The Effects of Galvanic Vestibular Stimulation on Motor Cortical Excitability

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Abstract

Galvanic vestibular stimulation (GVS) can reduce the symptoms of numerous neurological conditions including episodic migraine, parkinsonism, acquired prosopagnosia and hemi-spatial neglect. Despite these preliminary findings, the mechanism underlying these therapeutic effects are still poorly understood. Functional imaging conducted during GVS indicates a potential change in cortical activation across widespread regions of the brain. However, these imaging studies are limited because they lack a functional correlate and rely on a relatively crude and poorly localised measure of excitability. The current study aimed to investigate the effects of GVS on cortical excitability via the more precise markers of TMS-induced motor-evoked potentials (MEPs) and movement-related cortical potentials (MRCPs), surrogate markers of long-term potentiation (LTP) and long-term depression (LTD) which are often compromised in neurological patients. Experiment 1 (N = 40) examined the effects of 25 minutes 1mA noisy, bipolar GVS on MEPs in the minutes and subsequent day after stimulation. Relative to sham, GVS reduced MEP amplitude 24 hours following stimulation for all participants who showed high cortical excitability at baseline. Experiment 2 (N = 24) followed a similar pre-post design to Experiment 1, however, the effects of GVS on the MRCP were measured via the Bereitschaftspotential (BP) while participants performed voluntary finger movements. Most likely owing to methodological shortcomings, Experiment 2 failed to obtain a BP response at baseline so did not enable the study hypothesis to be assessed. Experiment 3 was designed to both address these potential shortcomings and increase clinical relevance, recruiting a single individual with right hemisphere chronic stroke. A BP at baseline was observed but there was no evidence of GVS modulation. In sum, whilst Experiments 2 and 3 yielded few novel insights, the results of Experiment 1 indicate that GVS inhibits cortical excitability, potentially reflecting LTD-like effects. This observation may help explain its reported therapeutic benefits and also suggests that is should be applied to other disorders that involve cortical hyper-excitability.

The Effects of Galvanic Vestibular Stimulation on Motor Cortical Excitability

The vestibular system within the inner ear consists of the fluid-filled semi-circular canals, which detect rotational head movements, and the otolith organs (the saccule and utricle), which detect linear acceleration and gravitational forces (Fitzpatrick & Day, 2005). It is traditionally known for its role in maintaining balance, postural control and stabilising eye movements during head rotation (Grabherr, Macauda & Lenggenhager, 2015; Clark, 1970). Unlike the five primary senses whose perceptual influences are readily accessible to us, the vestibular sense functions largely outside of our awareness. Despite this, its fundamental contribution is demonstrated when there is a loss of vestibular function. There is a growing body of evidence indicating that the role of the vestibular system extends beyond autonomic postural, gait and ocular control to encompass cognitive functions (Smith & Zheng, 2013; Bigelow & Agrawal, 2015). The artificial stimulation of the vestibular organs follows from neuropsychological and anatomical evidence that links vestibular function to cognition and affect (Smith, 2017; Balaban & Beryozkin, 1994). Several studies have demonstrated that vestibular stimulation can significantly reduce symptoms across a range of acquired and degenerative neurological conditions, such as hemi-spatial neglect, prosopagnosia, parkinsonism and episodic migraine (Zubko, Wilkinson, Langston & Sakel, 2013; Wilkinson, Kilduff, McGlinchey & Milberg, 2005; Yamamoto et al., 2005; Wilkinson et al., 2017). However, the mechanisms through which vestibular stimulation produces these benefits remain largely unclear. Identifying such mechanisms of effect would be valuable not only for understanding how the vestibular system influences cognition, but for the development of vestibular stimulation protocols that provide maximal benefits for patients with cognitive disorders.

Several neuropsychological studies demonstrate that the vestibular system is strongly associated with cognitive functions (Smith, 2016; Smith & Zheng, 2013). For example,

epidemiological surveys show that patients with vestibular dysfunctions perform significantly worse on the Weschley Adult Intelligence Scale and they also experience more difficulties in everyday activities, such as managing finances (Semenov et al., 2016; Harun, Semenov & Agrawal, 2015). Vestibular loss has also been shown to negatively affect memory and attention (Bigelow & Agrawal, 2015). For instance, patients with bilateral vestibular loss (BVL) show significant spatial memory deficits as indicated by poorer performance on the virtual Morris water maze task compared to healthy controls (Brandt, Dieterich & Strupp, 2005). Another study found that patients with unilateral vestibular loss (UVL) consistently experienced more difficulties compared to controls when completing tasks designed to divide participants' attention between cognitive and postural tasks (Redfern, Talkowski, Jennings & Furman, 2004). Furthermore, studies on neurologically healthy individuals indicate that artificially stimulating the vestibular nerves can alter performance in visual memory, egocentric mental rotations and short-term spatial memory (Wikinson et al., 2008; Dilda, MacDougall, Curthoys & Moore, 2012; Lenggenhager, Lopez & Blanke, 2008). These findings raise the possibility that via appropriate stimulation, it may be possible to remediate aspects of cognition in neurological patients that seem strongly affected by changes in vestibular input.

There are several ways through which to stimulate the vestibular organs, for example, via whole-body rotation, caloric vestibular stimulation (CVS) and optokinetic stimulation (Grabherr et al., 2015; Kerkhoff, Keller, Ritter & Marquardt, 2006). However, perhaps the most amenable and promising technique to emerge is galvanic vestibular stimulation (GVS). This involves the delivery of gentle electrical currents via electrodes placed on the skin overlaying the mastoid processes. GVS functions by modulating the firing rate of vestibular neurons within the eighth cranial nerve projecting from both the semi-circular canals and otolith organs in the same manner as natural head movement (Fitzpatrick & Day, 2004;

Goldberg, Smith & Fernandez, 1984). Recently, GVS has been shown to produce at least transient alleviation of symptoms for patients with hemi-spatial neglect, parkinsonism, multi-system atrophy (MSA) and prosopagnosia (Zubko et al., 2013; Wilkinson et al., 2005; Yamamoto et al., 2005). For example, 24 hours of noisy GVS has been reported to improve heart-rate variability and rest-to-active transitions in patients with MSA and parkinsonism, respectively (Yamamoto et al., 2005). This suggests that GVS may be capable of improving motor functions in neurodegenerative motor disorders. Additionally, a single case study on a patient with a face perception deficit following right hemisphere stroke reported that GVS improved his performance to above-chance levels on a face-matching task compared to pre-stimulation performance (Wilkinson et al., 2005).

Galvanic vestibular stimulation (GVS) has shown strongest success in remediating the symptoms of hemi-spatial neglect following stroke. Hemi-spatial neglect is characterised by an impaired ability to attend and spontaneously orient oneself to contralesional space (Robertson & Halligan, 1999; Vallar & Perani, 1986). This results in various difficulties during daily living such as bumping into objects and failing to notice people in contralesional space. Several studies have demonstrated that GVS can transiently relieve neglect in patients (Rorsman, Magnusson & Johansson, 1999; Saj, Honore & Rousseaux, 2006; Utz, Keller, Kardinal & Kerkhoff, 2011) and recent investigations have demonstrated carryover effects. A case study of two neglect patients who received repeated sessions of GVS over five consecutive days reported improvements in the letter and star cancellation tasks of the Behavioural Inattention Test (BIT) that were still evident three days following the end of the treatment (Zubko et al., 2013). More recently, Wilkinson et al. (2014) conducted the only randomised control trial (RCT) to date investigating the long-term clinical effects of GVS. Fifty-two stroke patients suffering from left-sided neglect were randomly assigned to either one, five or ten sessions of noisy, bipolar, 1mA GVS lasting 25 minutes. The results revealed

a significant reduction in neglect following all three treatment arms, as defined by improvements in the BIT and in the Barthel index (BI) for activities of daily living. These improvements were shown to last up to a month following the last stimulation session. This RCT confirms the efficacy of GVS in reducing neglect as shown by clinically relevant changes in behavioural measures.

Despite the success of these studies, there has been no attempt to explain these clinical improvements in the form of a functionally relevant physiological correlate. That is, it is clear that GVS can improve cognitive symptoms but it is unclear why this occurs. Findings from functional magnetic resonance imaging (fMRI) studies have hinted at a potential mechanism by revealing cortical activations and deactivations of the haemodynamic response following GVS. Activations have been observed in the insula, temporo-parietal junction, central sulcus, thalamus, hippocampus and the premotor regions of the frontal lobe (Lobel et al., 1998; Bucher et al., 1998; Stephan et al., 2005; Bense et al., 2001; Bottini et al., 1994). Deactivations have been observed mainly in the visual and somatosensory cortices (Bense et al., 2001). These studies suggest that GVS alters haemodynamic activity in key cortical areas as shown by changes in the blood oxygenation level-dependent (BOLD) response. However, the relevance of these findings is unclear because they have been obtained from neurologically healthy participants receiving GVS while at rest rather than engaging in a task. In addition, the BOLD signal provides an indirect measure of neural activity, relying on changes in blood flow to discrete areas and it is still unclear how these haemodynamic changes relate to the functional role of these brain areas (Crosson et al., 2010). Thus, in the context of the clinical changes following GVS, the functional role of these activations is not well understood.

Nevertheless, these functional imaging data do suggest that GVS may be affecting cortical excitability, which is considered a surrogate marker of synaptic plasticity in the

human brain in vivo (Badawy, Loetscher, Macdonell & Brodtmann, 2012). Synaptic plasticity refers to the brain's fundamental ability to strengthen and weaken synaptic transmission (Jedlicka, 2002) and is known to underpin several forms of memory and learning as well as functional restoration following injury (Bear, 1996; Bliss & Collingridge, 1993). There are two main types of synaptic plasticity: long-term potentiation (LTP) and long-term depression (LTD; Bear, 1995). LTP is defined as the lasting enhancement in the efficiency of synaptic transmission that outlasts the stimulation period (Bear, 1995). LTD is the opposite of this with a reduction in synaptic efficiency that outlasts the stimulation period (Berretta & Cherubini, 1998). LTP and LTD were first observed in animal studies of the hippocampus. LTP was induced and lasted up to ten hours when researchers applied a high-frequency stimulation protocol at synapses between the medial perforant path and the dentate gyrus of an anaesthetised rabbit (Bliss & Lømo, 1973). Subsequent studies utilizing chronically implanted stimulating and recording electrodes demonstrated that LTP can be maintained for up to one year in rats (Abraham, Logan, Greenwood & Dragunow, 2002). LTD was later found following low frequency stimulation in the hippocampus and other cortical pathways (Wöhrl, von Haebler & Heinemann, 2007). Various molecular mechanisms have been shown to underlie LTP and LTD in different regions. Hippocampal LTP and LTD appear to share a common mechanism mediated by the activity *N*-methyl-D-aspartate (NMDA) receptors (Nowak et al., 1984). Other molecular mechanisms may underlie LTP and LTD in other parts of the central nervous system (Bliss & Cooke, 2011). For example, in the cerebellar cortex, LTD may be mediated by activity of gamma-aminobutyric acid (GABA) receptors at Purkinje cell synapses (Ito & Kano, 1982).

There is substantial indirect evidence to suggest that stimulation-induced changes in cortical excitability may reflect LTP- and LTD-like plasticity in the human brain. Increases and decreases in cortical excitability following non-invasive brain stimulation (NIBS)

resemble LTP- and LTD-like synaptic plasticity in both duration and direction (Vallance & Ridding, 2013). For example, studies investigating the effects of transcranial direct current stimulation (tDCS) and repetitive transcranial magnetic stimulation (rTMS) on cortical excitability show changes that outlast the stimulation period (Berardelli et al., 1998; Fritsch et al., 2010). Moreover, high and low frequency stimulation increase and decrease cortical excitability in a way that resembles LTP- and LTD-like effects induced by similar stimulation protocols in animal studies (Hoogendam, Ramakers & Di Lazzaro., 2010). NIBS techniques have also been shown to affect motor learning, with facilitating stimulation protocols improving performance on learned motor tasks (Jung & Ziemann, 2009). Given that synaptic plasticity, and LTP in particular, are considered prime candidate mechanisms for learning and memory, these findings further indicate that synaptic plasticity may underlie the effects of NIBS techniques in the human brain. Finally, several studies have demonstrated that stimulation-induced changes in cortical excitability, as with LTP/LTD induction, are dependent on NMDA receptor activity. Pharmacological interventions utilizing NMDA receptor antagonists have been shown to abolish the effects of NIBS techniques on cortical excitability (Huang, Chen, Rothwell & Wen, 2007; Nitsche et al., 2003b). Thus, several lines of evidence support the notion that LTP- and LTD-like plasticity mediates stimulation-induced changes in cortical excitability.

However, it is important to emphasise that the features of cortical excitability following stimulation only correlate with those relating to synaptic plasticity observed in animal studies. These findings do not provide causal proof that LTP/LTD underlies the cortical effects of NIBS techniques in humans (Hoogendam et al., 2010). Indeed, there are some clear distinctions between the cortical effects observed in humans and LTP/LTD observed in animals and slice preparations. Cortical excitability changes in humans only seem to last hours following stimulation compared to the synaptic changes observed days and

months following stimulation (Vallance & Ridding, 2013). Additionally, cortical effects may not necessarily reflect plasticity changes at the cellular level and may instead reflect excitability changes between broader neuronal networks (Bestmann et al., 2003). Thus, cortical excitability changes provide an indirect measure of synaptic plasticity in the human brain in vivo and may instead be more accurately interpreted as LTP-like or LTD-like plasticity.

Synaptic plasticity may provide a useful, clinical framework to explain the functional changes that occur following GVS. There is direct evidence from animal models that reveal naturally-occurring synaptic plasticity within neural tissue following lesions, such as stroke and spinal-cord injuries (Murphy & Corbett, 2009; Darian-Smith, 2009). These mechanisms are analogous to those occurring in the intact brain during experience-dependent synaptic plasticity (Kleim & Jones, 2008). The difference is that in functional restoration, it is the neighbouring, spared circuitry that engage in synaptic strengthening or weakening to compensate for the loss of pre-injury connections (Murphy & Corbett, 2009). For example, animal models of stroke have demonstrated that the coincidental activation of neurons located in the spared tissue surrounding the damaged site can facilitate the induction of action potentials, thereby increasing the likelihood of synaptic plasticity occurring (Brown et al., 2009). Despite the lack of direct evidence linking synaptic plasticity and functional recovery in humans, there is sufficient indirect evidence to suggest that similar synaptic mechanisms may underlie human recovery. This includes studies demonstrating that NIBS protocols can ameliorate symptoms of neurological disorders, which suggests that LTP and LTD may indeed underlie recovery. An important implication of this may be that neurological conditions possibly arise from cortical hyper-excitability or hypo-excitability, which may reflect aberrant synaptic plasticity in the form of uncontrolled LTP/LTD induction (Ziemann & Siebner, 2008; Bliss & Cooke, 2011; Hoffman & Cavus, 2002).

There is evidence indicating that certain neurological disorders reflect pathological LTP/LTD induction. For example, several studies have reported hyper-excitability in the cerebral cortex of patients with epilepsy, which may reflect excessive LTP induction (McCormick & Contrerass, 2001; Bliss & Cooke, 2011). It follows that patients with epilepsy may benefit from low-frequency stimulation protocols that intend to decrease cortical excitability, possibly reflecting LTD-like effects (Bliss & Cook, 2011). Indeed, a recent study showed that 20 minutes of cathodal tDCS significantly reduced the weekly number of seizures experienced by patient with drug-resistant epilepsy (Assenza et al., 2017). Moreover, studies that have modulated cortical excitability using NIBS techniques in patients with neurological disorders also suggest that these may reflect pathological synaptic plasticity (Bunse et al., 2014; Radhu, Ravindran, Levinson, Daskalakis, 2012). For example, high-frequency rTMS applied over the primary motor cortex in patients with Parkinson's disease (PD) has been shown to increase cortical excitability (Lefaucher et al., 2004). This may suggest that PD arises from excessively low cortical excitability in the motor cortex, which is in line with animal and neuroimaging studies that show reduced activity in the motor cortex in PD (Lindenbach & Bishop, 2013). It is possible that this reflects uncontrolled LTD in the motor cortex and that inducing LTP-like effects via high-frequency NIBS protocols may restore cortical excitability to normal levels.

Such observations raise the possibility that GVS may also affect LTP/LTD induction, as measured by cortical excitability. It may be speculated that GVS can modulate motor cortical activity by strengthening or weakening pathways that run from the vestibular nuclei, via thalamus and parieto-insular vestibular cortex (PIVC), to areas in the primary motor and premotor cortices that are associated with motor and ocular-motor control (Fukushima & Kaneko, 1995; Fukushima, Takahashi, Ohno & Kato, 1984). Motor function aside, the other domain that may be most affected by GVS (but not probed in this thesis) is short-term

memory; studies on animal slice preparations demonstrate that vestibular input is important for normal hippocampal theta rhythm (Smith, 1997) while temporarily abolishing vestibular signals can disrupt the firing properties of head direction cells in the rat hippocampus (Stackman, Clark & Taube, 2002). In the present context, the inhibitory/excitatory effects of GVS on cortical excitability must first be established before justifying investigation of specific anatomical mechanisms through which they may occur. To this end, the aim of the current experiments is to investigate, via surrogate markers, if GVS either increases or decreases cortical excitability.

Measuring synaptic plasticity in humans

Typically, cortical excitability studies target the primary motor cortex and surrounding areas as these can be readily assessed using single-pulse TMS-elicited motor-evoked potential (MEPs) and movement-related cortical potentials (MRCPs) elicited by voluntary movements. (Bestmann & Krakauer, 2015; Colebatch, 2007). MEPs are the most common measure used to assess cortical excitability as they provide an overall quantification of the excitability of the cortico-spinal tract. Changes in MEP amplitude can be attributed not only to activity in the primary motor cortex, but also to processes occurring in other cortical areas. Increases in MEP amplitude following a stimulation protocol can be interpreted as LTP-like effects, whereas decreases in MEP amplitude are interpreted as LTD-like effects (Ziemann et al., 2008). Several non-invasive brain stimulation (NIBS) methods have been shown to modulate MEP amplitude, which potentially reflect induction of LTP and LTD in the motor cortex (Müller-Dahlhaus & Vlachos, 2013).

The induction of LTP- or LTD-like plasticity, as measured by increases and decreases in MEP amplitude, depends on the properties of the stimulation protocol. In the case of rTMS, high-frequency stimulation (>5Hz) is shown to increase MEP amplitude and low-frequency stimulation (=<1Hz) decreases MEP amplitude, likely reflecting LTP- and LTD-

like effects, respectively (Hoadayer et al., 2008). Chen et al. (1997) showed significant reductions in cortical excitability, as defined by diminished MEP amplitude, following fifteen minutes of rTMS at a frequency of 0.9Hz that lasted for roughly 15 minutes. Alternatively, Jung, Shin, Jeong and Shin (2008) increased MEP amplitude for up to 120 minutes after administering 10Hz rTMS. Similarly, the effects of tDCS on cortical excitability has been shown to depend on whether cathodal or anodal stimulation is applied. Nitsche et al. (2003a) showed that nine minutes of cathodal tDCS over the primary motor cortex led to significant reductions in MEP amplitude for up to an hour after stimulation. Finally, an increase in MEP amplitude for several minutes after anodal tDCS was shown by Nitsche and Paulus (2000, 2001). These findings support the use of MEPs as an indirect measure of synaptic plasticity in the human brain in vivo.

Compared to the MEP literature, there are markedly fewer investigations into the effects of NIBS protocols on MRCPs. Of particular interest in MRCP studies is a component called the Bereitschaftspotential (BP), which refers to a slow rising negative slope present in the back-averaged electroencephalogram (EEG) preceding voluntary movement (Kornhuber, Scheid & Deecke, 1969; Shibasaki & Hallet, 2006). It is often associated with the planning and readiness for movement (Birbaumer, Elbert, Canavan & Rockstroh, 1990). The BP component can be divided into an ‘early BP’ and ‘late BP’, which show different onsets and properties. The ‘early BP’ starts approximately two seconds prior to movement onset indicated by an electromyogram (EMG) signal. It is maximal over centro-parietal areas as well as being widely distributed across the scalp. The ‘late BP’ follows the ‘early BP’ initiating approximately 400-500 milliseconds prior to movement. It is characterised by a sharp increase in the gradient of the slope in central areas contralateral to movement (Colebatch, 2007). The findings from studies investigating the effects of NIBS techniques on the BP corroborate those observed in the MEP literature.

The difference between these two measures of cortical excitability is that MEPs measure the entire excitability in the cortico-spinal tract, whereas the BP reflects the overall excitability in cortical areas such as the supplementary motor area (SMA) and premotor cortices (Kristeva-Feige et al., 1997). Despite this, NIBS techniques seem to modulate both the BP and MEPs in similar ways. For example, Rossi et al. (2000) showed that fifteen minutes of rTMS at a low frequency of 1Hz significantly reduced the amplitude of the 'late BP' component compared to sham stimulation, which potentially reflects reduced cortical excitability in SMA and premotor areas. Similarly, Ortu, Ruge, Deriu and Rothwell (2009) showed that a variant of rTMS termed continuous theta burst stimulation (cTBS; Huang et al., 2005) can decrease the amplitude of the 'late BP' for up to 30 minutes following stimulation. These findings are in line with studies showing that low-frequency rTMS and cTBS cause a reduction in MEPs (Di Lazzaro et al., 2010), thus they may reflect LTD-like effects. There are fewer studies demonstrating increased BP amplitude following stimulation, and thus LTP-like effects. However, one recent study found that ten minutes of GVS applied at 70% of the participants' sensory threshold significantly increased BP amplitude compared to sham stimulation (Lee, 2015). However, the scarcity of methodological detail reported in this study precludes an appropriate evaluation of its methodological rigour.

Despite the limited number of studies investigating the effects of brain stimulation protocols on the BP, there is evidence to suggest that it is clinically important. For example, it is well-documented that the BP is abnormal in patients with PD (Dick et al., 1989). A recent study recording the BPs of patients with PD showed that they were reduced in amplitude compared to controls, and this reduction increased with severity of PD, despite the use of dopaminergic medications (Patil, Sood, Goyal & Kochnar, 2017). This may indicate defective activation of the SMA and premotor cortices as a result of excessive basal ganglia inhibition which ultimately affects planning and initiation of movement. Likewise, prefrontal

traumatic brain injury (TBI) in the acute phase has been associated with a significantly reduced amplitude and delayed onset of BP compared to age-matched controls (Wiese et al., 2004). Moreover, one recent study showed that applying low-frequency rTMS to the contralesional motor cortex in stroke patients increased BP amplitude over the ipsilesional cortex (Matsuura, Onoda, Oguro & Yamaguchi, 2015). This increase in BP amplitude over the ipsilesional hemisphere was associated with improved motor functions as assessed by the Fulg-Meyer assessment (FMA) and the Purdue Pegboard Test (PPT). This finding suggests that the BP may be an important physiological marker of functional change that may be correlated with behavioural responses following NIBS.

Another advantage of employing both the BP and MEPs to measure cortical excitability is that the BP overcomes some of the intrinsic noise in MEPs by removing the single-pulse TMS protocol. With TMS-elicited MEPs, there is the possibility that the TMS paradigm itself may mask any effects on cortical excitability induced by GVS. For example, recent research has cast doubt on the assumption that MEPs are invariant over time (Julkunen et al., 2012). Pellicciari et al. (2016) measured the amplitude of successive MEPs elicited by TMS at a random or fixed rate for 10 blocks of 20 pulses. They found that MEP amplitudes increased over time regardless of whether their inter-trial interval was fixed or random. The authors explained this increase as a cumulative effect of several single pulses of TMS on neuronal depolarisation over time, that is greater than the effect of a single pulse. Thus, caution is required when interpreting changes in MEP amplitude following stimulation protocols as these may be attributed to the single-pulse TMS. Similarly, single-pulse TMS protocols with fixed inter-trial intervals often closely resemble low-frequency rTMS protocols (Hallett, Wassermann, Pascual-Leone & Valls-Sole, 1999). It is well-documented that low-frequency rTMS protocols produce significant reductions in MEP amplitudes that possibly reflect LTD induction (Hoogendam et al., 2010). Typically, single-pulse TMS is

delivered at a frequency of 0.25Hz to measure changes in cortical excitability (Rossi, Hallet, Rossini & Pascual-Leone, 2009). One study showed that a rTMS protocol delivered at a frequency of 0.2Hz was capable of decreasing MEP amplitudes for 20 minutes following stimulation (Ikeguchi et al., 2005). This suggests that single-pulse TMS may have significant effects on corticospinal excitability if studies are not properly sham-controlled. Hence, by also measuring the BP, any confounding effects of the TMS protocol may be eliminated.

Finally, most studies investigating synaptic plasticity in the human cortex induced by NIBS techniques utilised a normative sample. Although this may provide preliminary clinical insights, utilising a clinical sample may be more relevant. In the case of GVS, it may be that the use of a healthy sample is limiting because its effects on a healthy brain are smaller than on the injured brain. In support of this, a meta-analysis conducted by Bastani and Jaberzadeh (2012) demonstrated that increases in motor cortex excitability induced by anodal tDCS were larger for stroke patients, compared to healthy participants. This may be because compared to healthy brains, the injured brain is less resilient to sensory perturbation. This is consistent with research showing that lasting changes in synaptic transmission may underlie functional recovery from brain injury through rehabilitation (Mang, Campbell, Ross & Boyd, 2013; Warraich & Kleim, 2010). Demonstrating changes in cortical excitability in even just one patient following GVS would mean that findings from healthy participants are indeed clinically relevant.

Summary

Various epidemiological, clinical and functional imaging studies link vestibular function to higher-order cortical functioning which in turn has led to research demonstrating that GVS can reduce neurological deficits. The main aim of this project is to determine a potential mechanism of effect behind the positive, therapeutic outcomes of GVS; utilising cortical excitability which is a potential physiological marker of functional change. Two

types of synaptic plasticity are said to underpin changes in cortical excitability: LTP and LTD. Many neurological disorders potentially reflect pathological induction of LTP and LTD. Thus, if the present study shows that GVS modulates cortical excitability, either in the direction of LTP or LTD, then synaptic plasticity may be considered a mechanism of action for the therapeutic benefits following GVS. If this can be shown, it will better define the disease targets for GVS and facilitate the development of effective stimulation protocols.

Experiment 1 investigated the effects of 25 minutes binaural, bipolar 1mA GVS on cortical excitability as measured by MEP amplitude in a neurologically healthy sample. This GVS protocol was chosen because it has been shown to produce persistent clinical improvements in hemi-spatial neglect (Wilkinson et al., 2014), thus is perhaps likely to produce robust changes in cortical excitability. Stimulation was applied following a baseline recording of 25 MEPs measuring using EMG of the left abductor pollicis brevis (APB). The APB was chosen because of its large representational area in the primary motor cortex facilitating the localisation of the 'hot spot' on the scalp (Menon, Kiernan & Vucic, 2014). Moreover, this muscle has been routinely used to measure cortical excitability in previous research (Delvendahl et al., 2012). Changes in cortical excitability were measured by MEPs recorded in blocks of 25 pulses following stimulation. Blocks of 25 MEPs were recorded 0, 15, 30 and 60 minutes following stimulation to observe the short-term effects of GVS on cortical excitability. Of particular clinical importance was follow-up recording of 25 MEPs 24 hours following stimulation to determine any persistent changes in cortical excitability. Such a finding would help explain the clinical improvements that have been observed for weeks following GVS (Zubko et al., 2013; Wilkinson et al., 2014). Another factor that was considered is that cortical excitability may vary between and within individuals such that some participants may present with high cortical excitability (as indicated by high MEP amplitudes at baseline) and others may present with low cortical excitability (as indicated by

low MEP amplitudes at baseline). Indeed, there is evidence to suggest that stable intra- and inter-individual differences in cortical excitability exist (Wasserman, 2002; Krause & Kadosh, 2014). Given that this may influence GVS modulation of MEPs, participants were separated into two groups according to high and low excitability at baseline during exploratory analyses.

The design and GVS parameters of Experiment 2 mirrored those of Experiment 1. However, it focused on the effects of GVS on cortical excitability as measured by the BP component of MRCPs. The stimulation period followed a baseline EEG recording of participants performing a self-paced motor task, in which they tapped their left middle finger at irregular intervals of approximately five seconds. They were not provided with any external, time-related cues and were instructed to move of their own volition. This task was selected because the BP is more strongly associated with internally generated movements and is absent during externally voluntary movements, such as in cued reaction time tasks (Papa, Artieda & Obeso, 1991; Birbaumer et al., 1990). EEG was recorded whilst the participant performed the task at 0 and 30 minutes following stimulation. A follow-up recording was also conducted 24 hours following stimulation.

Experiment 3 was conducted as a single case study of a patient who suffered a right middle cerebral artery infarct 9 years ago. The patient was selected because he has previously shown a favourable outcome following GVS administration (Wilkinson et al., 2014) and to examine if any observed effect was evident in a lesioned, more fragile brain. The experimental protocol for Experiment 3 was largely the same as Experiment 2 with a few technical modifications to overcome the methodological flaws identified in Experiment 2. In all cases, the aim was to determine whether GVS affects cortical excitability, and if so, in what way.

Experiment 1: The Effects of a Single Session of Galvanic Vestibular Stimulation on Motor-evoked Potentials

Methods

Participants

Fifty-eight students (41 females, 17 males) aged 18-52 (M= 21.69, SD = 5.62) from the University of Kent were recruited via the Research Participation Scheme (RPS) and Jobshop in exchange for either course credits or money. Participants were screened to ensure they were free from any neurological/psychiatric conditions (see Appendix A), skin abrasions behind the ears or metal plates in the upper body. All participants provided written informed consent prior to participating. Ethical approval was obtained by the Psychology Research Ethics Committee at the University of Kent.

Design

A mixed, pre-post design was used whereby Time was a within-participants variable and Stimulation was a between-participant variable. Time comprised of six levels measuring a baseline of 25 MEPs preceding stimulation and five blocks of 25 MEPs at intervals following stimulation. The Stimulation variable consisted of whether the participant had received active or sham GVS.

Materials

Galvanic vestibular stimulation (GVS). A Gaussian noise, bipolar current was applied to the mastoid processes for 25 minutes using a pair of rubber, self-adhesive disposable electrodes (5.1cm x 10.2cm; ComfortEase, Empi Inc.) connected to a *Neuroconn DC Stimulator* (see Appendix B). The stimulus intensity varied randomly from 0.5-1.5mA with a mean intensity of 1mA at a frequency of 1000Hz. The anode was placed behind the left ear and the cathode behind the right ear. These GVS parameters were selected as they

have been shown to be efficacious in reducing hemi-spatial neglect (Wilkinson et al., 2014). The skin over the mastoids was prepared using sterilising wipes and Nuprep exfoliant gel prior to electrode placement to ensure reduced impedance. Preparation for sham stimulation was conducted as described above, however, during stimulation the device remained turned off and was placed out of sight of the participant.

Measuring cortical excitability. TMS-elicited MEPs were recorded using surface EMG from the left APB. TMS pulses were generated by a *PowerMAG* stimulator (Brain Products) with a figure-of-eight magnetic coil (diameter of one winding = 90mm, peak magnetic field = 2T) that was placed tangentially and at a 45° angle from the skull midline with the handle pointing backwards (see Appendix C). A few minutes were spent locating the representational 'hot spot' for the APB on the scalp, from which the largest MEPs were observed upon firing a TMS pulse. A sticker was placed on the cap worn by the participant to mark the area on which the coil was to be positioned for all three sessions of the experiment.

Self-adhesive, disposable (28mm x 20mm) Ag/AgCl duck foot electrodes (Ambu® Neuroline 710) in a belly-tendon montage were used to monitor EMG responses. The recording electrode was placed over the APB, whilst the reference and ground were placed over the skin of the proximal phalanx of the thumb and the digiti minini brevis muscle, respectively (see Appendix D). Following preparation of the skin, the electrodes were connected to a BrainAmp amplifier and BrainVision recording software (Brain Products, GmbH, Gilching, Germany) was used to record MEPs. A 50Hz notch display filter was used.

Three sets of PsychoPy version 2 (Peirce, 2007) scripts were developed to enable TMS pulses to be triggered at fixed intervals during different phases of the experiment. This was initiated by pressing a button on the keyboard of a stimulus computer that was connected to both the TMS unit and the EMG amplifier. This enabled EMG waveforms signalling MEPs to be correlated with TMS pulses within a limited time interval of 80 milliseconds (-

20ms before and 60ms after the TMS trigger). Script 1 enabled experimenters to manually control pulses by pressing the spacebar on the stimulus computer during motor threshold determination. Script 2 was used for the baseline and 24h follow-up phases of the experiment, whereby 25 pulses were automatically delivered at a frequency of 0.25Hz (approximately every four seconds). Script 3 was designed for the post-stimulation phase of the experiment (on the same day as stimulation) whereby four blocks of 25 pulses were delivered 0, 15, 30 and 60 minutes following GVS.

Procedure

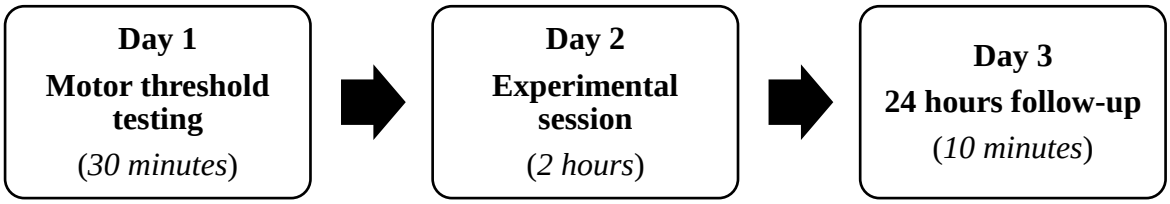

Figure 1. Schematic of experiment over three days.

The experiment was conducted over three days (see Figure 1). For all sessions participants were seated in a standard Fowler's position (tilted back 45°) with their legs straight in front of them in a comfortable chair (Cosmo Radi+ Beauty Couch). A *MAG&More* vacuum cushion was compressed using a vacuum pump behind the participant's neck to ensure that head movement was minimal during TMS administration (see Appendix E for participant set-up). They were asked to rest their left arm over a cushion beside them and to relax their forearm and hand for the duration of the experiment.

Motor threshold determination. The first session was conducted on the first day and usually lasted approximately 30 minutes. Once the participant was set up, a few minutes were spent locating the area on the scalp that elicited an observable muscle response on the APB. Individual motor threshold was determined by selecting the minimum TMS intensity (%) to elicit MEPs with peak-to-peak amplitudes greater than 50μV in three out of five trials.

Although this method is conventional within the cortical excitability literature (Bastani & Jaberzadeh, 2013; Di Lazzaro et al., 2008), 14 participants were excluded during this session because their MEPs failed to reach an amplitude of 50μV in three out of five trials. As a result, this experiment proved labour-intensive and time-consuming as limited resources, such as laboratory and equipment access, were spent on participants who were not able to continue with the second and third phases of the experiment.

Experimental session. The second session comprised the experimental manipulation and usually lasted approximately two hours. A few minutes were spent relocating the representational area of the APB. Next, a baseline of 25 MEPs were recorded, which lasted roughly five minutes. Immediately following baseline recording, 25 minutes of either sham or active GVS was administered during which the participant relaxed.

During sham stimulation, participants were falsely informed that they were receiving stimulation. All participants were informed that any sensation they felt would not be a reliable guide to their assigned stimulation condition, as the GVS protocol is subsensory. Despite this, all participants in the active condition reported in a stimulation perception questionnaire either a prickling or tingling sensation at the stimulation site that receded after the first few minutes of stimulation (see Appendix F). The participants in the sham group did not report any sensation. No adverse sensations such as nausea were reported by any of the participants, which is in line with previous research (Utz et al., 2011). Next, four blocks of 25 MEPs were recorded 0, 15, 30 and 60 minutes following GVS (see Figure 2).

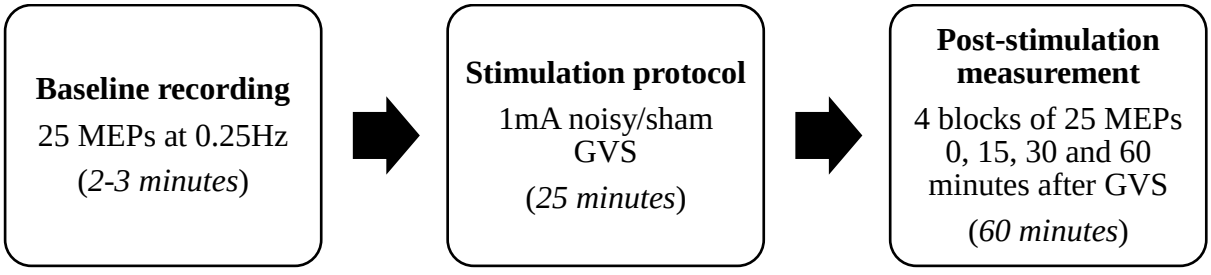

Figure 2. Schematic of experimental session during second day.

Follow-up session. The final session took place approximately 24 hours post-stimulation on the final day and usually lasted roughly ten minutes. This session consisted of relocating the APB area and a recording of 25 MEPs (see Figure 1). The participants were then debriefed and compensated for their time.

Data analysis and statistics

A total of 18 participants were excluded from data analyses. Four participants were excluded upon completion of the TMS safety screening questionnaire: three were excluded due to severe, episodic migraines and one other participant was excluded because of the use of anti-anxiety medication. A further 14 participants were excluded in the motor threshold determination session due to insufficient size of MEP amplitudes. In total, the data from 40 participants (29 females, 11 males) aged 18-52 (M= 21.55, SD = 5.93) were included in the analyses. Both the sham and active groups consisted of 20 participants. The mean stimulation intensity for the TMS across both groups was 54.4% (see Table 1 for a breakdown of mean intensity across groups).

Table 1

Mean TMS Intensity for Stimulation Conditions

Stimulation conditions	Mean TMS intensity (in %)
Active	52.7
Sham	56.1

Electromyogram data was filtered, segmented, baseline corrected and averaged using BrainVision Analyser 2 software (Brain Products, GmbH, Gilching, Germany). Artefacts were removed upon visual inspection of each MEP waveform by experimenters during offline analysis. Averaged MEPs from all blocks of 25 pulses from each phase of the experiment were calculated by obtaining the difference value of minimum and maximum waveform peaks.

Standardised post-stimulation MEPs were computed by normalising MEP amplitudes to baseline intra-individually, resulting in values that represented a change from baseline. Negative values indicated a reduction in MEP amplitude and positive values indicated an increase in MEP amplitude. An 2x5 analysis of variance (ANOVA) with mixed design was utilised to establish any effects of the between-subjects variable Stimulation (active or sham stimulation) and within-subjects variable Time (0 minutes, 15 minutes, 30 minutes, 60 minutes, 24 hours) on MEP amplitude. Subsequent exploratory analyses consisted of further subdividing the sample via a median split into groups of 10 participants, one group showing high MEP amplitudes at baseline and the other showing low MEP amplitudes at baseline. A 2x2x5 mixed ANOVA was performed with two between-subjects variables (Stimulation and High vs Low MEP at baseline) and Time as a within-subjects variable. Interaction effects were explored first utilising two- and then one-way ANOVAs followed by Bonferroni corrected pairwise comparisons. A p value of <.05 was considered statistically significant for all omnibus analyses. The Bonferroni correction was applied to all post-hoc analyses with a p value of <.01 considered statistically significant. Mauchly's test of sphericity was performed for ANOVAs and Huyhn-Feldt correction values were reported for all analyses.

Results

Mean MEP amplitudes for change from baseline values in both active and sham conditions are graphically presented in Figure 3. The results of the 2x5 ANOVA with Stimulation as a between-subjects variable and Time (0 minutes, 15 minutes, 30 minutes, 60 minutes, 24 hours) as a within-subjects variable yielded a main effect of Time, $F(5, 64336.71) = 3.01$, $p < .05$, however, no significant interaction was observed between Stimulation and Time, $F(5, 64336.71) = 1.04$, $p = .37$.

In the next analysis, a one-way repeated measures ANOVA to investigate effects across time in only the active stimulation group. Baseline absolute values were added to this

ANOVA to compare pre-stimulation MEPs with those in post-stimulation, thus it had 6 levels (Pre-stimulation, 0 minutes, 15 minutes, 30 minutes, 60 minutes, 24 hours). The post-stimulation time points remained change from baseline values. This was found to be found to be significant, $F(5, 308712.34) = 3.53$, $p < .05$. Pairwise comparisons between Pre-stimulation values and the values from the time points following stimulation revealed that MEP amplitude was significantly reduced from Pre-stimulation to the follow-up time point (24 hours following stimulation), $t(39) = 3.52$, $p < .01$.

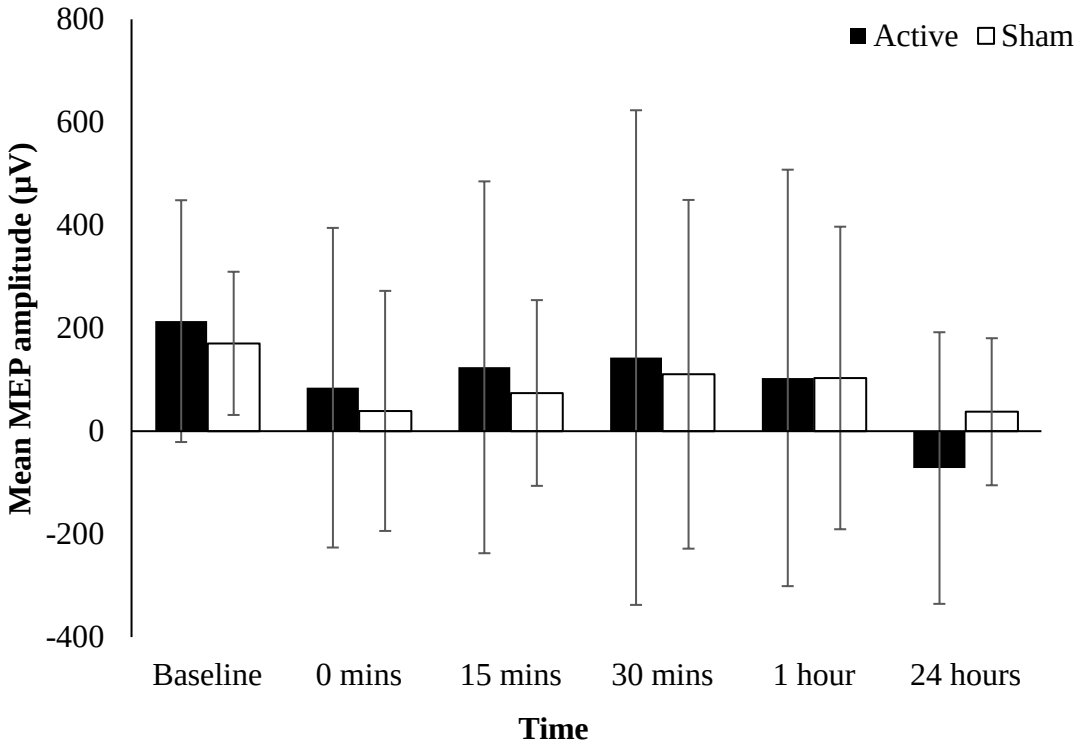

Figure 3. Mean change in MEP amplitude from baseline to post-stimulation blocks for active and sham groups with standard deviations.

Exploratory analyses. To further investigate whether GVS affected individuals with varying levels of cortical excitability differently, the sample was divided via a median split into even groups of participants who showed high versus low MEP amplitude at baseline. A 2x2x5 ANOVA with High versus Low MEPs at baseline as the new between-subjects variable was then conducted. A main effect of Time, $F(4, 51519.37) = 3.28$, $p < .05$, and a three-way interaction between Time, High versus Low MEPs and Stimulation, $F(4, 51519.37) = 3.14$, $p < .05$, were obtained.

To further investigate these effects separately, two separate 2x5 ANOVAs were conducted for the active and sham groups. High versus Low MEPs was the between-subjects variables whilst Time was the within-subjects variables. For the sham group, there was no main effect of Time, $F(4, 32263.07) = 0.81$, $p = .49$, nor was there an interaction between Time and High versus Low MEPs, $F(4, 14857.29) = 0.37$, $p = .77$. However, in the active group, there was both a main effect of Time, $F(4, 194211.39) = 3.03$, $p < .05$, and an interaction between Time and High versus Low MEPs, $F(4, 256724.91) = 4.01$, $p < .05$.

A further 2x5 ANOVA was conducted only on the High MEP at baseline group with Stimulation as a between-subjects variable and Time as a within-subjects variables which yielded a main effect of Time, $F(4, 99118.17) = 3.45$, $p < .05$ and an interaction between Stimulation and Time of marginal significance, $F(4, 99118.17) = 2.63$, $p = .07$. To further investigate this marginal significance, two one-way repeated measures ANOVAs were conducted for the active and sham conditions within the High MEP as baseline group. For the active stimulation group who showed High MEPs at baseline, a one-way repeated measures ANOVA (with six levels, Pre-stimulation, 0 minutes, 15 minutes, 30 minutes, 60 minutes, 24 hours) was found to be significant, $F(5, 18899.50) = 3.22$, $p = .05$. Post-hoc comparisons between Pre-stimulation MEPs and post-stimulation MEPs revealed a significant reduction from baseline to 24 hours following GVS, $t(39) = 3.45$, $p < .01$. The same was conducted for the sham stimulation group who showed High MEPs at baseline and a marginally significant results was found, $F(5, 4201.40) = 2.35$, $p = .08$.

Additionally, a one-way repeated measures ANOVA with Time as a within-subjects variable (with six levels, Pre-stimulation, 0 minutes, 15 minutes, 30 minutes, 60 minutes, 24 hours) was conducted for only the active group values. As before, Pre-stimulation values consisted of the baseline absolute values. A significant effect of Time was found, $F(5, 642763.11) = 4.42$, $p < .05$. Post-hoc t-tests showed that there was a significant reduction in

MEP amplitude from Pre-stimulation to the follow-up session (24 hours following GVS), $t(9) = 3.42$, $p < .01$, for the high MEP at baseline group who had received active stimulation (see Figure 4). All ten participants in the active high MEP at baseline group show a reduction in MEP amplitude 24 hours following GVS. This change was not present for participants in the Active group who showed low MEPs at baseline or those in the Sham group with either high or low MEPs at baseline.

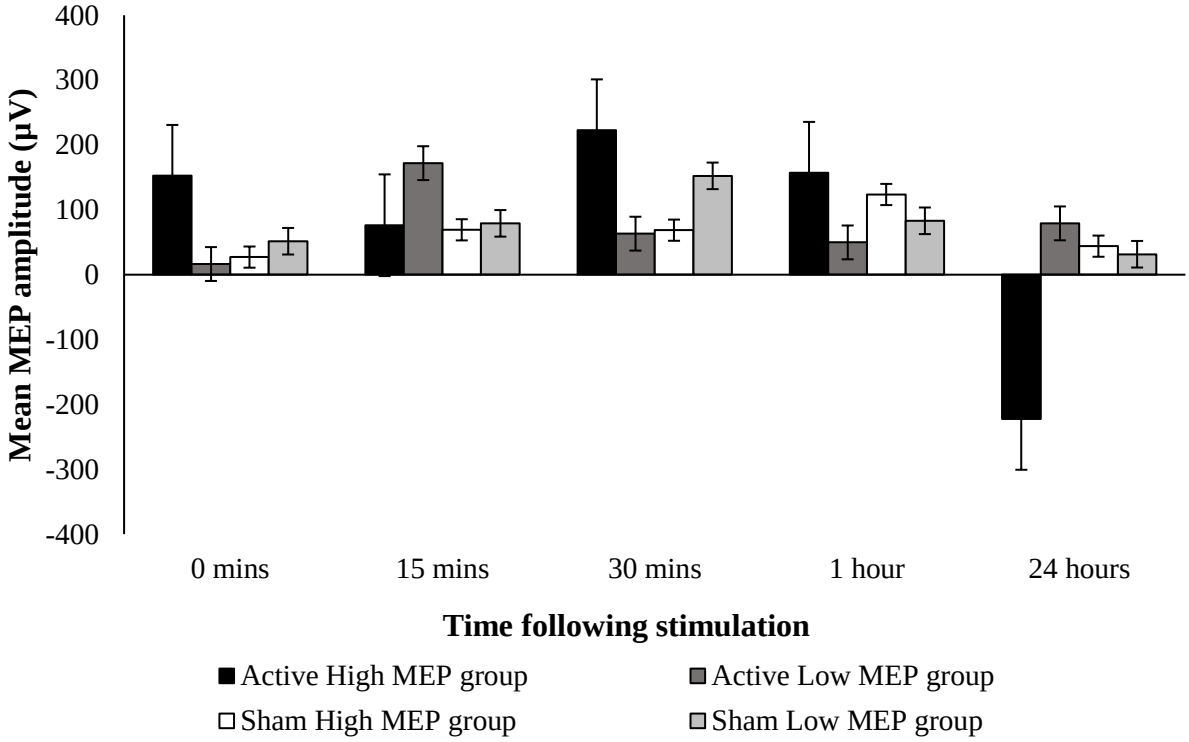

Figure 4. Mean change in MEP amplitude across time for all groups with standard deviations.

To assess whether this effect was driven by higher intensities of TMS delivered to the group who showed high MEPs at baseline compared to the other groups, a one-way ANOVA was conducted with Group as a between-subjects variable (Active High, Active Low, Sham High, Sham Low) and TMS Intensity (in percentage) as a dependent variable (see Table 2 for a breakdown of mean TMS intensity for each group). The one-way ANOVA was not significant, $F(3, 51.867) = 0.80$, $p = .50$, suggesting that all groups received comparable levels of TMS, i.e. participants in the High MEPs at baseline group did not receive higher percentages of TMS.

Table 2

Mean Stimulation Intensities for all Groups

Stimulation Groups	Mean TMS intensity (in %)
Active High MEP at baseline	53.7
Active Low MEP at baseline	55.1
Sham High MEP at baseline	51.7
Sham Low MEP at baseline	57.1

Discussion

Experiment 1 assessed the effects of GVS on MEPs, a measure of cortical excitability. The results for the sample as a whole showed no significant effect of active GVS on MEP amplitude over time compared to sham stimulation. However, when the sample was split into even groups according to their baseline level of cortical excitability, all participants who present with high cortical excitability at baseline (defined by high MEP amplitude at baseline) show a significant reduction in excitability following active GVS, compared to only half who present with low cortical excitability (defined by low MEP amplitude at baseline). These preliminary findings suggest for the first time that GVS may have an inhibitory effect on cortical excitability, which may reflect a delayed induction of LTD-like plasticity in the human motor cortex (Vallance & Ridding, 2014). Therefore, it may be that the clinical effects of GVS may partly reflect an enhancement of inhibitory activity.

One potential limitation of the current experiment concerns the high variability inherent in measuring TMS-elicited MEPs, demonstrated by the large error margins in mean MEP amplitudes (see Figure 3). This is a well-reported property of MEPs which may affect their reliability as a measure of cortical excitability (Kiers, Cros, Chiappa & Fang, 1993; Ellaway et al., 1998; Darling, Wolf & Butler, 2006). Practical factors such as accuracy of coil positioning, particularly when replicating positioning on a separate day, have been known to influence the variability of MEPs (Thickbroom, Byrnes & Mastaglia, 1999; Amassian, Cracco & Maccabee, 1989). Hence, special measures were taken to prevent this known

problem. A vacuum pillow was placed around the back of the participant's neck to prevent any involuntary head movement that may displace the coil from its position and to maintain the participant's head and neck erect throughout the experiment. Moreover, the sticker placed on the cap over the participant's head during the motor threshold session was not removed until the end of their participation to ensure that the correct coil positioning was replicated on subsequent days. Despite taking these measures, our MEP amplitudes still showed high variability. However, even efforts to increase spatial accuracy using functional imaging techniques as a guide in navigated TMS have failed to decrease MEP variability, showing similar variability to non-navigated methods (Gugino et al., 2001; Jung et al., 2010). It is likely that MEP variability may reflect less controllable factors such as the natural fluctuations in excitability pertaining to the cortico-spinal tract (Magistris, Rosler, Truffert & Myers, 1998).

Another potential problem of employing TMS-elicited MEPs is the confounding effect that the TMS protocol may have on MEPs over time. Single-pulse TMS is regularly utilised as solely a stimulus to measure cortico-spinal excitability with the assumption that they do not vary over time. Despite this, recent investigations have provided evidence that single-pulse TMS can increase MEP amplitudes over time (Julkunen et al., 2012; Pellicciari et al., 2016). Given that both active and sham GVS groups received TMS, it is possible that the inhibitory effect observed in the 24 hour follow-up does not represent the isolated effects of GVS on cortical excitability. To help disentangle the effects of GVS and TMS, an alternative measure of cortical excitability, which does not involve TMS, could therefore be employed.

Finally, it is possible that the sham stimulation applied in this experiment was not adequate given that participants' sensations were different in the active and placebo conditions, with prickling sensations being reported by all participants in the active group and

none in the sham group. However, it would be expected that any confounding effects of the sham stimulation would emerge in the first hour following stimulation. Our main significant change in cortical excitability emerges 24 hours following GVS in only a subgroup of participants with high cortical excitability at baseline whereas the first minutes post-stimulation failed to reach significance. Therefore, it is unlikely that an inadequate control condition produced the effects demonstrated in this experiment.

Experiment 2: The Effects of a Single Session of Galvanic Vestibular Stimulation on the Bereitschaftspotential

Experiment 2 was designed to corroborate the findings from Experiment 1 by employing MRCPs as an alternative measure of cortical excitability, which are uncontaminated by the potential effects of TMS. It also addressed the inherent variability in MEP data and the possibility that the TMS protocol may have had a confounding effect on cortical excitability beyond the effects of GVS. Although the BP was employed in this experiment to overcome the problems in MEP research, it is also worth noting that several movement-related factors can influence BP acquisition (Lang, 2003). In light of this, special care was taken to ensure participants performed consistent movements that were initiated from complete muscle relaxation. In line with the findings from Experiment 1, the prediction was that BP amplitude would decrease 24 hours following GVS. This would potentially indicate a reduction in the cortical excitability associated with pre-movement planning.

Methods

Participants

Twenty-four participants (14 females, 10 males) aged 19-43 (M= 24.25, SD = 5.86) from the University of Kent participated as part of the Research Participation Scheme (RPS) or through Jobshop. They were screened to ensure they had no skin abrasions behind the ears

or metal plates in their bodies. Participants provided written informed consent prior to participation. Upon completion of study, they were debriefed and compensated with either course credits or money. Ethical approval was obtained by the Psychology Research Ethics Committee at the University of Kent.

Design

A 2x3 mixed design was employed with Time as the within-subjects variable and Stimulation as the between-subjects variable. The within-subjects variables consisted of a baseline BP measurement, then a measurement at 0 and 30 minutes following stimulation as well as 24 hours post-stimulation. The between-subjects variable was whether participants had received active or sham stimulation. Active and sham conditions consisted of even groups of 12 participants.

Materials

Recording movement-related cortical potentials. Upon preparation of the scalp using sterilising wipes, eight cup electrodes were attached directly to the scalp with Elefix paste to record EEG at placements Fz, Cz, Cpz, Pz, C1, C2, C3, C4, according to the International 10-20 system. Linked A1 and A2 electrodes were used as references (see Appendix G). EEG and EMG were recorded simultaneously using Brain Vision Recorder software on a BrainAmp amplifier (Brain Products, GmbH, Gilching, Germany) with band pass filters of 0.05-70Hz for scalp recording and 20-70Hz for EMG as well as a 50Hz notch filter to reduce electrical noise. Impedance was maintained below 5kΩ and special care was taken during the entire experiment to prevent EEG artefacts resulting from swallowing, masticatory or other movements as well as cable movement. Remaining artefacts were excluded by experimenters upon visual inspection of EEG during offline analysis. Surface EMG was recorded using self-adhesive, disposable (28mm x 20mm) Ag/AgCl duck foot

electrodes (Ambu® Neuroline 710) in a bipolar montage over the extensor digitorum muscle of the left forearm (see Appendix H).

All preprocessing analyses were undertaken using BrainVision Analyser 2.0 (Brain Products, GmbH, Gilching, Germany). Averaged, rectified EMG signals were used to trigger back-averaging of EEG epochs by manually placing markers at EMG onset upon visual inspection. Duration of EEG epochs was set to four seconds (3 seconds prior to EMG onset and 1 second after EMG signal). The first 500 milliseconds of each epoch was baseline corrected and EEG data from 80 trials in each block were averaged to obtain BPs. To quantify the BP, information about the area under the slope is obtained from 2 seconds prior to EMG onset.

Galvanic vestibular stimulation. GVS parameters and set-up for this experiment were identical to those in Experiment 1.

Procedure

The experiment was conducted over two days and all participants provided written informed consent prior to participation. To ensure that no confounding effects relating to handedness affected performance on the left finger tapping task, all participants were required to be right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971; see Appendix I). Participants were then asked to sit in a comfortable chair and rest their left arm on a table provided. The time taken for set-up of EEG, EMG and GVS electrode placement was approximately 15-20 minutes (see Figure 5).

Participants were then provided with detailed albeit simple oral instructions on the motor task. They were instructed to perform voluntary extensions of the left middle finger at intervals of roughly 5 seconds with no external time-related cues (clocks, watches, etc. were removed from the laboratory). They were asked to perform these extensions at their own

pace. They were given 5 minutes within which to practice this task whilst monitoring their EMG trace on the computer screen and receiving auditory feedback from the experimenter. Only movements that were abrupt and commencing from complete muscular relaxation (silent EMG) were considered appropriate (this in turn facilitates the placement of markers on the EMG signal during offline processing). Special care was also taken to instruct the participants to avoid other movements such as chewing and fidgeting during EEG recording of the task.

Following this practice phase, baseline EEG recording was initiated whilst the participant performed 80 movements, during which they fixed their gaze to a fixation cross on the computer screen in front of them. Time taken to complete this motor task was usually 8-10 minutes depending on the speed with which participants decided to move their finger. Active or sham GVS was then applied for 25 minutes during which the participants were asked to relax. As in Experiment 1, all participants in the active stimulation group reported a prickling or tingling sensation behind the ears during the first few minutes of GVS onset. Sham stimulation was conducted in the same manner as in Experiment 1. Post-stimulation recording of EEG during the motor task was initiated immediately following GVS and then again 30 minutes after the stimulation period. During the break between blocks 1 and 2, participants were asked to relax (see Figure 5).

On the next day, approximately 24 hours following GVS, participants were again asked to perform the task in identical fashion to the previous day whilst recording EEG. A total of 320 movements (80 movement within each block) was completed by each participant at the end of the experiment. Upon completion of this session, participants were debriefed and compensated for their time.

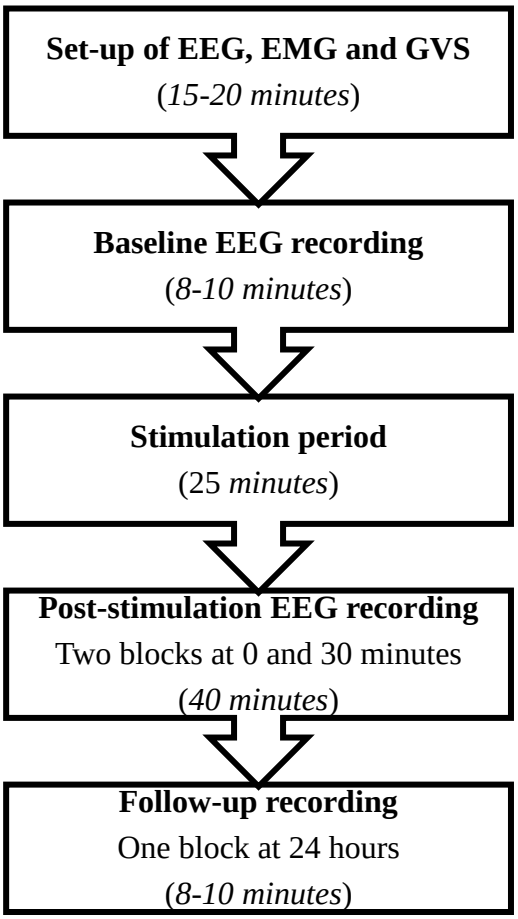

Figure 5. Schematic of Experiment 2 procedure.

Results and Discussion

Due to several methodological issues discussed later, analysable EEG data was obtained from only seven participants (three from Active group, four from Sham group) whose baseline BP waveforms are presented in Figures 6a-g. EEG data from 17 participants were excluded due to a high number of artefacts in the form of noise and drift from muscle movement, electrode/lead movement and perspiration. The EEG data from the remaining seven participants also failed to satisfy the criteria required for the presence of MRCPs. The absence of a BP component at baseline precluded the interrogation of BPs at later post-stimulation time points.

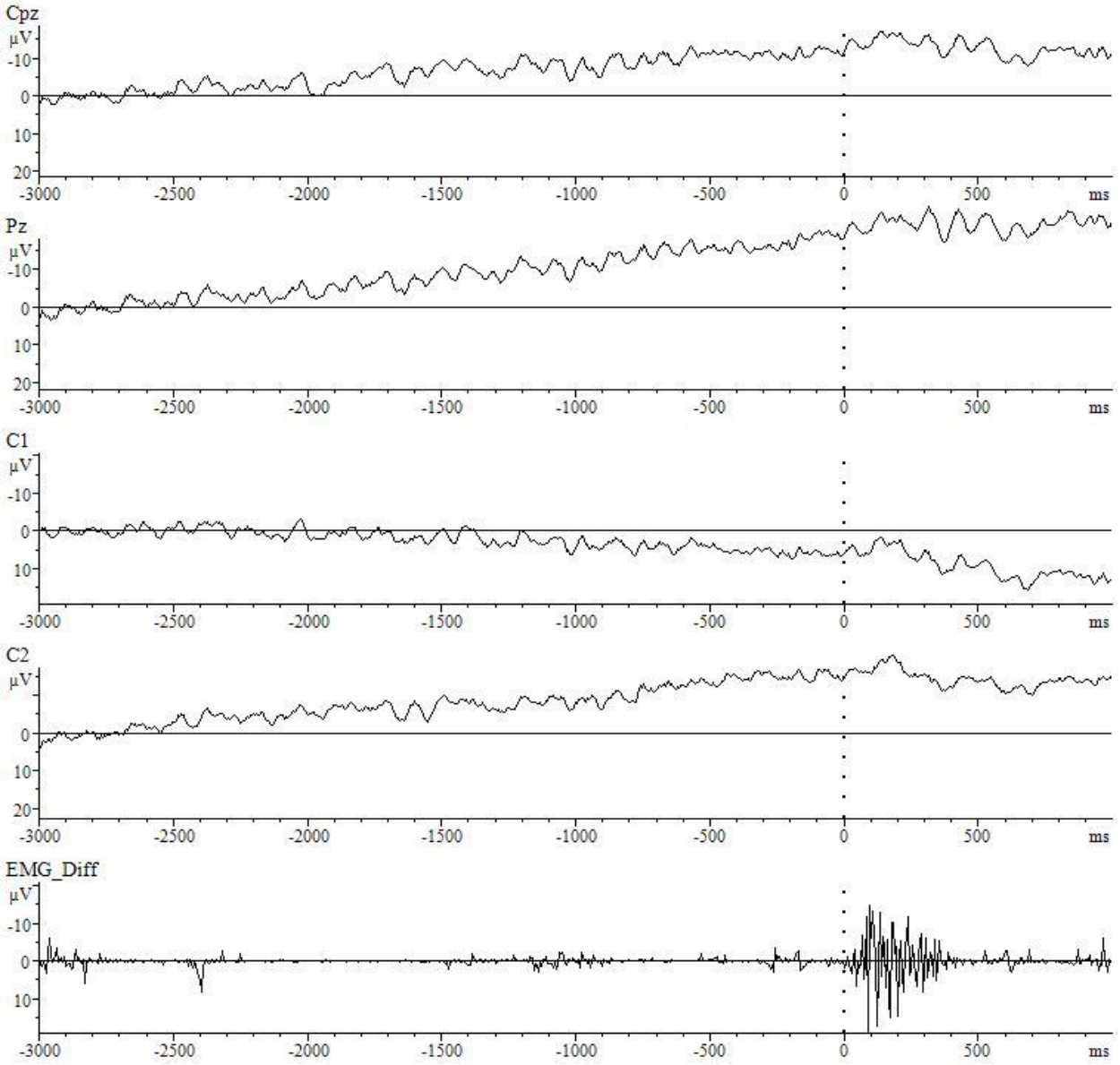

Figure 6a. Participant 01 baseline data.

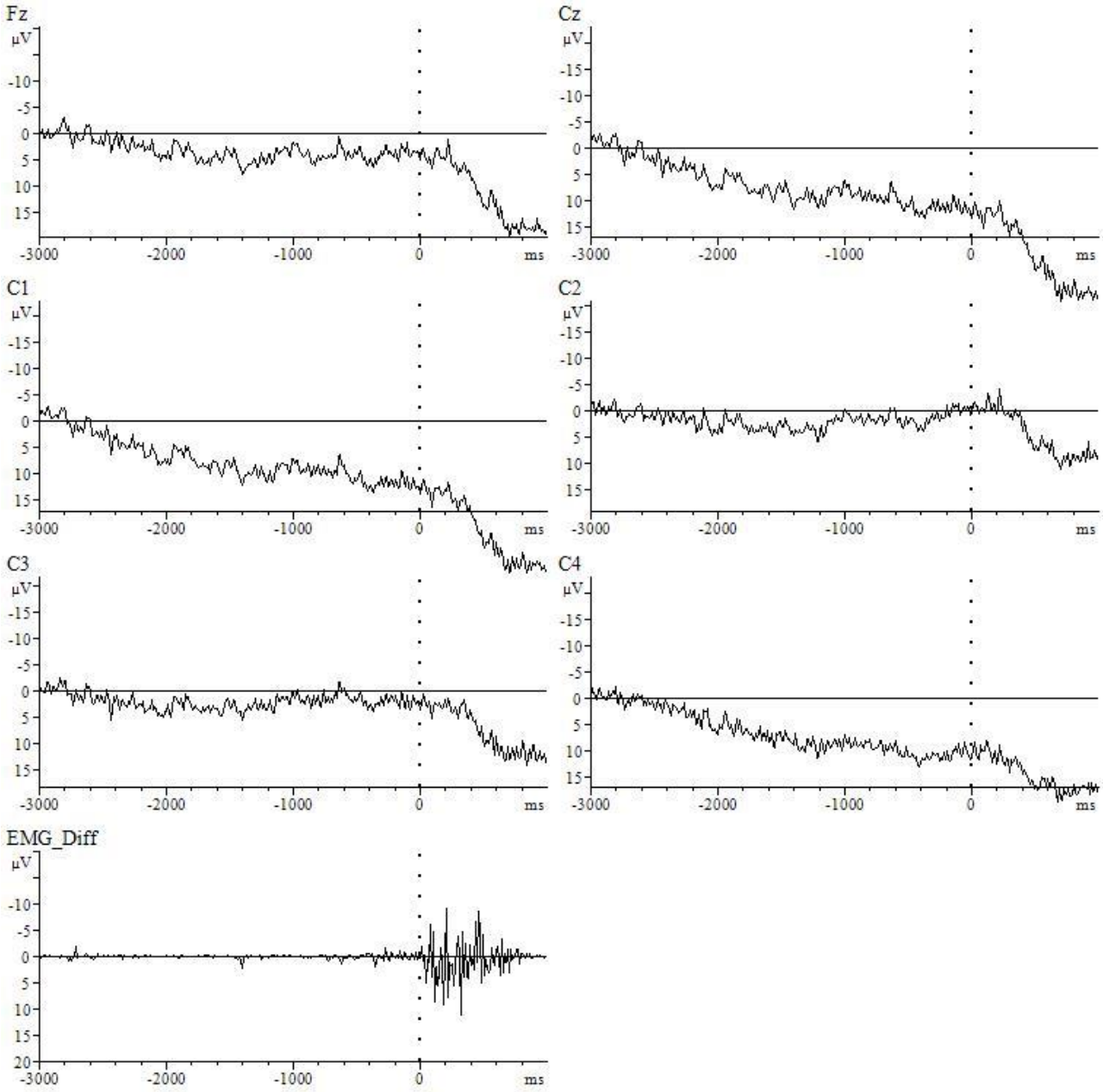

Figure 6b. Participant 04 baseline data.

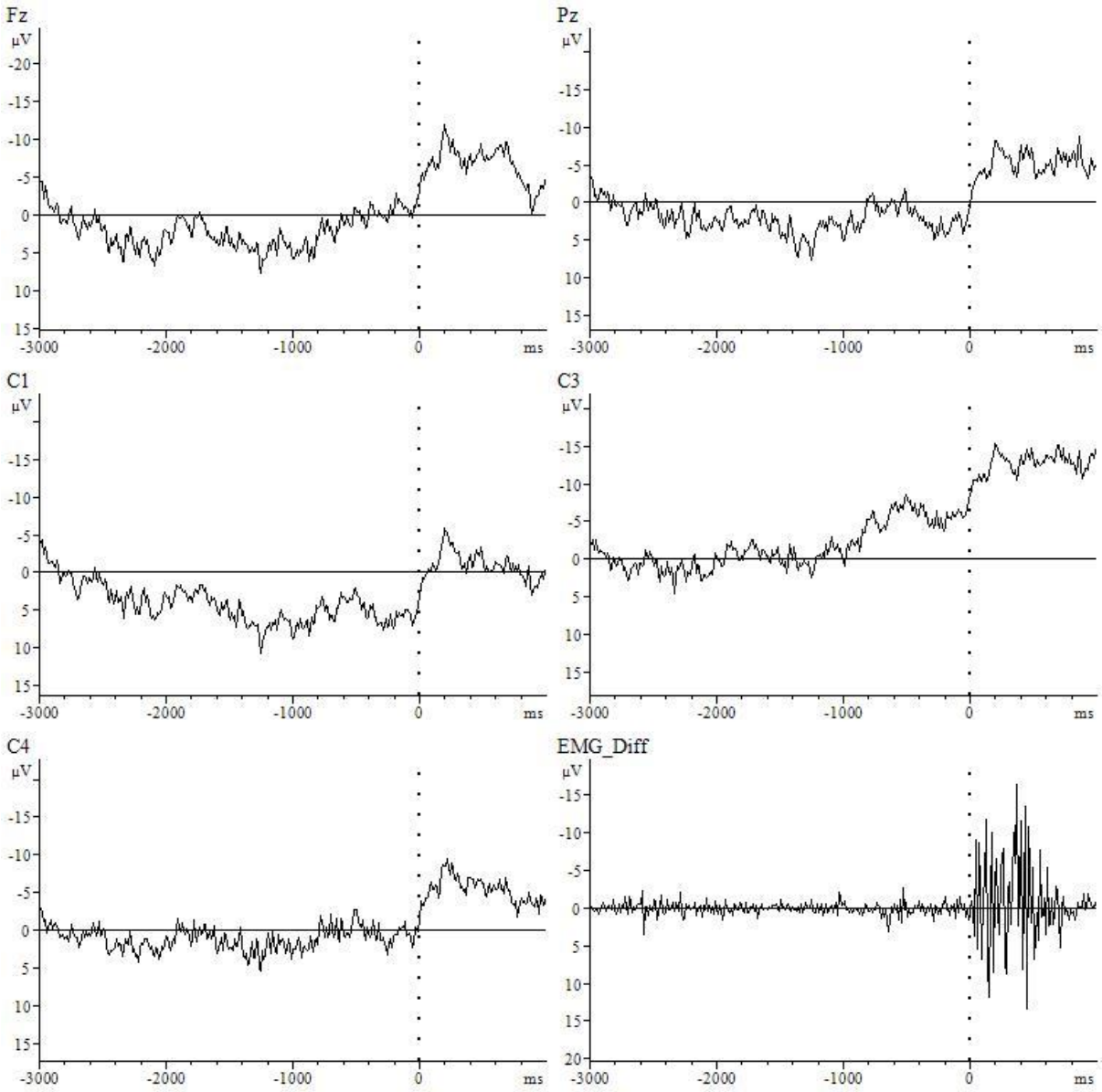

Figure 6c. Participant 05 baseline data.

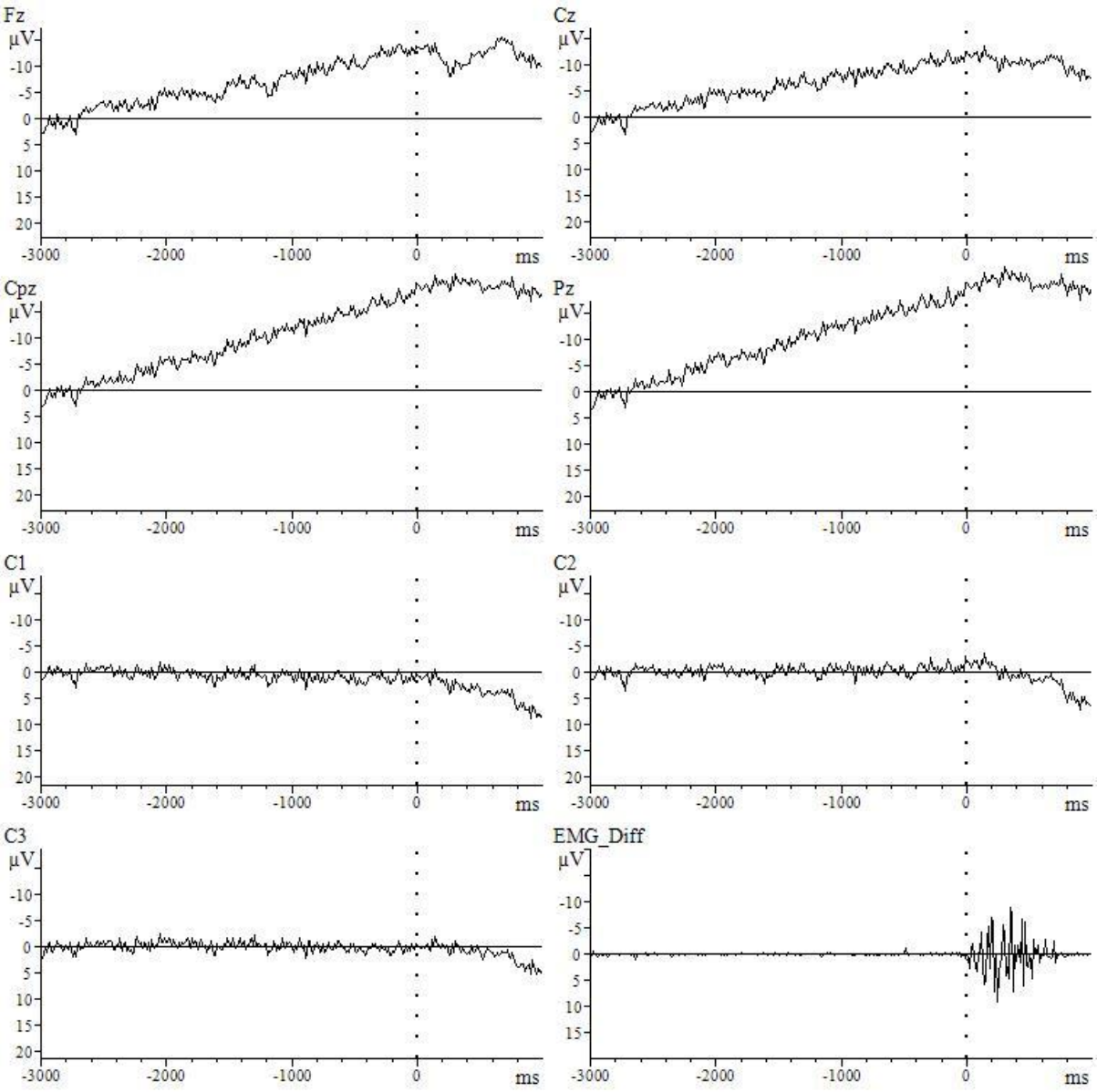

Figure 6d. Participant 07 baseline data.

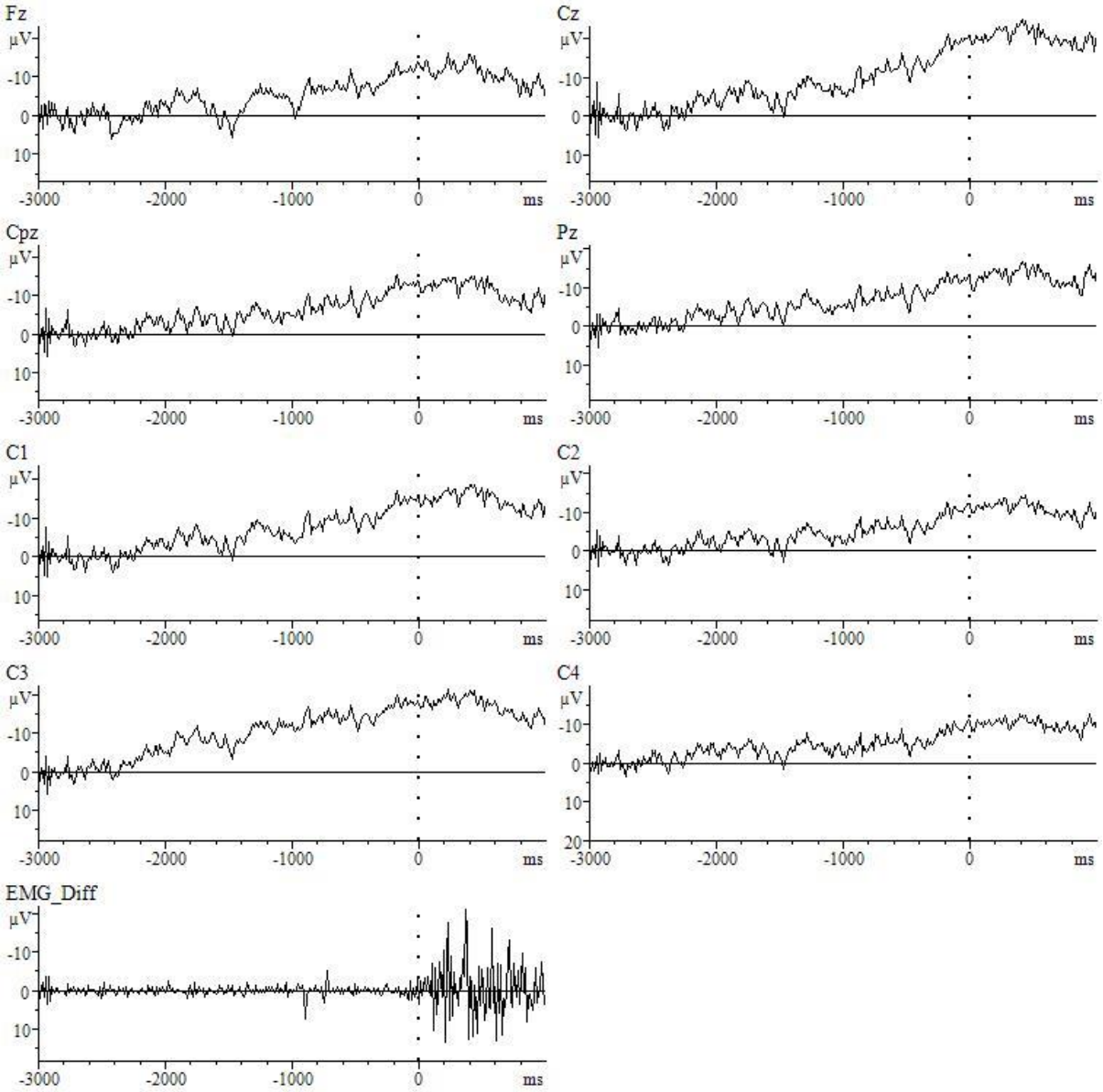

Figure 6e. Participant 08 baseline data.

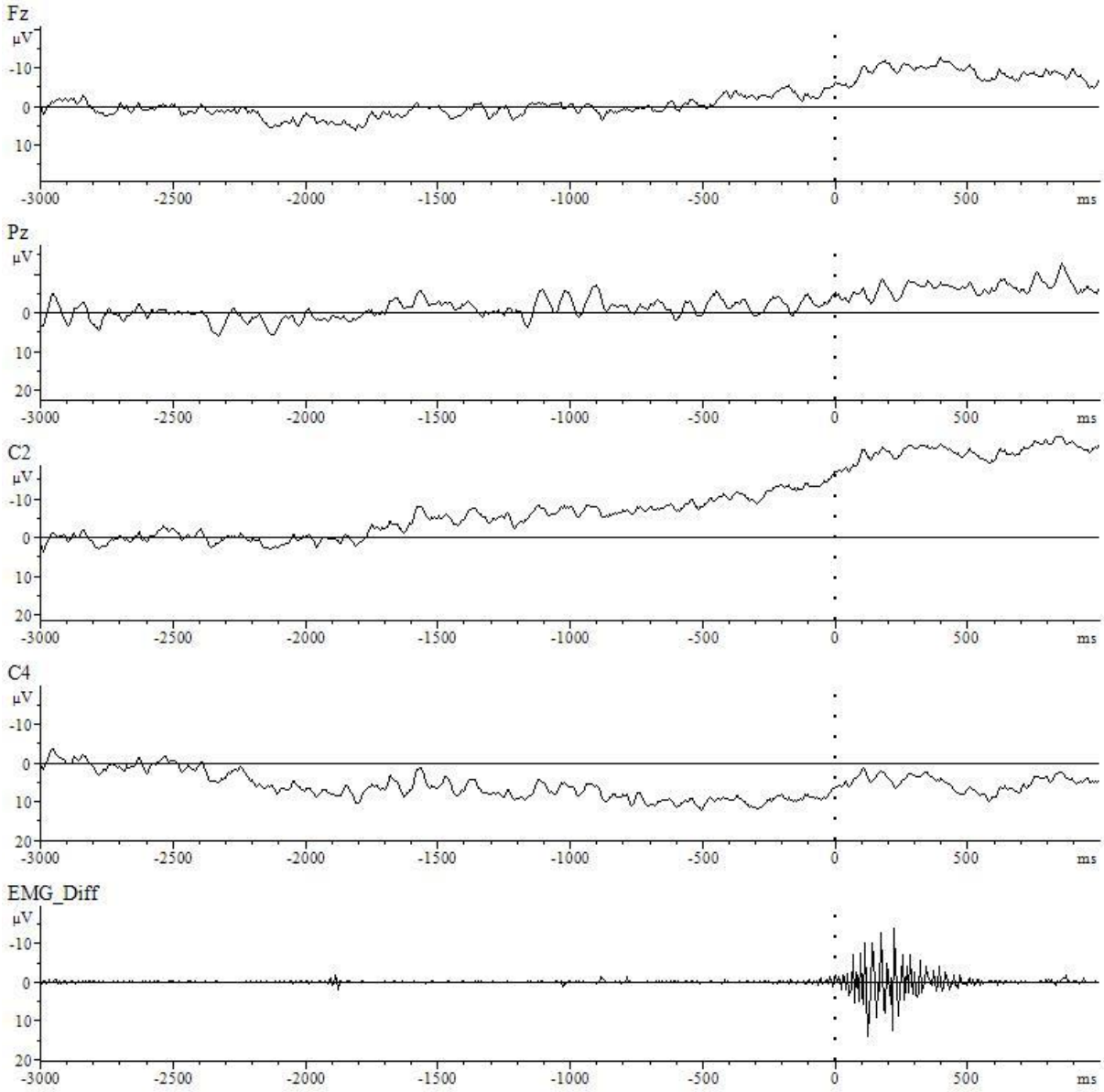

Figure 6f. Participant 17 baseline data.

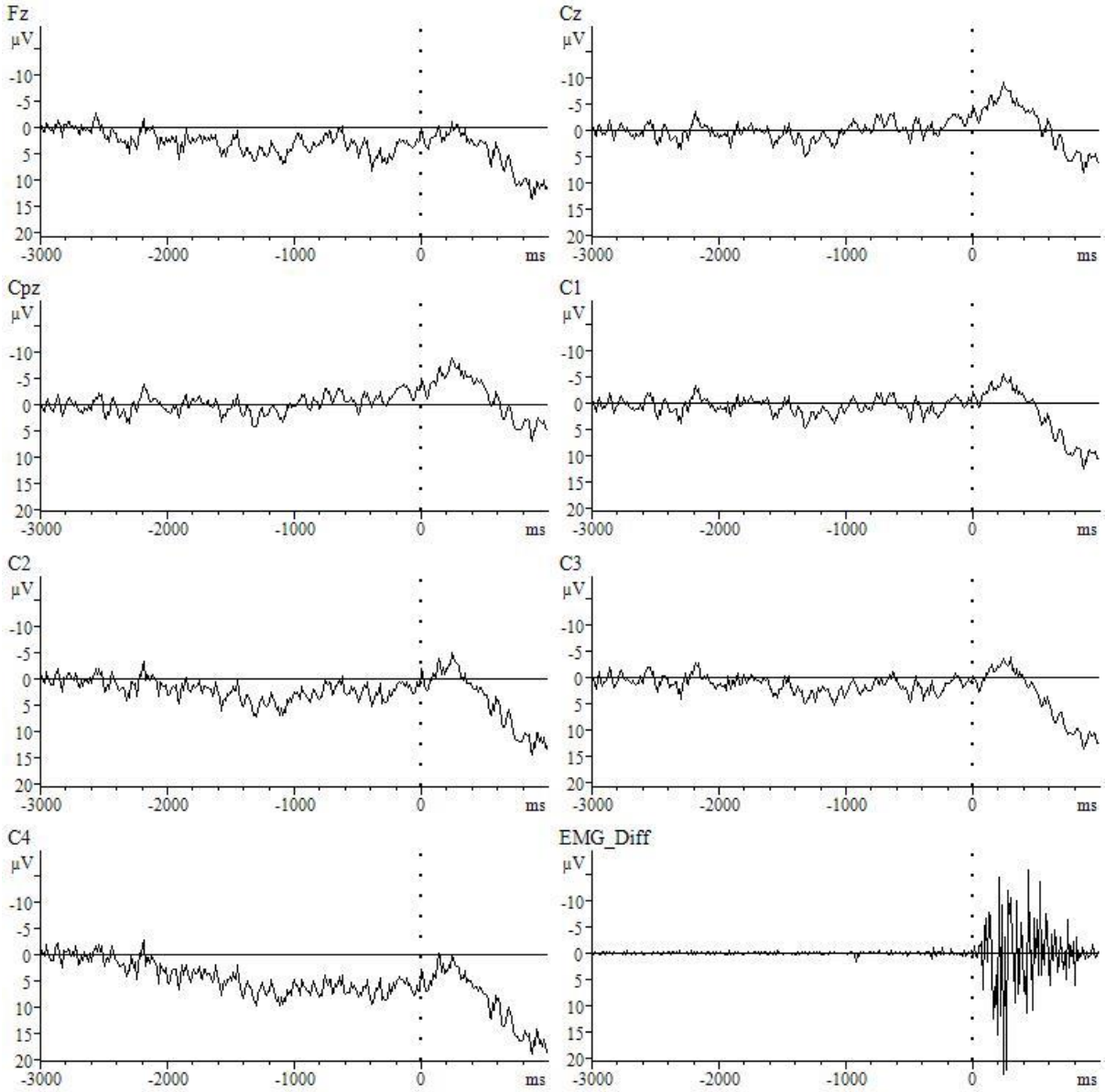

Figure 6g. Participant 23 baseline data.

Figures 6a to g reveal that the signals obtained from our sample did not constitute complete MRCPs, according to the criteria reported in the literature (Maurits, 2011; Shibasaki & Hallett, 2006; Deecke, Grözinger & Kornhuber, 1976). According to these criteria, a slow rising negative slope begins 2 to 1.5 seconds prior to the averaged, rectified EMG onset, which comprises the 'early BP'. It is maximal over Cz and varies from -3 and -5µV in amplitude. Following this, the 'late BP' is a rapidly increasing negativity starting approximately 500-300 milliseconds prior to the EMG trace. For left finger movements, the 'late BP' is often lateralised to the right central region (C2 and C4) and can reach -10 to -15µV in amplitude. Immediately before the peak EMG is a component known as the motor

potential (MP). It is characterised as the highest negative peak reaching -15 to -20μV in amplitude. The recorded activity then returns to baseline levels following the end of the EMG trace. As presented in Figures 6a to g, the segments from our EEG data demonstrate the presence of some MRCP components whilst others are absent. None show a complete pattern of components as outlined above.

Many participants did not show signs of MRCP components at any of the key sites. For example, Participants 01 and 07 show no signs of an MRCP pattern at sites C1, C2, C3 and Cpz (see Figures 6a and 6d). A few participants showed a potential early BP but in the absence of a late BP, for example, Participant 08 at all sites and Participant 01 at Pz and C2 (see Figures 6e and 6a). For some participants, there were signs of an MP, but no indication of a BP, early or late, such as in channels C2, C4 and Pz for Participants 04 and 05 (see Figures 6b and 6c). Participant 23 shows an MP at all sites but there is no indication of a BP (see Figure 6g). Participants 07 and 17 did show a rising negativity before EMG onset at sites Cpz, Pz and C2 (see Figures 6d and 6f). However, these were in the range of -10 to -20μV, which is considered too large for a BP component. Moreover, the negativity shown in these sites did not return to baseline levels following the end of the EMG trace, suggesting contamination from artefacts such as drift present in the raw EEG data. These examples demonstrate the failure to obtain MRCPs in our sample.

The failure to obtain an appropriate BP component during baseline analyses was unexpected as several steps were taken to ensure rigorous methodology and analysis. The self-paced finger extension task was selected as it has been used frequently in previous studies and is considered the most suitable to elicit pre-movement negativity (Jahanshahi et al., 1995; Papa et al., 1991). Additionally, participants were required to practice the task prior to recording to ensure they had mastered the finger movement. Mastery of the task consisted of performing brisk, consistent movements that initiated from complete muscle relaxation, as

is demonstrated in previous studies (Maurits, 2011). Finally, to ensure that our preprocessing method was not producing spurious signals within the EEG data analysis of our self-paced movement data, we applied this method to analyse control data from a participant sitting passively while EEG was recorded. No BP was derived from this analysis (see Figure 7), thus it is unlikely that our preprocessing method was influencing the analysis of our self-paced movement data.

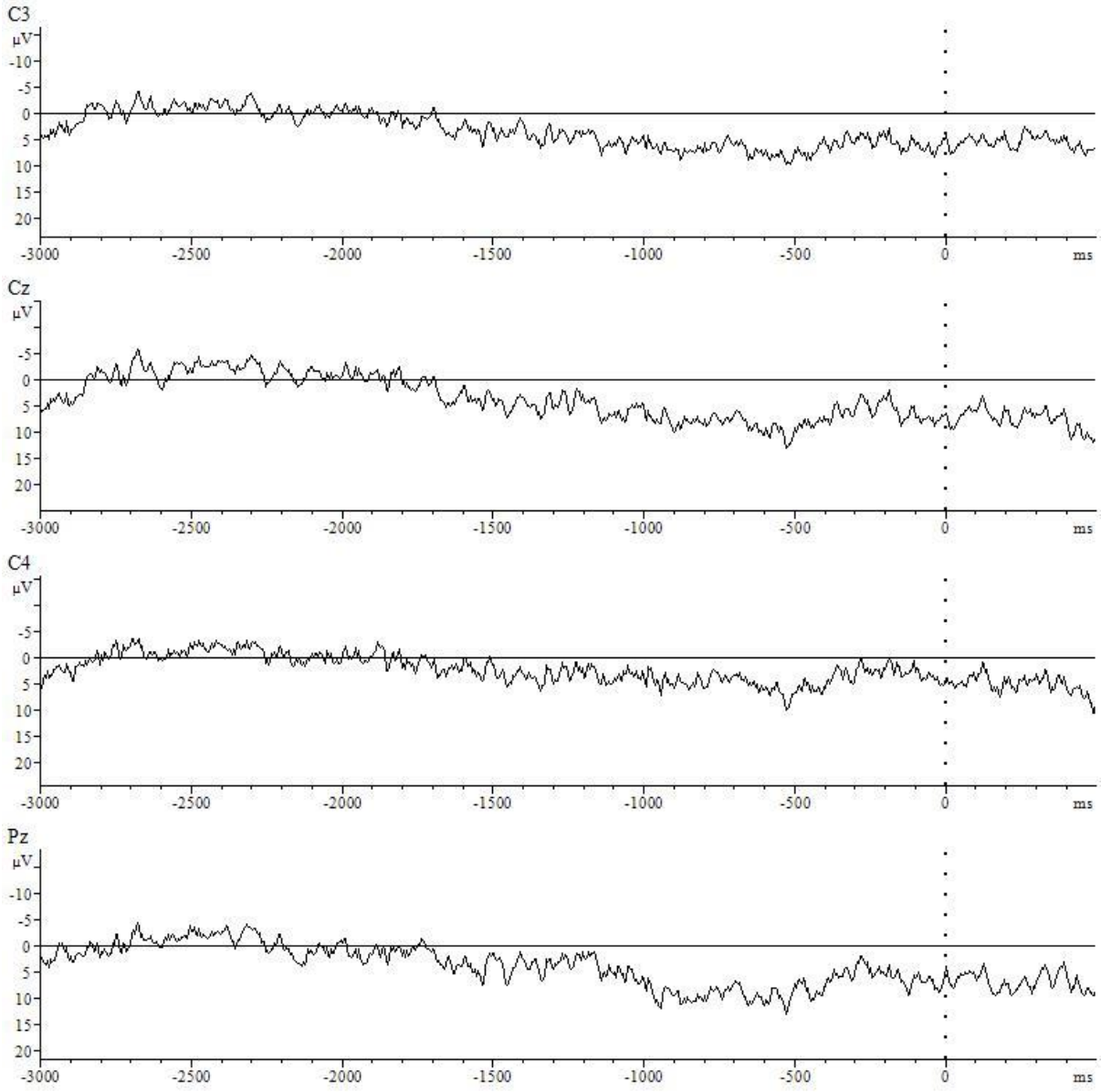

Figure 7. Control data from a participant sitting passively during EEG recording.

Despite taking these precautions, a number of practical factors were identified that may have impeded the acquisition of the BP component. First, suboptimal EEG electrode

placement directly over the scalp may have precluded signal acquisition from critical sources such as the SMA and premotor cortex. The use of an electrode cap may have facilitated the optimal placement of electrodes over the SMA and premotor cortex. Second, the experiment was conducted during a heatwave in the months of June and July, thus there were unexpectedly high temperatures within the laboratory in which the experiment was taking place. In the absence of an air conditioning unit, the experimenters were unable to maintain the temperature at a comfortable level for participants, thus leading to the acquisition of poor quality data. The raw EEG data contained several artefacts related to movement and perspiration. Several EEG epochs were then excluded leading to an average of 45.6 trials for the seven participants with analysable data. This may not have been a sufficient number of trials required to capture a BP component. Previous research has reported at least 80 to 100 movements for the successful acquisition of the BP (Deecke et al., 1976). These are factors relating to our experimental methodology that may have hampered the detection of suitable MRCPs.

Alternatively, the failure to obtain a BP component may have been caused by an unusual characteristic in our sample. Several authors have reported the 'extreme inter-individual variability' in BPs with some even suggesting their absence in some participants (Dick et al., 1987; Colebatch, 2007). Poor intra-individual reproducibility has also been reported with one study suggesting that only the late component of the BP is reproducible across time (Evidente et al., 1999). Intra- and inter-individual variability in eliciting the BP may have significant implications for pre-post designs across separate days such as the one used in the current experiment. Other factors have also been known to affect the magnitude and time course of the BP. Lang (2003) identified several factors which influence the BP including level of intention and perceived effort; force exerted; speed and precision of movement as well as discreteness and complexity of movement. Slobonov, Hallett and

Newell (2004) found that greater perceived effort was associated with a larger amplitude of the late BP. Masaki, Takasawa and Yamazaki (1998) also found that the faster the movement is performed, the later the BP commences. These factors were not accounted for in the current experiment, therefore it is possible that they may have influenced the BP. In sum, failure to record a BP at baseline in Experiment 2 prevented the assessment of how GVS affects the BP in the minutes and hours following stimulation.

Experiment 3: The Effects of a Single Session of Galvanic Vestibular Stimulation on the Bereitschaftspotential in an Individual with Chronic Stroke

Experiment 3 addressed two important issues that were not accounted for in Experiment 2. First, it attempted to correct some of the methodological flaws present in Experiment 2 that may have prevented the detection of an appropriate BP component. Three important modifications in the method were implemented to improve the chances of capturing the BP. First, the number of movements was increased from 80 to 100 in an effort to increase the number of artefact-free trials. Second, an electrode cap was used to ensure accurate localisation of key sites from which the BP is derived. These include the SMA and the premotor cortices. Third, the experiment was conducted in a temperature-controlled laboratory to prevent raw EEG data contamination from movement- and sweat-related artefacts.

A second aim of Experiment 3 was to investigate the effects of GVS on the BP in a lesioned brain. There is some evidence to suggest that brain-damaged individuals are more susceptible to sensory perturbation than healthy-brained participants (Bastani & Jaberzadeh, 2012), which makes it more possible that greater changes in cortical excitability will be observed. If a BP is obtained at baseline, the use of a clinical sample may increase the chances that GVS can modulate it at later time points. Thus, Experiment 3 recruited a single patient who suffered from a right hemisphere stroke. If an effect of GVS is shown in this

patient then it will provide proof-of-concept that will justify a larger clinical group study. The patient was selected as he has previously shown benefits from receiving GVS (Wilkinson et al., 2014).

As in Experiment 2, the prediction was that the BP would be reduced in amplitude 24 hours following stimulation. This would corroborate the delayed inhibitory effect observed in Experiment 1.

Method

Patient case history

Patient 001 is a 65-year old right-handed male who was admitted to hospital after he suffered a large right-temporal haematoma on November of 2009. Initial presentation of symptoms was a sudden pain behind his right eye. Later the same day he collapsed following a headache and left-sided weakness. A CT scan revealed a large right hemisphere haemorrhage (8.5cm x 4.4cm) extending from the anterior temporal lobe superiorly into the parietal lobe. The haemorrhage caused further effacement over the right hemisphere and midline shift to left of 10mm. Clinician's impression of possible aetiology consisted of either hypertension or amyloid angiopathy.

Subsequent intervention involved a right hemisphere craniotomy to evacuate the temporal haematoma. At post-surgery, patient 001 presented with absent sensation to the left upper and lower limbs and left-side hemi-spatial neglect with left homonymous hemianopia. His attention and orientation to the left side of space was significantly impaired. Following one month of bed-based rehabilitation, he showed significant improvements in limb function and was able to perform fine motor movements. Upon discharge, the patient was fully orientated in space with no working or long-term memory problems and was able to carry out mental manipulation tasks. However, he still presented with significant hemianopia and left-

sided neglect such that he regularly needed prompting to attend to tasks on the left side of space.

Two years following his stroke, patient 001 was enrolled into an RCT at the University of Kent which probed the efficacy of GVS in the amelioration of hemi-spatial neglect (Wilkinson et al., 2014). Following five active GVS sessions of 1 mA noise current for 25 minutes on consecutive days, patient 001 showed significant improvements in BIT scores from 119 out of 146 at baseline, to 145 out of 146 at a 4 week post-stimulation follow-up. The administration of GVS was well-tolerated by patient 001 and no adverse side effects were reported. Indeed, the patient was not able to distinguish between when he was receiving an active or a sham dosage. A self-report measure also revealed that patient 001 was "absolutely willing" to undertake the treatment again. Coupled with responsiveness to GVS, patient 001 was considered a suitable candidate to take part in the current experiment.

Currently, patient 001 shows an almost complete recovery from stroke with no indication of limb weakness or spasticity although he reported pins and needles as well as diminished sensation in the left limbs. He is able to carry out most activities of daily living independently and any residual neglect has been well-compensated. The most difficult remaining problem for patient 001 is the residual left-sided hemianopia, which has prevented him from fulfilling his goal of regaining his driving licence. He is currently taking irbesartan (150mg oral tablet once a day) for high blood pressure and pregabalin (50mg orally twice a day) for nerve pain.

Patient 001 provided written informed consent prior to his participation in the study. Ethical approval was obtained by the Psychology Research Ethics Committee at the University of Kent.

Materials

Materials utilised were identical to those in Experiment 2 (see Experiment 2 Materials section for reference) with one modification. An electrode cap was fitted to the patient's head instead of directly attaching cup electrodes to the scalp.

Procedure

Experiment 3 was conducted in an air-conditioned laboratory to ensure a comfortable temperature for the duration of the experiment. Thus, preventing the problems caused by high temperatures in Experiment 2. Experiment procedure was identical to that in Experiment 2 (see Experiment 2 Procedure section for reference) with a few exceptions. The patient was asked to perform 100 self-paced extensions of the left index finger with intervals of approximately 4 to 5 seconds. The total number of movements equated to 400 (100 within each recording block) for the entire experiment. To prevent fatigue on the part of the patient, breaks lasting 25 seconds were introduced within each block (see Figure 8). These modifications were implemented to both tailor the task to the patient's abilities and to increase the number of artefact-free trials for analysis.

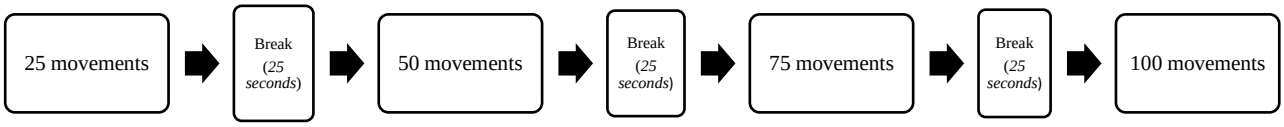

Figure 8. Schematic of motor task performed by patient 001.

Results

Successful acquisition of MRCPs in EEG segments across time blocks is presented in Figures 9a to d. BP amplitudes obtained from the mean area under the slope for each channel across time is presented in Figure 10. Due to poor quality EEG data on five time points, only

time points and EEG channels that yielded good quality data are presented in Figure 10. The average number of artefact-free trials for across time blocks was 65.25. BP amplitudes are presented and discussed descriptively as the use of a single case study precluded statistical inferential testing. The use of bootstrapping to determine significant differences was also precluded because of the insufficient number of raw data points. BP amplitudes as measured by mean area under the curve are extracted from the back-averaged EEG preceding several movement trials, thus only four values (one for each time point) were obtained for each electrode.

At most electrode sites, there is no indication of a marked reduction in BP amplitude with the notable exceptions of Fz and C4 (see Figure 10). BP amplitude at Fz decreases from -5.42μV at baseline to -1.17μV immediately post-stimulation and to -0.07μV twenty-four hours following GVS. For electrode C4, BP amplitude shows a slight reduction immediately post-GVS (-3.50μV) from baseline (-4.90μV), then a larger reduction 30 minutes following stimulation (-1.15μV) before finally returning to baseline levels the next day (-4.75μV).

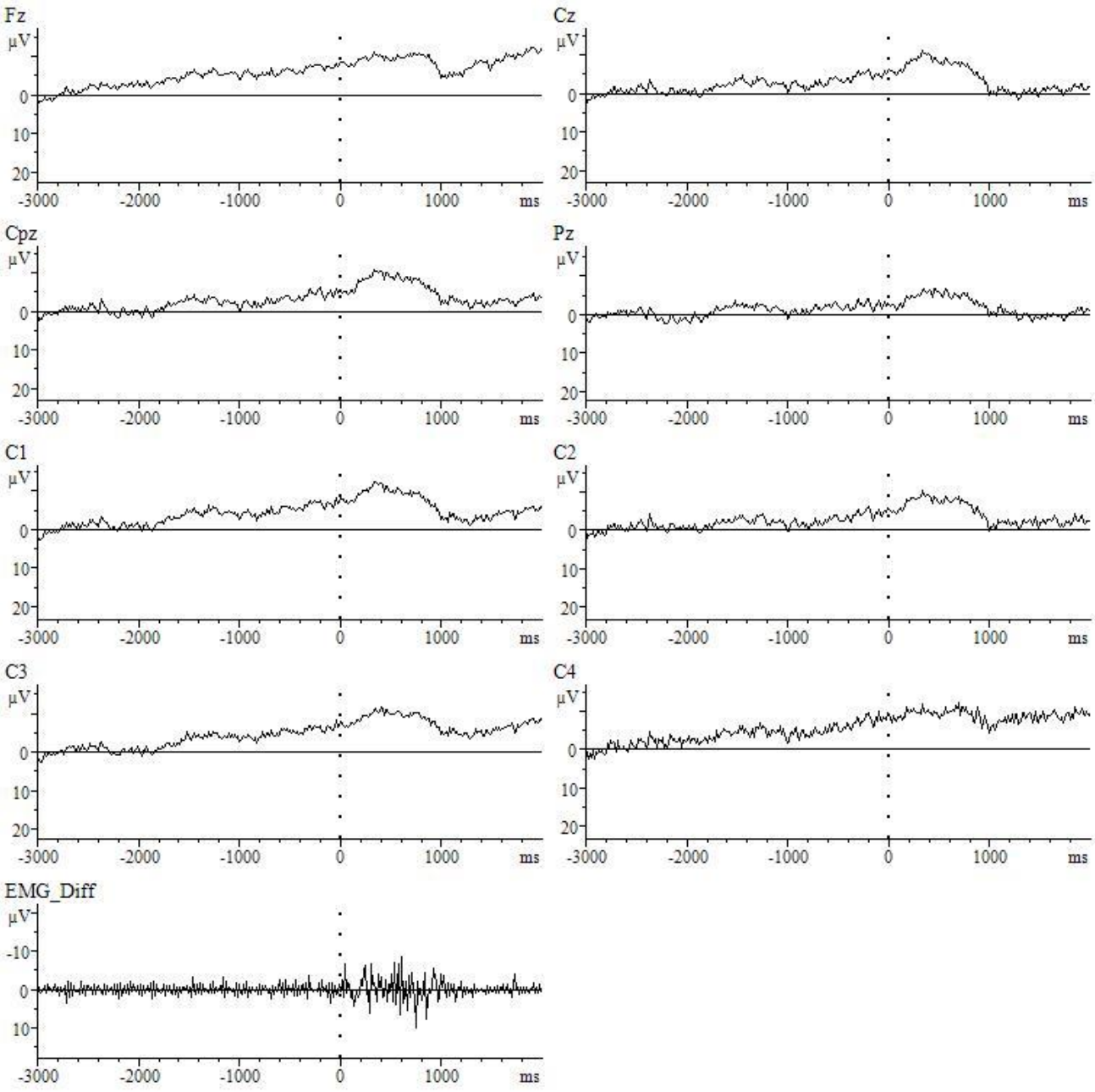

Figure 9a. Baseline BPs for patient 001.

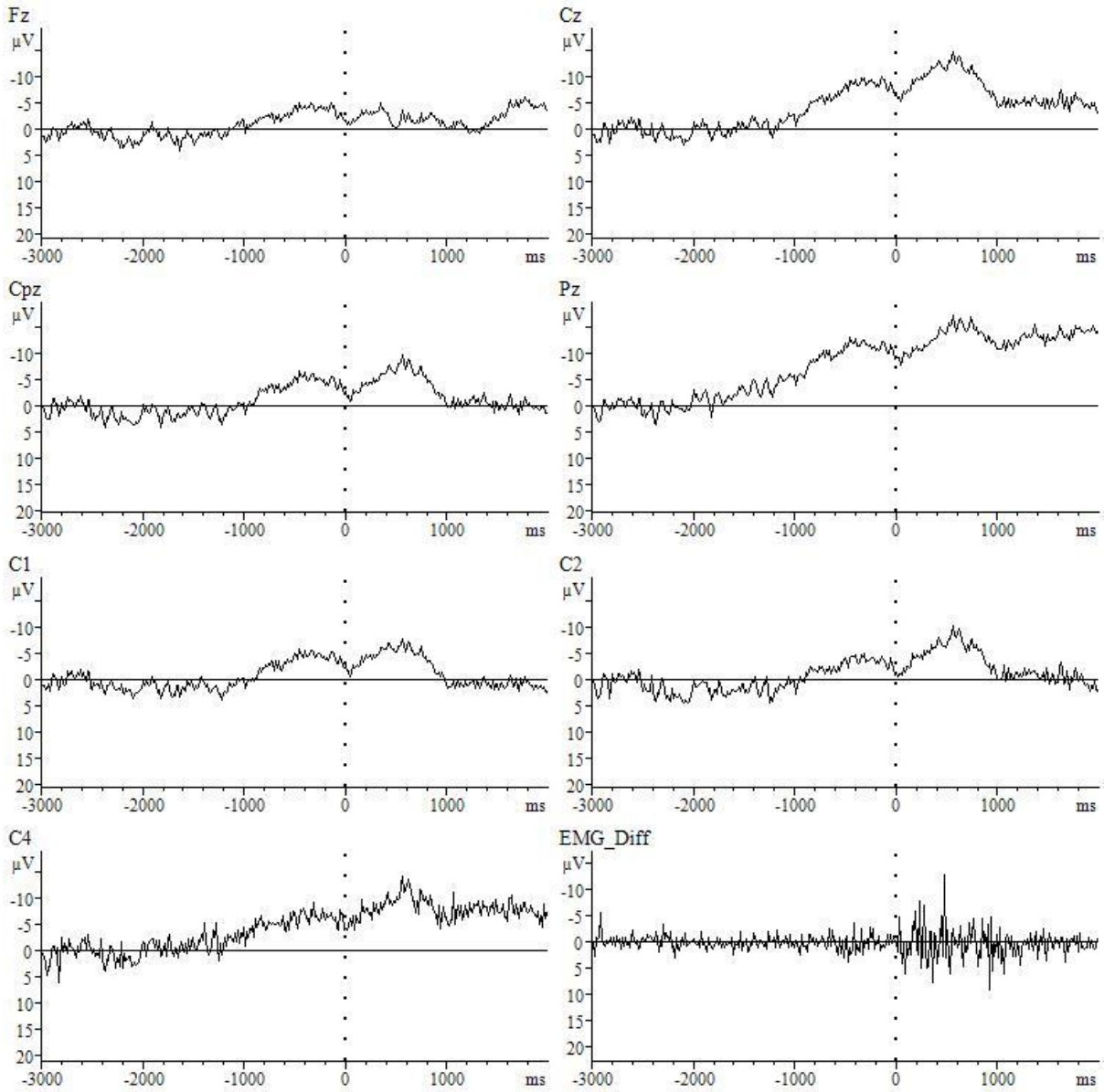

Figure 9b. BPs immediately following GVS for patient 001.

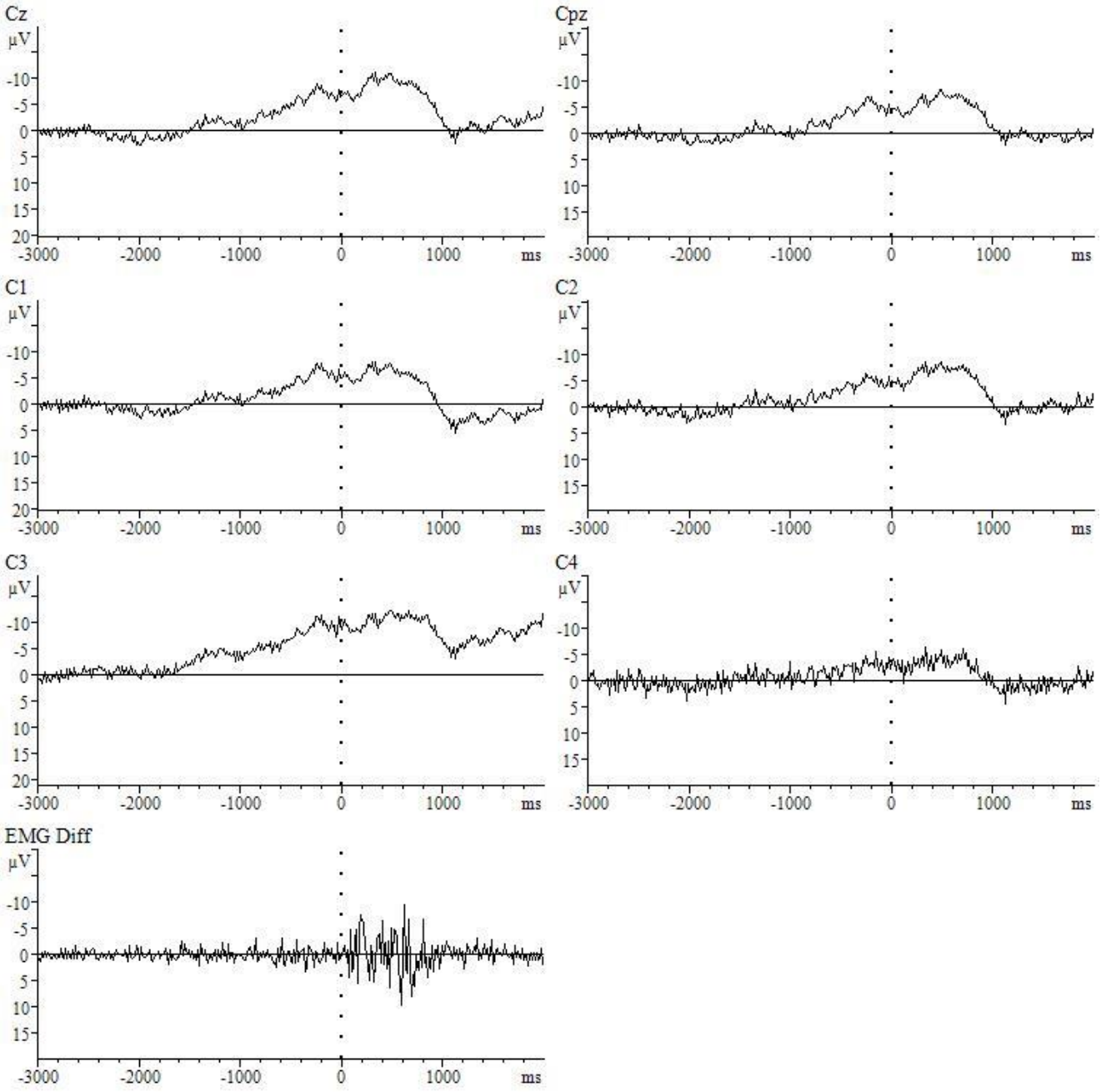

Figure 9c. BPs 30 minutes following GVS for patient 001.

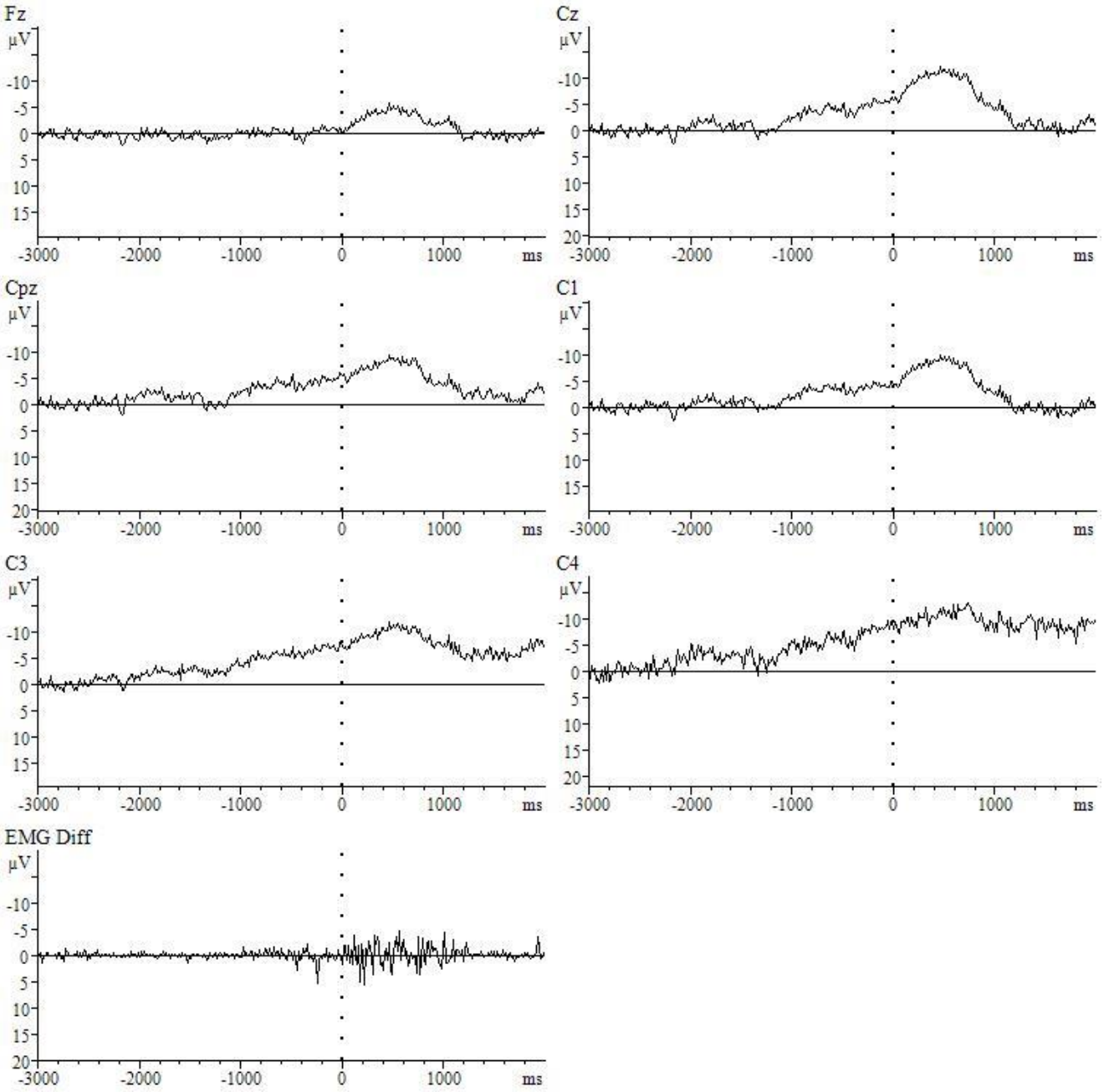

Figure 9d. BPs 24 hours following GVS for patient 001.

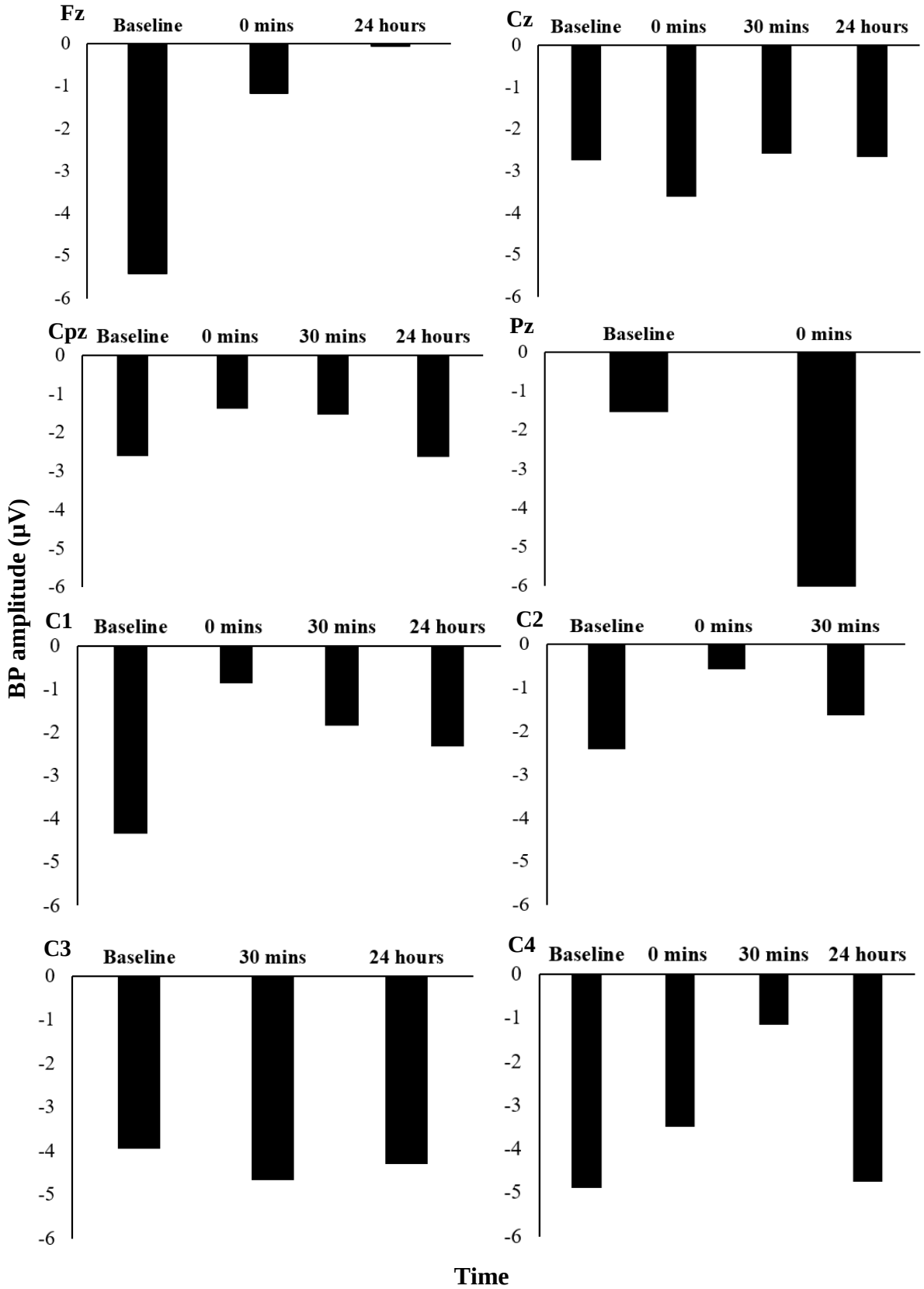

Figure 10. Mean area under the curve amplitude for all channels across time for patient 001.

Discussion

Experiment 3 aimed to correct the methodological limitations present in Experiment 2 thereby increasing the likelihood of obtaining a BP and to also test this hypothesis in a neurologically impaired rather than healthy individuals. The former aim was achieved by altering two aspects of the methodology: utilising an electrode cap; increasing the number of movements performed by the subject and conducting the experiment in a temperature-controlled laboratory. As is demonstrated in the baseline data (see Figure 9a), all necessary MRCP components were obtained.

The expectation was that BP amplitude would be reduced 24 hours following GVS, as was the case with the MEPs in Experiment 1. Reductions were observed only in channels Fz and C4 whilst all other sites showed no pattern of change following GVS. It is possible that the reduction observed at Fz 24 hours following GVS may reflect an inhibitory effect in frontal lobe areas, however, the relevance of this finding is unclear as changes to BP amplitude is most frequently reported at central sites such as Cz (Matsuura et al., 2015; Lee, 2015). The reduction observed in channel C4 at 30 minutes after GVS may be more relevant as it is both a central area and contralateral to movement. This may indicate reduced cortical excitability of motor and premotor areas and therefore may partially corroborate the results of Experiment 1. However, BP amplitude, and potentially cortical excitability, returns to baseline levels 24 hours following GVS, which contrasts with the reduction in MEP amplitude observed in some participants in Experiment 1. This finding warrants further investigation possibly in a future patient group study utilising identical methodology with the addition of a normative control group. This would be useful to uncover any further effects of GVS and to potentially replicate the effects observed in this experiment.

General discussion

The current set of experiments aimed to investigate the effects of GVS on cortical excitability with the goal of uncovering a potential mechanism of action for the therapeutic benefits observed following this type of stimulation. To date, no such mechanism has been established. Changes in cortical excitability following GVS may indicate the induction of synaptic plasticity mechanisms, such as LTP and LTD. The findings from Experiment 1 revealed an inhibitory effect of GVS on cortical excitability 24 hours following stimulation, which may suggest the delayed induction of LTD-like effects. Despite this intriguing finding, the isolated effects of GVS on cortical excitability could not be fully disentangled from the potential effects of the TMS protocol utilised to elicit MEPs since both active and sham conditions involved TMS application. Experiment 2 was conducted to eliminate the potential confounding effects of TMS by employing an alternative measure of cortical excitability, the BP. However, perhaps due to flaws in the methodology, Experiment 2 was unable to elicit an appropriate BP response during baseline recording. Thus, no meaningful changes in cortical excitability post-GVS could be established. Experiment 3, a single case study was therefore designed to correct a few of the methodological limitations present in Experiment 2 and to increase the likelihood of showing GVS modulation of the BP by using a stroke patient who had previously shown clinical benefits following GVS. An appropriate BP response was obtained at baseline and some changes in BP amplitude following stimulation were observed, albeit only descriptively.

Interestingly, the delayed inhibitory effect of GVS on MEPs at the 24-hour post-stimulation follow-up in Experiment 1 was observed only in participants with high cortical excitability at baseline. One potential explanation for this finding stems from the theory of homeostatic metaplasticity, proposed by Bienenstock, Cooper and Munro (1982). It is often used to explain why the same brain stimulation protocol can induce both LTP- and LTD-like

effects. According to the BCM model, homeostatic metaplasticity is the mechanism through which the brain maintains its level of synaptic plasticity within a natural physiological range (Cooper & Bear, 2012). It enables control of synaptic plasticity by preventing excessive LTP or LTD (Ziemann & Siebner, 2008) which is associated with several neurological disorders. An important consequence of this theory is that the induction of LTP and LTD necessarily depends on the history of activation within neuronal circuits (Rioult-Pedotti, Friedman & Donoghue, 2000; Müller-Dahlhaus & Ziemann, 2015). It follows then that a single stimulation protocol can produce differential effects on synaptic plasticity. A synaptic history of high activation will facilitate the subsequent induction of LTD by a stimulation protocol. Conversely, a history of low synaptic activation will increase the chances of LTP induction by a stimulation protocol. Studies that have documented this effect in humans generally utilise a 'priming' stimulation protocol to trigger the homeostatic response followed by a subsequent 'test' protocol, which captures the response. For example, Siebner et al. (2004) showed that a 1Hz rTMS protocol could induce both an inhibitory and excitatory effect depending on the priming protocol applied. A priming session with facilitating, anodal tDCS led to reductions in cortical excitability following a subsequent session of 1Hz rTMS. On the other hand, a priming session of inhibitory, cathodal tDCS caused the subsequent rTMS protocol to increase cortical excitability. These findings suggest that when synaptic activity is in a state of low activation, it favours LTP induction and conversely when there is high excitability, LTD induction is favoured (Karabanov et al., 2015). Thus, it is possible that the pre-existing high level of cortical excitability present in the subgroup of our sample may have interacted with the effects of GVS such that it favoured an inhibitory effect. This may suggest a potential homeostatic mechanism underlying the clinical effects of GVS that may be specific to high cortical excitability states.

Reducing high cortical excitability is of clinical relevance as it has been associated with cognitive impairment. A recent study investigating individual differences in cortical excitability in healthy participants has shown that high cortical excitability is strongly correlated with attentional problems and mood disturbances as measured by a battery of neuropsychological tests (Bolden, Griffis, Pati & Szaflarski, 2017). This is consistent with evidence from patients with neuropsychological conditions such as attention-deficit/hyperactivity disorder (ADHD), epilepsy, and schizophrenia who present with comorbid motor cortical hyperexcitability and cognitive deficits (Badawy et al., 2012; Hasan et al., 2013). It is possible that the synaptic mechanism underlying this hyperexcitability may reflect unchecked LTP-like plasticity. The findings from Experiment 1 warrant future investigations into the clinical effects of GVS on these hyperexcitability disorders. Given that GVS has already been shown to ameliorate attentional deficits in hemi-spatial neglect (Wilkinson et al., 2014; Zubko et al., 2013), it is also likely to reduce the attentional problems associated with these disorders. Moreover, future studies should not only examine the effects of GVS on neuropsychological tests but concomitantly assess cortical excitability to investigate whether reductions in cortical excitability accompany reductions in attentional and mood-related problems. This would provide further evidence that GVS may elicit LTD-like effects in a homeostatic fashion to cortical activity that may reflect excessive LTP in the human cortex.

One important feature of the results from Experiment 1 that previous research investigating homeostatic plasticity in humans does not necessarily explain is the delayed inhibitory effect that emerged only after 24 hours following stimulation. Previous studies investigating the effects of brain stimulation protocols on homeostatic metaplasticity and cortical excitability in general tend to monitor only the first few hours after stimulation, thereby missing important effects potentially still evident for days following stimulation

(Krause & Kadosh, 2014; Hoogendam, et al., 2010). These studies have focused primarily on early-phase long-term plasticity, which accounts for the maintenance of LTP and LTD within minutes and/or hours following stimulation. By contrast, studies of animal slice preparations distinguishing early and late phase plasticity have demonstrated that late-phase LTP and LTD can start after a delay of up to four hours after multiple stimulation sessions and can last for days and weeks (Abraham, 2003; Linden, 1998). Early- and late-phase plasticity are further differentiated by distinct molecular mechanisms. For instance, changes in gene expression and synthesis of new proteins appear to underlie late-phase mechanisms whereas early-phase plasticity depends mainly on NMDA receptor activity (Clopath et al., 2008; Reymann & Frey, 2007). The few studies that investigate early- and late-phase LTP and LTD in humans corroborate the findings from animal studies, suggesting similar processes occur in the human cortex. One study showed that administration of repeated sessions of anodal tDCS with an inter-stimulation interval of 20 minutes produced persistent increases in cortical excitability that were still evident 24 hours following stimulation, which may indicate the induction of late LTP (Monte-Silva et al., 2013). Reductions in cortical excitability, possibly indicating late LTD, have been shown to a lesser extent by the same authors (Monte-Silva et al., 2010). Similarly, a decrease in cortical excitability was evident until late evening following repeated administrations of tDCS in the morning. Thus, it is possible that GVS may trigger a delayed response on cortical excitability, reflecting late-phase LTD. This may be consistent with clinical studies investigating the clinical carryover effects of GVS (Zubko et al., 2013; Wilkinson et al., 2014).

One question that may arise is that induction of late-phase long-term plasticity generally follows repeated sessions of stimulation whereas only a single session of GVS was administered in Experiment 1. However, it has already been shown clinically that a single session of GVS is as efficacious in treating a neurological condition as several sessions

(Wilkinson et al., 2014). The carryover effects on behavioural symptoms of hemi-spatial neglect from a single session of GVS were comparable to five and ten sessions, lasting up to four weeks following treatment. This contradicts previous findings demonstrating the superior effects of repeated stimulation over single administrations on lasting functional recovery (Kleinjung et al., 2005; Shindo et al., 2006). The induction of late-phase plasticity in this experiment helps to explain the surprising results from this RCT. Both Experiment 1 and the RCT conducted by Wilkinson et al. (2014) suggest that a single session of GVS may be sufficient to induce lasting changes in synaptic plasticity (Cooke & Bliss, 2006). To further correlate the clinical effects of GVS with the findings from Experiment 1, a longer time frame assessing its effects on cortical excitability is required. For example, future studies should measure cortical excitability for days and weeks following GVS.

Experiment 2 sought to overcome the problems inherent in using TMS-elicited MEPs in Experiment 1. However, factors related to methodology may have prevented the acquisition of BP at baseline in the sample. First, the acquisition of poor quality data that contained a high number of artefacts in the form of drift and noise led to exclusion of 17 participants. The analysable data obtained from the remaining 7 participants also showed a high number of artefacts which led to the exclusion of roughly half of all trials. This was perhaps due to uncontrollably high temperatures in the laboratory in which the experiment was taking place. Participants were therefore more likely to move and perspire causing drift and noise in the raw EEG data. Second, it was questionable whether the cup electrode were placed in the optimum locations to detect signals from the SMA and premotor cortices. Several authors have also reported the high intra-individual variability and low reproducibility of the BP component (Dick et al., 1987; Evidente et al., 1999). Several other factors not taken into account in this experiment are also known to influence the amplitude and latency of the BP, including level of intention; force exerted; speed and complexity of

movements (Lang, 2003). Therefore, Experiment 2 warranted further investigation in a third experiment which implemented some methodological changes that potentially increased the chances of eliciting a BP.

Experiment 3 involved a single case study of a stroke patient using an identical methodology to Experiment 2 with the exception of a few important alterations. These included utilising an electrode cap; increasing the number of movements performed by the subject and conducting the experiment in an air-conditioned laboratory. The findings of Experiment 3 revealed the successful acquisition of MRCPs from a stroke patient and a potential reduction in BP amplitude at Fz and C4 following GVS. Activity in Fz is often associated with decision making and attentional processes often reported in experiments investigating the P300 (Polich, 2003). However, the relevance of Fz to the BP is unclear. Given that the P300 represents a largely distinct activation of Fz to the BP, it is unlikely that it provides a relevant explanation for the reduction observed in Fz. The reduction observed at C4 30 minutes following GVS is more in line with results from the BP literature as these more frequently report changes at central electrodes overlaying motor regions (Shibaski & Hallett, 2006). This result is also consistent with those obtained in Experiment 1 in that they both demonstrate a decrease in cortical excitability following GVS, which may indicate an inhibitory effect that resembles LTD-like plasticity. However, levels of cortical excitability in patient 001 return to baseline levels 24 hours after GVS, unlike in Experiment 1 where this reduction is only observed at the 24 hour time point. This may be attributed to the known differences in BP presentation between brain-damaged and healthy individuals (Shibasaki & Hallett, 2006). Nevertheless, it is important to note that no definitive conclusions can be drawn from these descriptive results. Instead these findings demonstrate the need to further investigate the effects of GVS on the BP in neurological disease.

One other consideration is that the BPs obtained from patient 001 may not have been abnormal as is frequently the case with stroke patients. Previous studies have found that the BP in stroke patients can be smaller in amplitude compared to controls, but only in the acute phase. Indeed, BP recovery in stroke patients can be observed from eight to ten months following stroke (Gerloff et al., 1996). Given that patient 001 suffered a stroke almost eight years ago and has since presented with an almost full recovery, it is possible that his BPs may have recovered to levels akin to those of healthy participants. They may therefore be less amenable to change from GVS. Nevertheless, obtaining the BP in Experiment 3 provides sufficient justification to apply this experimental paradigm to a clinical or healthy group study.

Conclusions

The set of experiments presented here provide preliminary evidence that synaptic plasticity underlies the clinical benefits of GVS. Specifically, the findings of Experiment 1 hint that GVS may engage homeostatic plasticity to inhibit high cortical excitability. This effect was delayed to 24 hours following stimulation potentially indicating late-phase plasticity, which may correlate with previous clinical findings (Wilkinsons et al., 2014). The potential clinical implications of this finding relate to the application of GVS to treat neurological and psychiatric disorders that present with cortical hyperexcitability. These include epilepsy, schizophrenia, Tourette's syndrome, ADHD and possibly autism (Badawy et al., 2012; Hasan et al., 2013; Bolden et al., 2017). The therapeutic application of GVS to hemi-spatial neglect, Parkinson's disease and prosopagnosia has already shown promise. Therefore, the findings from Experiment 1 extend the repertoire of potential clinical applications for GVS. Future clinical research should investigate the clinical effects of GVS on these hyperexcitability disorders. The findings from Experiments 2 and 3 are less clear, however, they warrant further investigations to corroborate the results obtained from

Experiment 1. Indeed, the demonstration that an appropriate BP response can be elicited in a single patient using the methodology in Experiment 3 leads to the conclusion that future group studies must be conducted to further investigate whether GVS can modulate the BP.

Although this is the first attempt to measure synaptic plasticity changes in humans following vestibular stimulation, it has provided valuable new knowledge for understanding the clinical effects of GVS. To provide further evidence of GVS as a useful treatment tool, mechanisms of effect must be established. Uncovering such mechanisms would guide future research in assessing effectiveness of GVS dosages and the application of GVS to a wider range of neurological disorders.

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Appendix A

TMS Safety screening questionnaire

University of Kent

School of Psychology
Transcranial Magnetic Stimulation Laboratory
PI: Dr Joseph Brooks, J.L.Brooks@kent.ac.uk

Safety Screening Questionnaire for Transcranial Magnetic Stimulation (TMS)
(Version 1.5, 06 Nov 2014)

Participant Name/ID: _____ **Date:** _____

Current Age: _____ (in years) **Handedness:** Left Right Ambi **Sex:** M F Other

ALL INFORMATION WILL BE TREATED CONFIDENTIALLY

(1) Have you ever had an adverse reaction to TMS? If so, please describe _____	☐ Yes	☐ No	
(2) Do you have epilepsy or have you ever had a seizure/convulsion?	☐ Yes	☐ No	
(3) Have you ever had a fainting spell or syncope? If yes, describe.	☐ Yes	☐ No	
(4) Have you ever had a stroke?	☐ Yes	☐ No	
(5) Have you ever had a serious head injury (with loss of consciousness)?	☐ Yes	☐ No	
(6) Have you ever had neurosurgery of any type (including brain or spinal cord)?	☐ Yes	☐ No	
(7) Do you have hearing problems or ringing in your ears?	☐ Yes	☐ No	
(8) Do you have any metal in your body such as shrapnel, surgical clips, or fragments from welding or metalwork?	☐ Yes	☐ No	
(9) Do you have any implanted devices such as cardiac pacemakers, aneurysm clips, cochlear implants, medical pumps, deep brain stimulators, or intracardiac lines?	☐ Yes	☐ No	
(10) Do you have a medication infusion device?	☐ Yes	☐ No	
(11) Do you suffer from frequent or severe headaches?	☐ Yes	☐ No	
(12) Have you ever had any other brain-related condition (including Psychiatric diagnoses)?	☐ Yes	☐ No	
(13) Have you ever had any illness that caused brain injury?	☐ Yes	☐ No	
(14) Are you taking any psychiatric or neuroactive medications? For instance, anti-depressants, anti-anxiety, anti-psychotics, anti-convulsants, or anything else with nervous system effects? (please list) _____	☐ Yes	☐ No	
(15) Are you taking any other medications or other drugs/substances? Please list. If any of these substances are illegal, please do still mark "yes" but do not write the name. We will contact you to confidentially discuss this in person to see whether TMS will be safe for you. _____	☐ Yes	☐ No	
(16) Are you pregnant or do you have any reason to believe that you may be?	☐ Yes	☐ No	
(17) Do you, or does any family member, have epilepsy/history of seizures?	☐ Yes	☐ No	
(18) Do you hold a heavy goods vehicle driving license or bus license?	☐ Yes	☐ No	
(19) Have you consumed alcohol in the past 24 hours?	☐ Yes	☐ No	
(20) Did you have adequate sleep last night?	☐ Yes	☐ No	
(21) Have you participated in a TMS study within the past 24 hours?	☐ Yes	☐ No	

Potential Contraindication Drugs

Strong Potential Hazard: imipramine, amitriptyline, doxepine, nortriptyline, maprotiline, chlorpromazine, clozapine, foscarnet, ganciclovir, ritonavir, amphetamines, cocaine, (MDMA, ecstasy), phencyclidine (PCP, angel's dust), ketamine, gamma-hydroxybutyrate (GHB), alcohol, theophylline

Relative Potential Hazard: mianserin, fluoxetine, fluvoxamine, paroxetine, sertraline, citalopram, reboxetine, venlafaxine, duloxetine, bupropion, mirtazapine, fluphenazine, pimozide, haloperidol, olanzapine, quetiapine, aripiprazole, ziprasidone, risperidone, chloroquine, mefloquine, imipenem, penicillin, ampicillin, cephalosporins, metronidazole, isoniazid, levofloxacin, cyclosporin, chlorambucil, vincristine, methotrexate, cytosine arabinoside, BCNU, lithium, anticholinergics, antihistamines, sympathomimetics

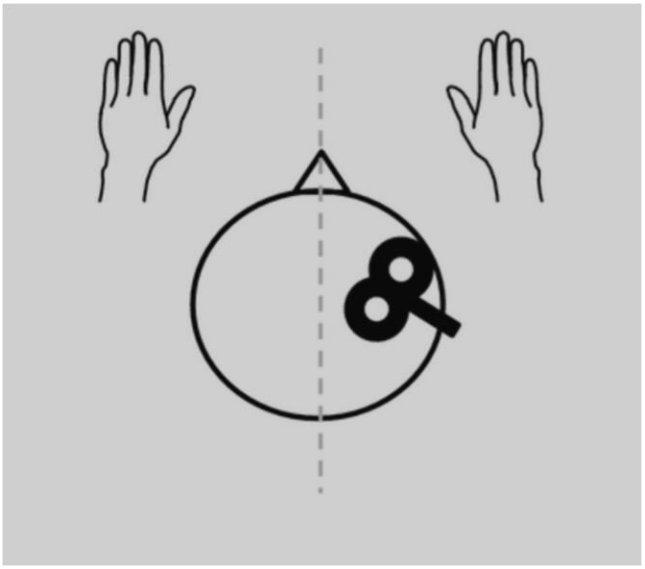
Withdrawl Hazard: alcohol, barbiturates, benzodiazepines, meprobamate, chloral hydrate.

Appendix B

GVS device

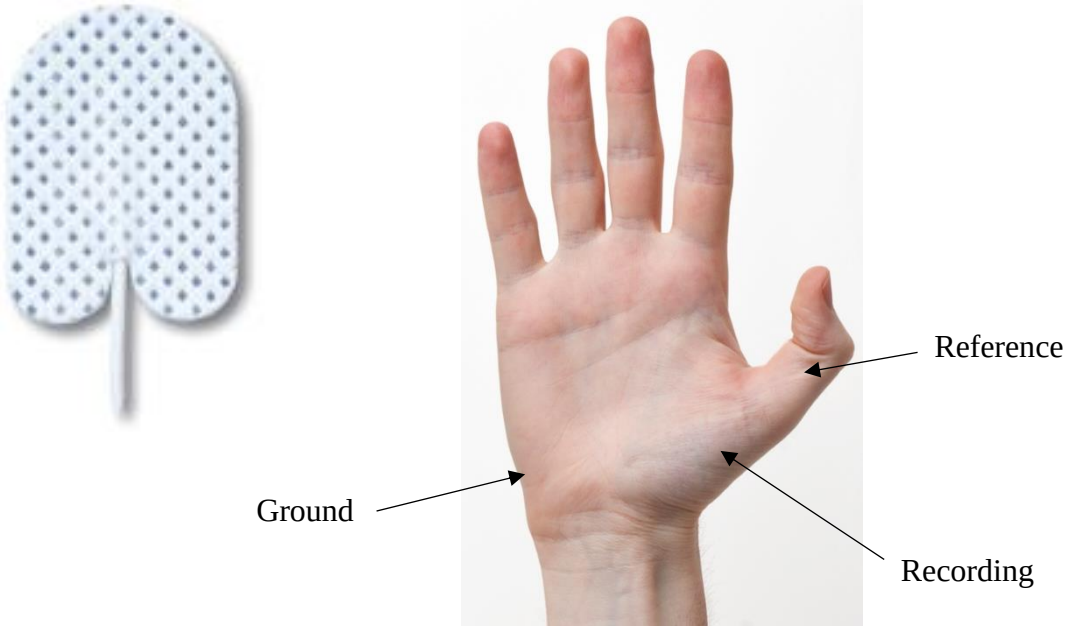
Appendix C

TMS coil positioning



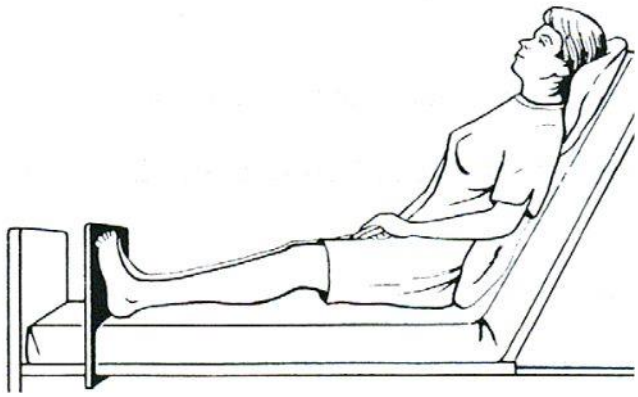
Appendix D

EMG electrode placement



Appendix E

Participant set-up



Standard Fowler's position

Cosmo Radi+ Beauty Couch

MAG&More vacuum cushion and pump

Appendix F

Perception of Stimulation Questionnaire

Participant number: _____.

Session Number:_____.

Condition:_____.

Did you notice the stimulation? **Yes / No**

If yes please answer the following:

Did it feel like you were moving?			
Sway	Vibration	Rotation	Other

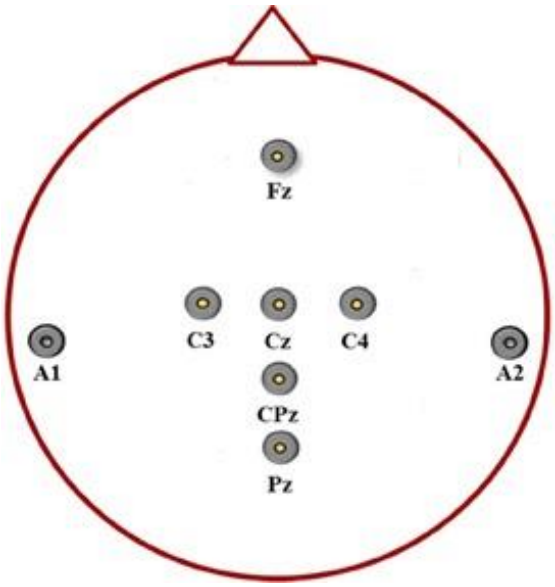
Which way were you moving?			
Through the head and feet (= Yaw)	Through the nose and occiput (= Roll)	Through both ears (= pitch)	Other Axis?

Was it just your head or your whole body that felt like it was moving?	
a) Your whole body?	b) Only your head?

Did you feel anything else?

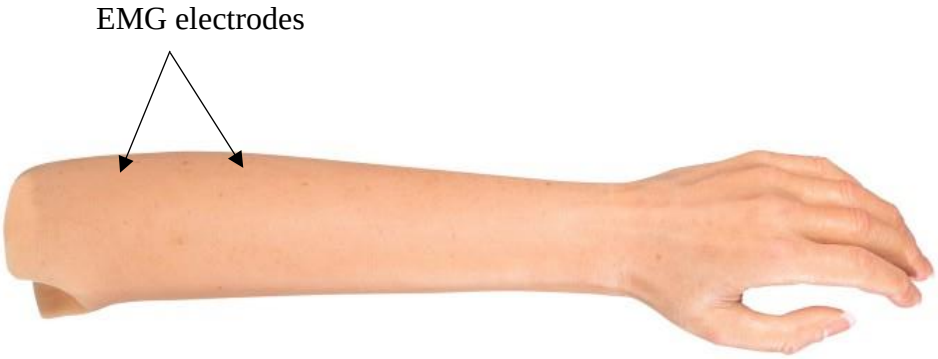
Appendix G

EEG cup electrodes and configuration



Appendix H

EMG electrode configuration (Experiment 2)



Appendix I

Edinburgh Handedness Inventory

Name: **Age:** **Sex:**

Please tick the appropriate box for each of the following questions:

Which hand do you use:					
	Always left	Usually left	No preference	Usually right	Always right
To write a letter legibly?					
To throw a ball to hit a target?					
To hold a racquet?					
To hold a match while striking it?					
To cut with scissors?					
To guide a thread through the eye of a needle (or guide needle onto thread)?					
At the top of a broom while sweeping?					
At the top of a shovel when moving sand?					
To deal playing cards?					
To hammer a nail into wood?					
To hold a toothbrush while cleaning teeth?					
To unscrew the lid of a jar?					