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LOW-INTENSITY REPETITIVE MAGNETIC STIMULATION LOWERS ACTION POTENTIAL THRESHOLD AND INCREASES SPIKE FIRING IN LAYER 5 PYRAMIDAL NEURONS *IN VITRO*

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Abstract—Repetitive transcranial magnetic stimulation (rTMS) has become a popular method of modulating neural plasticity in humans. Clinically, rTMS is delivered at high intensities to modulate neuronal excitability. While the high-intensity magnetic field can be targeted to stimulate specific cortical regions, areas adjacent to the targeted area receive stimulation at a lower intensity and may contribute to the overall plasticity induced by rTMS. We have previously shown that low-intensity rTMS induces molecular and structural plasticity *in vivo,* but the effects on membrane properties and neural excitability have not been investigated. Here we investigated the acute effect of low-intensity repetitive magnetic stimulation (LI-rMS) on neuronal excitability and potential changes on the passive and active electrophysiological properties of layer 5 pyramidal neurons *in vitro*. Whole-cell current clamp recordings were made at baseline prior to subthreshold LI-rMS (600 pulses of iTBS, $n = 9$ cells from 7 animals) or sham ($n = 10$ cells from 9 animals), immediately after stimulation, as well as 10 and 20 min post-stimulation. Our results show that LI-rMS does not alter passive membrane properties (resting membrane potential and input resistance) but hyperpolarises action potential threshold and increases evoked spike-firing frequency. Increases in spike firing frequency were present throughout the 20 min post-stimulation whereas action potential (AP) threshold hyperpolarization was present immediately after stimulation and at 20 min post-stimulation. These results provide evidence that LI-rMS alters neuronal excitability of excitatory neurons. We suggest that regions outside the targeted region of high-intensity rTMS are susceptible to neuromodulation and may contribute to rTMS-induced plasticity.

Key words: low-intensity rMS, action potential threshold, spike firing frequency, Intermittent Theta Burst Stimulation.

INTRODUCTION

Repetitive transcranial magnetic stimulation (rTMS) is a popular form of non-invasive brain stimulation used to induce neural plasticity in both clinical and non-clinical populations. rTMS delivers trains of magnetic fields over the scalp which in turn induce electrical currents in the underlying brain. The high-intensity magnetic fields delivered are of the same magnitude of MRI scanners (> 1T) (Ridding and Rothwell, 2007) and can be targeted to stimulate specific brain regions (e.g. motor cortex) and to alter neuronal excitability (e.g. corticospinal excitability). The onset of rTMS-induced changes in corticospinal excitability occurs immediately after stimulation and the effects persist for minutes to hours after stimulation (Huang et al., 2005; Ziemann et al., 2008; Wischnewski and Schutter, 2015). The mechanisms underlying rTMS neuromodulation are unclear, but are believed to involve changes in neuronal membrane properties (Hoppenrath et al., 2016), synaptic and non-synaptic mechanisms (Tang et al., 2015).

While specific regions can be targeted, such that the maximal current induced occurs at the targeted region, regions adjacent also receive stimulation with weaker induced electrical currents and the spread of electrical current from the targeted region (Wagner et al., 2009). The role of low-intensity stimulation in the overall rTMS-induced plasticity remains unclear but studies using extremely low magnetic fields (~0.002 T) have shown changes to neurophysiology (for a review see (Di Lazzaro et al., 2013)) and possibly to cortical excitatory neurotransmission (Capone et al., 2009). In mouse models, we have previously shown that low-intensity rTMS (0.01 T) induces molecular and functional plasticity (Rodger et al., 2012; Makowiecki et al., 2014).

Experimental models of repetitive magnetic stimulation (LI-rMS) using organotypic tissue cultures or brain slices from animals provide a useful adjunct to human studies as they allow direct measurement of

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, after hyperpolarization; AP, action potential; LI-rMS, low-intensity repetitive magnetic stimulation; RMP, resting membrane potential; rTMS, repetitive transcranial magnetic stimulation.

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plasticity at the single-cell level and provide insights into the cellular changes occurring after rTMS (for a review see (Müller-Dahlhaus and Vlachos, 2013; Tang et al., 2015)). Single-cell electrophysiological studies on brain slices of rats that received high-intensity rTMS show changes in the resting membrane potential and evoked spike firing of layer 2/3 fast spiking interneurons two hours after stimulation (Hoppenrath et al., 2016). However the effects of LI-rMS on the electrophysiological properties of cortical excitatory neurons are unknown. To investigate these effects, we employed *in vitro* whole-cell patch clamp electrophysiology on layer 5 pyramidal neurons from mouse motor and somatosensory brain slices. We investigated both passive and active membrane properties and evoked spiking properties following LI-rMS or sham stimulation over a 20-min period post-stimulation. Our results show that LI-rMS does not alter passive membrane properties but increases neural excitability by inducing hyperpolarized action potential thresholds and increases the evoked spike firing rate.

EXPERIMENTAL PROCEDURES

Ethics approval

All procedures were approved by the University of Western Australia animal ethics committee (RA/3/100/1229) which is in accordance with the Australian code of practice for the care and use of animals for scientific purposes.

Slice preparation

C57Bl/6J mice (post-natal days 12–15, of either sex, $n = 11$) were acquired from the Animal Resource Centre (Murdoch, Australia). Juvenile animals were chosen due to the high quality and longevity of the slices that they provide. Mice were terminally anaesthetized with an intra-peritoneal injection of pentabarbitone (> 160 mg/kg) followed by rapid dissection of the brain. Acute brain slices (300 μm thick) were prepared from the motor and somatosensory cortex. Coronal slices of cortex were prepared with a vibrating slicer (Campden Instruments 5000-mz) and ice-cold cutting solution comprising (mM) 125 NaCl, 3 KCl, 0.5 $CaCl_2$, 6 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4 and 10 glucose bubbled with carbogen (5% CO_2/95% O_2). Slices were kept at 35 °C for 1 h in a holding chamber containing carbogen-bubbled artificial CSF (artificial cerebrospinal fluid (ACSF), see below for composition), after which they were held at room temperature until required.

Electrophysiology

Slices received continuous perfusion (~1.5 mL/min) with ACSF comprising (mM) 125 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4 and 25 glucose bubbled with carbogen and maintained at 35 ± 2 °C (Warner Instruments TC-324B). For whole-cell current clamp recordings 6-10 MΩ borosilicate glass patch electrodes (Harvard apparatus GC150F-15, 1.5 mm outer dimeter × 0.86 inner diameter, SDR scientific, Australia) were filled with an internal solution comprising (mM) 135 potassium gluconate, 10 HEPES, 7 NaCl, 2 Na_2ATP, 0.3 Na_3GTP, 2 $MgCl_2$.

Slices were visualized at 40× magnification under bright field and infrared differential interference contrast video microscopy (Olympus BX51-WI). Somatic recordings were made using a Multiclamp 700B (Axon Instruments) and digitized with a Digidata 1440A, under the control of Axograph (Axograph X 1.5.4) and data acquired at a sampling rate of 50 kHz.

Whole-cell current clamp recordings were conducted on layer 5 (L5) pyramidal neurons. As we hypothesized *a priori* that stimulation would alter neuronal excitability and membrane properties, whole-cell recordings were made without applying holding currents during experimental procedures.

To investigate action potential (AP) properties (AP threshold, spike rise time, spike height, fast after hyperpolarization), single AP's were evoked with a 5-ms long depolarizing current step of +800 pA (Fig. 1D), repeated every second for a total of 10 s (i.e. 10 single AP's per recording).

To investigate the spike firing properties, spikes were evoked with an AP family protocol (Fig. 1C) consisting of 500-ms current steps ranging from −200 to +500 pA (20 current steps, with a 30-second interstep interval), which was repeated once more after a 30-s delay.

Cells were discarded and excluded from analysis if the series resistance changed by > 20% of baseline value and/or exceeded 30 MΩ. Current clamp bridge balance was adjusted prior to each AP family and single AP recording.

Repetitive magnetic stimulation (LI-rMS)

The LI-rMS protocol delivered was iTBS (Huang et al., 2005) (Fig. 1B) and consisted of trains of three 50-Hz pulses, repeated every 200 ms for 2 s. Trains were repeated once every 10 s for a total of 20 repetitions (total of 190 s). Monophasic pulses (400 μs rise time) were delivered with a custom circular coil (described in (Tang et al., 2016) (8 mm outer diameter, with an iron core). Coils were fixed to an electronic micro-manipulator and positioned in-between the slice chamber and microscope condenser (Fig. 1A). The coil was placed at a distance of approximately 1 mm from the slice, with the coil edge placed below the cortical layers; therefore apical dendrites from layer 5 pyramidal neurons were oriented perpendicular to the coil. The peak magnetic field at a distance of 1 mm from the base of the coil was measured with a Hall-effect probe (Honeywell, SS94A2D, USA) to be 85.4 mT and had a dB/dT of ~285 T/s. Coils were controlled by an arbitrary waveform generator (Agilent 33551B, Measurement innovation, Australia) and a programmable DC power supply (Kepco BOP 100-4 M, TMG test equipment, Australia). Sham stimulation consisted of placing a coil detached from the power supply beneath the slice as described above for 190 s before beginning the post-stimulation$_{+0}$ recordings.

Data analysis

Three single AP traces at each time point were analyzed for (i) AP threshold (change in membrane potential at a

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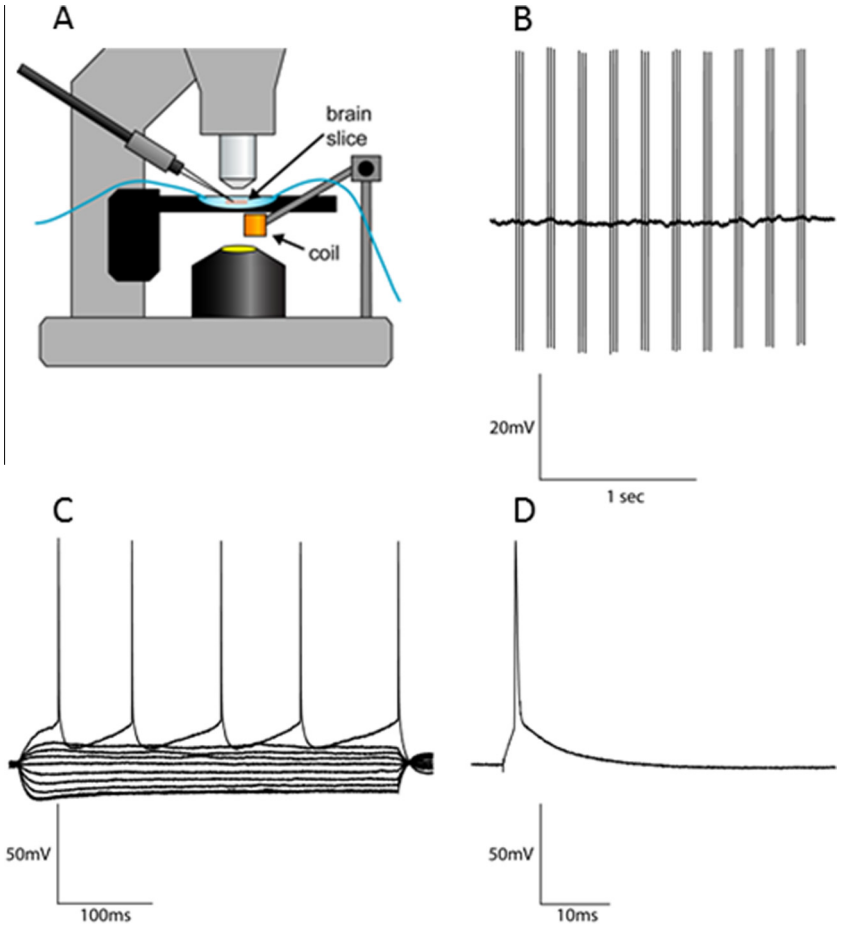

Fig. 1. *In vitro* LI-rMS delivery setup and representative traces of evoked spiking. LI-rMS was delivered with a custom 8 mm LI-rMS coil attached to a motorized head stage placed below slice chamber (A). Membrane potential recording during iTBS (B). Spike trains (C), and single AP's (D) evoked with electrical stimulation.

rate of 50 V/s) (ii) Spike height above resting membrane potential (iii) 20–80% rise time of spike peak (iv) spike half-width and (v) peak fast after hyperpolarization (AHP). AP family recordings were analyzed for (i) average resting membrane potential (RMP) (ii) rheobase (lowest current step that induced 1 or more APs), (iii) input resistance (iv) spike frequency. For spike frequency analysis, the number of spikes evoked by the 500-ms depolarizing current steps was quantified.

To confirm that LI-rMS did not trigger action potential firing with direct activation or through inducing suprathreshold currents in the electrode wire (Mueller et al., 2014; Pashut et al., 2014), we analyzed the membrane potential recorded during LI-rMS. There was no evidence of membrane after hyperpolarization following single or trains of LI-rMS pulses in any of the traces. Furthermore, the stimulus artifact had a peak voltage of 58.02 mV ± 0.07 relative to the resting membrane potential, which is below the mean action potential spike height (~110 mV), suggesting that LI-rMS did not induce action potential firing.

Statistical analysis

Statistical analysis was completed with IBM SPSS statistics 20 and data graphed with Graphpad Prism 6. Only cells contributing data for each of the 4 time points were included in the analysis. Initial analyses were completed on the raw values and confirmed that there were no significant differences in the baseline values between iTBS and sham for all the outcome measures. Further analysis on the raw values showed that although the mean baseline values were not significantly different, small differences in the baseline means obscured the detection of significant differences when running *post-hoc* tests. Therefore, we analyzed the data using an internal control method where the data at each of the post-stimulation time points was expressed as a *change relative to baseline for that cell* to account for any small differences in the baseline means between groups.

Normality was verified with Q–Q plots and homogeneity of variance tested with Levene's test. Data were analyzed with repeated measures ANOVAs. Degrees of freedom were corrected with Greenhouse-Geisser estimates when the assumptions of sphericity were violated (Mauchly's test). *Post-hoc* testing was performed using Sidak-corrected multiple comparisons tests and p values less than 0.05 were considered statistically significant. All data are represented as mean ± standard error of the mean.

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RESULTS

LI-rMS induces a hyperpolarized AP threshold and increases spike firing frequency

LI-rMS induced a more hyperpolarized AP threshold (Fig. 2A) relative to sham stimulation ($F_{1,\ 17} = 4.52$, $p = 0.048$). LI-rMS reduced the mean AP threshold by 1.76 mV ± 1.13 (post-stimulation$_{+0}$), 0.77 mV ± 1.10 (post-stimulation$_{+10}$) and 2.08 mV ± 1.60 (post-stimulation$_{+20}$). Post-hoc analysis showed a significant AP threshold hyperpolarization with LI-rMS at post-stimulation$_{+0}$ ($p = 0.025$) and post-stimulation$_{+20}$ ($p = 0.045$). There was no significant interaction between time and stimulation ($F_{2,\ 34} = 2.70$, $p = 0.082$).

Similarly, LI-rMS increased evoked spike firing frequency relative to sham stimulation ($F_{1,238} = 14.813$, $p = 0.001$) (Fig. 2B). LI-rMS increased the mean spike-firing frequency by 1.74 Hz ± 0.32 (post-stimulation$_{+0}$), 3.33 Hz ± 0.54 (post-stimulation$_{+10}$) and 4.44 Hz ± 0.61 (post-stimulation$_{+20}$) (Fig. 2C–E). Post-hoc analysis showed a significant increase in spike firing induced by LI-rMS at post-stimulation$_{+0}$ ($F_{1,264} = 20.681$, $p = 0.001$) post-stimulation$_{+10}$ ($F_{1,264} = 18.781$, $p = 0.001$) and post-stimulation$_{+20}$ ($F_{1,\ 264} = 5.683$, $p = 0.018$). There was no significant effect of current step amplitude ($F_{13,138} = 1.603$, $p = 0.085$) or significant interactions between time and stimulation ($F_{1.486,\ 353.746} = 3.359$, $p = 0.068$) or stimulation and current step amplitude ($F_{1,\ 13} = 0.503$, $p = 0.922$).

LI-rMS does not alter passive membrane properties, spike shape properties or fast after-hyperpolarization

In contrast to AP threshold and spike firing frequency, LI-rMS did not significantly change RMP ($F_{1,\ 17} = 0.56$, $p = 0.46$), rheobase ($F_{1,\ 17} = 1.02$, $p = 0.328$), spike height ($F_{1,\ 17} = 0.54$, $p = 0.473$), spike rise time ($F_{1,\ 17} = 0.001$, $p = 0.983$), spike half width ($F_{1,\ 17} = 2.320$, $p = 0.146$) or fast AHP ($F_{1,\ 17} = 0.848$, $p = 0.370$) (Fig. 3).

LI-rMS did not alter the input resistance (Fig. 3B) ($F_{1,\ 17} = 0.50$, $p = 0.49$) and was similar to sham for the first 10 min post-stimulation but trended toward a difference at 20 min post-stimulation. However, follow up analysis of the input resistance showed no significant differences in input resistance between sham and LI-rMS at post-

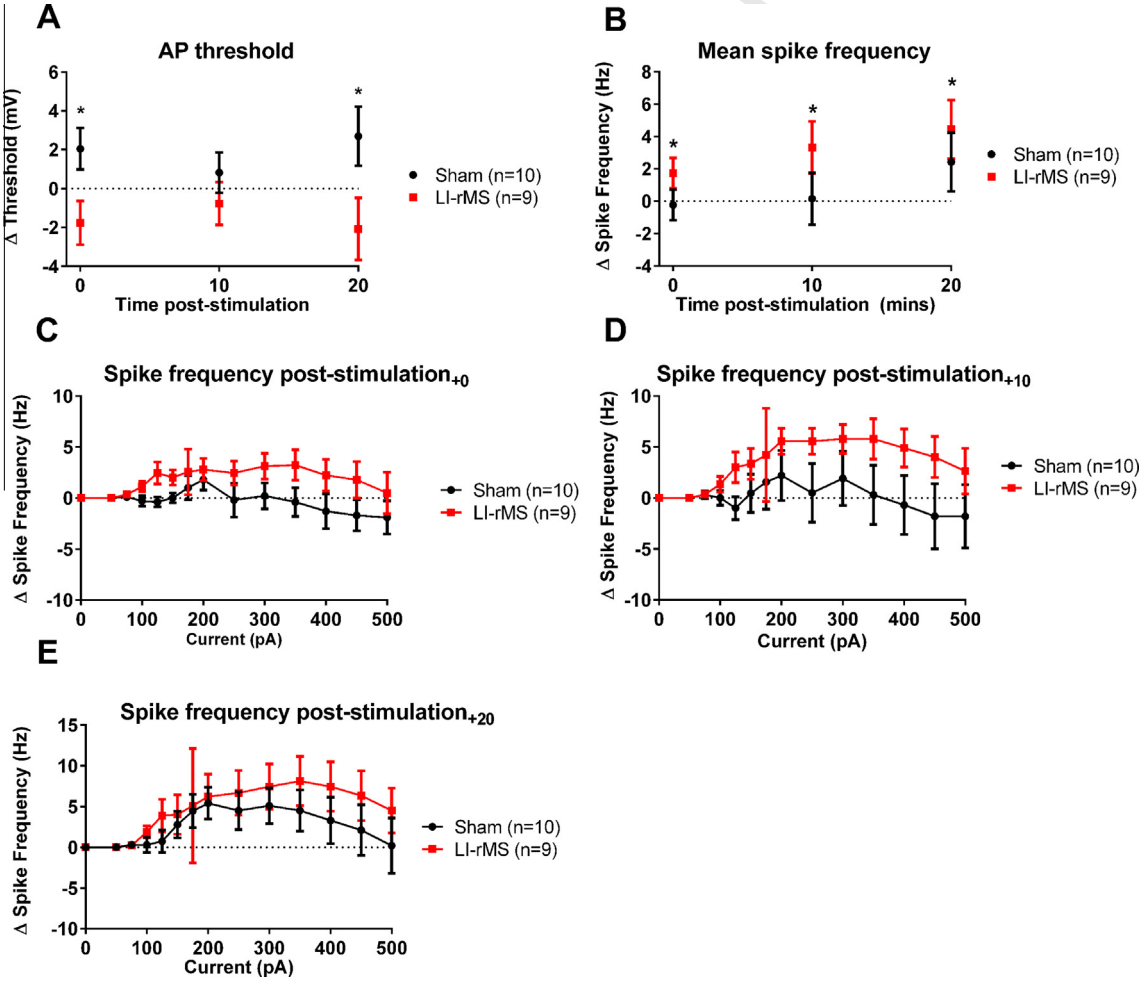

Fig. 2. LI-rMS alters AP threshold and spike firing frequency. LI-rMS significantly hyperpolarized the AP threshold (A) and increased spike firing frequency (B). Changes in spike frequency as function of current step amplitude at post-stimulation$_{+0}$ (C), post-stimulation$_{+10}$ (D), post-stimulation$_{+20}$ (E). $^{*}p < 0.05$, error bars represent SEM.

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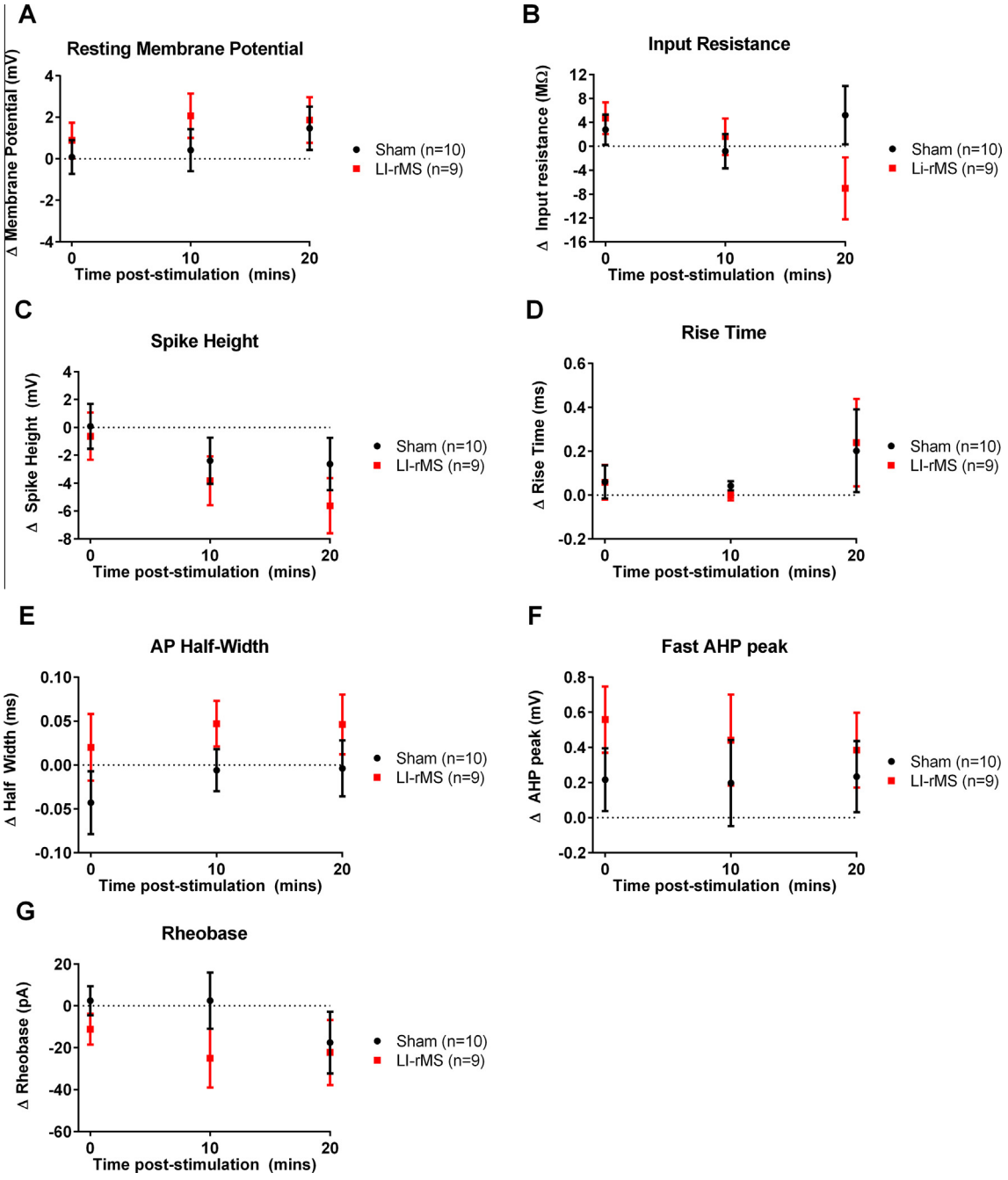

Fig. 3. LI-rMS does not alter passive membrane properties, AP shape or the rheobase. LI-rMS does not alter RMP (A), input resistance (B), spike height (C), AP rise time (D), AP half width (E), fast AHP (F) or rheobase (G) ($p > 0.05$). Error bars represent SEM.

stimulation$_{+0}$ ($p = 0.608$), post-stimulation$_{+10}$ ($p = 0.572$) and post-stimulation$_{+20}$ ($p = 0.106$).

DISCUSSION

To our knowledge, this is the first study to investigate the acute effects of LI-rMS at the single-cell level in cortical excitatory neurons. The main findings of our study show that LI-rMS does not alter passive membrane properties (RMP and input resistance) but increases neuronal excitability by inducing a more hyperpolarized AP threshold and increased evoked spike firing frequency relative to sham stimulation. Changes in AP threshold were present immediately after and 20 min post-stimulation whereas spike frequency changes were found immediately after stimulation and persisted to 20 min post-stimulation.

Given that the RMP remained unchanged, a hyperpolarized AP threshold is evidence of an LI-rMS-induced increase in neuronal excitability, due to modulation of membrane potential mechanisms at depolarized levels. Mechanisms affecting AP threshold/

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AP initiation include changes in fiber thickness and in the density and properties of voltage-gated sodium channels (Stuart et al., 1997; Kole et al., 2008). However, the changes in AP threshold were observed immediately after stimulation (190 s after onset), and changes in fiber thickness and the density of voltage-gated sodium channels (VGSCs) are unlikely to have occurred within such a short timeframe. Moreover, high-intensity rMS-induced structural changes, including changes in receptor density, have previously been shown to take greater than two hours post-stimulation (Vlachos et al., 2012). Rather, it is more likely that LI-rMS-induced changes in the properties of VGSCs, and has been suggested previously for voltage-gated calcium channels (for a review see reference (Pall, 2013)). Such a mechanism may underlie the change in AP threshold, probably through a direct modulation of the voltage-sensing mechanism, resulting in the opening of the VGSCs at more hyperpolarized voltages. Interestingly, the changes in AP threshold were present immediately and at 20 min post-stimulation but were not at 10 min post-stimulation. The apparent cyclical nature of AP threshold hyperpolarization may be due to LI-rMS altering the voltage-sensing mechanism by two different pathways, each with different times of onset. The immediate AP threshold hyperpolarization may be due to a direct interaction with LI-rMS (e.g. with the induced electric field during stimulation) whereas the AP threshold hyperpolarization 20 min post-stimulation may be due to activation of a biochemical/signaling pathway with a longer onset. As LI-rMS has previously been shown to increase intracellular calcium release in cortical neurons (Grehl et al., 2015), one such pathway that may underlie the longer onset change to AP threshold is through calcium signaling/-calmodulin which is known to alter VGSC function (Herzog et al., 2003).

Our second line of evidence that LI-rMS increases neuronal excitability is the increase in evoked spike firing following stimulation. Multiple channels are known to regulate spike firing frequency through alterations in the after hyperpolarization that follows spike firing (Hille, 2001), including A-type potassium channels (K_A) for L5 pyramidal neurons (Kang et al., 2000) which may have been modulated by LI-rMS. Interestingly, analysis of our single AP data revealed no change in the peak fast AHP accompanying the significant increases in spike firing frequency (see Fig. 3F). The involvement of K_A channels varies between single and repetitive firing, with a greater role of K_A channels during repetitive firing (Kang et al., 2000). Therefore LI-rMS-induced increases in spike frequency may be due to modulation of specific K_A channel properties, which would require separate pharmacological investigation.

Since LI-rMS lowered the AP threshold, we expected to observe a concurrent reduction in the rheobase current. While the mean rheobase current decreased over time following LI-rMS, it did not reach statistical significance. It is possible that our increments in current steps (25-pA steps in the 50–200-pA range) may have been too large to detect subtle changes in rheobase (i.e. changes < 25 pA) underlying a reduction in AP threshold of ~2 mV.

Our results are in part, similar to a recent study using high-intensity rTMS (Hoppenrath et al., 2016). The authors also show that rTMS increases neuronal excitability, with increased spike frequency in fast-spiking interneurons when probed at two hours post-stimulation. Interesting the authors also found changes to the resting membrane potential. Therefore it is possible that LI-rTMS and rTMS share common effects (e.g. changes in spike frequency) with high-intensity rTMS capable of more profound effects (e.g. altered resting membrane) due to increased intensity.

Recordings of the membrane potential during LI-rMS confirmed that the delivered stimulation intensity did not directly induce AP firing (i.e. subthreshold stimulation). In contrast to high-intensity rTMS where stimulation is believed to result in neuronal firing through transsynaptic (Labedi et al., 2014; Lenz et al., 2016) or direct activation (Lenz et al., 2016). Therefore our results provide further evidence that subthreshold stimulation induced by LI-rMS is capable of modulating neural plasticity. These results are in line with previous studies from our laboratory that used 12 mT rTMS in mice (approximately 2 orders of magnitude lower than suprathreshold rTMS) to induce structural and molecular plasticity (Rodger et al., 2012; Makowiecki et al., 2014). Although we provide evidence that LI-rMS modulates certain electrophysiological properties of cortical pyramidal neurons, further studies are needed to determine whether LI-rMS-induced plasticity is neuron subtype specific (e.g. pyramidal vs interneurons) or brain region specific (e.g. cerebellum (Morellini et al., 2014) vs hippocampus etc.) as well as whether non-neuronal cells such as glia (Cullen and Young, 2016) can be modulated.

It is well established that the endogenous electrophysiological properties of pyramidal neurons differ between young and adult animals (Zhang, 2004; Etherington and Williams, 2011). In our study, we used slices from developing mice (12–15 days post-natal) whereas previous high-intensity rMS studies have mostly used adult animals (~3 months old) to demonstrate plasticity of both inhibitory and excitatory networks using electrophysiological (Hsieh et al., 2014; Thimm and Funke, 2015) and molecular methods (Trippe et al., 2009; Hoppenrath and Funke, 2013). Interestingly, the recent study by Hoppenrath et al. showed a significant age effect on high-intensity rMS-induced plasticity in fast-spiking interneurons, with increases in evoked spike firing frequency present in young adult animals (post-natal days 29–38) but absent in juvenile (post-natal days 26–28) and older adult animals (post-natal days 40–62) (Hoppenrath et al., 2016). In contrast, our results show LI-rMS alters the excitability properties (including evoked spike firing frequency) in motor and somatosensory slices from developing animals (post-natal days 12–15). At this age, pyramidal neurons are in a heightened state of plasticity as they are approaching the end of the critical periods for both motor and somatosensory systems (Hensch, 2004). The intrinsic properties of the developing neurons, including their heightened plasticity state, may affect the capacity of LI-rMS to induce plasticity and the mechanism whereby it does so. Therefore, future studies

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in older animals are needed to determine whether the changes observed in developing tissue also occur in adult tissue. However, we have previously shown that LI-rTMS increases corticospinal excitability in anaesthetized adult rats, providing evidence of LI-rTMS-induced plasticity in adult animals (Tang et al., 2016).

We have provided insight into the acute effects of LI-rMS on single excitatory cortical neurons. Our results show that LI-rMS increases excitability of L5 pyramidal neurons from motor and somatosensory brain slices, by modulating specific active and spiking properties without altering passive membrane properties. These results further our understanding of LI-rMS-induced plasticity and highlight the capability of subthreshold magnetic stimulation to induce functional plasticity.

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REFERENCES

Capone F, Dileone M, Profice P, Pilato F, Musumeci G, Minicuci G, Ranieri F, Cadossi R, Setti S, Tonali P, Di Lazzaro V (2009) Does exposure to extremely low frequency magnetic fields produce functional changes in human brain? J Neural Transm 116:257–265.

Cullen CL, Young KM (2016) How does transcranial magnetic stimulation influence glial cells in the central nervous system? Front Neural Circuits 10:26.

Di Lazzaro V, Capone F, Apollonio F, Borea PA, Cadossi R, Fassina L, Grassi C, Liberti M, Paffi A, Parazzini M (2013) A consensus panel review of central nervous system effects of the exposure to low-intensity extremely low-frequency magnetic fields. Brain Stimul 6:469–476.

Etherington SJ, Williams SR (2011) Postnatal development of intrinsic and synaptic properties transforms signaling in the layer 5 excitatory neural network of the visual cortex. J Neurosci 31:9526–9537.

Grehl S, Viola H, Fuller-Carter PI, Carter KW, Dunlop SA, Hool L, Sherrard RM, Rodger J (2015) Cellular and molecular changes to cortical neurons following low intensity repetitive magnetic stimulation at different frequencies. Brain Stimul 8:114–123.

Hensch TK (2004) Critical period regulation. Annu Rev Neurosci 27:549–579.

Herzog RI, Liu C, Waxman SG, Cummins TR (2003) Calmodulin binds to the C terminus of sodium channels Nav1. 4 and Nav1. 6 and differentially modulates their functional properties. J Neurosci 23:8261–8270.

Hille B (2001) Ion channels of excitable membranes. MA: Sinauer Sunderland.

Hoppenrath K, Funke K (2013) Time-course of changes in neuronal activity markers following iTBS-TMS of the rat neocortex. Neurosci Lett 536:19–23.

Hoppenrath K, Härtig W, Funke K (2016) Intermittent theta-burst transcranial magnetic stimulation alters electrical properties of fast-spiking neocortical interneurons in an age-dependent fashion. Front Neural Circuits 10:22.

Hsieh T-H, Huang Y-Z, Rotenberg A, Pascual-Leone A, Chiang Y-H, Wang J-Y, Chen J-JJ (2014) Functional dopaminergic neurons in substantia nigra are required for transcranial magnetic stimulation-induced motor plasticity. Cereb Cortex.

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

Kang J, Huguenard JR, Prince DA (2000) Voltage-gated potassium channels activated during action potentials in layer v neocortical pyramidal neurons. J Neurophysiol 83:70–80.

Kole MH, Ilschner SU, Kampa BM, Williams SR, Ruben PC, Stuart GJ (2008) Action potential generation requires a high sodium channel density in the axon initial segment. Nat Neurosci 11:178–186.

Labedi A, Benali A, Mix A, Neubacher U, Funke K (2014) Modulation of inhibitory activity markers by intermittent theta-burst stimulation in rat cortex is NMDA-receptor dependent. Brain Stimul 7:394–400.

Lenz M, Galanis C, Muller-Dahlhaus F, Opitz A, Wierenga CJ, Szabo G, Ziemann U, Deller T, Funke K, Vlachos A (2016) Repetitive magnetic stimulation induces plasticity of inhibitory synapses. Nat Commun 7:10020.

Makowiecki K, Harvey A, Sherrard R, Rodger J (2014) Low-intensity repetitive transcranial magnetic stimulation improves abnormal visual cortical circuit topography and upregulates BDNF in mice. J Neurosci 34:10780–10792.

Morellini N, Grehl S, Tang A, Rodger J, Mariani J, Lohof AM, Sherrard RM (2014) What does low-intensity rTMS do to the cerebellum? Cerebellum 14:23–26.

Mueller JK, Grigsby EM, Prevosto V, Petraglia Iii FW, Rao H, Deng Z-D, Peterchev AV, Sommer MA, Egner T, Platt ML, Grill WM (2014) Simultaneous transcranial magnetic stimulation and single-neuron recording in alert non-human primates. Nat Neurosci 17:1130–1136.

Müller-Dahlhaus F, Vlachos A (2013) Unraveling the cellular and molecular mechanisms of repetitive magnetic stimulation. Front Mol Neurosci 6:50.

Pall ML (2013) Electromagnetic fields act via activation of voltage-gated calcium channels to produce beneficial or adverse effects. J Cell Mol Med 17:958–965.

Pashut T, Magidov D, Ben-Porat H, Wolfus S, Friedman A, Perel E, Lavidor M, Bar-Gad I, Yeshurun Y, Korngreen A (2014) Patch-clamp recordings of rat neurons from acute brain slices of the somatosensory cortex during magnetic stimulation. Front Cell Neurosci 8.

Ridding MC, Rothwell JC (2007) Is there a future for therapeutic use of transcranial magnetic stimulation? Nat Rev Neurosci 8:559–567.

Rodger J, Mo C, Wilks T, Dunlop SA, Sherrard RM (2012) Transcranial pulsed magnetic field stimulation facilitates reorganization of abnormal neural circuits and corrects behavioral deficits without disrupting normal connectivity. FASEB J 26:1593–1606.

Stuart G, Spruston N, Sakmann B, Häusser M (1997) Action potential initiation and backpropagation in neurons of the mammalian CNS. Trends Neurosci 20:125–131.

Tang A, Thickbroom G, Rodger J (2015) Repetitive transcranial magnetic stimulation of the brain: mechanisms from animal and experimental models. Neuroscientist. http://dx.doi.org/10.1177/1073858415618897.

Tang AD, Lowe AS, Garrett AR, Woodward R, Bennett W, Canty AJ, Garry MI, Hinder MR, Summers JJ, Gersner R, Rotenberg A, Thickbroom G, Walton J, Rodger J (2016) Construction and evaluation of rodent-specific rTMS coils. Front Neural Circuits 10. http://dx.doi.org/10.3389/fncir.2016.00047.

Thimm A, Funke K (2015) Multiple blocks of intermittent and continuous theta-burst stimulation applied via transcranial magnetic stimulation differently affect sensory responses in rat barrel cortex. J Physiol 593:967–985.

Trippe J, Mix A, Aydin Abidin S, Funke K, Benali A (2009) Theta burst and conventional low-frequency rTMS differentially affect GABAergic neurotransmission in the rat cortex. Exp Brain Res 199:411–421.

Vlachos A, Muller Dahlhaus F, Rosskopp J, Lenz M, Ziemann U, Deller T (2012) Repetitive magnetic stimulation induces functional and structural plasticity of excitatory postsynapses in mouse organotypic hippocampal slice cultures. J Neurosci 32:17514–17523.

Wagner T, Rushmore J, Eden U, Valero-Cabre A (2009) Biophysical foundations underlying TMS: setting the stage for an effective use of neurostimulation in the cognitive neurosciences. Cortex 45:1025–1034.

Wischnewski M, Schutter DJ (2015) Efficacy and time course of theta burst stimulation in healthy humans. Brain Stimul 8:685–692.

Z-w Zhang (2004) Maturation of layer V pyramidal neurons in the rat prefrontal cortex: intrinsic properties and synaptic function. J Neurophysiol 91:1171–1182.

Ziemann U, Paulus W, Nitsche MA, Pascual-Leone A, Byblow WD, Berardelli A, Siebner HR, Classen J, Cohen LG, Rothwell JC (2008) Consensus: motor cortex plasticity protocols. Brain Stimul 1:164–182.

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