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Research Paper

Low intensity rTMS has sex-dependent effects on the local response of glia following a penetrating cortical stab injury

Darren Clarke [a,b], Marissa A. Penrose [a], Alan R. Harvey [b,c], Jennifer Rodger [a,c], Kristyn A. Bates [a,*]

[a] School of Biological Sciences, The University of Western Australia, 35 Stirling Hwy, Perth, WA, Crawley, Western Australia 6009, Australia
[b] School of Human Sciences, The University of Western Australia, 35 Stirling Hwy, Perth, WA, Crawley, Western Australia 6009, Australia
[c] Perron Institute for Neurological and Translational Science, Perth, WA, Australia

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ABSTRACT

Repetitive transcranial magnetic stimulation (rTMS), a non-invasive form of brain stimulation, has shown experimental and clinical efficacy in a range of neuromodulatory models, even when delivered at low intensity (i.e. subthreshold for action potential generation). After central nervous system (CNS) injury, studies suggest that reactive astrocytes and microglia can have detrimental but also beneficial effects; thus modulating glial activity, for example through application of rTMS, could potentially be a useful therapeutic tool following neurotrauma. Immunohistochemistry was used to measure the effect of low intensity rTMS (LI-rTMS) on GFAP (astrocyte), IBA1 (microglial), and CS56 (proteoglycan) expression in a unilateral penetrating cortical stab injury model of glial scarring in young adult and aged male and female C57BL6/J mice. Mice received contralateral low frequency, ipsilateral low frequency, ipsilateral high frequency or sham LI-rTMS (4–5 mT intensity), for two weeks following injury. There was no significant difference in the overall volume of tissue containing GFAP positive ($^+$) astrocytes, IBA1$^+$ microglia, or proteoglycan expression, between sham and LI-rTMS-treated mice of all ages and sex. Importantly however, the density of GFAP$^+$ astrocytes and IBA1$^+$ microglia immediately adjacent to the injury was significantly reduced following ipsilateral low and high frequency stimulation in adult and aged females ($p \leq 0.05$), but was significantly increased in adult and aged males ($p \leq 0.05$). LI-rTMS effects were generally of greater magnitude in aged mice compared to young adult mice. These results suggest that sex differences need to be factored into therapeutic rTMS protocols. In particular, more work analyzing frequency and intensity specific effects, especially in relation to age and sex, is required to determine how rTMS can best be used to modify glial reactivity and phenotype following neurotrauma.

1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a form of non-invasive brain stimulation that harnesses the process of electromagnetic induction (Pell et al., 2011). rTMS modulates cortical excitability and has shown some experimental and clinical efficacy in a range of neurological and psychological conditions, such as depression (George et al., 1995; Martiny et al., 2010; Pascual-Leone et al., 1996) and stroke (Khedr et al., 2005; Takeuchi et al., 2005). rTMS modulates neuroplasticity (Siebner and Rothwell, 2003), possibly through modulating neurotransmitter release (Yue et al., 2009) and post-synaptic strength (Vlachos et al., 2012). While most rTMS research focuses on the effects of electromagnetic pulses on neurons, recent studies have shown that electromagnetic fields also have the capability to influence glial cell biology (as reviewed in Cullen and Young, 2016).

Many factors influence the phenotypic state of CNS glia. Altered reactivity of astrocytes and microglia can have both beneficial and inhibitory effects on plasticity and repair following brain injury. Glial cell activation is beneficial via facilitating blood brain barrier repair and attenuating secondary degeneration (Burda and Sofroniew, 2014; Bush et al., 1999). Conversely, reactive astrocytes and microglia ultimately contribute to neuroinflammation and inhibit axon regrowth (Busch and Silver, 2007; McKeon et al., 1991); activated astrocytes produce extracellular matrix, which is deposited into a fibrous scar, a major impediment to neuronal growth in and around the injury site (Wiese et al., 2012). Glial reactivity and scarring is also influenced by factors such as age and sex (Anderson et al., 2014; Colton, 2009). Endogenous male and female sex hormones have shown various levels of neuroprotective and gliosis-restricting properties after brain injury (Garcia-Estrada et al., 1993; García-Estrada et al., 1999; Pettus et al., 2005; Wagner et al., 2004). Finally, accelerated reactive astrocyte and microglial activity is

* Corresponding author at: MBDP 317 School of Biological Sciences, The University of Western Australia, 35 Stirling Hwy, Perth, WA 6009, Australia.
E-mail address: kristyn.bates@uwa.edu.au (K.A. Bates).

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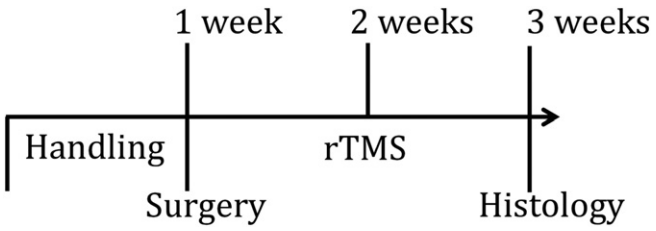

Fig. 1. Experimental timeline. A) After a week of daily handling, mice received a unilateral cortical stab. rTMS (ipsilateral sham, 1 Hz, BHFS, or contralateral 1 Hz) was applied daily from 24 h after injury for 10 min for two weeks.

observed in aged rodents after injury, which in turn, is associated with poorer functional outcome (Kumar et al., 2013; Kyrkanides et al., 2001).

Modulation of glial activity and enhancing neuroplasticity after CNS injury are therefore potentially attractive therapeutic targets (Yiu and He, 2006). Application of high field intensity rTMS following a cerebellar lesion in rats has been shown to reduce glial activation in the pontine nuclei (Sasso et al., 2016), however it is unclear whether rTMS is directly or indirectly affecting glia. We have previously found low and high rTMS frequencies, applied at low intensity (LI-rTMS) to rodents after stroke, increased macrophage infiltration, suggestive of altered blood brain barrier repair (Bates et al., 2012). Recently, we found that 1 Hz low intensity magnetic stimulation (LI-rMS: in vitro, no cranium) increased astrocytic intracellular calcium levels in astrocyte-enriched cultures (Penrose et al., Unpublished results). The present study examined the effects of LI-rTMS in adult (3 month) and aged (18 month) male and female mice following a penetrating cortical stab injury. Post-injury, we applied LI-rTMS daily for two weeks and then used immunohistochemistry to characterize LI-rTMS-induced changes in the phenotype of cells involved in glial scarring. We used well-established markers of astrocyte (GFAP) and microglia (IBA1) reactivity, and also tested for changes in proteoglycan immunoreactivity. The volume and density of these markers was quantified, revealing differential age- and sex-specific effects on glial cell reactivity elicited by different LI-rTMS frequencies.

2. Materials and methods

2.1. Animal source and habituation procedure

C57BL/6 J mice of 3 months of age (20 male, 20 female) and 18 months of age (20 male, 20 female) were sourced from the Animal Resource Centre (Canning Vale, WA, Australia) and housed at the Pre-Clinical Facility, University of Western Australia. Mice were housed under controlled conditions, with a 12 h light–dark cycle and food and water ad libitum. A maximum of 5 mice of a single sex were housed in each open top cage and animals were monitored daily for general health and wellbeing. Mice were allowed to acclimatize to the animal facility for one week. Because anaesthesia can alter the efficacy of rTMS induction of neuroplasticity mechanisms (Gersner et al., 2011; Sykes et al., 2016), we chose to conduct our stimulation on awake, freely moving mice. The mice were handled for up to 10 min per day for a week in preparation for rTMS delivery similar to previous studies (Makowiecki et al., 2014; Rodger et al., 2012). Mice were separated into eight experimental treatments based on age, sex, and rTMS intervention. Experimental procedures were approved by The University of Western Australia (UWA) Animal Ethics Committee (03/100/1380), under guidelines set by the National Health and Medical Research Council (NHMRC).

2.2. Surgical procedure – penetrating cortical stab injury

Mice were anaesthetised with ketamine and medetomidine (75 mg/kg and 1 mg/kg i.p. respectively, Troy Laboratories, NSW, Australia) and all mice received a unilateral penetrating cortical stab wound to the right hemisphere (Wang et al., 2007). Briefly, anaesthetized mice were placed in a stereotaxic frame and underwent a craniotomy at 1.8 mm posterior and 1.5 mm right of midline from bregma (Paxinos and Franklin, 2004). This location provided a reproducible injury site, and allowed the hippocampal dentate gyrus to be used as a reference point for histological analysis of the stab injury. A 23-gauge needle (BD PrecisionGlide™ #305143, NSW, Australia) attached to a

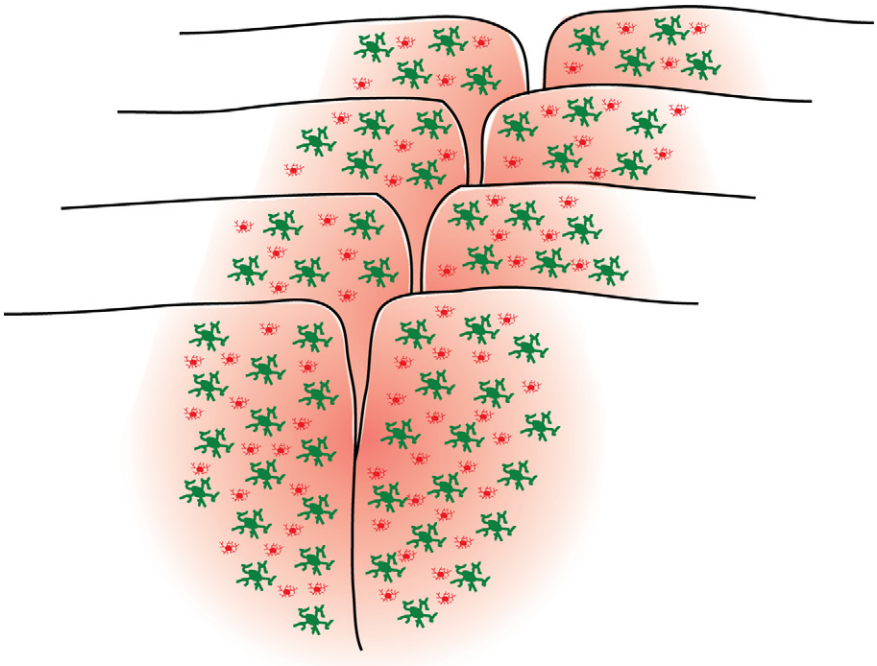

Fig. 2. Diagram of image analysis. Representation of lesion volume, astrocyte (green), microglial (red) and proteoglycan (shading) expression around the cortical stab on consecutive sections.