RESEARCH PAPER

Acta Neurobiol Exp 2019, 79: 290–301
DOI: 10.21307/ane-2019-027

since 1928
ACTA NEUROBIOLOGIÆ EXPERIMENTALIS

Anodal transcranial direct current stimulation with monopolar pulses improves limb use after stroke by enhancing inter-hemispheric coherence

Andrea G. P. Schjetnan[1,2], Darryl C. Gidyk[1], Gerlinde A.S. Metz[1] and Artur Luczak[1]*

[1] Canadian Centre for Behavioural Neuroscience, Department of Neuroscience, University of Lethbridge, Lethbridge, Canada,
[2] Krembil Research Institute, Toronto Western Hospital, Toronto, Canada,
** Email: Luczak@uleth.ca*

Post-stroke neurological deficits, such as sensorimotor impairments, are often permanent and a leading cause of disability. Stroke is also associated with changes in neuronal synchrony among different brain areas. Multiple studies demonstrated that non-invasive brain stimulation, such as transcranial direct current stimulation (tDCS), enhances the efficacy of existing rehabilitative therapies. We hypothesized that the therapeutic effects of tDCS could be due to its influence on neuronal synchrony. To study this, we recorded local field potentials in rats treated with anodal tDCS (a-tDCS) after unilateral ischemic motor cortex lesion. To enhance the effect of a-tDCS on neuronal synchrony, we added monopolar pulses (a-tDCSmp) during a treatment. We found that ischemic lesions reduced interhemispheric coherence in the low gamma frequency range. By contrast, a-tDCSmp treatment increased interhemispheric coherence along with motor improvement in a skilled reaching task. These observations indicate that increased neuronal coherence is a likely mechanism by which tDCS improves stroke recovery. Moreover, this work adds to previous evidence that measures of brain coherence could be used as a biomarker of stroke recovery, which may help in the design of more effective tDCS protocols for stroke rehabilitation.

Key words: stroke, electrophysiology, sensorimotor cortex

INTRODUCTION

Stroke is characterized by reduced blood flow to the brain. It represents the leading cause of chronic adult disability and the third leading cause of death in North America (Benjamin et al., 2017). Although considerable spontaneous rearrangement of connections after stroke occurs (Murphy and Corbett, 2009; Lim et al., 2014), spontaneous recovery is limited and mainly mediated by compensational adjustments (Buckner et al., 1996; Metz et al., 2005; Alaverdashvili and Whishaw, 2010). Post-stroke neuroplasticity includes regeneration of damaged pathways, axonal and dendritic sprouting, and remapping of functional representations (Adkins et al., 2002; Winship and Murphy, 2009; Westlake and Nagarajan, 2011). Studies using resting state functional magnetic resonance imaging (fMRI) reported that post-stroke loss and recovery of sensorimotor function is associated with acute deterioration and subsequent retrieval of interhemispheric functional connectivity within the sensorimotor system (van Meer et al., 2010; 2012; Westlake and Nagarajan, 2011). Similarly, large-scale phase synchrony in electroencephalography (EEG) was shown to correlate with post-stroke motor assessment (Kawano et al., 2017; 2018). Clinical rehabilitative strategies take advantage of this information, for example by using bilateral arm training (Coupar et al., 2010; Choo et al., 2015), and improvements in stroke rehabilitation correlated with increases in inter-hemispheric coupling in EEG (Pellegrino et al., 2012).

Received 26 January 2019, accepted 27 June 2019

Studies using non-invasive brain stimulation have shown promising results in enhancing effectiveness of stroke therapies. Transcranial direct current stimulation (tDCS) has been shown to improve recovery both in animal models and patients with ischemic brain injury (for review, see Floel and Cohen, 2010; Gomez Palacio Schjetnan et al., 2013; Gall et al., 2015; Lefebvre and Liew, 2017; Boonzaier et al., 2018). tDCS was also shown to affect cognitive and motor functions in intact animals/humans; however, its effect crucially depends on stimulation parameters (e.g., electrode polarity, stimulation site, current intensity) (Nitsche et al., 2007; Jacobson et al., 2012). This method is a noninvasive, low-cost and easy-to-use technique that can be applied to modify cerebral excitability. This is achieved by using weak direct currents to shift the resting potential of cortical neurons (Floel and Cohen, 2010). The direct currents are applied by attaching two electrodes (usually one anode and one cathode) to distinct areas of the scalp (Utz et al., 2010; Lefebvre and Liew, 2017). The degree of motor improvement for tDCS therapy depends on the interval between the infarct and the time of initiation of therapy, as well as, the type of stimulation (monopolar or bipolar), frequency of pulses, and duration of the stimulation (Reis and Fritsch, 2011). In addition to improving stroke outcomes, tDCS has been shown to promote a variety of motor and cognitive skills in healthy subjects (Ukueberuwa and Wassermann, 2010), demonstrating the broad applicability of tDCS for the enhancement of brain function (Fox, 2011; Fox et al., 2014).

Despite the demonstrated beneficial effects of tDCS, little is known about the underlying mechanisms. It has been proposed that tDCS provides a sub-threshold stimulus to modulate the likelihood that neurons will fire (Nitsche et al., 2003). The prolonged sensory, motor, and cognitive effects of tDCS have been attributed to a persistent bidirectional modification of post-synaptic connections, similar to long-term potentiation and long-term depression (Teskey et al., 2003; Fritsch et al., 2010; Koo et al., 2016). The N-methyl-D-aspartate (NMDA) antagonist dextromethorphan suppresses both anodal and cathodal tDCS effects, strongly suggesting an involvement of NMDA receptors in both types of DC-induced neuroplasticity (Liebetanz et al., 2002). Similarly, a study demonstrated that neuroplastic changes elicited by tDCS depend on brain-derived neurotrophic factor (BDNF)-TrkB interactions (Fritsch et al., 2010). Plastic modifications elicited by tDCS have also been attributed to the actions of glial cells (Gellner et al., 2016). In a previous study conducted by our group, we show that bilateral somatosensory stimulation enhances skilled movement quality and neural density in intact behaving rats (Faraji et al., 2013). Nevertheless, the underlying mechanisms and neuronal correlates mediating the benefit of tDCS require further exploration to serve as a viable technique in the clinical context.

Here, we investigated the interactions between cortical networks in rats with focal cerebral ischemia wherein the rehabilitation process was aided by anodal tDCS with added monopolar pulses (a-tDCSmp). Our findings show that animals with larger coherent activity between neuronal populations exhibit greater recovery from ischemia, as measured by skilled motor performance. In addition, we conducted intracortical local field potentials (LFP) recordings in anesthetized rats, which demonstrated that application of a-tDCSmp induced an increase in synchronization between hemispheres. All those results together suggest that increased interhemispheric coherence in the ~10-40Hz band represents a mechanism by which tDCS promotes sensorimotor recovery and enhances the efficacy of rehabilitation training after stroke.

METHODS

All procedures were performed in accordance with the guidelines set by the Canadian Council for Animal Care and approved by the University of Lethbridge animal welfare committee.

Skilled reaching task

Fifteen male Long-Evans hooded rats received from Charles River (Ontario, Canada), aged 70-80 days (400 g - 500 g), were habituated to the experimenter for seven days. After habituation, all rats were trained in the skilled reaching task, which is a reach-to-eat task designed to quantify and qualify skilled forelimb use (Whishaw and Pellis, 1990; Metz et al., 2005). The skilled reaching apparatus was a rectangular Plexiglas box, 40 cm long × 45 cm high × 13 cm wide with a 1 cm slot at one end, and a shelf fixed to the outside of the box which was accessible through the slot (Fig. 3A). The shelf had two symmetric indentations which were 1.5 mm deep, 1.5 cm from the slot, parallel to each edge of the slot to provide stability and a consistent position for the food pellets to be placed. Small 45 mg food pellets were used (Bioserve, USA; Product # F0021).

Once trained to asymptote success levels with their preferred paw, rats received 20 pellets per day for five consecutive days in the reaching task to establish baseline reaching success (RS) values. In each trial, animals were required to walk to the rear end of the box prior to reaching for a pellet to readjust their body position

(Metz and Whishaw, 2000). A successful reach was defined as an animal reaching for the pellet, grasping it and placing it in its mouth on the first attempt. RS for each rat and each session were calculated using the following formula: RS=number of successful reaches / 20 × 100%. The data were scored by group-blind observers from recorded videos.

Before inducing lesions and implanting stimulating electrodes (see next section), animals were assigned to experimental groups to match paw preference and RS between groups. Six days following surgery, animals were tested in skilled reaching for three weeks. After a four-week break, rats were re-tested for four consecutive days to evaluate the possible long-term effects of a-tDCSmp.

Photothrombosis and electrode implantation

Focal photothrombosis was induced in the forelimb area of primary motor cortex contralateral to the forelimb preferred in the reaching task (Metz et al., 2005). Animals were anesthetized using 4% isoflurane in a mixture of 1.5% oxygen and secured in a stereotaxic frame (David Kopf, Germany). The skull over the motor cortex was thinned using a fine dental burr in a rectangular shape from Bregma -1.0 to 4.0 anterior/posterior and Bregma 1.0 to 4.0 medial/lateral. A cold light source (Schott KL 1500, Germany) with an aperture of the same size and shape as the partial craniotomy was positioned over the skull. The skull was illuminated at maximum light settings for 20 min. During the first 2 min of illumination, Bengal Rose dye solution was injected through a tail vein (20 mg/kg, 10% solution in 0.9% saline). When the illumination period was completed, animals were implanted with stainless steel screws used for electrical stimulation (1 mm diameter; Small Parts, USA). Pilot holes were drilled in the skull bilaterally at coordinates: +4.5 A/P, ±1.0 M/L (Paxinos and Watson, 1998) (Fig. 1A). A dental acrylic skull cap was fashioned on the exposed skull, engulfing the electrodes, connecting wires, and connecting plug (Ginder Scientific, ON, Canada). Once the acrylic was hardened, animals were removed from the stereotaxic frame, placed on a heating pad, and monitored until they were awake.

Lesion analysis

DAPI (4',6-diamidino-2-phenylindole; Vector Labs Inc., CA) stained coronal sections (40 µm thickness), cut on a freezing microtome and mounted on microscope slides, were digitally scanned at 40x magnification (Nanozoomer, Hammatsu Photonics, Japan). The images were transferred to Image J software (NIH, USA) and the lesion volumes were quantified. Volumes were measured by tracing lesion borders, and then multiplying the lesion area by section thickness and number of sections in the series. Lesion area was determined by superimposing a straight-line connecting the lesion boundaries, as previously reported by our group (Metz et al., 2005; Knieling et al., 2009).

Transcranial direct current stimulation

The a-tDCSmp stimulation protocol consisted of 10 min of an anodal 65 µA direct current with superimposed 65 µA monopolar pulses of 30 ms duration, applied every 5 seconds during a-tDCS (Fig. 1C). The pulses were included in the protocol in an attempt to increase synchrony in interhemispheric activity. The stimulation was applied only during the first week of rehabilitation, which occurred concurrently with the skilled reaching task, 6 days after stroke and electrode implantation. The time point of stimulation was selected in order to: 1) allow a recovery period after surgical implantation as requested by the by the Canadian Council for Animal Care; 2) allow a time course of ischemic stroke to stabilize (Karl et al., 2010); and 3) evaluate the electrical stimulation effects in conjunction with motor rehabilitation as performed in many rehabilitation protocols that use this strategy (Alsharidah et al., 2018). Rats in all groups were connected to the stimulator during all reaching trials, but the stimulator was switched on only for the Stroke + Stim group.

Acute surgery and recordings

Detailed descriptions of the surgery and recording procedures have been published in (Luczak et al., 2007; 2009; Schjetnan and Luczak, 2011; Luczak and Barthó, 2012). Briefly, after the last session of re-testing, the animals were anaesthetized with urethane (1.5 g/kg), injected i.p. Then, rats were placed in a stereotaxic frame, and two 3 mm^2 craniotomies were prepared over the forelimb somatosensory cortex of each hemisphere (AP: +2.7 to -0.3; ML: ±2.5 to 5) (Paxinos and Watson, 1998). One rat from the Stroke + Stim group died during the electrophysiological recordings, and data from this experiment were excluded from analyses. Extracellular signals were recorded with silicon probes (NeuroNexus Technologies, Ann Arbor, MI) consisting of eight shanks (Fig. 1A), and each shank was separated by 200 µm. The location of the recording sites was determined to be layer V by histological reconstruction of the electrode tracks and electrode depth. Local field potentials were

referenced to a titanium screw implanted over the cerebellar cortex and sampled at 32 kHz and were band-pass filtered between 0.1 - 8000 Hz (Neuralynx, Inc., Bozeman, Montana, USA). Ten min of spontaneous activity was recorded, followed by 20 min of electrical stimulation with the same parameters (65 µA direct current with an additional 30 ms duration and 65 µA pulses applied every 5 seconds), as in training (Fig. 1B). This was followed by 10 min of recording of spontaneous activity. Stimulation during the acute electrophysiological re-

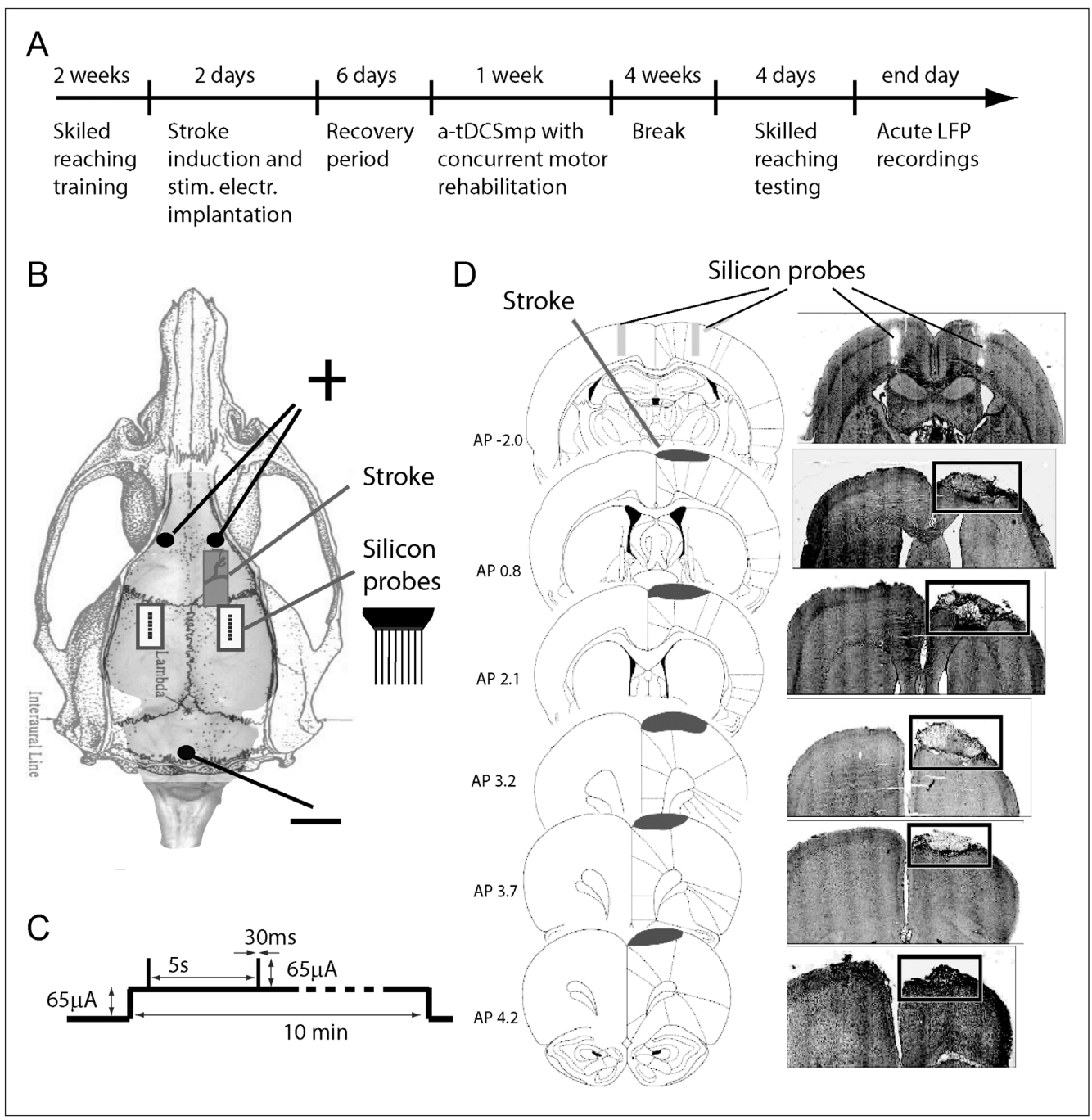

Fig. 1. Experimental procedures. (A) Timeline of experimental stages. (B) Illustration of the position of the stimulation electrodes (black dots, anode '+', cathode '-'), location of the photothrombotic lesion (dark rectangle), areas with inserted silicon probes (boxes with dots representing electrode locations), and schematic of the silicon probe with 8 shanks (right side). (C) Schematic illustration of stimulation parameters used for a-tDCSmp. (D) Representative coronal sections (40 µm) of the brain of an animal with ischemic stroke. Localization of the stroke area (gray blobs) corresponds to the primary motor cortex (M1). Top section shows silicon probe traces in the deeper layers of the limb somatosensory cortex in both hemispheres. AP=Anteroposterior coordinates (Paxinos and Watson, 1998).

cordings was applied to all experimental groups and the duration increased (to 20 min) in order to have a direct assessment of the effects of stimulation in LFP coherence. Following acute electrophysiological recordings, animals were deeply anaesthetized with pentobarbital and perfused. Histological analysis on the extracted brains was performed using DAPI mounting medium.

Coherence analysis

Coherence was calculated as the magnitude squared coherence estimate using Welch's averaged, modified periodogram method (Rabiner and Gold, 1975) implemented in Matlab (MathWorks, Inc.; mscohere function). The magnitude squared coherence estimate is

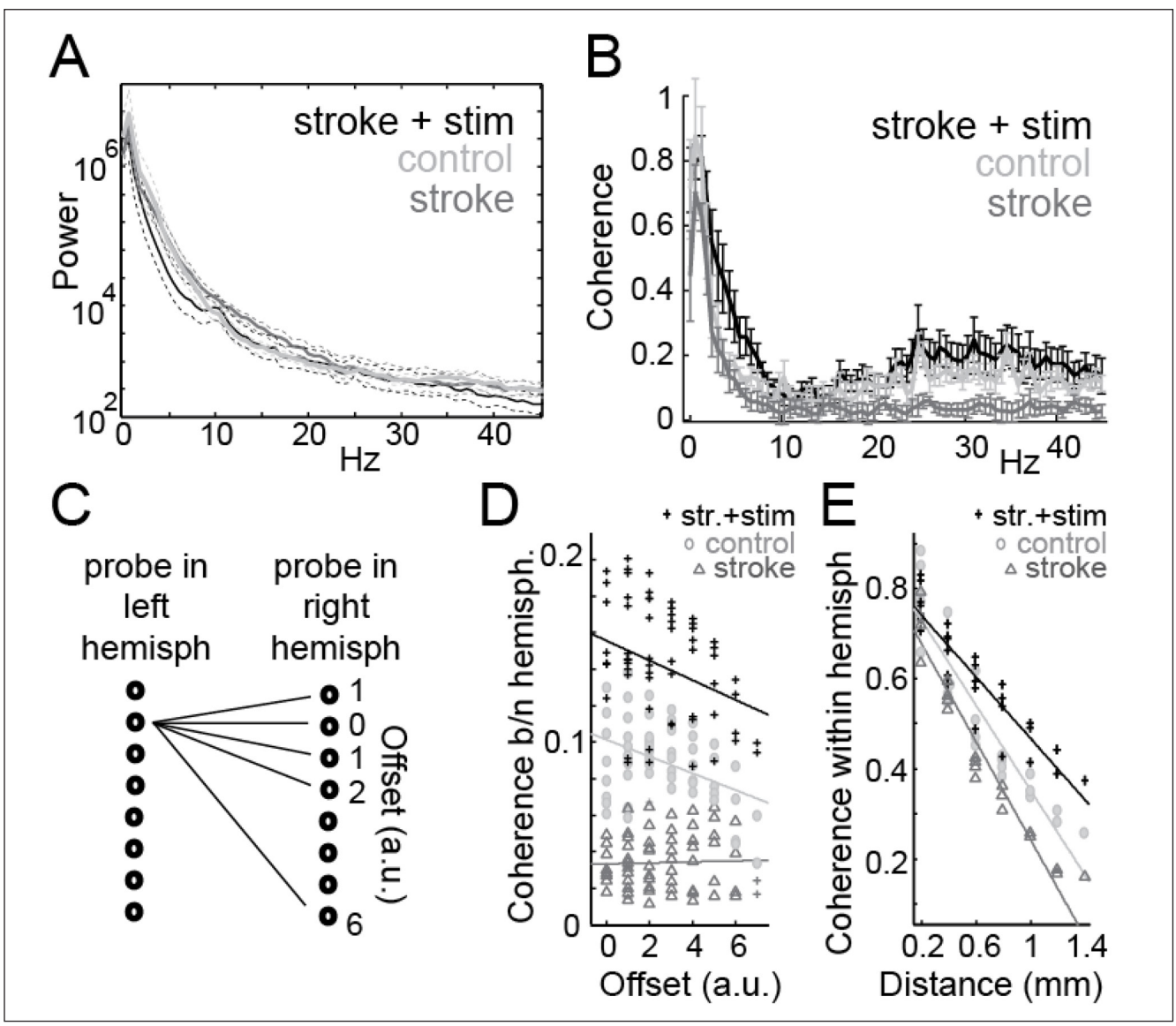

Fig. 2. Changes in LFP after stroke and electrical stimulation. (A) Power spectra in sensory cortex in the Control group (light gray), the Stroke group (dark gray), and Stroke+Stim group (black). Dashed lines show standard error. For each animal, data were averaged over both hemispheres. (B) Coherence between hemispheres for the same experimental groups that are shown in panel A. Error bars denote standard error. Note: higher coherence in the 20-40 Hz band for Control and Stroke + Stim groups. (C) Illustration of the definition of offset between homologous areas between hemispheres. Blue circles represent recording sites. (D) Coherence as a function of distance between homologous areas. Each point represents an average coherence between 20-40 Hz across animals, for each pair of shanks. Symbols represent experimental groups: cross=Stroke + Stim; triangle=Stroke group; circles=Control group. Lines show linear fit for each group. For Control and Stroke+Stim groups, the coherence decreased with offset between areas. (E) Coherence as a function of distance between shanks between hemisphere. Each cross represents an average coherence between 20-40 Hz across animals, for each pair of shanks. Symbols represent the same experimental groups that are shown in panel D. Lines show linear fit for each group. For all groups, the coherence decreased with distance between recording shanks. LFP=Local field potential.

a function of frequency, with values between 0 and 1, that indicates how well input signals x and y correspond to each other at each frequency. The magnitude squared coherence $C_{xy}(f)$ is a function of the power spectral densities ($P_{xx}(f)$ and $P_{yy}(f)$) of x and y, and the cross power spectral density ($P_{xy}(f)$) of x and y: $C_{xy}(f)=|P_{xy}(f)|/(P_{xx}(f)\cdot P_{yy}(f))$.

RESULTS

Decrease in local field potential (LFP) coherence after stroke. To investigate how interactions between sensory areas change after stroke and how this is modified by the application of electrical stimulation, three groups of rats were studied: Control (n=5), Stroke (n=5), and a group with stroke treated with direct current stimulation (Stroke+Stim, n=5). All rats were implanted bilaterally with stimulating electrodes anterior to the cortical motor areas and one reference electrode over the cerebellum (Fig. 1A), but stimulation was applied only in the Stroke+Stim group (Fig. 1B; Methods). During the same surgery, focal cortical ischemia was induced by photothrombosis in the forelimb area of motor cortex in the hemisphere contralateral to the preferred paw in the Stroke + Stim and Stroke groups. Ischemia was largely constrained to cortical areas (Fig. 1C). Average infarct volumes (V_{Stroke}=5.42 mm³ ± 2.01; $V_{Stroke+Stim}$=4.92 mm³ ± 1.80) did not statistically differ between groups (p=0.9, t-test). The stroke affected primarily the area corresponding to M1, which was verified with a histological examination.

Persistent effects of stroke and electrical stimulation on cortical activity were measured 8 weeks following the infarct. Animals were anesthetized with urethane and LFPs were recorded in the deep cortical layers. Two electrodes with 8 recording shanks each were inserted in sensory areas in both hemispheres (Fig. 1A). In all experimental groups, the LFP power spectra was highest around 0.5-2 Hz, with a systematic decrease in the higher frequencies (Fig. 2A). There were no significant differences between experimental groups ($p_{contr\text{-}strok}$>0.2; $p_{contr\text{-}strok+stim}$>0.2; $p_{Stroke\text{-}Strok+Stim}$>0.1; Kolmogorov-Smirnov test), or between lesion and intact hemispheres (p>0.2; Kolmogorov-Smirnov test) at any frequency band between 0.5-40 Hz.

Despite the lack of differences in LFP power spectra, LFP coherence showed notable differences between groups (Fig. 2B) which were most pronounced in the low gamma frequency range (20-40Hz). Inter-hemispheric coherence was significantly lower in the Stroke group as compared to the Control and Stroke+Stim groups ($p_{Control\text{-}Strok}$=0.044; $p_{Stroke+Stim\text{-}Strok}$=0.032; Kolmogorov-Smirnov test). This effect was consistent across different pairs of electrodes, although the values of coherence systematically changed with distance. For example, coherence between hemispheres was usually the highest between homologous areas and decreased with spatial offset between areas (Fig. 2C,D; R_{Contr}=–0.41; p<0.001; $R_{Stroke+Stim}$=–0.31; p=0.012; R_{Stroke}=0.03; p=0.78). In the Stroke

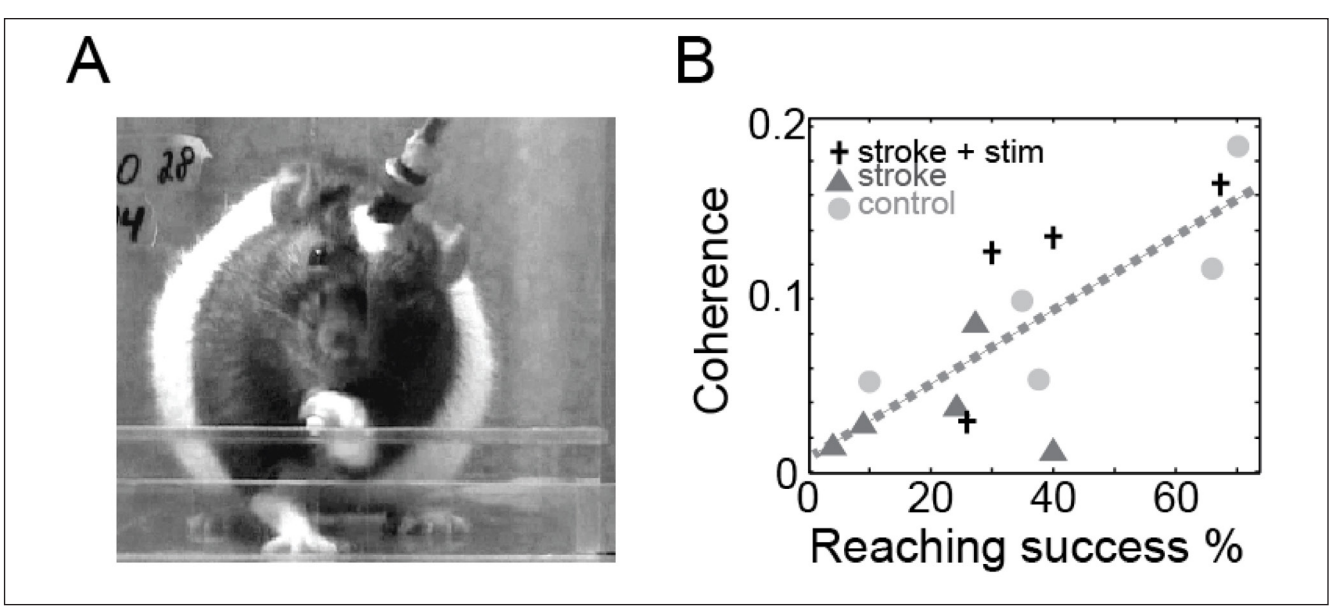

Fig. 3. (A) Picture of a rat performing a skilled reaching movement. (B) Relationship between coherence in the low gamma range and reaching success (circles=Control group; triangles=Stroke group; crosses=Stroke + Stim group). Dashed line represents the least-squares fit for the points combined from all groups.

group, the correlation was not significant, most likely due to insufficient coherence to detect this effect. Similarly, within-hemisphere coherence decreased significantly as a function of distance between electrodes (Fig. 2E; R_{Contr}=–0.93; p<0.001; $R_{Stroke+Stim}$=–0.92; p<0.001; R_{Stroke}=–0.96; p<0.0001). There were no systematic differences between the intact and lesion hemispheres concerning the relation between coherence and distance. Altogether, these results suggest that focal ischemia reduces the coherence between cortical areas, and electrical stimulation restores coherence in the gamma band.

Coherence in low gamma band correlates with behavior

A skilled forelimb reaching task was used to provide a behavioral measure of motor recovery after stroke (Metz et al., 2005) (Fig. 3A). On the last day of testing (one day prior to electrophysiological recordings), RS was calculated for each animal ($RS_{Control}$= 43.76 + 11.05, RS_{Stroke}= 20.92 + 6.31, $RS_{Stroke+Stim}$= 40.81 + 8.3). These results are consistent with previous studies showing decreased RS in rats after focal ischemia (Metz et al., 2005; Alaver-

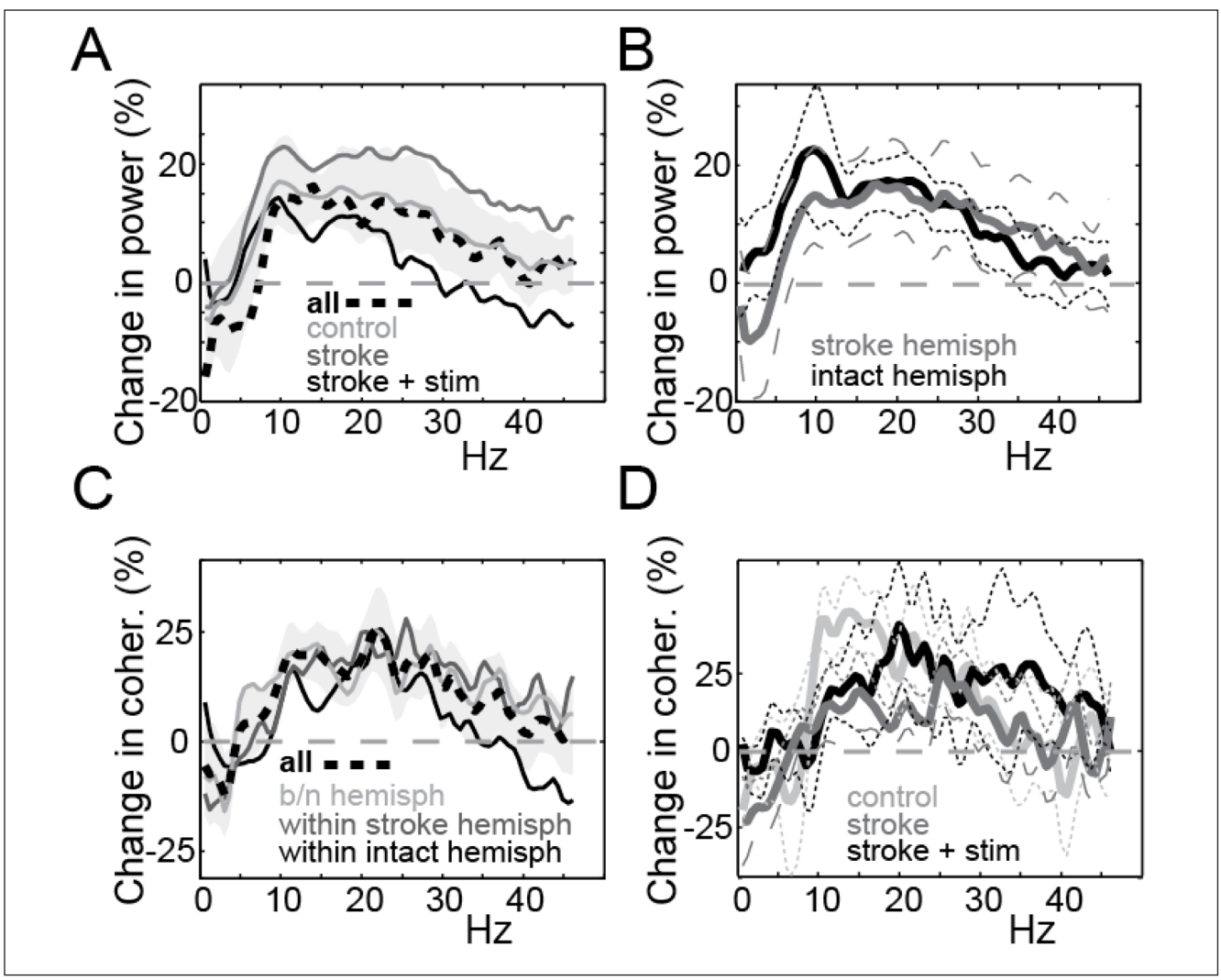

Fig. 4. Changes in brain activity after electrical stimulation. (A) Difference in LFP power spectra (subtracting power in the periods immediately before stimulation from power in periods immediately after stimulation). The dashed trace shows average and standard error (shaded area), for all animals. Note an increase in power after stimulation in the 10-40 Hz band. Light gray, dark gray, and black lines show the average for Control, Stroke, and Stroke + Stim groups, respectively. (B) A change in LFP power spectra after electrical stimulation in intact (black) and stroke (gray) hemispheres. (C) Increase in LFP coherence after electrical stimulation in the 10-40 Hz band. The dashed line shows average change in coherence across all recording sites for all animals, and the shaded area shows standard error. Light gray, dark gray, and black lines represent an average change in coherence between hemispheres, within the stroke hemisphere, and within the intact hemisphere, respectively. (D) Change in LFP coherence in the stroke hemisphere (dark gray=Stroke group; black=Stroke+Stim), and in control group in the left hemisphere (light gray). Differences between groups were not significant in the 10-40 Hz band (p>0.8 for all between groups comparisons; t-test). LFP=Local field potential.

dashvili and Whishaw, 2010) and improvements due to anodal electrical stimulation (Adkins et al., 2008; Ukueberuwa and Wassermann, 2010; Faraji et al., 2013). RS and coherence in the gamma band were strongly correlated (Fig. 3B; R=0.78; p=0.001). Notably, even within individual experimental groups, correlations tended to be positive, suggesting a general positive relationship between coherence and skilled movements (R_{Contr}=0.83; p=0.08; $R_{Stroke+Stim}$=0.75; p=0.24; R_{Stroke}=0.18; p=0.77). These findings suggest that low gamma band coherence may be important for competency in motor tasks.

Anodal stimulation increases LFP coherence.

The data above shows that the Stroke+Stim group had higher gamma band coherence than the Stroke group. To investigate if enhanced gamma band coherence is causally linked to electrical stimulation, we examined the effect of electrical stimulation on brain activity. During the acute LFP recordings in anesthetized animals, we applied 20 min of anodal stimulation in the animals from all of our experimental groups. This was aimed to compare brain activity directly before and after electrical stimulation. The stimulation significantly increased LFP power in the 10-40 Hz band (p=0.016; t-test). Of note, for each animal, the power spectra calculated in 2 min window before stimulation was subtracted from the power spectra calculated in a 2 min window after stimulation. Next, the average difference in the 10-40 Hz band was calculated, and we tested the null hypothesis that the results come from a normal distribution with a mean equal to zero, meaning no change in power. This effect was consistent across all experimental groups (Fig. 4A). Moreover, LFP power increased similarly in the intact and lesion hemispheres (Fig. 4B; p=0.34; paired t-test between the intact and stroke hemispheres in the 10-40 Hz band; of note, results were consistent when instead using the non-parametric sign test).

Importantly, the observed increased LFP power after electrical stimulation was associated with increased coherence in the corresponding frequency band (Fig. 4C; p=0.038; t-test; 10-40 Hz). Again, this increase was consistent within the intact and lesion hemispheres and between hemispheres (Fig. 4C). This effect was similar across experimental groups (Fig. 4D), suggesting that anodal stimulation directly caused a general increase in coherence among cortical areas.

DISCUSSION

In this study, we performed electrophysiological recordings to investigate interactions between cortical networks in rats treated with a-tDCSmp after a focal ischemic motor cortex lesion. We observed a widespread decrease in low gamma band coherence between cortical areas following ischemia. We also showed that coherence in the low gamma band range was positively correlated with performance on a skilled motor task. Importantly, we demonstrated that anodal stimulation enhanced gamma band coherence between cortical areas, which may explain the reported therapeutic effect of tDCS on stroke recovery. Our results suggest that maximizing synchronization among cortical areas with electrical stimulation could facilitate neuroplastic mechanisms and translate to more effective stroke therapies.

Although very few studies investigated changes in low gamma band coherence after stroke, we hypothesize that its decrease relates to a general reduction in connectivity among brain areas following stroke. Consistent with our results are reports of a decrease in interhemispheric functional connectivity in a rat model of stroke (van Meer et al., 2010; 2012). Crofts and Higham (2009), using data from diffusion magnetic resonance imaging, compared connectivity between 56 brain areas in stroke patients and healthy controls, and showed decreased communication among a number of brain regions in stroke patients. Similar results were obtained by Muehlschlegel and colleagues who, using near-infrared spectroscopy, showed that the interhemispheric correlation coefficient at frequency bands 0.15-3 Hz was smaller in stroke patients (Muehlschlegel et al., 2009). Analysis of functional connectivity with EEG also revealed reduced interhemispheric coherence in stroke patients (Strens et al., 2004; Gerloff et al., 2006). An interesting interpretation of this decline in interactions among brain areas after stroke was described by Wang et al. (Wang et al., 2010). Based on analysis of the topological configuration of the resting-state networks, Wang and coauthors found that brain networks shifted towards a more random and less optimized mode of function after stroke (Wang et al., 2010). Thus, our finding of decreased coherence in the low gamma band could be another manifestation of reduced efficiency of communication among brain networks after stroke.

Cortical gamma activity has been implicated in a variety of functions including attention, working memory, object recognition, feature binding, information processing, and sensorimotor performance (for review, see Herrmann et al., 2009). In general, 20-40 Hz activity is associated with top–down attentional processing by which the brain may be controlling the flow of sensorimotor information (Wróbel et al., 1994; Debener et al., 2003). Our results correspond well with this general framework. It should be noted that our electrophysiological data were recorded under anesthesia, which means that observed changes reflect long-lasting prop-

erties of cortical circuitry rather than task-related changes in neuronal synchronization. Thus, increased 20-40 Hz activity observed in rats with better skilled movements could indicate that cortical networks in those animals may be able to synchronize more readily, which may contribute to better control over motor tasks. Such interpretation is supported by experiments demonstrating that higher baseline levels of gamma activity correspond to shorter response times (Gonzalez Andino et al., 2005; Womelsdorf et al., 2006; Schoffelen et al., 2011) and greater task performance efficiency (Kaiser et al., 2009; Joundi et al., 2012). Although the mechanisms of tDCS are not well understood, we hypothesize that a long-term increase in low gamma band coherence could be the result of an increase in strength of neuronal connections. It has also been shown that direct current stimulation facilitates LTP (Kronberg et al., 2017). Thus, application of a-tDCSmp concurrently with a reaching task may promote a strengthening of connections between active motor cortex neurons. Previous studies have shown that, following stroke, reduced gamma band coherence relates to a general reduction in connectivity among brain areas (van Meer et al., 2010; Crofts and Higham, 2009; Muehlschlegel et al., 2009). Therefore, tDCS facilitation of LTP may strengthen motor cortex connectivity and may thus lead to a long-lasting increase in gamma coherence.

In this study, we show that anodal stimulation enhances gamma activity and coherence. This result is of interest because the electrophysiological effect of direct current stimulation on neuronal activity *in vivo* is poorly understood. Reato et al. previously reported that hippocampal gamma activity can be enhanced with weak electrical currents (Reato et al., 2014). Here, we report that direct current stimulation increases gamma power immediately after stimulation and has long-lasting modifications in gamma coherence. Interestingly, the increase in power above 10 Hz also resembles characteristics of an activated state (Luczak et al., 2013), which is known to enhance neuronal plasticity (Bermudez Contreras et al., 2013) and increase information transfer rate by generating more neuronal packets (Luczak et al., 2015). For example, during the attentive state, brain activity decreases in the low frequencies (<10 Hz) with a simultaneous increase in gamma range, which characterizes the activated state and is also linked to attention (Harris and Thiele, 2011). Thus, one might argue that increasing gamma activity with anodal stimulation may emulate the effect of attention. This idea is consistent with other studies in which the application of anodal tDCS had a striking resemblance to the effects of increased attention (Gladwin et al., 2012; Roe et al., 2016). For example, anodal tDCS applied over the prefrontal cortex can improve working memory and visual attention (Javadi and Walsh, 2011; Roy et al., 2015), and tDCS applied over the temporal lobe has been shown to facilitate problem solving (Chi and Snyder, 2011). Further, motor performance is improved when tDCS is applied over the motor cortex (Reis and Fritsch, 2011; Tanaka et al., 2011). Although the proposed idea linking the effect of tDCS to enhanced attention is speculative, we believe that it is important to state it here because it could lead to better conceptual understanding of tDCS mechanisms and possibly to better treatment strategies. It is also worth noting that stroke patients show attention deficits (Barker-Collo et al., 2011). Thus, one may argue that the therapeutic effects of tDCS when combined with physical rehabilitation may work by helping motor networks to "pay attention" to an exercise, thereby enhancing its efficacy.

From a translational research perspective, our data suggest that identifying the parameters of electrical stimulation that maximize 20-40 Hz coherence could lead to more effective stroke therapies. In this study, we used constant anodal stimulation at 65 μA with additional 30 ms long 65 μA pulses every 5 seconds, applied over both hemispheres. This stimulus was chosen because we hypothesized that anodal currents will excite motor cortices and additional short pulses may assist synchronization between the hemispheres. However, other types of stimuli with more translational implications and adherence to recent safety guidelines could be designed to activate and synchronize cortical networks more effectively, potentially leading to better stroke therapy and assure tDCS application (Gomez Palacio Schjetnan et al., 2013; Aparício et al., 2016; Bikson et al., 2016; Jackson et al., 2017). Studies in humans using EEG and fMRI suggest an increase in neural synchronization and functional connectivity induced by tDCS (Suppa and Berardelli, 2011; Sehm et al., 2013; Roy et al., 2014). A recent clinical study measuring resting state functional connectivity supports the present findings of increased connectivity after stroke rehabilitation in relevant motor control areas (Lefebvre et al., 2017).

In summary, our findings add to evidence that improved coherent activity between neuronal populations facilitates recovery after ischemic lesion. Importantly, our study suggests that a-tDCS mediates sensorimotor recovery by increasing the synchronization between cortical networks.

ACKNOWLEDGEMENTS

The authors thank Hiroe Yamazaki for help with the experiments and Dr. Mariam Alaverdashvili, Dr. Bruce McNaughton, and Amanda Mauthe-Kadoura for comments on the manuscript.

This research was supported by Alberta Innovates-Health Solutions (GM, AL), the Canadian Institutes of Health Research (GM), and the Natural Sciences and Engineering Research Council of Canada (GM, AL).

REFERENCES

Adkins DL, Bury SD, Jones TA (2002) Laminar-dependent dendritic spine alterations in the motor cortex of adult rats following callosal transection and forced forelimb use. Neurobiol Learn Mem 78: 35–52.

Adkins DL, Hsu JE, Jones TA (2008) Motor cortical stimulation promotes synaptic plasticity and behavioral improvements following sensorimotor cortex lesions. Exp Neurol 212: 14–28.

Alaverdashvili M, Whishaw IQ (2010) Compensation aids skilled reaching in aging and in recovery from forelimb motor cortex stroke in the rat. Neuroscience 167: 21–30.

Alsharidah M, Al-Hussain F, Iqbal M, Hamza A, Yoo W, Bashir S (2018) The effect of transcranial direct current stimulation combined with functional task training on motor recovery in stroke patients. Eur Rev Med Pharmacol Sci 22: 7385–7392.

Aparício LV, Guarienti F, Razza LB, Carvalho AF, Fregni F, Brunoni AR (2016) A systematic review on the acceptability and tolerability of transcranial direct current stimulation treatment in neuropsychiatry trials. Brain Stimul 9: 671–681.

Barker-Collo SL, Feigin VL, Lawes CM, Parag V, Senior H (2011) Attention deficits after incident stroke in the acute period: frequency across types of attention and relationships to patient characteristics and functional outcomes. Top Stroke Rehabil 17: 463–476.

Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jiménez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P; American Heart Association Statistics Committee and Stroke Statistics Subcommittee (2017) Heart disease and stroke statistics-2017 update: a report from the American Heart Association. Circulation 135: e146-e603.

Bermudez Contreras EJ, Schjetnan AG, Muhammad A, Bartho P, McNaughton BL, Kolb B, Gruber AJ, Luczak A (2013) Formation and reverberation of sequential neural activity patterns evoked by sensory stimulation are enhanced during cortical desynchronization. Neuron 79: 555–566.

Bikson M, Grossman P, Thomas C, Zannou AL, Jiang J, Adnan T, Mourdoukoutas AP, Kronberg G, Truong D, Boggio P (2016) Safety of transcranial direct current stimulation: evidence based update 2016. Brain Stimul 9: 641–661.

Boonzaier J, van Tilborg GA, Neggers SF, Dijkhuizen RM (2018) Noninvasive brain stimulation to enhance functional recovery after stroke: studies in animal models. Neurorehabilitation Neural Repair 32: 927–940.

Buckner RL, Corbetta M, Schatz J, Raichle ME, Petersen SE (1996) Preserved speech abilities and compensation following prefrontal damage. Proc Natl Acad Sci U S A 93: 1249–1253.

Chi RP, Snyder AW (2011) Facilitate insight by non-invasive brain stimulation. PLoS One 6: e16655.

Choo PL, Gallagher HL, Morris J, Pomeroy VM, van Wijck F (2015) Correlations between arm motor behavior and brain function following bilateral arm training after stroke: a systematic review. Brain Behav 5: e00411.

Coupar F, Pollock A, van Wijck F, Morris J, Langhorne P (2010) Simultaneous bilateral training for improving arm function after stroke. Cochrane Database Syst Rev: CD006432.

Crofts JJ, Higham DJ (2009) A weighted communicability measure applied to complex brain networks. J R Soc Interface 6: 411–414.

Debener S, Herrmann CS, Kranczioch C, Gembris D, Engel AK (2003) Top-down attentional processing enhances auditory evoked gamma band activity. Neuroreport 14: 683–686.

Faraji J, Gomez-Palacio-Schjetnan A, Luczak A, Metz GA (2013) Beyond the silence: bilateral somatosensory stimulation enhances skilled movement quality and neural density in intact behaving rats. Behav Brain Res 253: 78–89.

Floel A, Cohen LG (2010) Recovery of function in humans: cortical stimulation and pharmacological treatments after stroke. Neurobiol Dis 37: 243–251.

Fox D (2011) Neuroscience: Brain buzz. Nature 472: 156–158.

Fox MD, Buckner RL, Liu H, Chakravarty MM, Lozano AM, Pascual-Leone A (2014) Resting-state networks link invasive and noninvasive brain stimulation across diverse psychiatric and neurological diseases. Proc Natl Acad Sci 111: E4367-E4375.

Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, Lu B (2010) Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron 66: 198–204.

Gall C, Silvennoinen K, Granata G, de Rossi F, Vecchio F, Brösel D, Bola M, Sailer M, Waleszczyk WJ, Rossini PM (2015) Non-invasive electric current stimulation for restoration of vision after unilateral occipital stroke. Contemp Clin Trials 43: 231–236.

Gellner AK, Reis J, Fritsch B (2016) Glia: a neglected player in non-invasive direct current brain stimulation. Front Cell Neurosci 10: 188.

Gerloff C, Bushara K, Sailer A, Wassermann EM, Chen R, Matsuoka T, Waldvogel D, Wittenberg GF, Ishii K, Cohen LG, Hallett M (2006) Multimodal imaging of brain reorganization in motor areas of the contralesional hemisphere of well recovered patients after capsular stroke. Brain 129: 791–808.

Gladwin TE, den Uyl TE, Fregni FF, Wiers RW (2012) Enhancement of selective attention by tDCS: interaction with interference in a Sternberg task. Neurosci Lett 512: 33–37.

Gomez Palacio Schjetnan A, Faraji J, Metz GA, Tatsuno M, Luczak A (2013) Transcranial direct current stimulation in stroke rehabilitation: a review of recent advancements. Stroke Res Treat 2013: 170256.

Gonzalez Andino SL, Michel CM, Thut G, Landis T, Grave de Peralta R (2005) Prediction of response speed by anticipatory high-frequency (gamma band) oscillations in the human brain. Hum Brain Mapp 24: 50–58.

Harris KD, Thiele A (2011) Cortical state and attention. Nat Revi Neurosci 12: 509–523.

Herrmann CS, Frund I, Lenz D (2009) Human gamma-band activity: a review on cognitive and behavioral correlates and network models. Neurosci Biobehav Rev 34: 981–992.

Jackson MP, Bikson M, Liebetanz D, Nitsche M (2017) How to consider animal data in tDCS safety standards. Brain Stimul 10: 1141.

Jacobson L, Koslowsky M, Lavidor M (2012) tDCS polarity effects in motor and cognitive domains: a meta-analytical review. Exp Brain Res 216: 1–10.

Javadi AH, Walsh V (2011) Transcranial direct current stimulation (tDCS) of the left dorsolateral prefrontal cortex modulates declarative memory. Brain Stimul 5: 231–241.

Joundi RA, Jenkinson N, Brittain JS, Aziz TZ, Brown P (2012) Driving oscillatory activity in the human cortex enhances motor performance. Curr Biol 22: 403–407.

Kaiser J, Lutzenberger W, Decker C, Wibral M, Rahm B (2009) Task- and performance-related modulation of domain-specific auditory short-term memory representations in the gamma-band. Neuroimage 46: 1127–1136.

Karl JM, Alaverdashvili M, Cross A, Whishaw I (2010) Thinning, movement, and volume loss of residual cortical tissue occurs after stroke in the adult rat as identified by histological and magnetic resonance imaging analysis. Neuroscience 170: 123–137.

Kawano T, Hattori N, Uno Y, Kitajo K, Hatakenaka M, Yagura H, Fujimoto H, Yoshioka T, Nagasako M, Otomune H (2017) Large-scale phase synchro-

ny reflects clinical status after stroke: An EEG study. Neurorehabil Neural Repair 31: 561–570.
Kawano T, Hattori N, Hatakenaka M, Uno Y, Yagura H, Fujimoto H, Yoshioka T, Nagasako M, Otomune H, Kitajo K (2018) Comparison of EEG Synchrony Measures for Post-Stroke Neurorehabilitation. In: 2018 IEEE International Conference on Systems, Man, and Cybernetics (SMC), pp 35–38: IEEE.
Knieling M, Metz G, Antonow-Schlorke I, Witte O (2009) Enriched environment promotes efficiency of compensatory movements after cerebral ischemia in rats. Neuroscience 163: 759–769.
Koo H, Kim MS, Han SW, Paulus W, Nitche MA, Kim YH, Kim HI, Ko SH, Shin YI (2016) After-effects of anodal transcranial direct current stimulation on the excitability of the motor cortex in rats. Restor Neurol Neurosci 34: 859–868.
Kronberg G, Bridi M, Abel T, Bikson M, Parra LC (2017) Direct current stimulation modulates LTP and LTD: activity dependence and dendritic effects. Brain Stimul 10: 51–58.
Lefebvre S, Liew SL (2017) Anatomical parameters of tDCS to modulate the motor system after stroke: a review. Front Neurol 8: 29.
Lefebvre S, Dricot L, Laloux P, Desfontaines P, Evrard F, Peeters A, Jamart J, Vandermeeren Y (2017) Increased functional connectivity one week after motor learning and tDCS in stroke patients. Neuroscience 340: 424–435.
Liebetanz D, Nitsche MA, Tergau F, Paulus W (2002) Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain 125: 2238–2247.
Lim DH, LeDue JM, Mohajerani MH, Murphy TH (2014) Optogenetic mapping after stroke reveals network-wide scaling of functional connections and heterogeneous recovery of the peri-infarct. J Neurosci 34: 16455–16466.
Luczak A, Barthó P (2012) Consistent sequential activity across diverse forms of UP states under ketamine anesthesia. Eur J Neurosci 36: 2830–2838.
Luczak A, Barthó P, Harris KD (2009) Spontaneous events outline the realm of possible sensory responses in neocortical populations. Neuron 62: 413–425.
Luczak A, Bartho P, Harris KD (2013) Gating of sensory input by spontaneous cortical activity. J Neurosci 33: 1684–1695.
Luczak A, McNaughton BL, Harris KD (2015) Packet-based communication in the cortex. Nat Rev Neurosci 16: 745–755.
Luczak A, Barthó P, Marguet SL, Buzsáki G, Harris KD (2007) Sequential structure of neocortical spontaneous activity in vivo. Proceedings of the National Academy of Sciences 104: 347–352.
Metz GA, Whishaw IQ (2000) Skilled reaching an action pattern: stability in rat (Rattus norvegicus) grasping movements as a function of changing food pellet size. Behav Brain Res 116: 111–122.
Metz GA, Antonow-Schlorke I, Witte OW (2005) Motor improvements after focal cortical ischemia in adult rats are mediated by compensatory mechanisms. Behav Brain Res 162: 71–82.
Muehlschlegel S, Selb J, Patel M, Diamond SG, Franceschini MA, Sorensen AG, Boas DA, Schwamm LH (2009) Feasibility of NIRS in the neurointensive care unit: a pilot study in stroke using physiological oscillations. Neurocrit Care 11: 288–295.
Murphy TH, Corbett D (2009) Plasticity during stroke recovery: from synapse to behaviour. Nat Rev Neurosci 10: 861–872.
Nitsche M, Doemkes S, Karakose T, Antal A, Liebetanz D, Lang N, Tergau F, Paulus W (2007) Shaping the effects of transcranial direct current stimulation of the human motor cortex. J Neurophysiol 97: 3109–3117.
Nitsche MA, Liebetanz D, Antal A, Lang N, Tergau F, Paulus W (2003) Modulation of cortical excitability by weak direct current stimulation-technical, safety and functional aspects. Suppl Clin Neurophysiol 56: 255–276.
Paxinos G, Watson A (1998) The rat brain: in stereotaxic coordinates., 4th Edition Edition. San Diego: Academic Press.
Pellegrino G, Tomasevic L, Tombini M, Assenza G, Bravi M, Sterzi S, Giacobbe V, Zollo L, Guglielmelli E, Cavallo G (2012) Inter-hemispheric coupling changes associate with motor improvements after robotic stroke rehabilitation. Restor Neurol Neurosci 30: 497–510.
Rabiner LR, Gold B (1975) Theory and Application of Digital Signal Processing. Englewood Cliffs, NJ Prentice-Hall, Inc.
Reato D, Bikson M, Parra LC (2014) Lasting modulation of in vitro oscillatory activity with weak direct current stimulation. J Neurophysiol 113: 1334–1341.
Reis J, Fritsch B (2011) Modulation of motor performance and motor learning by transcranial direct current stimulation. Curr Opin Neurol 24: 590–596.
Roe JM, Nesheim M, Mathiesen NC, Moberget T, Alnaes D, Sneve MH (2016) The effects of tDCS upon sustained visual attention are dependent on cognitive load. Neuropsychologia 80: 1–8.
Roy A, Baxter B, He B (2014) High-definition transcranial direct current stimulation induces both acute and persistent changes in broadband cortical synchronization: a simultaneous tDCS-EEG study. IEEE Trans Biomed Eng 61: 1967–1978.
Roy LB, Sparing R, Fink GR, Hesse MD (2015) Modulation of attention functions by anodal tDCS on right PPC. Neuropsychologia 74: 96–107.
Schjetnan AG, Luczak A (2011) Recording large-scale neuronal ensembles with silicon probes in the anesthetized rat. J Vis Exp 56: e3282.
Schoffelen JM, Poort J, Oostenveld R, Fries P (2011) Selective movement preparation is subserved by selective increases in corticomuscular gamma-band coherence. J Neurosci 31: 6750–6758.
Sehm B, Kipping J, Schafer A, Villringer A, Ragert P (2013) A Comparison between uni- and bilateral tDCS effects on functional connectivity of the human motor cortex. Front Hum Neurosci 7: 183.
Strens LH, Asselman P, Pogosyan A, Loukas C, Thompson AJ, Brown P (2004) Corticocortical coupling in chronic stroke: its relevance to recovery. Neurology 63: 475–484.
Suppa A, Berardelli A (2011) Functional connectivity between non-primary motor cortex and primary motor and sensory areas investigated in humans with TDCS and rTMS. Clin Neurophysiol 122: 643–644.
Tanaka S, Takeda K, Otaka Y, Kita K, Osu R, Honda M, Sadato N, Hanakawa T, Watanabe K (2011) Single session of transcranial direct current stimulation transiently increases knee extensor force in patients with hemiparetic stroke. Neurorehabil Neural Repair 25: 565–569.
Teskey GC, Flynn C, Goertzen CD, Monfils MH, Young NA (2003) Cortical stimulation improves skilled forelimb use following a focal ischemic infarct in the rat. Neurol Res 25: 794–800.
Ukueberuwa D, Wassermann EM (2010) Direct current brain polarization: a simple, noninvasive technique for human neuromodulation. Neuromodulation 13: 168–173.
Utz KS, Dimova V, Oppenlander K, Kerkhoff G (2010) Electrified minds: transcranial direct current stimulation (tDCS) and galvanic vestibular stimulation (GVS) as methods of non-invasive brain stimulation in neuropsychology - a review of current data and future implications. Neuropsychologia 48: 2789–2810.
van Meer MP, Otte WM, van der Marel K, Nijboer CH, Kavelaars A, van der Sprenkel JWB, Viergever MA, Dijkhuizen RM (2012) Extent of bilateral neuronal network reorganization and functional recovery in relation to stroke severity. J Neurosci 32: 4495–4507.
van Meer MP, van der Marel K, Wang K, Otte WM, El Bouazati S, Roeling TA, Viergever MA, Berkelbach van der Sprenkel JW, Dijkhuizen RM (2010) Recovery of sensorimotor function after experimental stroke correlates with restoration of resting-state interhemispheric functional connectivity. J Neurosci 30: 3964–3972.
Wang L, Yu C, Chen H, Qin W, He Y, Fan F, Zhang Y, Wang M, Li K, Zang Y, Woodward TS, Zhu C (2010) Dynamic functional reorganization of the motor execution network after stroke. Brain 133: 1224–1238.
Westlake KP, Nagarajan SS (2011) Functional connectivity in relation to motor performance and recovery after stroke. Front Syst Neurosci 5: 8.

Whishaw IQ, Pellis SM (1990) The structure of skilled forelimb reaching in the rat: a proximally driven movement with a single distal rotatory component. Behav Brain Res 41: 49–59.

Winship IR, Murphy TH (2009) Remapping the somatosensory cortex after stroke: insight from imaging the synapse to network. Neuroscientist 15: 507–524.

Womelsdorf T, Fries P, Mitra PP, Desimone R (2006) Gamma-band synchronization in visual cortex predicts speed of change detection. Nature 439: 733–736.

Wróbel A, Bekisz M, Kublik E, Waleszczyk W (1994) 20 Hz bursting beta activity in the cortico-thalamic system of visually attending cats. Acta Neurobiol Exp 54: 95–107.