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HIGH FREQUENCY STIMULATION ALTERS MOTOR MAPS, IMPAIRS SKILLED REACHING PERFORMANCE AND IS ACCOMPANIED BY AN UPREGULATION OF SPECIFIC GABA, GLUTAMATE AND NMDA RECEPTOR SUBUNITS

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Abstract—High frequency stimulation (HFS) has the potential to interfere with learning and memory. HFS and motor skill training both lead to potentiation of the stimulated network and alter motor map expression. However, the extent to which HFS can interfere with the learning and performance of a skilled motor task and the resulting effect on the representation of movement has not been examined. Moreover, the molecular mechanisms associated with HFS and skilled motor training on the motor cortex are not known. We hypothesized that HFS would impair performance on a skilled reaching task, and would be associated with alterations in motor map expression and protein levels compared to non-stimulated and untrained controls. Long Evans Hooded rats were chronically implanted with stimulating and recording electrodes in the corpus callosum and frontal neocortex, respectively. High frequency theta burst stimulation or sham stimulation was applied once daily for 20 sessions. The rats were divided into five groups: control, HFS and assessed at 1 week post stimulation, HFS and assessed 3 weeks post stimulation, reach trained, and HFS and reach trained. A subset of rats from each group was assessed with either intracortical microstimulation (ICMS) to examine motor map expression or Western blot techniques to determine protein expression of several excitatory and inhibitory receptor subunits. Firstly, we found that HFS resulted in larger and reorganized motor maps, and lower movement thresholds compared to controls. This was associated with an up-regulation of the $GABA_A\alpha1$ and NR1 receptor subunits 3 weeks after the last stimulation session only. Stimulation affected skilled reaching performance in a subset of all stimulated rats. Rats that were poor performers had larger rostral forelimb areas, higher proximal and lower distal movement thresholds compared to rats that were good performers after stimulation. Reach training alone was associated with an up-regulation of $GABA_A\alpha1$, $\alpha2$, GluR2, NR1 and NR2A compared to controls. HFS and reach-trained rats showed an up-regulation of $GABA_A\alpha2$ compared to stimulated rats that were not reach-trained. Therefore, we have shown that HFS induces significant plasticity in the motor cortex, and has the potential to disrupt performance on a skilled motor task.

Key words: high frequency stimulation, cortex, movement representation, skilled reach training, plasticity, Long Evans Hooded rat.

INTRODUCTION

It is widely accepted that centrally applied electrical stimulation has the potential to interfere with learning and memory processes. For example, it has been shown in rodents that high frequency stimulation (HFS) applied to the perforant path sufficient to induce long-term potentiation (LTP) in the hippocampus results in the inability to find a hidden platform in the water maze test (Castro et al., 1989; Moser et al., 1998; McNaughton et al., 1986), suggesting a deficit in spatial learning and memory. Similar behavioural deficits have been shown on the Barnes circular platform task (Barnes et al., 1994) after bilateral perforant path stimulation and on procedural memory task strategy after dorsal striatum stimulation (Schumacher et al., 2011). Recently, this phenomenon was demonstrated in human populations as well, where stimulation of the right inferior parietal cortex via transcranial direct current stimulation produced selective impairments on a working memory task (Berryhill et al., 2010).

Despite the many studies examining the effect of stimulation on primarily spatial learning tasks, few studies have examined how skilled motor behaviour is affected after callosal stimulation. Skilled motor learning may occur through an LTP-like process in the motor cortex (Rioult-Pedotti et al., 2000; Monfils and Teskey, 2004), and the application of both HFS and low frequency stimulation (LFS) can disrupt motor skill acquisition when applied during the training phase (Hodgson et al., 2005). However it is not known how the application of HFS affects subsequent skilled motor learning.

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Abbreviations: HFS, High frequency stimulation; ICMS, intracortical microstimulation; LFS, low frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; RFA, rostral forelimb area.

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Motor maps are a representation of how movement is organized in the cortex, and their expression can be altered after cortical injury (Nudo and Milliken, 1996), tactile experience (Keller et al., 1996), seizures (Teskey et al., 2002; Henderson et al., 2011), the induction of cortical LTP (Monfils et al., 2004), cortical long-term depression (LTD) (Teskey et al., 2007) and motor skill learning (Kleim et al., 1998, 2002, 2004). Neocortical LTP induction with HFS has been shown to result in the enlargement of the caudal forelimb area (Monfils et al., 2004) of the motor map, but other characteristics of motor map expression (movement representation over-lap, movement thresholds) and the associated molecular changes at the level of receptor subunit expression have not been reported. Additionally, determining the behavioural consequences and associated molecular mechanisms of electrical stimulation on skilled reaching performance has received little attention.

Thus the objectives of this study were to; firstly (in Experiment 1) determine how electrical stimulation can change the organization of the motor cortex by applying HFS and examining the expression of motor maps, and by examining the associated expression of several excitatory and inhibitory receptor subunits. Secondly (in Experiment 2), examine how skilled reach-training is affected after HFS, and how skilled motor behaviour can induce further plasticity and molecular changes after stimulation. We hypothesized that HFS would result in larger and reorganized motor maps, and would be associated with molecular changes reflecting increased excitability. We also hypothesized that HFS would result in an impairment in skilled reaching behaviour. Using HFS which leads to a well-characterized increase in synaptic strength, and examining the resulting effects on plasticity and skilled motor performance may enhance our knowledge of brain and behaviour relationships, and could potentially contribute to treatments for various behavioural and neurological disorders where brain stimulation is a therapeutic option.

Experiment 1. Effect of HFS on motor map and receptor subunit protein expression

EXPERIMENTAL PROCEDURES

Animals

Forty adult male Long-Evans Hooded rats were used in this experiment. They were obtained from the University of Calgary breeding colonies and Charles River (Saint-Constant, QC). They were housed individually under standard laboratory conditions where food and water were always available. The colony room was maintained on a 12 h on/off cycle, with lights on at 07:00, and all experimentation was conducted during the light phase. All protocols were approved by the University of Calgary animal care committee and were in accordance with the Canadian Council on Animal Care guidelines.

Treatment groups

The rats were divided into three groups, where all groups received chronically implanted electrodes. The control group received no stimulation (control) and was divided to receive ICMS to map movement representations ($n = 9$) or tissue was taken for Western blotting experiments ($n = 6$). Twenty five rats received HFS and were divided as follows: a subset (HFS 1 week, $n = 5$) was mapped with ICMS within 1 week of the last stimulation session, or tissue was taken for Western blotting experiments within 1 week of the last stimulation session (HFS 1 week, $n = 6$). An additional subset of stimulated rats was mapped 3 weeks after the last stimulation session (HFS 3 week, $n = 10$) or tissue was taken 3 weeks after the last stimulation session for Western blotting experiments (HFS 3 week, $n = 4$). Data from the rats in Experiments 1 and 2 (described below) were collected at approximately the same time (within weeks).

Chronic electrode implantation

Teflon-coated stainless steel wire (178 μm in diameter, A-M Systems, Everett, WA, USA) was twisted to produce bipolar stimulating and recording electrodes. Gold-plated amphenol pins were connected to one end of the wires that were stripped of insulation, and the other ends were separated by 1.0 mm.

During the surgical procedure rats were anaesthetized using isoflurane (3% induction and 1.5–2.5% maintenance), and anaesthesia levels were monitored by checking the response to a tail pinch. Lidocaine 2% was administered subcutaneously at the incision site to further minimize any discomfort. Two bipolar electrodes were permanently implanted into the right hemisphere according to the stereotaxic coordinates of Swanson, 1992. One electrode was placed in the corpus callosum (1.0 mm anterior to bregma, 0.5 mm lateral to midline and lowered to a depth that optimized the evoked potential ~3 mm), and one in the sensorimotor neocortex (1.0 mm anterior to bregma, 4.0 mm lateral to midline, and 1.5 mm ventral to the brain surface). The amphenol pins were then inserted into a nine-pin McIntyre connector plug (Ginder Science, Ottawa, ON, Canada) and secured to the skull with dental cement and five stainless steel screws. One of the screws served as the ground reference. The left hemisphere remained free of electrodes, screws and cement to permit the craniotomy required during the ICMS procedure. Experimental procedures commenced 7 days after electrode implantation. All rats were implanted with chronic electrodes.

HFS

High-frequency theta patterned stimulation was delivered to the corpus callosum for 20 days (once a day, five times a week, 4 weeks), as this has been shown to reliably induce potentiation (Racine et al., 1995; Teskey and Valentine, 1998). The HFS consisted of eight biphasic square wave pulses, with each pulse 200 μs in duration, at an intensity of 1000 μA, applied at 100 Hz. The pulses were delivered as two-paired trains of four pulses, each separated by 150 ms. Thirty sessions were delivered at each session, at 0.1 Hz. Following the HFS sessions, I/O measures were collected once more to assess the changes in evoked potential amplitude and shape. Animals remained quiet and immobile during each session.

Intracortical microstimulation (ICMS)

Twenty-four rats (control: $n = 9$, HFS 1 week: $n = 5$, HFS 3 week: $n = 10$) underwent standard ICMS procedures to derive detailed maps of forelimb movement representations (Nudo et al., 1990; Kleim et al., 1998; Teskey et al., 2007). Twelve hours prior to surgery, rats were food restricted, but had water *ad libitum*. On the day of surgery, an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/kg) was given. Subsequent injections of ketamine (25 mg/kg), or a cocktail of both ketamine (17 mg/kg) and xylazine (2 mg/kg) were delivered as needed during the surgery to maintain a constant level of anaesthesia, monitored by observing changes in breathing rate, vibrissae whisking, and response to a pinch of the hindlimb or tail.