Published in final edited form as:

Neuromodulation. 2012 July ; 15(4): 283–295. doi:10.1111/j.1525-1403.2012.00478.x.

Analgesic effects of noninvasive brain stimulation in rodent animal models: A systematic review of translational findings

Magdalena Sarah Volz^{1,2,3}, Theresa Sophie Volz¹, Andre Russowsky Brunoni, MD⁴, João Paulo Vaz Tostes Ribeiro de Oliveira^{1,4}, and Felipe Fregni, MD, PhD^{1,3,4,*}

¹Laboratory of Neuromodulation, Department of Physical Medicine & Rehabilitation, Spaulding Rehabilitation Hospital and Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

²Charité University Medicine Berlin, Berlin, Germany

³Spaulding-Harvard Spinal Cord Injury Model System, Spaulding Rehabilitation Hospital, Boston, MA, USA

⁴University Hospital, University of São Paulo, São Paulo, Brazil

Abstract

Objectives—Noninvasive brain stimulation (NIBS) interventions have demonstrated promising results in the clinical treatment of pain, according to several preliminary trials, although the results have been mixed. The limitations of clinical research on NIBS are the insufficient understanding of its mechanisms of action, a lack of adequate safety data, and several disparities with regard to stimulation parameters, which have hindered the generalizability of such studies. Thus, experimental animal research that allows the use of more invasive interventions and creates additional control of independent variables and confounders is desirable. To this end, we systematically reviewed animal studies investigating the analgesic effects of NIBS. In addition we also explored the investigation of NIBS in animal models of stroke as to compare these findings with NIBS animal pain research.

Methods—Of 1916 articles that were found initially, we identified 15 studies (stroke and pain studies) per our eligibility criteria that used NIBS methods, such as transcranial direct current stimulation (tDCS), paired associative stimulation (PAS), transcranial magnetic stimulation (TMS), and transcranial electrostimulation (TES). We extracted the main outcomes on stroke and pain, as well as the methods and electrical parameters of each technique.

*Corresponding author: Felipe Fregni, MD, PhD, MPH, Laboratory of Neuromodulation, Spaulding Rehabilitation Hospital, 125 Nashua Street, #727, Boston, MA, USA 02114, fregni.felipe@mgh.harvard.edu, Phone: 617-573-2195 Fax: 617-573-7198.

Author's contributions

Magdalena S. Volz: designed the study; data collection; data analysis; discussed the results; drafting of the manuscript

Theresa S. Volz: designed the study; data collection; discussed the results; commented on the manuscript

Andre Russowsky Brunoni: discussed the results; commented on the manuscript

João Paulo Vaz Tostes Ribeiro de Oliveira: data collection; discussed the results; commented on the manuscript

Felipe Fregni: conceived and designed the study; discussed the results; commented and finalized the manuscript

Disclosure

The authors have nothing to disclose.

Results—NIBS techniques are effective in alleviating pain. Similar beneficial clinical effects are observed in stroke. The main insights from these animal studies are: (i) combination of NIBS with analgesic drugs has a synergistic effect; (ii) effects are dependent on the parameters of stimulation, and in fact, not necessarily the strongest stimulation parameter (i.e., the largest intensity of stimulation) is associated with the largest benefit; (iii) pain studies show an overall good quality as indexed by ARRIVE guidelines of the reporting of animal experiments, but insufficient with regard to the reporting of safety data for brain stimulation; (iv) these studies suggest that NIBS techniques have a primary effect on synaptic plasticity, but they also suggest other mechanisms of action such as via neurovascular modulation.

Conclusions—We found a limited number of animal studies for both pain and stroke NIBS experimental research. There is a lack of safety data in animal studies in these two topics and results from these studies have not been yet fully tested and translated to human research. We discuss the challenges and limitations of translating experimental animal research on NIBS into clinical studies.

Keywords

brain stimulation; pain; stroke; translational research; systematic review

Introduction

Noninvasive brain stimulation (NIBS) interventions are techniques that are being increasingly tested to induce neuroplasticity to effect clinical changes and guide plasticity in neuropsychiatric disorders. The most frequently used methods are transcranial direct current stimulation (tDCS), paired associative stimulation (PAS), transcranial magnetic stimulation (TMS), and transcranial electrical stimulation (TES).

TES is an established NIBS technique where high-frequency, intermittent, balanced current is delivered to the cortex via scalp electrodes ¹. tDCS and TMS have received significant attention in the past decade. In tDCS, a weak, electric direct current is applied to the brain through relatively large electrodes. TMS generates a varying magnetic field that secondarily induces a transient electrical change, predominantly in superficial cortical areas. In contrast, PAS utilizes the mechanisms of a combined stimulation of a peripheral nerve and the motor cortex. It is a relatively novel technique, which was firstly described in 2000 by Stefan et al.

Transcranial electrical stimulation techniques (such as TES and tDCS) have been used and tested for several decades. By introducing a new electrical stimulation protocol in the sixties called *Limoge's current*, TES has become safer, which led to an increased interest of researchers in the field ¹. In contrast, tDCS has a long history, with anecdotal use reported in the past 200 to 300 years ², whereas TMS was first introduced in 1985 ³. Especially the techniques of tDCS and TMS have risen in relevance and significance in the past 25 years, because they have been effective against various forms of pain, including neuropathic and chronic pain syndromes and fibromyalgia and pain after spinal cord injury ⁴⁻⁹. Both techniques also alleviate poststroke symptoms and facilitate recovery after central insults, particularly motor recovery ¹⁰⁻¹³.

Yet, the principles and efficacy of NIBS with regard to its underlying therapeutic effects are unknown. In addition, NIBS research in human subjects is often challenging to perform for many reasons, such as lack of recruitment, ethical concerns, and methodological difficulties¹⁴. In brain research in humans, it is often not possible to perform invasive experiments or collect objective markers of safety.

In contrast to human trials, NIBS research with experimental animals enables histological and molecular analyses, stimulation-pharmacotherapy studies, and more explicit testing of safety limits. Despite its elegance, translational research creates challenges and open-ended questions in pain and stroke research^{15, 16}. Can we translate the results from experimental research to humans? What limitations and challenges need to be addressed? Given that most experimental research in brain stimulation uses invasive montages; it is necessary to analyze results from research in animals using noninvasive approaches of brain stimulation to facilitate its translation to humans and increase its applicability.

Thus, we performed a systematic review to identify translational NIBS studies in rodent pain models. We also applied the same strategy for studies in rodent stroke models as to compare with pain studies. The primary aim was to collect and summarize the outcomes from these studies. In addition, we aimed to describe the procedures of various NIBS techniques. We collected data on stimulation parameters and electrical variables of brain stimulation techniques, including intensity, duration, frequency, and polarity. We also reviewed all identified articles with regard to safety, after-effects, attrition, and histological analysis.

Methods

Eligibility criteria

We included studies per the following inclusion criteria: (1) articles that were published before December 2011; (2) studies that measured at least 1 stroke- or pain-related outcome; (3) studies that delivered transcranial brain stimulation; (4) experimental studies in animals; and (5) studies that were published in English, German, or Portuguese.

The exclusion criteria were: (1) use of invasive brain stimulation (eg, intracerebral/epidural stimulation, invasive electrostimulation, deep brain stimulation); (2) use of techniques other than tDCS, TMS, PAS, and TES (eg, electroacupuncture, vagus/trigeminus nerve stimulation, peripheral nerve stimulation); (3) studies in humans; (4) studies in vitro; and (5) articles that were reviews or editorials or that reported duplicate data or data that were extracted from original articles (Figure 1).

Literature search

We searched the Medline database using the following keywords:

- “brain stimulation” OR
- “noninvasive brain stimulation” OR
- “transcranial direct current stimulation” OR
- “transcranial magnetic stimulation” OR

- “transcranial electrostimulation”

AND

- “rat(s)” OR
- “rodent(s)” OR
- “mouse” OR
- “mice”

AND

- “stroke” OR
- “pain.”

Additionally, the reference lists from the articles were screened, and researchers in the field of experimental NIBS were consulted.

Data extraction

Each article was screened for the following data:

1. Experimental results on transcranial brain stimulation, including its effects on stroke recovery and pain perception and safety, lesion, and histological analyses;
2. Technique of brain stimulation, including details on the method and application, such as the location and duration of stimulation, electrode size, intensity, and frequency;
3. Sample characteristics, such as sample size (with active, sham or without stimulation), gender, weight, and animal species.

Qualitative analysis

We assessed the quality of reporting per the ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines on the reporting of animals ^{17, 18}. To analyze these data, the following aspects were extracted from each study: methods [randomization, blinding, control (no stimulation vs. sham stimulation)], experimental procedures (technique of brain stimulation, details of experiment, and measurements/assessment of pain or stroke), experimental animals (species, sample, gender, weight), housing and husbandry (light/dark cycle, number of cage companions, access to food and water), and analysis (outcomes, attrition/safety, histological analyses).

Quantitative analysis

We anticipated that studies of NIBS in animals would be scarce, spread out over decades, and heterogeneous; thus, we initially chose not to perform a meta-analysis, limiting the quantitative analysis to descriptive statistics, such as means and frequencies. Further, when studies included several experiments using various parameters of stimulation, we used the standard value to calculate the statistics.

Results reporting

Because the majority of articles that met our inclusion criteria was on pain and this was our main goal in this study, we reported the results for rat models of pain in greater detail. Although this filter resulted in 5 articles on rat models of stroke, we included them in a summarized manner.

Results

Our search initially resulted in 1916 articles (Figure 1). Most articles were not included in this review per the eligibility criteria, primarily due to (i) use of invasive brain stimulation; (ii) investigation of conditions other than stroke or pain; and (iii) failure to describe original data (Figure 1). After reviewing 20 full-text articles, we determined 15 reports to meet our overall criteria (Table 1-I and 1-II).

Three of the 20 articles were excluded, because they did not use any measurement of pain and focused on anesthesia¹⁹, opiate abstinence syndrome²⁰, and levels of opioids and neurotransmitters in rat brains²¹. Moreover, 2 of the 20 articles were excluded, because they used experimental models of transient, induced ischemia rather than a permanent stroke model^{22, 23}. Ultimately, we identified 10 articles that reported the effects of NIBS on pain perception in rats and 5 articles that were related to stroke and its outcomes.

Sample

All statistical analyses are shown in Table 2. Notably, all experiments used only male rats. The total number of rats in all studies was 1503. The mean sample size was 100 (SD = 85.6; range 24–334). Eleven studies used Sprague-Dawley rats (total 1187 rats), 3 studies used Wistar rats (total 224 rats), and 1 study used Simonsen Albino (S/A) rats (total 92 rats).

The mean weight was 253.1 g (SD = 83; range 140–553). All studies that examined pain used only wild-type rats. Further, the reports on stroke used disparate models on their animals, only 1 of which had a healthy control group.

Animal models of pain

We identified 9 pain-related studies that used TES and 1 study that combined tDCS and TES. The total sample size was 1244 male rats. The main outcomes and results of each study are described in Table 3.

Eight studies demonstrated that TES significantly relieved acute pain sensations, whereas 2 studies did not show any difference between sham and active stimulation. All studies that examined the combination of TES with pain medications found that it enhanced and potentiated the analgesic effects.

All studies measured pain by behavioral assessments. Nine studies used the tail flick test (TFL), which measures the latency between painful exposure of a rat's tail and its retraction; 1 study used the hot plate test (HP) (which also measures an animal's reaction time to a hot plate while standing on it); and 1 study used tolerated peak pressure (TPP) as the main outcome measure. All experiments were performed in male, wild-type rats.

Pain outcomes versus electrical stimulation parameters

Warner et al. showed that the verum TES analgesic effect was 613% higher than sham stimulation²⁴, using the following stimulation parameters: frequency 10 Hz, intensity 10 μ A, pulse width 2 msec, electrode positioning bilaterally at the pinnae of the apex, and duration 30 min - the standard stimulation parameters in all studies (Table 1-I and 1-II).

Consistent with this finding, Wilson et al. investigated the effects of a range of stimulation parameters, 5 – 50 Hz, 0.1 – 8 ms pulse-width, and electrode positioning (e.g., 1 electrode in each ear, or 2 electrodes in either the left or the right ear - the electrode size is not reported). Analgesia had a maximum stimulation intensity of 10 μ A, 10 Hz frequency, and pulse width of 2 msec²⁵. In another study, this group aimed to determine the most effective stimulation intensity (5 - 20 μ A) and duration (10 - 60 min), observing the maximum analgesic effect at 10 μ A intensity and 30 min duration. In this study, TES had long-lasting effects, up to 200 min after stimulation²⁶.

Similar analgesic effects were confirmed in Nekhendzy et al., who also demonstrated that the effects were significantly altered by TES frequency but not by stimulation period²⁷. Although, in contrast to the prior studies above, they concluded that a frequency between 40 and 65 Hz induced the greatest antinociceptive effect.

Combination of transcranial electrostimulation and pharmacotherapy: insights into mechanisms of action in animal models of pain

Skolnick et al. observed that naloxone, an opiate antagonist, abolishes the TES analgesic effects; thus, implicating an opiate-mediated pathway of TES-induced analgesia²⁶. A separate study generated similar evidence, demonstrating that TES, combined with morphine, tripled the analgesic effects, as assessed by TFL. In this study, TES potentiated opioid-induced analgesia, and intriguingly, attenuated opiate abstinence syndrome by 48%²⁸.

Further insight into the TES mechanism of action comes from Warner et al., who found that pretreatment with p-chlorophenylalanine, a serotonin synthesis inhibitor, diminished TES-mediated analgesic effects, as evidenced by a decrease in pain assessment (tolerated peak pressure) of 91.5%²⁴, indicating that serotonin is involved in TES-induced effects. Similarly, Malin et al. found that rats receiving both L-tryptophan and TES experienced significantly greater analgesia than rats administered either placebo drug and TES, or active drug and sham stimulation, or placebo drug and sham stimulation²⁹.

Yet another controlled study demonstrated the adjunctive potential of TES, which significantly augmented the effects of thiorphan and acetorphan, suggesting that the analgesic effects of TES can be potentiated by enkephalinase inhibitors. Notably, these effects were elicited by either intraventricular and intraperitoneal injections³⁰.

Kabalak et al. (2004) found that TES, combined with remifentanyl, increased the peak of analgesia 150% higher compared with the drug-only group and 251% greater than the stimulation-only group. TES prolonged analgesia for 20 min versus all other groups.

Although this finding is additional evidence that TES is linked to the opioid system, TES alone fails to modify pain thresholds during stimulation ³¹.

This finding of effective combined treatment of TES and drugs is similar to another study, in which 6-hour application of TES did not modulate pain thresholds. However, this report observed that TES potentiated morphine-induced analgesia, peaking with stimulation 3 hours prior to drug injection. This potentiation depended on morphine dose and TES intensity, with maximum analgesic effects at 100 mA (peak to peak value) intensity and a morphine dose of 10 µg. There were no significant effects when stimulation was not maintained after drug injection ³².

In yet another study showing a synergistic analgesic effect of TES and pharmacotherapy, Kabalak et al. showed that TES decreased the requirements for thiopental and remifentanyl. Specifically, TES, in combination with these agents, led to an analgesic effect that lasted for up to 90 min., increasing by 30% to 40% compared with baseline, 160% versus control, and 50% to 75% compared with TES or thiopental alone ³³.

Animal models of stroke – comparison with animal pain studies

We identified 5 stroke studies that used animal models—2 tDCS, 1 PAS, and 2 TMS—totaling 259 male rats (see Table 1-I and 1-II for details). All studies showed significant effects and changes from brain stimulation (Table 3).

Shin et al. investigated motor recovery and MEPs after stroke using middle cerebral artery occlusion (MCAO) with PAS. Amplitudes of MEPs after PAS sessions increased in the active group, which had significantly higher Garcia's scores on the Day 7 and stable MEPs ³⁴. Another tDCS study showed that anodal stimulation improved motor scores on postoperative Day 16, which also occurred in the exercise group. In contrast, cathodal stimulation resulted in deterioration of motor function on postoperative Day 16. There was no significant difference in mean infarct volume between groups ³⁵.

Zhang et al. studied the effects of TMS, which significantly improved motor function on Days 7, 14, 21, and 28 after infarct using MCAO model compared with the untreated group ³⁶.

Kaga et al. evaluated MEPs and measured their amplitudes, observing that the group with MCAO experienced an increase in amplitude by 200% over preischemic values, remaining significantly high for 60 min. The group with basilar artery occlusion (BAO) had MEPs that were 10% of preischemic values, staying significantly low for 60 min ³⁷.

Another study, which did not report the stroke model used properly, examined the effects of tDCS on cortical blood flow (CBF), noting that anodal stimulation at 50 and 100 µA increased CBF up to 30 min and that 100 µA intensity led to a rise in CBF of 25%, whereas 50 µA effected a CBF of 18%. Conversely, cathodal stimulation decreased cortical blood flow, also in proportion to current intensity ³⁸.

In summary, NIBS studies in animal models of stroke have used other techniques of NIBS such as TMS and tDCS. These studies, in contrast with pain studies, used more

neurophysiological outcomes such as MEP and CBF and also more histological analysis. Similar to pain studies, stroke studies in animals also assessed the effects of NIBS in the acute phase. Also similarly, both groups of studies assessed different parameters of stimulation.

Stimulation parameters

We assessed all stimulation parameters variables for each brain stimulation technique separately (Table 2). Regarding TES, the applied stimulation regimens differed between studies, so that the pulse width range was 0.1 ms to 8 ms and the duration range was 10 min to 5760 min. However, across the studies there were two stimulation regimens most commonly used, which utilized following parameters: first regimen: 10 Hz frequency with 0.01 mA intensity (exception: one study used 60 Hz and 2.25 mA), and second regimen: high-frequency stimulation using 166 kHz with intermittent low-frequency utilizing 100 Hz. The intensity of the second regimen is described to be peak-to-peak of 100 mA with an effective current of 17.5 mA (Limoge's current). Since the first stimulation regimen has less intensity but longer effective duration of stimulation and the second stimulation regimen the reversed protocol, both regimens can be considered to induce similar effects, which makes comparability possible. In 5 studies, the electrode for TES was set bilaterally to the ears (exception: one study additionally used a montage where both electrodes were placed in the same ear). The other 5 studies used three electrodes (2 electrodes on the mastoid and 1 over the frontal cortex or between the eyes).

Two studies used the technique of TMS. Kaga et al. placed the coil in the center of the cranium and delivered stimuli at 120% of the MEP threshold³⁷. Zhang et al. stimulated animals twice daily with a frequency of 0.5 Hz³⁶.

Three different tDCS montages were used. Kim et al. applied tDCS with 0.1 mA intensity and 30 min duration. The active electrode (0.785 cm²) was placed in front of interaural line, whereas the reference electrode (9 cm²) was attached to the trunk³⁵. Wachter et al. applied 25 - 100 μ A intensity and 15 min duration. The montage was set with the active electrode (3.5 mm²) behind the coronal suture and the reference electrode (10.5 cm²) over the thorax³⁸. Nekhendzy et al. applied TES with combined direct and alternating current with 2.25 mA intensity and 45 min duration. The electrodes were placed over the frontal cortex (cathode) and attached to the mastoid (anode)²⁷.

Quality assessment

We used a modified checklist of the ARRIVE guidelines to assess study quality (Table 4-I and 4-II). Randomization processes were reported in 9 studies; 6 did not describe them. Blinding methods were applied in 10 studies; 4 did not describe the blinding assessment, and 1 study was not blinded. Fourteen studies had control groups, and 11 studies applied sham stimulation (total, 434 rats with placebo stimulation). Housing and husbandry were not described in 4 studies; 6 studies provided an incomplete description, and 5 studies described housing and husbandry protocols correctly.

Six studies did not report attrition or safety aspects. Nine studies reported safety and attrition, primarily limiting the description to the number of deaths during the surgical

procedure (ie, not directly related to brain stimulation). Three of these 9 studies reported the absence of adverse effects due to brain stimulation, and another 3 incompletely described adverse effects as no seizures observed³⁶, no “catalepsy or sedation”³², and no skin burns²⁷.

Histological analysis were performed in 4 studies, all of which described pathologies due to induced stroke, with 1 study reporting an animal with colliquative necrosis in the left parieto-occipital region³⁸; 11 studies did not include a histological examination.

When comparing quality assessment between the two groups (pain vs. stroke), it can be observed that pain studies have an overall better quality as shown by better reporting of blinding and randomization as compared with stroke studies (see Table 4-I and 4-II).

Discussion

After a systematic literature review, we included and reviewed 15 articles on noninvasive brain stimulation techniques, including TES, TMS, tDCS, and PAS, in experimental animals for treating pain and stroke.

TES was the most frequent method that was examined—3 studies investigated TES only and observed that it was effective in ameliorating acute pain. Further, 7 studies administered a combination of TES and pain medication, which significantly augmented and potentiated the analgesic effects; 3 of these studies described a significant effect of TES alone, comparing sham and real stimulation, whereas 2 trials could not confirm the analgesic effect of TES. Three studies, all examining stroke, noted a beneficial effect of PAS, tDCS, and TMS on motor recovery.

Thus, nearly all preclinical studies demonstrated significant effects of noninvasive neuromodulatory techniques for pain and stroke.

Pain

Mechanisms of brain stimulation in relieving pain—The studies on pain and TES proffer interesting suggestions on the involvement of neurotransmitters associated with effects of TES. Pretreatment with pCPA (a serotonin antagonist) reversed analgesic effects, whereas pre-treatment with L-tryptophan (a biochemical precursor of serotonin) enhanced the analgesia, implicating serotonin in TES-induced analgesia^{24, 29}.

The opiate system also appears to play a role in the mechanisms of TES. For instance, naloxone (an opioid receptor antagonist) abolishes the analgesic effects of TES^{26, 29}, and thiorphan and acetorphan, which are antagonists of endogenous opioid peptide receptors, intensify the analgesic effects of TES³⁰, consistent with the hypothesis that the effects of TES are related to the opiate system^{31, 32}. Similarly, another study noted that TES significantly attenuates morphine abstinence syndrome²⁸.

Nekhendzy et al. compared TES with diffuse noxious inhibition, because they function in the same time frame and depend on stimulation intensity²⁷. Further, TES is not always

effective in inducing analgesia in every subject, raising the possibility of other pathways of pain that are not modulated by TES ²⁶.

Translation of stimulation parameters—The effects of TES on antinociception and analgesia depend on frequency and intensity. A current intensity of 0.01 mA and a frequency of 10 Hz induce maximum analgesia in a TES paradigm ²⁵, whereas TES, coupled with DC, is most effective in pain relief at frequencies between 40 to 65 Hz ²⁷. One study observed the maximum effect with stimulation 3 hours prior to drug injection ³². Without a drug adjunct, the optimal stimulation was 30 min ²⁶. Further, no effects of morning or nighttime stimulation were observed ²⁵.

One challenging issue in the field of brain stimulation is to compare parameters across studies; especially due to the fact that it is not known which parameters exactly induce most of the biological effects (or whether it is a combination of them). For instance, when describing AC stimulation, some important parameters are: peak to peak current, DC component (that may be zero if positive and negative deflections are equal) and effective current. In fact, if the DC component is the most important parameter, two studies with same DC components but with different peak to peak currents (for instance 0.1 vs. 100 mA) may induce similar biological effects.

The after-effects varied between studies and lasted for 20 min after the end of TES in combination with remifentanyl ³¹ and 60 min with a combination of TES and opiates ³²; another study reported significant analgesia that persisted for 200 min after stimulation ²⁶.

Mechanisms of brain stimulation as evidenced by animal stroke model studies—As most of the stroke studies, differently than pain studies, assessed the mechanisms of brain stimulation, they provide interesting findings to understand the mechanisms underlying the therapeutic effects of NIBS. One study showed that tDCS, with a current intensity of 0.1 mA, for 15 min induced sustained changes in, which lasted for at least 30 min after the stimulation ceased. This effect was polarity-specific; anodal tDCS increased the CBF, whereas cathodal tDCS decrease it. The authors discussed several possibilities to explain mechanisms of stimulation, including direct action on blood vessels and indirect effects of the induced modulation in neurovascular coupling. The latter hypothesis, which suggests that neurons modulate cortical blood flow in regions of activation, appears to be more likely ³⁸.

Another study observed that TMS using 0.5 Hz frequency upregulates c-Fos and BDNF, prominently in the peri-infarct area, implying that TMS affects neural cells in the penumbral area or the direct effects are localized under the area of stimulation ³⁶. Further, Kim et al. demonstrated that transcranial anodal stimulation reduces white matter axonal damage after ischemia, suggesting that the effects of tDCS are linked to the modulation of calcium channel and NMDA receptor activity, which might cause white matter injury due to excessive glutamate release following acute stroke ³⁵. Yet, Kim et al. generated mixed evidence, including a paradoxical deterioration in behavioral scores after cathodal stimulation.

Safety and quality of studies—We used the ARRIVE guidelines to evaluate study quality¹⁷. With regard to blinding, randomization, and presence of a control group, the studies showed satisfactory to moderate reporting, although some groups failed to describe all methods in detail; 14 studies used control animals, and 11 experiments included sham stimulation.

The quality of experimental procedures varied widely between studies—some reports had large control groups with sham stimulation²⁶ or without stimulation³⁶ and implemented a disease model at a high standard³⁷, whereas other studies did not use a control group and did not discuss how they induced stroke³⁸. Overall, sample sizes were adequate by having mean size of 100, and all studies used male rodents only.

With regard to stimulation parameters, TES was the best-reported technique, whereas TMS was incompletely described; the partial description of tDCS had missing variables. Housing and husbandry practices were appropriately conducted when studies reported these conditions, but some values, such as the number of cage companions, were generally not stated. Finally, when comparing pain vs. stroke studies, pain studies were better reported in most of the quality category items such as blinding and randomization.

In contrast, the quality of the evaluation and reporting of safety aspects of brain stimulation was not satisfactory, wherein only generalized statements or partial descriptions of the observed effects were given. The chief criticism with regard to safety reporting is that the studies failed to distinguish between attrition of the surgery (experimental procedure) and brain stimulation, precluding valuable safety data on noninvasive brain stimulation to be gleaned. Similarly, the histological analyses were performed primarily to evaluate the stroke model—not to assess the safety of brain stimulation.

Limitations and translation of results to humans—A significant limitation of the studies that we reviewed concerns the potential for their results to be translated (Table 1-II: see “challenges”). For instance, all experiments were conducted in male rats, complicating the translation of the results to both genders in humans, particularly because the effects of NIBS are altered by modulations in hormone state^{39, 40}. In addition, only 1 species was evaluated, preventing interspecies interpretation of the results.

No study evaluated chronic models of stroke or pain. Yet, clinical brain stimulation studies are performed primarily for chronic pain and subacute or chronic stroke^{41, 42}; thus, this difference also limits interspecies comparisons from being made. Further, in animal models of stroke, lesions are induced under controlled conditions and anesthesia, which might have a neuroprotective effect and thus accelerate recovery. On the other hand, based on these findings one would expect that human studies would explore NIBS for acute pain or acute stroke; however it is minimal the number of studies in the acute stage of these conditions. Similarly, given the results of pain studies showing that NIBS has a synergistic effect to analgesic drugs, it would be expected that this strategy had been tested in humans; but there is a lack of studies exploring this approach. Though, in the field of major depression, this approach – combination with NIBS and drugs - has been tested before in several human trials.

The application of NIBS methods is not the same in animals and humans with regard to montages and stimulation parameters. For example, the application of TMS to animals involves relatively larger coils⁴³, and current density, particularly in tDCS, is usually set higher in animal models compared with humans, as shown in a recent systematic review¹⁴.

There are several minor aspects that can complicate the interpretation of the results. Although the electrode positioning was well described in the included articles (Tables 1 and 2: see “*electrode positioning*”), the brain areas that were stimulated are unknown, constituting a significant limitation in comparing results between studies, because the neuronal mechanisms that mediate these effects might differ. Moreover, the stroke studies used disparate methods of NIBS (tDCS, PAS and TMS) and differed in rationale, methodology, and outcome measures. Thus, the conclusion that NIBS is a beneficial therapy for recovery after stroke needs to be interpreted cautiously.

Future Directions—Our review has demonstrated that existing animal NIBS reports on pain and also stroke are limited in certain aspects that should be addressed in future studies—no study focused on chronic stroke or pain, which are the most prevalent conditions that are encountered in clinical practice in physical rehabilitation. In addition, there was little assessment of adverse effects—an important aspect that can be investigated in animal research—in the reviewed studies. The safety data of brain stimulation were not provided, due to erratic reporting.

Moreover, the results of future studies should also increase our understanding of the underlying mechanisms of TES and facilitate their translation into clinical treatments, especially because most studies in humans have only examined therapies for chronic pain syndromes and chronic or subacute stroke. Better models of noninvasive brain stimulation should be developed—for instance, using small TMS coils in rodents and tDCS/TES models that mimic those in humans better.

Acknowledgments

Volz MS was funded by a grant scholarship from the German Academic Exchange Service (DAAD). This work was partially supported by a NIH grant (5R21DK081773 - 03), Christopher and Dana Reeve Foundation and NIDRR SCI model systems program.

Financial Support

Volz MS was funded by a grant scholarship from the German Academic Exchange Service (DAAD).

Abbreviation list

BA(O)	basilar artery (occlusion)
BDNF	brain-derived neurotrophic factor
CBF	cortical blood flow
HP	hot plate latency
LDF	Laser Doppler flowmetry

MCA(O)	middle cerebral artery (occlusion)
MEP	motor evoked potential
n	micro
N/A	not applicable
NIBS	Non-invasive brain stimulation
NSS	Neurological Severity Scores
PAS	paired associative stimulation
pCPA	p-chlorophenylalanine
RMT	resting motor threshold
tDCS	transcranial direct current stimulation
TES	transcranial electrostimulation
TFL	tail flick latency
TMS	transcranial magnetic stimulation
TPP	tolerated peak pressure

References

1. Limoge A, Robert C, Stanley TH. Transcutaneous cranial electrical stimulation (TCES): a review 1998. *Neurosci Biobehav Rev.* 1999; 23:529–38. [PubMed: 10073891]
2. Priori A. Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability. *Clin Neurophysiol.* 2003; 114:589–95. [PubMed: 12686266]
3. Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. *Lancet.* 1985; 1:1106–7. [PubMed: 2860322]
4. Sampson SM, Rome JD, Rummans TA. Slow-frequency rTMS reduces fibromyalgia pain. *Pain Med.* 2006; 7:115–8. [PubMed: 16634724]
5. Lima MC, Fregni F. Motor cortex stimulation for chronic pain: systematic review and meta-analysis of the literature. *Neurology.* 2008; 70:2329–37. [PubMed: 18541887]
6. Fregni F, Freedman S, Pascual-Leone A. Recent advances in the treatment of chronic pain with non-invasive brain stimulation techniques. *Lancet Neurol.* 2007; 6:188–91. [PubMed: 17239806]
7. Pleger B, Janssen F, Schwenkreis P, Volker B, Maier C, Tegenthoff M. Repetitive transcranial magnetic stimulation of the motor cortex attenuates pain perception in complex regional pain syndrome type I. *Neurosci Lett.* 2004; 356:87–90. [PubMed: 14746870]
8. Nguyen JP, Lefaucher JP, Le Guerinel C, et al. Motor cortex stimulation in the treatment of central and neuropathic pain. *Arch Med Res.* 2000; 31:263–5. [PubMed: 11036176]
9. Fregni F, Boggio PS, Lima MC, et al. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. *Pain.* 2006; 122:197–209. [PubMed: 16564618]
10. Alonso-Alonso M, Fregni F, Pascual-Leone A. Brain stimulation in poststroke rehabilitation. *Cerebrovasc Dis.* 2007; 24(Suppl 1):157–66. [PubMed: 17971652]
11. Fregni F, Pascual-Leone A. Hand motor recovery after stroke: tuning the orchestra to improve hand motor function. *Cogn Behav Neurol.* 2006; 19:21–33. [PubMed: 16633016]
12. Fregni F, Boggio PS, Mansur CG, et al. Transcranial direct current stimulation of the unaffected hemisphere in stroke patients. *Neuroreport.* 2005; 16:1551–5. [PubMed: 16148743]

13. Mahmoudi H, Haghighi AB, Petramfar P, Jahanshahi S, Salehi Z, Fregni F. Transcranial direct current stimulation: electrode montage in stroke. *Disabil Rehabil.* 2011
14. Brunoni AR, Amadera J, Berbel B, Volz MS, Rizzerio BG, Fregni F. A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *Int J Neuropsychopharmacol.* 2011;1–13.
15. Mao J. Translational pain research: achievements and challenges. *J Pain.* 2009; 10:1001–11. [PubMed: 19628433]
16. Zaleska MM, Mercado ML, Chavez J, Feuerstein GZ, Pangalos MN, Wood A. The development of stroke therapeutics: promising mechanisms and translational challenges. *Neuropharmacology.* 2009; 56:329–41. [PubMed: 19007799]
17. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol.* 2010; 8:e1000412. [PubMed: 20613859]
18. Hooijmans CR, Leenaars M, Ritskes-Hoitinga M. A gold standard publication checklist to improve the quality of animal studies, to fully integrate the Three Rs, and to make systematic reviews more feasible. *Altern Lab Anim.* 2010; 38:167–82. [PubMed: 20507187]
19. Mantz J, Azerad J, Limoge A, Desmonts JM. Transcranial electrical stimulation with Limoge's currents decreases halothane requirements in rats. Evidence for the involvement of endogenous opioids. *Anesthesiology.* 1992; 76:253–60. [PubMed: 1736702]
20. Malin DH, Murray JB, Crucian GP, Schweitzer FC, Cook RE, Skolnick MH. Auricular microelectrostimulation: naloxone-reversible attenuation of opiate abstinence syndrome. *Biol Psychiatry.* 1988; 24:886–90. [PubMed: 2852967]
21. Warner RL, Johnston C, Hamilton R, Skolnick MH, Wilson OB. Transcranial electrostimulation effects on rat opioid and neurotransmitter levels. *Life Sci.* 1994; 54:481–90. [PubMed: 7906003]
22. Jang DK, Park SI, Han YM, et al. Motor-evoked potential confirmation of functional improvement by transplanted bone marrow mesenchymal stem cell in the ischemic rat brain. *J Biomed Biotechnol.* 2011; 2011:238409. [PubMed: 21772790]
23. Yoon KJ, Lee YT, Han TR. Mechanism of functional recovery after repetitive transcranial magnetic stimulation (rTMS) in the subacute cerebral ischemic rat model: neural plasticity or anti-apoptosis? *Exp Brain Res.* 2011; 214:549–56. [PubMed: 21904929]
24. Warner R, Hudson-Howard L, Johnston C, Skolnick M. Serotonin involvement in analgesia induced by transcranial electrostimulation. *Life Sci.* 1990; 46:1131–8. [PubMed: 2140423]
25. Wilson OB, Hamilton RF, Warner RL, et al. The influence of electrical variables on analgesia produced by low current transcranial electrostimulation of rats. *Anesth Analg.* 1989; 68:673–81. [PubMed: 2719297]
26. Skolnick MH, Wilson OB, Hamilton RF, et al. Low current electrostimulation produces naloxone-reversible analgesia in rats. *Stereotact Funct Neurosurg.* 1989; 53:125–40. [PubMed: 2626603]
27. Nekhendzy V, Fender CP, Davies MF, et al. The antinociceptive effect of transcranial electrostimulation with combined direct and alternating current in freely moving rats. *Anesth Analg.* 2004; 98:730–7. table of contents. [PubMed: 14980928]
28. Auriacombe M, Tignol J, Le Moal M, Stinus L. Transcutaneous electrical stimulation with Limoge current potentiates morphine analgesia and attenuates opiate abstinence syndrome. *Biol Psychiatry.* 1990; 28:650–6. [PubMed: 2173629]
29. Malin DH, Lake JR, Hamilton RF, Skolnick MH. Augmented analgesic effects of L-tryptophan combined with low current transcranial electrostimulation. *Life Sci.* 1990; 47:263–7. [PubMed: 2388530]
30. Malin DH, Lake JR, Hamilton RF, Skolnick MH. Augmented analgesic effects of enkephalinase inhibitors combined with transcranial electrostimulation. *Life Sci.* 1989; 44:1371–6. [PubMed: 2716474]
31. Kabalak AA, Senel OO, Gogus N. The effects of transcranial electrical stimulation on opiate-induced analgesia in rats. *Pain Res Manag.* 2004; 9:203–6. [PubMed: 15605134]
32. Stinus L, Auriacombe M, Tignol J, Limoge A, Le Moal M. Transcranial electrical stimulation with high frequency intermittent current (Limoge's) potentiates opiate-induced analgesia: blind studies. *Pain.* 1990; 42:351–63. [PubMed: 2250924]

33. Kabalak AA, Akcay M, Ceylan A, Senel OO, Gogus N. The effects of transcranial electrical stimulation on anaesthesia and analgesia in rats. *J Vet Med Sci.* 2005; 67:433–6. [PubMed: 15876795]
34. Shin HI, Han TR, Paik NJ. Effect of consecutive application of paired associative stimulation on motor recovery in a rat stroke model: a preliminary study. *Int J Neurosci.* 2008; 118:807–20. [PubMed: 18465426]
35. Kim SJ, Kim BK, Ko YJ, Bang MS, Kim MH, Han TR. Functional and histologic changes after repeated transcranial direct current stimulation in rat stroke model. *J Korean Med Sci.* 2010; 25:1499–505. [PubMed: 20890433]
36. Zhang X, Mei Y, Liu C, Yu S. Effect of transcranial magnetic stimulation on the expression of c-Fos and brain-derived neurotrophic factor of the cerebral cortex in rats with cerebral infarct. *J Huazhong Univ Sci Technolog Med Sci.* 2007; 27:415–8. [PubMed: 17828499]
37. Kaga A, Fujiki M, Hori S, Nakano T, Isono M. Motor evoked potentials following transcranial magnetic stimulation after middle cerebral artery and/or basilar artery occlusions in rats. *J Clin Neurosci.* 2003; 10:470–5. [PubMed: 12852889]
38. Wachter D, Wrede A, Schulz-Schaeffer W, et al. Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. *Exp Neurol.* 2011; 227:322–7. [PubMed: 21147105]
39. Hattemer K, Knake S, Reis J, et al. Excitability of the motor cortex during ovulatory and anovulatory cycles: a transcranial magnetic stimulation study. *Clin Endocrinol (Oxf).* 2007; 66:387–93. [PubMed: 17302873]
40. Hausmann M, Tegenthoff M, Sanger J, Janssen F, Gunturkun O, Schwenkreis P. Transcallosal inhibition across the menstrual cycle: a TMS study. *Clin Neurophysiol.* 2006; 117:26–32. [PubMed: 16337187]
41. O'Connell NE, Wand BM, Marston L, Spencer S, Desouza LH. Non-invasive brain stimulation techniques for chronic pain. A report of a Cochrane systematic review and meta-analysis. *Eur J Phys Rehabil Med.* 2011
42. Nowak DA, Bosl K, Podubecka J, Carey JR. Noninvasive brain stimulation and motor recovery after stroke. *Restor Neurol Neurosci.* 2010; 28:531–44. [PubMed: 20714076]
43. Tischler H, Wolfus S, Friedman A, et al. Mini-coil for magnetic stimulation in the behaving primate. *J Neurosci Methods.* 2010; 194:242–51. [PubMed: 20974177]

Figure 1.
Flowchart of literature search

Main characteristics of each study

TPP = tolerated peak pressure; N/A = not applicable; n = micro; TFL= tail flick latency; HP = hot plate latency; tDCS = transcranial direct current stimulation; PAS = paired associative stimulation; TMS = transcranial magnetic stimulation; TES = transcranial electrostimulation; MCA(O) = middle cerebral artery (occlusion); BA(O) = basilar artery (occlusion); CBF = cortical blood flow; LDF = Laser Doppler flowmetry; NSS = Neurological Severity Scores; MEP = motor evoked potential; RMT = resting motor threshold; BDNF = brain-derived neurotrophic factor

Table 1 (I and II)

Author (Year)	Title	TMS/TES/PAS				TDCS			Duration
		Location of electrodes	Frequency	Intensity	Interval	Electrode Positioning	Size of electrode	Intensity	
Malin, D.H., et al (1989)	Augmented analgesic effects of enkephalinase inhibitors combined with transcranial electrostimulation	bilaterally pinnae at apex of antihelix	10 Hz	10 nA	2 ms				30 min
Nekhendzy, Fender et al. (2004)	The antinociceptive effect of transcranial electrostimulation with combined direct and alternating current in freely moving rats.	Anterior pole of the frontal cortex and bilaterally mastoid	standard 60 Hz (10–100 Hz)	standard 2.25 mA (1.5, 1.9 mA)	4 ms of stimulation and 6 ms of inter-stimulus interval	Cathodal: anterior pole of the frontal cortex, Anodal: bilaterally mastoid	N/A	standard 2.25 mA (1.5, 1.9 mA)	45 min
Kabalak, Senel et al. (2004)	The effects of transcranial electrical stimulation on opiate-induced analgesia in rats.	between eyes on metopic suture and be-mastoid	high-frequency: 166 kHz; intermittent low frequency: 100 Hz	100 mA	high-frequency 2 ns and 4 ns of stimulation; intermittent low frequency: 4 ms stimulation and 6 ms inter-stimulus interval				60 min (with an-and-off cycles of 5 min)
Warner, Hudson-Howard et al. (1990)	Serotonin involvement in analgesia induced by transcranial electrostimulation.	bilaterally pinnae at apex of antihelix	10 Hz	10 nA	2 ms				30 min

Author (Year)	Title	TMS/TES/PAS				TDCS			Duration
		Location of electrodes	Frequency	Intensity	Interval	Electrode Positioning	Size of electrode	Intensity	
Kabalak, Akcay et al. (2005)	The effects of transcranial electrical stimulation on anaesthesia and analgesia in rats.	between eyes on metopic suture and be-mastoid	high-frequency: 166 kHz; intermittent low frequency: 100 Hz	100 mA	high-frequency 2 ns and 4 ns of stimulation; intermittent low frequency: 4 ms stimulation and 6 ms inter-stimulus interval				75 - 95 min
Malin, Lake et al. (1990)	Augmented analgesic effects of L-tryptophan combined with low current transcranial electrostimulation	bilaterally pinnae at apex of anthelix	10 Hz	10 nA	2 ms				30 min
Stinus, Auriacombe et al. (1990)	Transcranial electrical stimulation with high frequency intermittent current (Limoge's) potentiates opiate-induced analgesia: blind studies	between eyes on mesopic suture and bilaterally behind mastoid bones	high-frequency: 166 kHz; intermittent low frequency: 100 Hz	100 mA (12.5, 25, 50 mA)	high-frequency 2 ns and 4 ns of stimulation; intermittent low frequency: 4 ms stimulation and 6 ms inter-stimulus interval				standard 180 min plus duration until end of session (60, 120, 180, 240 min, 24 hours)
Auriacombe, Tignol et al. (1990)	Transcutaneous electrical stimulation with Limoge current potentiates morphine analgesia and attenuates opiate abstinence syndrome.	between eyes on metopic suture and bilaterally behind ears	high-frequency: 166 kHz; intermittent low frequency: 100 Hz	100 mA (17.5 mA effective current)	high-frequency 2 ns and 4 ns of stimulation; intermittent low frequency: 4 ms stimulation and 6 ms inter-stimulus interval				1. group: 720 min once a week for 3 weeks; 2. group: once continuously for 4 days

Author (Year)	Title	TMS/TES/PAS				TDCS			Duration
		Location of electrodes	Frequency	Intensity	Interval	Electrode Positioning	Size of electrode	Intensity	
Wilson, Hamilton et al. (1989)	The influence of electrical variables on analgesia produced by low current transcranial electrostimulation of rats	monolateral (both electrodes on left or right ear); bilaterally (pinnae at the apex of the anthelix)	standard = 10 Hz (5, 5.7, 10.0, 15, 20, 50 Hz)	10 nA	standard = 2 ms (0.1, 0.5, 1, 2, 4 and 8 ms)				30 min
Skolnick, Wilson et al. (1989)	Low current electrostimulation produces naloxone-reversible analgesia in rats.	bilaterally pinnae at apex of anthelix	10 Hz	standard = 10 nA (5, 7.5, 10, 12.5, 15, 20 nA)	0.1ms				standard = 30 min (10, 20, 30, 40, 50, 60 min)
Shin HI, et al.(2008)	Effect of consecutive application of paired associative stimulation on motor recovery in a rat stroke model: a preliminary study	Soleus muscle and motor cortex (0.5 cm lateral to bregma)	0.05 Hz	120% RMT (TMS); 6mA (electrical stimulation)	20 s				30 min (5 sessions, 5 days)
Zhang X, et al. (2007)	Effect of transcranial magnetic stimulation on the expression of c-Fos and brain-derived neurotrophic factor of the cerebral cortex in rats with cerebral infarct	not reported	0.5 Hz	Not reported (1.33 T)	2 s				1 min (two times a day)
Kim SJ et al.(2010)	Functional and histologic changes after repeated transcranial direct current stimulation in rat stroke model.					Active: 3mm to left and 2mm in front of interaural line Reference: attached to trunk	0.785 cm ² (Active); 9 cm ² (Reference)	0.1 mA	30 min

Author (Year)	Title	TMS/TES/PAS				TDCS			Duration
		Location of electrodes	Frequency	Intensity	Interval	Electrode Positioning	Size of electrode	Intensity	
Kaga A et al. (2003)	Motor evoked potentials following transcranial magnetic stimulation after middle cerebral artery and/or basilar artery occlusions in rats	Center of Cranium	N/A	120% of MEP threshold	N/A				MEPs recorded 5, 10, 15, 30, 45 and 60 min
Watcher D (2011)	Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat.					Active: 2mm behind coronal suture and 4mm lateral to sagittal suture Reference: ventral thorax	3.5 mm2 (Active); 10.5 cm2 (Reference)	15, 25 and 100 nA	15 min (6 times = 90 min; 48 hour interval)

Author (year)	Experiment				Methods			Translation	
	Measurement of outcome	Specimen	Gender	Sample Size	Randomization	Blinding	Control (number of control animals with sham stimulation)	Challenges	Insights
Malin, D.H., et al (1989)									
Nekhendzy, Fender et al. (2004)	TFL	Sprague-Dawley rats	male	104	not reported	yes	yes(52)	Montage (positioning); Stimulation paradigm (focality); Investigation in acute pain	Mechanisms of stimulation; Intensification of TES-induced analgesic effects with enkephalinase inhibitors
Kabalak, Senel et al. (2004)	HP; TFL	Sprague-Dawley rats	male	31	yes (all experiments)	Yes (only first experiment)	yes (not reported)	Distinction between different techniques; Montage (three electrodes, positioning); Stimulation	Significance of frequency; Antinociceptive effect of stimulation

Author (year)	Experiment				Methods			Translation	
								paradigm; Investigation in acute pain; Intensity (relatively)	
Warner, Hudson-Howard et al. (1990)	TFL	Albino Wistar rats	male	80	yes	not reported	yes (40)	Montage (three electrodes, positioning); Stimulation paradigm (Limoge current); Investigation in acute pain	Linkage of TES effects to opioid system; Augmentation of analgesic effect
Kabalak, Akcay et al. (2005)	TPP	S/A Simonsen Albino	male	92	not reported	yes	yes (not reported)	Montage (positioning); Stimulation paradigm (Limoge current); Investigation in acute pain	Serotonin involvement in TES-induced effects
Malin, Lake et al. (1990)	TFL	Albino Wistar rats	male	120	yes	not reported	yes (60)	Montage (three electrodes, positioning); Stimulation paradigm (Limoge current); Investigation in acute pain	Time- dependence of different stimulation groups; Augmentation of analgesic effect
Stinus, Auriacombe et al. (1990)	TFL	Sprague- Dawley rats	male	40	not reported	yes	yes (20)	Montage (positioning); Stimulation paradigm (Limoge current); Investigation in acute pain	Linkage to opioid and serotonergic system; Augmentation of analgesic effect with L- tryptophan
Auriacombe, Tignol et al. (1990)	TFL	Sprague- Dawley rats	male	213	yes	Yes	yes (96)	Montage (three electrodes, positioning); Stimulation paradigm (Limoge current); Investigation in acute pain	Potentiation of morphine- induced analgesia; Onset of stimulation (3 hours prior)
Wilson, Hamilton et al. (1989)	TFL	Sprague- Dawley rats	male	41	yes	Yes	yes (10)	Montage (three electrodes, positioning); Stimulation	Linkage to opioid system; Potentiation of morphine

Author (year)	Experiment				Methods		Translation	
							paradigm (Limoge current); Investigation in acute pain	analgesia; Attenuation of opiate abstinence syndrome
Skolnick, Wilson et al. (1989)	TFL	Sprague- Dawley rats	male	189	yes	yes	Montage (positioning); Investigation in acute pain	Optimized stimulation parameters (frequency, pulse width and electrode positioning)
Shin HI, et al.(2008)	TFL	Sprague- Dawley rats	male	334	yes	yes	Montage (positioning); Investigation in acute pain	Analgesia is naloxone- reversible; Optimal stimulation parameters (intensity, duration and after effects)
Zhang X, et al. (2007)	Garcia's score	Sprague- Dawley rats	male	34	not reported	Yes	Intensity (relatively); Treatment of acute stroke phase; Size of TMS coil (relatively large)	Relevance of time frame (consecutive) of stimulation; Improvement in motor function scores
Kim SJ et al.(2010)	NSS; c-Fos and BDNF expressions	Sprague- Dawley rats	male	80	yes	no	Size of TMS coil (relatively large); Treatment of acute/subacute stroke phase; Intensity (relatively high)	Increased c- Fos and BDNF expressions after TMS; Improvement in motor function scores
Kaga A et al. (2003)	Garcia's, Modified Foot Fault, Rota-rod scores	Sprague- Dawley rats	male	41	not reported	Yes	Size of the electrodes (relatively large); Location/ montage of electrodes (extracephalic)	Relevance of time frame (consecutive, multiple) and duration of stimulation; Improvement in motor function scores; Polarity- specific effects of tDCS

Author (year)	Experiment				Methods		Translation	
	MEP; rCBF	Wistar rats	male	24	not reported	yes (6 without stroke, but MEP measurement)	Size of TMS coil (relatively large); Focality of stimulation (relatively broad)	Altered amplitudes of MEPs after stroke
Watcher D (2011)	CBF; LDF	Sprague-Dawley rats	male	80	yes	no (0)	Size of the electrodes (relatively large); Location of electrodes (extracephalic); Intensity (relatively high); Experiment (continuously anaesthetised)	TDCS induces polarity-specific changes

Statistics

SD = standard deviation; N/A = not applicable; RMT = resting motor threshold; tDCS = transcranial direct current stimulation; PAS = paired associative stimulation; TMS = transcranial magnetic stimulation; TES = transcranial electrostimulation

Table 2

	Studies on Pain	Studies on Stroke	All Studies
Studies (number)	-	-	
<i>Studies</i>	10	5	15
<i>Studies using control groups</i>	10	4	14
<i>Studies using sham stimulation</i>	10	1	11
<i>Studies using Sprague-Dawley rats</i>	7	4	11
<i>Studies using Albino Wistar rats</i>	2	1	3
<i>Studies using Simonsen Albino rats</i>	1	0	1
Animals (number)			
<i>Total sample size</i>	1244	259	1503
<i>Sham stimulated animals</i>	419	15	434
<i>Healthy/ Wilde type</i>	1244	6	1250
<i>Diseas model animals</i>	0	253	253
<i>Male/ female</i>	1244/0	259/0	1503/0
<i>Mean sample size</i>	124.4 (SD = 95.58, range: 31 - 334)	51.8 (SD = 26.44; range 24 - 80)	100.2 (SD = 85.6 ; range 24 - 334)
<i>Mean weight (g)</i>	245.9 (SD = 95.90; range 140 - 553)	271.25 (SD = 40.49; range 200 - 325)	253.14 (SD = 82.99; range 140 - 553)
<i>Sprague- Dawley rats</i>	952	235	1187
<i>Albino Wistar rats</i>	200	24	224
<i>S/A Simonsen Albino</i>	92	0	92
Stimulation Parameters			
TES			
<i>Mean frequency (Hz)</i>	5 studies: 10 Hz frequency 4 studies: high-frequency (166		5 studies: 10 Hz frequency 4 studies: high-frequency (166 kHz)

	<u>Studies on Pain</u>	<u>Studies on Stroke</u>	<u>All Studies</u>
	kHz) intermittent low frequency (100 Hz) (Limoge current). Mean: 51 (SD = 44.83; range 5 - 100)		intermittent low frequency (100 Hz) (Limoge current). Mean: 51 (SD = 44.83; range 5 - 100)
<i>Mean intensity (mA)</i>	5 studies: low intensity; Mean: 0.38 (SD = 0.91; range 0.005 - 2.25) 4 studies: high intensity (100 mA, but 17.5 mA effective current)		5 studies: low intensity; Mean: 0.38 (SD = 0.91; range 0.005 - 2.25) 4 studies: high intensity (100 mA, but 17.5 mA effective current)
<i>Mean duration (min)</i>	376 (SD = 1007.39; range 10 to 5760)		376 (SD = 1007.39; range 10 to 5760)
<i>Mean interval (ms)</i>	3.81 (SD = 2.38; range 0.1 - 8)		3.81 (SD = 2.38; range 0.1 - 8)
<i>Mean electrode positioning</i>	5 studies: bilaterally pinnae at apex of anthelix (1 study additional: monolateral with both electrodes on either the left or the right ear). 5 studies: bi-mastoidal and third anterior pole of frontal cortex /between eyes on metopic suture		5 studies: bilaterally pinnae at apex of anthelix (1 study additional: monolateral with both electrodes on either the left or the right ear). 5 studies: bi-mastoidal and third anterior pole of frontal cortex /between eyes on metopic suture
PAS (1 study)			
<i>Frequency (Hz)</i>	0.05	0.05	0.05
<i>Intensity (% of RMT; mA)</i>	120; 6	120; 6	120; 6
<i>Duration (min)</i>	30	30	30
<i>Interval (s)</i>	20	20	20
<i>Electrode positioning</i>	Soleus muscle and motor cortex	Soleus muscle and motor cortex	Soleus muscle and motor cortex
TMS *			
<i>Mean frequency (Hz)</i>	0.5	0.5	0.5
<i>Mean intensity (% of MEP threshold)</i>	120	120	120
<i>Mean duration (min)</i>	1	1	1
<i>Mean interval (s)</i>	2	2	2
<i>Electrode positioning</i>	center of cranium	center of cranium	center of cranium
tDCS			
<i>Mean intensity (mA)</i>	1.88 (SD = 0.38; range 1.5 - 2.25)	0.06 (SD = 0.046; range 0.015 - 0.1)	0.97 (SD = 1.287; range 0.015 - 2.25)

	<u>Studies on Pain</u>	<u>Studies on Stroke</u>	<u>All Studies</u>
<i>Mean duration (min)</i>	45	60 (SD = 42.43; range 30 -90)	45 (SD 15; range 30 - 90)
<i>Mean electrode size (cm²)</i>	N/A	active: 0.5675 (SD = 0.31; range 0.35 - 0.785), reference: 9.75 (SD = 1.06; range 9 - 10.5)	active: 0.5675 (SD = 0.31; range 0.35 - 0.785), reference: 9.75 (SD = 1.06; range 9 - 10.5)
<i>Electrode positioning</i>	Active: bilaterally mastoid; Reference: anterior pole of the frontal cortex	Active: 1. 2mm behind coronal suture/ 4mm lateral to sagittal suture; 2. 3mm to left and 2mm in front of interaural line Reference: 1. ventral thorax; 2. attached to trunk	Active: 1. 2mm behind coronal suture/ 4mm lateral to sagittal suture; 2. 3mm to left and 2mm in front of interaural line; 3. bilaterally mastoid Reference: 1. ventral thorax; 2. attached to trunk; 3. anterior pole of the frontal cortex

* only two studies applied TMS and had incomplete reports, mean calculations were not possible.

Results

TPP = tolerated peak pressure; TFL= tail flick latency; HP = hot plate latency; tDCS = transcranial direct current stimulation; PAS = paired associative stimulation; TMS = transcranial magnetic stimulation; TES = transcranial electrostimulation; MCA(O) = middle cerebral artery (occlusion); BA(O) = basilar artery (occlusion); CBF = cortical blood flow; LDF = Laser Doppler flowmetry; NSS = Neurological Severity Scores; MEP = motor evoked potential; RMT = resting motor threshold; BDNF = brain-derived neurotrophic factor; pCPA = p-chlorophenylalanine

Table 3

Author (Year)	Title	Results
Malin, D.H., et al (1989)	Augmented analgesic effects of enkephalinase inhibitors combined with transcranial electrostimulation	Significantly augmented analgesia effect of TES and thiophan/acetorphan compared to controls. Significant TES effect (TES vs. sham).
Nekhendzy, Fender et al. (2004)	The antinociceptive effect of transcranial electrostimulation with combined direct and alternating current in freely moving rats.	Demonstration of antinociceptive effect of cutaneously administered TES. The antinociceptive effect of TES is significantly altered by the TES frequency, but not by different stimulation durations.
Kabalak, Senel et al. (2004)	The effects of transcranial electrical stimulation on opiate-induced analgesia in rats.	TES increases the duration and analgesic potency of remifentanyl. Combination of TES with remifentanyl has an increased peak of analgesia, which is 150 % higher compared to the drug group and 251 % higher compared to TES group or control.
Warner, Hudson-Howard et al. (1990)	Serotonin involvement in analgesia induced by transcranial electrostimulation.	TES produced an increased analgesic effect of 613 % compared with sham group. Pretreatment with pCPA/5HTP decreased pain assessment by 91.5 percent.
Kabalak, Akcay et al. (2005)	The effects of transcranial electrical stimulation on anaesthesia and analgesia in rats.	Combination of TES with thiopental or thiopental+remifentanyl augments the analgesic effect. Triple combination: analgesia of 90 min; 30-40 % increased compared to baseline; 160 % increased compared to control; 50-75% increased compared to TES and thiopental alone
Malin, Lake et al. (1990)	Augmented analgesic effects of L-tryptophan combined with low current transcranial electrostimulation	TES augments the analgesic effect of L-tryptophan. Active TES has a significant effect compared to sham. Group receiving both TES and L-tryptophan displayed significantly more analgesia than all other groups.
Stinus, Auriacombe et al. (1990)	Transcranial electrical stimulation with high frequency intermittent current (Limoge's) potentiates opiate-induced analgesia: blind studies	TES did not modify the pain threshold in drug-free rats, but it potentiated morphine-induced analgesia. A maximal effect was found with stimulation 3 hours prior to morphine injection. TES potentiation is depended on drug dosage and the intensity of stimulation.
Auriacombe, Tignol et al. (1990)	Transcutaneous electrical stimulation with Limoge current potentiates morphine analgesia and attenuates opiate abstinence syndrome.	TES increases morphine analgesia by three times assessed by TFL and attenuates opiate abstinence syndrome by 48 %.

Author (Year)	Title	Results
Wilson, Hamilton et al. (1989)	The influence of electrical variables on analgesia produced by low current transcranial electrostimulation of rats	The study investigated the effects of various stimulation parameters including frequency, pulse width and electrode positioning. Analgesia was maximized with a stimulation paradigm of 10 nA intensity, 10 Hz frequency and pulse width of 2 msec
Skolnick, Wilson et al. (1989)	Low current electrostimulation produces naloxone-reversible analgesia in rats.	Investigation of various stimulation parameters (intensity, duration and after effects). Analgesia was maximized with a stimulation paradigm of 10 nA intensity and 30 min duration. After effects of analgesia persisted until 200 min after the end of stimulation.
Shin HI, et al.(2008)	Effect of consecutive application of paired associative stimulation on motor recovery in a rat stroke model: a preliminary study	Garcia's score: PAS group had significantly higher scores on the 7th day. MEP amplitude did not decrease in PAS group. In sham group, mEp amplitude decreased on day 1 and got back to preoperative levels at day 4.
Zhang X, et al. (2007)	Effect of transcranial magnetic stimulation on the expression of c-Fos and brain-derived neurotrophic factor of the cerebral cortex in rats with cerebral infarct	NSS scores: TMS group had significantly lower scores at day 7, 14, 21, 28 after MCAO. c-Fos: became significantly higher at TMS group at day 7, 14, 21, 28. BDNF: Significantly higher in TMS group at day 7, 14, 21
Kim SJ et al.(2010)	Functional and histologic changes after repeated transcranial direct current stimulation in rat stroke model.	Anodal: improvement of Garcia's and Foot Fault scores 16 days postoperatively. No effect on infarct size, but decreased axonal degeneration. Cathodal: deterioration of motor function. No significant difference between all groups in mean infarct volume.
Kaga A et al. (2003)	Motor evoked potentials following transcranial magnetic stimulation after middle cerebral artery and/or basilar artery occlusions in rats	MCA: amplitude of MEPs was 200% increased compared to preschismic value, stayed significantly high for 60 min. BA: MEP amplitudes were 10% of preschismic value, stayed significantly low for 60 min. MCA+BA: similar to BA
Watcher D (2011)	Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat.	Anodal: 50 and 100 μ A intensity increased CBF up to 30 min; 100 μ A increased CBF by 25%, 50 μ A by 18%. Cathodal: decreased CBF; 100 μ A had effects of 25% of baseline levels, persisted for 30 min; at 25 and 50 μ A, baseline-levels were re-established within 30 min.

Quality assessment

TPP = tolerated peak pressure; N/A = not applicable; TFL= tail flick latency; HP = hot plate latency; tDCS = transcranial direct current stimulation; PAS = paired associative stimulation; TMS = transcranial magnetic stimulation; TES = transcranial electrostimulation; MCA(O) = middle cerebral artery (occlusion); BA(O) = basilar artery (occlusion); CBF = cortical blood flow; LDF = Laser Doppler flowmetry; NSS = Neurological Severity Scores; MEP = motor evoked potential; RMT = resting motor threshold; BDNF = brain-derived

Table 4 (I and II)

Author (Year)	Methods			Experimental Procedures			Experimental Animals			
	Randomization	Blinding	Control (number of control animals with sham stimulation)	Brain stimulation technique	Details of experiment: type/ diseases model of animal (number)	Assessment technique of pain or stroke	Species	Sample Size	Gender	Weight (g)
Malin, D.H., et al (1989)	not reported	Yes	Yes (52)	TES	wild type	TFL	Sprague-Dawley rats	104	male	180-225
Nekhendzy, Fender et al. (2004)	yes (all experiments)	Yes (only first experiment)	Yes (not reported)	tDCS and TES	wild type	HP, TFL	Sprague-Dawley rats	31	male	390–553
Kabalak, Senel et al. (2004)	yes	not reported	yes (40)	TES	wild type	TFL	Albino Wistar rats	80	male	140-180
Warner, Hudson-Howard et al. (1990)	not reported	yes	Yes (not reported)	TES	wild type	TPP	S/A Simonsen Albino/ male	92	male	200 - 250
Kabalak, Akcay et al. (2005)	yes	not reported	yes (60)	TES	wild type	TFL	Albino Wistar rats	120	male	140-180
Malin, Lake et al. (1990)	not reported	yes	yes (20)	TES	wild type	TFL	Sprague-Dawley rats	40	male	200-250
Stinus, Auriacombe et al. (1990)	yes	Yes	yes (96)	TES	wild type	TFL	Sprague-Dawley rats	213	male	300
Auriacombe, Tignol et al. (1990)	yes	Yes	yes (10)	TES	wild type	TFL	Sprague-Dawley rats	41	male	300 - 350
Wilson, Hamilton et al. (1989)	yes	yes	yes (59)	TES	wild type	TFL	Sprague-Dawley rats	189	male	180 – 210

Author (Year)	Methods			Experimental Procedures			Experimental Animals			
	Randomization	Blinding	Control (number of control animals with sham stimulation)	Brain stimulation technique	Details of experiment: type/ diseases model of animal (number)	Assessment technique of pain or stroke	Species	Sample Size	Gender	Weight (g)
Skolnick, Wilson et al. (1989)	yes	yes	yes (82)	TES	wild type	TFL	Sprague- Dawley rats	334	male	180 – 210
Shin HI, et al.(2008)	not reported	Yes	Yes (15)	PAS	MCAO (34)	Garcia's score	Sprague- Dawley rats	34	male	275-325
Zhang X, et al. (2007)	yes	no	Yes (no sham stimulation, 40 no stimulation)	TMS	MCAO (80)	NSS; c-Fos and BDNF expressions	Sprague- Dawley rats	80	male	200-250
Kim SJ et al.(2010)	not reported	Yes	Yes (21 no stimulation, no sham group)	tDCS	MCAO (41)	Garcia's, Modified Foot Fault, Rota-rod scores	Sprague- Dawley rats	41	not reported	not reported
Kaga A et al. (2003)	not reported	not reported	Yes(N/A)	TMS	MCAO (6), BAO (6), MCAO+BAO (6)	MEP; CBF	Wistar rats	24	male	200-300
Watcher D (2011)	yes	not reported	no (0)	tDCS	not reported (80)	CBF; LDF	Sprague- Dawley rats	80	male	310

Author (Year)	Housing & Husbandry			Analysis		
	Light/ dark cycle (hours)	Number of cage companions	Access to food and water	Outcomes	Attrition/ Safety	Histological Analysis
Malin, D.H., et al (1989)	12	not reported	ad libitum	Significantly augmented analgesia effect of TES and thiorphan/acetorphan compared to controls.	not reported	no
Nekhendzy, Fender et al. (2004)	12	0 (housed individually)	ad libitum	Demonstration of antinociceptive effect of cutaneously administered TES.	No skin burns were observed	no

Author (Year)	Housing & Husbandry			Analysis		
	Light/ dark cycle (hours)	Number of cage companions	Access to food and water	Outcomes	Attrition/ Safety	Histological Analysis
Kabalak, Senel et al. (2004)	12	not reported	ad libitum	TES increases the duration and analgesic potency of remifentanyl.	No major adverse effects were observed throughout the procedures	no
Warner, Hudson-Howard et al. (1990)	12	groups of six	ad libitum	Serotonin seems to be involved with TES induced analgesia.	not reported	no
Kabalak, Akcay et al. (2005)	12	not reported	ad libitum	TES decreases the anaesthetic and analgesic requirements for thiopental and remifentanyl	No major adverse effects were observed throughout the procedures	no
Malin, Lake et al. (1990)	12	not reported	ad libitum	TES augments the analgesic effect of L- tryptophan	not reported	no
Stinus, Auriacombe et al. (1990)	not reported	not reported	ad libitum	Potentiation of opiate-induced analgesia	Absence of catalepsy and sedation	no
Auriacombe, Tignol et al. (1990)	12	not reported	ad libitum	TES potentiates analgesic effect of morphine and attenuates opiate abstinence syndrome	not reported	no
Wilson, Hamilton et al. (1989)	12	groups of four	ad libitum	Influence of electrical variables on analgesia	not reported	no
Skolnick, Wilson et al. (1989)	12	groups of four	ad libitum	Electrical variables on analgesia and abolishing the analgesia effects with naloxone	not reported	no
Shin HI, et al.(2008)	not reported	not reported	not reported	Motor recovery in stroke model under a PAS treatment scheme	15 rats were excluded for scoring above 12 on Garcia's motor behavior index 12 hours after MCAO. Five rats died during follow-up period.	no
Zhang X, et al. (2007)	not reported	not reported	not reported	Expression of BDNF and c-Fos in a stroke model treated with TMS	No notice of seizures and consciousness change in rats treated with TMS	yes

Author (Year)	Housing & Husbandry			Analysis		
	Light/ dark cycle (hours)	Number of cage companions	Access to food and water	Outcomes	Attrition/ Safety	Histological Analysis
Kim SJ et al.(2010)	not reported	not reported	not reported	Effects of tDCS on function and histology of a stroke model	20 rats died during the experiment (control 4; anodal 5; cathodal; 6; exercise 5)	yes
Kaga A et al. (2003)	not reported	not reported	not reported	Assessment of MEP in a stroke model	There was no evidence of contusion or damage due to operative procedure of the brainstem beneath BA	yes
Watcher D (2011)	12	single	ad libitum	Effects of tDCS on cortical blood perfusion	All animals coped well with the surgical and stimulation procedures. One animal showed colliquative necrosis in the left parieto-occipital region.	yes