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Authors

Bahr Hosseini, Mersedeh
Hou, Jesse
Bikson, Marom
et al.

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Central Nervous System Electrical Stimulation for Neuroprotection in Acute Cerebral Ischemia: Meta-analysis of Preclinical Studies

Mersedeh Bahr Hosseini, MD[1], **Jesse Hou, BS**[1], **Marom Bikson, PhD**[2], **Marco Iacoboni, MD. PhD**[3], **Jeffrey Gornbein, PhD**[4], **Jeffrey L Saver, MD**[1]

[1]Department of Neurology and Comprehensive Stroke Center, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

[2]Department of Biomedical Engineering, The City College of New York (CCNY), New York, NY, USA

[3]Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

[4]Department of Biomathematics and Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Abstract

Background and Purpose—Brain electrical stimulation, widely studied to facilitate recovery from stroke, has also been reported to confer direct neuroprotection in preclinical models of acute cerebral ischemia. Systematic review of controlled preclinical acute cerebral ischemia studies would aid planning for initial human clinical trials.

Methods—A systematic Medline search identified controlled, preclinical studies of central nervous system electrical stimulation in acute cerebral ischemia. Studies were categorized among 6 stimulation strategies. Three strategies applied different stimulation types to tissues within the ischemic zone [cathodal hemispheric stimulation (CHS), anodal hemispheric stimulation (AHS), and pulsed hemispheric stimulation (PHS)] and three strategies applied deep brain stimulation to different neuronal targets remote from the ischemic zone [fastigial nucleus stimulation (FNS), subthalamic vasodilator area stimulation (SVAS), and dorsal periaqueductal gray stimulation (DPAGS)]. Random effects meta-analysis assessed electrical stimulation modification of final

Correspondence Mersedeh Bahr Hosseini, MD, UCLA Comprehensive Stroke Center, Geffen School of Medicine at UCLA, 710 Westwood Plaza, Los Angeles, CA 90095, Tel 310-794-6379, Fax 310-267-2063, mbahrhosseini@mednet.ucla.edu.

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infarct volume. Study-level risk-of-bias and intervention-level readiness-for-translation were assessed using formal rating scales.

Results—Systematic search identified 28 experiments in 21 studies, including a total of 350 animals, of electrical stimulation in preclinical acute cerebral ischemia. Overall, in animals undergoing electrical stimulation final infarct volumes were reduced by 37% (CI 95%; 34-40% P< 0.001), compared with control. There was evidence of heterogeneity of efficacy among stimulation strategies (I^2 = 93.1%, $p_{heterogeneity}$ < 0.001). Amongst the within-ischemic zone stimulation strategies, only CHS significantly reduced the infarct volume (27 %, CI 95%; 22-33%, P< 0.001); amongst the remote-from ischemic zone approaches, all (FNS, SVAS, and DPAGS) reduced infarct volumes by approximately half. On formal rating scales, CHS studies had the lowest risk-of-bias and CHS had the highest overall quality of intervention-level evidence supporting readiness to proceed to clinical testing.

Conclusions—Electrical stimulation reduces final infarct volume across preclinical studies. Cathodal hemispheric stimulation shows the most robust evidence and is potentially appropriate for progression to early stage human clinical trial testing as a promising neuroprotective intervention.

Keywords

Electrical stimulation; neuroprotection; acute cerebral ischemia

Introduction

Central nervous system electrical stimulation has been used as a neuromodulatory technique for diverse neurological and neuropsychiatric diseases and stroke recovery.[1, 2] In case of non-invasive transcranial electrical stimulation, a low voltage electrical current is delivered to the brain via scalp electrodes such as in transcranial direct current stimulation (tDCS) (unidirectional current applied continuously or pulsed) and transcranial alternating current stimulation (tACS) (alternating pulsed electrical current).[3] On the other hand, in more invasive methods such as in direct deep brain stimulation (DBS) electrical current is delivered to the brain via deep electrodes.[4]

In addition, electrical stimulation has been investigated as a potential acute neuroprotective intervention in preclinical models of acute ischemic stroke.[5-15] While reperfusion therapy for acute ischemic stroke with intravenous thrombolysis and endovascular thrombectomy is highly effective, many patients still have poor outcomes, due to failure to reperfuse or reperfusion only after substantial irreversible injury has already occurred.[16, 17] Neuroprotection interventions that could be started prior to, or concomitant with intravenous thrombolysis, could substantially further improve outcome from acute ischemic stroke. Neuroprotective interventions that could be started prior to start of cardiac, abdominal, and cerebral surgical and endovascular interventions with high risk of intra-procedural cerebral ischemia or prior to the onset of delayed cerebral ischemia after subarachnoid hemorrhage also could provide benefit in those special settings of expected, imminent ischemic insult.

In preclinical studies using electrical stimulation as a neuroprotective method, two different approaches and targets of electrical stimulation have been explored. In one approach, electrical stimulation is applied directly and broadly to ischemic tissues and will be referred to as “hemispheric” approach.[5, 10, 11] The electrical stimulation may be cathodal, anodal, or pulsed with the greatest number of studies addressing cathodal stimulation. Cathodal hemispheric stimulation with largely inhibitory effects, applied to ischemic and peri-ischemic fields, has the potential to exert a direct neuroprotective effects through multiple mechanisms of action, including reduction of peri-infarct depolarizations, down-regulation of N-methyl-D-aspartate receptor, and decrease peri-ischemic inflammatory response.[10, 11]

In the other general approach, stimulation is applied focally to target nuclei remote from the ischemic field. Targets have included the fastigial nucleus of cerebellum, subthalamic vasodilator area, and dorsal periaqueductal gray.[6-9, 12-15] Stimulation of these regions with electrical stimulation may be beneficial in acute ischemia by evoking pressor and/or cerebral vasodilatory responses, resulting in an increase in cerebral blood flow, and by mediating a long-lasting conditioned central neuroprotective effect via inhibition of peri-infarct depolarization, brain inflammatory response, and apoptosis, independent of cerebral blood flow.[18-21]

In addition to pleiotropic neuroprotective effects, electrical stimulation delivered to cerebral tissues has further potentially advantageous properties compared with many of the prior neuroprotective agents for ischemic stroke that have failed in translation.[22] Systemically administered pharmacologic agents are dependent on cerebral blood flow to reach target cerebral regions and, by definition, cerebral blood flow is impaired in acute cerebral ischemia. In addition, even when systemically delivered agents do arrive at ischemic fields, they must pass through the blood-brain barrier to achieve effective concentrations within the neural parenchyma, and many agents have slow trafficking into the central nervous system compartments.[22, 23] In contrast, in electrical stimulation the electrical current reaches the target, independent of anterograde cerebral blood flow and of blood-brain barrier status.[24] Moreover, in addition to assured delivery to target cerebral tissues, electrical stimulation’s independence from the systemic circulation substantially avoids exposure of other organs to the intervention, reducing dose-limiting constraints of systemic side effects.[25]

Given these potential advantages of electrical stimulation over many prior tested neuroprotective therapies, several research groups worldwide have investigated acute electrical stimulation in preclinical stroke models. Study findings have generally suggested promise, with some individual studies independently positive and others formally neutral but with favorable point estimates. In addition, outcomes were analyzed in a variety of ways, and effect magnitudes were accordingly variable. We, therefore, undertook a formal meta-analysis of preclinical studies investigating the neuroprotective effect of central nervous system electrical stimulation in acute cerebral ischemia, to characterize and quantify the preclinical evidence supporting initiation of translational human clinical trials of electrical stimulation as a neuroprotective therapy in patients with acute ischemic stroke. Of note, peripheral nervous system stimulation to enhance collateral circulation is another neuromodulatory intervention that has been tested for acute stroke in several preclinical and

early clinical studies, and has been the subject of reviews elsewhere.[26] The current study's focus is upon central nervous system electrical stimulation.

Methods

A systematic review of the literature and meta-analysis was performed using the methodology recommended by the CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies).[27-30] The review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. [31]

Data Search and Selection

PubMed/Medline was searched through December 31, 2017, using the following search strategy: electrical stimulation <or> transcranial direct current stimulation <and> acute stroke. Citations were screened by the lead author at the title and abstract level and retrieved for full-text evaluation if they were considered possibly relevant.

Inclusion criteria and outcome measures

Inclusion criteria for this study were: 1) animal models of focal cerebral ischemia, 2) treatment applied in the acute period, before, during, or up to 6 hours after the start of ischemia, 3) intervention consisted of electrical stimulation. We included any stimulation protocol (type of electrical stimulation, intensity, location, and duration of stimulation). The endpoint analyzed was final infarct volume as a proportion of hemispheric volume.

Data Extraction

The following data were extracted from the studies: Type and total number of animal subjects, type of anesthetics, occlusion model type and time of stimulation relative to the ischemia induction (treatment time epoch), polarity and location of the center electrode, location of the reference electrode, electrodes size, intensity of stimulation, total duration of the stimulation. Studies were categorized among 6 treatment strategies. The first three were different stimulation types applied within the ischemic zone: 1) cathodal hemispheric stimulation (CHS), 2) anodal hemispheric stimulation (AHS), and 3) pulsed hemispheric stimulation (PHS). The remaining three were deep brain stimulation applied to different neuronal targets remote from the ischemic zone: 4) cerebellar fastigial nucleus stimulation (FNS), 5) subthalamic vasodilator area stimulation (SVAS), and 6) dorsal periaqueductal gray stimulation (DPAGS).

The authors declare that all supporting data are available within the article and its online Data Supplement files.

Risk of Bias / Quality Assessment

We assessed methodological risk of bias / quality of the pre-clinical investigations using 2 scales: 1) a study-level risk of bias / quality scale, and 2) an intervention-level evidence

quality scale. Detailed criteria for item scoring are shown in online-only Data Supplement, Tables I and II.

The study-level risk of bias / quality scale was applied to individual controlled studies and was comprised of 12 items, based upon study design recommendations of two consensus groups: the Stroke Therapy Academic Industry Roundtable (STAIR)[32] and the CAMARADES.[27, 29] The items assessed: blinding; randomization; dose response exploration; inclusion of behavioural outcome measures; inclusion of long-term outcomes; well-defined entry criteria; power analysis; disclosure of conflicts of interest; attention to temperature control; avoidance of anesthetic with neuroprotective properties; compliance with animal welfare regulattions; and peer-reviewed publication. The quality scale ranges from 0 to 24. We defined the studies with score of 0–7 as studies with high risk of bias, 8–15 as having intemediate risk of bias, and 16–24 as studies with low risk of bias.

The intervention-level evidence quality scale was applied to each treatment strategy, as an index of the cumulative strength of all pre-clinical work testing that strategy. The intervention-level scale was based upon STAIR recommendations for neuroprotective agent development programs,[32] and comprised 9 items for which positive scores were given if the intervention showed benefit, including: testing in both males and females; testing in older animals; testing in more than 1 species (preferably primates in addition to rodents); testing of at least two strains within a species; testing in one or more treatment time epochs; testing in animals with comorbidities; feasible time window; dose response exploration; feasible route of administration. For detailed description of treatment time epoch scoring method, see Data Supplement.

Overall, the intervention-level readiness-for-translation score ranges from 0 to 18. We defined the the scores of 0–5 as low readiness-for-translation, 6–11 as intemediate readiness-for-translation, and 12–18 as high readiness-for-translation of the stimulation strategy.

Statistical Analysis

We calculated the reduction proportion in infarct volumes for each study as 1- (mean infarct volume of stimulation arm (mm^3)/ mean infarct volume of control arm (mm^3)). For a given study, the standard errors of mean infarct volume were calculated by dividing the standard deviations by the square root of sample size. The standard error for the reduction proportion was computed using the ratio variance formula.[33] The overall reduction proportion estimates were computed under a random effects model.

For the two studies which compared two interventional group regimens with a shared control group,[9, 11] the sample size of the control group was apportioned equally to the different active interventions, as recommended by the Cochrane collaboration.[30, 35] For the one study that compared four interventional group regimens with a shared control group,[5] the weighted average of the results of the four interventional groups was compared to the control result. Heterogeneity was assesses using I^2, the percentage of the residual variation that is attributable to between-study heterogeneity. The presence of potential publication bias was

assessed using funnel plot visual inspection analysis, and Egger's and Peters' regression tests. Statistical analysis was performed using Review Manager 5 software.

Results

The systematic search identified 3247 publications for screening, among which 11 studies containing 28 experiments met inclusion criteria as controlled studies of electrical stimulation in preclinical acute cerebral ischemia models (Data Supplemental, Figure I). Across the 28 experiments, a total of 350 animals were investigated, all with middle cerebral artery occlusion. Table 1 shows the detailed characteristics of the studies, and for highlights of the studies characteristics, see online-only Data Supplement.

Overall, electrical stimulation, compared with control, significantly reduced infarct volumes, by 37% (CI 95%; 34–40% P< 0.00001) (Fig 1). There was a strong evidence of treatment effect heterogeneity according to stimulation strategy, with subgroup I^2 = 93.1%, p (heterogeneity) < 0.0001. There was evidence of a greater magnitude of benefit with the 4 stimulation strategies of cathodal hemispheric stimulation (CHS), fastigial nucleus stimulation (FNS), subthalamic vasodilator area stimulation (SVAS), and dorsal periaqueductal gray area stimulation (DPAGS); and a lesser magnitude or no benefit with the 2 treatment strategies of anodal hemispheric stimulation (AHS) and pulsed hemispheric stimulation (PHS).

Electrical Stimulations with Within-ischemic Zone Targets

Cathodal Hemispheric Stimulation (CHS)—Two publications were identified testing CHS in 4 different experiments (40 animals)[10, 11]. CHS was associated with a significant reduction in the final infarct volume by 27 % (CI 95%; 22–33%, P< 0.00001) (Fig 1). Moderate heterogeneity was noted amongst the experiments using CHS (I^2=62%).

Anodal Hemispheric Stimulation (AHS)—One publication was found assessing AHS used in 2 experiments (16 animals)[11]. AHS resulted in a non-significant, non-substantial reduction of 9.8% in the final infarct volume (95% CI; −1 to 17%, P= 0.09) (Fig 1).

Pulsed Hemispheric Stimulation (PHS)—One publication was found using PHS in 4 different experiments (16 animals)[5] which measured final infarct volume as their outcome. No significant neuroprotective effect of PHS was observed (CI 95%; −11 to 37%; P= 0.28) (Fig 1).

Electrical Stimulations with Remote-from-Ischemic-Zone Targets

Fastigial Nucleus Stimulation (FNS)—A total of 7 publications reporting 14 controlled experiments (91animals)[6, 7, 9, 12-15] were found using FNS as a neuroprotective method while measuring final infarct volume as their outcome. FNS exhibited a significant neuroprotective effect resulting in reduction of final infarct volume by 45 % (40–50%; 95% CI; P< 0.00001) (Fig 1). No substantial heterogeneity was noted amongst the FNS experiments (I^2=37%).

Subthalamic Vasodilator Area Stimulation (SVAS)—One publication consisting of two controlled experiments (13 animals)[9] was found using SVAS as a neuroprotection method. A significant neuroprotective effect of SVA stimulation was observed, resulting in a 52% reduction of final infarct volume (CI 95%; 29–74%, P< 0.00001). No heterogeneity was noted amongst the SVAS experiments.

Dorsal Periaqueductal Gray Stimulation (DPAGS)—The search identified one publication reporting two controlled experiments (12 animals)[8] of stimulating DPAG for neuroprotection. There was a significant reduction of final infarct volume by 48% (35–60%; 95% CI; P< 0.00001). No heterogeneity was noted amongst the DPAGS comparisons.

In assessments for publication bias, there was no evidence of substantial non-reporting of study data. Visual inspection of the funnel plot suggested perhaps a small degree of missingness of smaller, non-positive trials (Fig 2). However, formal, quantitative testing did not indicate the presence of demonstrable publication bias on either Egger's test (p = 0.63) or Peters' test (p = 0.45).

Study-Level Quality / Risk of Bias—Several sources of risk of bias were identified in the analyzed studies (Figure 3). None of the studies indicated that randomization was employed to allocate animals to active versus control groups. Use of blinding was explicitly stated for only 1 of the 11 studies. Assessment of a behavioral outcome in addition to infarct volume outcome was indicated for only 2 of the 11 studies. In the single study with both a behavioral outcome and a statistically significant reduction in infarct volume, the neurological severity score behavioral outcome also showed statistically significant benefit.[11] On the other hand, 8 of the 11 studies did indicate control of temperature during the experimental period. Overall, the median study-level quality score was 4 (IQR 4–8). Among the stimulation strategies showing beneficial effects, the highest quality scores were for studies of cathodal hemispheric stimulation (8 and 13).[10, 11]

Intervention-Level Evidence Quality Assessment/Readiness-for-Translation Score—At the intervention-level, the mean readiness-for-translation score was 4.3 (±3) [median 5.5 (IQR 0–7.2)] (Figure 4). Amongst all stimulation strategies, cathodal hemispheric stimulation was the strategy with the strongest, intermediate-level, quality evidence supporting readiness to proceed to clinical testing (readiness-for-translation score of 8 of 18) (Figure 4). Weaker, intermediate-level, quality evidence supported FNS and DPAGS (readiness-for-translation score of 7 and 6 of 18). The evidence quality supporting readiness to proceed to clinical testing for other stimulation strategies was low, ranging from 0 to 5 of 18 (Figure 4).

Discussion

In this formal meta-analysis of preclinical studies, electrical stimulation therapies substantially reduced final infarct volumes in acute ischemic stroke rodent models. Amongst stimulation strategies applying stimulation over the ischemic zone, substantial benefit was observed with cathodal hemispheric stimulation (CHS), which reduced infarct volumes by one-quarter, while no benefit was noted for anodal hemispheric stimulation (AHS) or pulsed

hemispheric stimulation (PHS). Amongst strategies applying deep brain stimulation to targets remote from the ischemic zone, substantial benefit was observed for all assessed techniques, including subthalamic vasodilator area stimulation (SVAS), dorsal periaqueductal gray stimulation (DPAGS), and fastigial nucleus stimulation (FNS), all reducing infarct volumes by approximately one-half. Formal funnel plot analysis did not show evidence of publication bias. Considering multiple dimensions of therapy translational appropriateness, including feasibility (e.g. stimulation by external rather than implanted electrodes), time windows assessed in preclinical studies, and demonstration of dose-response effects, cathodal hemispheric stimulation showed the greatest overall readiness to advance to early stage clinical testing.

A diverse range of electrical stimulation strategies were analyzed in this meta-analysis. A broad, overall analytic framework was employed as electrical stimulation may have biologic effects, especially safety effects that pertain across all variations in stimulation delivery. However, we expected that there would be important differences in treatment effect among different strategies, and that core analyses would best be pursued within, rather than across, stimulation approaches. Formal heterogeneity testing confirmed differential effects for individual treatment strategy. Accordingly, readiness for escalation to human testing was assessed for each stimulation strategy individually, rather than for undifferentiated electrical stimulation.

The analytic approach undertaken in this study used novel study-level and intervention-level assessments, based upon recent recommendations from expert consensus groups calling for more stringent, formalized assessment of preclinical acute stroke treatment studies. To assess study-level risk of bias/quality, a twelve-item score was developed, incorporating recommended content items advanced by the CAMARADES (general preclinical science) and STAIR (stroke-specific preclinical science) expert bodies,[28-30, 32] with scoring format based on the risk of bias tool of the Cochrane Collaboration (general clinical science).[30, 34] With this tool, the majority of analyzed preclinical studies were found to have substantial risk of bias. Quality criteria frequently not reported in study manuscripts included: blinded treatment administration and outcome assessment; use of randomization in allocating animals to study treatment groups; well-defined entry criteria; and avoidance of anesthetics with competing neuroprotective properties. Two of the analyzed studies had better, intermediate risk of bias/quality scores.[5, 11] Distinctive features of these study manuscripts included: assessment of both infarct volume and behavioral outcomes; testing electrical stimulation in different doses; and use of blinding. The overall high to intermediate risk of bias scores for analyzed studies suggest caution in interpreting meta-analysis results, and indicate that routine use of a formal scoring tool to assess study risk of bias may be helpful in assessing preclinical, controlled, therapeutic studies.

To assess intervention-level readiness for advancement to clinical testing, a nine item score was developed, based on STAIR consensus group recommendations[32] with scoring format based on the risk of bias tool of the Cochrane Collaboration.[30, 34] With this tool, although 4 of the 6 electrical stimulation strategies were found to have neuroprotective effects in formal-meta analysis, only CHS was deemed to demonstrate the strongest intermediate readiness for proceeding to clinical testing. The evidence supporting CHS indicated efficacy

in 3 different treatment time epochs (bridging neuroprotective therapy for transient ischemia, durable neuroprotective therapy for permanent ischemia, and reperfusion injury therapy); efficacy in later post-onset time windows achievable in the clinical setting; presence of a dose-response curve providing additional evidence of genuine therapeutic effect; testing in multiple species (rat and mouse); and having a feasible, external route of delivery. However, desirable evidence for advancement currently missing in CHS studies includes evidence of efficacy in animals with baseline comorbidities, female sex, and older age. Nonetheless, the presence of important intervention-level readiness for translation characteristics for CHS provides grounding for initial pilot trials that have been launched in human stroke patients in France and the United States. [35, 36] In contrast, the other stimulation strategies with neuroprotective effects had several unreadiness features, including: testing the strategies beyond clinically feasible therapeutic windows (pre-conditioning or immediately upon onset of ischemia); testing in only one specie (rats only); and especially using a clinically infeasible means of stimulation delivery (implanted deep electrodes rather than external epicranial source).

The overall low to intermediate readiness for translation scores for analyzed stimulation strategies highlights the usefulness of a formal scoring tool to identify additional experimental settings that are desirable to fully qualify an intervention for advancement.

Limitations

This study has limitations. First, the analyzed experimental studies generally had intermediate to high risk of bias scores, due to absence of testing in female animals, absence of long-term functional outcome assessment, and other infelicities, indicating caution in interpreting the findings of the overall meta-analysis. Second, diverse types of electrical stimulation strategies were analyzed, and heterogeneity of treatment effects by treatment strategy were noted. Accordingly, emphasis should be place upon the analyses of each strategy individually, rather than overall summary effect. Third, some of the individual experiments were performed with stimulation before or immediately upon the start of cerebral ischemia, which would lead to over-estimation of treatment effects achievable in the clinical setting with a delayed start of therapy from ischemia onset. In human clinical trials of neuroprotection for acute ischemic stroke, the earliest start time of therapy achieved in large pivotal trials was a median of 45 minutes after ischemia onset.[37]

Conclusion

Electrical stimulation reduces final infarct volume across preclinical studies. While most techniques have evidential weaknesses and delivery challenges for translation to human studies meriting further preclinical investigation, cathodal hemispheric stimulation shows the most robust evidence and is potentially appropriate for progression to early stage human clinical trial testing as a promising neuroprotective intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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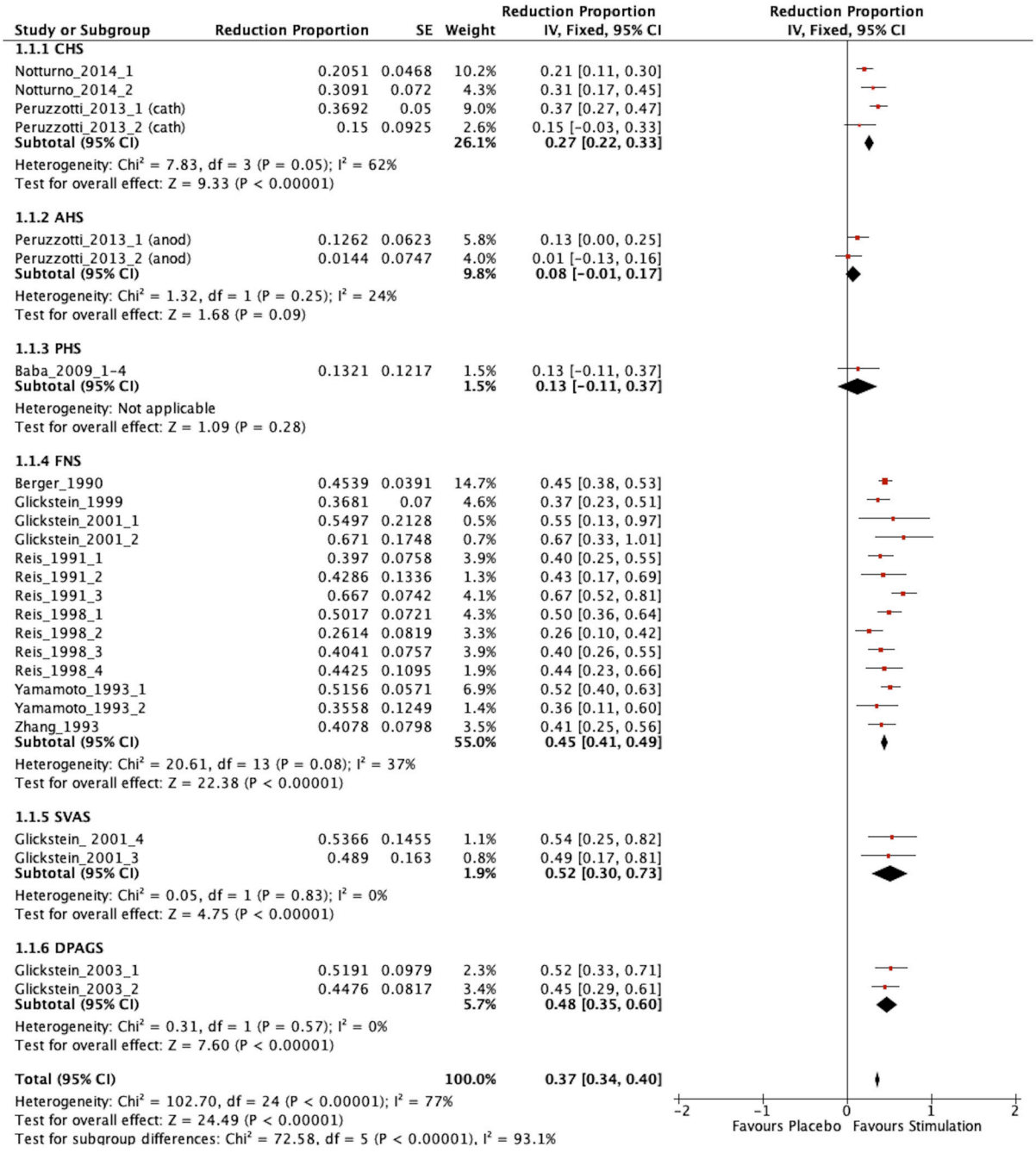

Figure 1.

Forest plot shows the neuroprotective effect of electrical stimulation across multiple preclinical studies

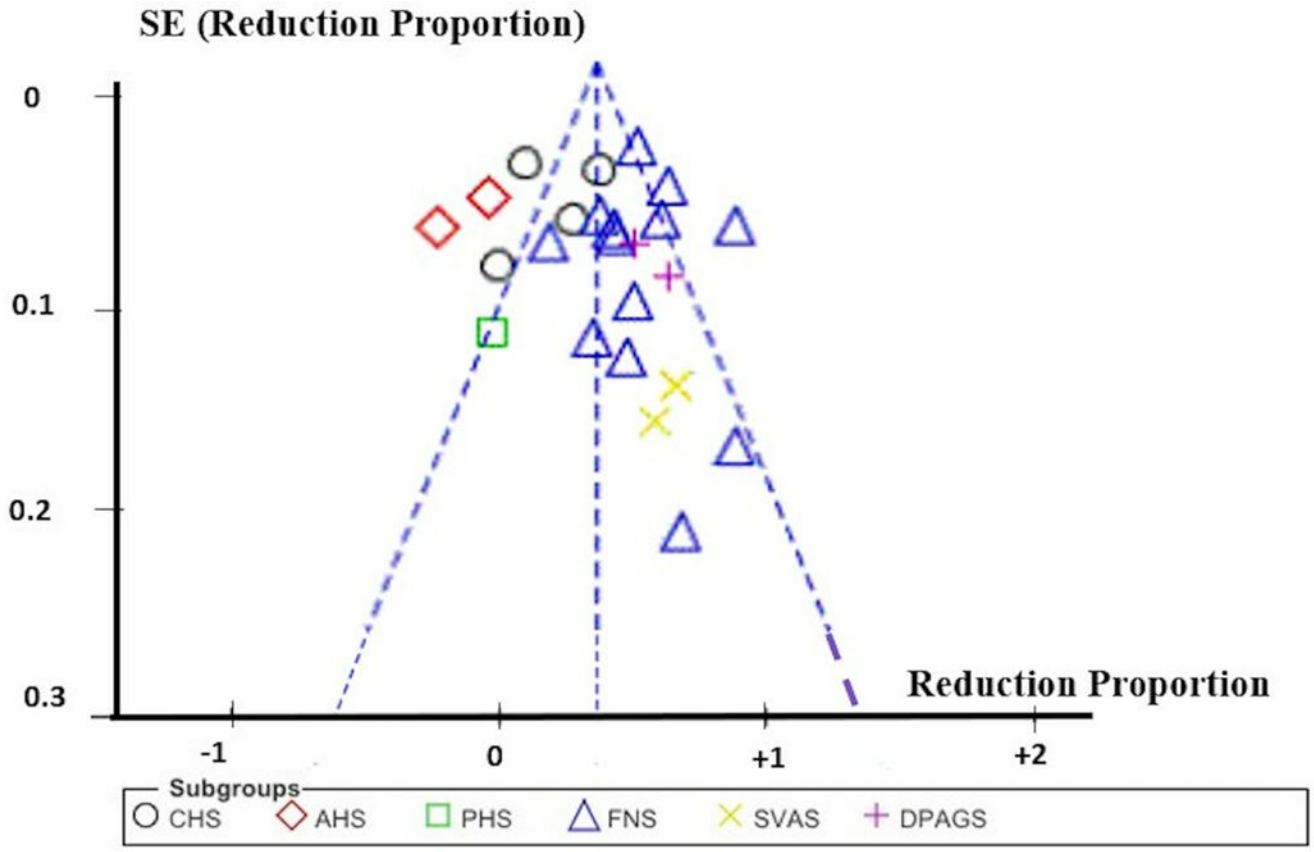

Figure 2.

Shows an asymmetric Funnel plot likely due to in-between studies heterogeneity and over-representation of positive effects among smaller fastigial nucleus stimulation (FNS) studies. Due to some missing studies over the non-significant right lower area of the plot, the presence of publication bias was suggested, although was not statistically significant based on regression models (P=0.63 based on Egger's and P= 0.45 based on Peters' regression tests for bias).

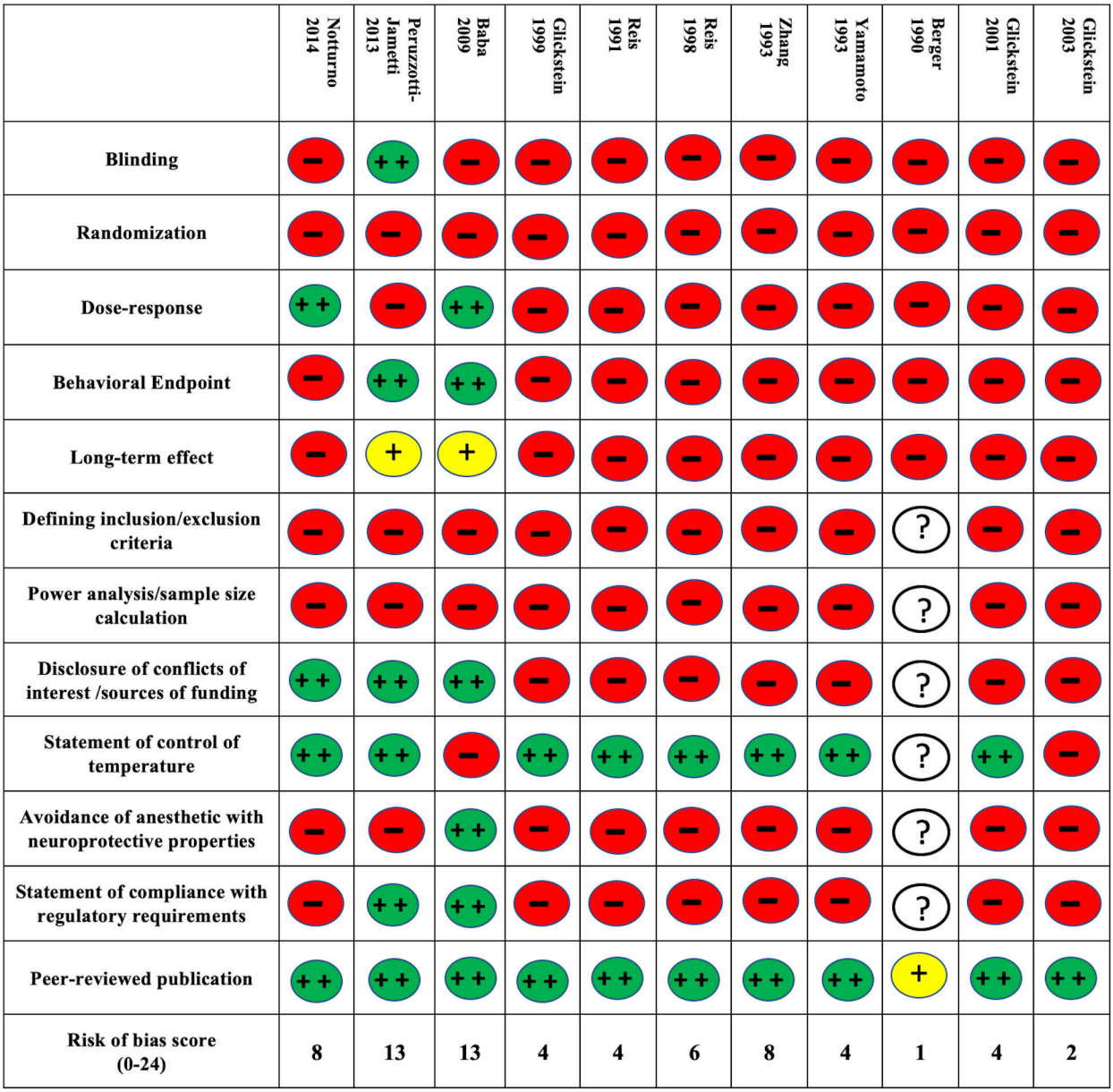

Figure 3.

Study-level risk of bias ratings. Risk of bias items based on CAMARADES and STAIR recommendations[28-30, 32]. For individual items: green indicates low risk of bias; yellow indicates some concerns; red indicates high risk of bias; white indicates unclear risk of bias. Total scores can range from 0 to 24, with scores of 16-24 indicating low risk of bias; scores of 8-15 indicating intermediate risk of bias; and scores of 0-7 indicating high risk of bias.

	Cathodal Hemispheric Stimulation	Anodal Hemispheric Stimulation	Pulsed Hemispheric Stimulation	Fastigial Nucleus Stimulation	Subthalamic Vasodilator Area Stimulation	Dorsal Periaqueductal Gray Stimulation
Sex of animals	−	−	−	−	−	−
Age of animals	−	−	−	+ +	+ +	+ +
Species of animals	+	−	−	−	−	−
Strains of animals	−	−	−	+ +	+ +	+ +
Treatment time epoch	+ +	−	−	+ +	+	+
Baseline comorbidities	−	−	−	+	−	+
Feasible time window	+ +	−	−	−	−	−
Dose-response	+ +	−	−	−	−	−
Feasible route of delivery	+	−	−	−	−	−
Readiness-for-translation score (0-18)	8	0	0	7	5	6

Figure 4.

Intervention-level evidence quality ratings and readiness-for-translation scoring. Quality items based on STAIR recommendations[32]. Green indicates high evidence quality; Yellow indicates intermediate evidence quality; Red indicates low evidence quality. Note that for the two stimulation subtypes of anodal and electrical hemispheric stimulations, red was allotted to all the quality items due to lack of benefit of the two simulation strategies. Total scores can range from 0 to 18, with scores of 12-18 indicating high readiness-for-translation; scores of 6-11 indicating intermediate readiness-for-translation; and scores of 0-5 indicating low readiness-for-translation.

Table 1.

Features of included studies

Study	Number, type, and sex of animals	Co-morbid status	Occlusion method	Timing	Treatment time epoch	Polarity and location of center electrode	Electrode size	Intensity (mA)	Duration of stimulation	Anesthetic	Outcome measure relative to MCAO onset
Cathodal Hemispheric Stimulation/ Anodal Hemispheric Stimulation											
Notturno et al 2014[10]	48 Young Sprague-Dawley Male Rats	Healthy	Bipolar electrocoagulation	45 min after pMCAO	Permanent	Cathode over the skull, 2mm left and 1mm posterior to the bregma. Anode over the chest	10.5 cm2	0.2 (density of 2.86 mA/cm^2)	120 and 180min (alternating 15'on-15'off)	2% isoflurane	Histological (infarct volume corrected for cerebral edema) 48hr, Electrographic during stimulation
Peruzzotti-Jametti et al 2013[11]	49 Young Charles River Italy Male Mice	Healthy	Silicon coated 8-0 nylon filament	30 min and 4.5hr after start of tMCAO (90min)	Transient – bridging and Reperfusion injury	Cathode over the skull, 2.5mm left and 0.5mm posterior to the bregma, anode over the chest and vice versa in anodal experiments	5.2 cm2	0.25 (density of 5.5 mA/cm^2)	40min (alternating 20'on-20'off)	1.5% isoflurane (Merial, Assago, Italy) in 30% O2 (remainder N2O)	Histological (infarct volume corrected for cerebral edema) 24hr and 72hr, Behavioral 24hr and 72hr, Metabolic 90min
Pulsed Hemispheric Stimulation											
Baba et al 2009[5]	20 Adult Wister Male Rats	Healthy	Intraluminal Suture	60 min after end of tMCAO (90min)	Reperfusion injury	Cathode on the parietal epidural space, 4-4.5mm lateral from the bregma	Unknown	0.1 and 0.2 with frequencies of 0, 2, 10, 50 Hz	3 Days or 1 week	1.0% halothane in 70% N2O and 30% O2, pentobarbital	Histological (infarct volume corrected for cerebral edema) 3d, Behavioral 30min 3d and 1wk
Fastigial Nucleus Stimulation											
Glickstein et al 1999[7]	6 Adult SHR Male Rats	Hyper-tensive	Cauterization	5 days before pMCAO	Pre-conditioning	Deep electrodes (cathode) Over the fastigial nucleus of cerebellum and anode over the neck	150 μm	Alternating 1 sec on-1 sec off of 0.5msec duration at 50Hz with intensity 5x threshold (31.8+−1.18 μA) required	60min	Halothane (1.8–2.5% in $100\%O_{2)}$	Histological (infarct volume corrected for cerebral edema) 24hr, Hemodynamic during stimulation

Study	Number, type, and sex of animals	Co-morbid status	Occlusion method	Timing	Treatment time epoch	Polarity and location of center electrode	Electrode size	Intensity (mA)	Duration of stimulation	Anesthetic	Outcome measure relative to MCAO onset
								to elevate BP by 10 mmHg			
Reis et al 1991[12]	58 Adult Wister, SHR and Sprague-Dawley Male Rats	Hyper-tensive	Cauterization	Immediately after pMCAO	Permanent	Deep electrodes (cathode) Over the fastigial nucleus of cerebellum and anode over the neck	150 μm	Alternating 1 sec on-1 sec off of square-wave pulses, 0.5msec duration at 50Hz with intensity 5x threshold (10-20μA) required to elevated BP by 10 mmHg	60min	Isoflurane (1-3% in 100% O2)	Histological 24hr, Hemodynamic during stimulation
Reis et al 1998[13]	40 Adult SHR Male Rats	Hyper-tensive	Cauterization	Immediately before, up to 30 days prior to pMCAO	Pre-conditioning	Deep electrodes (cathode) Over the fastigial nucleus of cerebellum and anode over the neck	outer diameter of 150 μm	Alternating 1 sec on-1 sec off of square-wave pulses, 0.5msec duration at 50Hz with intensity 5x threshold (18.5+−0.7 μA) required to elevate BP by 10 mmHg	60min	Halothane (5% in 100% O_2	Histological (infarct volume corrected for cerebral edema) 24hr, Hemodynamic during stimulation
Zhang et al 1993[15]	19 Adult Sprague-Dawley Male Rats		Cauterization	3-5 min after pMCAO	Permanent	Deep electrodes (cathode) Over the fastigial nucleus of cerebellum and anode over the neck	outer diameter of 150 μm	Alternating 1 sec on-1 sec off of square-wave pulses, 0.5msec duration at 50Hz with intensity of 75-100 μA	60min (alternating 1 sec on-1 sec off)	Halothane (5% in 100% O2)	Histological 24hr, Hemodynamic and Electrographic during the stimulation
Yamamoto et al 1993[14]	19 Adult SHR Male Rats	Hyper-tensive	Cauterization	Immediately after pMCAO	Permanent	Deep electrodes (cathode) Over the fastigial nucleus of cerebellum and anode over the neck	outer diameter of 150 μm	Alternating 1 sec on-1 sec off of square-wave pulses, 0.5msec duration at 50Hz with intensity of 70-100 μA	60min (alternating 1 sec on-1 sec off)	Halothane (1.8–2.5% in 100%$O_{2)}$	Histological 24hr, Hemodynamic during stimulation

Study	Number, type, and sex of animals	Co-morbid status	Occlusion method	Timing	Treatment time epoch	Polarity and location of center electrode	Electrode size	Intensity (mA)	Duration of stimulation	Anesthetic	Outcome measure relative to MCAO onset
Berger et al 1990[6]	11 Adult SHR Male Rats	Hyper-tensive	Unknown	Immediately after pMCAO	Permanent	Stimulation of the fastigial nucleus of cerebellum	Unknown	Unknown	60min	Unknown	Histological and Imaging 24hr
Subthalamic Vasodilator Area Stimulation											
Glickstein et al 2001[9]	47 Adult Sprague-Dawley and Fisher Male Rats	Healthy	Cauterization	Immediately up to 10 days before pMCAO	Pre-conditioning	Deep electrodes (cathode) Over the fastigial nucleus of cerebellum or subthalamic region and anode over the neck	outer diameter of 150 μm	Alternating 1 sec on-1 sec off of square-wave pulses, 0.5msec duration at 50Hz with intensity of 75-150 μA	60min	Isoflurane (2-2.5% in 100% O2)	Histological (infarct volume corrected for cerebral edema) 24hr, Hemodynamic and Electrographic during stimulation
Dorsal Periaqueductal Gray Stimulation											
Glickstein et al 2003[8]	28 Adult Sprague-Dawley, Fisher and SHR Male Rats	Healthy and Hyper-tensive	Cauterization	Three days before pMCAO	Pre-conditioning	Deep electrodes (cathode) Over the fastigial nucleus of midbrain region and anode over the neck	outer diameter of 150 μm	Alternating 1 sec on-1 sec off of square-wave pulses, 0.5msec duration at 50Hz with intensity of 100 μA	60min	Isoflurane (1.8-2% in 100% O2)	Histological (infarct volume corrected for cerebral edema) 24hr, Hemodynamic and Electrographic during stimulation

BP indicates blood pressure; pMCAO, permanent middle cerebral artery occlusion; tMCAO, temporary middle cerebral artery occlusion; SHR, spontaneously hypertensive rat.