of the "associated professional sleep societies". The 5th experiment was successful as well and it defined the theme of the rest of my studies on the contribution of passage of time (with or without sleep) on memory consolidation. Later I ran the 6th experiment to explore the extendibility of the findings of the 5th experiment. I am going to discuss the results of the last two experiments in the first part of this thesis.

Working in a group with highly sophisticated skills in brain stimulation as well as my engineering background, electronics and mechatronics engineering, drove me into studies with transcranial electrical stimulation and electroencephalography (EEG). I aimed at modulating long-term memory using transcranial direct current stimulation. I conducted two experiments with different stimulation protocols as follows:

1. Modulatory effect of Long-duration transcranial direct current stimulation (tDCS) (20min constant stimulation) on dorsolateral prefrontal cortex (DLPFC) on long-term verbal memory (Javadi & Walsh, Submitted); 34 subjects were tested in 2 groups of experimental conditions with different stimulation phase (encoding/retrieval), 3 sessions each with different stimulation types (anodal/cathodal/sham).

2. Modulatory effect of short-duration tDCS (1.6s per trial) on DLPFC on long-term verbal memory (Javadi, Cheng, & Walsh, submitted); 17 subjects were tested in 2 sessions with different stimulation types (anodal/cathodal).

Both the experiments were successful and showed significant modulatory effects of tDCS in verbal long-term memory. To address the optimal timing of administration of stimulation to effectively modulate long-term verbal memory I ran a pilot study with 9 participants in which I employed EEG to record their cortical activity when they were encoding words for long-term memory. I noted that for 4-second trials in which participants had to imagine a word in order to memorise it, the left DLPFC displayed higher activity in the first half of a trial than in the second half. I therefore postulated that tDCS will have a different effect on long-term memory depending on whether it is administered early or late on in a trial. I discuss the results of these two experiments in the 2nd part of this thesis.

Chapter 1. Introduction to Memory Consolidation and Sleep

The study of memory relates to three distinct phases: acquisition (encoding), consolidation and retrieval. *Acquisition* refers to the processes by which new information is encoded into a memory trace or representation, whereas *retrieval* refers to the subsequent recall of memory. This report focuses on *consolidation*, which refers to post-encoding processes that stabilise or enhance an initially weak and labile memory trace into a stable and enduring representation.

Consolidation is a term used to describe two processes, separated on the basis of distinct neurophysiological properties (Dudai, 2004). Synaptic consolidation refers to the stabilisation of *synaptic changes* or *plasticity* and takes place within minutes or hours after encoding (Born, Rasch, & Gais, 2006). *Systems consolidation* however, may take days, months or years to complete and is thought to involve the reorganisation of memory traces such that they become represented by different neural networks (Dudai, 2004). It is intended to focus on the systems consolidation, with particular emphasis on memories that are neutral or memories carrying emotional salience. This study area covers a vast quantity of literature with numerous sub-topics spanning over many years. However, although the significance of the investigative literature is without question, it is important to first describe the theory of human memory systems that has provided the basis for consolidation research.

Due to the diversity of human memory, a number of classification systems have been proposed, the most generally accepted of which involves the distinction between declarative and nondeclarative memory (Squire & Zola, 1996). *Declarative memory* relates to representations for facts (semantic memory) and events (episodic memory) that are subject to conscious recollection (Eichenbaum, 1997). Neural models of declarative memory highlight the importance of the medial temporal lobe (MTL) with particular emphasis placed on the hippocampus (Eichenbaum, 2000). Conversely, *nondeclarative memory* is unconscious and expressed through behavioural changes, which includes procedural memory such as the learning of skills or actions, as well as conditioning and priming (M. Walker, 2008). Nondeclarative memory appears to rely on striatal and cerebellar function rather than the MTL region (Doyon, Penhune, & Ungerleider, 2003; Squire, Knowlton, & Musen, 1993). As a broad domain, memory consolidation encompasses both declarative and nondeclarative memory systems. However, in contrast to the wealth of research demonstrating an influence of sleep upon the formation of declarative memory this thesis is concerned with nondeclarative memory consolidation, which has received less attention.

1.1 Memory

As a topic of psychological research, human memory has continued to attract curiosity, fascination and excitement over several decades. Despite an extensive wealth of literature, memory remains at the forefront of cognitive neuroscience, with investigators relentlessly contributing to the understanding of this complex phenomenon. Since this topic encompasses a substantial proportion of one's everyday function, it can by no means be considered a single entity. Accordingly, research has branched out in different directions, focusing on a variety of memory systems, and specific stages of the memory process. Figure 1-1 shows the outline of different types of memory.

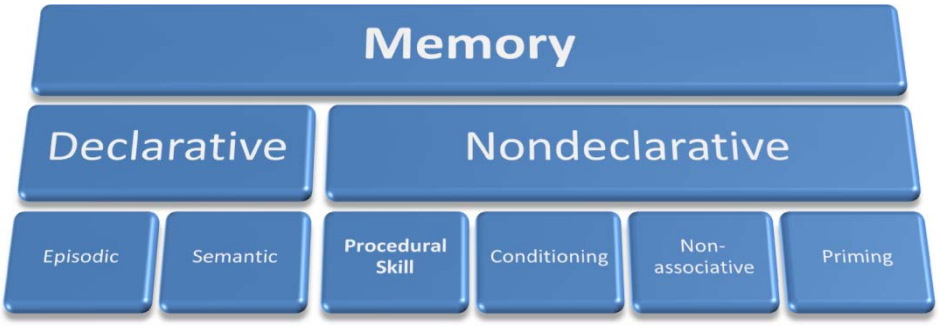


Figure 1-1. A diagram illustrating the distinction between the declarative and nondeclarative memory systems including their various subsystems (M. Walker & Stickgold, 2004).

1.1.1 Emotional Memory

Emotional arousal, like sleep, tends to exert a substantial influence upon declarative memory consolidation (McGaugh, 2004). A large body of evidence has shown arousing experiences to be far better remembered than non-arousing experiences (Bradley, Greenwald, Petry, & Lang, 1992). According to the memory modulation hypothesis (McGaugh, Cahill, & Roozendaal, 1996), the memory enhancing effect of emotional arousal is a result of the basolateral amygdala (BLA) modulating the encoding and consolidation processes occurring within the medial temporal lobe (MTL), including the hippocampus and related parahippocampal areas.

Although animal studies using drugs, neurotransmitters and brain lesions (Hatfield & McGaugh, 1999; Liang, McGaugh, & Yao, 1990; McGaugh & Roozendaal, 2002; Roozendaal & McGaugh, 1996) have provided evidence for the memory modulation hypothesis; this chapter and thesis will focus on research with humans. Human brain lesion studies have lent support to modulatory influences of the amygdala, although organic syndromes selectively impairing the amygdala are very rare (LaBar & Cabeza, 2006). Research has shown patients with unilateral and bilateral amygdala damage to be impaired on a number of emotional declarative memory tasks (Adolphs, Cahill, Schul, & Babinsky, 1997; Adolphs, Tranel, & Denburg, 2000). However, such studies are unable to determine whether memory deficits reflect an impairment of amygdala modulation processes or damage to the nearby MTL (Dolcos, LaBar, & Cabeza, 2004).

Human imaging research has advanced knowledge by helping to distinguish the effects of emotion at different stages of memory processing: acquisition, retrieval or consolidation. A number of functional imaging studies have found that amygdala activity during encoding correlates with long term memory for emotionally arousing but not neutral material (Canli,

Zhao, Brewer, Gabrieli, & Cahill, 2000). Additionally, a later study using positron emission tomography (PET) found amygdala influences upon the ipsilateral parahippocampal gyrus and ventrolateral prefrontal cortex to be increased during emotional relative to neutral film viewing, reflecting a memory modulating role of the amygdala during emotionally arousing experiences (Kilpatrick & Cahill, 2003). Moreover, a study by Pelletier et al. (Pelletier, Likhtik, Filali, & Pare, 2005) demonstrated that emotional arousal increased the firing rate of BLA neurons, persisting for up to two hours after encoding, which was proposed to facilitate emotional memory consolidation.

The subsequent memory paradigm (Paller & Wagner, 2002), used in event related fMRI, analyses encoding activity according to whether items are subsequently remembered or forgotten (LaBar & Cabeza, 2006). Using this method, researchers can identify the difference to memory effect which is an index of brain activity thought to reflect successful encoding operations (Paller & Wagner, 2002). Accordingly, an event related fMRI study found the difference to memory effect for emotional items to be larger than that of neutral items in the amygdala and MTL, and the correlation between the amygdala and MTL was also greater for the emotional difference to memory, providing yet further evidence for the memory modulation hypothesis (Dolcos, et al., 2004).

In comparison to a neutral tone, the effects of emotional arousal upon declarative memory increase as the delay between learning and retrieval increases (Hu, Stylos-Allan, & Walker, 2006). Research has been shown this to be the case over numerous periods of time including twenty minutes to one week (Kleinsmith & Kaplan, 1963), one day to two weeks (Anderson, Yamaguchi, Grabski, & Lacka, 2006) and immediate to twenty-four hours (Sharot & Phelps, 2004). Using the subsequent memory paradigm, Ritchey, Dolcos & Cabeza (2008) sought to demonstrate the temporal nature of emotional enhancement in memory with event related fMRI. The authors proposed that an analysis of brain activity during encoding could predict the persistence of subjects' memory for emotionally arousing and neutral pictures over short (20 minutes) and long (1 week) delays. Amygdala activity during encoding was a significantly better predictor of memory for emotional relative to neutral pictures, but this pattern was no different for memory over short and long delays. However, during encoding, greater levels of connectivity between the left amygdala and bilateral anterior MTL regions predicted memory performance for emotional pictures over long relative to short delays.

1.1.2 Emotional Arousal and Valence

Despite a wealth of literature focusing on emotional arousal, another dimension of emotion "valence" has also been subject to research in cognitive neuroscience, although not to the same extent. Emotional valence refers to how positive or negative an item is and has shown to be dissociable from arousal in a number of ways. Ritchey et al (2008) described arousal and valence as having respectively "direct" and "indirect" routes to emotional memory enhancement. As the modulatory influence of the amygdala upon the MTL can only occur through arousal it is known as the direct route. Contrastingly, the indirect route to emotional memory, namely changes in attentional and perceptual resources can be recruited by valence alone (Kensinger & Corkin, 2004). Accordingly, research has demonstrated that distinct neural networks process arousal and valence of words (Lewis, Critchley, Rotshtein, & Dolan, 2007). Using the subsequent memory paradigm it was reported that successful encoding of arousing words correlated with activity in the left amygdala and left hippocampus, whereas successful memory for non-arousing negatively valenced words was supported by activity in the left inferior pre-frontal cortex (PFC) and left hippocampus (Kensinger & Corkin, 2004). These results concur with previous research indicating that it is the level of arousal, rather than valence that modulates memory consolidation (Anderson & Sobel, 2003). Interestingly, Kesinger & Corkin (2004) also found that the PFC-hippocampal network which supported the formation of non-arousing negatively valenced words also supported the encoding of neutral words.

1.2 Sleep

In 1924, Jenkins & Dallenbach (1924) reported an improved retention of nonsense syllables following a period of sleep relative to a corresponding period of wakefulness. Since then, a substantial body of evidence has accumulated to support the hypothesis that sleep is actively involved in memory consolidation, known as sleep dependent memory processing. The pioneering findings of Jenkins & Dallenbach (1924) were consistently replicated in a number of studies, also demonstrating an enhanced recall of declarative memories following a period of sleep relative to wakefulness (Newman, 1939; Van Ormer, 1933). Over the following years, research continued to report sleep dependent memory effects, with authors using a wider range of study materials and more precise methodological controls to account for

confounding factors such as time of day and practice effects (Lovatt & Warr, 1968). However, it was not long before investigations moved away from studying consolidation across an entire period of sleep. Instead, researchers sought to expand on previous findings by linking memory consolidation to specific stages of sleep.

1.2.1 The Standard Sleep Cycle

Like memory, sleep is not a unitary phenomenon, but is broadly divided into two main stages. One stage is readily distinguishable due to its characteristic rapid eye movements (REM) and global muscle atonia, thus known as REM sleep (Peigneux, Laureys, Delbeuck, & Maquet, 2001), whilst the other is known as non rapid eye movement (NREM) sleep. These principal stages of sleep are highly dissociable on the basis of several features including, distinct electrophysiology and neurochemistry (Pace-Schott & Hobson, 2002), temporal regulation (Borbely & Achermann, 1999) and regional brain activity (Maquet et al., 1996). In primates and felines, NREM sleep can be subdivided into stages one to four which, in the same ascending order, correspond to deeper forms of sleep. In the electroencephalogram (EEG) stage one sleep is characterised by an oscillation at the frequency of approximately 9 to 12 Hz known as the alpha rhythm and sharp waves called vertex spikes (Rosenzweig, Breedlove, & Watson, 2005). Stage two sleep however, is recognisable through the presence of K complexes and sleep spindles (10-16Hz) (Born, et al., 2006). NREM stages three and four are collectively known as slow-wave sleep (SWS). During the transition into human SWS, slow oscillations (< 1 Hz) , delta activity (1 -4 Hz) and spindle activity predominate (Steriade, 2003). The occurrence of slow and delta wave activity is collectively known as slow wave activity. Throughout the night, NREM and REM sleep periods occur in ultradian cycles, each of which last approximately ninety minutes (Peigneux, et al., 2001).

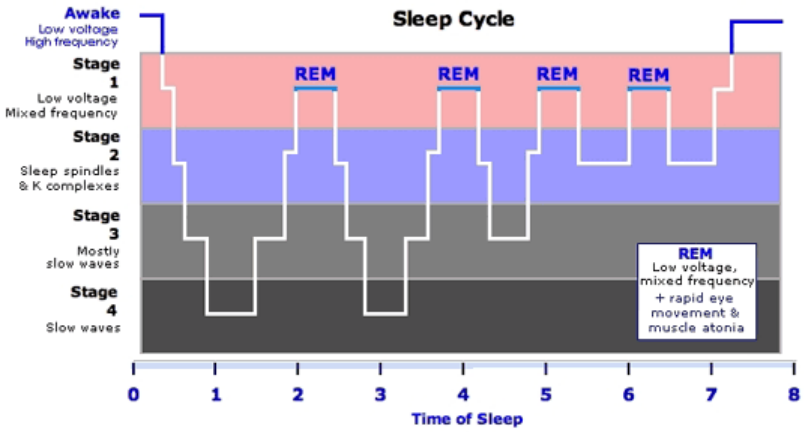


Figure 1-2. A diagram demonstrating the standard sleep cycle. As illustrated above, the early part of the night is dominated by slow wave sleep (SWS) with small periods of rapid eye movement (REM) sleep. However, the opposite pattern is observed in the later part of sleep which is characterised by more REM sleep and less SWS. This is relevant to the dual process model discussed shortly.

1.2.2 Sleep Stages and Memory Systems: Two Hypotheses

The Dual Process Hypothesis

Due to its links with vivid dreams and cognitive processing, initial research into sleep stages and consolidation focused on REM sleep. These studies produced mixed results, with some studies showing that REM sleep deprivation impaired declarative memory (Empson & Clarke, 1970; Tilley & Empson, 1978) and others showing no change in performance whatsoever (Greenberg, Pearlman, Schwartz, & Grossman, 1983). Studies of post-learning REM sleep changes also revealed inconsistent results (Castaldo, Krynicki, & Goldstein, 1974; De Koninck, Lorrain, Christ, Proulx, & Coulombe, 1989). Accordingly, the direction of research changed and investigators became concerned with comparing the contributions of SWS relative to REM sleep in declarative memory formation. Ekstrand and colleagues (Fowler, Sullivan, & Ekstrand, 1973; Yaroush, Sullivan, & Ekstrand, 1971) developed an experimental design to address this issue, which controlled for the stress linked to REM sleep deprivation. Subjects would learn a list of paired associates and then sleep in either the early or late half of the night, after which retrieval was tested. Due to a natural circadian rhythm, the first half of nocturnal sleep contains greater amounts of SWS and relatively little REM sleep, whereas the reverse effect is observed during the late half of nocturnal sleep when REM sleep prevails (see Figure 1-2). In using this method, cyclic patterns of sleep are not disturbed and circadian confounds can be excluded by examining memory over corresponding periods of wakefulness (Marshall & Born, 2007).

The results of this research suggested that memory for declarative word pairs is significantly better following a four hour retention period of early SWS-rich nocturnal sleep compared to an equivalent period of late REM-rich nocturnal sleep. These findings demonstrated, for the first time, the importance of SWS for declarative memory consolidation. Subsequent research has replicated these results for word pairs (Plihal & Born, 1997a), and spatial tasks (Plihal & Born, 1999), which also utilise declarative memory. This method was also extended to investigations of nondeclarative memory, which included studies of procedural memory tasks (Plihal & Born, 1997a; Smith, 1995) and word stem priming (Plihal & Born, 1999). As a result the 'dual process hypothesis' was formed (Peigneux, et al., 2001), which proposes that the beneficial effects of sleep upon memory are task dependent. Specifically, declarative memory appears to benefit from SWS, whereas retention periods containing REM rich late sleep facilitate the consolidation of nondeclarative memory. Throughout this research, the amount of time spent awake and in the lighter stages of sleep was very similar for subjects in the early or late sleep conditions. Thus, the double dissociation between procedural and declarative memory could not be attributed to these states (Born, et al., 2006).

However, the dual process hypothesis has been criticised on the basis of inconsistent research findings. One study found that a four hour retention period of REM-rich late sleep facilitated episodic declarative memory (Rauchs et al., 2004), whereas another found performance on a procedural visual texture discrimination task to improve across an early SWS-rich period of sleep (Gais, Plihal, Wagner, & Born, 2000). Furthermore, a study by Walker et al. (2002) reported associations between improved procedural motor skill performance and stage two NREM sleep, rather than REM sleep. Such equivocal findings present difficulty for the dual process hypothesis in linking specific forms of memory to particular stages of sleep.

Nevertheless, it is important to mention that study tasks cannot be purely declarative or nondeclarative, thus contamination from another memory system may occur (Peigneux, et al., 2001). For example, language learning requires nondeclarative procedural resources for articulating speech, whilst word selection requires aspects of declarative memory (M. Walker, 2008). Accordingly, declarative aspects of procedural skill learning may be consolidated during slow wave sleep, whilst procedural associations used during encoding in a declarative task may be strengthened by REM sleep, ultimately improving performance. Nevertheless, the dual process hypothesis supports an opposite role for SWS and REM sleep in the consolidation of specific forms of memory, but does not consider that the two sleep stages may offer complimentary contributions to memory processing (Ficca & Salzarulo, 2004).

The Two-Step Hypothesis

Alternatively, the 'two step hypothesis' (Ambrosini & Giuditta, 2001; Giuditta et al., 1995), suggests that memory consolidation is equally reliant on SWS and REM sleep. Accordingly, a preliminary step involving the weakening of non-adaptive memory representations takes place in SWS, whilst during subsequent REM sleep, the remaining memory traces are stored again in a more efficient formation. The two-step hypothesis corresponds to the sequential appearance of SWS and REM sleep and distinct EEG wave forms that occur during these stages (Giuditta, et al., 1995).

Human behavioural research, although smaller in volume than research on the dual process hypothesis, has provided some evidence in support of the two-step hypothesis. Stickgold James, & Hobson (2000a) reported that a visual texture discrimination task was facilitated across an entire period of sleep, rather than an interval of SWS or REM sleep. Similarly, although performance gains in visual texture discrimination were associated with SWS, Gais et al. (2000) found even greater enhancements after a whole night of sleep. Moreover, Stickgold et al. (2000) found that performance improvements in a visual search task were correlated to both the amount of SWS and REM sleep in the first and last quarter of the night respectively. The authors here also discuss a possibility of a two-step model, with equally important roles of SWS and REM sleep for memory consolidation. However, the research discussed above has involved nondeclarative, rather than declarative memory tasks. Further investigations into both forms of memory are required in order to strengthen the validity of this hypothesis.

1.2.3 Theories of Sleep Dependent Consolidation

A variety of memory systems are dependent on diverse cortical and subcortical networks. It is therefore unlikely that these systems rely on a 'stand alone' neural mechanism, or sleep stage physiology for their modulation (M. Walker, 2009). Currently, two theories of sleep dependent consolidation of declarative memory have been put forward to account for the overnight facilitation of retrieval consistently reported in the literature. These are known as (1) The hippocampal-neocortical dialogue (McClelland, McNaughton, & O'Reilly, 1995; Squire & Alvarez, 1995) and (2) The synaptic homeostasis hypothesis (Tononi & Cirelli, 2003, 2006)

The Hippocampal-Neocortical Dialogue

When encoding new information, one is faced with the problem of doing so without overwriting pre-existing knowledge. Accordingly, the process of consolidation for declarative

memories can be considered as an interaction between neocortical and MTL circuitry, particularly the hippocampus. This hypothesis states that information is initially encoded into both the hippocampal and neocortical networks. However, the hippocampus is believed to be of critical importance for the formation and retrieval of new declarative memories (M. Walker, 2009), When retrieving recently formed memory representations, the hippocampus integrates patterns of activation within distributed neocortical networks which were present at encoding and represent various features of an experience (Figure 1-3). In other words, the hippocampus 'fuses' neocortical memory traces to form a coherent memory representation (Eichenbaum, 2004; Morris et al., 2003). According to this model of memory consolidation, memory retrieval initially requires hippocampal 'binding' of neocortical representations. However, over time and by way of slow offline processes, incremental strengthening of cortico-cortical connections allows new memory representations to become independent of the hippocampus and become integrated into pre-existing long term memory networks across neocortical regions (McClelland, et al., 1995; Squire & Alvarez, 1995). Accordingly, neocortical structures become the storage site for consolidated episodic memories via cross-cortical connections and the hippocampus is no longer required for retrieval (M. Walker, 2009) (Figure 1-3).

This consolidation process is thought to involve covert reactivations of this hippocampal-neocortical network, which drives the progressive strengthening of cortico-cortical connections. Marr (1970) and McClelland et al. (1995) suggested that the hippocampus plays a vital role in reactivating neocortical networks, specifically during sleep. Hippocampal reactivation is believed to reinstate experience dependent patterns of neural activity in the neocortex, and thus stabilise connections between distributed cortical modules. A wealth of research (Sutherland & McNaughton, 2000; Wilson, 2002) (as described in the 'evidence' section below) has demonstrated learning associated neural reactivations in the hippocampus and in both hippocampal-cortical and cortico-cortical networks during sleep, particularly SWS.

Hippocampal reactivations are reported to occur mostly during bursts of activity in the EEG known as sharp wave ripple events (Kudrimoti, Barnes, & McNaughton, 1999). Sharp waves are fast depolarising potentials generated in CA3 which are superimposed upon a high frequency local field potential (100-300Hz) generated in CA1 known as ripple activity (Born, et al., 2006). Since sharp wave ripples have been found to occur in temporal correlation with thalamo-neocortical spindle activity (Siapas & Wilson, 1998), it is quite likely that sharp wave ripple activity may not only increase synaptic strength in the hippocampus, but also direct consolidation processes in relevant neocortical regions (Frankland & Bontempi, 2005).

Moreover, it is thought that the rhythmic processes underlying declarative consolidation are driven by the synchronising effects of slow oscillations which dominate SWS (McClelland, et al., 1995).

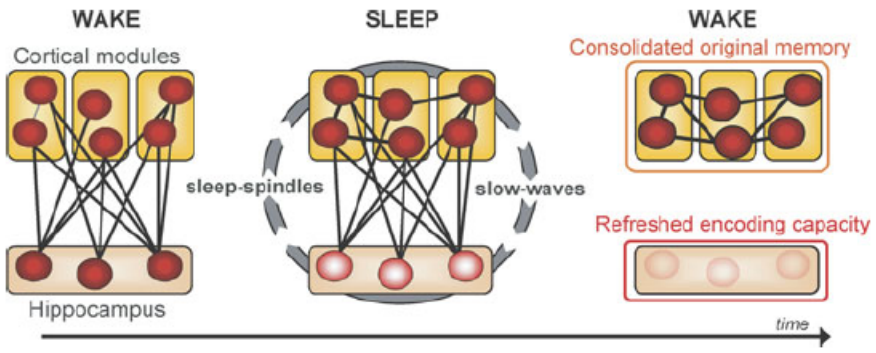


Figure 1-3. A diagram illustrating the hippocampal-neocortical dialogue theory of sleep dependent memory consolidation for declarative memory. Initial retrieval of new declarative memories is highly dependent on the hippocampus. The hippocampus binds together information from distributed neocortical modules representing various aspects of an experience. Driven by slow oscillations that characterise slow wave sleep (SWS), subsequent reactivation of this hippocampal-neocortical network strengthens cortico-cortical connections. Eventually this process allows new memories to become independent of the hippocampus and integrated within pre-existing neocortical networks through cross-cortical connections (M. Walker, 2009)

The Synaptic Homeostasis Hypothesis (Tononi & Cirelli, 2003, 2006)

In more recent years an alternative hypothesis for the role of SWS in memory consolidation has emerged. As described above, during SWS, neurons in the neocortex fire in waves of activity with frequencies of less than 4.5 Hz. This slow wave activity (SWA) increases linearly with previous wakefulness and gradually decreases during the course of sleep (Borbely & Achermann, 1999) Such a homeostatic regulation of SWA forms a critical part of the synaptic homeostasis hypothesis, as it may be linked to some restorative function of sleep for memory.

This hypothesis makes three main predictions which will be described in order. Firstly, it is stated that plastic processes such as learning that occur during wakefulness are accompanied by synaptic potentiation within a large proportion of cortical regions, which results in an overall increase in synaptic weight. Moreover, plastic changes occurring during wakefulness are more likely to result in long term potentiation (LTP) than in long term depression (LTD), resulting in a net potentiation of synaptic strength. Secondly, the hypothesis claims that the homeostatic regulation of SWA is correlated with the level of synaptic potentiation that has occurred during previous wakefulness. Specifically, larger amounts of cortical synaptic potentiation during waking experience will result in increased levels of SWA during

subsequent sleep. The third prediction of this hypothesis claims that rather than being a mere epiphenomenon of synaptic potentiation occurring during wakefulness, slow waves have a critical function to perform. It is suggested that through repeated sequences of depolarisation and hyperpolarisation, a process known as synaptic downscaling promotes a generalised proportional reduction in the strength of synapses until the total synaptic weight converging on each neuron returns to baseline levels. Thus, greater levels of learning or environmental interaction during waking experience lead to increased amounts of SWA during sleep as a higher degree of synaptic downscaling is required. If synapses are proportionally downscaled then the relative differences in the strength of cortical synapses, and therefore memory representations can be maintained. Accordingly, weak and inefficient synapses (i.e. the red synapse in Figure 1-4) are removed as downscaling reduces their strength below threshold and they become silent or ineffective. Therefore, when one wakes up cortical circuits maintain a representation of a previous experience, but with a more efficient trace affording improved recall. Moreover, as a result of downscaling, total synaptic weight is reduced to prevent synaptic 'over-potentiation' the next day, so new learning can take place and this cyclic process can begin again.

The hippocampal-neocortical theory also postulates sleep as critical for learning and memory, but through quite a different, if not a completely opposite mechanism to the synaptic homeostasis hypothesis. Accordingly, this model predicts that overnight improvements in memory result from patterns of neural activity present in the original learning episode being 'replayed' during SWS, which has been attributed to synaptic potentiation (Sejnowski & Destexhe, 2000). Conversely the synaptic homeostasis hypothesis suggests that sleep enhances learning and memory through a proportional downscaling of synapses impinging on each neuron, thus creating a more efficient memory trace. Therefore, uninterrupted synaptic plasticity and thus a lack of downscaling would eventually lead to an inability of the brain to acquire new information (Tononi & Cirelli, 2006).

Collectively, the main points of the synaptic homeostasis hypothesis are supported via a diverse wealth of research at the behavioural, molecular and neurophysiological level. However, numerous questions remain unanswered, for example, unlike the hippocampal-neocortical dialogue theory; the synaptic homeostasis hypothesis does not address its application to neural regions other than the cortex, such as the hippocampus where sleep rhythms are different, or other species (Tononi & Cirelli, 2006). Nevertheless this does not mean to say that the hippocampal-neocortical dialogue model is flawless; it shares a

fundamental problem with the synaptic homeostasis model in that the mnemonic role of REM sleep is not accounted for. Accordingly, the synaptic homeostasis hypothesis offers a new perspective for sleep and memory research, but as with the hippocampal-neocortical dialogue theory, despite a large body of supportive evidence, further investigation is unquestionably required to address a number of tentative points.

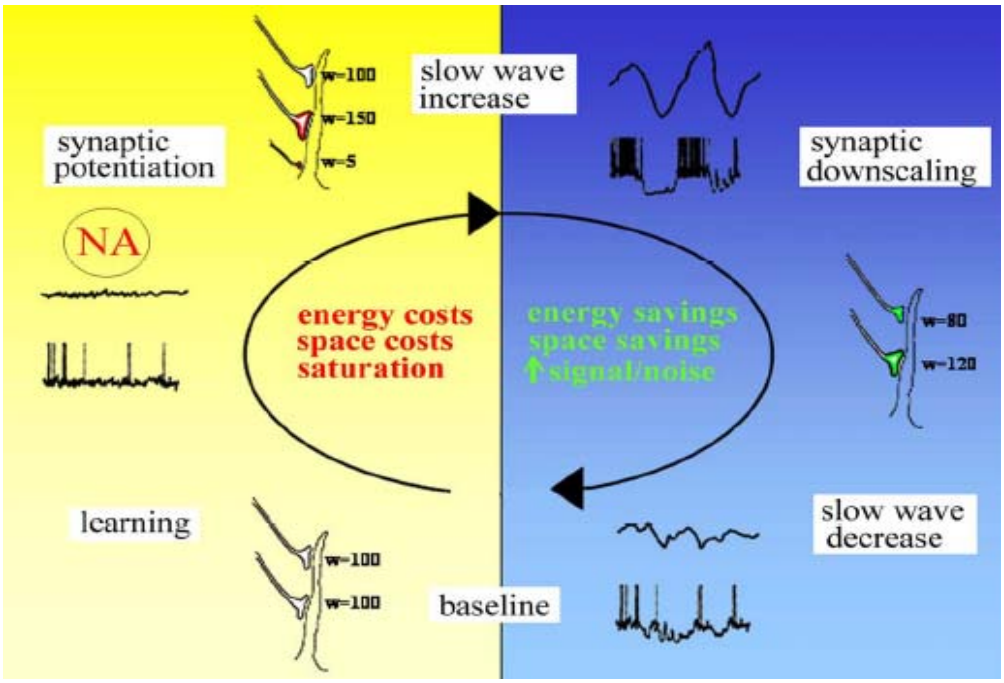


Figure 1-4. The Synaptic Homeostasis Hypothesis. This model postulates SWA as promoting a decrease of synaptic connections rather than an increase. Accordingly, during wakefulness plastic processes such as learning result in a net increase of synaptic strength within cortical circuits. It is proposed that the levels of SWA occurring during sleep are homeostatically regulated by the amount of synaptic potentiation that took place in previous wakefulness (i.e. more synaptic potentiation = more SWA). The role of SWA is to proportionally downscale the strength of synapses until the levels of synaptic weight converging on the same neuron reach baseline levels. Such synaptic downscaling would remove weak and inefficient synapses (red synapse) and leave behind a more efficient memory trace the next day. Moreover, synaptic strength would be recalibrated and this process can take place again (Tononi & Cirelli, 2006).

1.2.4 Sleep Dependent Emotional Memory Consolidation

Based on the evidence described above, it is reasonable to assume that emotionally arousing memories would also benefit from a period of sleep (Sterpenich et al., 2007). Using the remember/know (R/K) paradigm (Tulving, 1985), a study by Hu Stylos-Allan & Walker (Hu, et al., 2006) found that recognition accuracy for K judgements (but not R judgements) of emotional stimuli was greater after sleep, suggesting that sleep enhances the familiarity of

emotional memories but not their conscious recollection. Similarly, another study found that late rapid eye movement (REM) rich sleep improved retention of texts with emotional salience, suggesting a crucial role for REM in the formation of emotional memories (U. Wagner, Gais, & Born, 2001). Strikingly, this superior retention of emotional texts was maintained four years later (U. Wagner, Hallschmid, Rasch, & Born, 2006).

The Contributions of REM Sleep

Due to its unique biology, it has been suggested that REM sleep is a brain state that facilitates emotional memory consolidation (Hu, et al., 2006). Acetylcholine (ACh) has shown to be associated with the consolidation of declarative memories (McGaugh, 2004). However, unlike neutral declarative memory, research has shown that experimental enhancements of ACh levels boost the formation of emotional memory representations (Power, 2004). During REM sleep levels of hippocampal ACh release are four times higher than those in SWS and two times higher than waking levels (Marrosu et al., 1995). Therefore, the procholinergic REM state may result in a selective facilitation of emotional arousing experiences (Hu, et al., 2006). This variation in ACh levels during REM sleep may be caused, or reflected by changes in synchronised oscillatory activity between the amygdala and other limbic regions, including the hippocampus (Pare, Collins, & Pelletier, 2002). Since the memory modulation hypothesis postulates emotional memory enhancement as being a result of cooperation between these brain circuits, it is possible that this synchronised oscillatory activity during REM sleep modulates the neural plasticity required for emotional memory consolidation (Hu, et al., 2006). Accordingly, in a recent study using a nap paradigm, a sleep dependent benefit of emotional memory was not only correlated with the amount of time subjects spent in REM sleep, but also with the amount of right dominant prefrontal theta power during REM sleep (Nishida, Pearsall, Buckner, & Walker, 2009). It is claimed that correlated theta oscillations represent a functional process in which distinct brain circuits that originally encoded the information interact selectively, and support the strengthening of specific memory traces over distributed neural networks (Buzsáki, 2002). Therefore, REM sleep theta activity may constitute a large cooperation between connected limbic and prefrontal circuits (Jones & Wilson, 2005), the amount of which predicting subsequent emotional memory facilitation (Nishida, et al., 2009).

Imaging Studies

An fMRI study by Sterpenich et al. (2007) investigated the neural correlates of sleep dependent emotional memory consolidation. It was reported that the successful recollection of emotional valenced (positive & negative) versus neutral pictures elicited greater activation of the hippocampus and various cortical areas in subjects who slept after learning relative to those who were awake, suggesting the emotional meaning of the stimuli enhanced sleep dependent memory consolidation. Furthermore, unlike positive pictures, the recollection of negative pictures after sleep consistently elicited the hippocampal-neocortical response pattern, suggesting a superiority of negatively-valenced material for enhancing consolidation. However, this neurological contrast between negative and positive items may have been due to the negative items containing a higher degree of emotional arousal. Additionally, the specific contributions of SWS or REM sleep to these effects were not investigated. Interestingly, this study also reported that following sleep deprivation, subjects recruited an alternative amygdalo-cortical network during the recollection of negative items. It is therefore possible that during the cognitive repercussions of sleep deprivation, subjects utilised an unconventional method to maintain a hold of negative and potential dangerous memories (Sterpenich, et al., 2007). The activation of the amygdala in these subjects also indicates that positive and negative stimuli were not matched in terms of emotional arousal, a strong modulator of memory consolidation as described above.

The Interaction of Sleep and Arousal vs. Valence

More recently, research has begun to address the effects of sleep upon the consolidation of emotional memories separated in terms of arousal and valence. Using picture stimuli, Atienza & Cantero (Atienza & Cantero, 2008) reported that arousal exerted a stronger effect upon memory than valence alone, although there was no significant interaction between sleep and either arousal or valence. However, this study did not examine the specific effects of SWS or REM sleep upon these two dimensions of emotion.

Chapter 2. Off-Line Consolidation of Procedural Skill Learning is Enhanced by Negative Emotional Content

2.1 Abstract

In this study it is shown that the background presence of negative emotional content during the encoding phase of a classic procedural learning task led to significantly greater improvements in tracing accuracy across a 12 hour retention interval than the background presence of positive or neutral emotional content. It is studied this by training participants on a mirror rotation task in which they repeatedly traced the outlines of images which were positive, negative, or neutral in content. Following training, participants consolidated their new skill across 12 hours of daytime wakefulness, 12 hours including a night of sleep, or 24 hours including normal wake and sleep. In a subsequent retest, those learning in a negative context demonstrated enhanced mirror tracing accuracy when compared to those learning in

positive or neutral contexts. Furthermore, those who had consolidated across a night of sleep performed better than those who had only consolidated across wake, but the wake-related impairment was less apparent in participants who had encoded in a negative context. These novel findings show that the emotional context in which a procedural skill is learned can interact with subsequent off-line consolidation.

2.2 Introduction

One of the most potent factors known to modulate memory is emotion, and numerous studies across phylogeny have demonstrated that experiences which elicit emotion at the time of learning lead to superior subsequent recollection (Cahill, 2000; Cahill & Alkire, 2003; Maddock, 1999; McGaugh, 2004; McGaugh & Roozendaal, 2002; Phelps, 2004; Richardson, Strange, & Dolan, 2004). Furthermore, these effects of emotion on memory appear to increase as the delay between learning and testing increases (Kleinsmith & Kaplan, 1963; LaBar & Phelps, 1998; Levonian, 1972; Sharot & Phelps, 2004). For example, LaBar and Phelps (1998) showed that healthy participants remembered significantly more of the arousing words compared to neutral words after an hour of retention interval. Sharot and Phelps (2004) showed a similar effect after 24 hours. This has lead to the suggestion that emotional content may not only facilitate the initial encoding of information, but may also enhance the subsequent “offline” consolidation of memory processing (McGaugh, 2004; Payne, Stickgold, Swanberg, & Kensinger, 2008; Payne, Stickgold, Swanberg, Kensinger, & Ave, 2008; U. Wagner, et al., 2001; M. Walker, 2009). To date, the investigation of how emotional content impacts upon consolidation has focused principally on declarative memory (Brown & Kulik, 1977; Kensinger, Garoff-Eaton, & Schacter, 2006; Ochsner, 2000; Plihal & Born, 1997b). These studies have demonstrated an intimate relationship between emotional memory, stress hormones, and hippocampal-based learning, and the amygdala (Cahill, 2000; McGaugh & Roozendaal, 2002; Phelps, 2004) (for review see (McGaugh, 2004)). While these studies have deepened the understanding of the effects of emotion on consolidation, the possible modulatory role emotions may have on procedural learning and off-line consolidation remains unknown. How emotion modulates the consolidation of a procedural skill governed by memory systems known to be distinct from those involved in the types of declarative memory

that is more commonly associated with emotional influences is investigated in this thesis (Milner, 1985; Polster, Nadel, & Schacter, 1991; Squire, et al., 1993).

Mirror tracing is a procedural learning task in which subjects trace a figure with a stylus while viewing their hand, the stylus, and the figure reflected in a mirror. With practice, subjects trace the figure more quickly and make fewer errors (departures from the figure). Such sensorimotor skill tasks are used to study motor learning in patients with declarative memory problems due to amnesia (Milner, 1962), Alzheimer's Disease (Gabrieli, Corkin, Mickel, & Growdon, 1993), Huntington's disease (Gabrieli, Stebbins, Singh, Willingham, & Goetz, 1997) or patients with cerebellar dysfunction (Sanes, Dimitrov, & Hallett, 1990). Mirror tracing has also been used to study learning and the effects of distributed practice (Adams, 1987; Snoddy, 1920). In the current study, the mirror tracing task is embedded in negative, positive and neutral contexts in order to determine whether this emotional content influenced consolidation. The primary aim was to examine the effect of emotion on consolidation of the mirror tracing task.

Previous studies have shown offline consolidation of procedural memory without any emotion manipulation (Cohen & Robertson, 2007; Nishida & Walker, 2007; Press, Casement, Pascual-Leone, & Robertson, 2005; Robertson & Cohen, 2006; Robertson, Pascual-Leone, & Miall, 2004; M. Walker, et al., 2002) during wakeful retention intervals as well as an interval including sleep (Fischer, Hallschmid, Elsner, & Born, 2002; Laureys, Peigneux, Perrin, & Maquet, 2002; Maquet, et al., 1996; Plihal & Born, 1997b; R. Stickgold, James, & Hobson, 2000; M. Walker & Stickgold, 2004). Interestingly, within declarative memory, the emotional content of stimuli such as texts (U. Wagner, et al., 2001) or scenes (Nishida, et al., 2009; Payne, Stickgold, Swanberg, & Kensinger, 2008) has been shown to interact with sleep-dependent consolidation such that emotional items are more strongly consolidated across sleep than neutral items (for review see (M. Walker, 2009). As the secondary aim of the study, the contribution of sleep in the offline consolidation of a learnt skill, searching for interactions between this consolidation and the emotional valence presented during training is examined.

2.3 Materials and Methods

2.3.1 Participants

99 participants (mean age 25.63, SD 3.08) took part in the study in 9 groups, comprised of the combination of day/night/control and negative/positive/neutral emotional content conditions. Table 2-1 shows the number of participants in each group. All participants had normal or corrected-to-normal vision, and all were screened to exclude those with a history of neurological trauma or psychiatric disorder. They agreed to be drug, alcohol and caffeine free for 24 hours prior to and during the study period. All subjects were right-handed. No participant was taking any centrally acting medications. Informed consent according to the declaration of Helsinki was obtained from all participants. The local institutional ethics committee approved general procedures.

Table 2-1. Number of participants in each group; Participants were assigned to one of 9 different groups according to retention type (day/night/control) and the emotional content of the experiment (negative/positive/neutral).

Emotion	Negative			Positive			Neutral		
Retention type	Male	Female	Total	Male	Female	Total	Male	Female	Total
Day	5	7	12	5	7	12	6	6	12
Night	6	6	12	6	6	12	6	6	12
Control	5	4	9	4	5	9	5	4	9

2.3.2 Test Procedure

The experiment was composed of two sessions - training and testing - with either a 12 or 24 hour retention interval in between these. The timeline and design of the experiment is shown in Figure 2-1(a). Participants' activity during the retention interval and before the first session was monitored using log books in which they recorded sleep/wake times, consumed foods and drinks and the quality of the dreams they might have had at night.

At the beginning of each session participants were asked to complete a Stanford Sleepiness Scale (SSS) (Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973), a standard measure of subjective alertness, and to perform a simple finger tapping task to evaluate their alertness and reaction time (Zimmermann & Fimm, 1995).

The finger tapping task was composed of two different configurations, Figure 2-1(b). Participants were instructed to respond as fast and as accurately as possible. Finger tapping

continued for 70 repetitions in which the first 10 repetitions were discarded. Two participants who performed with less than 90% accuracy on the remaining 60 repetitions were withdrawn from further analysis. These participants are not included in Table 2-1. These two tasks were used as measures of the alertness and sleepiness of the participants in each group in each session.

The procedure for the first session is shown in Figure 2-1(c).

The second (testing) session consisted of tracing all 5 neutral stimulus shapes for once in a fixed order from 1 to 5. The final value of the performance for the second session is calculated as in session one, the mean of the performance of the last four tracings $P_{final} = (\Sigma P_{n-3..n})/4$ in which $n = 5$. All three groups of participants (day/night/control) traced the emotionally neutral stimuli, the last column of Figure 2-2, in the second session. Importantly, because all participants traced the same novel stimulus in this test session this assessment was comparable for those who had encoded with each of the three emotional types.

Participants were asked to fill in a sleep and activity-log diary after each session.

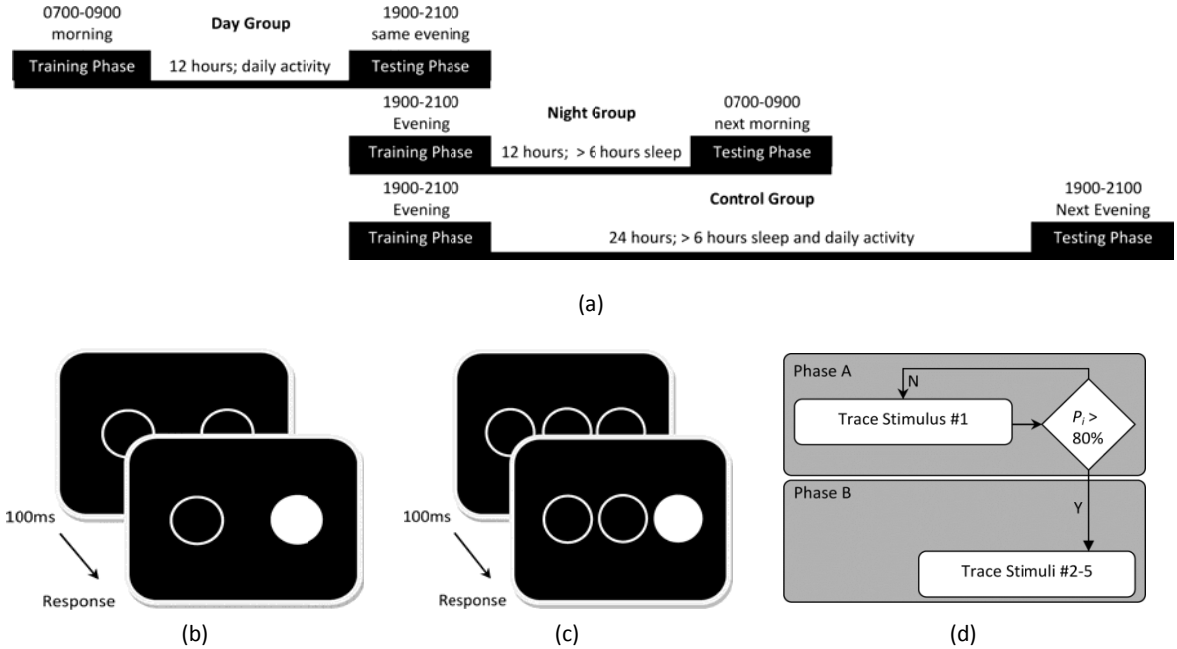


Figure 2-1. (a) The timing of the three groups; Participants were assigned to one of three different groups - day, night and control. The retention interval was 12 hours for the day and night groups and 24 hours for the control group. Participants were asked to leave the lab after the first session to have regular daily activity with no nap, for the day and control groups, and have at least 6 hours of nocturnal sleep, for the night and control groups. Participants could attend the test anytime between 7-9am and for the morning and evening sessions, respectively (b) shows the first configuration in which participants were asked to press a key corresponding to each of the circles using both index fingers. In this configuration participants were asked to press a key corresponding to each circle. (c) In this task participants were asked to use their right hand index, middle and ring fingers. (d) Procedure of the 1st session. The session was composed of two phases, phase A and phase B. Phase A repeated with a constant stimulus, stimulus #1, until the participant's performance passed 80% accuracy. Phase B was composed of four tracings, one each of stimuli #2-5. The mean value of the performance of the last four tracings was considered as the final performance value of the participant, P_{final}. P_i denotes the performance for the *i*th repetition. *n* denotes the total number of repetitions. $P_{final} = (\Sigma P_{n-3..n})/4$.

2.3.3 Stimuli

The stimuli had three different emotional contents, negative, positive and neutral. Each participant was assigned to one of these sets. Figure 2-2 shows all the stimuli in three different sets. To ensure that these differed significantly in valence, but not in arousal, 34 participants (healthy participants, age 18-26 years old, 18 female) rated the images for valence and arousal using the scales developed by Bradley and Lang (1994). Stimuli were presented serially for 1 s and participants were given 15 s to respond. The exact instruction given to the participants on the screen was as following:

> For about the next 2 minutes, you will be looking at different pictures projected on the screen, and you will be rating each picture in terms of how it made you feel while viewing it. Here are no right or wrong answers, so simply respond as honestly as you can. In this rating study you will be asked to rate the images on two scales, happy-unhappy scale and excited-calm scale. You will first see an image and then you are asked to rate on happy-unhappy scale and then excited-calm scale. The images will be shown for 1 s and then you have 15 s to respond to the rating.
>
> At one extreme of the happy vs. unhappy scale is happy, pleased, satisfied, contented or hopeful. If you felt completely happy while viewing the picture, you can indicate this by number 1. The other end of the scale is when you felt completely, unhappy, annoyed, unsatisfied, melancholic, despaired or bored. You can indicate feeling completely unhappy by number 9. The intermediate numbers also allow you to describe intermediate feelings of pleasure, by selecting the appropriate number. If you felt completely neutral, neither happy nor sad, press 5. The excited vs. calm dimension is the second type of feeling asked here. At one extreme of the scale is stimulated, excited, frenzied, jittery, wide-awake or aroused. If you felt completely aroused while viewing the picture, press 1. On the other hand, at the other end of the scale is relaxed, calm, sluggish, dull, sleepy or unaroused. You can indicate you felt completely calm by pressing 9. As with the happy-unhappy scale, you can represent intermediate levels by pressing an intermediate number. If you are not at all excited nor at all calm, press 5.

Independent sample t-tests revealed a significant difference between the valence scores of positive and negative images ($t(338) = 18.43$, $p < 0.001$) but no significant difference in the arousal score ($t(338) = 0.922$, $p = 0.35$). Table 2-2 shows the mean and SD of valence and arousal rating of each stimulus. Figure 2-3 shows histogram of number of responses for valence and arousal rating.

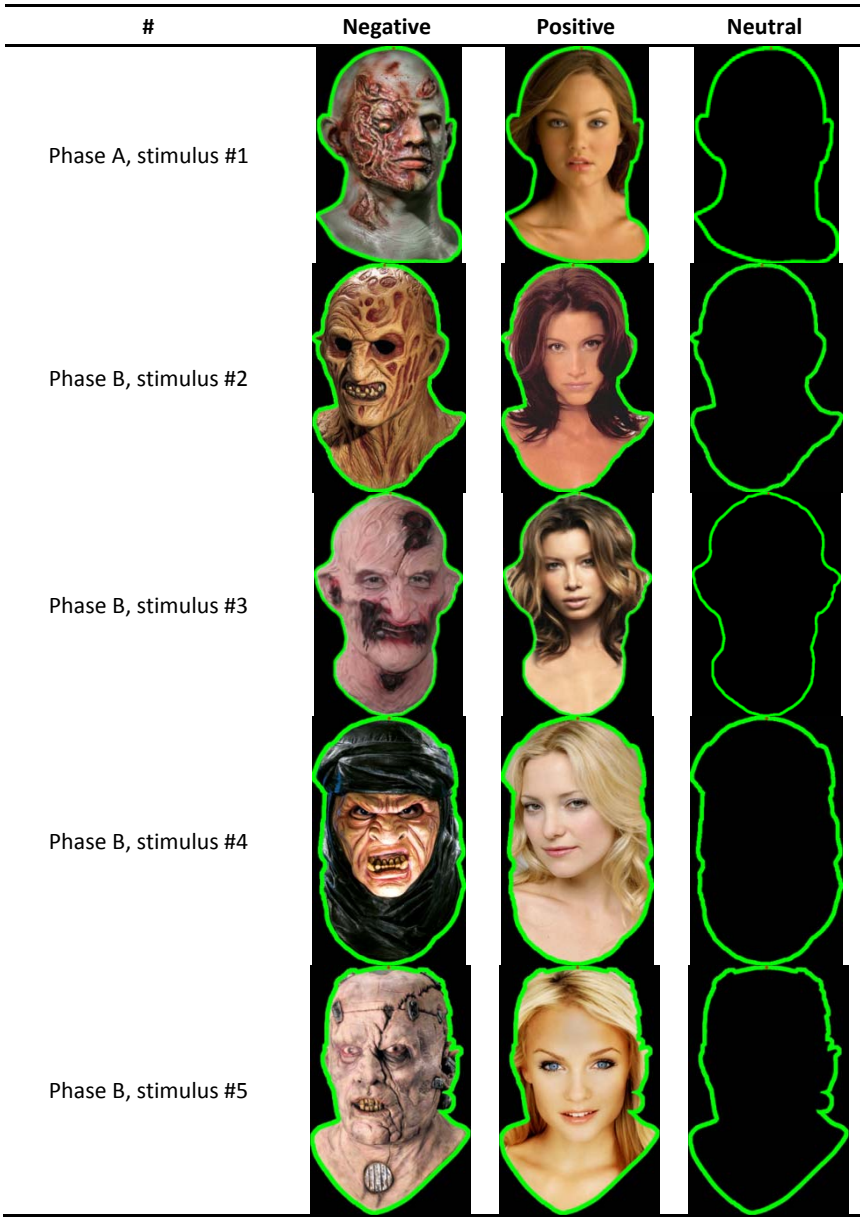


Figure 2-2. Different stimuli in three sets of emotional contents; Images of horror masks, female faces and blank content were used for negative, positive and neutral emotional contents, respectively. The border of the horror masks was used as the tracing template in all the three sets of emotional contents. In total 5 different tracing templates were used. The first template was just used for rehearsal, in phase A of the first session and the first trial of the second session as practice. Participants, in the first session, had to repeatedly trace the border of this template until their performance passed 80% accuracy. The tracing performance for this template was not considered in the overall performance of the participant. The mean value of performance of the remaining 4 templates was considered as the measure of the final ability of the participant in the mirror tracing task.

Table 2-2. Table showing the mean (SD) value of each stimulus' valence and arousal rating.

	Valence		Arousal	
#	Negative	Positive	Negative	Positive
Phase A, stimulus #1	7.14 (1.12)	3.25 (1.72)	3.62 (1.23)	4.62 (2.25)
Phase B, stimulus #2	7.52 (0.71)	2.87 (1.46)	3.75 (1.87)	4.51 (1.95)

Phase B, stimulus #3	7.29 (1.04)	2.62 (0.86)	3.87 (1.91)	4.11 (1.42)
Phase B, stimulus #4	7.39 (1.10)	2.25 (0.67)	3.62 (1.85)	3.62 (2.01)
Phase B, stimulus #5	6.25 (1.48)	3.01 (1.59)	4.12 (1.51)	3.43 (1.49)
Total	7.12 (1.39)	2.80 (1.22)	3.79 (1.48)	4.06 (1.67)

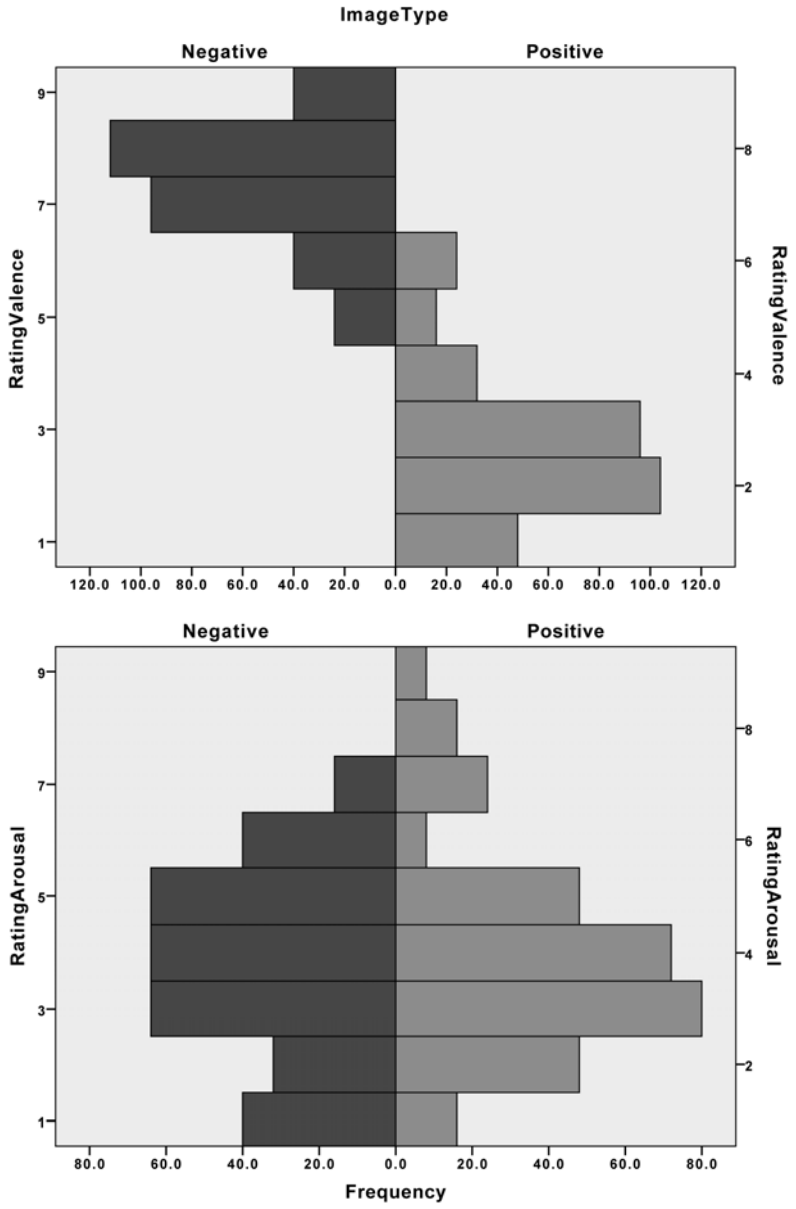


Figure 2-3. Histograms showing number of responses for arousal and valence rating of the images. The histogram on top shows valence rating and the histogram on bottom shows arousal rating.

The mirror tracing task was performed using a PC computer. Stimuli were presented on a 17" colour monitor, 75Hz refresh rate, subtending approximately 8 x 13 degrees of visual angle. Stimuli were presented on a black background and 53cm from participants' eyes. The border of the images was 8 pixels wide, ~0.25 degrees of visual angle. Participants used a regular

mouse to trace the border. The sampling frequency of mouse position was 100Hz. The movement of the pointer on the screen was in the opposite direction of the movement of the hand of the participant in both horizontal and vertical directions, rather than just the vertical direction as in mirror tracing tasks that use a mirror and mechanical apparatus. Performance was calculated as:

Performance (%) = 100 x (number of pixels traced on the green border / total number of traced pixels)

Eye tracking was used to trace participants' eye movements during both the first and second sessions. Eyes of participants in the control group were not tracked. The eyes of 8 of the 72 experimental participants were not tracked due to incompatibility of their glasses to the eye-tracker.

2.4 Results

A number of control analyses were performed to ensure that the between-group comparisons were not confounded by gender, alertness, or levels of initial performance.

2.4.1 Gender

Considering the masculine and feminine background of the stimuli for negative and positive emotional content, the performance of male and female participants is first compared. A 2x3 ANOVA with gender (female/male) and emotion (negative/positive/neutral) as between subject factors for performance improvement revealed no significant effects ($p > 0.5$ for all the comparisons). As there were no effects of gender, data from male and female participants were combined in all subsequent analyses. While some studies have reported effects of gender on memory for emotional information (Bremner et al., 2001; Burton et al., 2004; Cahill, Gorski, Belcher, & Huynh, 2004) (for review see (Hamann & Canli, 2004)), others have not. It may be that gender-related traits, rather than gender per se, influence memory (Cahill, et al., 2004) or that only particular paradigms elicit robust effects of gender on emotional memory.

2.4.2 Alertness

To examine the alertness of the participants at the beginning of each session, a 3x3x2 ANOVA with retention type (day/night/control), emotion (negative/positive/neutral) and session number (first and second sessions) as between subject factors for the Stanford Sleepiness Scale (SSS) rating and finger tapping performance was conducted. This revealed no significant effects, showing that neither sleepiness nor alertness differed across groups or sessions (p > 0.5 for all the comparisons).

Participants in the night and control groups reported two nocturnal sleeps, one the night before the first session and one in between the two sessions. Participants in the day group reported one nocturnal sleep before the first session. To ensure that these sleep episodes were of comparable duration prior to each session, a 5x3 ANOVA with retention type – day group (night before the 1st session)/night group (night before the 1st session)/night group (night in between the two sessions)/control group (night before the first session)/control group (night in between the two sessions – and emotion (negative/positive/neutral) as between subject factors for duration of nocturnal sleep acquired from sleep and activity-log diaries was conducted. This analysis showed no significant effect in any of the comparisons (p > 0.5 in all the comparisons). None of the participants reported nocturnal sleep of less than 6 hours, and none reported unpleasant dreams. Participants in day and control groups did not nap during the day in between their two sessions.

Overall, these measures show that participants in all the groups were well matched for alertness.

2.4.3 Initial performance and learning

The procedure of the first session required participants to repeat the rehearsal using a single stimulus until their performance passed a certain criterion (80% accuracy). To compare the participants' baselines on the learnt skill, final performance at the end of the first session, learning rate (measured as number of repetitions of the first phase of training needed to reach the 80% criteria), and tracing time (as the mean value of the tracing time of the last 4 tracing trials in each session) was examined using a 3x3 ANOVA with retention type (day/night/control) and emotion (negative/positive/neutral) as between subject factors. None of these analyses revealed a significant result (see Table 2-3), showing that the experimental groups were balanced for learning ability, or the level of skill obtained during training, 3 x 3

ANOVA with retention type and emotional content as independent variables ($p > 0.5$ in all the comparisons). Finally, examination of the relationship between learning rate and subsequent improvement between the first and second sessions revealed no significant correlation.

Table 2-3. Summary of performance (%) and tracing time (s) of participants in different groups at the end of the first session. The first number is the mean value and the second number is the standard deviation.

	Performance (%)			Tracing Time (s)		
Configuration	**Emotional Type**			**Emotional Type**		
Retention Type	**Negative**	**Positive**	**Neutral**	**Negative**	**Positive**	**Neutral**
Day	81.9, 4.8	80.2, 5.0	78.7, 5.8	48.6, 8.1	50.2, 6.8	48.2, 6.9
Night	79.4, 5.1	78.5, 5.3	81.4, 4.7	45.9, 7.5	47.8, 7.2	49.7, 6.6
Control	78.8, 5.0	81.1, 4.8	80.3, 5.1	49.1, 7.5	48.4, 8.2	52.3, 7.1

2.4.4 Performance accuracy

The main goal of the study was to investigate the effects of emotion at encoding on consolidation of a procedural task as a function of time. Mean change in performance accuracy from the first to second session is shown in Figure 2-4(a) for each experimental group. A 3x3 ANOVA with the retention type (day/night/control) and emotion (negative/positive/neutral) as between subject factors for performance improvement indicated an effect of retention type ($F(2, 90) = 5.10$, $p < 0.01$), and an effect of emotional content ($F(2, 90) = 4.97$, $p < 0.01$), but no significant effect of interaction ($F(4, 90) = 0.45$, $p > 0.2$). Post-hoc Bonferroni corrected two-tailed T-tests comparing performance changes across the stimulus type revealed greater improvements in those who had encoded in a negative context than in those who had encoded in positive ($t(64) = 2.98$, $p = 0.004$) or neutral ($t(64) = 2.84$, $p = 0.006$) contexts, Figure 2-4(b). Post-hoc Bonferroni corrected two-tailed T-tests for different retention intervals also showed significant improvements in night ($t(70) = 2.90$, $p = 0.005$) and control groups ($t(61) = 2.91$, $p = 0.005$), Figure 2-4(c).

Because participants traced the border of the novel stimuli just once in phase B of the 1st and 2nd sessions, Figure 2-1(d), the higher ability of the negative emotional group shows that they learnt the mirror tracing skill rather than simply memorising the shape of boarders which were traced in the 1st session. Improvements therefore relate to their skill in performing the task, rather than in retracing a learned pattern.

2.4.5 Tracing time

In addition to accuracy of performance, tracing time was also altered after the offline retention interval. To study the impact of the three emotional backgrounds and of retention type upon this change (2^{nd} session minus 1^{st} session), a 3x3 ANOVA with retention type (day/night/control) and emotion (negative/positive/neutral) as factors was conducted. This revealed a significant effect of retention type ($F(2, 90) = 3.32$, $p < 0.05$), with tracing time markedly impaired after consolidation across 12 hours of wake, but not across 12 or 24 hours including sleep, Figure 2-5(a). There was no effect of emotional content, and no interaction between retention type and emotion. Post-hoc Bonferroni corrected two-tailed T-tests revealed a difference between the tracing time change of negative group and positive ($t(64) = 2.16$, $p = 0.034$) and neutral ($t(64) = 2.08$, $p = 0.041$) groups, Figure 2-5(b). Tracing time was significantly more impaired across retention in the day group than in the night ($t(70) = 2.36$, $p = 0.021$) and control ($t(61) = 2.41$, $p = 0.019$) groups (using Bonferroni corrected two-tailed T-test), Figure 2-5(c)**.** Overall, these data show that the speed of mirror tracing is reduced after an off-line period of wake if the skill was learned in a neutral or positive context, but not if it was learned in a negative context.

Finally, examination of the relationship between performance improvement and tracing time impairment revealed a significant correlation (Day group $r = -0.61$, $p < 0.05$, Night group $r = -0.68$, $p < 0.01$, Control group $r = -0.66$, $p < 0.05$) showing that accuracy and speed changed together, e.g. the less accuracy improved the more speed decreased.

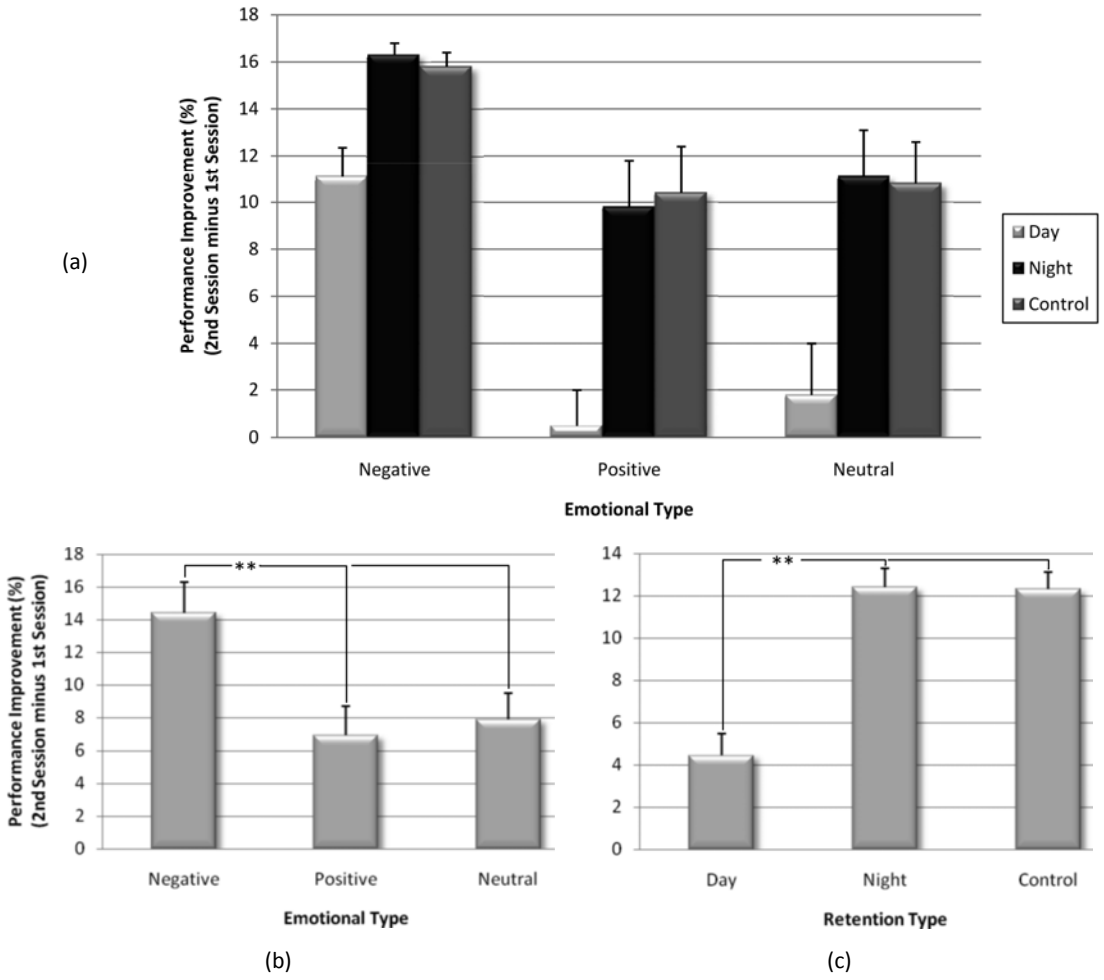


Figure 2-4. Performance improvements across an offline delay. (a-c) show data on accuracy. (a) Performance improvement from the first session to the second session, comparison between different emotional contents and different retention types. (b) Comparison between groups experiencing different background emotions at encoding shows that improvement on the procedural task is significantly greater when negative content is present at encoding compared to positive or neutral emotional content conditions. (c) comparison between the day, night and control groups shows that having a period of sleep (> 6 hours) benefits consolidation of procedural task more than having no sleep, although negative emotional content enhances the consolidation despite the retention type. ** $p < 0.01$, Error Bars represent one standard deviation (SD).

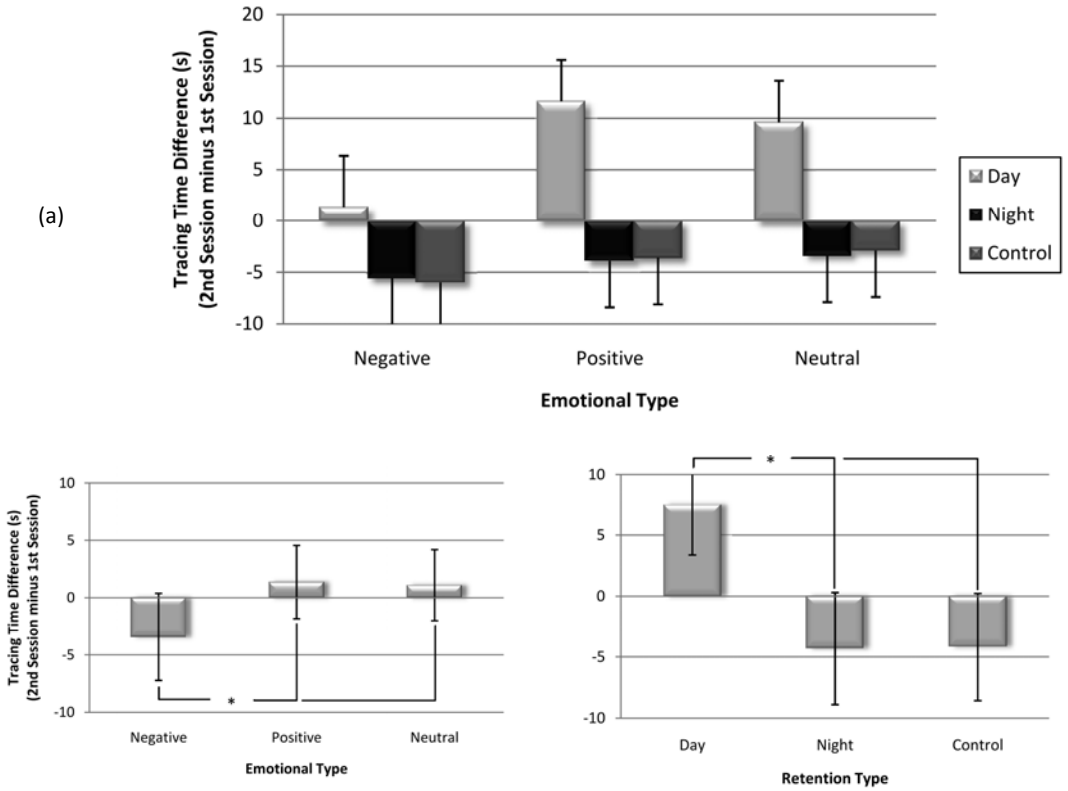


Figure 2-5. Performance improvements across an offline delay. (a-c) show alterations in tracing time. (a) Changes in tracing time from the first to the second session. (b) Tracing time difference for groups with different retention type. (c) Tracing time difference for different emotional content for the day group. The positive values show reduction of tracing speed, indicating longer tracing time for the 1st session compared to the 2nd session. * $p < 0.05$, Error Bars represent one standard deviation (SD).

2.5 Discussion

In contrast to the diversity of studies on the contribution of emotion to explicit memory, studies on interaction of emotion and implicit memory have mostly focused on conditioning (Adolphs, 2008; Adolphs, Tranel, Damasio, & Damasio, 1995; Ehrlich et al., 2009; Maren, 2008; Sah, Westbrook, & Luthi, 2008). The main goal of the current investigation was to examine the effect of different emotions as a background context during encoding upon the subsequent off-line consolidation of procedural learning. To investigate this, three different types of emotional stimuli, negative, positive and neutral, as background images during the encoding phase of a mirror tracing task was embedded. The result extended the literature by showing that the presence of negative content at encoding enhances subsequent performance on this

procedural task in terms of both speed and accuracy. Participants who learned this new skill with a negative image in the background demonstrated significantly greater enhancement of this skill across an offline retention interval than participants who trained with neutral or positive images in the background.

2.5.1 Arousal influences procedural learning

The observation of superior post-consolidation performance by subjects exposed to negative stimuli during training supports the possibility that the presence of negatively arousing emotions at encoding may contribute to the off-line consolidation of this procedural task. It has been shown that the amygdala responds strongly to fear and negatively arousing emotions (LeDoux, 2000) (for review see (Adolphs, 2008; Ehrlich, et al., 2009; Roozendaal, McEwen, & Chattarji, 2009; Seymour & Dolan, 2008)), for instance in the recognition of fearful facial expressions (Adolphs, et al., 1995; Calder, 1996). In addition to its role in encoding fear, converging findings of animal and human studies using methods such as brain lesions (Kluver & Bucy, 1937; Weiskrantz, 1956), electrical stimulation (Goddard, 1964; Kesner & Wilburn, 1974; McGaugh & Gold, 1976) , and post-training drug infusions (Ellis & Kesner, 1983; Gallagher, Kapp, Pascoe, & Rapp, 1981), provide compelling evidence that the amygdala is critically involved in enabling us to acquire and retain lasting memories of emotional experiences (LaBar & Cabeza, 2006) (for review see (McGaugh, 2004)). Cortical and subcortical connections between amygdala and Basal Ganglia, a key player in the motor system which is also known to be critical for procedural memory (Gazzaniga, 2004; Kandel, Schwartz, & Jessell, 2000), mean the amygdala is ideally situated to mediate an interaction between negative emotion experienced during the learning of a procedural task and subsequent enhancements in the off-line consolidation of that task.

Amygdala based mediation of striatal responses is anatomically plausible and could occur via connections through cortical areas, within subcortical areas, or cortico-subcortical re-entrant circuits. Morecraft and Van Hoesen (1998) have shown that, in rhesus monkey, Brodmann areas 24c (M3) and 23c (M4) receive inputs from all parts of the limbic lobe, as a cortical entry point, including the basolateral complex of the amygdala. These connections as well as those between areas 24c, 23c, and diverse parts of the granular prefrontal cortex (Bates & Goldman-Rakic, 1993; Lu, Preston, & Strick, 1994; Morecraft & van Hoesen, 1993), and topographic projection from these regions to the primary and supplementary motor cortices (Darian-Smith, Darian-Smith, Burman, & Ratcliffe, 1993; Dum & Strick, 1991; M. P. Galea & Darian-

Smith, 1994; Morecraft & van Hoesen, 1992; Muakkassa & Strick, 1979; Murray & Coulter, 1981) provide a possible anatomical basis for the connection between emotion and motor learning. Other possible pathways are through subcortical areas. There are several known pathways between limbic lobe regions and subcortical areas of the motor system. For instance, the ventral striatum receives prominent afferents from other nonisocortical limbic lobe regions, including the entorhinal cortex, anterior cingulate cortex, large parts of orbitomedial prefrontal cortex, and insula (Chikama, McFarland, Amaral, & Haber, 1997; Ferry, Ongur, An, & Price, 2000; Haber, Kunishio, Mizobuchi, & Lynd-Balta, 1995). Finally, interactions between limbic regions and basal ganglia also occur through 'basal ganglia loops' (Haber, Groenewegen, Grove, & Nauta, 1985; Heimer, 1978; Heimer, Switzer, & Hoesen, 1982; Young, Alheid, & Heimer, 1984). For example, ventral regions of the basal ganglia receive input from many different parts of the limbic lobe, including orbitomedial prefrontal cortex, anterior cingulate cortex, insula, hippocampus, basolateral amygdala (Groenewegen & Berendse, 1994; Haber, 2003).

2.5.2 The role of sleep

In addition to investigation of the effects of emotionally arousing stimuli upon subsequent consolidation, the extent to which sleep contributed to this effect was examined. The results showed that participants who obtained at least 6 hours of nocturnal sleep (night and control groups), performed mirror tracing faster and more accurately than participants who did not sleep (the day group). Interestingly, this enhancement was more apparent in the negative emotional content group (t-test $p < 0.05$), suggesting that encoding in a negative context lead to greater consolidation during subsequent sleep.

Interactions between the limbic and motor systems have been studied extensively (Haegelen, Rouaud, Darnault, & Morandi, 2009) but, to the knowledge of author, the impact of such interactions upon the off-line consolidation of a procedural task have not examined previously. Here it is shown that the presence of negative stimuli at encoding boosts the subsequent off-line enhancement of procedural memory. In contrast to previous studies which did not report improvement of the learnt skill with simple passage of time (e.g. without sleep) (Cohen & Robertson, 2007; Nishida & Walker, 2007; Plihal & Born, 1997b; Press, et al., 2005; Robertson & Cohen, 2006; Robertson, Pascual-Leone, & Miall, 2004; M. Walker, et al., 2002), the result showed that negative emotional content at encoding enhances subsequent off-line consolidation and improves performance, Figure 2-4(a).

In addition to simple passage of time, the contribution of sleep to the off-line consolidation of a procedural task was studied. In line with previous studies showing that sleep selectively enhances memory for emotionally arousing stimuli (Kleinsmith & Kaplan, 1963; LaBar & Phelps, 1998; Levonian, 1972; Nishida & Walker, 2007; Plihal & Born, 1997b; Sharot & Phelps, 2004; M. Walker, et al., 2002), the results showed selective off-line enhancement in the performance of participants exposed to negative stimuli during training, although performance of participants exposed to positive and neutral stimuli at encoding also improved to a lesser extent. The observation that negative emotional content boosts consolidation across periods containing sleep and prevents deterioration across periods of wakefulness is in-line with work by Sterpenich et al. (2007), showing that while memory for positive and neutral episodes is impaired by sleep deprivation on the post-encoding night, memory for negative episodes remains unaffected. These authors showed that an amygdalo-neocortical network is recruited during recollection of negative but not positive stimuli in subjects who had experienced sleep deprivation after encoding, but who were fully refreshed at the time of retrieval. Levels of arousal were balanced between positive and negative stimuli, hence the observed effects are most parsimoniously linked to differences in valence. These findings suggest that negative information is preserved across retention periods when neutral and positive information is lost, even if this preferential retention requires the recruitment of additional neural systems.

In contrast to previous studies (Cahill, 2000; Cahill & Alkire, 2003; Maddock, 1999; McGaugh, 2004; McGaugh & Roozendaal, 2002; Phelps, 2004; Richardson, et al., 2004), no performance enhancement in the positive emotion group across groups with different retention types was found. This could be because the emotional content of the positive stimuli is not sufficiently arousing. Further investigation is therefore necessary to study the effect of positively arousing stimuli in the consolidation of procedural memory with standard valenced images, e.g. Eckman faces. Three possible neuronal mechanisms establishing the link between limbic system and procedural memory could be suggested. The exact neural mechanism(s) responsible for this interaction, however, remains open to further investigation.

Contribution of emotion in stability of memory has been studied before. It has been shown that this modulatory effect lasts for a long time, sometimes up to years (LaBar & Cabeza, 2006) (for review see (McGaugh, 2004)). It has also been shown that memory consolidation continues days and even years after the initial encoding of memory (M. Walker et al., 2003; M. Walker & Stickgold, 2004). As mentioned above, their combination has also been studied

(Sterpenich, et al., 2007; E. Walker & Tarte, 1963; M. Walker, 2009). But the time-course of each of the factors in relation to each other has not been studied systematically.

2.5.3 Circadian influences on performance

To exclude the possibility that differences between groups were due to differences in time of the sessions as an explanation of the learning, two groups of participants had a 12 hour retention interval (day and night groups) and the other group had 24 hours retention interval (control group). The second session of the control group and the second session of the day group were both conducted at the same time of the day. The comparison between the second session of the day group with the second session of the control group is therefore independent of the time of the day, tiredness or lack of attention. Performance of the participants in the control group was comparable with the performance of the participants in the night group in both accuracy and speed, which were both significantly higher in this group than in the day group. In combination with the non-significant difference between the groups in the SSS ratings, finger tapping speed and accuracy, number of training tracing trials and performance base-line in the first session, this evidence discounts the possibility of the time of the session affecting the performance of the participants in the day group. Instead, these data suggest that the > 6 hours of sleep obtained after the training session by both night and control groups was associated with superior subsequent performance of the learnt skill. The data additionally suggest that sleep stabilised memory for the learnt skill and protected it from slowing down during wakefulness since the control (24 hour) group, who experienced 12 hours of wakeful retention after their nocturnal sleep, showed no slowing in tracing speed see Figure 2-4.

2.6 Acknowledgement

I would like to thank Iroise Dumontheil for critical reading of this chapter and Bahador Bahrami for technical assistance.

Chapter 3. Contribution of Emotional Stimuli to Procedural Memory Consolidation; Evidence from A Modified Serial Reaction Time Task (SRTT)

3.1 Abstract

Researchers in the field of memory have been interested in discovering factors that influence memory consolidation. Various studies have examined the effects of sleep and emotion on memory consolidation. The majority of these studies have focused on episodic memory and only ones study so far has showed the effect of emotion in procedural memory. A serial reaction time task has been used to study the modulatory effect of sleep on procedural memory consolidation. To have a better understanding of this interaction, this study aimed to investigate whether the presentation of negative, high valence stimuli and sleep between training and testing sessions, would facilitate procedural memory consolidation of SRTT. 78

participants were tested twice within two experiments. In one experiment participants (*n* = 48) were presented with a modified SRTT in four conditions where retention time (with/without sleep) and stimulus type (neutral/negative) were manipulated. In another experiment participants (*n* = 30) were tested with a standard SRTT in two groups with different retention time periods. Unknown to the participants, the order of presentation of the images followed a repeated sequence of 12 items. Results showed that participants in all the groups got faster and more accurate and there was no difference between the conditions. Some of the participants acquired explicit knowledge of the sequence which was higher in the first experiment with modified SRTT. Response time and accuracy on the second session for those participants who acquired explicit knowledge of the sequence were significantly better than the participants without explicit knowledge although the later participants also benefited from memory consolidation. The results show that excessive training enhances the memory consolidation regardless of retention type and implicit or explicit knowledge of the sequence.

3.2 Introduction

Memory consolidation refers to the processes of brain plasticity by which experiences result in more or less enduring changes in adaptive behaviour (Fischer, et al., 2002). With regards to motor skills, practicing a motor task leads to the generation of an internal model that codes corresponding motor outputs in response to different stimuli in the task (Shadmehr & Brashers-Krug, 1997). Some researchers have suggested that development of this internal model continues after practice, which strengthens memory of this model. This has been termed as the process of “offline memory consolidation” (Press, et al., 2005).

A large body of research provides evidence for the significant contribution sleep has towards the formation of various memories (Cohen, Pascual-Leone, Press, & Robertson, 2005; Hauptmann, Reinhart, Brandt, & Karni, 2005). Although earlier studies proposed the influence of sleep on only declarative memory consolidation due to its hippocampal dependence, a similar effect has been found for procedural memories and also in the slow consolidation of latent motor skills (Fischer, et al., 2002; Sutherland & McNaughton, 2000; M. Walker, et al., 2002). In this study, it is sought to confirm the enhancement effect of sleep on memory consolidation of an implicit motor task.

Another powerful factor known to affect memory consolidation is emotion. A multitude of studies have shown that experiences that elicit emotions at the time of learning will lead to significant improvements in subsequent recollection relative to neutral experiences (McGaugh, 2004) (see also the previous chapter). Also, these effects of emotion on memory increase in impact as the duration between the learning and testing phases increases (Sharot & Phelps, 2004). It has since been suggested that emotion may also facilitate subsequent offline memory consolidation in addition to enhancing the encoding process (McGaugh, 2004).

Researchers investigating this effect of emotion on encoding and memory consolidation have suggested three possible reasons. Firstly, emotionally arousing stimuli may increase the attention paid to the experience during the process of encoding (McGaugh, 2003). Secondly, Guy and Cahill (1999) suggested that people usually rehearse emotionally arousing experiences, which strengthens memories. Thirdly, a similar explanation to that of Fischer et al (2002) was suggested in Muller and Pilzecker's hypothesis (1900). They postulated that emotional experiences initiate neural processes that persist and consolidate over time that are involved in memory consolidation for emotional processes. Furthermore, brain-imaging studies found increased amygdala activity during the training phase of this experiment. Anderson, Wais & Gabrieli (2006) suggested that arousal levels of stimuli were significant in influencing memory enhancement through firing of neurons in the amygdala. Thus, this study investigates the effect of emotion on the consolidation of an implicit motor skill task.

The contribution of emotion in consolidation of procedural memory during wakefulness or sleep was the main interest of this study. A serial reaction time task (SRTT) was selected as the procedural task. It has previously been widely employed both to assess implicit skill acquisition (Cleeremans & McClelland, 1991; Nissen & Bullemer, 1987) and to study the consolidation of procedural memory by several researchers (Cohen, et al., 2005; Press, et al., 2005; M. Walker, et al., 2002). SRTT has been employed in various studies to explore cognitive and biological processes underlying learning and memory (Robertson, 2007). It is a choice reaction task employed where participants are required to key in a response as quickly and accurately as possible to the appearance of a target stimulus on a screen. Stimuli are consistently matched to corresponding keys and participants are or are not informed that the sequence follows a particular order, explicit and implicit sequence learning respectively. With some practice in the SRTT, participants begin to acquire implicit knowledge of the sequence in addition to decrements in reaction time due to improved visuomotor skills. Participants are tested in a second phase of experiments to determine changes in performance levels due to

memory consolidation. In order to check whether participants had any knowledge of the sequence Fischer, Drosopoulos, Tsen and Born (2006) recommended applying a generation task to the participants. In this task participants were asked to try to repeat the sequence without any feedback (see below).

In this study two experiments were run. In the 1st experiment a modified version of SRTT (mSRTT) was used in which participants had to respond to images rather than spatial location of cues. In this experiment the target stimuli are either all neutral or all negative, high valence, to test if emotional content of the stimuli will influence memory consolidation as reflected by performance levels in the testing session. In the 2nd experiment the standard SRTT (sSRTT) was used.

As such, the general aim of this study was to investigate whether varying retention type with fixed delay (with/without sleep between sessions – night and day groups respectively) and emotional content of stimuli (neutral/negative) would affect memory consolidation and hence performance levels on the SRTT from the first session to the second session.

Given prior researches conducted by Fischer et al (2002) and Anderson et al (2006), it was predicted that a main effect of sleep and emotional content of stimuli will be found. It was expected that participants would perform better on the SRTT if they slept between the two sessions and were assigned the negative stimuli. An interaction effect between retention and stimuli type is also predicted, participants in the night-emotional condition would perform better than those in the day-neutral condition. The independent variables would hence be retention period- without sleep (day) and with sleep (night) and emotional content of target stimuli- neutral and negative. The dependent variables would be the response time and accuracy to target stimuli in the SRTT and score in generation task.

3.3 Methods

3.3.1 Experiment 1

Participants

48 participants (14 males, 34 females, mean 21.3, range 18 to 24 years, SD= 1.26) took part in the study in 4 groups, comprising the combination of day/night and neutral/negative content conditions. Table 3-1 shows the number of participants in each group.

Table 3-1. Number of Participants in each group. Participants were assigned to one of 4 different groups according to retention type (day/night) and stimuli type (Neutral/Negative) of the experiment.

Condition	Emotional Content	
Retention Type	Neutral	Negative
Day	13	11
Night	9	15

All participants had normal or corrected-to-normal vision and agreed to be drug, alcohol and caffeine free for 24 hours prior to and during the study period. Participants had normal or corrected to normal vision. All participants were right-handed yielding a laterality quotient of at least +50 on Edinburgh Handedness Inventory (Oldfield, 1971). Informed consent was obtained from all participants and they were paid or given course credits for their participation.

Stimuli

Two sets of four images each were used as stimuli in the experiment. They were drawn from the International Affective Picture System (IAPS); (P. Lang, Bradley, & Cuthbert, 1997). All the images of each set had either a neutral or an arousing emotional valence (see Appendix i).

Apparatus

A modified version of the SRTT was created for the study (Nissen & Bullemer, 1987) using MATLAB v 7.0 and Psychtoolbox v3 (Brainard, 1997; Pelli, 1997). It was performed on PC computers with 17-inch LCD monitors, 1024x768 resolution and 75Hz refresh rate.

Design

The experiments comprised two sessions (training and testing). Participants in the day group were trained in the morning and tested at night 12 hours later, while participants in the night group were trained at night and tested next morning 12 hours later, Figure 3-1. Day group participants were asked to not sleep between the two sessions and night group participants were asked to have at least 6 hours of nocturnal sleep. All the participants were asked to have at least 6 hours of night sleep before the first session.

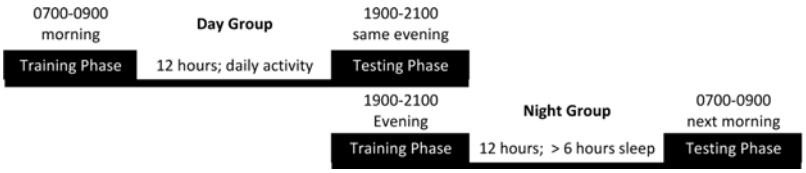


Figure 3-1. Experiments comprised two sessions in two retention types – day and night groups – with 12 hours retention interval in between the two sessions.

The experiment used a 2x2 mixed-model design. There were two independent variables and three dependent variables. The independent variables were the retention type, which did or did not include sleep – night and day respectively, and the emotional valence of the stimuli, which had either a neutral or an arousing valence – neutral or negative respectively. Thus, there were four experimental conditions: day/neutral, day/negative, night/neutral and night/negative. The dependent variables were the reaction time, accuracy and amount of explicit knowledge acquired by participants. Participants were randomly assigned to one of two emotional conditions (neutral and negative conditions) in the training session.

Experimental Procedure

At the beginning of each session, participants were asked to complete a Stanford Sleepiness Scale (SSS) (Hoddes, Dement, & Zarcone, 1972; Hoddes, et al., 1973) to measure subjective alertness.

In the training session, participants were required to fill in a Morningness-Eveningness questionnaire (Horne & Ostberg, 1976), a Handedness test from the Edinburgh Handedness Inventory (Oldfield, 1971) and a consent form. They then proceeded with the training session and were informed that all instructions would be on the screen.

In the testing session, participants were given log sheets to indicate sleep/wake times, food and drinks consumed and quality of dreams during sleep. This was done to monitor their activity during the retention interval and before the first session. The second part of the experiment was subsequently administered.

Training Session

The difference between the two experiments lay in the way they were cued in the tapping task. For the 1st experiment they were cued with images and for the 2nd experiment they were cued with spatial location of a circle showed on the monitor. For the 1st experiment the participants' task in the training session was to memorise the association of each image to one

of the 4 fingers of their non-dominant hand, which were placed on 4 different keys on the computer keyboard. For the 2nd experiment the participants' task in the training session was to strike one of the keys associated with the position of the circle showed on the screen. The keys were A, S, D and F respectively from small finger to index finger of participants' non-dominant hand (left hand).

When a stimulus appeared, participants were required to press the corresponding key as quickly and as accurately as possible. Upon making a correct response, the stimulus disappeared and the next one would appear. If an incorrect response was made, the stimulus would remain until the correct key was pressed. The participants were informed that they would be presented with each stimulus separately and that they should press the correct key as quickly and accurately as possible. The maximum amount of time a participant would take on each block is 30 seconds regardless of the number of stimuli responded to. Response time was defined as the time lapse between presentation of stimulus and tapping of the correct response. The task was introduced to the participants as a test of accuracy and reaction time. Without their knowledge, a specific sequence comprising of 12 items, 2 3 1 4 3 2 4 1 3 4 2 1, was given to all participants, allowing them to acquire the skill implicitly. This sequence was kept constant throughout the first session and it was the same for all the participants. Each item corresponds to one finger as index, ring, middle and small fingers respectively from 1 to 4. The sequence was created by dividing the 12 items into 3 sets of 4 items, so that no item was repeated in each set or with less than 1 item separation. Figure 3-2 depicts the procedure of the training session.

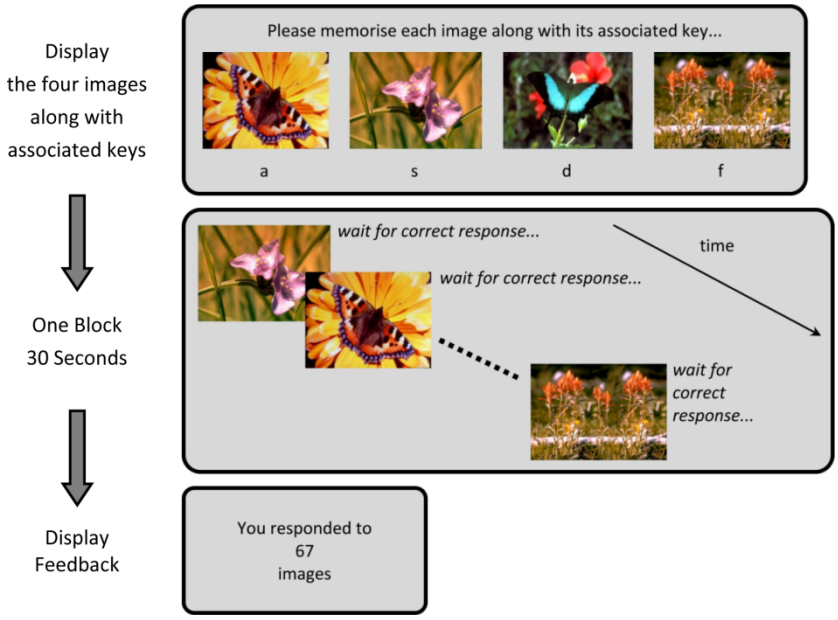


Figure 3-2. Procedure of the training session (1st session) of the 1st experiment;

Testing Session

The second session had three phases, SRTT with the same order of items in the first session, generation task and SRTT with random order of items. In the first phase, participants were given the same task as in the training session. However, they were only required to complete 4 blocks of the SRTT and were told that the test depends on their performance.

On the generation task, participants were asked to perform a free recall of the sequence used in the training and testing sessions. Performance on this task was influenced by implicit memory processes such as a sense of familiarity of finger tapping pattern. However, as a direct test of particular sequence knowledge, participants were asked to generate as many numbers, equivalent to each finger tapping, as they wanted to form the sequence they felt was used and it was recorded. There was no time limit to perform this phase.

For the random sequence phase, images were shown in random order with equal presentation chance with the exception that no image was repeated twice in a row. Figure 3-3 shows the procedure of the testing session. At the beginning of this phase participants were informed that there is no sequence embedded in the sequence anymore.

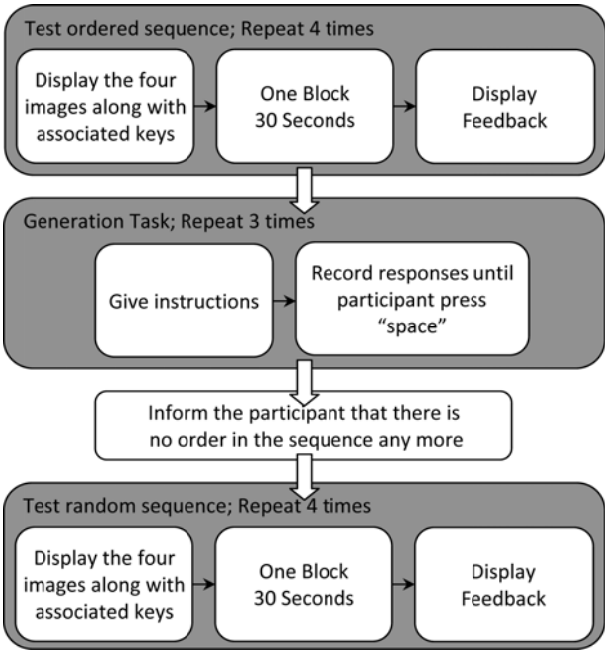


Figure 3-3. Procedure of the testing session, 2nd session.

Generation Task

Considering the rules for the used sequence all the possible permutations of items were created in sets of 2 items, 3 items, 4 items and so forth till 12 items and possibility of occurrence of each set with certain length was calculated. A score equivalent to $(1 - \text{possibility})^{-1}$ was assigned to each sequence length. The scores were as follows, 0, 0, 2.5, 5, 10, 20, 40, 80, 160, 320, 640 respectively for 2 correct items in a row, 3 correct items in a row and so forth till 12 correct items in a row which represents the whole sequence.

The score of each participant in each block was calculated by summing the score of all correct sequences in the first 12 tapped items. The maximum achieved score in the three blocks was considered the final score of each participant.

Distribution of scores in the generation task is neither normal nor uniform. A 1-stage randomisation test using 200,000 permutation runs was conducted. In this randomisation procedure sequences consisted of 12 items with no item repeated immediately were generated and the score for each sequence was calculated. The distribution of scores can then be used to determine the *P*-level of a given score. This method is based on the randomisation procedure proposed by Blair and Karniski (1993) and was already used in several studies in the statistical analysis of EEG/MEG data (Bäuml, Hanslmayr, Pastötter, & Klimesch, 2008; Hanslmayr et al., 2007; Hanslmayr, Spitzer, & Bauml, 2008; B. Pastötter, K. Bäuml, & S. Hanslmayr, 2008).

3.3.2 Experiment 2

30 participants (14 males, 16 females, mean 21.4, range 18 to 24 years, SD= 1.85) took part in the experiment in 2 groups with different retention types – day (n = 16) and night (n = 14).

The experiment pursued a mixed-model design. There was one independent variable and three dependent variables. The independent variable was the retention type, which did or did not include sleep – night and day respectively. The dependent variables were the reaction time, accuracy and amount of explicit knowledge acquired by participants.

The experimental groups were similar to the 1st experiment with day and night retention types, Figure 3-1. The procedure of the training session (Figure 3-2) and testing session (Figure 3-3) was also the same as the 1st experiment. The participants' task, however, was to tap one of the keys on the computer keyboard associated to the position of a circle showed in 4 rectangles centrally aligned on the screen rather than responding to an image, Figure 3-4.

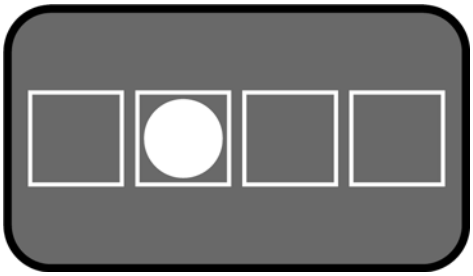


Figure 3-4. Sample screen shot of the standard SRTT used in the 2nd experiment; In this example the cueing circle is placed on the 2nd rectangle associated with ring finger of the left hand.

3.3.3 General Methods

Participants did not differ in scores either in the Morningness-Eveningness questionnaire (Horne & Ostberg, 1976). Analysis of variance of morningness-evening scores of participants in different groups showed non-significant difference, $p > 0.8$ for both experiments for all the conditions (two 2x2 ANOVAs for the 1st and 2nd experiments with retention type and stimulus type as independent factors and morningness-evening score as a dependent variable). Analysis of variance of the Stanford Sleepiness Scale also showed a non-significant difference between different conditions, $p > 0.7$ for both experiments for all the conditions (two 2x2x2 ANOVA for the 1st and 2nd experiments with session number, retention type and stimulus type as independent factors and SSS score as dependent factor).

All night condition participants had more than 6 hours of sleep and none in the day condition took a nap. Also, none of the night group participants had negative dreams and reported pleasant sleep. None of the participants consumed alcoholic or caffeinated drinks.

3.4 Results, 1st Experiment

Performance of the 1st and 2nd session

To make sure participants began the retention interval with comparable acquired skill two ANOVAs were conducted with response time and accuracy as dependant variables and retention type and stimulus type as between subject factors (Table 3-2). The mean accuracy scores and the mean response times of the last three blocks of the training session were used for each participant. Figure 3-5 shows mean response time and accuracy of participants at the end of the 1st session across retention and stimulus types.

Table 3-2. Two separate 2x2 ANOVA conducted to analyse the accuracy and response time of participants at the end of the first session in different groups. The first number in each column is the *F* value.

Configuration	Comparison of performance at the end of the 1st session	
Effect	**Response Time**	**Accuracy**
Retention Type, *F*(1, 44)	0.41, $p = 0.522$	3.38, $p = 0.073$
Stimulus Type, *F*(1, 44)	0.02, $p = 0.887$	0.19, $p = 0.666$
Retention x Emotion, *F*(1, 44)	0.02, $p = 0.890$	2.56, $p = 0.116$

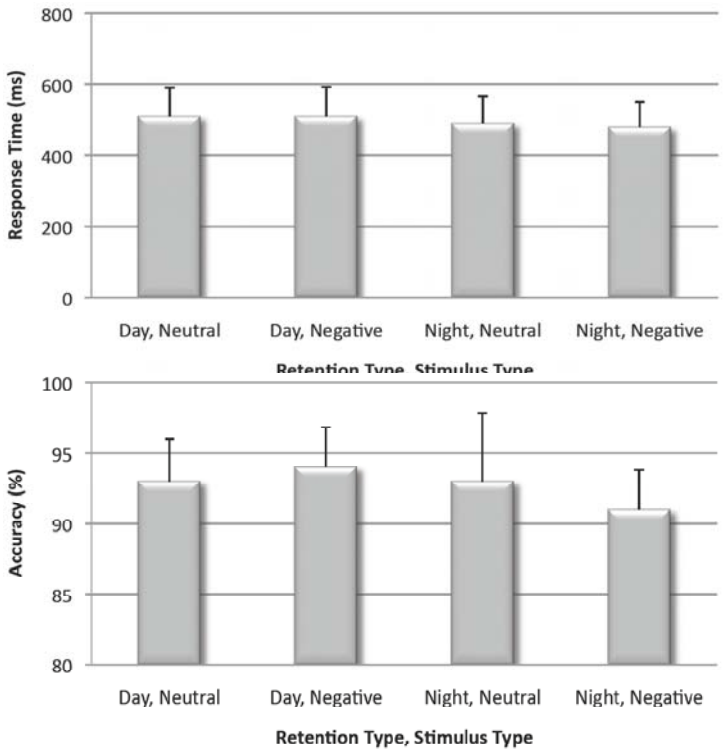


Figure 3-5. Response time, top, and accuracy, bottom, of the participants at the end of the 1st session in different groups. Error bars show 95% confidence interval.

To investigate the amount of memory consolidation in between the two sessions, the mean accuracy scores and the mean response times of the last three blocks of the training session were compared with the mean accuracy scores and the mean response times of the last three blocks of the first phase of the testing session. This provides a test of accuracy and speed across sessions on the blocks testing the ordered sequence, Figure 3-6.

Two separate mixed model tests were conducted with session number as within subject and retention and stimulus types as between subject factors. Table 3-3 summarises the results. Results showed that participants were more accurate and faster in the second session compared to the first session which is evidence of memory consolidation in between the two sessions.

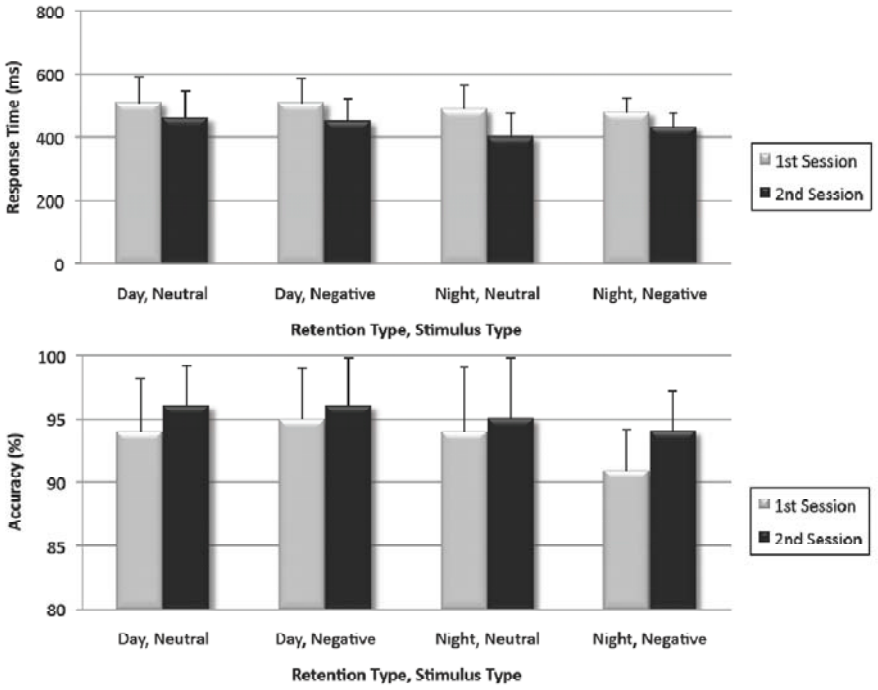


Figure 3-6. The response time, top, and accuracy, bottom, of participants in different groups comparing the first session with the second session. Error bars show 95% confidence interval.

Table 3-3. Mixed model test with session number as within subject and retention type and stimulus type as between subject factors. The first number in each column is the *F* value.

Configuration	Comparison between the 1st and the 2nd sessions	
Effect	**Response Time**	**Accuracy**
Session Number, *F*(1, 44)	51.4, $p < 0.001$	7.87, $p = 0.007$
Session x Retention Type, *F*(1, 44)	1.33, $p = 0.255$	2.74, $p = 0.105$
Session x Stimulus Type, *F*(1, 44)	0.49, $p = 0.487$	1.46, $p = 0.232$
Session x Retention x Emotion, *F*(1, 44)	1.66, $p = 0.204$	0.12, $p = 0.723$

The mean response time and accuracy in the last three blocks of the first phase was compared with the mean response time and mean accuracy of the last three blocks of the third phase of the testing session. As in the first phase participants were tested on the ordered sequence, whereas in the third phase they were tested on a random sequence (see Figure 3-7), this comparison tests for implicit knowledge of the sequence. A significant decrease in response time and accuracy in the blocks with a random sequence would indicate that participants implicitly retained the ordered sequence. Two mixed model tests with ordered/random as within subject and retention type and stimulus type as between subject factors were conducted, Table 3-4.

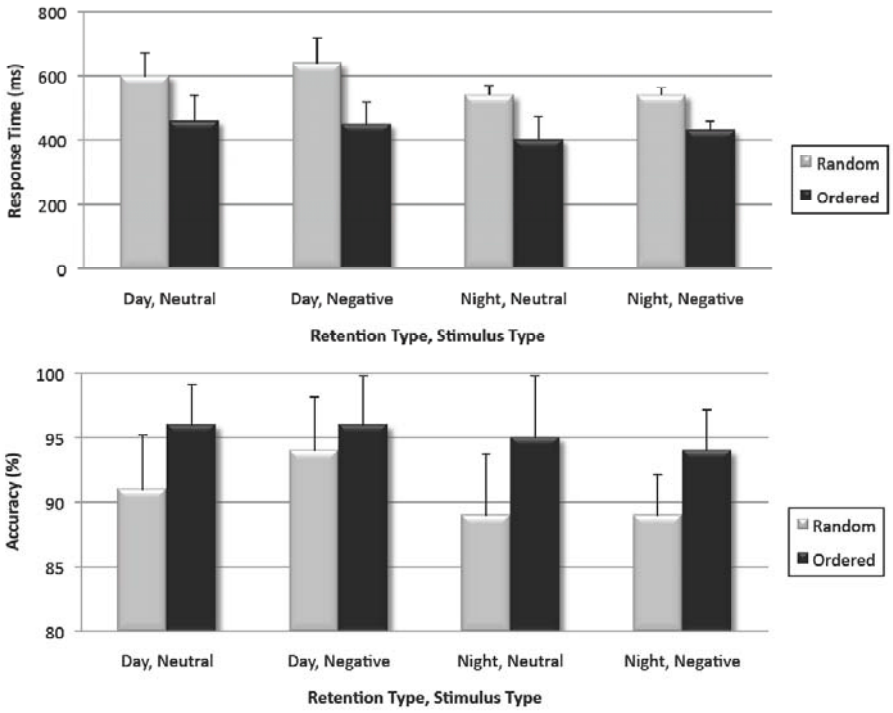


Figure 3-7. The mean response time, top, and mean accuracy, bottom, of participants in the random and ordered sequences in the testing session across stimulus and retention type. Error bars show 95% confidence interval.

Table 3-4. Mixed model test with random/ordered conditions as within subject and retention type and stimulus type as between subject factors. The first number in each column is the *F* value.

Configuration	Comparison between Random and Ordered Conditions	
Effect	**Response Time**	**Accuracy**
Ordered/Random Condition, *F*(1, 31)	97.7, $p < 0.001$	39.2, $p < 0.001$
Ordered/Random x Retention Type, *F*(1, 31)	0.03, $p = 0.869$	4.26, $p = 0.048$
Ordered/Random x Stimulus Type, *F*(1, 31)	0.02, $p = 0.875$	1.18, $p = 0.285$
Ordered/Random x Retention x Emotion, *F*(1, 31)	0.08, $p = 0.778$	0.06, $p = 0.805$

The interaction between sequence type and retention type became significant for the accuracy measurement. For further investigation of the effect of retention type on ordered/random condition two independent samples t-test on mean accuracy data were run (Table 3-5). A significant difference between day and night groups in the random sequence condition shows that participants in the night group in addition to implicitly learning the sequence also acquired a general skill on SRTT. Figure 3-8 shows mean accuracy of participants in day and night groups across type of sequence collapsing across the stimulus type.

Table 3-5. Independent samples t-test comparing the performance between participants. The first number in each column is the *t* value.

Configuration	Comparison between Day and Night groups
Effect	**Accuracy**
Ordered, *t*(46)	1.43, $p = 0.160$
Random, *t*(46)	2.16, $p = 0.036$

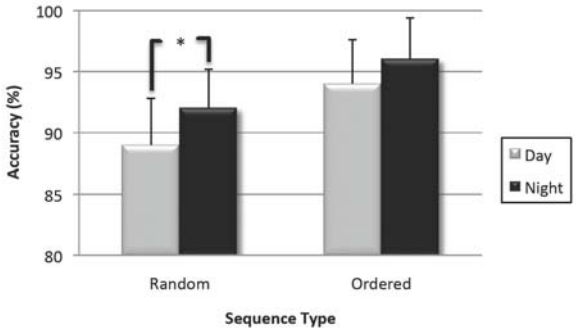


Figure 3-8. Comparison of performance of participants on the 2nd session in the day and night groups; * $p < 0.05$; Error bars show 95% confidence interval.

Generation Task

Participants were split into two groups according to their generation task scores: participants who acquired explicit knowledge of the sequence – explicit group – and those who did not – implicit group (Table 3-6). Chi square analysis showed a significant difference between the two groups (day/night) in the negative stimulus type condition, $\chi^2(26) = 5.37$, $p = 0.053$ (two-tailed).

Table 3-6. Percentage of participants acquiring explicit knowledge of the embedded sequence in different retention and stimulus types. The numbers in the parentheses are the number of participants in each group acquiring explicit knowledge over total number of participants in that group.

Condition	Emotional Content	
Retention Type	**Neutral**	**Negative**
Day	30.76% (4 / 13)	45.45% (5 / 11)
Night	33.33% (3 / 9)	6.66% (1 / 15)

Having split participants into two groups – explicit and implicit groups – the previous analyses were conducted to compare them.

Table 3-7 summarises two 2x2x2 ANOVAs conducted on response time and accuracy measurements at the end of the first session with knowledge, retention type and stimulus type as between subject analysis. This analysis shows that participants at the end of the 1st session began the retention interval with significantly different acquired skills.

Table 3-7. Two 2x2x2 ANOVAs with knowledge (Explicit/Implicit), retention and stimulus types as independent factors and response time and accuracy as between subject factors comparing the response time and accuracy of participants at the end of the 1st session.

Configuration	Comparison at the end of the 1st sessions	
Effect	**Response Time**	**Accuracy**
Knowledge Type, *F*(1, 40)	14.5, $p < 0.001$	3.14, $p = 0.084$
Retention Type, *F*(1, 40)	1.45, $p = 0.235$	1.21, $p = 0.278$
Stimulus Type, *F*(1, 40)	0.07, $p = 0.792$	0.10, $p = 0.754$
Knowledge x Retention, *F*(1, 40)	0.02, $p = 0.873$	0.29, $p = 0.592$
Knowledge x Stimulus, *F*(1, 40)	0.06, $p = 0.811$	1.30, $p = 0.261$
Retention x Stimulus, *F*(1, 40)	0.03, $p = 0.566$	0.18, $p = 0.671$
Knowledge x Retention x Stimulus, *F*(1, 40)	0.78, $p = 0.382$	2.76, $p = 0.105$

Figure 3-9 shows the progress of participants in the two explicit and implicit groups. As shown in the figure participants with later acquired explicit knowledge about the sequence began the task with significantly shorter response times. Figure 3-10 shows performance of the participants in the explicit and implicit groups at the end of the 1st session.

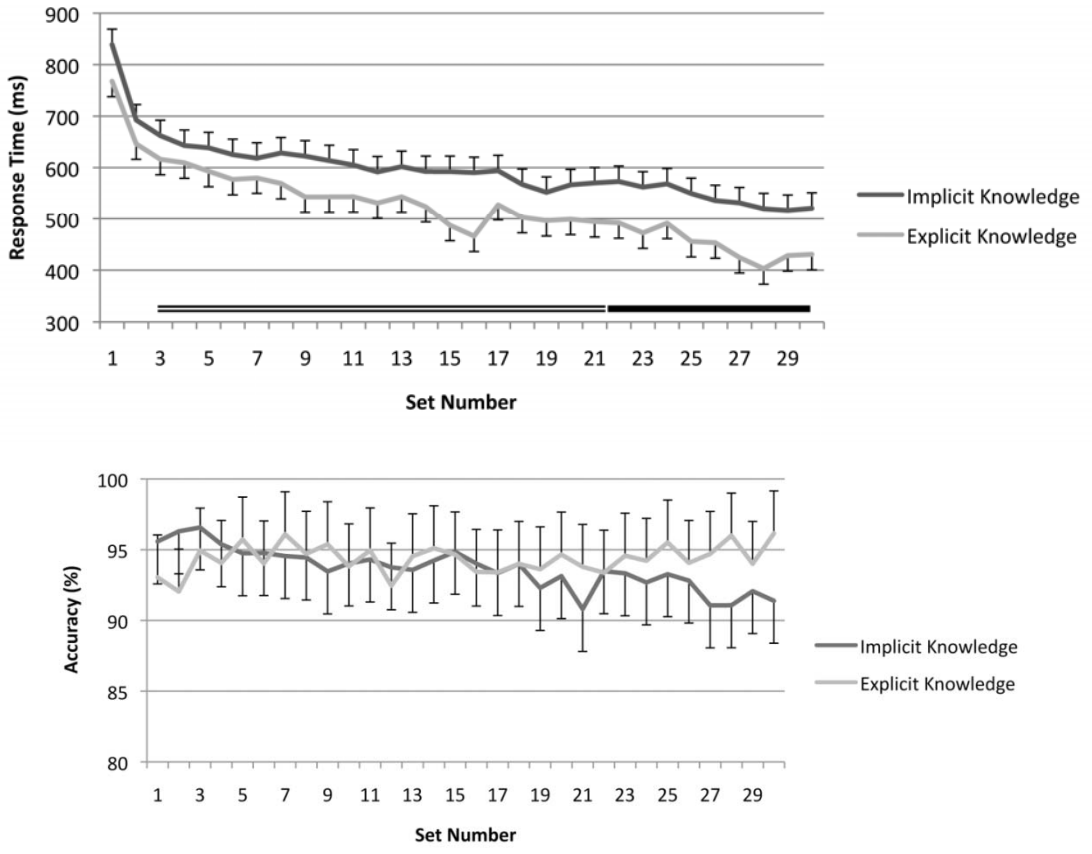


Figure 3-9. Progress of the participants in the two explicit and implicit groups. The double line shows $p < 0.05$ (uncorrected) and the solid line shows $p < 0.01$ (uncorrected) using independent samples t-test analysis comparing the performance in the two groups; Error bars show 95% confidence interval.

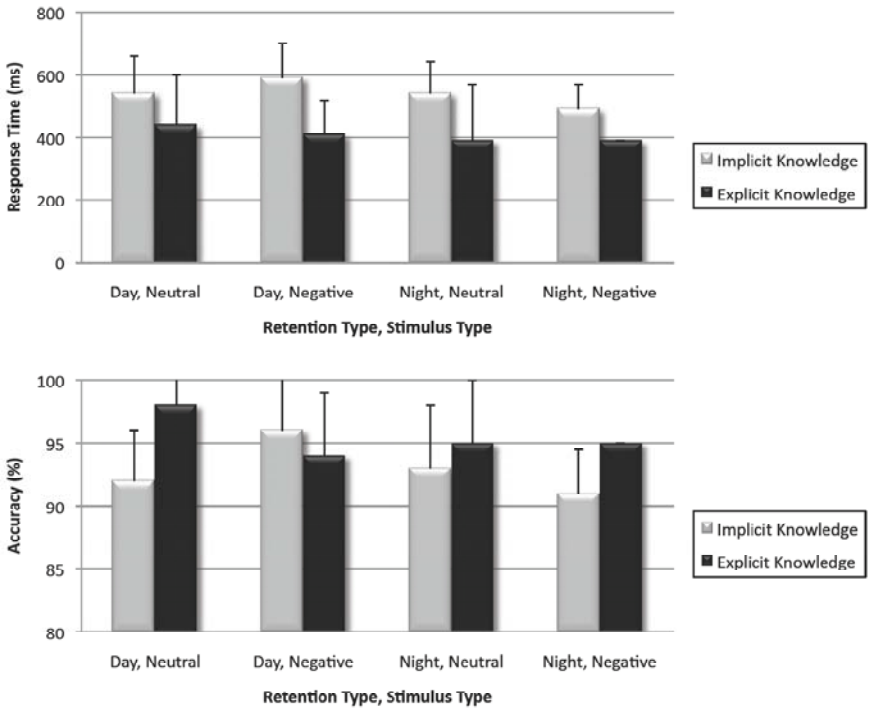


Figure 3-10. Comparison of performance of the participants in the explicit and implicit groups at the end of the 1st session, Error bars show 95% confidence interval.

The results of this experiment did not support the hypotheses about memory consolidation enhancement either due to negative emotional content of the stimuli nor due to sleep. The results showed that the response time and accuracy of the participant on the second session in all the categories were comparable in both ordered and random sequence types while the performance of the participants was comparable at the end of the first session except a significant difference between accuracy of the participants between day and night groups in the random sequence type. It was not evident whether this similarity in between the conditions is due to association between fingers and images or merely because of excessive number of training blocks. To study the effect of association of fingers and images and excessive training separately another experiment in which participants responded to the special location of a cueing circle rather than images was conducted.

3.5 Results, 2nd Experiment

Performance of the 1st and 2nd session

To make sure that participants began the retention interval with comparable acquired skill two independent samples t-tests were conducted with response time and accuracy as dependant variables and retention type as between subject factors. The mean accuracy scores and the mean response times of the last three blocks of the training session were considered as accuracy score and response time score for each participant. The two tests showed no significant difference between the two retention types – $t(28) = 0.150$, $p = 0.882$ and $t(28) = 1.809$, $p = 0.081$ for response time and accuracy respectively. The accuracy of the first session of the participants in the night group was however slightly lower than the accuracy of the participants in the day group, Table 3-8.

Table 3-8. Performance of the participants at the end of the first session in the two retention types. Values in the parentheses show standard deviation.

Retention Type	Response Time (ms)	Accuracy (%)
Day	410 (11.47)	96.23 (2.31)
Night	416 (8.95)	94.34 (3.36)

To investigate the amount of memory consolidation in between the two sessions, the mean accuracy scores and the mean response times of the last three blocks of the training session were compared with the mean accuracy scores and the mean response times of the last three blocks of the first phase of the testing session. This provides a test of accuracy and speed across sessions on the blocks testing the ordered sequence, Figure 3-11.

Two separate mixed model tests were conducted with session number as within subject and retention as between subject factor. Table 3-9 summarises the results. Results showed that participants were faster and that there was a trend towards higher accuracy on the second session compared to the first session. This is evidence of memory consolidation in between the two sessions.

The mean response time and accuracy in the last three blocks of the first phase was compared with the mean response time and mean accuracy of the last three blocks of the third phase of the testing session. As in the first phase participants were tested on the ordered sequence, whereas in the third phase they were tested on a random sequence (see Table 3-10), this comparison tests for implicit knowledge of the sequence. A significant decrease in response

time and accuracy in the blocks with a random sequence would indicate that participants implicitly retained the ordered sequence. Two mixed model tests with ordered/random as within subject and retention type as between subject factors were conducted, Table 3-11.

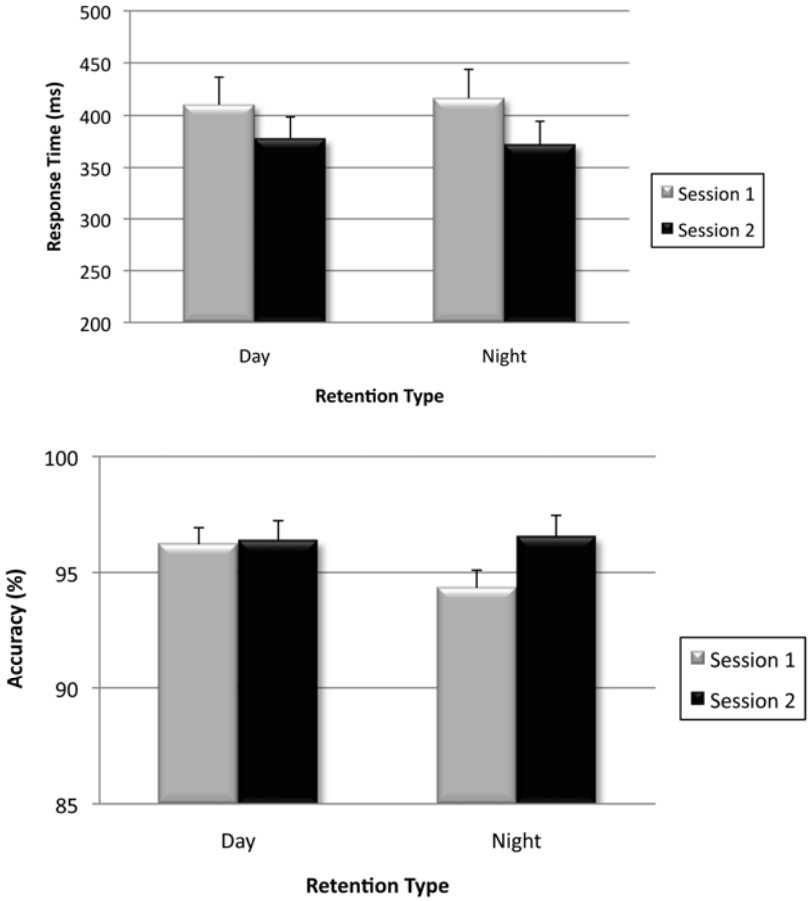


Figure 3-11. The response time, top, and accuracy, bottom, of participants in different groups comparing the first session with the second session. Error bars show one standard error.

Table 3-9. Mixed model test with session number as within subject and retention type as between subject factor. The first number in each column is the *F* value.

Configuration	Comparison between the 1st and the 2nd sessions	
Effect	**Response Time**	**Accuracy**
Session Number, *F*(1, 28)	32.57, $p < 0.001$	3.36, $p = 0.077$
Session x Retention Type, *F*(1, 28)	0.70, $p = 0.409$	2.59, $p = 0.119$

Table 3-10. The mean response time and mean accuracy of participants in the random and ordered sequences in the testing session across stimulus and retention type. Numbers in the parentheses represent standard error.

Measurement	Day	Night

	Random	Ordered	Random	Ordered
Response Time (ms)	444.27 (17.50)	324.20 (21.85)	432.28 (18.71)	322.10 (23.36)
Accuracy (%)	92.52 (1.59)	96.37 (0.86)	90.42 (1.70)	96.54 (0.92)

Table 3-11. Mixed model test with random/ordered conditions as within subject and retention type as between subject factor. The first number in each column is the *F* value.

Configuration	Comparison between Random and Ordered Conditions	
Effect	**Response Time**	**Accuracy**
Ordered/Random, *F*(1, 28)	162.8, $p < 0.001$	34.8, $p < 0.001$
Ordered/Random x Retention Type, *F*(1, 28)	0.296, $p = 0.591$	1.79, $p = 0.192$

The interaction of sequence type and retention type did not become significant which shows that the difference of performance of the participants in the night and days groups for the two sequence types (ordered and random) were comparable.

Generation Task

Due to generation task scores participants were split into two groups, participants who acquired explicit knowledge of the sequence – explicit group – and those who did not – implicit groups. Table 3-12 summarises the percentage of participants who acquired explicit knowledge in each groups.

Table 3-12. Percentage of participants acquiring explicit knowledge of the embedded sequence in different retention types. The numbers in the parentheses are the number of participants in each group acquiring or not acquiring explicit knowledge.

Retention Type	Implicit (%)	Explicit (%)
Day	81.25 (n = 13)	18.75 (n = 3)
Night	85.72 (n = 12)	14.28 (n = 2)

Having split the participants into two groups – explicit and implicit groups – the previous analyses were conducted to compare the two mentioned groups.

Table 3-13 summarises two 2x2 ANOVAs conducted on response time and accuracy measurements at the end of the first session with knowledge and retention type as between subject analysis. This analysis shows that participants at the end of the 1st session began the retention interval with significantly different acquired skills.

Figure 3-12 shows the progress of participants in the two explicit and implicit groups. As shown in the figure participants with later acquired explicit knowledge about the sequence began the task with significantly shorter response times.

Previous literature showed that performance improvement of the participants in the day group in between the two sessions to be much lower than the night group because of

beneficial effect of sleep (M. Walker, et al., 2002). So it was expected to see less memory enhancement for the day group compared to the night group. The results, however, showed no significant difference in between the two retention types. It is arguable that this is because of extensive number of training blocks.

Table 3-13. Two 2x2 ANOVAs with knowledge (Explicit/Implicit) and retention type as independent factors and response time and accuracy as between subject factors comparing the response time and accuracy of participants at the end of the 1st session.

Configuration	Comparison at the end of the 1st sessions	
Effect	**Response Time**	**Accuracy**
Knowledge Type, *F*(1, 26)	5.39, $p = 0.028$	0.48, $p = 0.495$
Retention Type, *F*(1, 26)	0.25, $p = 0.619$	3.82, $p = 0.061$
Knowledge x Retention Type, *F*(1, 26)	0.75, $p = 0.395$	0.98, $p = 0.330$

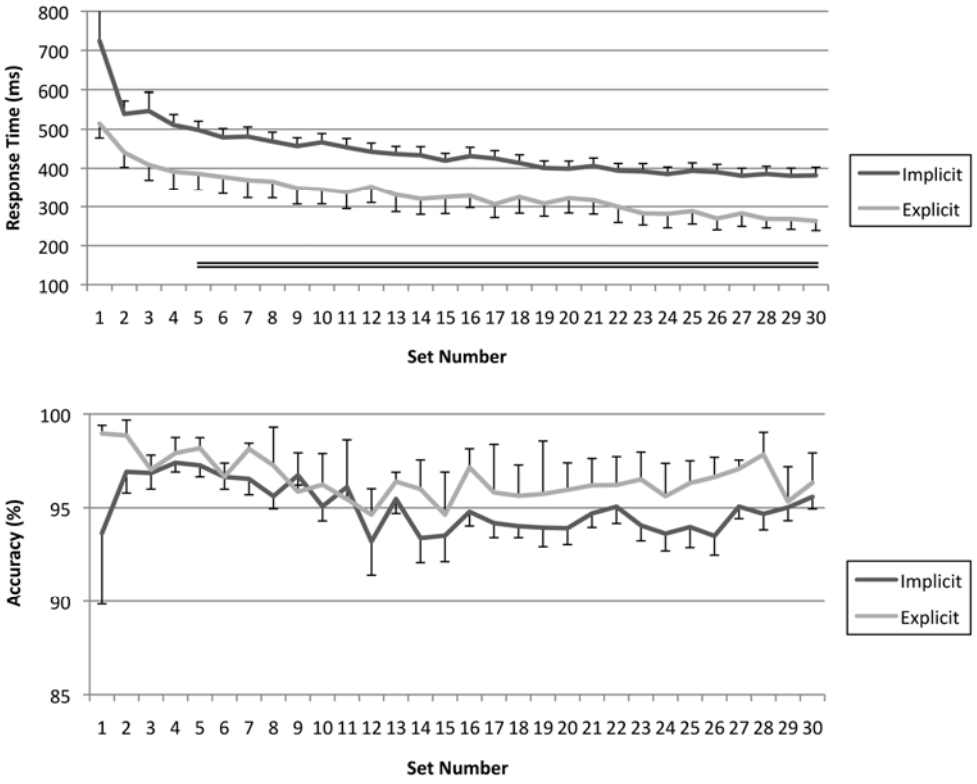


Figure 3-12. Progress of the participants in the two explicit and implicit groups. The double line shows $p < 0.05$ (uncorrected) using independent samples t-test analysis comparing the performance in the two groups; Error bars show one standard error.

3.6 Discussion

This study was comprised of two experiments. In the first experiment participants were subjected to a modified version of the SRTT, in which they associated different response keys with images having either neutral or arousing emotional valence. In the second experiment they were subjected to the standard version of the SRTT in which they were instructed to respond to the position of a cue. Unknown to them, the order of presentation of the stimuli followed a repeated sequence. Participants were tested in two sessions, separated by a twelve-hour interval which did or did not include sleep. It was found that all participants improved on reaction times and accuracy and acquired implicit knowledge of the sequence in the second session. There was no effect of emotional valence of the stimuli on performance and no difference in explicit knowledge between groups in the second experiment. Comparing the first and the second experiment showed that the modified version of the SRTT did not enhance memory consolidation. The hypothesis about potential boosting effects of negative emotional stimuli proved not to be right. Result of the first experiment showed that there was no difference between the performance of the participants trained with either of the stimulus types. An important finding was that contrary to previous literature (M. Walker, et al., 2002) participants in the day group in both the experiments benefited from offline memory consolidation comparable to participants in the night group. This shows that excessive training can trigger memory consolidation for the day group.

3.6.1 Performance and Implicit Knowledge

The first hypothesis of this study stated that all participants would present a gradual improvement in their performance and implicit knowledge of the sequence, but that only the sleep group would significantly improve in the second session, as a result of sleep-dependent consolidation. This prediction was partly supported by the findings. All the participants improved reaction times throughout practice and showed implicit knowledge of the sequence, reflected by a decrease in accuracy and an increase in response when the sequence was violated. However, not only the sleep group, but also the wake group, improved in the second session. This finding is only partly consistent with the study of Spencer et al. (2006), which found that when the SRTT is combined with contextual association, offline improvement is dependent on the presence of sleep in the interval between sessions. The inconsistency between this and the Spencer et al. (2006) study may due to the different type of contextual

associations. In fact, it may be argued that the images used in this study contained stronger contextual associations than the colour cues used by Spencer et al. (2006). Hence it may be proposed that strong contextual association induces offline improvements also during intervals of wakefulness. Further studies may support or disconfirm this suggestion by combining the SRTT with different types of contextual associations. The results of this experiment was also against the study of Walker et al. (2002). In their study, the memory consolidation of the participants in the night and day groups were significantly different. The difference between this study and theirs lies in the number of training blocks. Participants were asked to repeat the training blocks for 30 times but they asked their participants to repeat the training blocks for 12 times. It can be suggested that the excessive number of training blocks enforces the offline memory consolidation in a way that even participants in the day group benefited from the memory consolidation.

It should be mentioned that there was no improvement across sessions in accuracy for any of the groups, but only a general improvement for the wake group. This finding is not consistent with the experimental prediction. However, as the mean accuracy scores were all above 90% for all groups regardless of session, it may be argued that the task was already too easy for the participants to develop offline improvements in terms of accuracy. Moreover, most previous studies have preferred to focus on response times (Robertson, 2007; Robertson, Pascual-Leone, & Miall, 2004; Robertson, Pascual-Leone, & Press, 2004; Spencer, et al., 2006; M. Walker, et al., 2002).

As implicit knowledge was tested only in the second session, it is not possible to determine whether sleep was critical or not for its acquisition, as participants may have developed it before the interval between sessions. Previous studies suggest that the presence of sleep should be irrelevant for offline acquisition of implicit learning (Fischer, et al., 2006; Spencer, et al., 2006). In addition, as both groups significantly improved across session, it is reasonable to assume that implicit knowledge acquisition occurred in the interval. However, this limitation of the study may be overcome by further replications by subjecting participants to a test for implicit knowledge at the end of the training session. On the other hand, plotting the response time of the first session of the participants who had acquired explicit knowledge separated with those who did not, showed a significant different from the 3rd block for the 1st experiment and 5th block for the 2nd experiment which showed that participants who acquired explicit knowledge of the embedded sequence had a potential for realising the hidden sequence discriminating them from those who did not realise the hidden sequence.

3.6.2 Emotional valence

The absence of any effect of emotional valence of the stimuli on performance in the second session is inconsistent with the second hypothesis of the experiment. It was predicted that participants presented with emotionally arousing stimuli would show higher consolidation in the testing session than participants presented with neutral stimuli. This hypothesis was generated on the basis of previous studies showing that emotionally arousing stimuli enhance declarative memory (McGaugh, 2003, 2004), and, more specifically, on the experiment explained in the last chapter. Javadi, Walsh and Lewis (2011) found that the participants who performed on negative images in a mirror tracing task presented higher offline improvements than participants who performed on more neutral images. The inconsistency between the findings of this and the last experiment discussed in this section might be due to two reasons. First, it may be proposed that emotionally arousing images do not affect offline improvement of implicit procedural memory, or, at least, on the SRTT task. However, this position is rather implausible in the light of the last experiment and previous research supporting an interaction between the emotional valence of stimuli and declarative memory consolidation.

Secondly, and more plausibly, it may be suggested that the different type of images used in this study might have affected the study's outcome. The images used in this experiment were drawn from the IAPS and had a high emotionally arousing index, as they represented severely injured people. Many participants referred to the images as "really disgusting". On the other hand, the pictures used in the previous experiment were not drawn from a standard database and represented ugly masks, which are a more tolerable view than the stimuli of this study. Consequently, it may be argued that in this experiment participants may have tried to pay attention to the content of the images as little as possible, in order not to be emotionally disturbed or feel repulsion. This might have impaired the effect of emotional arousal on consolidation. A further study may test this suggestion by presenting participants with less macabre images, as those used by last experiment or by injecting them with adrenaline shortly after training.

3.6.3 Explicit knowledge

The absence of any effect of sleep on explicit knowledge disconfirms the third hypothesis of the study, which stated that the sleep group would present higher explicit knowledge than the wake group as a result of sleep-dependent consolidation. This hypothesis was based on

previous evidence that explicit, offline learning of procedural skill is dependent on sleep (Robertson, 2004; Spencer, et al., 2006), and, more crucially, on a study by Fischer et al. (2006). They tested participants on a SRTT in which the succession of items followed a set of probabilistic rules, and found that the presence of sleep in the interval between sessions had a considerable impact on accuracy at replicating the grammatically correct positions. The different finding of this study may be due to two different characteristics of the generation task used. First, in the study by Fischer et al. (2006), the sequence was based on a probabilistic grammar, thus participants learnt probability rules and not a precise sequence. Hence it might be argued that only grammar but not sequence learning benefits from sleep. Secondly, in the study of Fischer et al. (2006), participants were given feedback of their performance, after each trial of the generation task. This did not occur in the present experiment but might have helped participants to improve their performance. A further replication of this study might provide feedback to support or disconfirm the influence of this factor.

The results showed that some of the participants acquired explicit knowledge of the sequence after a retention interval of 12 hours. From the current design, it is not evident whether this is due to the retention interval or whether participants had realised the sequence throughout the first session before the retention interval. This could be investigated using another group of participants who were asked to do the generation task right after the first session.

In summary, in this study participants in two separate experiments performed a modified or standard version of the SRTT in which they pressed different keys in response to images which all had either a neutral or arousing emotional valence or in response to spatial location of a cueing circle respectively. Participants were unaware that the order of presentation followed a determined sequence. They were retested after an interval of 12-hours which did or did not include sleep. All participants improved response times in the second session and showed implicit knowledge of the sequence. There was no difference in explicit knowledge between the wake and sleep groups and no effect of emotional valence. These findings partly contradict previous research, but most differences might be due to the different characteristics of the design of this study. The number of training blocks was much higher than used in previous studies (Robertson, 2007; M. Walker, et al., 2002). The contextual associations and emotional valence of the stimuli were indeed stronger compared to the previous experiment mentioned in previous chapter, and stronger compared to previous research done by other researchers (Spencer, et al., 2006). Moreover, the explicit knowledge

test did not provide feedback and tested sequence, rather than grammar learning (Fischer, et al., 2006).

Further experiments needed to be carried on to (1) Compare the performance of participants with less number of training blocks with the result of current study which actually might answer the question of why participants in the day group experienced a procedural memory consolidation comparable to the night group, (2) Study the effect of different emotional stimuli in an experiment in which the offline procedural memory consolidation of the day and night groups differs.

Chapter 4. Introduction to Transcranial Direct Current Stimulation (tDCS)

Transcranial electrical current stimulation is a form of non-invasive brain stimulation in which weak electrical current is passed through two or more electrodes that are placed on the scalp or other sites, such as the leg or arm. Due to the direction of the flow of the electrical current, transcranial electrical current stimulation is mainly categorised into two types: transcranial *direct* current stimulation (tDCS) and transcranial *alternative* current stimulation (tACS). The main focus of this thesis is on tDCS. Electrodes in this type of stimulation are called *anode* (positive electrode) and *cathode* (negative electrode) and current flows from anode to cathode. tDCS is classed as *anodal* or *cathodal* according to the electrode which is placed over the target site of stimulation. For example in anodal stimulation of left dorsolateral prefrontal cortex (DLPFC), the anode electrode should be placed over left DLPFC and the cathode electrode should be placed somewhere distant, often over the contralateral supraorbital area.

Electrical brain stimulation has been used for decades but has just recently attracted considerable attention in clinical and cognitive applications. In the 1960s it was shown it that weak polarising currents applied to mammals' brain surface can produce lasting effects on cortical activity (Bindman, Lippold, & Redfearn, 1962, 1964; Creutzfeldt, Fromm, & Kapp,

1962; Hern, Landgren, Phillips, & Porter, 1962). Early animal experiments have revealed that depending on the direction of the current in the targeted brain region, cathodal tDCS reduces spontaneous firing rates of cortical cells, most likely by hyperpolarising the cell body, while anodal stimulation results in the opposite effect (Bindman, et al., 1964; Creutzfeldt, et al., 1962; Purpura & McMurtry, 1965).

For decades the application of transcranial electrical stimulation of the brain was limited to the treatment of depression. Recently researchers have studied and developed new applications for this method. Nitsche et al. (2008) summarises these studies since 1998. It has been shown that transcranial electrical brain stimulation can be effective in different perceptual, cognitive and behavioural functions such as short term memory and motor learning, as well as having clinical applications, such as the treatment of migraine and stroke.

In order to study the effect of electrical brain stimulation, tDCS was deployed to modulate long-term verbal memory. The results of these studies made a strong case for the viability of tDCS to study memory enhancement and impairment mechanisms in healthy participants. It is also shown that short tDCS can effectively be used in addressing temporal activity of different brain areas in long term verbal memorisation. In this section an introduction to tDCS is given and then the two studies that the author has conducted are discussed.

The effects and mechanism of action of transcranial electrical brain stimulation are of great debate. Mechanisms of action of tDCS are studied in many ways but there are still many questions that remain unanswered. The first chapter discusses different possible mechanisms contributing to the action of tDCS. Its effects appear to be due to several settings such as strength, stimulation length, and electrode montage that are discussed on the second chapter as a guideline for designing studies involving tDCS and as a tool to compare studies from different points of attention. And finally, safety consideration and different applications of tDCS are discussed in the third and fourth chapters, respectively.

4.1 Mechanisms of Action

The mechanisms by which tDCS may modulate cognitive and behavioural mechanisms are yet to be clarified. Below, existing knowledge of the effects of tDCS on cerebral function will be reviewed. A leading view is that changes in spontaneous neuronal firing rate contribute to intra-stimulation effects and synaptic neuroplasticity contribute to post-stimulation effects.

Postulated mechanisms of actions are generally based on the following four evidences (Arul-Anandam & Loo, 2009).

4.1.1 Spontaneous Neuronal Firing

It has been shown that tDCS can induce lasting changes in spontaneous neuronal activity without directly inducing action potentials during the period of stimulation. Using computer simulation, Wagner and colleagues (2007) showed that current densities between 0.77 and 2.00mA/cm^2 are well below the action potential threshold for cortical neurons (Tehovnik, 1996; T. Wagner, Valero-Cabre, et al., 2007). Although current densities below 2mA/cm^2 may not directly produce action potentials. Animal studies showed that even this amount of electrical current can change the firing rate of neurons. Bindman et al. (1964) and Purpura and McMurtry (1965) showed that anodal direct current stimulation increases, while cathodal direct current stimulation decreases spontaneous neuronal firing *in vivo* (Bindman, et al., 1964; Purpura & McMurtry, 1965). Moreover, Bindman et al. (1964) also showed that the effect of 5-10 minutes of constant stimulation lasts up to 5 hours. Overall, these experiments showed that subthreshold direct currents can alter neuronal activity.

4.1.2 Non-synaptic Mechanisms

'non-synaptic mechanisms' refers to the changes in resting membrane potential of pre- and post-synaptic neurons. Purpura and McMurtry (1965), using intracellular recordings from animal studies, showed that 30-400 μA/mm^2 anodal stimulation for 5-40 s caused neuronal depolarisation and cathodal stimulation caused hyperpolarisation (Purpura & McMurtry, 1965).

In human studies, the same effects were found. Nitsche and Paulus (2000), recording motor evoked potentials (MEP) from peripheral muscles, showed that 0.2-5.0mA anodal tDCS to the motor cortex for 4s-5min increased MEP size, while cathodal tDCS reduced MEP size. However, MEP testing cannot reject the possibility of a contribution of synaptic mechanisms in tDCS effects. TMS of the motor cortex does not only stimulate corticospinal tract, but there is some stimulation of cortico-cortical circuitry as well. Therefore changes in cortico-cortical synapses contribute to the modulation of MEP after tDCS. Ardonlino et al. (2005) using a technique called transcutaneous electrical stimulation (TES) showed that even in the absence of cortico-cortical input, 1.5mA cathodal tDCS for 10 min still changes MEP. This suggests that

non-synaptic mechanisms do contribute to the observed effects of tDCS. In another study, Antal et al. (2006) showed tDCS to the visual cortex changed visual evoked potentials, contrast sensitivity and motion detection threshold which are also polarity dependant.

4.1.3 Synaptic Mechanisms

Another method of action of tDCS is altering the strength of synaptic transmission. There are studies strongly showing the contribution of this mechanism. As showed above, however, it is unlikely that this method is the only method of action of tDCS. Although long-term potentiation (LTP) and long-term depression (LTD) are frequency cited as candidates (Nitsche, Fricke, et al., 2003), the mechanisms of action of these changes have not yet been fully described. LTP and LTD refer to prolonged enhancement and reduction in neuronal activities that can last hours to months, respectively (Malenka & Bear, 2004). Cooke and Bliss showed (2006) that LTP is induced by concurrent activity of pre- and post-synaptic neurons. The mechanism of action of LTD is poorly understood. It is suggested that anodal tDCS could induce LTP by increasing pre-synaptic activity that is coupled with post-synaptic depolarisation. Conversely, it was shown that cathodal tDCS could induce LTD through reduced pre-synaptic discharge and post-synaptic hyperpolarisation (Nitsche, Fricke, et al., 2003). Some of these phenomena are validated by animal studies (Bindman, et al., 1964) and are known to potentiate and depotentiate synaptic strength, respectively (Frégnac, Smith, & Friedlander, 1990; Froc, Chapman, Trepel, & Racine, 2000).

There are also some studies on modulatory effect of tDCS on TMS which suggest that tDCS may stimulate neurons which makes them, in a way, more or less sensitive to subsequent TMS. Lang et al., and Siebner and colleagues showed that 1mA tDCS over motor cortex for 10 min enhances subsequent effects with 1 to 10 min repetitive TMS (rTMS) (N. Lang et al., 2004; Siebner et al., 2004). Moreover, the direction of action is polarity dependant. Siebner et al. (2004) argue that these changes may reflect homeostatic synaptic plasticity, whereby after a period of excitatory tDCS, neurons are more susceptible to LTD by TMS, while inhibitatory tDCS would make neurons more susceptible to LTP by TMS.

There are other neuroplastic synaptic mechanisms of action of tDCS such as changes of extracellular calcium (Hardingham et al., 2006; Hattori, Moriwaki, & Hori, 1990; Islam, Aftabuddin, Moriwaki, Hattori, & Hori, 1995; Nitsche, Fricke, et al., 2003), protein synthesis during and after stimulation (Cooke & Bliss, 2006; Gartside, 1968), modulation of glutamate levels and glutamate receptors (Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche et al.,

2004) and contribution of its NMDA and AMPA receptors on neuronal plasticity (Antal, et al., 2006; Nitsche, Fricke, et al., 2003; Pittenger & Duman, 2007).

4.1.4 Neuroimaging Findings

Lang et al. (2005) used PET to study the effect of anodal and cathodal tDCS on the primary motor cortex. They showed that 10 min of 1mA stimulation of left M1 can alter widespread cortical and subcortical regional cerebral blood flow (rCBF) with increase and decrease in response to anodal and cathodal stimulation, respectively. The effect was seen in the left M1, right frontal pole, right primary sensorimotor cortex and posterior brain regions. The effect of stimulation remained stable throughout the PET scanning session (50min), demonstrating the long-lasting effects of tDCS. Their study also highlighted the complexity of distribution of tDCS effects. Surprisingly, their results showed an increased rCBF in cortical areas directly underlying the electrodes, which is in contrast to well-described polarity-specific differences in electrophysiological studies (Nitsche & Paulus, 2000). Polarity-specific differences in rCBF, however, were found in distal cortical areas and other brain structures, including the contralateral motor cortex, caudal anterior cingulate cortex, right parieto-occipital junction and cerebellum due to cathodal tDCS that cause reduced rCBF.

The following studies by Lang et al., and Baudewig et al. (2001), using blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI), showed that the related cortico-cortical connections were weakened compared to the connections' strength prior to the stimulation rather than the direct cortical representation of functional challenges that were reduced in mean vowels (Baudewig, Nitsche, Paulus, & Frahm, 2001). For example, they showed that in response to 1mA cathodal tDCS for 5min, a significant reduction in number of activated vowels were recorded in the supplementary motor area (SMA) whereas only negligible changes were found in the M1 hand area. In addition, they showed while anodal tDCS produced no significant changes, cathodal tDCS decreased the mean number of activated voxels for 38% ($p < 0.01$). These findings are in contrast with electrophysiological studies that demonstrated that anodal tDCS increases neuronal excitability (Nitsche & Paulus, 2000). This contradiction, though, may be due to the mechanism of fMRI. fMRI works based on BOLD changes. The BOLD fMRI signal is based on the paramagnetic resonance properties of the deoxyhaemoglobin compound contained with red blood cells. BOLD signal reflects the deoxyhaemoglobin levels. During focal brain activation, cerebral blood flow (CBF) to that region changes by a factor which is different from oxygen consumption which consequently is

recorded by the MRI. These changes in CBF appear to be in response to changes in the excitation-inhibition balance of large neuronal populations in a given region of interest (Logothetis, 2008), which may not correlate well with the modulatory effect of tDCS.

In an electroencephalographic (EEG) study, Antal et al. (2004) showed that 1mA cathodal tDCS to the occipital area for 10min significantly reduced power while anodal stimulation slightly increased the normalised beta and gamma frequency powers. Ardolino et al. (2005) also found that 1.5mA cathodal tDCS for 10min to the motor cortex can produce focally increased low frequency rhythms, especially theta and delta frequencies.

4.2 Use of tDCS

The efficacy of tDCS to induce effective modifications depends on several factors such as current density, which is the quotient of current strength and electrode size (Purpura & McMurtry, 1965), electrode montage, duration of stimulation and phase of stimulation. An overview of the factors that are necessary to control for an acute modification is given.

4.2.1 Electrodes Size

The focality of tDCS is mostly limited to the size of the electrodes (Gandiga, Hummel, & Cohen, 2006). The smallest electrode size used in tDCS experiments is about 2.5cm x 2.5cm. Because of the large electrode size, tDCS might stimulate adjacent cortical areas in addition to the intended area. Moreover, a cephalic reference electrode might also stimulate the brain area beneath it and this electrode is therefore not physiologically inert. Since both electrodes (stimulation and reference electrodes) have similar current and both are placed on the scalp, there is the possibility that both brain areas are stimulated. The issue of an active reference is less important when the hypothesis under study is anatomically constrained; for example by imaging or TMS studies. There are, however, a couple of methods to reduce the stimulating effect of the reference electrode, for example by using bigger electrodes to reduce the current density and consequently reducing its efficacy. This method has previously been used effectively (Fregni, Liguori, et al., 2008; Knoch et al., 2008). Another method is placing the reference electrode on the periphery such as the shoulders (Accornero, Li Voti, La Riccia, & Gregori, 2007; Ferrucci, Marceglia, et al., 2008). It should be mentioned that results achieved by these protocols might differ from those with cephalic references (Accornero, et al., 2007;

Antal, Kincses, Nitsche, Bartfai, & Paulus, 2004; Nitsche & Paulus, 2000; Priori, Berardelli, Rona, Accornero, & Manfredi, 1998).

By using computer simulation, Wagner et al. (2007; 2007) showed the distribution of tDCS over grey matter surface. They revealed that the overall percentage of electrical current affecting the cortex and deeper area is fairly small. The shunting (i.e., the flow of current along the scalp surface as opposed to the cortex) effects were considerably larger for the 1 cm^2 electrodes compared to the larger electrodes. Using different electrode sizes they showed that greater shunting occurs with smaller electrode areas, indicative of the varied resistive paths. This is important when one wants to select the electrode sizes as electrode sizes and shunting effect are inversely related.

Focality of the stimulation can be increased by reducing the electrode size. However, small electrodes could have qualitatively different effects due to: (a) different shunting of current in the scalp; (b) greater edge-effect relative to the overall electrode area (Roth, 1994) and some other factors (T. Wagner, Valero-Cabre, et al., 2007). Boros et al. (2008) studied the effect of stimulation of the motor cortex with different electrode sizes. They showed the effects of stimulation with smaller and larger electrodes are the same for corticospinal excitability, but different on intracortical inhibition and facilitation and the effects were larger for the smaller electrode.

4.2.2 Electrode Montage

Correct placement of the electrodes is important to achieve effective stimulation of desired brain areas. Just two of six different electrode position-combinations so far were effective in stimulating the human motor cortex. The effective combinations may have modulated different neuronal populations (Antal, Nitsche, Kincses, et al., 2004; Antal, Nitsche, Kruse, et al., 2004; Boggio, Castro, et al., 2006; Kuo et al., 2008; N. Lang, Nitsche, Sommer, Tergau, & Paulus, 2003; Nitsche & Paulus, 2000; Nitsche, Schauenburg, et al., 2003; Priori, et al., 1998; Rosenkranz, Nitsche, Tergau, & Paulus, 2000). In two other studies, in which the primary visual cortex was stimulated, the placement of the second electrode over the vertex or the neck resulted in qualitatively different effects on visual-evoked potentials (Accornero, et al., 2007; Antal, Kincses, et al., 2004).

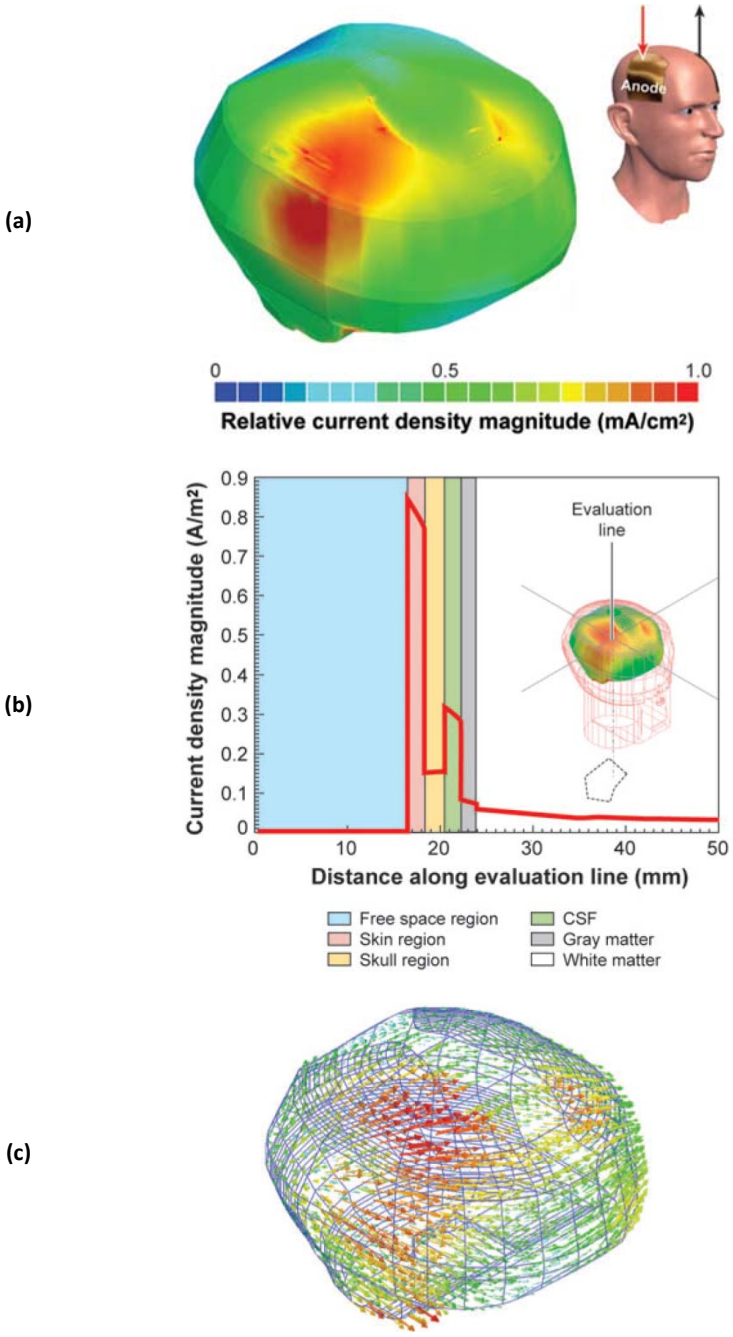


Figure 4-1. Plots of the current density magnitudes on the cortical surface for transcranial direct current stimulation (tDCS). The location of the two electrodes is shown in (a) with the anode (golden) and the cathode (black) represent the tDCS electrodes. (b) Current density magnitude evaluated along the evaluation line in the. (c) Current density vector plots on the grey matter surface. (T. Wagner, Valero-Cabre, et al., 2007)

Rosenkranz et al. (2000) used a training-induced plasticity paradigm to show differential effects of anodal and cathodal tDCS on different neuronal networks. They trained subjects to move their thumb in the opposite direction to a twitch induced by TMS to the motor cortex. This training-induced plasticity led to a temporary change in the direction of the induced twitch toward the training direction. By using 1mA tDCS over the motor cortex for 5min they

diminished this 'directional' change, which surprisingly was not polarity dependant. Since both anodal and cathodal stimulation produced the same effect (inhibition), they suggested that anodal tDCS could activate networks that encode pre-training movements, whereas cathodal tDCS could diminish those networks encoding for new training-induced movements.

Considering the experiments so far, three suitable electrode positions have been suggested (Figure 4-2). In these montage combinations, the positive electrode (anode, red colour) is placed over the target area and the negative electrode (cathode, blue colour) is positioned so that the resulting current flow allows effective modulation of neuronal activity under the anode electrode.

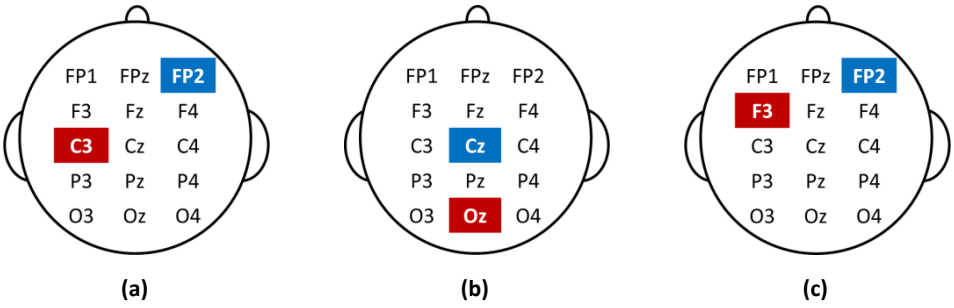


Figure 4-2. Schematic drawing of electrode positions primarily used in transcranial direct current stimulation (tDCS) studies. (a) stimulation of motor cortex, (b) stimulation of visual cortex, (c) stimulation of left dorsolateral prefrontal cortex (DLPFC). Figures (a-c) show anodal (positively charged electrode, red colour) stimulation of the respective area according to the 10-20 system of electrode placement.

4.2.3 Current Strength

Priori et al. (1998) found that 0.3mA anodal stimulation of the motor cortex actually reduced MEP size. This finding is in contradiction with other studies showing that larger anodal currents increase MEP size (Nitsche & Paulus, 2000). This suggests that the direction of change is highly amplitude dependant, and small currents may depolarise superficial inhibitatory neurons or hyperpolarise excitatory neurons. This phenomena might explain the inconsistent findings of the early clinical studies in which much weaker electrical currents (less than 0.5mA) were used compared to recently used amplitudes (1 and 2mA) (Arfai, Theano, Montagu, & Robin, 1970; Costain, Redfearn, & Lippold, 1964).

It has been shown in humans that larger current densities induce stronger effects of tDCS (Iyer et al., 2005; Nitsche & Paulus, 2000). Furthermore, it was found that it is necessary to apply enough current strength to have detectable effects (Boggio, Ferrucci, et al., 2006; Fregni,

Boggio, Nitsche, et al., 2005; Iyer, et al., 2005). Fregni et al. (2005) and Iyer et al. (2005) showed that while a current strength of 2mA was effective in their task, 1mA was not.

4.2.4 Duration of Stimulation

Stimulation duration is one of the most important factors in lasting effect of tDCS. It has been shown that residual effects of tDCS have a strong dependency on the duration of stimulation (Nitsche, Nitsche, et al., 2003; Nitsche & Paulus, 2001b). Nitsche and Paulus (2000) showed that short duration stimulation of primary motor cortex has short-lasting effect on motor cortical excitability which does not outlast the stimulation itself. These short-lived effects, which do not last after the stimulation, are called intra-tDCS effects (Nitsche, Antal, et al., 2008). In a study on long-term verbal memory task the author showed that 1.6s of tDCS did not lead to after-effects (discussed in the following chapters).

The stimulation effect lasts longer for stimulation durations of more than a few seconds. In a series of experiments, Nitsche and colleagues (2000-2003) studied the long-lasting effects of tDCS (Nitsche, Nitsche, et al., 2003; Nitsche & Paulus, 2000, 2001b). They showed that the effect of 9 minutes of stimulation can last up to half an hour and the effect of 11 minutes of stimulation can last for an hour (Figure 4-3).

Although there are several studies on the lasting effects of tDCS over the motor cortex, measured by MEP, there is no study on these lasting effects on the other brain areas, nor on the higher level cognitive tasks such as memory. Javadi and Walsh (Submitted) showed that the effects of 20 minutes of tDCS diminishes after 60 minutes of retention interval.

4.2.5 Phase of Stimulation

In almost all the studies so far, tDCS is applied regardless of each trial. Stimulation is delivered constantly either before the test or during the test. This is mostly due to the duration of the stimulation which is in the range of minutes. An experiment, however, was conducted with 1.6s stimulation that was synched with the onset of each trial, discussed in the following chapters.

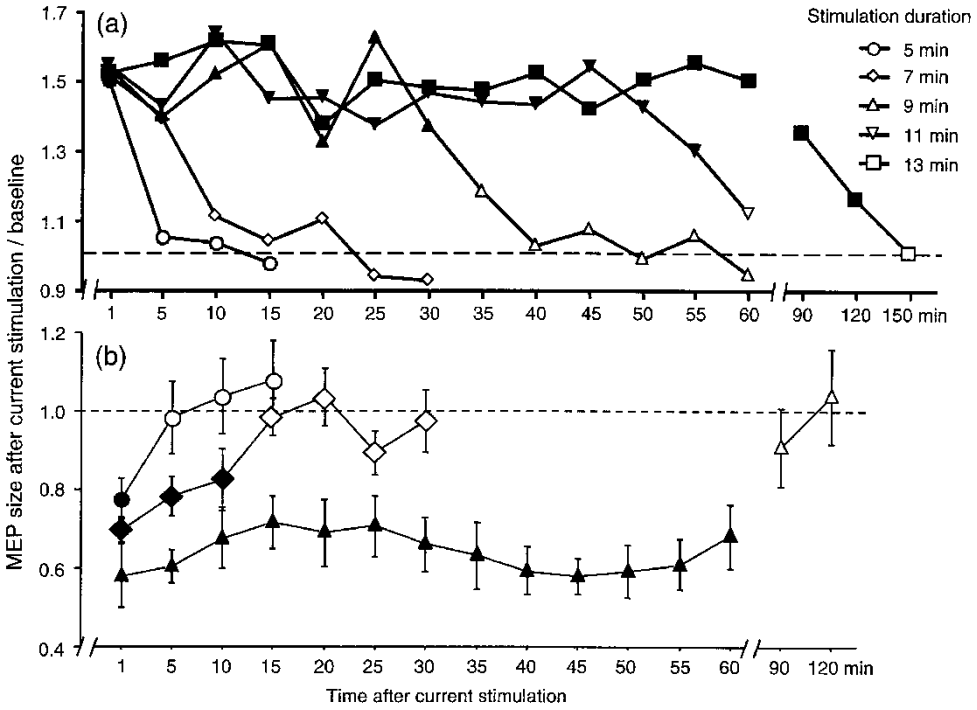


Figure 4-3. Lasting effect of transcranial direct current stimulation (tDCS) on the human motor cortex elicited by motor-evoked potential (MEP). (a) shows enhancing effect of cortical excitability due to anodal stimulation while (b) shows diminishing effect of cortical excitability due to cathodal stimulation (Nitsche, Antal, et al., 2008).

4.3 Safety Considerations

Animal and human studies as well as theoretical knowledge has extensively shown that tDCS, under current protocols, is safe. However, the knowledge about safe limits of duration and stimulation strength is still limited (Iyer, et al., 2005; Nitsche, Cohen, et al., 2008; Nitsche, Liebetanz, Lang, et al., 2003).

Some exclusion criteria must be applied for tDCS studies with healthy participants such as: subjects should be free of unstable medical conditions, or any neurological diseases such as epilepsy or acute exzema under the electrodes. They should have no metallic implants close to the electrodes. Subjects also have to be informed about the possible side effects of the stimulation such as dizziness, headache, nausea, itching sensation and skin irritation under the electrodes (Poreisz, Boros, Antal, & Paulus, 2007).

Safety of stimulation for patients is also important. Generally, all the exclusion criteria mentioned above must be considered. However, because the altered physiology in neuropsychiatric diseases might make the brain more vulnerable to stimulation, safety

measures such as cognitive tests, imaging techniques and questionnaires should be undertaken. Safety considerations are especially important for protocols containing stimulation parameters which are significantly more intense than those used conventionally for healthy participants. It has been shown that tDCS neither causes epileptic seizures nor reduces the seizure threshold in animals (Liebetanz et al., 2006), thus seizures do not appear to be a risk for healthy participants. Nevertheless, this might not apply to patients with epilepsy or a history of seizures.

4.4 Applications

Lippold and Redfearn (1964) were two of the frontiers in studying different aspects of electrical stimulation of neurons and the brain. They showed for the first time that passage of small direct currents through the human brain can change mental states (Lippold & Redfearn, 1964). They applied electrical current using two electrodes that were placed above the two eyebrows of 32 participants and a third electrode above their right knee. The amplitude of the electrical current was between 100 and 250 μA both in anodal and cathodal conditions. The two electrodes on the head were circular with about 1.5cm diameter. The duration of stimulation was between 1 and 5 hours. They showed that 26 of the participants experienced quietness and withdrawal due to negative stimulation (cathodal) and alertness and liveliness due to positive stimulation (anodal) ($p < 0.001$). In two other studies Costain, Lippold and Redfearn (1964) showed the therapeutic effects of polarisation of the brain in depressive illness (Costain, et al., 1964) and in certain psychiatric disorders (Redfearn, Lippold, & Costain, 1964). In the latter two studies, they used the same stimulation strength and electrodes' placement. Duration of the stimulation was up to 8 hours per day for 12 days and 5 times a week. Results demonstrated significant clinical improvement. For about 10 years, the same electrode montage was used in different studies on the effect of electrical stimulation of the brain on depression (Arfai, et al., 1970; Baker, 1970; Carney, Cashman, & Sheffield, 1970; Herjanic & Moss-Herjanic, 1967; Nias & Shapiro, 1974; Ramsay & Schlagenhauf, 1966). These results, however, were not replicated in later studies conducted in the United Kingdom. This inconsistency might be due to differences in stimulation parameters, different patient subgroups or other factors that are not systematically controlled (Lolas, 1977; Nitsche, Liebetanz, Antal, et al., 2003b; Priori, 2003).

In the following years, transcranial current stimulation was almost forgotten, which might be due to the inconsistency in findings and lack of methods (for an overview see (Lolas, 1977)). Recently, the transcranial electrical and magnetic stimulation (TES and TMS) methods, as well as imaging methods such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), have evolved as suitable tools to monitor changes of brain activity and neuronal excitability. Consequently, transcranial electrical current stimulation has been reconsidered as a reliable tool for modulating neuroplasticity in human cerebral cortex (Nitsche, Cohen, et al., 2008; Nitsche, Liebetanz, Antal, et al., 2003a; Nitsche & Paulus, 2000, 2001b; Priori, 2003; Priori, et al., 1998). tDCS allows diagnostic and interventional applications (Fregni & Pascual-Leone, 2007; Liebetanz, et al., 2002; Nitsche & Paulus, 2000; Webster, Celnik, & Cohen, 2006). They also provide a potential therapeutic use in neuro-rehabilitation, chronic pain, focal epilepsy and neuropsychiatric disorders (Antal, Brepohl, et al., 2008; Fregni, Boggio, Lima, et al., 2006; Liebetanz, et al., 2006; Webster, et al., 2006).

In the following, an overview of the implication of tDCS in different applications is given.

4.4.1 Clinical

Transcranial electrical stimulation was first used in clinical trials, especially for treating depression as a tool for modulating human behaviour (Lippold & Redfearn, 1964). It has been used in several studies and has been effectively applied achieving long lasting effects. Table 4-1 summarises the studies on clinical trials of tDCS for depression. Early studies produced inconsistent findings, possibly due to significant variations in the stimulation method. They differed in many aspects, especially current strength, electrode montage and duration of stimulation.

Table 4-1. Summary of clinical trials of tDCS for depression. Reproduced from (Arul-Anandam & Loo, 2009).

Study		Design		Findings
N	**Electrode Montage**	**Current Strength**	**Duration of Stimulation**	
(Redfearn, et al., 1964)	Pilot study applying anodal tDCS in treatment refractory depressed subjects. No sham group.			13 subjects demonstrated clinical improvement, 11 showed either mild or no improvement, and 5 subjects dropped out
29	Two electrodes placed over eyebrows, third placed over leg	20-250μA	Up to 8h/day, 5 times a week, for 6 months. Parameters varied considerably based on side effects and convenience	

(Lippold & Redfearn, 1964)		Double-blinded crossover trial in mostly mildly depressed subjects, applying both cathodal and anodal tDCS at different times, in randomised order. No sham group.		Anodal polarisation increased alertness, mood elevation, tendency to giggle. Cathodal polarisation caused silence, light retardation, withdrawal. Blind raters were able to distinguish between anodal and polarisation in 26 out of 32 cases (p = 0.001)
32	Two electrodes placed over eyebrows, third placed over leg		500µA	Duration not standardised across subjects, due to safety concerns about cathodal stimulation. Duration thus varied widely from 2 min up to 5 hours.
(Costain, et al., 1964)		Double-blinded sham-controlled trial with parallel design, in depressed inpatient subjects		Ratings by nurses and psychiatrists were able to detect significant differences between the two groups at 1-2% level of confidence. Anxiety, agitation and somatic symptoms best responded to treatment. However, self-ratings showed no statistically significant improvement.
24	Two electrodes placed over eyebrows, third placed over leg		250µA	8h/day for 12 days
(Ramsay & Schlagenhauf, 1966)		Open label trial in depressed subjects		14 subjects experienced definite improvement, 4 equivocal improvement, and 2 no improvement
20	Two electrodes placed over eyebrows, third placed over leg		250µA	Approximately 6h/day for 6-8 treatment sessions
(Herjanic & Moss-Herjanic, 1967)		Uncontrolled trial in depressed and schizophrenic patients		Noticeable improvement in all 20 subjects, lasting up to 9 months in some. Some effects indistinguishable from concomitant medications and psychotherapy.
20	Two electrodes placed over eyebrows, third placed over leg		100-500µA	1 to 8h/day, on alternate days, until desired results obtained.
(Arfai, et al., 1970)		Double-blinded sham-controlled trial with parallel design, in depressed inpatient subjects		Concluded that tDCS is therapeutically inert, as no differences were observed between n active and sham conditions
19	Two positive electrodes placed over the eyebrows, one electrode one each thigh		250µA	8h/day for 12 days
(Baker, 1970)		Open-label trial in depressed subjects who received ancillary medications and psychotherapy		Suggests tDCS is efficacious, but findings limited by lack of control group, and concomitant treatment effects (medications and psychotherapy)
107	Two electrodes placed over eyebrows, third placed over leg		200-400µA	Approximately 6h/day for 6-8 treatment sessions
(Carney, et al., 1970)		Uncontrolled trial in depressed outpatient subjects		Based on observations over 5 years, authors suggest that tDCS is most effective in 'atypical' depressives with chronic neurotic symptoms, including phobic and hysterical symptoms
119	Unreported		Unreported	Up to two or three times/week. For various durations. Up to 290 applications of tDCS in one subject
(Nias & Shapiro, 1974)		Double-blinded, sham controlled pilot study comparing effects of anodal, cathodal and sham polarisation in depressed subjects		One subject displayed improvement with anodal polarisation compared to sham, while the other improved with cathodal polarisation
2	Two electrodes placed over eyebrows, third placed over leg		400-500µA	3-4h/day for 14-20 treatment sessions, over several weeks
(Fregni, Boggio, Nitsche, et al., 2006)		Double-blinded, sham controlled trial of anodal tDCS for the treatment of depression		Four out of five in the active group responded, compared to no responders in sham group (p < 0.05). Hamilton Depression Rating Scale and Beck Depression Inventory.
10	Anode placed over left DLPFC, cathode placed over right supraorbital region		1mA	20min/day for five alternated days

(Boggio, Rigonatti, et al., 2007)	Double-blinded, sham controlled trial comparing prefrontal, occipital and sham tDCS for the treatment of depression		Significantly greater reductions in depression rating scores after DLPFC tDCS compared to occipital tDCS and sham tDCS. Beneficial effects in the DLPFC group persisted on one-month follow up. An extension of this study suggested that effects of tDCS are similar to 6 weeks of 20mg/day fluoxetine (Rigonatti et al., 2008)
40	Anode placed over left DLPFC or occipital cortex. Cathode placed over right supraorbital region	2mA	20min/day for 10 sessions, over 2 weeks

Recent clinical studies targeted the M1, the unilateral DLPFC (left or right) or the bilateral DLPFC (left and right) area. In most of the cases anodal stimulation resulted in an improvement of performance (Boggio, Nunes, et al., 2007; Fregni, Boggio, Santos, et al., 2006; Hummel & Cohen, 2005; Nitsche et al., 2005), a decrease in depression scores (Boggio, Rigonatti, et al., 2007; Fregni, Boggio, Nitsche, Marcolin, et al., 2006; Rigonatti, et al., 2008) and less pain perception (Fregni, Boggio, Lima, et al., 2006; Fregni, Gimenes, et al., 2006). A few studies used bilateral stimulation of left and right DLPFC (Boggio et al., 2008; Fregni, Orsati, et al., 2008). These studies showed significant reduction of craving for anode right/cathode left and anode left/cathode right tDCS. Fregni et al. (2008) showed unilateral anodal stimulation of left or right DLPFC reduced smoking craving. Hesse et al. (2007) showed that there was an improvement of aphasia for the patients with paresis after stroke, but only when the stimulation was combined with arm training. Additionally, Hummel et al. (2006) found stimulation to be more effective for patients with more severely impaired. Monti et al. (2008) showed cathodal stimulation could improve naming in patients with chronic non-fluent aphasia. Roizenblatt et al. (2007) showed differential effect of stimulation of M1 and DLPFC. They showed that stimulation over M1 increased sleep efficiency and decreased arousals, while stimulation over DLPFC decreased sleep efficiency, increased rapid eye movement and increased sleep latency. Most of the studies used anodal stimulation; in some studies cathodal stimulation was delivered as well. Most of these studies showed no significant effect of cathodal stimulation (Chadaide et al., 2007; Fregni, Boggio, Santos, et al., 2006). Ferrucci et al. (2008) showed cathodal stimulation significantly worsened word recognition in Alzheimer patients, while anodal stimulation significantly improved performance. In a couple of studies both anodal and cathodal stimulation showed the same effects. Table 4-2 summarises recent clinical studies performed in humans.

Table 4-2. Synopsis of tDCS clinical studies performed in humans since 1998 (Nitsche, Cohen, et al., 2008). A = anodal tDCS; C = cathodal tDCS; S = sham tDCS. Electrode position refers to the international 10-20 system, if appropriate. M1 = primary motor cortex; S1 = primary somatosensory cortex; DLPFC = dorsolateral prefrontal cortex.

Studies	Polarity Stimulation elec. pos. Reference elec. pos.	Duration Current density (mA/cm^2)	Effects
Migraine			
(Antal, Lang, et al., 2008)	A/C M1 Contralateral orbit	10 min 0.029	Short term homeostatic plasticity is altered in patients with migraine
(Chadaide, et al., 2007)	A/C/S Oz Cz	10 min 0.029	Cathodal stimulation had no effect on phosphene thresholds in migraineurs
Depression			
(Fregni, Boggio, Nitsche, Marcolin, et al., 2006)	A/S Left DLPFC Contralateral orbit	20 min (5 days) 0.029	Anodal tDCS leads to a significant decrease in depression scores.
(Boggio, Rigonatti, et al., 2007)	A/S Left DLPFC, occipital cortex Contralateral supraorbital area	20 min (10 days) 0.057	Anodal tDCS leads to a significant decrease in depression scores that lasts for at least 30 d after the end of treatment.
(Rigonatti, et al., 2008)	A/S Left DLPFC Contralateral supraorbital area	20 min (10 days) 0.057	Antidepressant effects of tDCS were similar to those of a 6-week course of fluoxetine (20 mg/day)
Stroke			
(Boggio, Nunes, et al., 2007)	A/C/S M1 (hand area) of the affected (anodal) or unaffected (cathodal) hemisphere Contralateral supraorbital area	20 min (4 weekly sessions or 5 consecutive daily sessions) 0.029	Anodal or cathodal tDCS leads to a motor improvement. Consecutive daily sessions but not weekly sessions were associated with a cumulative motor improvement that lasted for 2 weeks.
(Fregni, Boggio, Mansur, et al., 2005)	A/C/S M1 Contralateral orbit	20 min 0.029	Both cathodal stimulation of the unaffected hemisphere and anodal stimulation of the affected hemisphere improved motor performance.
(Hesse, et al., 2007)	A C3/C4 Contralateral orbit	7 min 0.04	Improvement of arm function in patients with paresis after stroke, when tDCS was combined with arm training, improvement of aphasia
(Hummel et al., 2005)	A/S M1, hand area Contralateral orbit	20 min 0.04	Anodal tDCS improved the performance of a test mimicking activities of daily living with the paretic hand of chronic stroke patients
(Hummel, et al., 2006)	A/S M1, hand area Contralateral orbit	20 min 0.04	Anodal tDCS improved the performance of simple motor functions such as pinch force and reaction times in chronic stroke patients. The improvement was more pronounced in the more impaired patients.

(Monti, et al., 2008)	A/C/S Left fronto-temporal area Right deltoid muscle	10 min 0.057	Improvement of naming in patients with chronic non-fluent aphasia by cathodal tDCS
Parkinson's disease			
(Fregni, Boggio, Santos, et al., 2006)	A/C/S M1, hand area DLPFC Contralateral orbit	20 min 0.029	Anodal tDCS of M1 but not cathodal or DLPFC tDCS improved motor function. Anodal stimulation of M1 increased MEP amplitude and area and cathodal stimulation of M1 decreased them.
Pain			
(Fregni, Boggio, Lima, et al., 2006)	A/S M1 Contralateral orbit	20 min (5 days) 0.057	Pain improvement after anodal stimulation over M1 of patients with central pain due to traumatic spinal cord injury.
(Fregni, Gimenes, et al., 2006)	A/S M1, DLPFC Contralateral orbit	20 min (5 days) 0.057	Anodal tDCS of M1 induced greater pain improvement compared with sham stimulation and stimulation of the DLPFC of patients with fibromyalgia. This effect was still significant after 3 weeks of follow up.
(Roizenblatt, et al., 2007)	A/S Left M1 or DLPFC Contralateral supraorbital area	20 min (5 days) 0.057	M1 tDCS increased sleep efficiency and decreased arousals. DLPFC tDCS was associated with a decreased sleep efficiency, an increase in rapid eye movement and sleep latency. The decrease in REM latency and sleep efficiency were associated with an improvement in fibromyalgia symptoms.
Craving			
(Boggio, et al., 2008)	A/C/S Left or right DLPFC Left or Right DLPFC	20 min 0.057	Both anodal left/cathodal right and anodal right/cathodal left decreased alcohol craving compared to sham stimulation. Following treatment, craving could not be further increased by alcohol cues.
(Fregni, Boggio, Mansur, et al., 2005)	A/C/S Left or right DLPFC Left or Right DLPFC	20 min 0.057	Craving for viewed foods was reduced by anode right/cathode left tDCS. Compared with sham stimulation, subjects fixated food-related pictures less frequently after anode right/cathode left tDCS and consumed less food after both active stimulation conditions.
(Fregni, Liguori, et al., 2008)	A/S Left or right DLPFC Homologue area. Cathodal electrode of 100 cm^2	20 min 0.057	Both left and right DLPFC tDCS, but not sham, reduced smoking craving after cue-exposition.
Diverse			
(Ferrucci, Mameli, et al., 2008)	A/C/S P3-T5, P4-T6 Deltoid muscle	15 min 0.06	Improved word recognition in Alzheimer´s disease by anodal and worsened performance by cathodal tDCS
(Fregni, Marcondes, et al., 2006)	A/C/S Left temporoparietal area Contralateral Supraorbital area	3 min 0.029	Anodal tDCS of LTA resulted in a reduction of tinnitus.

(Huey et al., 2007)	A/S F3 Contralateral orbit	40 min 0.08	No effect on verbal fluency in frontotemporal degeneration
(Quartarone et al., 2007)	A/C M1, hand area Contralateral orbit	10 min 0.029	Lack of tDCS after effects in ALS patients
(Quartarone et al., 2005)	A/C/S M1, hand area Contralateral orbit	10 min 0.029	Lack of inhibition by cathodal tDCS in patients with focal dystony, no clear homeostatic effect with consecutive rTMS

4.4.2 Cognitive/Behavioural

A huge body of literature has studied the modulatory effects of tDCS on different behavioural and cognitive tasks, such as learning and memory, social cognition, perception, decision making etc (Table 4-3). Most of the studies stimulated the left DLPFC (F3) (Fregni, Boggio, Nitsche, et al., 2005; Fregni, Boggio, Nitsche, Rigonatti, & Pascual-Leone, 2006; Iyer, et al., 2005; Ohn et al., 2008), right DLPFC (F4) (Fecteau, Pascual-Leone, et al., 2007; Hecht, Walsh, & Lavidor, 2010; Knoch, et al., 2008), bilateral DLPFC (F3 and F4) (Fecteau, Knoch, et al., 2007; Marshall, Molle, Hallschmid, & Born, 2004; Marshall, Molle, Siebner, & Born, 2005; Priori et al., 2008) and M1 (Boggio, Castro, et al., 2006; Kuo, et al., 2008; N. Lang, et al., 2003; Nitsche, Schauenburg, et al., 2003; Rosenkranz, et al., 2000). In most of the cases, while DLPFC (left or right) was targeted, the motor cortex is used as control area and has been shown that the effects of tDCS are site specific (Boggio, Ferrucci, et al., 2006; Fregni, Boggio, Nitsche, et al., 2005). Sham conditions in all the studies showed no effect, thus the effects of tDCS are genuine. The reference electrode in most of the studies targeting DLPFC was placed over contralateral orbit (Boggio, Bermpohl, et al., 2007; Fecteau, Knoch, et al., 2007; Fregni, Boggio, Nitsche, Rigonatti, et al., 2006; Iyer, et al., 2005; Knoch, et al., 2008; Kuo, et al., 2008; N. Lang, et al., 2003; Ohn, et al., 2008). For studies targeting M1, contralateral orbit was selected for the reference electrode as well (Antal, Nitsche, Kincses, et al., 2004; Antal, Nitsche, Kruse, et al., 2004; Boggio, Castro, et al., 2006; Boggio, Ferrucci, et al., 2006; Fregni, Boggio, Nitsche, et al., 2005; Kuo, et al., 2008; N. Lang, et al., 2003; Nitsche, Schauenburg, et al., 2003; Rosenkranz, et al., 2000). A few studies stimulated the V5 (Antal, Nitsche, Kincses, et al., 2004; Antal, Nitsche, Kruse, et al., 2004), cerebellum (Ferrucci, Marceglia, et al., 2008), CP5 (Flöel, Rösser, Michka, Knecht, & Breitenstein, 2008; Sparing, Dafotakis, Meister, Thirugnanasambandam, & Fink, 2008), FP3 (Kincses, Antal, Nitsche, Bártfai, & Paulus, 2004), P6 and P8 (Varga et al., 2007). In most cases, anodal stimulation resulted in positive results, such as improved learning (Antal, Nitsche, Kincses, et al., 2004; Flöel, et al., 2008; Fregni, Boggio, Nitsche, et al., 2005; Iyer, et al., 2005; Kincses, et al., 2004) and memory (Boggio,

Bermpohl, et al., 2007; Fregni, Boggio, Nitsche, Rigonatti, et al., 2006; Marshall, et al., 2004; Ohn, et al., 2008), higher accuracy (Boggio, Castro, et al., 2006; Marshall, et al., 2005; Nitsche, Schauenburg, et al., 2003; Sparing, et al., 2008) and in some studies it resulted in negative results (Ferrucci, Marceglia, et al., 2008; Marshall, et al., 2005). Anodal and cathodal stimulation in a few studies showed similar effects (Ferrucci, Marceglia, et al., 2008; Marshall, et al., 2005; Rosenkranz, et al., 2000). Cathodal stimulation was shown to be less effective or showed negative effects (Ferrucci, Marceglia, et al., 2008; Marshall, et al., 2005).

Antal et al. (2004) showed an improving effect of cathodal tDCS on visuo-motor performance and modified motion perception threshold by anodal and cathodal stimulation. This is one of the few experiments in which a low level perceptual task was targeted by tDCS. Boggio et al. (2006) showed that 2mA tDCS over left DLPFC improved working memory of Parkinson's patients, while 1mA tDCS did not. This differential effect of 1mA and 2mA stimulation amplitudes underscore that stimulation with a higher intensity might yield a greater beneficial effect on some aspects of cognition (Boggio, Ferrucci, et al., 2006; Fregni, Boggio, Nitsche, et al., 2005; Iyer, et al., 2005). Ferrucci et al. (2008) provided the only study in which the cerebellum (2cm under the inion, 1cm posterior to the mastoid process) was stimulated to study the effect of stimulation on working memory. Interestingly, they showed that both anodal and cathodal tDCS impairs the practice-dependent proficiency of working memory. They used relatively high current density, 0.095mA/cm2. Fregni and colleagues (2005; 2006) showed improving effect of anodal tDCS over left DLPFC on working memory on healthy and depressive patients. Kang et al. (2009) showed anodal tDCS of DLPFC improved stroke patients' response accuracy in Go/No-Go task, whereas it did not show any improvement in healthy participants. The nonsignificant effect of stimulation in healthy participants in this study does not necessarily exclude the possibility of having a significant effect of stimulation in patients. Actually stimulation might be more effective due to the possibly greater plasticity of the patients' brain (Hummel, et al., 2005). de Vries et al. (2009) investigated the causal relationship between Broca's area and learning of an artificial grammar by means of tDCS. They showed that participants who received anodal tDCS had higher accuracy in a subsequent classification task, detecting syntactic violations and rule-based decisions. Marshall and colleague (2004; 2005) used an alternating stimulation protocol with 15s off and 15s on to stimulate left and right DLPFCs bilaterally. In both their studies they used a relatively high current density (0.52mA/cm^2), which is about 18 times higher than most of the studies conducted on healthy participants. They showed that anodal tDCS during slow wave sleep

improved declarative verbal memory. They also showed an impairment effect of bilateral stimulation of DLPFC in the Sternberg-task (Marshall, et al., 2005). Fregni et al. (2005) and Ohn et al. (2003) showed a beneficial effect of anodal tDCS over the left DLPFC. Hecht et al. (2010), in a recent study, showed bilateral stimulation with anode over left DLPFC and cathode over right DLPFC facilitated decision making in a probabilistic guessing task. Andrews et al. (2010) showed the effect of combining cognitive activity and anodal tDCS to the left DLPFC. They demonstrated that tDCS applied while performing a n-back task was more effective, compared to tDCS applied at rest. Their result is in line with Floel and Cohen (2007), who suggested that noninvasive cortical stimulation, in combination with memory training, may enhance the effects of training.

Table 4-3. Synopsis of tDCS cognitive/behavioural studies performed in humans since 1998 (Nitsche, Cohen, et al., 2008). A = anodal tDCS; C = cathodal tDCS; S = sham tDCS. Electrode position refers to the international 10-20 system, if appropriate. M1 = primary motor cortex; S1 = primary somatosensory cortex; DLPFC = dorsolateral prefrontal cortex.

Studies	Polarity Stimulation elec. pos. Reference elec. pos.	Duration Current density (mA/cm^2)	Effects
Learning/memory			
(Antal, Nitsche, Kruse, et al., 2004)	A/C Left V5, M1 Cz, Contralateral orbit	7 min 0.029	Improved visuo-motor performance by cathodal tDCS, modified motion perception threshold by anodal and cathodal tDCS
(Antal, Nitsche, Kincses, et al., 2004)	A/C Left V5, M1 Cz, Contralateral orbit	10 min 0.029	Improved visuo-motor learning by anodal tDCS
(Boggio, Castro, et al., 2006)	A/S M1, hand area Contralateral orbit	20 min 0.029	Anodal tDCS on non-dominant M1 improved motor function.
(Boggio, Ferrucci, et al., 2006)	A/S M1, left DLPFC Contralateral orbit	20 min 0.029 or 0.057	Improvement in working memory of Parkinson´s disease patients after anodal tDCS of the LDLPFC with 2 mA but not with 1 mA.
(Boggio, Bermpohl, et al., 2007)	A/S DLPFC, Occipital cortex Supraorbital area	20 min 0.057	Left DLPFC anodal stimulation of depressive patients induced an improvement in an affective go-no-go task.
(Fecteau, Knoch, et al., 2007)	A/C/S left or right DLPFC Left, right DLPFC, or Contralateral orbit	20 min 0.057	Bilateral DLPFC tDCS with an anodal electrode over the right or the left DLPFC (with cathodal electrode over the homologous area of the contralateral hemisphere) resulted in a risk-averse response style compared to those with sham or unilateral DLPFC stimulation.
(Fecteau, Pascual-Leone, et al., 2007)	A/C/S left or right DLPFC right or left DLPFC	15 min 0.057	Right anodal/left cathodal tDCS resulted in safer responses.

(Ferrucci, Marceglia, et al., 2008)	A/C/S Cerebellum (2 cm under the inion, 1 cm posterior to the mastoid process) Right deltoid muscle	15 min 0.095	Anodal and cathodal tDCS impairs the practice-dependent proficiency in working memory
(Flöel, et al., 2008)	A/C/S Cp5 Contralateral orbit	20 min 0.029	Enhanced language learning by anodal tDCS
(Fregni, Boggio, Nitsche, et al., 2005)	A/C/S M1, DLPFC Contralateral orbit	10 min 0.029	Left DLPFC anodal tDCS leads to an enhancement of working memory performance.
(Fregni, Boggio, Nitsche, Rigonatti, et al., 2006)	A/S Left DLPFC Contralateral orbit	20 min (5days) 0.029	Working memory improvement after anodal tDCS on depressive patients.
(Iyer, et al., 2005)	A/C/sham F3 Contralateral orbit	20 min up to 0.08	Enhanced verbal fluency by anodal tDCS
(Kincses, et al., 2004)	A/C/no Fp3 Cz	10 min 0.029	Anodal tDCS enhanced probabilistic classification learning
(Kuo, et al., 2008)	A/C M1, hand area Contralateral orbit	10 min 0.029	No Impact of tDCS on SRTT and in a simple reaction time task, if tDCS applied before task performance
(N. Lang, et al., 2003)	A/C M1, hand area Contralateral orbit	app. 10 min 0.029	Anodal tDCS affects recall performance after motor sequence learning
(Marshall, et al., 2004)	A/non F3 and F4 Both mastoids	15 sec off/15 sec on over 30 min 0.52	Anodal tDCS during slow wave sleep improves declarative verbal memory
(Marshall, et al., 2005)	A/C/non F3 and F4 Both mastoids	15 sec off/15 sec on over 15 min 0.52	Impaired performance in Sternberg-task by anodal and cathodal tDCS
(Nitsche, Schauenburg, et al., 2003)	A/C M1, hand area premotor, prefrontal, frontopolar cortex Contralateral orbit	About 10 min 0.029	Anodal stimulation of the primary motor cortex during SRTT ans RTT performance resulted in increased performance, whereas stimulation of the remaining cortices had no effect.
(Ohn, et al., 2008)	A/S F3 Contralateral orbit	30 min 0.04	Anodal tDCS enhanced performance in a 3 letter back working memory task
(Rosenkranz, et al., 2000)	A/C M1, hand area Contralateral orbit	5 min 0.029	With tDCS of anodal and cathodal polarity motor training-induced directional change of thumb movements was reduced during a 10 min post-training interval
(Sparing, et al., 2008)	A/C/S Cp5 Cz	7 min 0.06	Improved picture naming by anodal tDCS
Social cognition			
(Knoch, et al., 2008)	C right DLPFC (F4) Contralateral orbit	About 14 min (4 min before and during task performance 0.043 (stimulation electrode) 0.015 (reference)	Less propensity to punish unfair behaviour

(Priori, et al., 2008)	A/C/S Bilateral DLPFC Right deltoid muscle	10 min 0.046	Anodal tDCS over DLPFC influences experimental deception
Perception			
(Varga, et al., 2007)	A/C/S P6-P8 Cz	10 min 0.029	Cathodal stimulation reduced the duration of gender specific after-effect

Many studies have been mentioned in this section. The majority of the studies regarding the differential effects of anodal and cathodal tDCS confirms earlier in vitro and animal studies (Bindman, et al., 1964; Frégnac, et al., 1990; Froc, et al., 2000; Malenka & Bear, 2004; Nitsche, Fricke, et al., 2003). It is shown that the effects of cathodal tDCS (if any) are generally in the opposite direction of anodal tDCS. There are, however, some studies that show similar effect for cathodal and anodal tDCS (Ferrucci, Marceglia, et al., 2008). From the tables it is clear that in studying memory, anodal stimulation is more effective than cathodal tDCS (Boggio, Ferrucci, et al., 2006; Flöel, et al., 2008; Fregni, Boggio, Nitsche, et al., 2005; Fregni, Boggio, Nitsche, Rigonatti, et al., 2006; Kincses, et al., 2004; Ohn, et al., 2008). In the following two sections two studies on verbal memory are discussed, one with long duration of tDCS and the other one with short duration of tDCS. We showed that anodal tDCS over left DLPFC improves memory and cathodal tDCS impairs memory.

Chapter 5. Transcranial Direct Current Stimulation (tDCS) Applied Over Left Dorsolateral Prefrontal Cortex during Encoding or Retrieval Modulates Episodic Verbal Memory

5.1 Abstract

Previous studies have claimed that weak transcranial direct current stimulation (tDCS) induces persisting activity changes in the human motor cortex and working memory, but to date no studies have evaluated the effects of tDCS on episodic memory. Its aim was to determine whether anodal and cathodal transcranial direct current stimulation would differentially modify performance in a word memorisation task during encoding or recall when administered over the left dorsolateral prefrontal cortex (DLPFC). In two experiments, thirty

two participants underwent a series of word memorisation tasks. This task was performed during sham, anodal and cathodal stimulation applied over the left DLPFC. Moreover, participants in the first experiment performed the same task with anodal tDCS of the primary motor cortex (M1). During encoding, anodal stimulation of the left DLPRC improved memory while cathodal stimulation of the same area impaired memory performance in later recognition. Cathodal stimulation of the left DLPRC during recall impaired recognition compared to sham stimulation of the same area. Anodal stimulation of the left DLPFC during recall and anodal stimulation of M1 had no effect on episodic memory. These changes during active stimulation cannot be accounted for by changes in response speed, as response times were not changed by stimulation. The results indicate that active stimulation of the left DLPFC leads to an enhancement or impairment of verbal memorisation depending on the polarity of the stimulation. Furthermore, this effect is specific to the site of stimulation.

5.2 Introduction

Recent studies have highlighted the importance of noninvasive brain stimulation as a means of modulating cortical excitability. Transcranial direct current stimulation (tDCS) is a noninvasive technique for brain stimulation that induces prolonged functional changes in the cerebral cortex through the application of a weak direct current on the scalp (Gandiga, et al., 2006; Kesner & Wilburn, 1974; McGaugh & Gold, 1976; Paulus, 2003; Wassermann, 2008). Safety aspects of this kind of stimulation have been addressed in several studies, which demonstrates that this technique can be safely used in human subjects (Iyer, et al., 2005; Nitsche, Liebetanz, Antal, et al., 2003a; Poreisz, et al., 2007).The effect of tDCS varies depending on the polarity of the electrode - anodal polarisation increases cortical excitability; whereas cathodal polarisation decreases it (J. M. Galea, Jayaram, Ajagbe, & Celnik, 2009; Miranda, Lomarev, & Hallett, 2006; Nitsche & Paulus, 2000; Terney, Chaieb, Moliadze, Antal, & Paulus, 2008). tDCS performed on humans induces sustained elevations in cortical excitability beyond the period of stimulation (Ardolino, et al., 2005; Nitsche & Paulus, 2001a; Priori, 2003; Quartarone et al., 2004). A number of studies using tDCS in humans have been published ((Boggio et al., 2009; Cerruti & Schlaug, 2008; Dockery, Hueckel-Weng, Birbaumer, & Plewnia, 2009; Elmer, Burkard, Renz, Meyer, & Jancke, 2009; Kincses, et al., 2004; Rosenkranz, et al., 2000; Sparing & Mottaghy, 2008; Stone & Tesche, 2009; Tanaka,

Hanakawa, Honda, & Watanabe, 2009; Wassermann & Grafman, 2005), for review see (Been, Ngo, Miller, & Fitzgerald, 2007; Flöel & Cohen, 2007; Hummel & Cohen, 2005; Nitsche, Cohen, et al., 2008)). Several studies have shown that this technique might modulate cortical excitability in the human motor cortex (Di Lazzaro et al., 2004; Kwon et al., 2008; Nitsche, Nitsche, et al., 2003) and visual cortex (Antal, Kincses, et al., 2004; Antal, Nitsche, & Paulus, 2001; Sparing et al., 2009), can have beneficial effects on motor learning (Bolognini, Pascual-Leone, & Fregni, 2009; Hunter, Sacco, Nitsche, & Turner, 2009; Nitsche, Schauenburg, et al., 2003) and visuo-motor coordination tasks (Antal, Nitsche, Kincses, et al., 2004; Antal, Nitsche, Kruse, et al., 2004), and can have clinical applications ((Arul-Anandam & Loo, 2009; Ferrucci et al., 2009; Ferrucci, Bortolomasi, et al., 2008; Ferrucci, Mameli, et al., 2008), for review see (Murphy, Boggio, & Fregni, 2009; Nitsche, Boggio, Fregni, & Pascual-Leone, 2009; Rosen, Ramkumar, Nguyen, & Hoeft, 2009; Schlaug & Renga, 2008)).

In addition to motor and visual learning tasks, working memory in both healthy participants (Ferrucci, Marceglia, et al., 2008; Fregni, Boggio, Nitsche, et al., 2005; Marshall, et al., 2005; Ohn, et al., 2008) and patients (Boggio, Ferrucci, et al., 2006; Fregni, Boggio, Nitsche, Rigonatti, et al., 2006; Jo et al., 2009) has also attracted much attention recently. In all of these experiments the left DLPFC was targeted for stimulation, except for Ferrucci et al. (2008) in which two sites were used, one over the cerebellum and the other over the prefrontal cortex and Marshal et al. (2005), in which stimulation was bilateral on the left and right DLPFC. Fregni et al. (2005) showed that anodal tDCS over DLPFC significantly enhances performance in 3 back letter working memory compared to sham and anodal stimulation of the primary motor cortex (M1). Marshall et al. (2005) studied the effect of bilateral stimulation over two fronto-lateral locations (F3 and F4) on a modified visual Sternberg task (Sternberg, 1966). They did not improve participants' behaviour using active stimulation and observed slower reaction times both for anodal and cathodal stimulation. Ohn et al. (2008) used the paradigm used by Fregni et al. (2005) to study the time dependency effect of tDCS and showed that working memory performance is enhanced with longer stimulation.

So far most of the attention in memory and learning field has been attracted toward working memory. Marshall et al. (2006) applied intermittent slow oscillatory stimulation over frontal cortex during early stage-two sleep to boost slow oscillations and slow spindle activity. Using this method they could enhance hippocampus-dependent declarative memory but not nondeclarative memory. Recently one paper has been published in which the effect of tDCS on episodic verbal short- and long-term memorisation with auditory stimuli is studied (Elmer,

et al., 2009). Elmer et al. administered anodal, cathodal and sham tDCS over left DLPFC or right DLPFC. They showed that only cathodal tDCS over left DLPFC could impair short-term verbal memory when compared to the sham.

The aim of this study was to investigate the effects of tDCS on episodic verbal memory. Based on previous neuroimaging studies on episodic memory (Cabeza & Nyberg, 2000a, 2000b; Martin, 2001) and previous studies on working memory (Boggio, Ferrucci, et al., 2006; Fregni, Boggio, Nitsche, et al., 2005; Jo, et al., 2009; Ohn, et al., 2008), left DLPFC was selected as the main site of stimulation. The location of the other electrode was selected as the contralateral right supraorbital area as suggested by Nitsche et al. (2008) and Im et al. (2008). Anodal, cathodal and sham stimulation types were administered both during encoding and retrieval phases, in two separate experiments, to study effects of stimulation on different stages of memorisation and recognition. To investigate the location specificity of the effects primary motor area (M1) was stimulated as a control site. It was postulated that anodal stimulation of left DLPFC during encoding and retrieval would improve episodic verbal memory and cathodal stimulation of the same site during encoding and retrieval would impair episodic verbal memory.

5.3 Materials and Methods

5.3.1 Participants

In total 32 participants (mean age 22.46, SD 2.31, 19 females) took part in the study comprising of two separates experiments: stimulation during encoding (*n* = 16) and stimulation during recognition (*n* = 16). All participants were university students enrolled at the University of London. All participants were naïve to the study, fluent English speakers and right-handed yielding a laterality quotient of at least +50 on the Edinburgh Handedness Inventory (Oldfield, 1971). All participants had normal or corrected-to-normal vision, and all were screened to exclude those with a history of neurological trauma or psychiatric disorder. No participant was taking any centrally acting medications. Informed consent was obtained from all participants.

5.3.2 Experimental Design

This study was designed as a single-blind, sham and cortical-site controlled experiment. Participants were recruited separately for the first and second experiment. In the first experiment participants were stimulated during the encoding phase, 4 sessions, and in the second experiment participants were stimulated during the recognition phase, 3 sessions. The experiment for the former group was conducted first. All participants attended the whole sessions. Each session contained a different type of stimulation; left DLPFC anodal; left DLPFC cathodal; M1; sham. Table 5-1 summarises the stimulation types in the two experiments and Figure 5-1 shows the schematic presentation of the electrode positions in different stimulation conditions. Order of conditions was randomised. To minimise carryover effects, the interval between sessions was at least 48 hours (Boggio, Ferrucci, et al., 2006).

Table 5-1. Full list of conditions used in this experiment.

Experiment	Stimulation			Electrode Placement	
	Phase	Name	Site	Anode	Cathode
Experiment 1	Encoding	Anodal	Left DLPFC	Left DLPFC	Supraorbital Area
		Cathodal	Left DLPFC	Supraorbital Area	Left DLPFC
		Sham	Left DLPFC	Left DLPFC	Supraorbital Area
		Control	Primary motor cortex	Primary motor cortex (M1)	Supraorbital Area
Experiment 2	Retrieval	Anodal	Left DLPFC	Left DLPFC	Supraorbital Area
		Cathodal	Left DLPFC	Supraorbital Area	Left DLPFC
		Sham	Left DLPFC	Left DLPFC	Supraorbital Area

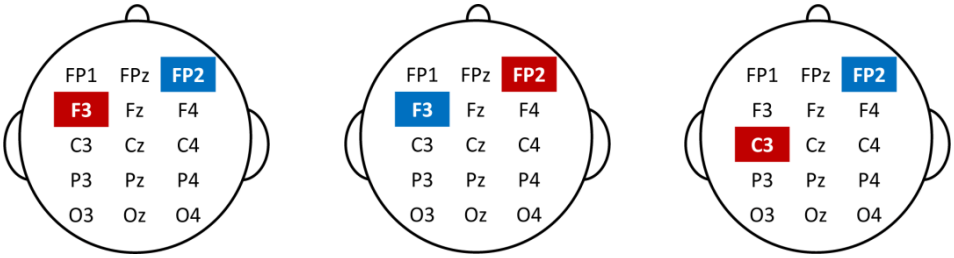


Figure 5-1. Schematic drawing of electrode positions in the study. (a) Anodal and Sham stimulation of left dorsolateral prefrontal cortex (DLPFC) (b) cathodal stimulation of left DLPFC, (c) stimulation of primary motor cortex. Red rectangles show anode electrode and blue rectangle show cathode electrode.

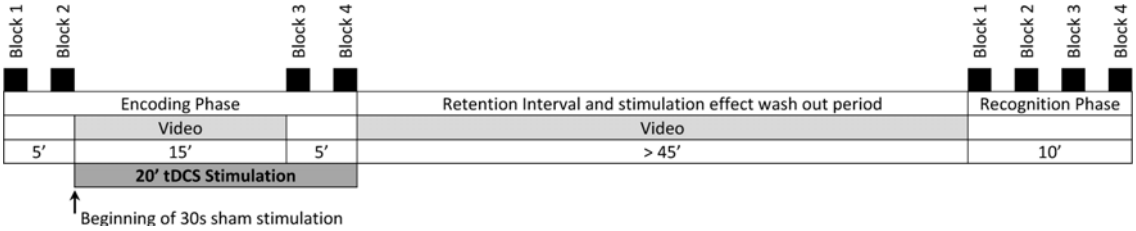
Each session was composed of two phases, an encoding phase and a recognition phase. At the beginning of each phase participants were asked to complete a Stanford Sleepiness Scale (SSS) (Hoddes, et al., 1973), a standard measure of subjective alertness. In the encoding phase participants were shown words, one at a time, and they were asked to first judge the number of syllables of the word as quickly and as accurate as possible and then to memorise it. Participants were instructed to imagine the words in order to memorise them. They were

asked to use imagery method in order to make the memorisation strategy consistence in between the participants. Further, it aimed to reduce the possibility of participants changing their memorisation strategy throughout the session. The instruction given to the participants was as follows:

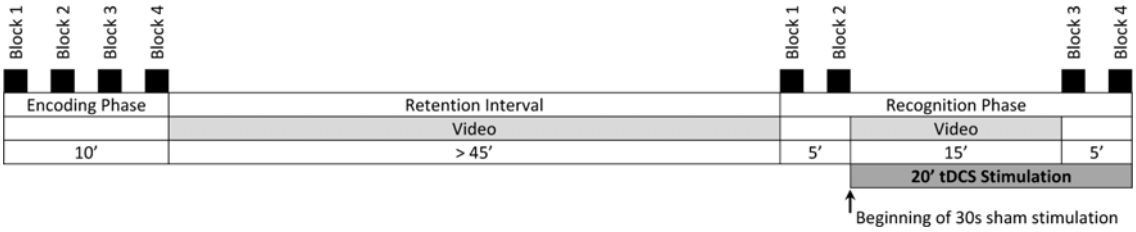
> *"Words will be shown one by one on the screen. Your task is to first judge the number of syllables of the words. Words are either one syllable or two syllables. Please use the mouse with your left hand to specify the number of syllables. The left button of the mouse goes for words with one syllable and the right button of the mouse goes for words with two syllables. Please respond as quickly and as accurately as possible.*
>
> *After responding to the number of syllables, try to imagine the word to memorise it. All the words are selected in such a way that they are easily imaginable. Words are either nouns such as 'apple' or verbs such as 'walking' or adjectives such as 'happy'. Try to imagine the words to memorise them. Later you will be asked to discriminate words which you are going to memorise from new words."*

To exclude the possible influence of interference with the stimulation on motor cortex in the stimulated hemisphere, participants were instructed to perform the tasks during both encoding and recognition phases with their left hand while the left hemisphere was being stimulated. In addition to the above instructions they were debriefed about the procedure of each session and each phase separately, relative to their experiment number. Figure 5-2 shows the procedure of each session.

Each session was composed of two phases, an encoding phase and a recognition phase. The encoding phase was composed of 4 blocks. The first block contained 35 words; the remaining 3 blocks contained 30 words. The first 5 words of the 1st block were considered as practice trials to ensure that participants were familiar with the procedure of each trial. These words were later discarded and were not used in the recognition phase. The timeline of a trial in the encoding phase is shown in Figure 5-3. At the end of each block the percentage of each participant's correct response to the number of syllables of the words was feedback to the subject for 3 seconds. There was a 15s rest interval in between the blocks.



(a) First experiment; stimulation during encoding.



(b) Second experiment; stimulation during retrieval.

Figure 5-2. Procedure of each session; Participants were assigned to one of two experiments that differed in the period during which tDCS was applied. Stimulation was either active or sham. In sham stimulation type, stimulation was stopped after 30' of stimulation. In the active stimulation type, stimulation was initiated after the 2nd block, either in encoding phase or recognition phase, and continued for 15' until the beginning the 3rd block and continued for 5 more minutes to the end of the 4th block.

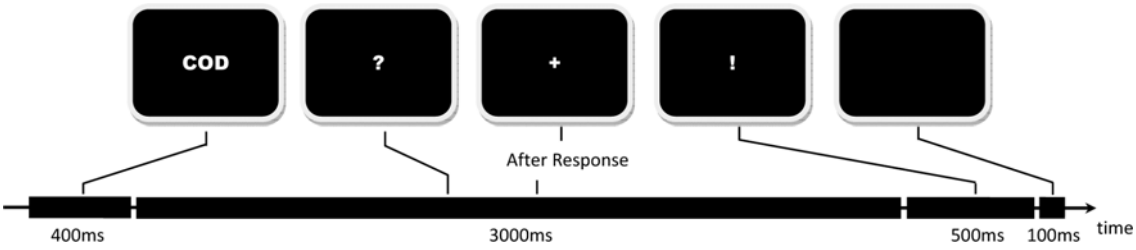


Figure 5-3. Procedure during the encoding phase; Participants were asked to quickly respond to the number of syllables of the words and then try to imagine the words to memorise them. A question mark appeared on the screen after the word presentation. It changed into a cross after participants' responded to the number of syllables. Participants were told that while the cross is on the screen they have time to memorise the word. An exclamation mark was shown to inform the participant that the next word is about to be presented. They were asked to use the mouse with their left hand using the left button for words with one syllable and the right button for words with two syllables.

The mean retention interval was 49.32' (SD 3.40') enough time for the brain to recover from the tDCS (Cerruti & Schlaug, 2008). In between the two encoding and retrieval phases and during the 15' within encoding phase period, in order to engage the participants with a simple task and keep their activity consistence throughout the sessions and between participants, sketches of the Mr. Bean TV Series (Universal Pictures Ltd) were shown. This TV series was

selected to avoid any possible interference of the verbal communications as the amount of verbal communications in this TV series is very little.

The recognition phase was also composed of 4 blocks. Each block consisted of 30 pairs of words. In each trial two words were shown in which one was an old word previously presented in the encoding phase, and one was a new word. The order of the words in the recognition phase was not the same as the order of words in the encoding phase and they were again randomised. The procedure of each trial of the recognition phase is shown in Figure 5-4. Participants were instructed to select the word they saw during the encoding phase, as quickly and as accurate as possible. Accuracy was stressed in the instruction. A question mark appeared on the screen after the words presentation. It changed into a cross after participants responded. An exclamation mark was shown to inform the participant that the next pair of words is about to be presented. For the selection of words appearing on the left hand side, they were asked to use the left button of the mouse with their left hand and for words on the right hand side they used the right button. Participants were not given any feedback of their performance. There was a 15s rest interval in between the blocks.

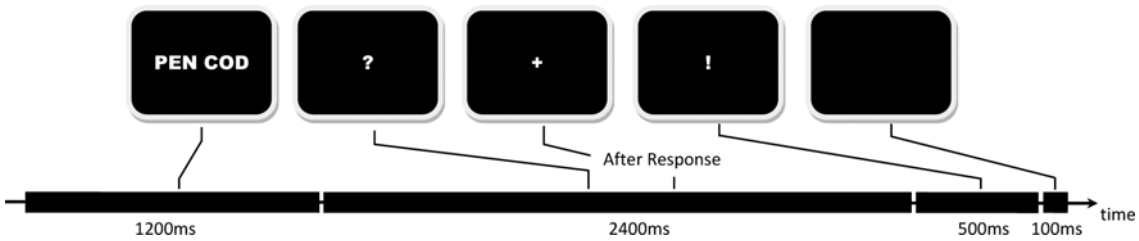


Figure 5-4. Procedure of the recognition phase;

The experiment was performed using a PC computer. Stimuli were presented on a 17" monitor, 75Hz refresh rate, subtending approximately 3-6 degrees of horizontal visual angle. Stimuli were presented on a black background with Arial font, white colour, in capital letters and 53cm from participants' eyes. Stimulus presentation and timing of all stimuli and response events were achieved using MATLAB (MathWorks company) and the Psychtoolbox v3 (Brainard, 1997).

5.3.3 Transcranial Direct Current Stimulation (tDCS)

Direct current was transferred by a saline-soaked pair of surface sponge electrodes (35 x 35 mm) and delivered by Magstim DC Brain Stimulator Plus (Magstim Company). Two different types of stimulation were used to stimulation left DLPFC: anodal and cathodal. In order to test

if the effects of left DLPFC stimulation were location specific, in the first experiment, tDCS was applied over primary motor cortex (M1) in a separate session (Boggio, Ferrucci, et al., 2006; Fregni, Boggio, Nitsche, et al., 2005). Sham stimulation was also delivered to control for somatosensory effects.

5.3.4 Electrode placement

1. For anodal stimulation of the left DLPFC, the anode electrode was placed over F3 (according to the 10-20 international system for electroencephalogram electrode placement (Herwig, Satrapi, & Schönfeldt-Lecuona, 2003; Homan, Herman, & Purdy, 1987)), and the cathode electrode was placed over the contralateral right supraorbital area.
2. For cathodal stimulation the cathode electrode was place over F3 and the anode electrode was placed over the contralateral right supraorbital area.
3. For sham stimulation, the placement of electrodes was the same as anodal stimulation (1) above.
4. For primary motor cortex stimulation the anode electrode was placed over M1 (C3) rather than Left DLPFC (F3) and the cathode electrode was maintained on the contralateral right supraorbital area.

A constant current of 1mA with 15s fade in/fade out was applied for either 20’ for anodal, cathodal and control conditions or 30s for the sham condition, as shown in Figure 5-2. For sham stimulation, the stimulator was turned off after 30s of stimulation as previously described being a reliable method of blinding (Gandiga, et al., 2006). It has been shown that 1mA tDCS for 20’ is safe for human subjects (Iyer, et al., 2005).

5.3.5 Stimuli

A bank of words consisting of 1200 words was extracted from The MRC psycholinguistic database (Coltheart, 1981). The words were verb, noun or adjective. Words were controlled for number of letters (min 3, max 8, mean 4.8897, SD 1.24), number of syllables (min 1, max 2, mean 1.49, SD 0.502), printed familiarity (mean 558.48, SD 31.41), concreteness (mean 542.51, SD 67.73) and imagability (mean 555.60, SD 55.21).

The words which were used in the instructions, i.e. ‘apple’, ‘table’, ‘word’, ‘old’ and ‘new’ were excluded from the list. At the beginning of the first session a set of words was randomly assigned to each participant consisting of 980 words, (30 x 4 + 5 + 30 x 4) x 4, with 50% of the words with one syllable. Participants in each session were presented with new words to prevent any interference with previous sessions.

5.3.6 Statistical Analysis

The effect of tDCS was assessed with a two-way repeated measure ANOVA with stimulation condition (pre-stimulation/post-stimulation) and stimulation type (anodal/cathodal/sham /control) as within-subject factors. Performance percentage and reaction time were measured as dependent variables. A significance level of $p < 0.05$ was used. Bonferroni-corrected post-hoc paired-samples *t*-tests were used to study the difference between conditions. The dependent variables were checked for normal distribution.

5.4 Results

All participants completed the entire experiment. All participants tolerated the stimulation well and there was no complaint of pain or discomfort during the stimulation. Subjects felt the current as an itching sensation at both electrodes at the beginning of the stimulation. Subjects could not distinguish between real and sham stimulation as they felt the initial itching in both conditions. Moreover, explicit questioning at the end of the last session showed that they did not realise that in one session they were stimulated just for the first 30s.

5.4.1 Experiment 1; Stimulation during the encoding phase

Participants took part in this experiment over 4 sessions with different stimulation types (anodal/cathodal/sham/control). To analyse the response accuracy in the recognition phase, we conducted a repeated measures 2 x 4 ANOVA with stimulation condition (pre-stimulation/post-stimulation) and stimulation type (anodal/cathodal/sham/control) as within subject factors for performance. This repeated measure ANOVA indicated a non-significant effect of stimulation condition ($F(1, 15) = 0.52$, $p = 0.48$), significant effect of stimulation type ($F(3, 45) = 3.62$, $p = 0.02$) and significant interaction between the two factors stimulation

condition and stimulation type ($F(3, 45) = 5.37$, $p = 0.003$). Post-hoc comparison with Bonferroni correction showed that there was significant difference between pre- and post-stimulation for left and right DLPFC anodal and cathodal stimulation types but no significant difference between pre- and post-stimulation for sham or control stimulation types, Figure 5-5.

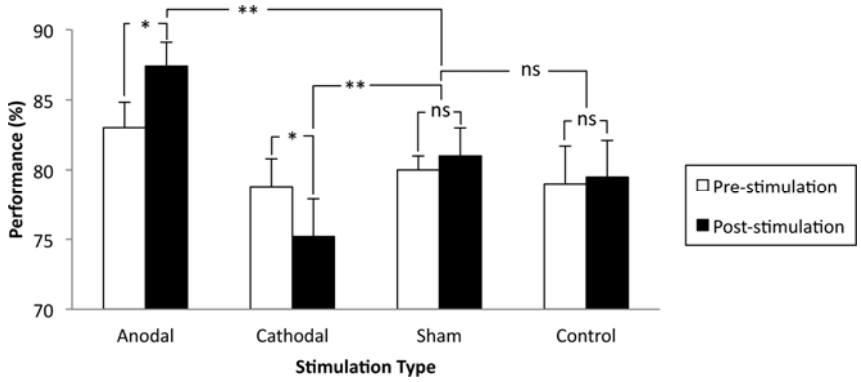


Figure 5-5. Comparison of pre- and post-stimulation conditions of percentage of recognition accuracy when stimulation was administered during the encoding phase; *p < 0.01, ** p < 0.001, ^{ns} not significant. Pre-stimulation stands for the recognition percentage of the words which were presented in the 1st half of the encoding phase. Post-stimulation stands for the recognition percentage of the words which were shown in the 2nd half of the encoding phase while stimulation was administered. Control stands for anodal tDCS over primary motor area (M1). This figure shows that active anodal and cathodal stimulation of left DLPFC during the encoding phase significantly affect the later recognition of the words in the recognition phase. All other comparisons were non-significant. represent one standard deviation (SD).

We analysed the recognition response time (RT) in the recognition phase with a repeated measure 2 x 4 ANOVA with stimulation condition (pre-stimulation/post-stimulation) and stimulation type (anodal/cathodal/sham/control) as within subject factors for mean RT. This repeated measure ANOVA indicated no significant effect of stimulation condition ($F(1, 15) = 0.33$, $p = 0.57$), no significant effect of stimulation type ($F(3, 45) = 0.61$, $p = 0.61$) and no significant interaction between the two factors stimulation condition x stimulation type ($F(3, 45) = 1.54$, $p = 0.22$). This shows that higher performance with anodal stimulation or lower performance with cathodal stimulation is not due to changes of reaction time.

The effect of stimulation on participants' accuracy and RT in the syllable judgment task was also investigated. A repeated measure 2 x 4 ANOVA with stimulation condition (pre-stimulation/post-stimulation) and stimulation type (anodal/cathodal/sham/ control) as within subject factors for mean syllable judgment accuracy and mean RT was conducted. This indicated no significant effect of stimulation condition ($F(1, 15) = 0.45$, $p = 0.51$ for accuracy;

$F(1, 15) = 0.28$, $p = 0.60$ for RT), no significant effect of stimulation type ($F(3, 45) = 0.30$, $p = 0.82$ for accuracy; $F(3, 45) = 0.35$, $p = 0.79$ for RT) and no significant interaction between the two factors stimulation condition x stimulation type ($F(3, 45) = 0.58$, $p = 0.63$ for accuracy; $F(3, 45) = 0.37$, $p = 0.77$ for RT).

To analyse the alertness of the participants at the beginning of each phase, we conducted a repeated measures 2 x 4 ANOVA with phase (training/testing) and stimulation type (anodal/cathodal/ sham/control) as within subject factors for the Stanford Sleepiness Scale (SSS) rating. This ANOVA indicated no significant effect of phase ($F(1, 15) = 0.04$, $p = 0.84$), no significant effect of stimulation type ($F(3, 45) = 0.35$, $p = 0.79$) and no significant interaction between the two factors phase x stimulation type ($F(3, 45) = 0.64$, $p = 0.59$).

5.4.2 Experiment 2; Stimulation during the recognition phase

Sixteen participants (9 female) attended this group with 3 sessions, each session with different type of stimulation, anodal/cathodal/sham. As there were no effects on memory performance in the control stimulation type in the first experiment, control stimulation was not administered in this experiment. To analyse the response accuracy in the recognition phase, a repeated measures 2 x 3 ANOVA was conducted with stimulation condition (pre-stimulation/post-stimulation) and stimulation type (anodal/cathodal/sham) as within subject factors for performance. This repeated measure ANOVA indicated no significant effect of stimulation condition ($F(1, 15) = 0.15$, $p > 0.7$), no significant effect of stimulation type ($F(2, 30) = 1.126$, $p > 0.4$) but significant interaction between the two factors stimulation condition x stimulation type ($F(2, 30) = 13.89$, $p < 0.05$). Post-hoc comparisons with Bonferroni correction showed a significant difference between the two conditions of cathodal stimulation type, see Figure 5-6.

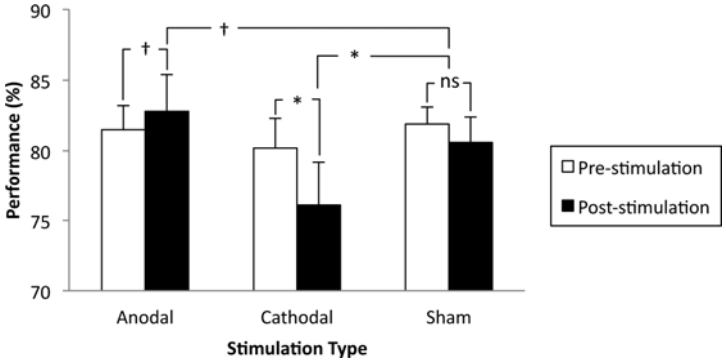


Figure 5-6. Comparison of pre- and post-stimulation conditions of percentage of recognition accuracy when stimulation was administered during the recognition phase; *$p < 0.01$, † $p < 0.2$, ^{ns} not significant. Pre-stimulation stands for the recognition percentage of the words which were presented in the 1st half of the recognition phase. Post-stimulation stands for the recognition percentage of the words which were shown in the 2nd half of the recognition phase while stimulation was administered. All other comparisons were non-significant. Error Bars represent one standard deviation (SD).

To analyse any possible effect on the recognition RT in the recognition phase a repeated measures 2 x 3 ANOVA was conducted with stimulation condition (pre-stimulation/post-stimulation) and stimulation type (anodal/cathodal/sham) as within subject factors for mean RT. This repeated measure ANOVA indicated no significant effect of stimulation condition (F(1, 15) = 1.2, p > 0.4), no significant effect of stimulation type (F(2, 30) = 0.87, p > 0.6) and no significant interaction between the two factors stimulation condition x stimulation type (F(2, 30) = 0.64, p > 0.6). This shows that different types of stimulation did not change participants' response speed which means lower performance in cathodal stimulation type is not due to faster reaction times.

To analyse the alertness of the participants at the beginning of each phase, a 2 x 3 repeated measure ANOVA was conducted with phase (training/testing) and stimulation type (anodal/cathodal/sham) as within subject factors for the Stanford Sleepiness Scale (SSS) rating. This ANOVA indicated no significant effect of phase (F(1, 15) = 0.17, p > 0.8), no significant effect of stimulation type (F(2, 30) = 0.19, p > 0.8) and no significant interaction between the two factors phase x stimulation type (F(2, 30) = 0.22, p > 0.7).

5.5 Discussion

The results showed that transcranial direct current stimulation (tDCS) of left dorsolateral prefrontal cortex (DLPFC) can significantly modulate memory performance of verbal memory while administered during encoding (first experiment) or recognition (second experiment) and its effects were location specific and polarity dependent. The results of the first experiment showed that anodal stimulation of left DLPFC during the encoding phase enhanced the memory performance in a later recognition task. Cathodal stimulation, however, impaired the later recognition of stimuli. Sham stimulation and stimulation of primary motor cortex did not affect the memory performance. The results of the second experiment showed that anodal stimulation of left DLPFC during recognition did not affect the recognition of studied words and only cathodal stimulation impaired the recognition performance. Reaction time (RT) didn't change in any of the stimulation conditions. The results are considerable in three aspects: improving effect of anodal and impairing effect of cathodal stimulations during encoding and impairing effect of cathodal stimulation during recognition.

5.5.1 Encoding or Retention Improvement due to Anodal Stimulation during Encoding

Considering the beneficial effects of anodal tDCS over left DLPFC in previous studies on working memory (Boggio, Ferrucci, et al., 2006; Ferrucci, Marceglia, et al., 2008; Fregni, Boggio, Nitsche, et al., 2005; Jo, et al., 2009; Ohn, et al., 2008), it was plausible to hypothesise that anodal stimulation of left DLPFC will enhance the episodic verbal memorisation. Transcranial direct current stimulation in the human is able to induce sustained cortical excitability elevations beyond the period of stimulation (Ardolino, et al., 2005; Nitsche & Paulus, 2001a; Priori, 2003). Using transcranial magnetic stimulation (TMS), Nitsche and Paulus (2001a) showed that the effect of tDCS over motor cortical area last up to 90 minutes after the end of stimulation. This duration is not consistent between different subjective statuses even in the same area though. Quartarone et al. (2004) showed that two different subjective states changes the duration of lasting of the effects of tDCS applied over the primary motor cortex. So far, lasting effect of tDCS applied over DLPFC has not been studied yet. Post-hoc tests comparing the recognition accuracy in sham and control stimulation types with recognition accuracy in pre-stimulation condition in anodal stimulation type showed that recognition accuracy in pre-stimulation condition in these three stimulation types are

comparable, which shows that the possible residual effect of anodal stimulation over left DLPFC did not affect later recognition accuracy in pre-stimulation condition per se or the effect has been diminished and performance improvement in later recognition accuracy in post-stimulation condition is not due to enhanced retrieval ability.

It is, however, unclear whether observed enhancement in anodal stimulation type is due to stronger encoding of the studied words or better retention of the studied words. Further experiments need to be conducted to address this question.

Furthermore, one might argue that the higher accuracy performance on later recognition of the words presented during the second half of the encoding phase is due to 15' shorter retention interval as the second half of the encoding phase was conducted after 15' of stimulation. Shorter retention interval cannot be the cause of higher accuracy performance though, since the same timing is used for sham or control stimulation types in which no significant enhancement has been observed.

5.5.2 Recognition Impairment due to Cathodal Stimulation during Retrieval

Effects of tDCS are polarity dependant. Depending on the polarity of the applied current, neural firing rates increase (anodal) or decrease (cathodal) (Flöel & Cohen, 2007; Nitsche & Paulus, 2001a). The results show that administration of cathodal stimulation over left DLPFC during recognition of episodic memory impairs the retrieval accuracy. This effect might be because of less neural activity in left DLPFC.

5.5.3 Disruption of Retention of the Words Encoded prior to Cathodal Stimulation

It should be stressed that observed impairment in recognition phase cannot be due to lasting effects of cathodal stimulation during the second half of the encoding phase because the words in the recognition phase was randomised and the order of presentation is not as in encoding phase. So the words are shuffled and even if effects of cathodal stimulation lasts until the recognition phase it would affect recognition of both word groups studied before and after the administration of stimulation.

5.5.4 General discussion

Considering the fact that left prefrontal cortex is highly active in so many cognitive tasks (for review see (Desgranges, Baron, & Eustache, 1998; Fletcher, Frith, & Rugg, 1997; Fletcher & Henson, 2001; Fletcher, Shallice, & Dolan, 1998; Fletcher, Shallice, Frith, Frackowiak, & Dolan, 1998)) none of previous studies which studied effect of stimulation on frontal cortex has mentioned or completely rejected the possibility of more or less engagement of other systems modulation due to stimulation (Boggio, Ferrucci, et al., 2006; Cerruti & Schlaug, 2008; Elmer, et al., 2009; Ferrucci, Marceglia, et al., 2008; Jo, et al., 2009; Ohn, et al., 2008; Wassermann & Grafman, 2005), such as attention which has to be addressed in future studies. Two evidences in this study partly reject the possibility of functional change of other systems due to the stimulation, accuracy and reaction time (RT) in syllable judgment in the encoding phase and RT in the recognition during recognition phase. As shown all these measurements are comparable in between different conditions and stimulation types within each participant, which can reject the possibility of engagement of attentional shift or single word processing systems under the influence of stimulation.

The closest study to this investigation is the study done by Elmer et al. (2009). The result, however, differ with their results in two points. Administration of anodal tDCS over left DLPFC during encoding enhanced the recognition during later retrieval. Moreover, using cathodal tDCS over left DLPFC during encoding impairs the recognition in later retrieval. Elmer et al. did not observe any significant effect of stimulation during encoding. They, however, found significant effect of cathodal stimulation during retrieval. The result in line with their finding shows that cathodal stimulation over left DLPFC impairs recognition of episodic verbal memory. This study, though, differs from theirs in several ways as following. In this study, depending on anodal or cathodal stimulation type either anode or cathode electrode was placed on F3 and contralateral right supraorbital area vice versa, which, in their study, they put one of the electrodes on F3 or F4 and the other one on mastoid which vastly affects the flow of current (Im, et al., 2008; Nitsche, Doemkes, et al., 2007). They used auditory stimuli and here visual stimuli were used. The duration of stimulation in their study was 5’ and in mine was 15’ prior and 5’ during the presentation of the words. Possibly in their case the targeted brain area did not undergo enough stimulation to see behavioural effects as has been shown that it takes a while to see behavioural changes due to stimulation (Ohn, et al., 2008). Considering the wider area of stimulation in their study, the stimulation intensity per cm^2 was lower. They applied 1.5mA electrical current over 28 cm^2 compared to mine which

was 1mA electrical current applied over 12.25 cm^2, 53 $\mu A/cm^2$ in their study vs. 81 $\mu A/cm^2$ in this study. It has been shown that the intensity of the stimulation is a critical parameter. Boggio et al. (2006) showed that 2mA anodal tDCS over left DLPFC significantly improved working memory accuracy in patients with Parkinson's disease while stimulation with half of the intensity, 1mA, did not show any significant behavioural effect. This can also be the case with the finding on anodal stimulation of left DLPFC during retrieval phase in which a trend toward better recognition was observed. Possibly higher electrical current intensity can significantly improve the recognition accuracy.

In conclusion, the results demonstrated that active stimulation of left DLPFC affects later recognition of words in episodic verbal memorisation paradigm. It is shown that anodal tDCS over left DLPFC can improve later recognition and cathodal tDCS over the same area impairs the recognition of the words. Moreover, that cathodal stimulation of left DLPFC during retrieval affected the recognition of the encoded episodic verbal memory. The study failed to show any significant effect of anodal tDCS over left DLPFC during retrieval, although it tends to improve recognition task. Using stimulation of primary motor cortex it is shown that the affect of tDCS over left DLPFC is location specific. Several questions have been raised by the study which needs to be addressed in future studies.

5.6 Acknowledgements

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Chapter 6. Administration of Short Duration Transcranial Direct Current Stimulation (tDCS) at the Correct Time Modulates Performance of Episodic Verbal Memory

6.1 Abstract

According to a pilot EEG study, the left dorsolateral prefrontal cortex (DLPFC) displayed higher activity in the first half of a trial than in the second half. The aim of this study was to investigate the effects of tDCS on memory accuracy, and whether the effects were affected by stimulation administered early or late. Eleven participants memorised words under anodal and cathodal tDCS to the left DLPFC in two separate sessions in no-stimulation, early-

stimulation and late-stimulation trials. Early stimulation occurred during word presentation. Late stimulation occurred after word presentation. Early anodal tDCS led to significantly better accuracy in a subsequent recall test compared to cathodal tDCS. The effects cannot be attributed to differences in reaction times. The results are discussed in light of possible clinical applications of tDCS for patients with memory deficits.

6.2 Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive technique of brain stimulation that can induce prolonged changes in cerebral cortical excitability by applying a weak direct current (DC) on the scalp (Gandiga, et al., 2006; Paulus, 2003). The effects of tDCS can be relatively site-specific, with different behavioural changes observed following the stimulation of different cortical areas. For example stimulating the various areas of the motor cortex influences thumb and leg movements, while attentional shifts between global and local feature processing are modulated by stimulating the posterior parietal cortex (Rosenkranz, et al., 2000; Stone & Tesche, 2009; Tanaka, et al., 2009). Therefore, depending on where it is administered, tDCS may benefit motor learning and visual-motor coordination (Antal, Nitsche, Kincses, et al., 2004; Bolognini, et al., 2009), and has been suggested to have clinical applications for patients suffering from Alzheimer's disease, stroke and depression (Arul-Anandam & Loo, 2009; Ferrucci, Mameli, et al., 2008; Schlaug & Renga, 2008).

The effects of tDCS are polarity-dependent: anodal stimulation increases cortical excitability while cathodal stimulation decreases it (Miranda, et al., 2006). Nitsche and Paulus (2000) observed that administering anodal tDCS to the motor cortex raised its excitability, as reflected by increased motor-evoked potentials (MEPs) in the hand compared to a baseline condition. Conversely, cathodal tDCS to the motor cortex dampened its excitability and led to reduced MEPs. Similarly, a separate study that employed electromyography (EMG) to measure changes in cortical activity reported that anodal tDCS applied to the right cerebellar cortex significantly enhanced activity while cathodal stimulation decreased it (J. M. Galea, et al., 2009). Furthermore, about 20 minutes of tDCS has been found to induce sustained cortical changes that remain up to 60 minutes after stimulation has stopped (Nitsche & Paulus, 2001a; Priori, 2003).

Recent developments in tDCS research have focussed on its effects on working memory in healthy participants and patients (Boggio, Ferrucci, et al., 2006; Marshall, et al., 2004). In a study involving stroke patients who had cognitive deficits, administering 25 minutes of anodal tDCS to the left dorsolateral prefrontal cortex (DLPFC) was found to improve their accuracy in a 2-back working memory task (Jo, et al., 2009). Adopting a similar paradigm, Fregni et al. (2005) applied 10 minutes of anodal stimulation to the left DLPFC of healthy participants and reported that their performance on a subsequent 3-back memory test significantly improved compared to sham stimulation. Congruently, both studies reported that the response times of participants were not affected by tDCS stimulation, a result that is supported by other studies (Ferrucci, Marceglia, et al., 2008; Fregni, Boggio, Nitsche, Rigonatti, et al., 2006).

Ohn et al. (2008) extended the literature by demonstrating that 30 minutes of anodal tDCS improved working memory accuracy significantly more than 20 minutes of stimulation, indicating that the benefits of anodal tDCS increase with stimulation duration. Boggio et al. (2006) reported that while 2mA of anodal tDCS administered to the prefrontal cortex significantly improved working memory accuracy in patients with Parkinson's disease, a 1mA stimulation did not. It seems then that stimulation intensity is a critical parameter in eliciting the effects of tDCS (c.f. (Iyer, et al., 2005)).

In comparison, little attention has been devoted to the effects of tDCS on episodic memory. In one study, Elmer and colleagues (2009) presented participants with a word list to memorise and applied sham, anodal and cathodal tDCS to the right or left DLPFC. In a subsequent verbal recall test conducted 25 minutes after stimulation ended, it was reported that neither stimulation polarity had an effect on long-term memory retrieval. In contrast, the results showed that administering anodal tDCS to the left DLPFC when participants were learning words improved their memory accuracy on a word recall test performed 45 minutes after stimulation; while cathodal stimulation impaired subsequent memory performance. The divergence in findings may be attributed to the former study selecting the mastoid for the reference electrode's position, while the latter study chose the right supraorbital area as the reference. The placement of electrodes has been found to wield considerable influence over the flow of direct current, which may alter the effects of tDCS (Im, et al., 2008). Furthermore, participants in the first study were stimulated at a lower intensity and for a shorter duration, which may not have been sufficient to fully induce the effects of tDCS on long-term memory (Boggio, Ferrucci, et al., 2006; Ohn, et al., 2008).

In a further development electroencephalography (EEG) was employed to record the cortical activity of participants when they were encoding words for long-term memory (Appendix ii). It was noted that for 4-second trials in which participants had to imagine a word in order to memorise it, the left DLPFC displayed higher activity in the first half of a trial than in the second half. Therefore it was postulated that tDCS will have a different effect on memory depending on whether it is administered early or late on in a trial.

The aim of this study was to extend previous research by investigating the effects of administering tDCS during the encoding phase of episodic memory. The results of this study may potentially help to reconcile the differences in the literature. Participants attended 2 experimental sessions, each of which was split into a training phase and a testing phase. In the training phase, participants were required to memorise words for a subsequent memory test. In a repeated-measures design, the left DLPFC of participants was stimulated with anodal or cathodal tDCS in the first or second half of a trial, or not stimulated at all for a trial in the training phase. In the testing phase, participants were required to identify the old words that were presented among novel distracter words. The dependent variables recorded were the proportion of correct responses they made in this task and the time they took to respond.

Previous studies suggest that anodal tDCS administered to the left DLPFC boosts some kind of cognitive ability while cathodal stimulation dampens some kind of cognitive ability (Antal, Nitsche, Kruse, et al., 2004; Boggio, Castro, et al., 2006; Flöel, et al., 2008; Iyer, et al., 2005; Kincses, et al., 2004; N. Lang, et al., 2003; Ohn, et al., 2008). Therefore, the first prediction is that anodal tDCS will lead to higher memory accuracy on the memory recall task, and that cathodal tDCS will impair subsequent memory accuracy. Also, according to the EEG study, the DLPFC displays more activity in the first half of a trial than the second half when participants are required to remember words (Appendix ii). The second prediction is therefore that tDCS will have a greater effect on memory accuracy when it is administered in the first half of a trial rather than the second half.

6.3 Method

6.3.1 Participants

A total of 13 university students took part in the experiment. There were 6 females, and the mean age of participants was 22.42 years (range 18-26 years). All participants had normal or corrected-to-normal vision, and were right-handed, fluent English speakers who were naïve to the purpose of the study. In addition, none of the participants were taking any centrally-acting medication. Informed consent was obtained from all participants, and they were monetarily reimbursed for their participation in the study.

6.3.2 Design

The experiment adopted a 2 x 3 repeated-measures design. The first independent variable that was varied within subjects was stimulation polarity, which had 2 levels: anodal and cathodal stimulation. Participants attended 2 experimental sessions that were separated by at least 48 hours to minimise carryover effects (Boggio, Ferrucci, et al., 2006). Within one session, participants received either anodal or cathodal stimulation only. In order to eliminate potential order effects, 9 of the participants were assigned to receive anodal stimulation first while the others began with cathodal stimulation.

The second independent variable that was also varied within subjects was the time of stimulation onset during a trial in the training phase. Three levels were used: early onset in the first half of a trial; late onset in the second half of a trial; or no stimulation for that trial. As this study is primarily interested in examining the effects of tDCS on episodic memory encoding and not memory retrieval, tDCS was only administered during the training phase and not the testing phase.

The dependent variables recorded were the responses in a subsequent recall task that required participants to identify the old words that were presented amidst novel distracter words, and the time they took to make their responses.

6.3.3 Stimuli

A bank of 1354 words was extracted from the MRC psycholinguistic database (Coltheart, 1981). The words were controlled for number of letters (min. = 3, max. = 8, μ = 4.89, SD =

1.24), number of syllables (min. = 1, max. = 2, μ = 1.49, SD = 0.50), printed familiarity (μ = 558.48, SD = 31.41), concreteness (μ = 542.51, SD = 67.73) and imagability (μ = 555.60, SD = 55.21).

In the training phase, participants were presented with 8 blocks of 21 randomly-selected words. The first block had 5 additional words in front to allow participants to practise the memorisation task. These additional words were discarded and not tested in the testing phase. In the testing phase "quad word" presentation was used: each previously-displayed word was presented together with 3 new words randomly chosen from the bank. The 677 words remaining in the bank after the first session were then used in the second session to prevent interference between the words used in both sessions.

6.3.4 Apparatus

The experiment was run on a desktop computer with a 17-inch monitor and 75 Hz refresh rate with the resolution 1024 x 768. Stimuli were presented in capital letters using 16-point Arial font, subtending approximately 3 – 6 degrees of horizontal visual angle, and were displayed in white colour against a black background. Stimuli presentation and the recording of response time were achieved using MATLAB (v7.5; MathWorks Company) and the Psychtoolbox v3 (Brainard, 1997; Pelli, 1997). Data analyses were performed using SPSS (v17.0; LEAD Technologies, Inc.).

6.3.5 Brain Stimulation

Direct electrical current was administered using a MagStim DC Brain Stimulator Plus unit (MagStim Inc.) and delivered via a pair of saline-soaked surface sponge electrodes (35 x 35mm over target site and 55 x 55mm over non-target site) attached to the skin of participants. The left DLPFC was chosen as the main site of stimulation, while the contralateral supraorbital area was selected as the reference electrode position (Im, et al., 2008; Nitsche, Cohen, et al., 2008). For anodal stimulation of the left DLPFC, the anode was placed over F3 in accordance with the 10-20 international system for EEG electrode placement (Homan, et al., 1987) and the cathode was placed over the right supraorbital area (Figure 6-1). For cathodal stimulation of the DLPFC, the positions of the electrodes were swapped. This procedure has been shown to be relatively accurate at locating the left DLPFC in subjects (Herwig, et al., 2003).

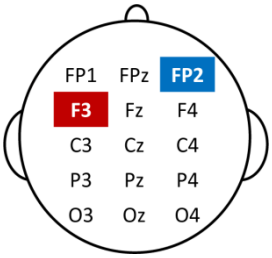


Figure 6-1. Electrode positions selected to stimulate left dorsolateral prefrontal cortex (DLFPC) according to the 10-20 international system. Red rectangle (F3) represents anode electrode and blue rectangle (FP2) represents cathode electrode over contralateral supraorbital.

Previous studies have reported that a 1-2mA DC stimulation can be safely applied over the DLPFC for up to 20 minutes (Iyer, et al., 2005; Poreisz, et al., 2007). As such, the current selected for this study was 1.5mA. A stronger current strength compared to 1mA that was used in the previous study was selected to increase the possible effects of the stimulation, as the duration of the stimulation was so short. However, the stimulation was unpleasant for some participants and the amplitude was lowered until it was comfortable for them. Eventually, 7 participants were stimulated at 1.5mA, 2 participants at 1.3mA, and 2 each at 1.2mA and 0.85mA. Regardless of the amplitude used, participants either received 1.6s DC stimulation or no stimulation in each trial. Furthermore, when stimulation was administered, it could be an early stimulation that appeared in the first half of a trial (onset at 0.1s before target word presentation) or a late stimulation that appeared in the second half of a trial (onset at 2.15s after target word presentation).

6.3.6 Procedure

Participants were brought to a quiet testing room for the experiment. Each session was blocked into three phases – training phase, retention interval and testing phase (Figure 6-2).

Training Phase	Retention Interval	Testing Phase
8 blocks of training 21 words per block + 5 practice words at the 1st block 3 stimulation types: No stimulation/early/late	About 60 minutes watching two episodes of American sitcom Friends	8 blocks of testing 21 quad-words per block No stimulation

Figure 6-2. Procedure of one session; Each session had 3 parts – training phase, retention interval and testing phase.

For the training phase, the respective electrodes were placed on the left DLPFC or right supraorbital area and stimulation amplitude was gradually increased to ensure that

participants were comfortable with the stimulation. After a suitable amplitude had been selected, participants were informed that they were required to perform a word memorisation task. They were told that individual words would be briefly presented, followed by a fixation cross that would appear for 3.5s to represent the "imagination period". Participants were asked to sustain their attention and use the entire "imagination period" to silently imagine the word in order to remember it for a subsequent memory test. They were also instructed to imagine each word separately from the others. Participants were then informed that there would be 8 blocks of trials, and that they were able to take a break between blocks. They were then allowed to clarify their queries before the task began. Figure 6-3 summarises the procedure in a typical training phase trial.

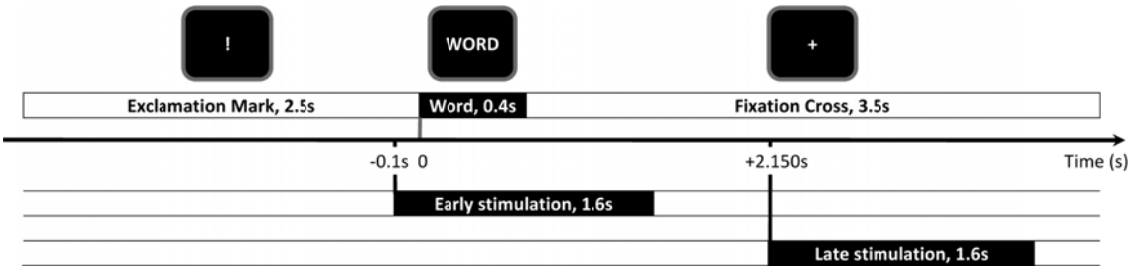


Figure 6-3. Procedure of trials in the training phase. The top row shows what participants see on screen, while the black bars below the timeline represent the onset of either early or late stimulation at 0.10s before or 2.15s after word presentation respectively.

The surface sponge electrodes were removed after the training phase was completed. Before the testing phase commenced, participants watched 2 episodes of the American sitcom Friends, which lasted for around 60 minutes. Contrary to the previous study, Friends TV series are very verbal communication intensive. Participants' engagement during the retention interval seems not to have a noticeable effect on their performance, based on author's personal communications with a few researchers. The memory retention interval was intentionally calibrated to this duration in order for the brain to recover its pre-stimulation state during the test (Cerruti & Schlaug, 2008). This is so that the observed results can be solely attributed to the effects of tDCS on the training phase and not on the testing phase.

In the testing phase, participants were told that 4 words would be presented in a line on the computer screen in each trial, out of which only one was an old word that they had seen in the training phase. Their task was to identify the old word by pressing a key on the keyboard that corresponded to the word's position along the line. Participants were told that the words would stay on screen for 6s, after which the words would disappear and they then had 6 more

seconds to make their response (Figure 6-4). They were reminded to be sure of the answer before making a response and to respond as soon as they were sure of the answer. Participants were encouraged to respond even if they could not select the target word with a high level of confidence. Participants were then informed that the target word was equally likely to appear in any of the 4 positions, and that none of the old or new words would be repeated within the whole study. The order of the old words in the testing phase was randomised and different from the order in which they appeared in the training phase. At the end of each block, the percentage accuracy for that block was shown on screen for 3s as feedback for participants.

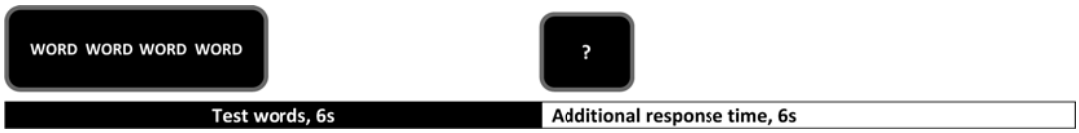


Figure 6-4. Procedure of the testing phase; The four words, one from the trained words and three distracter words, lasted on the screen for 6s and after that a question mark was shown for 6 additional seconds.

The entire procedure was repeated for the second experimental session with reversed stimulation polarity (anodal or cathodal) from the first session. Participants were debriefed and reimbursed at the end of the second session.

6.4 Results

All participants completed both experimental sessions. Trials in the testing phase were split according to whether the target word was presented with early stimulation, late stimulation or no stimulation during the training phase. The mean proportions of correct responses in the word recall task were calculated for both anodal and cathodal stimulation.

The interaction effect between stimulation polarity and time was highly significant, $F(2, 24) = 9.06$, $p < 0.001$, indicating that the difference between anodal and cathodal stimulation varied as a function of whether stimulation was administered early, late, or not administered at all in the training phase. The main effect of stimulation polarity was non-significant, $F(1, 12) = 2.22$, $p = 1.62$ and the main effect of stimulation time was non-significant, $F(2, 24) = 0.14$, $p = 0.87$.

A paired-samples t-test comparing participants' accuracies in the no-stimulation and early-stimulation trials under anodal stimulation was significance, $t(12) = 2.62$, $p = 0.022$, indicating

that administering anodal stimulation early in a trial led to an improvement in working memory accuracy over a non-stimulation trial. The same pattern of results was observed when comparing no-stimulation and early-stimulation trials under cathodal stimulation, $t(12) = 2.82$, $p = 0.015$, depicting an impaired memory accuracy following cathodal tDCS in the first half of a trial. Comparing early-stimulation and late-stimulation in anodal and cathodal conditions using paired-samples t-test analysis also showed a significant difference, $t(12) = 2.52$, $p = 0.027$ and $t(12) = 2.34$, $p = 0.037$ respectively, indicating the importance of proper timing of the stimulation. All other tests remained non-significant (Figure 6-5).

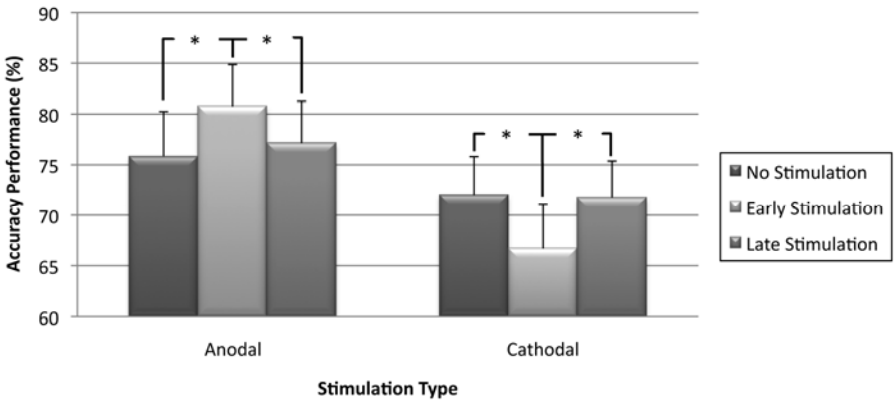


Figure 6-5. The proportion of correct responses made by the 13 participants who received 1.5mA of stimulation in the word recall task under the various conditions of stimulation polarity and time; * paired-samples t-test, $p < 0.05$; Error bars represent one standard error.

The reaction times of participants in the word recall task were also subjected to a 2 x 3 ANOVA, with stimulation polarity and time as within-subject factors. The main effect of stimulation polarity was non-significant, $F(1, 12) < 1$, indicating that participants were not significantly faster at responding in anodal-stimulation trials ($\mu = 3.09$s, SD = 0.08s) than in cathodal-stimulation trials ($\mu = 3.103$s, SD = 0.08s). The main effect of stimulation time was non-significant, $F(2, 24) < 1$, indicating that the response speeds were similar when participants responded to no-stimulation ($\mu = 3.091$s, SD = 0.07s), early-stimulation ($\mu = 3.089$s, SD = 0.08s) or late-stimulation ($\mu = 3.102$s, SD = 0.08s) trials. The interaction effect between stimulation polarity and time was however significant, $F(2, 24) = 9.291$, $p = 0.001$.

A paired-samples t-test comparing participants' reaction time in the no-stimulation and early-stimulation trials under anodal stimulation was significance, $t(12) = 2.40$, $p = 0.033$, indicating that administering anodal stimulation early in a trial led to a mean faster response time over a non-stimulation trial. The same pattern of results was observed when comparing no-

stimulation and early-stimulation trials under cathodal stimulation, $t(12) = 2.20$, $p = 0.048$, depicting a reduction in response time following cathodal tDCS in the first half of a trial. Comparing early-stimulation and late-stimulation in anodal condition using paired-samples t-test analysis also showed a significant difference, $t(12) = 2.51$, $p = 0.027$ but not in cathodal condition $t(12) = 1.72$, $p = 0.111$ indicating the importance of proper timing of the stimulation. All other tests remained non-significant (Figure 6-6).

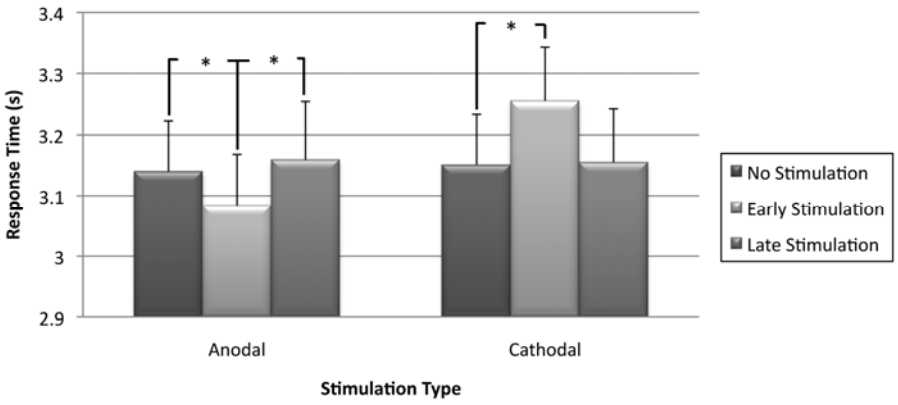


Figure 6-6. The mean reaction times made by the 13 participants who received 1.5mA of stimulation in the word recall task under the various conditions of stimulation polarity and time; * paired-samples t-test, $p < 0.05$; Error bars represent one standard error.

6.5 Discussion

The data showed that participants who received a stimulation of 1.5mA revealed a significant interaction between stimulation polarity and time of stimulation indicating that delivering anodal tDCS to the left DLPFC at the proper time led to significantly better memory accuracy as compared to cathodal stimulation which impaired memory. This supports the notion that a sufficiently high stimulation intensity is required to fully educe the effects of tDCS (Boggio, Ferrucci, et al., 2006). The advantage in memory performance under anodal stimulation cannot be attributed to response times in the word recall task, as higher performance was accompanied by faster reaction time and lower performance was accompanied by slower reaction time. These results support the first hypothesis, as well as the result of the previous experiment, that anodal tDCS aids memory accuracy while cathodal tDCS hampers it.

However, the exact functional role that anodal tDCS plays in improving memory accuracy remains unclear. Memory enhancement could have resulted from the stronger encoding of target words, or alternatively better retention of encoded words. Furthermore, other systems could have been engaged by left DLPFC stimulation, including learning capacity, planning skills and verbal fluency (Cerruti & Schlaug, 2008; Dockery, et al., 2009; Kincses, et al., 2004). These factors may have contributed to the memory advantage seen in anodal stimulation.

The results here seem to contradict previous literature by demonstrating that 1.6s of stimulation has a modulatory effect on verbal memory. Nitsche and colleagues (Nitsche, Liebetanz, Antal, et al., 2003b; Nitsche & Paulus, 2000, 2001b; Nitsche, Roth, et al., 2007) reported that the application of tDCS over the motor cortex for less than 5 minutes has no significant effects on motor-evoked potentials (MEPs) beyond the duration of the stimulation. However, the MEPs in these studies were only measured after and not during brain stimulation, which cannot be used to show that memory modulation did not occur because the latter does not necessarily require brain stimulation to have lasting effects as shown in this study. The effects of tDCS, however, are cumulative. The no-stimulation trials under both stimulation polarities recorded slightly different memory performance: the performance accuracy for anodal no-stimulation trials was non-significantly higher than cathodal no-stimulation trials, hinting at a general improvement and impairment for the anodal and cathodal stimulations respectively.

It should also be noted that the 1.6s of stimulation used here would have no behavioural effect as it is even shorter than the stimulation durations which have conventionally been used for sham stimulation that is considered as placebo stimulation condition and has appeared to have no effect on brain function (Nitsche, Cohen, et al., 2008). The stimulation protocol used in this study has one major difference with sham stimulation: periods of stimulation were carried out throughout the session rather than constantly at the beginning of the session. In this way each of the three stimulation types modulated memory for one-third of the whole stimuli, rather than 1 / 75 of the whole session in sham stimulation protocols (assuming 16 seconds of sham stimulation and a 20-minute session).

The results of this study point to the wider benefits that anodal tDCS may have. While cathodal stimulation suppresses cortical excitability, anodal tDCS has been shown to enhance cortical activity and subsequently improve behavioural patterns (J. M. Galea, et al., 2009; Miranda, et al., 2006; Nitsche, Schauenburg, et al., 2003). Depending on where it is administered, anodal tDCS offers advantages in tasks involving probabilistic classification

learning, motor learning and visuo-motor coordination (Antal, Nitsche, Kincses, et al., 2004; Bolognini, et al., 2009; Kincses, et al., 2004). As this and other studies have shown, anodal tDCS applied to the left DLPFC improves both short-term and long-term memory accuracy (Fregni, Boggio, Nitsche, et al., 2005; Jo, et al., 2009). Importantly, the effects of tDCS last beyond the period of stimulation, indicating its potential in helping patients recover in a clinical setting (Priori, 2003; Schlaug & Renga, 2008). Indeed, tDCS over left DLPFC has already been shown to improve the working memory of patients with Parkinson's or Alzheimer's disease (Boggio, Ferrucci, et al., 2006; Ferrucci, Bortolomasi, et al., 2008). The effect of tDCS on long term memory is not limited to verbal memory either. A study by Javadi, Ting and Walsh (in preparation) showed that anodal tDCS over left DLPFC enhances pictorial memory while having no significant effect on non-imaginable words. It will therefore be meaningful to conduct a study similar to this on elderly subjects or patients with memory deficits and ascertain if anodal tDCS similarly yields long-term memory advantages for them. For a more comprehensive understanding on the effects of tDCS, future studies need also consider what behavioural changes are observed when different areas of the brain are stimulated with tDCS. Stimulation protocols, such as the one used in this study, may be more effective for longer-lasting effects. In this study, tDCS was administered for 1.6s in stimulation trials, leading to a combined stimulation time of about 3 minutes keeping the same effect as 20-minute stimulation block. It has also been shown that stimulation during the second half of each trial was not as effective as in the first half. Therefore, this study has highlighted the importance of determining the appropriate stimulation duration and onset time in order to achieve the most effective results of tDCS.

In conclusion, when delivering DC stimulation at 1.5mA, the main effect of stimulation polarity was significant, indicating that anodal tDCS led to higher memory accuracy compared to cathodal stimulation. Compared to no-stimulation trials, the effects of early anodal and cathodal stimulation were significant but the effects of late stimulation were non-significant. These results suggest that administering anodal tDCS early on during memory encoding will yield the most benefits for memory accuracy. This study highlights the potential that tDCS has in a clinical setting for patients with memory impairments.

Chapter 7. General Discussion

In this document, two types of experiments are discussed: memory consolidation during sleep and wakefulness and memory modulation using electrical brain stimulation. All the studies and results, as well as summarising all the important findings are reviewed here.

7.1 Consolidation of procedural memory

Prior literature showed that stimuli with emotional content compared to stimuli with neutral emotional content lead to superior subsequent recollection (Cahill, 2000; Cahill & Alkire, 2003; Maddock, 1999; McGaugh, 2004; McGaugh & Roozendaal, 2002; Phelps, 2004; Richardson, et al., 2004); moreover, this superiority is enhanced by the delay between encoding and retrieving (Kleinsmith & Kaplan, 1963; LaBar & Phelps, 1998; Levonian, 1972; Sharot & Phelps, 2004). This evidence led many researchers to evaluate the contribution of emotion in offline memory consolidation. They showed that emotional content both facilitates the initial encoding of information as well as enhancing the subsequent memory consolidation (McGaugh, 2004; Payne, Stickgold, Swanberg, & Kensinger, 2008; Payne, Stickgold, Swanberg, Kensinger, et al., 2008; U. Wagner, et al., 2001; M. Walker, 2009). Most of these studies were

on declarative memory, whereas the aim was to extend the literature by studying the contribution of emotion in procedural memory.

Based on this evidence, the possibility of a contribution of emotion in procedural memory consolidation was investigated, in a series of studies.

7.1.1 Mirror tracing

Mirror tracing was used, which has been used effectively previously, to study motor learning in patients (Gabrieli, et al., 1993; Gabrieli, et al., 1997; Milner, 1962; Sanes, et al., 1990), as well as healthy participants (Adams, 1987; Snoddy, 1920). Furthermore, to investigate the effect of different emotional contexts during encoding upon the subsequent off-line procedural memory consolidation, three different types of emotional was embedded (negative, positive and neutral) in the stimuli. It was expected that participants, who were trained with stimuli with positive or negative emotional content, to perform better than participants trained with stimuli with neutral emotional content, after 12 or 24 hours of retention. The results showed higher performance in terms of speed and accuracy for participants who were trained with negative emotional content than participants who were trained with neutral emotional content, but they failed to show any difference in performance between participants, who were trained with stimuli with positive or neutral emotional content.

The results suggest the presence of negatively arousing emotions at encoding phase, which may enhance the off-line consolidation in the mirror tracing task. It was arguable that the amygdala might play a key role in this enhancement (LeDoux, 2000) (for review see (Adolphs, 2008; Ehrlich, et al., 2009; Seymour & Dolan, 2008)). The strong response of the amygdala to fear and negatively arousing emotions in addition to its role in encoding fear (Adolphs, et al., 1995; Calder, 1996; Ellis & Kesner, 1983; Gallagher, et al., 1981; Goddard, 1964; Kesner & Wilburn, 1974; Kluver & Bucy, 1937; McGaugh & Gold, 1976; Weiskrantz, 1956), provide compelling evidence that the amygdala is critically involved in encoding memories of emotional experiences (LaBar & Cabeza, 2006) (for review see (McGaugh, 2004)). Furthermore, cortical and subcortical connections between the amygdala and the basal ganglia, as a critical component of the motor system (Gazzaniga, 2004; Kandel, et al., 2000), make this contribution even more plausible. Other possible mechanisms contributing to the interaction between the negative emotional content of the stimuli and off-line procedural memory consolidation was also mentioned. These include the amygdala's connections

throughout the cortical areas, within subcortical areas, and cortico-subcortical re-entrant circuits (Bates & Goldman-Rakic, 1993; Darian-Smith, et al., 1993; Dum & Strick, 1991; M. P. Galea & Darian-Smith, 1994; Lu, et al., 1994; Morecraft & van Hoesen, 1992, 1993, 1998; Muakkassa & Strick, 1979; Murray & Coulter, 1981). Other possible pathways are via subcortical areas (Chikama, et al., 1997; Ferry, et al., 2000; Haber, et al., 1995) or interactions between limbic regions and basal ganglia through 'basal ganglia loops' (Groenewegen & Berendse, 1994; Haber, 2003; Haber, et al., 1985; Heimer, 1978; Heimer, et al., 1982; Young, et al., 1984).

In the same experiment, the extent to which sleep contributed to this effect was examined. Complying with prior literature the results showed that participants who obtained at least 6 hours of nocturnal sleep between the two sessions, performed mirror tracing faster and more accurately than participants who did not sleep. This enhancement was more apparent in the negative emotional content group. Interactions between the limbic and motor systems have been studied extensively (Haegelen, et al., 2009), but to the knowledge of the author, this study is the first to examine the impact of such interactions upon the off-line consolidation of a procedural task. In contrast to previous studies, which reported no significant improvement of learnt skill with a period of wake retention interval (Cohen & Robertson, 2007; Nishida & Walker, 2007; Plihal & Born, 1997b; Press, et al., 2005; Robertson & Cohen, 2006; Robertson, Pascual-Leone, & Miall, 2004; M. Walker, et al., 2002), participants in the negative emotional content had higher performance in the testing phase. This observation is in line with work by Sterpenich et al. (2007), showing that while memory for positive and neutral episodes is impaired by sleep deprivation on the post-encoding night, memory for negative episodes remains unaffected.

In contrast to previous studies (Cahill, 2000; Cahill & Alkire, 2003; Maddock, 1999; McGaugh, 2004; McGaugh & Roozendaal, 2002; Phelps, 2004; Richardson, et al., 2004), no performance enhancement in the positive emotion group. This could be due to the emotional content of the positive stimuli used, which might not have been sufficiently arousing.

Overall, the first study showed the first evidence of a contribution of emotion on consolidation of procedural memory.

7.1.2 Serial Reaction Time Task (SRTT)

The second experiment aimed to investigate the possibility of modulatory effect of stimuli with emotional content in another procedural task, serial reaction time task (SRTT) (Cleeremans & McClelland, 1991; Nissen & Bullemer, 1987; Robertson, 2007), which showed robust results in sleep dependant memory consolidation literature (Cohen, et al., 2005; Press, et al., 2005; M. Walker, et al., 2002). The standard SRTT (mSRTT) was modified in order to involve different types of emotions in the task. Participants were asked to respond to images with two types of emotional content, high valence and neutral. Furthermore, participants were instructed to memorise the association between 4 images with 4 fingers of their non-dominant hand and respond to each presented image as quickly and as accurately as possible. In the training session, participants were asked to repeat the training block for 30 times and each block lasted for 30 seconds. Unbeknown to the participants, the order of the images followed a 12-item sequence. Based on prior research, conducted by Fischer et al. (2002) and Anderson et al. (2006), it is predicted that a main effect of sleep and emotional content of stimuli will be found. It is expected that participants would perform better on the SRTT if they slept between the two sessions and were assigned to the negative stimuli (see the first study). An interaction effect between retention and stimuli type was also predicted; participants in the night-emotional condition would perform better than those in the day-neutral condition.

This experiment failed to achieve any main effect of emotional content, retention type or their interaction or their interaction with session number. The only significant effect was found for session number in which participant showed significantly higher performance. The first hypothesis of this study stated that only the sleep group would significantly improve in the second session, as a result of sleep-dependent consolidation. Nevertheless, the results showed not only for the sleep group, but also for the wake group, an improvement in the second session. These results were contrary to prior literature in two ways. First of all, the results showed no effect of sleep despite the huge body of literature showing an enhancing effect of sleep in memory consolidation. Secondly, there was no difference between the performance of the participants trained with emotional stimuli and the participants who were trained with stimuli with neutral emotional content, which was contrary to the findings of the first experiment, described in this document as well as prior literature. This could be because of the number of the excessive number of training blocks. Participants underwent 30 blocks of training rather than conventional number of 12 blocks of training. As a result of that,

participants were trained too much to see any effect of emotional valence on their performance after 12 hours of retention interval, including either sleep or not.

This finding is only partly consistent with the study of Spencer et al. (2006), which found that when the SRTT is combined with contextual association, offline improvement is dependent on the presence of sleep in the interval between sessions. This inconsistency might be due to the difference in the contextual associations. it was argued that the images used in this study contained stronger contextual associations than the colour cues used by Spencer et al. (2006). Hence, it was proposed that strong contextual association induce offline improvements, as well as during intervals of wakefulness.

The results of this experiment were also opposing to the study of Walker et al. (2002). In their study, the memory consolidation of the participants in the night and day groups were significantly different. The difference between this study and theirs lies in the (a) association between images and finger and (b) the number of training blocks. To make the study more consistent with their study, another experiment was ran with standard serial reaction time task (sSRTT) in which participants were asked to respond to the spatial location of a circle on the screen using 4 of the fingers of their non-dominant hand. Similar to the previous experiment, participants were asked to repeat the training blocks for 30 times but they asked their participants to repeat the training blocks for 12 times. The result of this experiment was not in line with Walker et al. (2002). The results showed no significant difference between the two groups (day and night groups). Thus, it was suggest that the excessive number of training blocks enforces the offline memory consolidation in a way that even participants in the day group benefited from it in the memory consolidation.

No effect of emotional valence of the stimuli on performance in the second session was observed, which was inconsistent with the second hypothesis of the experiment: enhancement of memory consolidation due to negative emotional valence of the associated images. This hypothesis was generated based on previous studies showing that emotionally arousing stimuli enhance declarative memory (McGaugh, 2003, 2004), and, more specifically, in the experiment explained previously. This inconsistency might be due to (a) emotionally arousing images do not affect offline improvement of implicit procedural memory, or, at least, on the SRTT task and (b) the different type of images used in this study might have affected the study's outcome as they represented severely injured people compared to the previous experiment which were more tolerable.

Based on previous studies (Fischer, et al., 2006; Robertson, 2004; Spencer, et al., 2006), it was hypothesised that explicit off-line learning of procedural skill is dependent on sleep. The result disconfirmed this hypothesis, which stated that the sleep group would present higher explicit knowledge than the wake group as a result of sleep-dependent consolidation, due to the absence of any effect of sleep on explicit knowledge.

It was speculated that the differing findings of this study may be due to two different characteristics of the generation task used in this and other studies, namely, (a) only grammar, based on (Fischer, et al., 2006), but not sequence learning benefits from sleep, and (b) absence of feedback after each response.

In summary, two experiments were run, one with a modified and the other with a standard serial reaction time task. For the mSRTT participants responded to four images which all had either a neutral or arousing emotional valence. For the sSRTT participants responded to spatial location of a cue. In the first experiment, the contribution of emotion in consolidation of SRTT was investigated. Participants were unaware that the order of presentation followed a determined sequence. They were retested after an interval of 12-hours which did or did not include sleep. All participants improved in response times in the second session and showed implicit knowledge of the sequence. There was no difference in explicit knowledge between the wake and sleep groups and no effect of emotional valence. Based on the results of this study, it was proposed that excessive training enhances the memory consolidation during wakefulness to the extent that its difference with memory consolidation during sleep vanishes.

The effects of different biological states, especially for females since, in the perception of emotion is also an important factor to consider and study. For example, Emotional perceptions change over the menstrual cycle of women. In a study Penton-Voak and Perrett (2000) showed that during preovulatory and ovulatory phases of the menstrual cycle, women prefer more masculinised faces than at other stages. It has been suggested that oestrogen plays a modulatory role in females' perception of fear. Oestrogen receptors have been identified in the hippocampus and the corpus callosum (Fitch & Denenberg, 1998) as well as the amygdala (Osterlund & Hurd, 2001). As the result, oestrogen can facilitate increased functioning of these areas, and consequently changes their perception of fearful and neutral faces. Pearson and Lewis (2005) confirming this and females' mean accuracy for recognition of fearful faces impairs significantly compared to happy, surprised, angry, sad and disgusted faces during menses phase while they are more accurate in recognition of sad faces, Figure

7-1. Therefore, it is better to control for this variation across participants when using emotional stimuli.

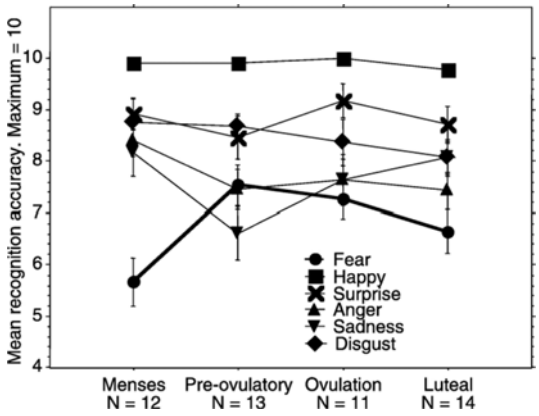


Figure 7-1. Mean accuracy for recognition of the different types of emotional faces by participants in different phases of menstrual cycle. (Pearson & Lewis, 2005)

Further experiments needed to be carried out (1) to compare the performance of participants with less number of training blocks with the result of current study, which actually might answer the question of why participants in the day group experienced a procedural memory consolidation comparable to the night group, and (2) to study the effect of different emotional stimuli in an experiment in which the offline procedural memory consolidation of the day and night groups differs. The first experiment is consisted of the sSRTT with 12 blocks of training. It was expected that this number of training was appropriate to find a significant difference between the day and night groups. In the second experiment, the mSRTT was run with 12 blocks of training and the performance of the participants in the day group, who were trained with stimuli with negative emotional content, was looked at.

7.2 Brain stimulation

Given recent studies highlighting the importance of noninvasive brain stimulation as a means of modulating cortical excitability, tDCS was used to modulate memory ((Boggio, et al., 2009; Cerruti & Schlaug, 2008; Dockery, et al., 2009; Elmer, et al., 2009; Kincses, et al., 2004; Rosenkranz, et al., 2000; Sparing & Mottaghy, 2008; Stone & Tesche, 2009; Tanaka, et al., 2009; Wassermann & Grafman, 2005), for review see (Been, et al., 2007; Flöel & Cohen, 2007; Hummel & Cohen, 2005; Nitsche, Cohen, et al., 2008)). It has been shown that transcranial direct current stimulation (tDCS) can be applied effectively to induce prolonged functional

changes in the cerebral cortex through the application of a weak direct current on the scalp (Gandiga, et al., 2006; Kesner & Wilburn, 1974; McGaugh & Gold, 1976; Paulus, 2003; Wassermann, 2008). It has also been studied that it is a safe tool for modulating behavioural and cognitive functions (Iyer, et al., 2005; Nitsche, Liebetanz, Antal, et al., 2003a; Poreisz, et al., 2007). One key feature of tDCS is its polarity dependency that allows the possibility of enhancing and reducing neuronal excitability (J. M. Galea, et al., 2009; Miranda, et al., 2006; Nitsche & Paulus, 2000; Terney, et al., 2008). Specifically, this method has attracted much attention in studies of working memory in both healthy participants (Ferrucci, Marceglia, et al., 2008; Fregni, Boggio, Nitsche, et al., 2005; Marshall, et al., 2005; Ohn, et al., 2008) and patients (Boggio, Ferrucci, et al., 2006; Fregni, Boggio, Nitsche, Rigonatti, et al., 2006; Jo, et al., 2009). In most of the studies, the application of tDCS showed significant effect on working memory of the participants.

The second section is devoted to the two experiments in which tDCS were used to modulate memory performance of the participants in a verbal episodic memorisation task. To date, to no studies have evaluated the effects of tDCS on episodic memory, and therefore, the effect of tDCS on verbal memory was investigated.

7.2.1 Long-duration tDCS

In the first experiment, long duration stimulation was used (20 minutes constant stimulation) to investigate the effect of anodal and cathodal stimulation on the performance of the participants in a verbal memory task. Left dorsolateral prefrontal cortex (DLPFC) was selected as the main site for stimulation, based on previous evidence of a contribution of the left DLPFC in episodic (Cabeza & Nyberg, 2000a, 2000b; Martin, 2001) and working memory (Boggio, Ferrucci, et al., 2006; Fregni, Boggio, Nitsche, et al., 2005; Jo, et al., 2009; Ohn, et al., 2008). Anodal, cathodal and sham stimulation types were administered in 3 separate sessions, both during encoding and retrieval phases, in two separate experiments, to study the effects of stimulation on different stages of memorisation and recognition (encoding and retrieval). Primary motor area (M1) was also stimulated as a control site to investigate the location specificity of the tDCS. It was expected that anodal stimulation of left DLPFC during encoding and retrieval would improve episodic verbal memory, whereas cathodal stimulation of the same site during encoding and retrieval would impair episodic verbal memory. Participants were shown words and asked to judge the number of syllables of each word, but also to try to imagine it in order to memorise it afterward for a later recognition task.

Results showed that anodal stimulation enhanced the memory performance while the stimulation was delivered during the encoding phase, whereas cathodal stimulation impaired the memory performance for the words that were encoded prior to the stimulation and impaired the recognition performance while cathodal stimulation was delivered during the testing phase. Sham stimulation and stimulation of primary motor cortex during encoding of the words did not affect the episodic verbal memorisation.

Considering the beneficial effects of anodal tDCS over left DLPFC in previous studies on working memory (Boggio, Ferrucci, et al., 2006; Ferrucci, Marceglia, et al., 2008; Fregni, Boggio, Nitsche, et al., 2005; Jo, et al., 2009; Ohn, et al., 2008), it was hypothesised that anodal stimulation of left DLPFC will enhance the episodic verbal memorisation. Results of the experiment were partly in line with this hypothesis, since administration of anodal tDCS over left DLPFC during encoding led to enhancement of memory in the subsequence test while anodal tDCS over left DLPFC during retrieval did not affect the memory performance.

Furthermore, it was discussed that this higher performance due to anodal stimulation of left DLPFC during the encoding is not due to 15' shorter retention interval since the same timing was used for sham and control stimulation types in which no significant enhancement was observed. Cathodal tDCS during the retrieval reduced the performance which it was discussed that this effect might be due to decrement of neural activity in the left DLPFC (Flöel & Cohen, 2007; Nitsche & Paulus, 2001a).

The most interesting result of the study was a disruption of retention of the words encoded prior to cathodal stimulation. Previously, a few studies investigated the effect of stimulation on materials encoded prior to the stimulation. Hotermans et al. (2008) used transcranial magnetic stimulation (TMS) over primary motor cortex (M1) to modulate performance gain in motor sequence learning. They showed that early stimulation depressed the early boost in performance. Gallate et al. (2009), as well, use TMS over left anterior temporal lobe to investigate the possibility of reduction of false memory. Their results showed significantly lower false memory after the administration of TMS, when compared to the group with no stimulation. Marshall et al. (2006) used slow oscillatory stimulation during early stage-two sleep. They successfully enhanced the consolidation of declarative memory. It was argued that this effect might be due to any or a combination of the following mechanisms: (a) a destruction of weak traces of memories and preventing them from being stabilised, (b) preventing retention of the encoded words or (c) an impairment of retrieval of memories.

In summary, tDCS over left DLPFC affected later recognition of words in episodic verbal memorisation paradigm. It is shown that anodal tDCS over left DLPFC can improve later recognition and cathodal tDCS over the same area impairs the recognition of the words encoded prior to stimulation. Moreover, cathodal stimulation of left DLPFC during retrieval affected the recognition of the encoded episodic verbal memory. Using stimulation of the primary motor cortex, it is shown that the affect of tDCS over the left DLPFC is location specific.

7.2.2 Short-duration tDCS

In the first study the effect of long-duration tDCS over left DLPFC on verbal episodic memory was investigated. It is shown that anodal stimulation during encoding enhanced while cathodal stimulation impaired the memory performance in a later recognition phase. In the first study the participants were stimulated for 20' constantly during the second part of the encoding phase. Using the second study, the effect of short-duration tDCS over the left DLPFC on verbal episodic memory was studied. It was investigated whether short-duration stimulation is effective enough to pick up any memory performance. Two points were addressed using this experiment, (a) the importance of a proper phase of stimulation and (b) the effectiveness of short-duration tDCS. None of these points has ever been investigated for tDCS before.

In this study, anodal and cathodal stimulation in separate sessions were delivered for 1.6s for each presented word in three different conditions: no stimulation, early-stimulation and late-stimulation. These numbers were selected based on a separate pilot study in which EEG recording was administered while participants were asked to memorise single words (Appendix ii). It was noted that for 4-second trials in which participants had to imagine a word in order to memorise it, the left DLPFC displayed higher activity in the first half of a trial than in the second half. It was therefore postulated that tDCS will have a different effect on memory depending on whether it is administered early or late on in a trial. Early-stimulation was initiated 0.100s before the onset of the word and late-stimulation was initiated 2.150s after the word onset.

Based on the previous study and prior literature, the first prediction was that anodal tDCS will lead to higher memory accuracy on the memory recall task, and that cathodal tDCS will impair subsequent memory accuracy. Also, according to the EEG study, the second prediction was therefore that tDCS will have a greater effect on memory accuracy when it is administered in

the first half of a trial rather than the second half. Results of this study confirmed both the predictions. Results showed that early stimulation has a significantly stronger effect on memory performance of the participants compared to no-stimulation and late-stimulation in both anodal and cathodal stimulation times. Results also showed that early anodal stimulation enhanced the memory performance and early cathodal stimulation impaired the memory performance.

Appendix i. Neutral and Emotionally Arousing Stimuli Used in the 2nd Study

Neutral Images	
	
	

Emotionally Arousing Images	
	
	

Appendix ii. EEG Frequency analysis of subsequent memory effect for longer duration of memorisation time

An experiment using electroencephalography (EEG) was carried out to investigate the patterns of brain activity during a relatively longer duration of memorisation time compared to previous studies. A within-subject design was used and 13 healthy participants took part in a two-session experiment. In the first session EEG was recorded while participants memorised 160 words, for 4 seconds each. In the second session, participants were tested on their memory of these words and 160 new words using an old-new recognition task. Results showed that frequency activity significantly differed for some time intervals throughout the memorisation period between remembered and forgotten words, specifically at the alpha frequency band (9-12 Hz). Analysis of results revealed that brain activity was not consistent throughout the 4 seconds of memorisation and it was more pronounced during the first half of the memorisation period to the second of the memorisation period.

ii.i Method

ii.i.i Participants

A total of 13 (10 females, 7 males) with a mean age of 19.05 years (range 18-21) healthy participants took part in this experiment through opportunity sampling. All participants had normal or corrected-to-normal vision and were right-handed. The inclusion criteria for participants were that they had to be fluent English speakers with no psychological or neurological disorders. Informed consent was obtained from all participants, and they were either monetarily reimbursed or given course-credits for their participation in the study.

ii.i.ii Stimuli

A bank of 320 highly familiar and highly imaginable words were extracted from the MRC psycholinguistic database (Coltheart, 1981). The words were controlled for their number of letters (min = 3, max = 6, μ = 4.54, SD = 1.01), number of syllables (min = 1, max = 2, μ = 1.24, SD = 0.42), printed familiarity (μ = 574.36, SD = 28.13), concreteness (μ = 581.15, SD = 40.67) and imaginability (μ = 593.59, SD = 23.91).

ii.i.iii Apparatus

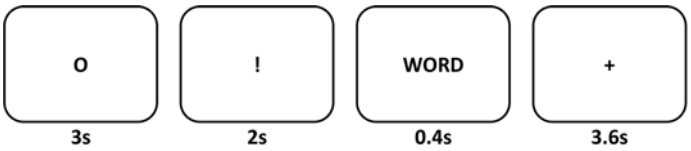
The experiment was run on a desktop computer with a 17-inch CRT monitor, 100 Hz refresh rate and a resolution of 1024 x 768 pixels. Stimuli were presented in capital letters using Arial font, subtending approximately 3-6 degrees of horizontal visual angle, and were displayed in black against a white background. Responses were entered on a computer keyboard. Stimulus presentation and the recording of response time were achieved using MATLAB (v7.5; MathWorks Company) and Psychtoolbox v3 (Brainard, 1997; Pelli, 1997).

ii.i.iv Procedure

The experiment adopted a within subject design with recognition accuracy (remembered/ forgotten) as the within subject factor. Subjects participated in two experimental sessions: encoding and retrieval. The encoding session took place in the morning and the retrieval session took place seven hours later in the evening. In the encoding session, participants were

asked to memorise 160 words which were randomly blocked into 8 sets of 20 words. Participants were given at least 30s break in between each of the 8 blocks.

Each word was shown for 0.4s followed by a fixation cross for 3.6s. This gave the participants a total of 4s to memorise each word, Appendix Figure ii-i. Subjects were instructed to imagine an image of the word in order to memorise it. A screen with a centrally presented circle was shown at the beginning of each trial. Participants were instructed to blink comfortably during this time interval. This screen was then proceeded by a screen with an exclamation mark in the centre of it warning the participant that the next word is about to be shown. This exclamation mark was a signal for them not to blink anymore.



Appendix Figure ii-i. Procedure of one trial in the encoding session.

Participants were then required to come back for the retrieval session seven hours later. During this session participants were asked to do an old/new recognition task. They were given a word and were asked to decide whether the word is 'old' or 'new' meaning whether they had seen the word previously in the encoding session or it is a new word they have not seen before. Words that were studied in the encoding session were randomly re-presented with another 160 words that were not presented before in the previous session. After each decision, they were also asked to rate their confidence in their selection. The rating had 3 levels: 'not sure', 'fairly sure' and 'sure'. Participants were instructed to respond as accurately and as quickly as possible. The 320 words were blocked in 8 sets of 40 words with at least 30s break in between the blocks.

ii.i.v EEG Recording

Electroencephalogram (EEG) was recorded continuously from 64 active electrodes with a BioSemi Active-Two amplifier system (BioSemi, Amsterdam, Netherlands) placed according to the 10-10 system. To monitor for eye movements and blinks, the horizontal and vertical electrooculogram (EOG) was recorded: left and right side of the left and right eyes, respectively for monitoring horizontal eye movements and bottom of the left eye for monitoring vertical eye movements. Raw EEG and EOG were sampled at 512 Hz with 12 bit resolution. Two additional electrodes, namely Common Mode Sense (CMS) and Driven Right

Leg (DRL), were used as reference and ground[*]. EEGLab (Delorme & Makeig, 2004), FASTER toolbox (Nolan, Whelan, & Reilly, 2010) and SPM 8[†] were used for data analysis.

ii.i.vi EEG Analysis

Recorded data were referenced to a nose-tip electrode, digitally band-pass filtered between 0.1 Hz and 30 Hz (Butterworth, -12 db/oct rolloff) and down-sampled to 128 Hz. Non-overlapping epochs were extracted between 2.3 s before the word onset and 4 s after the word onset. Accordingly, epochs were marked based on subsequent memory performance. Epochs were marked as 'remembered' only if participant judged a word correctly as old and gave a confidence rating of 'fairly sure' or 'sure'. To maximise the signal-to-noise ratio of the waveforms associated with failed episodic encoding, words were classified as 'forgotten' if they were judged incorrectly as new or rated as 'not sure' (Brewer, Zhao, Desmond, Glover, & Gabrieli, 1998; Hanslmayr, Spitzer, et al., 2008; Otten, Henson, & Rugg, 2001; Otten, Quayle, Akram, Ditewig, & Rugg, 2006; A. D. Wagner et al., 1998).

Using FASTER toolbox, data artefacts were detected and corrected in four aspects of the EEG data: channels, epochs, independent components (ICs) and single-channel single-epochs. Z-score of ±3 was considered as contaminated data for each parameter (Nolan, et al., 2010). EEG trials were filtered in the frequency range 2--18 Hz (0.5 Hz steps) by continuous wavelet transforms implementing Morlet wavelets of five-cycle length (Daubechies, 2002). The logarithm of event-related desynchronisation/event- related synchronization (ERD/ERS) method was used to calculate event-related power changes (Pfurtscheller & Aranibar, 1977). This method examines stimulus induced power decrease (ERD) or power increase (ERS) in relation to a baseline (set to 150 ms of the beginning of each epoch).

ii.i.vii Statistical Analysis of EEG Data

In order to compensate for the small number of samples and to account for multiple comparisons, a 2-stage randomisation procedure was used throughout the whole EEG analysis: participant level and group level (R. C. Blair & W. Karniski, 1993). At first, a randomisation test using 2000 permutation runs was conducted for each participant.

[*] see http://www.biosemi.com/faq/cms&drl.htm for details

[†] Wellcome Trust Centre for Neuroimaging, London; http://www.fil.ion.ucl.ac.uk/spm/

Considering the number of trials in each condition, certain number of epochs was randomly selected with replacement. For each randomisation run, mean ERD across epochs for each condition was calculated. Subsequently ERD-difference between remembered and forgotten conditions was calculated (ERD of remembered minus ERD of forgotten). For the second stage, 2000 permutation runs were conducted over previously generated ERD-differences for each participant to achieve a distribution of grand average of ERD-differences over the two conditions of all the participants. This distribution was then used to determine the P-value of a given data set. If the P-value of this comparison is below 0.05, less than 5% of the permutation runs exhibited equal or more ERD-difference with a significant difference between the two conditions (Bäuml, et al., 2008; Hanslmayr et al., 2008; Hanslmayr, Spitzer, et al., 2008; B. Pastötter, K. H. Bäuml, & S. Hanslmayr, 2008).

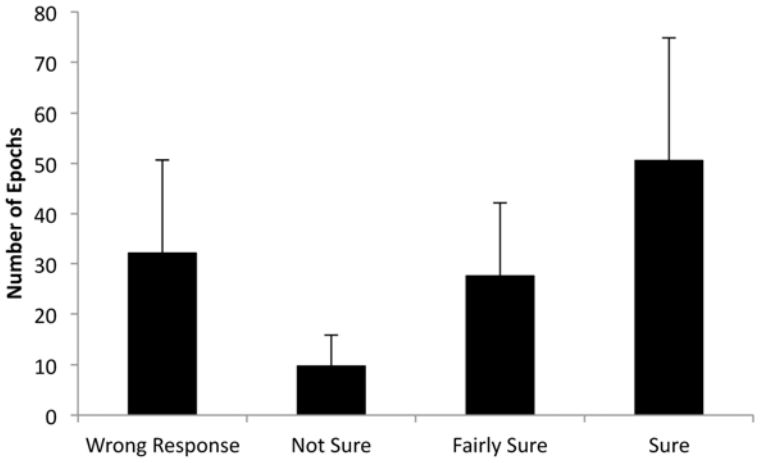
Time-frequency data was analysed in two aspects. First, it was analysed with all EEG channels collapsed to investigate the most prominent frequency band. Subsequently data was averaged over the selected frequency band to focus on the activity of individual channels. Warm colours in the plots indicate power increase (ERS) and cold colours indicate power decrease (ERD). The highlighted areas in the figures, area within the black border in Appendix Figure ii-iii and Appendix Figure ii-iv or shown as dots in Appendix Figure ii-v, shows areas with significantly synchronised frequency activity with $P < 0.05$.

All the participants had at least 15 trials in both of the two conditions (remembered/ forgotten). This number proved to be sufficient to achieve a stable baseline for each condition, using variance homogeneity tests (Leven tests) ran over variance of mean of certain number of randomly selected epochs (from 3 to 30 epochs) with replacement for 50 permutation runs. Alpha frequency band was used for this test was (9-12 Hz) (Hanslmayr, Spitzer, et al., 2008).

ii.ii Results

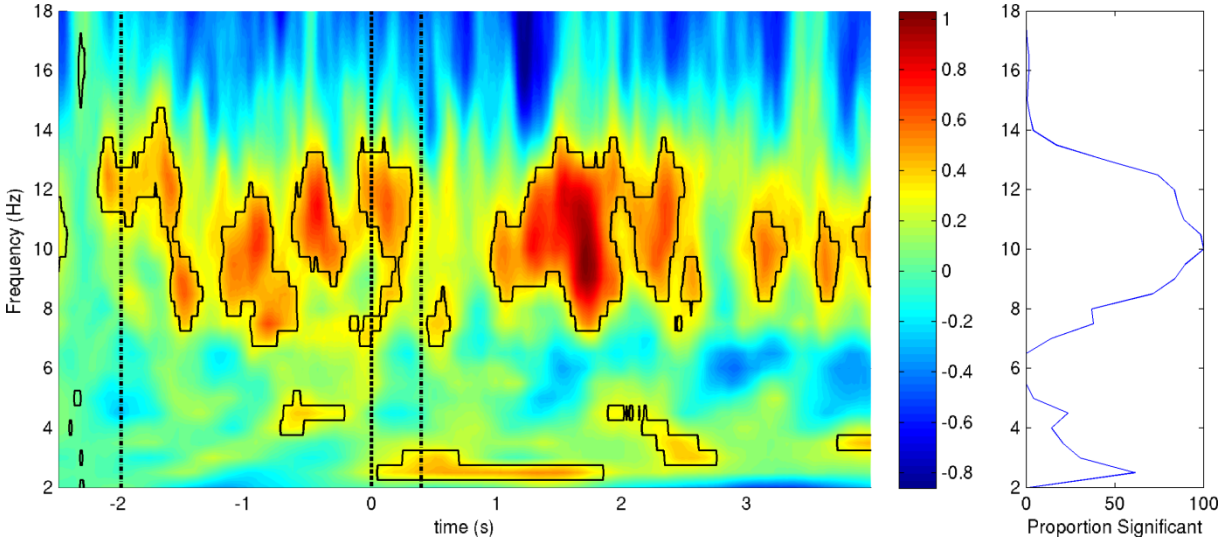
Participants' performance in the retrieval session showed that they attentively memorised the stimuli (accuracy: min = 67.08%, max = 93.75%, mean = 79.22%, SD = 9.10%; reaction time: min = 1.16 s, max = 1.95 s, mean = 1.66 s, SD = 0.20 s). Appendix Figure ii-ii shows the mean number of epochs separated for wrong responses and different confidence ratings for correct

responses. Epochs as 'wrong response' and 'not sure' were classified as 'forgotten' and epochs as 'fairly sure' and 'sure' were classified as 'remembered'.



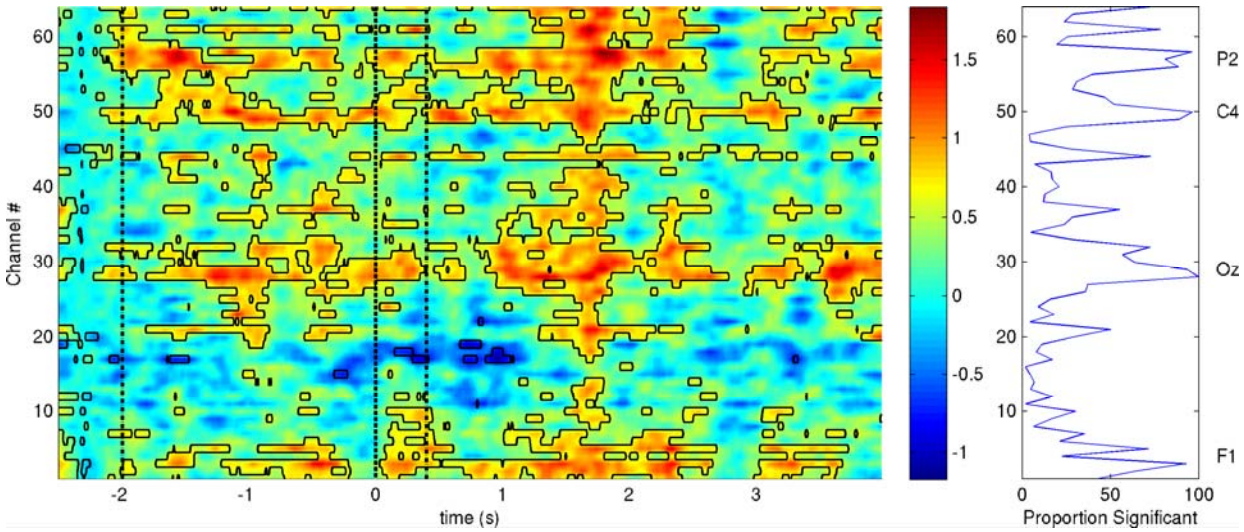
Appendix Figure ii-ii. Mean number of trials in different conditions categorised using subsequent memory effect. 'wrong response' and 'not sure' conditions and 'fairly sure' and 'sure' conditions were collapsed together to comprise 'forgotten' and 'remembered' conditions, respectively. Error bars represent one standard deviation (SD).

Appendix Figure ii-iii shows time-frequency data of ERD/ERS with all the channels collapsed. It shows that the most influential frequency bank in between 2-18 Hz is 9-12 Hz. The plot on the right shows the ratio of significant ERD/ERS of different frequencies over time compared to the most significantly synchronised (ERS) frequency (10 Hz).



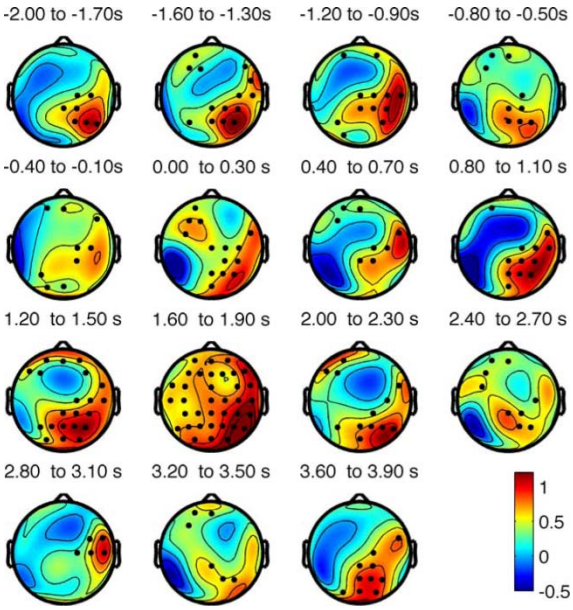
Appendix Figure ii-iii. Time-frequency map throughout a trial. The Black borders show the significant area with *P* < 0.05. The plot on the right, shows proportion significant ratio with activity in the 10 Hz being the most significantly effective frequency.

Considering the dominant frequency band (9-12 Hz) achieved from Appendix Figure ii-iii, a channel-time plot was created for mean activity of all the channels over the selected frequency band. Appendix Figure ii-iv shows this plot along with a summary of ratio of significantly synchronised (ERS) electrodes compared to the most significantly synchronised electrode (Oz). This figure shows that left-frontal area (representative electrode F1), occipital area (representative electrode Oz), right-central area (representative electrode C4) and right-parietal area (representative electrode P2) are the most indicative ones.



Appendix Figure ii-iv. Time-channel map throughout a trial. The Black borders show the significant area with *P* < 0.05. The plot on the right, shows proportion significant ratio for all the channels.

Appendix Figure ii-v shows the topographical maps of ERD/ERS for different time windows (each 300 ms) with the significantly synchronised electrodes highlighted.



Appendix Figure ii-v. Topographical maps of ERD/ERS for 300 ms time windows for the duration of one epoch. Dots show electrodes that showed significant ERS throughout each time window.

References

Accornero, N., Li Voti, P., La Riccia, M., & Gregori, B. (2007). Visual evoked potentials modulation during direct current cortical polarization. *Experimental Brain Research, 178*(2), 261-266.

Adams, J. A. (1987). Historical review and appraisal of research on the learning, retention, and transfer of human motor skills. *Psychological Bulletin, 101*(1), 41-74.

Adolphs, R. (2008). Fear, faces, and the human amygdala. *Current opinion in neurobiology, 18*(2), 166-172.

Adolphs, R., Cahill, L., Schul, R., & Babinsky, R. (1997). Impaired declarative memory for emotional material following bilateral amygdala damage in humans. *Learn Mem, 4*(3), 291-300.

Adolphs, R., Tranel, D., Damasio, H., & Damasio, A. (1995). Fear and the human amygdala. *Journal of Neuroscience, 15*(9), 5879-5891.

Adolphs, R., Tranel, D., & Denburg, N. (2000). Impaired emotional declarative memory following unilateral amygdala damage. *Learn Mem, 7*(3), 180-186.

Ambrosini, M. V., & Giuditta, A. (2001). Learning and sleep: the sequential hypothesis. *Sleep Med Rev, 5*(6), 477-490.

Anderson, A. K., & Sobel, N. (2003). Dissociating Intensity from Valence as Sensory Inputs to Emotion. *Neuron, 39*(4), 581-583.

Anderson, A. K., Yamaguchi, Y., Grabski, W., & Lacka, D. (2006). Emotional memories are not all created equal: evidence for selective memory enhancement. *Learn Mem, 13*(6), 711-718.

Andrews, S. C., Hoy, K. E., Enticott, P. G., Daskalakis, Z. J., & Fitzgerald, P. B. (2010). Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. *Brain Stimulation*.

Antal, A., Brepohl, N., Poreisz, C., Boros, K., Csifcsak, G., & Paulus, W. (2008). Transcranial Direct Current Stimulation Over Somatosensory Cortex Decreases Experimentally Induced Acute Pain Perception. *The Clinical journal of pain, 24*(1), 56.

Antal, A., Kincses, T., Nitsche, M., Bartfai, O., & Paulus, W. (2004). Excitability changes induced in the human primary visual cortex by transcranial direct current stimulation: direct electrophysiological evidence. *Investigative ophthalmology & visual science, 45*(2), 702.

Antal, A., Lang, N., Boros, K., Nitsche, M., Siebner, H., & Paulus, W. (2008). Homeostatic metaplasticity of the motor cortex is altered during headache-free intervals in migraine with aura. *Cerebral Cortex*.

Antal, A., Nitsche, M., Kincses, T., Kruse, W., Hoffmann, K., & Paulus, W. (2004). Facilitation of visuo-motor learning by transcranial direct current stimulation of the motor and extrastriate visual areas in humans. *European Journal of Neuroscience, 19*(10), 2888.

Antal, A., Nitsche, M., Kruse, W., Kincses, T., Hoffmann, K., & Paulus, W. (2004). Direct current stimulation over V5 enhances visuomotor coordination by improving motion perception in humans. *Journal of Cognitive Neuroscience, 16*(4), 521-527.

Antal, A., Nitsche, M., & Paulus, W. (2001). External modulation of visual perception in humans. *Neuroreport, 12*(16), 3553.

Antal, A., Nitsche, M., & Paulus, W. (2006). Transcranial direct current stimulation and the visual cortex. *Brain Research Bulletin, 68*(6), 459-463.

Antal, A., Varga, E., Kincses, T., Nitsche, M., & Paulus, W. (2004). Oscillatory brain activity and transcranial direct current stimulation in humans. *Neuroreport, 15*(8), 1307.

Ardolino, G., Bossi, B., Barbieri, S., & Priori, A. (2005). Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. *The Journal of physiology, 568*(2), 653.

Arfai, E., Theano, G., Montagu, J., & Robin, A. (1970). A controlled study of polarization in depression. *The British Journal of Psychiatry, 116*(533), 433.

Arul-Anandam, A., & Loo, C. (2009). Transcranial direct current stimulation: A new tool for the treatment of depression? *Journal of Affective Disorders, 117*(3), 137-145.

Atienza, M., & Cantero, J. L. (2008). Modulatory effects of emotion and sleep on recollection and familiarity. *J Sleep Res, 17*(3), 285-294.

Baker, A. (1970). Brain stem polarization in the treatment of depression. *South African medical journal (Suid-Afrikaanse tydskrif vir geneeskunde), 44*(16), 473.

Bates, J., & Goldman-Rakic, P. (1993). Prefrontal connections of medial motor areas in the rhesus monkey. *The Journal of comparative neurology, 336*(2), 211-228.

Baudewig, J., Nitsche, M., Paulus, W., & Frahm, J. (2001). Regional modulation of BOLD MRI responses to human sensorimotor activation by

transcranial direct current stimulation. *Magnetic Resonance in Medicine, 45*(2).

Bäuml, K., Hanslmayr, S., Pastötter, B., & Klimesch, W. (2008). Oscillatory correlates of intentional updating in episodic memory. *Neuroimage, 41*(2), 596-604.

Been, G., Ngo, T., Miller, S., & Fitzgerald, P. (2007). The use of tDCS and CVS as methods of non-invasive brain stimulation. *Brain research reviews, 56*(2), 346-361.

Bindman, L., Lippold, O., & Redfearn, J. (1962). Long-lasting changes in the level of the electrical activity of the cerebral cortex produced by polarizing currents. *Nature*.

Bindman, L., Lippold, O., & Redfearn, J. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *The Journal of physiology, 172*(3), 369.

Blair, R., & Karniski, W. (1993). An alternative method for significance testing of waveform difference potentials. *Psychophysiology, 30*(5), 518-524.

Blair, R. C., & Karniski, W. (1993). An alternative method for significance testing of waveform difference potentials. *Psychophysiology, 30*(5), 518-524.

Boggio, P., Bermpohl, F., Vergara, A., Muniz, A., Nahas, F., Leme, P., . . . Fregni, F. (2007). 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*(1-3), 91-98.

Boggio, P., Castro, L., Savagim, E., Braite, R., Cruz, V., Rocha, R., . . . Fregni, F. (2006). Enhancement of non-dominant hand motor function by anodal transcranial direct current stimulation. *Neuroscience letters, 404*(1-2), 232-236.

Boggio, P., Ferrucci, R., Rigonatti, S., Covre, P., Nitsche, M., Pascual-Leone, A., & Fregni, F. (2006). Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease. *Journal of the neurological sciences, 249*(1), 31-38.

Boggio, P., Khoury, L., Martins, D., Martins, O., de Macedo, E., & Fregni, F. (2009). Temporal cortex direct current stimulation enhances performance on a visual recognition memory task in Alzheimer disease. *British Medical Journal, 80*(4), 444.

Boggio, P., Nunes, A., Rigonatti, S., Nitsche, M., Pascual-Leone, A., & Fregni, F. (2007). Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. *Restorative neurology and neuroscience, 25*(2), 123-129.

Boggio, P., Rigonatti, S., Ribeiro, R., Myczkowski, M., Nitsche, M., Pascual-Leone, A., & Fregni, F. (2007). A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. *The International Journal of Neuropsychopharmacology, 11*(02), 249-254.

Boggio, P., Sultani, N., Fecteau, S., Merabet, L., Mecca, T., Pascual-Leone, A., . . . Fregni, F. (2008). Prefrontal cortex modulation using transcranial DC stimulation reduces alcohol craving: a double-blind, sham-controlled study. *Drug and alcohol dependence, 92*(1-3), 55-60.

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*(1).

Borbely, A. A., & Achermann, P. (1999). Sleep homeostasis and models of sleep regulation. *J Biol Rhythms, 14*(6), 557-568.

Born, J., Rasch, B., & Gais, S. (2006). Sleep to Remember. *Neuroscientist, 12*(5), 410-424. doi: 10.1177/1073858406292647

Boros, K., Poreisz, C., Münchau, A., Paulus, W., & Nitsche, M. (2008). Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. *European Journal of Neuroscience, 27*(5), 1292-1300.

Bradley, M. M., Greenwald, M. K., Petry, M. C., & Lang, P. J. (1992). Remembering pictures: pleasure and arousal in memory. *J Exp Psychol Learn Mem Cogn, 18*(2), 379-390.

Bradley, M. M., & Lang, P. J. (1994). Measuring emotion: The self-assessment manikin and the semantic differential. *Journal of behavior therapy and experimental psychiatry, 25*(1), 49-59.

Brainard, D. (1997). The psychophysics toolbox. *Spatial vision, 10*(4), 433-436.

Bremner, J., Soufer, R., McCarthy, G., Delaney, R., Staib, L., Duncan, J., & Charney, D. (2001). Gender differences in cognitive and neural correlates of remembrance of emotional words. *Psychopharmacology bulletin, 35*(3), 55.

Brewer, J. B., Zhao, Z., Desmond, J. E., Glover, G. H., & Gabrieli, J. D. E. (1998). Making memories: brain activity that predicts how well visual experience will be remembered. *Science, 281*(5380), 1185.

Brown, R., & Kulik, J. (1977). Flashbulb memories. *Cognition, 5*(1), 73-99.

Burton, L., Rabin, L., Vardy, S., Frohlich, J., Wyatt, G., Dimitri, D., . . . Guterman, E. (2004). Gender differences in implicit and explicit memory for affective passages. *Brain and Cognition, 54*(3), 218-224.

Buzsáki, G. (2002). Theta Oscillations in the Hippocampus. *Neuron, 33*(3), 325-340.

Cabeza, R., & Nyberg, L. (2000a). Imaging cognition II: An empirical review of 275 PET and fMRI studies. *Journal of Cognitive Neuroscience, 12*(1), 1-47.

Cabeza, R., & Nyberg, L. (2000b). Neural bases of learning and memory: functional neuroimaging evidence. *Current opinion in neurology, 13*(4), 415.

Cahill, L. (2000). Neurobiological mechanisms of emotionally influenced, long-term memory. *Progress in brain research, 126*, 29.

Cahill, L., & Alkire, M. (2003). Epinephrine enhancement of human memory consolidation: Interaction with arousal at encoding. *Neurobiology of Learning and Memory, 79*(2), 194-198.

Cahill, L., Gorski, L., Belcher, A., & Huynh, Q. (2004). The influence of sex versus sex-related traits on long-term memory for gist and detail from an emotional story. *Consciousness and Cognition, 13*(2), 391-400.

Calder, A. (1996). Facial emotion recognition after bilateral amygdala damage: Differentially severe

impairment of fear. *Cognitive Neuropsychology, 13*(5), 699-745.

Canli, T., Zhao, Z., Brewer, J., Gabrieli, J. D., & Cahill, L. (2000). Event-related activation in the human amygdala associates with later memory for individual emotional experience. *J Neurosci, 20*(19), RC99.

Carney, M., Cashman, M., & Sheffield, B. (1970). Polarization in depression. *The British Journal of Psychiatry, 117*(539), 474.

Castaldo, V., Krynicki, V., & Goldstein, J. (1974). Sleep stages and verbal memory. *Percept Mot Skills, 39*, 1023-1030.

Cerruti, C., & Schlaug, G. (2008). Anodal Transcranial Direct Current Stimulation of the Prefrontal Cortex Enhances Complex Verbal Associative Thought. *Journal of Cognitive Neuroscience, 21*(10), 1980-1987.

Chadaide, Z., Arlt, S., Antal, A., Nitsche, M., Lang, N., & Paulus, W. (2007). Transcranial direct current stimulation reveals inhibitory deficiency in migraine. *Cephalalgia, 27*(7), 833.

Chikama, M., McFarland, N., Amaral, D., & Haber, S. (1997). Insular cortical projections to functional regions of the striatum correlate with cortical cytoarchitectonic organization in the primate. *Journal of Neuroscience, 17*(24), 9686-9705.

Cleeremans, A., & McClelland, J. (1991). Learning the structure of event sequences. *Journal of Experimental Psychology: General, 120*(3), 235-253.

Cohen, D., Pascual-Leone, A., Press, D., & Robertson, E. (2005). Off-line learning of motor skill memory: A double dissociation of goal and movement. *Proceedings of the National Academy of Sciences, 102*(50), 18237-18241.

Cohen, D., & Robertson, E. (2007). Motor sequence consolidation: constrained by critical time windows or competing components. *Experimental Brain Research, 177*(4), 440-446.

Coltheart, M. (1981). The MRC psycholinguistic database. *The Quarterly Journal of Experimental Psychology A: Human Experimental Psychology, 33*(4), 497-505.

Cooke, S., & Bliss, T. (2006). Plasticity in the human central nervous system. *Brain, 129*(7), 1659.

Costain, R., Redfearn, J., & Lippold, O. (1964). A controlled trial of the therapeutic effect of polarization of the brain in depressive illness. *Br J Psychiatry, 110*, 786-799.

Creutzfeldt, O., Fromm, G., & Kapp, H. (1962). Influence of transcortical dc currents on cortical neuronal activity. *Experimental neurology, 5*(6), 436-452.

Darian-Smith, C., Darian-Smith, I., Burman, K., & Ratcliffe, N. (1993). Ipsilateral cortical projections to areas 3a, 3b, and 4 in the macaque monkey. *The Journal of comparative neurology, 335*(2).

Daubechies, I. (2002). The wavelet transform, time-frequency localization and signal analysis. *Information Theory, IEEE Transactions on, 36*(5), 961-1005.

De Koninck, J., Lorrain, D., Christ, G., Proulx, G., & Coulombe, D. (1989). Intensive language learning and increases in rapid eye movement sleep: evidence of a performance factor. *Int J Psychophysiol, 8*(1), 43-47.

de Vries, M., Barth, A., Maiworm, S., Knecht, S., Zwitserlood, P., & Flöel, A. (2009). Electrical Stimulation of Broca's Area Enhances Implicit Learning of an Artificial Grammar. *Journal of Cognitive Neuroscience*, 1-10.

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of neuroscience methods, 134*(1), 9-21.

Desgranges, B., Baron, J., & Eustache, F. (1998). The functional neuroanatomy of episodic memory: the role of the frontal lobes, the hippocampal formation, and other areas. *Neuroimage, 8*(2), 198-213.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., . . . Rothwell, J. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clinical Neurophysiology, 115*(2), 255-266.

Dockery, C., Hueckel-Weng, R., Birbaumer, N., & Plewnia, C. (2009). Enhancement of Planning Ability by Transcranial Direct Current Stimulation. *Journal of Neuroscience, 29*(22), 7271.

Dolcos, F., LaBar, K. S., & Cabeza, R. (2004). Interaction between the amygdala and the medial temporal lobe memory system predicts better memory for emotional events. *Neuron, 42*(5), 855-863.

Doyon, J., Penhune, V., & Ungerleider, L. G. (2003). Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. *Neuropsychologia, 41*(3), 252-262.

Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annu Rev Psychol, 55*, 51-86.

Dum, R., & Strick, P. (1991). The origin of corticospinal projections from the premotor areas in the frontal lobe. *Journal of Neuroscience, 11*(3), 667-689.

Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C., & Lüthi, A. (2009). Amygdala Inhibitory Circuits and the Control of Fear Memory. *Neuron, 62*(6), 757-771.

Eichenbaum, H. (1997). Declarative memory: insights from cognitive neurobiology. *Annu Rev Psychol, 48*, 547-572.

Eichenbaum, H. (2000). A cortical-hippocampal system for declarative memory. *Nat Rev Neurosci, 1*(1), 41-50.

Eichenbaum, H. (2004). Hippocampus: cognitive processes and neural representations that underlie declarative memory. *Neuron, 44*(1), 109-120.

Ellenbogen, J., Hu, P., Payne, J., Titone, D., & Walker, M. (2007). Human relational memory requires time and sleep. *Proceedings of the National Academy of Sciences, 104*(18), 7723.

Ellis, M., & Kesner, R. (1983). The noradrenergic system of the amygdala and aversive information processing. *Behavioral Neuroscience, 97*(3), 399.

Elmer, S., Burkard, M., Renz, B., Meyer, M., & Jancke, L. (2009). Direct current induced short-term modulation of the left dorsolateral prefrontal cortex while learning auditory presented nouns. *Behavioral and Brain Functions: BBF, 5*, 29.

Empson, J. A., & Clarke, P. R. (1970). Rapid eye movements and remembering. *Nature, 227*(5255), 287-288.

Fecteau, S., Knoch, D., Fregni, F., Sultani, N., Boggio, P., & Pascual-Leone, A. (2007). Diminishing risk-taking behavior by modulating activity in the prefrontal cortex: a direct current stimulation study. *Journal of Neuroscience, 27*(46), 12500.

Fecteau, S., Pascual-Leone, A., Zald, D., Liguori, P., Theoret, H., Boggio, P., & Fregni, F. (2007). Activation of prefrontal cortex by transcranial direct current stimulation reduces appetite for risk during ambiguous decision making. *Journal of Neuroscience, 27*(23), 6212.

Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Salvoro, B., Giacopuzzi, M., . . . Priori, A. (2009). Transcranial direct current stimulation in severe, drug-resistant major depression. *Journal of Affective Disorders, 118*(1-3), 215-219.

Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Salvoro, B., Giacopuzzi, M., & Priori, A. (2008). Transcranial direct current stimulation improves patients with severe major depression. *Brain Stimulation, 1*(3), 259-259.

Ferrucci, R., Mameli, F., Guidi, I., Mrakic-Sposta, S., Vergari, M., Marceglia, S., . . . Priori, A. (2008). Transcranial direct current stimulation improves recognition memory in Alzheimer disease. *Neurology, 71*(7), 493.

Ferrucci, R., Marceglia, S., Vergari, M., Cogiamanian, F., Mrakic-Sposta, S., Mameli, F., . . . Priori, A. (2008). Cerebellar transcranial direct current stimulation impairs the practice-dependent proficiency increase in working memory. *Journal of Cognitive Neuroscience, 20*(9), 1687-1697.

Ferry, A., Ongur, D., An, X., & Price, J. (2000). Prefrontal cortical projections to the striatum in macaque monkeys: evidence for an organization related to prefrontal networks. *The Journal of comparative neurology, 425*(3), 447-470.

Ficca, G., & Salzarulo, P. (2004). What in sleep is for memory. *Sleep Medicine, 5*(3), 225-230.

Fischer, S., Drosopoulos, S., Tsen, J., & Born, J. (2006). Implicit learning -- explicit knowing: a role for sleep in memory system interaction. *J Cogn Neurosci, 18*(3), 311-319.

Fischer, S., Hallschmid, M., Elsner, A. L., & Born, J. (2002). Sleep forms memory for finger skills. *Proceedings of the National Academy of Sciences, 99*(18), 11987-11991.

Fitch, R. H., & Denenberg, V. H. (1998). A role for ovarian hormones in sexual differentiation of the brain. *Behavioral and Brain Sciences, 21*(03), 311-327.

Fletcher, P., Frith, C., & Rugg, M. (1997). The functional neuroanatomy of episodic memory. *Trends in neurosciences, 20*(5), 213-218.

Fletcher, P., & Henson, R. (2001). Frontal lobes and human memory: insights from functional neuroimaging. *Brain, 124*(5), 849.

Fletcher, P., Shallice, T., & Dolan, R. (1998). The functional roles of prefrontal cortex in episodic memory. I. Encoding. *Brain, 121*(7), 1239.

Fletcher, P., Shallice, T., Frith, C., Frackowiak, R., & Dolan, R. (1998). The functional roles of prefrontal cortex in episodic memory. II. Retrieval. *Brain, 121*(7), 1249.

Flöel, A., & Cohen, L. (2007). Contribution of noninvasive cortical stimulation to the study of memory functions. *Brain research reviews, 53*(2), 250-259.

Flöel, A., Rösser, N., Michka, O., Knecht, S., & Breitenstein, C. (2008). Noninvasive brain stimulation improves language learning. *Journal of Cognitive Neuroscience, 20*(8), 1415-1422.

Fowler, M. J., Sullivan, M. J., & Ekstrand, B. R. (1973). Sleep and memory. *Science, 179*(70), 302-304.

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nat Rev Neurosci, 6*(2), 119-130.

Frégnac, Y., Smith, D., & Friedlander, M. (1990). *Postsynaptic membrane potential regulates synaptic potentiation and depression in visual cortical neurons.* Paper presented at the Society of Neuroscience Abstract.

Fregni, F., Boggio, P., Lima, M., Ferreira, M., Wagner, T., Rigonatti, S., . . . Freedman, S. (2006). A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. *Pain, 122*(1-2), 197-209.

Fregni, F., Boggio, P., Mansur, C., Wagner, T., Ferreira, M., Lima, M., . . . Nitsche, M. (2005). Transcranial direct current stimulation of the unaffected hemisphere in stroke patients. *Neuroreport, 16*(14), 1551.

Fregni, F., Boggio, P., Nitsche, M., Bermpohl, F., Antal, A., Feredoes, E., . . . Paulus, W. (2005). Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Experimental Brain Research, 166*(1), 23-30.

Fregni, F., Boggio, P., Nitsche, M., Marcolin, M., Rigonatti, S., & Pascual-Leone, A. (2006). Treatment of major depression with transcranial direct current stimulation. *Bipolar disorders, 8*(2), 203.

Fregni, F., Boggio, P., Nitsche, M., Rigonatti, S., & Pascual-Leone, A. (2006). Cognitive effects of repeated sessions of transcranial direct current stimulation in patients with depression. *Depression and anxiety, 23*(8), 482.

Fregni, F., Boggio, P., Santos, M., Lima, M., Vieira, A., Rigonatti, S., . . . Pascual-Leone, A. (2006). Noninvasive cortical stimulation with transcranial direct current stimulation in Parkinson's disease. *Movement Disorders, 21*(10).

Fregni, F., Gimenes, R., Valle, A., Ferreira, M., Rocha, R., Natalle, L., . . . Nitsche, M. (2006). A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. *Arthritis Care & Research, 54*(12), 3988-3998.

Fregni, F., Liguori, P., Fecteau, S., Nitsche, M., Pascual-Leone, A., & Boggio, P. (2008). Cortical stimulation of the prefrontal cortex with transcranial direct current stimulation reduces cue-provoked smoking craving: a randomized, sham-controlled study. *Journal of Clinical Psychiatry, 69*(1), 32.

Fregni, F., Marcondes, R., Boggio, P., Marcolin, M., Rigonatti, S., Sanchez, T., . . . Pascual-Leone, A. (2006). Transient tinnitus suppression induced by repetitive transcranial magnetic stimulation and transcranial direct current stimulation. *European Journal of Neurology, 13*(9), 996-1001.

Fregni, F., Orsati, F., Pedrosa, W., Fecteau, S., Tome, F., Nitsche, M., . . . Boggio, P. (2008). Transcranial direct current stimulation of the prefrontal cortex modulates the desire for specific foods. *Appetite, 51*(1), 34-41.

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*(7), 383-393.

Froc, D., Chapman, C., Trepel, C., & Racine, R. (2000). Long-term depression and depotentiation in the sensorimotor cortex of the freely moving rat. *Journal of Neuroscience, 20*(1), 438.

Gabrieli, J. D. E., Corkin, S., Mickel, S. F., & Growdon, J. H. (1993). Intact acquisition and long-term retention of mirror-tracing skill in Alzheimer's disease and in global amnesia. *Behavioral Neuroscience, 107*, 899-899.

Gabrieli, J. D. E., Stebbins, G. T., Singh, J., Willingham, D. B., & Goetz, C. G. (1997). Intact mirror-tracing and impaired rotary-pursuit skill learning in patients with Huntington's disease: evidence for dissociable memory systems in skill learning. *Neuropsychology, 11*, 272-281.

Gais, S., Plihal, W., Wagner, U., & Born, J. (2000). Early sleep triggers memory for early visual discrimination skills. *Nat Neurosci, 3*(12), 1335-1339. doi: 10.1038/81881

Galea, J. M., Jayaram, G., Ajagbe, L., & Celnik, P. (2009). Modulation of Cerebellar Excitability by Polarity-Specific Noninvasive Direct Current Stimulation. *Journal of Neuroscience, 29*(28), 9115.

Galea, M. P., & Darian-Smith, I. (1994). Multiple corticospinal neuron populations in the macaque monkey are specified by their unique cortical origins, spinal terminations, and connections. *Cerebral Cortex, 4*(2), 166-194.

Gallagher, M., Kapp, B. S., Pascoe, J. P., & Rapp, P. R. (1981). *A Neuropharmacolocy Of Amygdala Systems Which Contribute To Learning And Memory*: Elsevier/North-Holland Biomedical Press.

Gallate, J., Chi, R., Ellwood, S., & Snyder, A. (2009). Reducing false memories by magnetic pulse stimulation. *Neuroscience letters, 449*(3), 151-154.

Gandiga, P., Hummel, F., & Cohen, L. (2006). Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clinical Neurophysiology, 117*(4), 845-850.

Gartside, I. (1968). Mechanisms of sustained increases of firing rate of neurones in the rat cerebral cortex after polarization: role of protein synthesis. *Nature*.

Gazzaniga, M. (2004). *The cognitive neurosciences*: Bradford Books.

Giuditta, A., Ambrosini, M. V., Montagnese, P., Mandile, P., Cotugno, M., Grassi Zucconi, G., & Vescia, S. (1995). The sequential hypothesis of the function of sleep. *Behav Brain Res, 69*(1-2), 157-166.

Goddard, G. (1964). Amygdaloid stimulation and learning in the rat. *Journal of Comparative and Physiological Psychology, 58*, 23.

Greenberg, R., Pearlman, C., Schwartz, W. R., & Grossman, H. Y. (1983). Memory, emotion, and REM sleep. *J Abnorm Psychol, 92*(3), 378-381.

Groenewegen, H., & Berendse, H. (1994). Anatomical relationships between the prefrontal cortex and the basal ganglia in the rat. In A.-M. Thierry (Ed.), *Motor and cognitive functions of the prefrontal cortex* (pp. 51–77). Berlin: Springer.

Guy, S., & Cahill, L. (1999). The Role of Overt Rehearsal in Enhanced Conscious Memory for Emotional Events. *Consciousness and Cognition, 8*(1), 114-122.

Haber, S. (2003). Integrating cognition and motivation into the basal ganglia pathways of action. *Mental and Behavioral Dysfunction in Movement Disorders*, 35.

Haber, S., Groenewegen, H., Grove, E., & Nauta, W. (1985). Efferent connections of the ventral pallidum: evidence of a dual striato pallidofugal pathway. *The Journal of comparative neurology, 235*(3), 322-335.

Haber, S., Kunishio, K., Mizobuchi, M., & Lynd-Balta, E. (1995). The orbital and medial prefrontal circuit through the primate basal ganglia. *Journal of Neuroscience, 15*(7), 4851-4867.

Haegelen, C., Rouaud, T., Darnault, P., & Morandi, X. (2009). The subthalamic nucleus is a key-structure of limbic basal ganglia functions. *Medical Hypotheses, 72*(4), 421-426.

Hamann, S., & Canli, T. (2004). Individual differences in emotion processing. *Current opinion in neurobiology, 14*(2), 233-238.

Hanslmayr, S., Aslan, A., Staudigl, T., Klimesch, W., Herrmann, C., & Bäuml, K. (2007). Prestimulus oscillations predict visual perception performance between and within subjects. *Neuroimage, 37*(4), 1465-1473.

Hanslmayr, S., Pastötter, B., Bäuml, K., Gruber, S., Wimber, M., & Klimesch, W. (2008). The electrophysiological dynamics of interference during the Stroop task. *Journal of Cognitive Neuroscience, 20*(2), 215-225.

Hanslmayr, S., Spitzer, B., & Bauml, K. (2008). Brain oscillations dissociate between semantic and nonsemantic encoding of episodic memories. *Cerebral Cortex*.

Hardingham, N., Bannister, N., Read, J., Fox, K., Hardingham, G., & Jack, J. (2006). Extracellular calcium regulates postsynaptic efficacy through group 1 metabotropic glutamate receptors. *Journal of Neuroscience, 26*(23), 6337.

Hatfield, T., & McGaugh, J. L. (1999). Norepinephrine infused into the basolateral amygdala posttraining enhances retention in a spatial water maze task. *Neurobiol Learn Mem, 71*(2), 232-239.

Hattori, Y., Moriwaki, A., & Hori, Y. (1990). Biphasic effects of polarizing current on adenosine-sensitive generation of cyclic AMP in rat cerebral cortex. *Neuroscience letters, 116*(3), 320-324.

Hauptmann, B., Reinhart, E., Brandt, S., & Karni, A. (2005). The predictive value of the leveling off of within session performance for procedural memory consolidation. *Cognitive Brain Research, 24*(2), 181-189.

Hecht, D., Walsh, V., & Lavidor, M. (2010). Transcranial Direct Current Stimulation Facilitates

Decision Making in a Probabilistic Guessing Task. *Journal of Neuroscience, 30*(12), 4241.

Heimer, L. (1978). The olfactory cortex and the ventral striatum. *Livingston, KE:, O. Hornykiewitz (eds): Limbic Mechanisms: The Continuing Evolution of the Limbic System Concept: New York, Plenum*, 95–187.

Heimer, L., Switzer, R., & Hoesen, G. (1982). Ventral striatum and ventral pallidum: Components of the motor system. *Trends Neurosci, 5*, 83087.

Herjanic, M., & Moss-Herjanic, B. (1967). Clinical report on a new therapeutic technique: polarization. *Canadian Psychiatric Association journal, 12*(4), 423.

Hern, J., Landgren, S., Phillips, C., & Porter, R. (1962). Selective excitation of corticofugal neurones by surface-anodal stimulation of the baboon's motor cortex. *The Journal of physiology, 161*(1), 73.

Herwig, U., Satrapi, P., & Schönfeldt-Lecuona, C. (2003). Using the international 10-20 EEG system for positioning of transcranial magnetic stimulation. *Brain topography, 16*(2), 95-99.

Hesse, S., Werner, C., Schonhardt, E., Bardeleben, A., Jenrich, W., & Kirker, S. (2007). Combined transcranial direct current stimulation and robot-assisted arm training in subacute stroke patients: a pilot study. *Restorative neurology and neuroscience, 25*(1), 9-15.

Hoddes, E., Dement, W., & Zarcone, V. (1972). The development and use of the Stanford Sleepiness Scale (SSS). *Psychophysiology, 9*, 150.

Hoddes, E., Zarcone, V., Smythe, H., Phillips, R., & Dement, W. (1973). Quantification of sleepiness: a new approach. *Psychophysiology, 10*(4), 431-436.

Homan, R., Herman, J., & Purdy, P. (1987). Cerebral location of international 10-20 system electrode placement. *Electroencephalography and clinical Neurophysiology, 66*(4), 376.

Horne, J., & Ostberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology, 4*(2), 97.

Hotermans, C., Peigneux, P., de Noordhout, A. M., Moonen, G., & Maquet, P. (2008). Repetitive transcranial magnetic stimulation over the primary motor cortex disrupts early boost but not delayed gains in performance in motor sequence learning. *European Journal of Neuroscience, 28*(6), 1216-1221.

Hu, P., Stylos-Allan, M., & Walker, M. P. (2006). Sleep facilitates consolidation of emotional declarative memory. *Psychol Sci, 17*(10), 891-898.

Huey, E., Probasco, J., Moll, J., Stocking, J., Ko, M., Grafman, J., & Wassermann, E. (2007). No effect of DC brain polarization on verbal fluency in patients with advanced frontotemporal dementia. *Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology, 118*(6), 1417.

Hummel, F. C., Celnik, P., Giraux, P., Floel, A., Wu, W. H., Gerloff, C., & Cohen, L. G. (2005). Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain, 128*(3), 490.

Hummel, F. C., & Cohen, L. G. (2005). Drivers of brain plasticity. *Current opinion in neurology, 18*, 667-674.

Hummel, F. C., Voller, B., Celnik, P., Floel, A., Giraux, P., Gerloff, C., & Cohen, L. G. (2006). Effects of brain polarization on reaction times and pinch force in chronic stroke. *BMC Neuroscience, 7*(1), 73.

Hunter, T., Sacco, P., Nitsche, M., & Turner, D. (2009). Modulation of internal model formation during force field-induced motor learning by anodal transcranial direct current stimulation of primary motor cortex. *Experimental Physiology, 587*(12), 2949-2961.

Im, C., Jung, H., Choi, J., Lee, S., & Jung, K. (2008). Determination of optimal electrode positions for transcranial direct current stimulation (tDCS). *Physics in medicine and biology, 53*(11), 219.

Islam, N., Aftabuddin, M., Moriwaki, A., Hattori, Y., & Hori, Y. (1995). Increase in the calcium level following anodal polarization in the rat brain. *Brain Research, 684*(2), 206-208.

Iyer, M., Mattu, U., Grafman, J., Lomarev, M., Sato, S., & Wassermann, E. (2005). Safety and cognitive effect of frontal DC brain polarization in healthy individuals. *Neurology, 64*(5), 872-875.

Javadi, A. H., Cheng, P., & Walsh, V. (submitted). *Administration of short duration transcranial direct current stimulation (tDCS) at the correct time modulates performance of episodic memory*.

Javadi, A. H., Ting, L., & Walsh, V. (in preparation). *Electrical stimulation of left dorsolateral prefrontal cortex preferentially enhances pictorial memory*.

Javadi, A. H., & Walsh, V. (2009). *Memory consolidation of semantically related and unrelated word pair memorisation including emotion*. Paper presented at the Sleep 2009, 23rd annual meeting of the associated professional sleep societies, Seattle.

Javadi, A. H., & Walsh, V. (Submitted). *Transcranial direct current stimulation applied over left dorsolateral prefrontal cortex modulates declarative verbal memory*.

Javadi, A. H., Walsh, V., & Lewis, P. A. (2011). Offline consolidation of procedural skill learning is enhanced by negative emotional content. *Experimental Brain Research, 208*(4), 507-518. doi: 10.1007/s00221-010-2497-7

Javadi, A. H., Yang, J., Bellesi, G., Lewis, P., & Walsh, V. (2010). *Association between fingers and images enhances the consolidation of procedural memory in serial reaction time task*. Paper presented at the Training in sleep research and sleep medicine, Germany.

Javadi, A. H., Yang, J., Bellesi, G., Lewis, P., & Walsh, V. (In preparation). *Emotional stimuli enhance procedural memory consolidation; Evidence from a modified serial reaction time task*.

Jenkins, J. G., & Dallenbach, K. M. (1924). Obliviscence during Sleep and Waking. *The American Journal of Psychology, 35*(4), 605-612.

Jo, J., Kim, Y., Ko, M., Ohn, S., Joen, B., & Lee, K. (2009). Enhancing the Working Memory of Stroke Patients Using tDCS. *American Journal of Physical Medicine & Rehabilitation, 88*(5), 404.

Jones, M. W., & Wilson, M. A. (2005). Theta rhythms coordinate hippocampal-prefrontal

interactions in a spatial memory task. *PLoS Biol, 3*(12), e402.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (2000). *Principles of neural science* (4th ed.). New York: McGraw-Hill, Health Professions Division.

Kang, E., Baek, M., Kim, S., & Paik, N. (2009). Non-invasive cortical stimulation improves post-stroke attention decline. *Restorative neurology and neuroscience, 27*(6), 647-652.

Kensinger, E., & Corkin, S. (2004). Two routes to emotional memory: distinct neural processes for valence and arousal. *Proc Natl Acad Sci USA, 101*(9), 3310-3315.

Kensinger, E., Garoff-Eaton, R., & Schacter, D. (2006). Memory for specific visual details can be enhanced by negative arousing content. *Journal of Memory and Language, 54*(1), 99-112.

Kesner, R., & Wilburn, M. (1974). A review of electrical stimulation of the brain in context of learning and retention. *Behavioral biology, 10*(3), 259.

Kilpatrick, L., & Cahill, L. (2003). Amygdala modulation of parahippocampal and frontal regions during emotionally influenced memory storage. *NeuroImage, 20*(4), 2091-2099.

Kincses, T., Antal, A., Nitsche, M., Bártfai, O., & Paulus, W. (2004). Facilitation of probabilistic classification learning by transcranial direct current stimulation of the prefrontal cortex in the human. *Neuropsychologia, 42*(1), 113-117.

Kleinsmith, L. J., & Kaplan, S. (1963). Paired-associate learning as a function of arousal and interpolated interval. *Journal of Experimental Psychology, 65*(2), 190-193.

Kluver, H., & Bucy, P. (1937). Psychic blindness" and other symptoms following bilateral temporal lobectomy in rhesus monkeys. *Am J Physiol, 119*, 352-353.

Knoch, D., Nitsche, M., Fischbacher, U., Eisenegger, C., Pascual-Leone, A., & Fehr, E. (2008). Studying the Neurobiology of Social Interaction with Transcranial Direct Current Stimulation--The Example of Punishing Unfairness. *Cerebral Cortex, 18*(9), 1987-1990.

Kudrimoti, H. S., Barnes, C. A., & McNaughton, B. L. (1999). Reactivation of Hippocampal Cell Assemblies: Effects of Behavioral State, Experience, and EEG Dynamics. *J. Neurosci., 19*(10), 4090-4101.

Kuo, M., Unger, M., Liebetanz, D., Lang, N., Tergau, F., Paulus, W., & Nitsche, M. (2008). Limited impact of homeostatic plasticity on motor learning in humans. *Neuropsychologia, 46*(8), 2122-2128.

Kwon, Y., Ko, M., Ahn, S., Kim, Y., Song, J., Lee, C., . . . Jang, S. (2008). Primary motor cortex activation by transcranial direct current stimulation in the human brain. *Neuroscience letters, 435*(1), 56-59.

LaBar, K. S., & Cabeza, R. (2006). Cognitive neuroscience of emotional memory. *Nature Reviews Neuroscience, 7*(1), 54-64.

LaBar, K. S., & Phelps, E. A. (1998). Arousal-Mediated Memory Consolidation: Role of the Medial Temporal Lobe in Humans. *Psychological Science, 9*(6), 490-493.

Lang, N., Nitsche, M., Sommer, M., Tergau, F., & Paulus, W. (2003). Modulation of motor consolidation by external DC stimulation.

Supplements to Clinical neurophysiology, 56, 277-281.

Lang, N., Siebner, H., Ernst, D., Nitsche, M., Paulus, W., Lemon, R., & Rothwell, J. (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*(9), 634-639.

Lang, N., Siebner, H., Ward, N., Lee, L., Nitsche, M., Paulus, W., . . . Frackowiak, R. (2005). How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? *European Journal of Neuroscience, 22*(2), 495.

Lang, P., Bradley, M., & Cuthbert, B. (1997). International affective picture system (IAPS): Technical manual and affective ratings. *NIMH Center for the Study of Emotion and Attention*.

Laureys, S., Peigneux, P., Perrin, F., & Maquet, P. (2002). Sleep and motor skill learning. *Neuron, 35*(1), 5-7.

LeDoux, J. (2000). Emotion circuits in the brain. *Annual review of neuroscience, 23*(1), 155-184.

Levonian, E. (1972). Retention over time in relation to arousal during learning: An explanation of discrepant results. *Acta Psychologica, 36*(4), 290-321.

Lewis, P. A., Critchley, H. D., Rotshtein, P., & Dolan, R. J. (2007). Neural correlates of processing valence and arousal in affective words. *Cereb Cortex, 17*(3), 742-748.

Liang, K. C., McGaugh, J. L., & Yao, H. Y. (1990). Involvement of amygdala pathways in the influence of post-training intra-amygdala norepinephrine and peripheral epinephrine on memory storage. *Brain Research, 508*(2), 225-233.

Liebetanz, D., Klinker, F., Hering, D., Koch, R., Nitsche, M., Potschka, H., . . . Tergau, F. (2006). Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. *Epilepsia, 47*(7), 1216-1224.

Liebetanz, D., Nitsche, M., Tergau, F., & Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain, 125*(10), 2238.

Lippold, O., & Redfearn, J. (1964). Mental changes resulting from the passage of small direct currents through the human brain. *The British Journal of Psychiatry, 110*(469), 768.

Logothetis, N. K. (2008). What we can do and what we cannot do with fMRI. *Nature, 453*(7197), 869-878.

Lolas, F. (1977). Brain polarization: behavioral and therapeutic effects. *Biological Psychiatry, 12*(1), 37.

Lovatt, D. J., & Warr, P. B. (1968). Recall after Sleep. *The American Journal of Psychology, 81*(2), 253-257.

Lu, M., Preston, J., & Strick, P. (1994). Interconnections between the prefrontal cortex and the premotor areas in the frontal lobe. *The Journal of comparative neurology, 341*(3).

Maddock, R. (1999). The retrosplenial cortex and emotion: new insights from functional

neuroimaging of the human brain. *Trends in neurosciences, 22*(7), 310-316.

Malenka, R., & Bear, M. (2004). LTP and LTD: An Embarrassment of Riches. *Neuron, 44*(1), 5-21.

Maquet, P., Peters, J., Aerts, J., Delfiore, G., Degueldre, C., Luxen, A., & Franck, G. (1996). Functional neuroanatomy of human rapid-eye-movement sleep and dreaming. *Nature, 383*(6596), 163-166.

Maren, S. (2008). Pavlovian fear conditioning as a behavioral assay for hippocampus and amygdala function: cautions and caveats. *European Journal of Neuroscience, 28*(8), 1661.

Marr, D. (1970). A theory for cerebral neocortex. *Proc. R. Soc. Lond. B Biol. Sci., 176*, 161-234.

Marrosu, F., Portas, C., Mascia, M. S., Casu, M. A., Fa, M., Giagheddu, M., . . . Gessa, G. L. (1995). Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. *Brain Res, 671*(2), 329-332.

Marshall, L., & Born, J. (2007). The contribution of sleep to hippocampus-dependent memory consolidation. *Trends in Cognitive Sciences, 11*(10), 442-450.

Marshall, L., Helgadottir, H., Molle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature, 444*(7119), 610-613.

Marshall, L., Molle, M., Hallschmid, M., & Born, J. (2004). Transcranial direct current stimulation during sleep improves declarative memory. *J Neurosci, 24*, 9985 - 9992.

Marshall, L., Molle, M., Siebner, H., & Born, J. (2005). Bifrontal transcranial direct current stimulation slows reaction time in a working memory task. *BMC Neuroscience, 6*(1), 23.

Martin, A. (2001). Functional neuroimaging of semantic memory. *Handbook of functional neuroimaging of cognition*, 153–186.

McClelland, J. L., McNaughton, B. L., & O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev, 102*(3), 419-457.

McGaugh, J. L. (2003). *Memory and emotion: The making of lasting memories*: Columbia Univ Pr.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual review of neuroscience, 27*, 1-28.

McGaugh, J. L., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. *Proc Natl Acad Sci USA, 93*(24), 13508-13514.

McGaugh, J. L., & Gold, P. E. (1976). Modulation of memory by electrical stimulation of the brain. *Neural mechanisms of learning and memory*, 549-560.

McGaugh, J. L., & Roozendaal, B. (2002). Role of adrenal stress hormones in forming lasting memories in the brain. *Current opinion in neurobiology, 12*(2), 205-210.

Milner, B. (1962). Les troubles de la memoire accompagnant des lesions hippocampiques bilaterales. *Physiologie de l'hippocampe*, 257–272.

Milner, B. (1985). Memory and the human brain. *How We Know*, 1864–1869.

Miranda, P., Lomarev, M., & Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clinical Neurophysiology, 117*(7), 1623-1629.

Monti, A., Cogiamanian, F., Marceglia, S., Ferrucci, R., Mameli, F., Mrakic-Sposta, S., . . . Priori, A. (2008). Improved naming after transcranial direct current stimulation in aphasia. *British Medical Journal, 79*(4), 451.

Morecraft, R., & van Hoesen, G. (1992). Cingulate input to the primary and supplementary motor cortices in the rhesus monkey: evidence for somatotopy in areas 24c and 23c. *The Journal of comparative neurology, 322*(4).

Morecraft, R., & van Hoesen, G. (1993). Frontal granular cortex input to the cingulate (M3), supplementary (M2) and primary (M1) motor cortices in the rhesus monkey. *The Journal of comparative neurology, 337*(4).

Morecraft, R., & Van Hoesen, G. (1998). Convergence of limbic input to the cingulate motor cortex in the rhesus monkey. *Brain Research Bulletin, 45*(2), 209-232.

Morris, R. G., Moser, E. I., Riedel, G., Martin, S. J., Sandin, J., Day, M., & O'Carroll, C. (2003). Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. *Philos Trans R Soc Lond B Biol Sci, 358*(1432), 773-786.

Muakkassa, K., & Strick, P. (1979). Frontal lobe inputs to primate motor cortex: evidence for four somatotopically organized'premotor'areas. *Brain Research, 177*(1), 176.

Muller, G., & Pilzecker, A. (1900). Experimentelle beitrage zur lehre vom gedachtnis. *Z. Psychol, 1*, 1-300.

Murphy, D., Boggio, P., & Fregni, F. (2009). Transcranial direct current stimulation as a therapeutic tool for the treatment of major depression: insights from past and recent clinical studies. *Current Opinion in Psychiatry, 22*(3), 306.

Murray, E., & Coulter, J. (1981). Organization of corticospinal neurons in the monkey. *The Journal of comparative neurology, 195*(2).

Newman, E. B. (1939). Forgetting of Meaningful Material during Sleep and Waking. *The American Journal of Psychology, 52*(1), 65-71.

Nias, D., & Shapiro, M. (1974). The effects of small electrical currents upon depressive symptoms. *The British Journal of Psychiatry, 125*(587), 414.

Nishida, M., Pearsall, J., Buckner, R., & Walker, M. (2009). REM Sleep, Prefrontal Theta, and the Consolidation of Human Emotional Memory. *Cerebral Cortex, 19*(5), 1158.

Nishida, M., & Walker, M. (2007). Daytime naps, motor memory consolidation and regionally specific sleep spindles. *PloS One, 2*(4).

Nissen, M., & Bullemer, P. (1987). Attentional requirements of learning: Evidence from performance measures. *Cognitive Psychology, 19*(1), 1-32.

Nitsche, M., Antal, A., Liebetanz, D., Lang, N., Tergau, F., & Paulus, W. (2008). Neuroplasticity induced by transcranial direct current stimulation. *The Oxford Handbook of Transcranial Stimulation*, 201.

Nitsche, M., Boggio, P., Fregni, F., & Pascual-Leone, A. (2009). Treatment of depression with transcranial direct current stimulation (tDCS): A Review. *Experimental neurology, 219*(1), 14-19.

Nitsche, M., Cohen, L., Wassermann, E., Priori, A., Lang, N., Antal, A., . . . Fregni, F. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation, 1*(3), 206-223.

Nitsche, M., Doemkes, S., Karakose, T., Antal, A., Liebetanz, D., Lang, N., . . . Paulus, W. (2007). Shaping the effects of transcranial direct current stimulation of the human motor cortex. *Journal of neurophysiology, 97*(4), 3109.

Nitsche, M., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., . . . Paulus, W. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *The Journal of physiology, 553*(1), 293.

Nitsche, M., Jaussi, W., Liebetanz, D., Lang, N., Tergau, F., & Paulus, W. (2004). Consolidation of human motor cortical neuroplasticity by D-cycloserine. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology, 29*(8), 1573.

Nitsche, M., Liebetanz, D., Antal, A., Lang, N., Tergau, F., & Paulus, W. (2003a). Modulation of cortical excitability by weak direct current stimulation--technical, safety and functional aspects. *Supplements to Clinical neurophysiology, 56*, 255.

Nitsche, M., Liebetanz, D., Antal, A., Lang, N., Tergau, F., & Paulus, W. (2003b). Modulation of cortical excitability by weak direct current stimulation-technical, safety and functional aspects. *Supplements to Clinical neurophysiology, 56*, 255-276.

Nitsche, M., Liebetanz, D., Lang, N., Antal, A., Tergau, F., & Paulus, W. (2003). Safety criteria for transcranial direct current stimulation (tDCS) in humans. *Clinical Neurophysiology, 114*(11), 2220-2222.

Nitsche, M., Nitsche, M., Klein, C., Tergau, F., Rothwell, J., & Paulus, W. (2003). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clinical Neurophysiology, 114*(4), 600-604.

Nitsche, M., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of physiology, 527*(3), 633-639.

Nitsche, M., & Paulus, W. (2001a). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans (Vol. 57, pp. 1899-1901): AAN Enterprises.

Nitsche, M., & Paulus, W. (2001b). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology, 57*(10), 1899-1901.

Nitsche, M., Roth, A., Kuo, M., Fischer, A., Liebetanz, D., Lang, N., . . . Paulus, W. (2007). Timing-dependent modulation of associative plasticity by general network excitability in the human motor cortex. *Journal of Neuroscience, 27*(14), 3807.

Nitsche, M., Schauenburg, A., Lang, N., Liebetanz, D., Exner, C., Paulus, W., & Tergau, F. (2003). Facilitation of implicit motor learning by weak transcranial direct current stimulation of the

primary motor cortex in the human. *Journal of Cognitive Neuroscience, 15*(4), 619-626.

Nitsche, M., Seeber, A., Frommann, K., Klein, C., Rochford, C., Nitsche, M., . . . Antal, A. (2005). Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *The Journal of physiology, 568*(1), 291.

Nolan, H., Whelan, R., & Reilly, R. (2010). FASTER: Fully Automated Statistical Thresholding for EEG artifact Rejection. *Journal of neuroscience methods*.

Ochsner, K. (2000). Are affective events richly recollected or simply familiar? The experience and process of recognizing feelings past. *Journal of Experimental Psychology General, 129*(2), 242-261.

Ohn, S., Park, C., Yoo, W., Ko, M., Choi, K., Kim, G., . . . Kim, Y. (2008). Time-dependent effect of transcranial direct current stimulation on the enhancement of working memory. *Neuroreport, 19*(1), 43.

Oldfield, R. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia, 9*(1), 97-113.

Osterlund, M. K., & Hurd, Y. L. (2001). Estrogen receptors in the human forebrain and the relation to neuropsychiatric disorders. *Progress in Neurobiology, 64*(3), 251-267.

Otten, L., Henson, R., & Rugg, M. (2001). Depth of processing effects on neural correlates of memory encoding: relationship between findings from across-and within-task comparisons. *Brain, 124*(2), 399.

Otten, L., Quayle, A., Akram, S., Ditewig, T., & Rugg, M. (2006). Brain activity before an event predicts later recollection. *Nature Neuroscience, 9*(4), 489-491.

Pace-Schott, E. F., & Hobson, J. A. (2002). The neurobiology of sleep: genetics, cellular physiology and subcortical networks. *Nat Rev Neurosci, 3*(8), 591-605.

Paller, K. A., & Wagner, A. D. (2002). Observing the transformation of experience into memory. *Trends Cogn Sci, 6*(2), 93-102.

Pare, D., Collins, D. R., & Pelletier, J. G. (2002). Amygdala oscillations and the consolidation of emotional memories. *Trends Cogn Sci, 6*(7), 306-314.

Pastötter, B., Bäuml, K., & Hanslmayr, S. (2008). Oscillatory brain activity before and after an internal context change--Evidence for a reset of encoding processes. *Neuroimage, 43*(1), 173-181.

Pastötter, B., Bäuml, K. H., & Hanslmayr, S. (2008). Oscillatory brain activity before and after an internal context change--Evidence for a reset of encoding processes. *Neuroimage, 43*(1), 173-181.

Paulus, W. (2003). Transcranial direct current stimulation (tDCS). *Transcranial Magnetic Stimlation and Transcranial Direct Current Stimulation (Supplements to Clinical neurophysiology), 56*, 249.

Payne, J. D., Stickgold, R., Swanberg, K., & Kensinger, E. A. (2008). Sleep preferentially enhances memory for emotional components of scenes. *Psychological Science, 19*(8), 781-788.

Payne, J. D., Stickgold, R., Swanberg, K., Kensinger, E. A., & Ave, C. (2008). Changes in Emotional Memory Across Wake and Sleep. *Psychological Science*.

Pearson, R., & Lewis, M. B. (2005). Fear recognition across the menstrual cycle. *Hormones and behavior, 47*(3), 267-271.

Peigneux, P., Laureys, S., Delbeuck, X., & Maquet, P. (2001). Sleeping brain, learning brain. The role of sleep for memory systems. *Neuroreport, 12*(18), A111-124.

Pelletier, J. G., Likhtik, E., Filali, M., & Pare, D. (2005). Lasting increases in basolateral amygdala activity after emotional arousal: Implications for facilitated consolidation of emotional memories. *Learn. Mem., 12*(2), 96-102. doi: 10.1101/lm.88605

Pelli, D. (1997). The VideoToolbox software for visual psychophysics: Transforming numbers into movies. *Spatial vision, 10*(4), 437-442.

Penton-Voak, I. S., & Perrett, D. I. (2000). Female preference for male faces changes cyclically:: Further evidence. *Evolution and Human Behavior, 21*(1), 39-48.

Pfurtscheller, G., & Aranibar, A. (1977). Event-related cortical desynchronization detected by power measurements of scalp EEG. *Electroencephalography and clinical Neurophysiology, 42*(6), 817.

Phelps, E. A. (2004). Human emotion and memory: interactions of the amygdala and hippocampal complex. *Current opinion in neurobiology, 14*(2), 198-202.

Pittenger, C., & Duman, R. (2007). Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology, 33*(1), 88-109.

Plihal, W., & Born, J. (1997a). Effects of Early and Late Nocturnal Sleep on Declarative and Procedural Memory. *J. Cogn. Neurosci., 9*(4), 534-547.

Plihal, W., & Born, J. (1997b). Effects of early and late nocturnal sleep on declarative and procedural memory. *Journal of Cognitive Neuroscience, 9*(4), 534-547.

Plihal, W., & Born, J. (1999). Effects of early and late nocturnal sleep on priming and spatial memory. *Psychophysiology, 36*(5), 571-582.

Polster, M., Nadel, L., & Schacter, D. (1991). Cognitive neuroscience analyses of memory: A historical perspective. *Journal of Cognitive Neuroscience, 3*(2), 95-116.

Poreisz, C., Boros, K., Antal, A., & Paulus, W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain Research Bulletin, 72*(4-6), 208-214.

Power, A. E. (2004). Muscarinic cholinergic contribution to memory consolidation: with attention to involvement of the basolateral amygdala. *Curr Med Chem, 11*(8), 987-996.

Press, D., Casement, M., Pascual-Leone, A., & Robertson, E. (2005). The time course of off-line motor sequence learning. *Cognitive Brain Research, 25*(1), 375-378.

Priori, A. (2003). Brain polarization in humans: a reappraisal of an old tool for prolonged non-

invasive modulation of brain excitability. *Clinical Neurophysiology, 114*(4), 589-595.

Priori, A., Berardelli, A., Rona, S., Accornero, N., & Manfredi, M. (1998). Polarization of the human motor cortex through the scalp. *Neuroreport, 9*(10), 2257.

Priori, A., Mameli, F., Cogiamanian, F., Marceglia, S., Tiriticco, M., Mrakic-Sposta, S., . . . Sartori, G. (2008). Lie-specific involvement of dorsolateral prefrontal cortex in deception. *Cerebral Cortex, 18*(2), 451.

Purpura, D., & McMurtry, J. (1965). Intracellular activities and evoked potential changes during polarization of motor cortex. *Journal of neurophysiology, 28*(1), 166.

Quartarone, A., Lang, N., Rizzo, V., Bagnato, S., Morgante, F., Sant'Angelo, A., . . . Girlanda, P. (2007). Motor cortex abnormalities in amyotrophic lateral sclerosis with transcranial direct-current stimulation. *Muscle & nerve, 35*(5), 620-624.

Quartarone, A., Morgante, F., Bagnato, S., Rizzo, V., Sant'Angelo, A., Aiello, E., . . . Girlanda, P. (2004). Long lasting effects of transcranial direct current stimulation on motor imagery. *Neuroreport, 15*(8), 1287.

Quartarone, A., Rizzo, V., Bagnato, S., Morgante, F., Sant'Angelo, A., Romano, M., . . . Siebner, H. (2005). Homeostatic-like plasticity of the primary motor hand area is impaired in focal hand dystonia. *Brain, 128*(8), 1943.

Ramsay, J., & Schlagenhauf, G. (1966). Treatment of depression with low voltage direct current. *Southern Medical Journal, 59*(8), 932.

Rauchs, G., Bertran, F., Guillery-Girard, B., Desgranges, B., Kerrouche, N., Denise, P., . . . Eustache, F. (2004). Consolidation of strictly episodic memories mainly requires rapid eye movement sleep. *Sleep, 27*(3), 395-401.

Redfearn, J., Lippold, O., & Costain, R. (1964). A preliminary account of the clinical effects of polarizing the brain in certain psychiatric disorders. *Br J Psychiatry, 110*, 773-785.

Richardson, M., Strange, B., & Dolan, R. (2004). Encoding of emotional memories depends on amygdala and hippocampus and their interactions. *Nature Neuroscience, 7*(3), 278-285.

Rigonatti, S., Boggio, P., Myczkowski, M., Otta, E., Fiquer, J., Ribeiro, R., . . . Fregni, F. (2008). Transcranial direct stimulation and fluoxetine for the treatment of depression. *European psychiatry: the journal of the Association of European Psychiatrists, 23*(1), 74.

Ritchey, M., Dolcos, F., & Cabeza, R. (2008). Role of amygdala connectivity in the persistence of emotional memories over time: an event-related FMRI investigation. *Cereb Cortex, 18*(11), 2494-2504.

Robertson, E. (2004). Skill learning: putting procedural consolidation in context. *Current Biology, 14*(24), 1061-1063.

Robertson, E. (2007). The serial reaction time task: Implicit motor skill learning? *Journal of Neuroscience, 27*(38), 10073.

Robertson, E., & Cohen, D. (2006). Understanding consolidation through the architecture of memories. *The Neuroscientist, 12*(3), 261.

Robertson, E., Pascual-Leone, A., & Miall, R. (2004). Current concepts in procedural consolidation. *Nature Reviews Neuroscience, 5*(7), 576-582.

Robertson, E., Pascual-Leone, A., & Press, D. (2004). Awareness modifies the skill-learning benefits of sleep. *Current Biology, 14*(3), 208-212.

Roizenblatt, S., Fregni, F., Gimenez, R., Wetzel, T., Rigonatti, S., Tufik, S., . . . Valle, A. (2007). Site-specific effects of transcranial direct current stimulation on sleep and pain in fibromyalgia: a randomized, sham-controlled study. *Pain Practice, 7*(4), 297-306.

Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews Neuroscience, 10*(6), 423-433.

Roozendaal, B., & McGaugh, J. L. (1996). The memory-modulatory effects of glucocorticoids depend on an intact stria terminalis. *Brain Research, 709*(2), 243-250.

Rosen, A., Ramkumar, M., Nguyen, T., & Hoeft, F. (2009). Noninvasive transcranial brain stimulation and pain. *Current pain and headache reports, 13*(1), 12-17.

Rosenkranz, K., Nitsche, M., Tergau, F., & Paulus, W. (2000). Diminution of training-induced transient motor cortex plasticity by weak transcranial direct current stimulation in the human. *Neuroscience letters, 296*(1), 61-63.

Rosenzweig, M. R., Breedlove, M. S., & Watson, N. V. (2005). *Biological Psychology: An Introduction To Behavioral And Cognitive Neuroscience* (4th ed.): Sinauer.

Roth, B. (1994). Mechanisms for electrical stimulation of excitable tissue. *Critical reviews in biomedical engineering, 22*(3-4), 253.

Sah, P., Westbrook, R., & Luthi, A. (2008). Fear Conditioning and Long-term Potentiation in the Amygdala What Really Is the Connection? *Annals of the New York Academy of Science, 1129*, 88.

Sanes, J. N., Dimitrov, B., & Hallett, M. (1990). Motor learning in patients with cerebellar dysfunction. *Brain, 113*(1), 103.

Schlaug, G., & Renga, V. (2008). Transcranial direct current stimulation: a noninvasive tool to facilitate stroke recovery. *Expert Review of Medical Devices, 5*(6), 759-768.

Sejnowski, T. J., & Destexhe, A. (2000). Why do we sleep? *Brain Res, 886*(1-2), 208-223.

Seymour, B., & Dolan, R. (2008). Emotion, decision making, and the amygdala. *Neuron, 58*(5), 662-671.

Shadmehr, R., & Brashers-Krug, T. (1997). Functional stages in the formation of human long-term motor memory. *Journal of Neuroscience, 17*(1), 409.

Sharot, T., & Phelps, E. A. (2004). How arousal modulates memory: Disentangling the effects of attention and retention. *Cognitive, Affective, & Behavioral Neuroscience, 4*(3), 294–306.

Siapas, A. G., & Wilson, M. A. (1998). Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron, 21*(5), 1123-1128.

Siebner, H., Lang, N., Rizzo, V., Nitsche, M., Paulus, W., Lemon, R., & Rothwell, J. (2004). Preconditioning of low-frequency repetitive

transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *Journal of Neuroscience, 24*(13), 3379.

Smith, C. (1995). Sleep states and memory processes. *Behavioural Brain Research, 69*(1-2), 137-145.

Snoddy, G. S. (1920). *An experimental analysis of a case of trial and error learning in the human subject*: Psychological Review.

Sparing, R., Dafotakis, M., Meister, I., Thirugnanasambandam, N., & Fink, G. (2008). Enhancing language performance with non-invasive brain stimulation--A transcranial direct current stimulation study in healthy humans. *Neuropsychologia, 46*(1), 261-268.

Sparing, R., & Mottaghy, F. (2008). Noninvasive brain stimulation with transcranial magnetic or direct current stimulation (TMS/tDCS)—from insights into human memory to therapy of its dysfunction. *Methods, 44*(4), 329-337.

Sparing, R., Thimm, M., Hesse, M., Kust, J., Karbe, H., & Fink, G. (2009). Bidirectional alterations of interhemispheric parietal balance by non-invasive cortical stimulation. *Brain*.

Spencer, R., Sunm, M., & Ivry, R. (2006). Sleep-dependent consolidation of contextual learning. *Current Biology, 16*(10), 1001-1005.

Squire, L., & Alvarez, P. (1995). Retrograde amnesia and memory consolidation: a neurobiological perspective *Curr Biol, 5*, 169-177.

Squire, L., Knowlton, B., & Musen, G. (1993). The structure and organisation of memory. *Annu Rev Psychol, 44*, 453-495.

Squire, L., & Zola, S. (1996). Structure and function of declarative and nondeclarative memory systems. *Proc Natl Acad Sci U S A, 93*(24), 13515-13522.

Steriade, M. (2003). The corticothalamic system in sleep. *Front Biosci, 8*, d878-899.

Sternberg, S. (1966). High-speed scanning in human memory (Vol. 153, pp. 652-654): © 1966 by the American Association for the Advancement of Science.

Sterpenich, V., Albouy, G., Boly, M., Vandewalle, G., Darsaud, A., Balteau, E., . . . Maquet, P. (2007). Sleep-related hippocampo-cortical interplay during emotional memory recollection. *PLoS Biol, 5*(11), e282.

Stickgold, R., James, L., & Hobson, J. A. (2000a). Visual discrimination learning requires sleep after training. *Nat Neurosci, 3*(12), 1237-1238.

Stickgold, R., James, L. T., & Hobson, J. A. (2000). Visual discrimination learning requires sleep after training. *Nature Neuroscience, 3*, 1237-1238.

Stickgold, R., Whidbee, D., Schirmer, B., Patel, V., & Hobson, J. A. (2000). Visual Discrimination Task Improvement: A Multi-Step Process Occurring During Sleep. *J. Cogn. Neurosci., 12*(2), 246-254.

Stone, D., & Tesche, C. (2009). Transcranial direct current stimulation modulates shifts in global/local attention. *Neuroreport, 20*(12), 1115.

Sutherland, G. R., & McNaughton, B. (2000). Memory trace reactivation in hippocampal and neocortical neuronal ensembles. *Current Opinion in Neurobiology, 10*(2), 180-186.

Tanaka, S., Hanakawa, T., Honda, M., & Watanabe, K. (2009). Enhancement of pinch force

in the lower leg by anodal transcranial direct current stimulation. *Experimental Brain Research*, 1-7.

Tehovnik, E. (1996). Electrical stimulation of neural tissue to evoke behavioral responses. *Journal of neuroscience methods, 65*(1), 1-17.

Terney, D., Chaieb, L., Moliadze, V., Antal, A., & Paulus, W. (2008). Increasing Human Brain Excitability by Transcranial High-Frequency Random Noise Stimulation. *Journal of Neuroscience, 28*(52), 14147.

Tilley, A. J., & Empson, J. A. C. (1978). REM sleep and memory consolidation. *Biological Psychology, 6*(4), 293-300.

Tononi, G., & Cirelli, C. (2003). Sleep and synaptic homeostasis: a hypothesis. *Brain Res Bull, 62*(2), 143-150.

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Med Rev, 10*(1), 49-62.

Tulving, E. (1985). Memory and Consciousness. *Canadian Psychologist, 26*, 1-12.

Van Ormer, E. B. (1933). Sleep and retention. *Psychol. Bull., 30*(6), 415-439.

Varga, E., Elif, K., Antal, A., Zimmer, M., Harza, I., Paulus, W., & Gyula, K. (2007). Cathodal transcranial direct current stimulation over the parietal cortex modifies facial gender adaptation. *Ideggyogy Sz, 60*(11-12), 474-479.

Wagner, A. D., Schacter, D. L., Rotte, M., Koutstaal, W., Maril, A., Dale, A. M., . . . Buckner, R. L. (1998). Building memories: remembering and forgetting of verbal experiences as predicted by brain activity. *Science, 281*(5380), 1188.

Wagner, T., Fregni, F., Fecteau, S., Grodzinsky, A., Zahn, M., & Pascual-Leone, A. (2007). Transcranial direct current stimulation: a computer-based human model study. *Neuroimage, 35*(3), 1113-1124.

Wagner, T., Valero-Cabre, A., & Pascual-Leone, A. (2007). Noninvasive human brain stimulation. *Annual review of biomedical engineering, 9*, 527-565.

Wagner, U., Gais, S., & Born, J. (2001). Emotional memory formation is enhanced across sleep intervals with high amounts of rapid eye movement sleep. *Learning & Memory, 8*(2), 112-119.

Wagner, U., Hallschmid, M., Rasch, B., & Born, J. (2006). Brief sleep after learning keeps emotional memories alive for years. *Biol Psychiatry, 60*(7), 788-790.

Walker, E., & Tarte, R. (1963). Memory storage as a function of arousal and time with homogeneous and heterogeneous lists. *Journal of Verbal Learning and Verbal Behavior, 2*(1), 113-119.

Walker, M. (2008). Sleep-Dependent Memory Processing. *Harvard Review of Psychiatry, 16*(5), 287-298.

Walker, M. (2009). The Role of Sleep in Cognition and Emotion. *Annals of the New York Academy of Sciences, 1156*(1 The Year in Cognitive Neuroscience 2009), 168-197.

Walker, M., Brakefield, T., Morgan, A., Hobson, J., & Stickgold, R. (2002). Practice with Sleep Makes Perfect Sleep-Dependent Motor Skill Learning. *Neuron, 35*(1), 205-211.

Walker, M., Brakefield, T., Seidman, J., Morgan, A., Hobson, J., & Stickgold, R. (2003). Sleep and the Time Course of Motor Skill Learning. *Learn. Mem., 10*(4), 275-284. doi: 10.1101/lm.58503

Walker, M., & Stickgold, R. (2004). Sleep-Dependent Learning and Memory Consolidation. *Neuron, 44*(1), 121-133.

Wassermann, E. (2008). Direct current brain polarization. *The Oxford Handbook of Transcranial Stimulation*, 57.

Wassermann, E., & Grafman, J. (2005). Recharging cognition with DC brain polarization. *Trends in Cognitive Sciences, 9*(11), 503-505.

Webster, B., Celnik, P., & Cohen, L. (2006). Noninvasive brain stimulation in stroke rehabilitation. *NeuroRx, 3*(4), 474-481.

Weiskrantz, L. (1956). Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *Journal of Comparative and Physiological Psychology, 49*(4), 381.

Wilson, M. A. (2002). Hippocampal Memory Formation, Plasticity, and the Role of Sleep. *Neurobiology of Learning and Memory, 78*(3), 565-569.

Yaroush, R., Sullivan, M. J., & Ekstrand, B. R. (1971). Effect of sleep on memory. II. Differential effect of the first and second half of the night. *J Exp Psychol, 88*(3), 361-366.

Young, W., Alheid, G., & Heimer, L. (1984). The ventral pallidal projection to the mediodorsal thalamus: a study with fluorescent retrograde tracers and immunohistofluorescence. *Journal of Neuroscience, 4*(6), 1626-1638.

Zimmermann, P., & Fimm, B. (1995). Test for attentional performance (TAP). *Herzogenrath, Germany: PsyTest*.