Central Nervous System Electrical Stimulation for Neuroprotection in Acute Cerebral Ischemia

Meta-Analysis of Preclinical Studies

Mersedeh Bahr Hosseini, MD; Jesse Hou, BS; Marom Bikson, PhD; Marco Iacoboni, MD. PhD; Jeffrey Gornbein, PhD; Jeffrey L. Saver, MD

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 in 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, and pulsed hemispheric stimulation), and 3 strategies applied deep brain stimulation to different neuronal targets remote from the ischemic zone (fastigial nucleus stimulation, subthalamic vasodilator area stimulation, and dorsal periaqueductal gray stimulation). Random-effects meta-analysis assessed electrical stimulation modification of final 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% (95% CI, 34%–40%; $P<0.001$), compared with control. There was evidence of heterogeneity of efficacy among stimulation strategies ($I^2=93.1\%$, $P_{\text{heterogeneity}}<0.001$). Among the within-ischemic zone stimulation strategies, only CHS significantly reduced the infarct volume (27 %; 95% CI, 22%–33%; $P<0.001$); among the remote-from ischemic zone approaches, all (fastigial nucleus stimulation, subthalamic vasodilator area stimulation, and dorsal periaqueductal gray stimulation) 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. CHS shows the most robust evidence and is potentially appropriate for progression to early-stage human clinical trial testing as a promising neuroprotective intervention. **(*Stroke*. 2019;50:2892-2901. DOI: 10.1161/STROKEAHA.119.025364.)**

Key Words: acute stroke ■ central nervous system ■ electrical stimulation ■ meta-analysis ■ neuroprotection

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 noninvasive transcranial electrical stimulation, a low voltage electrical current is delivered to the brain via scalp electrodes, such as in transcranial direct current stimulation (unidirectional current applied continuously or pulsed) and transcranial alternating current stimulation (alternating pulsed electrical current).[3] However, in more invasive methods, such as in direct deep brain stimulation, 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] Although 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 before, or concomitant with intravenous thrombolysis, could substantially further improve outcome from acute ischemic stroke. Neuroprotective interventions that could be

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From the Department of Neurology and Comprehensive Stroke Center (M.B.H., J.H., J.L.S.), Department of Psychiatry and Biobehavioral Sciences (M.I.), and Department of Biomathematics and Medicine (J.G.), David Geffen School of Medicine at UCLA; and Department of Biomedical Engineering, The City College of New York (CCNY) (M.B.).

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Correspondence to Mersedeh Bahr Hosseini, MD, UCLA Comprehensive Stroke Center, Geffen School of Medicine at UCLA, 710 Westwood Plaza, Los Angeles, CA 90095. Email mbahrhosseini@mednet.ucla.edu

started before start of cardiac, abdominal, and cerebral surgical and endovascular interventions with high risk of intraprocedural cerebral ischemia or before 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, 2 different approaches and targets of electrical stimulation have been explored. In 1 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 (CHS) with largely inhibitory effects, applied to ischemic and peri-ischemic fields, has the potential to exert a direct neuroprotective effect through multiple mechanisms of action, including reduction of peri-infarct depolarizations, downregulation 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 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 pharmacological 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 on central nervous system electrical stimulation.

Methods

A systematic review of the literature and meta-analysis was performed using the methodology recommended by the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies.[27–30] The review followed the 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 end point 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, and total duration of the stimulation. Studies were categorized among 6 treatment strategies. The first 3 were different stimulation types applied within the ischemic zone: (1) CHS, (2) anodal hemispheric stimulation (AHS), and (3) pulsed hemispheric stimulation (PHS). The remaining 3 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-only Data Supplement.

Risk of Bias / Quality Assessment

We assessed methodological risk of bias / quality of the preclinical 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 Tables I and II in the online-only Data Supplement.

The study-level risk of bias / quality scale was applied to individual controlled studies and was comprised of 12 items, based on study design recommendations of 2 consensus groups: the Stroke Therapy Academic Industry Roundtable[32] and the Collaborative

Table. Features of Included Studies

Study	Number, Type, and Sex of Animals	Comorbid 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, 2 mm left and 1 mm posterior to the bregma. Anode over the chest	10.5 cm^2	0.2 (density of 2.86 mA/cm^2)	120 and 180 min (alternating 15′ on to 15′ off)	2% isoflurane	Histological (infarct volume corrected for cerebral edema) 48 h, 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 4.5 h after start of tMCAO (90 min)	Transient—bridging and reperfusion injury	Cathode over the skull, 2.5 mm left and 0.5 mm posterior to the bregma, anode over the chest and vice versa in anodal experiments	5.2 cm^2	0.25 (density of 5.5 mA/cm^2)	40 min (alternating 20′ on to 20′ off)	1.5% isoflurane (Merial, Assago, Italy) in 30% O_2 (remainder N_2O)	Histological (infarct volume corrected for cerebral edema) 24 and 72 h, behavioral 24 and 72 h, metabolic 90 min
Pulsed hemispheric stimulation											
Baba et al (2009)[5]	20 adult Wister male rats	Healthy	Intraluminal Suture	60 min after end of tMCAO (90 min)	Reperfusion injury	Cathode on the parietal epidural space, 4–4.5 mm lateral from the bregma	Unknown	0.1 and 0.2 with frequencies of 0, 2, 10, 50 Hz	3 d or 1 wk	1.0% halothane in 70% N_2O and 30% O_2, pentobarbital	Histological (infarct volume corrected for cerebral edema) 3 d, behavioral 30 min 3 d and 1 wk
Fastigial nucleus stimulation											
Glickstein et al (1999)[7]	6 adult SHR male rats	Hypertensive	Cauterization	5 d before pMCAO	Preconditioning	Deep electrodes (cathode) over the fastigial nucleus of cerebellum and anode over the neck	150 μm	Alternating 1 s on to 1 s off of 0.5 ms duration at 50 Hz with intensity 5× threshold (31.8+ to 1.18 μA) required to elevate BP by 10 mm Hg	60 min	Halothane (1.8%–2.5% in 100%$O_{2)}$	Histological (infarct volume corrected for cerebral edema) 24 h, hemodynamic during stimulation
Reis et al (1991)[12]	58 adult Wister, SHR and Sprague-Dawley male rats	Hypertensive	Cauterization	Immediately after pMCAO	Permanent	Deep electrodes (cathode) Over the fastigial nucleus of cerebellum and anode over the neck	150 μm	Alternating 1 s on to 1 s off of square-wave pulses, 0.5 ms duration at 50 Hz with intensity 5× threshold (10–20 μA) required to elevated BP by 10 mm Hg	60 min	Isoflurane (1%–3% in 100% O_2)	Histological 24 h, hemodynamic during stimulation
Reis et al (1998)[13]	40 adult SHR male rats	Hypertensive	Cauterization	Immediately before, up to 30 d before pMCAO	Preconditioning	Deep electrodes (cathode) over the fastigial nucleus of cerebellum and anode over the neck	Outer diameter of 150 μm	Alternating 1 s on to 1 s off of square-wave pulses, 0.5 ms duration at 50 Hz with intensity 5x threshold (18.5+ to 0.7 μA) required to elevate BP by 10 mm Hg	60 min	Halothane (5% in 100% O_2	Histological (infarct volume corrected for cerebral edema) 24 h, Hemodynamic during stimulation

(*Continued*)

Table. Continued

Study	Number, Type, and Sex of Animals	Comorbid 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
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 s on to 1 s off of square-wave pulses, 0.5 ms duration at 50 Hz with intensity of 75–100 μA	60 min (alternating 1 s on to 1 s off)	Halothane (5% in 100% O_2)	Histological 24 h, hemodynamic and electrographic during the stimulation
Yamamoto et al (1993)[14]	19 adult SHR male rats	Hypertensive	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 s on to 1 s off of square-wave pulses, 0.5 ms duration at 50 Hz with intensity of 70–100 μA	60 min (alternating 1 s on to 1 s off)	Halothane (1.8%–2.5% in 100%$O_{2)}$	Histological 24 h, hemodynamic during stimulation
Berger et al (1990)[6]	11 adult SHR male rats	Hypertensive	Unknown	Immediately after pMCAO	Permanent	Stimulation of the fastigial nucleus of cerebellum	Unknown	Unknown	60 min	Unknown	Histological and imaging 24 h
Subthalamic vasodilator area stimulation											
Glickstein et al (2001)[9]	47 adult Sprague-Dawley and Fisher male rats	Healthy	Cauterization	Immediately up to 10 d before pMCAO	Preconditioning	Deep electrodes (cathode) over the fastigial nucleus of cerebellum or subthalamic region and anode over the neck	Outer diameter of 150 μm	Alternating 1 s on to 1 s off of square-wave pulses, 0.5 ms duration at 50 Hz with intensity of 75–150 μA	60 min	Isoflurane (2%–2.5% in 100% O_2)	Histological (infarct volume corrected for cerebral edema) 24 h, 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 Hypertensive	Cauterization	3 d before pMCAO	Preconditioning	Deep electrodes (cathode) over the fastigial nucleus of midbrain region and anode over the neck	Outer diameter of 150 μm	Alternating 1 s on to 1 s off of square-wave pulses, 0.5 mss duration at 50 Hz with intensity of 100 μA	60 min	Isoflurane (1.8%–2% in 100% O_2)	Histological (infarct volume corrected for cerebral edema) 24 h, hemodynamic and electrographic during stimulation

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

Approach to Meta-Analysis and Review of Animal Data from Experimental Studies.[27,29] The items assessed: blinding; randomization; dose-response exploration; inclusion of behavioral 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 regulations; and peer-reviewed publication. The quality scale ranges from 0 to 24. We defined the studies with score of 0 to 7 as studies with high risk of bias, 8 to 15 as having intermediate risk of bias, and 16 to 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 preclinical work testing that strategy. The intervention-level scale was based on Stroke Therapy Academic Industry Roundtable 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 >1 species (preferably primates in addition to rodents); testing of at least 2 strains within a species; testing in ≥1 treatment time epochs; testing in animals with comorbidities; feasible time window; dose-response exploration; and feasible route of administration. For detailed description of treatment time epoch scoring method, see the online-only Data Supplement.

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

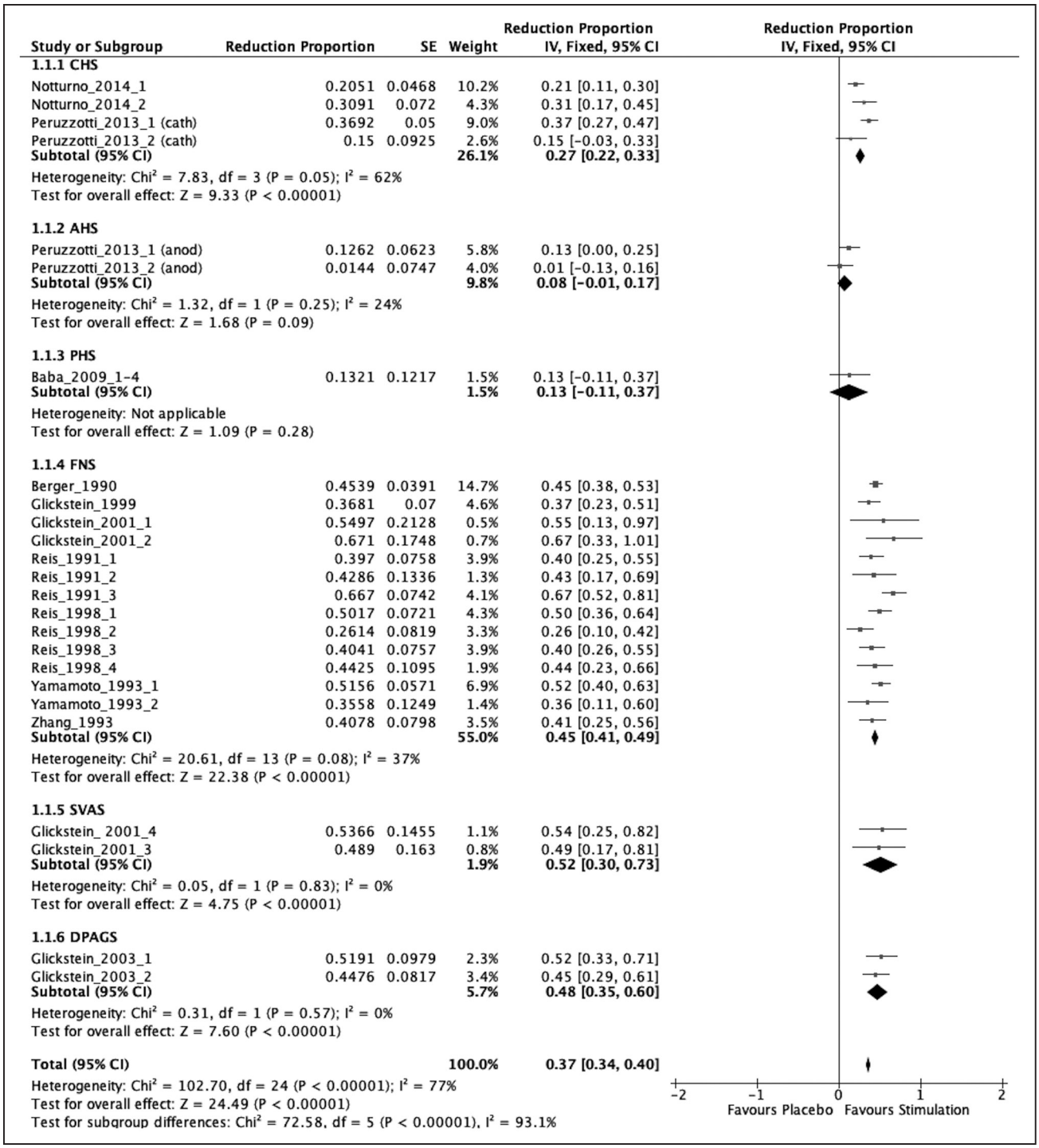

Figure 1. Forest plot shows the neuroprotective effect of electrical stimulation across multiple preclinical studies. AHS indicates anodal hemispheric stimulation; CHS, cathodal hemispheric stimulation; DPAGS, dorsal periaqueductal gray stimulation; FNS, fastigial nucleus stimulation; PHS, pulsed hemispheric stimulation; and SVAS, subthalamic vasodilator area stimulation.

For the 2 studies which compared 2 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.[30,34] For the 1 study that compared 4 interventional group regimens with a shared control group,[5] the weighted average of the results of the 4 interventional groups was compared with the control result. Heterogeneity was assessed 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 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

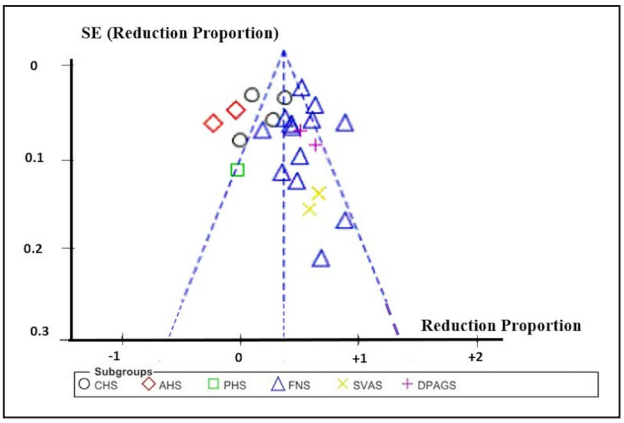

Figure 2. Shows an asymmetrical Funnel plot likely due to in-between studies heterogeneity and over-representation of positive effects among smaller fastigial nucleus stimulation (FNS) studies. Due to some missing studies over the nonsignificant right lower area of the plot, the presence of publication bias was suggested, although was not statistically significant based on regression models (*P*=0.63 based on Egger and *P*=0.45 based on Peters regression tests for bias). AHS indicates anodal hemispheric stimulation; CHS, cathodal hemispheric stimulation; DPAGS, dorsal periaqueductal gray stimulation; PHS, pulsed hemispheric stimulation; and SVAS, subthalamic vasodilator area stimulation.

in preclinical acute cerebral ischemia models (Figure I in the online-only Data Supplement). Across the 28 experiments, a total of 350 animals were investigated, all with middle cerebral artery occlusion. The Table shows the detailed characteristics of the studies, and for highlights of the studies characteristics, see the online-only Data Supplement.

Overall, electrical stimulation, compared with control, significantly reduced infarct volumes by 37% (95% CI, 34%–40%; *P*<0.00001; Figure 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 CHS, FNS, SVAS, and dorsal periaqueductal gray area stimulation (DPAGS) and a lesser magnitude or no benefit with the 2 treatment strategies of AHS and PHS.

Electrical Stimulations With Within-Ischemic Zone Targets

Cathodal Hemispheric Stimulation

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% (95% CI, 22%–33%; *P*<0.00001; Figure 1). Moderate heterogeneity was noted among the experiments using CHS (I^2=62%).

Anodal Hemispheric Stimulation

One publication was found assessing AHS used in 2 experiments (16 animals).[11] AHS resulted in a nonsignificant, nonsubstantial reduction of 9.8% in the final infarct volume (95% CI, −1% to 17%; *P*=0.09; Figure 1).

Pulsed Hemispheric Stimulation

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 (95% CI, −11% to 37%; *P*=0.28; Figure 1).

Electrical Stimulations With Remote-From-Ischemic-Zone Targets

Fastigial Nucleus Stimulation

A total of 7 publications reporting 14 controlled experiments (91 animals)[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 % (95% CI, 40%–50%; *P*<0.00001; Figure 1). No substantial heterogeneity was noted among the FNS experiments (I^2=37%).

Subthalamic Vasodilator Area Stimulation

One publication consisting of 2 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 (95% CI, 29%–74%; *P*<0.00001). No heterogeneity was noted among the SVAS experiments.

Dorsal Periaqueductal Gray Stimulation

The search identified 1 publication reporting 2 controlled experiments (12 animals)[8] of stimulating DPAG for neuroprotection. There was a significant reduction of final infarct volume by 48% (95% CI, 35%–60%; *P*<0.00001). No heterogeneity was noted among the DPAGS comparisons.

In assessments for publication bias, there was no evidence of substantial nonreporting of study data. Visual inspection of the funnel plot suggested perhaps a small degree of missingness of smaller, nonpositive trials (Figure 2). However, formal, quantitative testing did not indicate the presence of demonstrable publication bias on either Egger 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 used 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] However, 8 of the 11 studies did indicate control of temperature during the experimental period. Overall, the median study-level quality score was 4 (interquartile range, 4–8). Among the stimulation strategies showing beneficial effects, the highest quality scores were for studies of CHS (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 [interquartile range, 0–7.2];

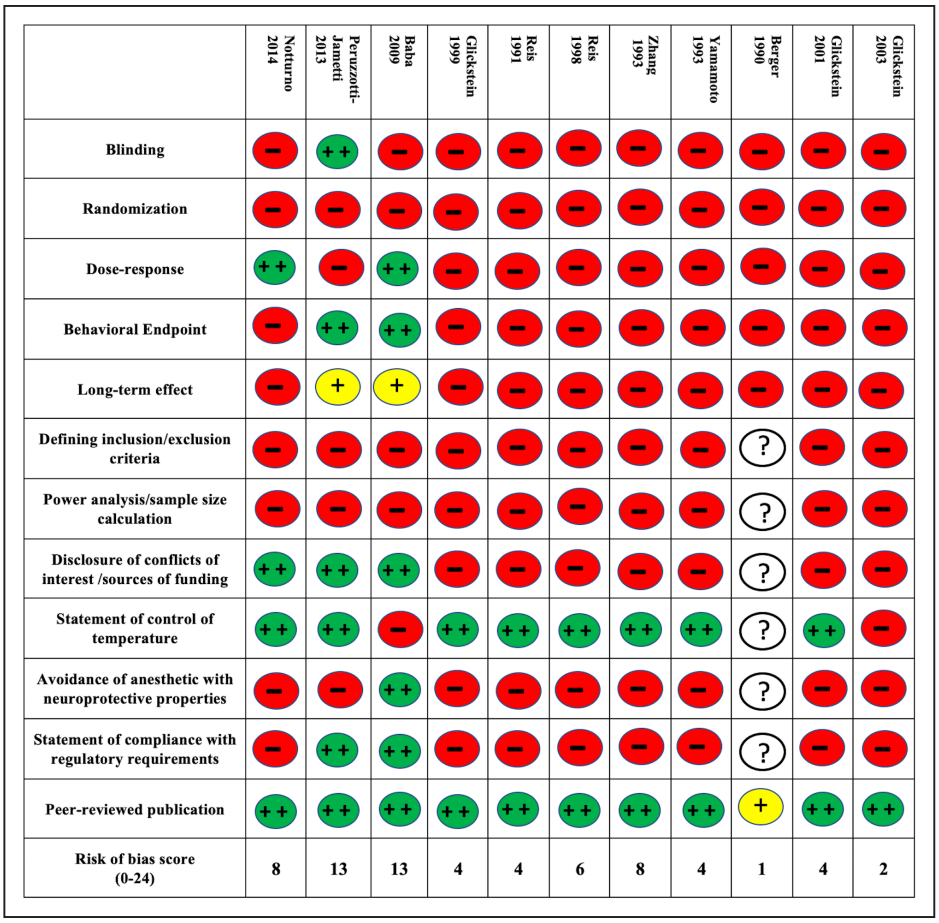

Figure 3. Study-level risk of bias ratings. Risk of bias items based on Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies and Stroke Therapy Academic Industry Roundtable recommendations.[28–30,32] For individual items: green indicates low risk of bias; yellow 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.

Figure 4). Among all stimulation strategies, CHS 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. Among stimulation strategies applying stimulation over the ischemic zone, substantial benefit was observed with CHS, which reduced infarct volumes by one-quarter, whereas no benefit was noted for AHS or PHS. Among strategies applying deep brain stimulation to targets remote from the ischemic zone, substantial benefit was observed for all assessed techniques, including SVAS, DPAGS, and 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 (eg, stimulation by external rather than implanted electrodes), time windows assessed in preclinical studies, and demonstration of dose-response effects, CHS 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 used 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 on 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 12-item score was developed, incorporating

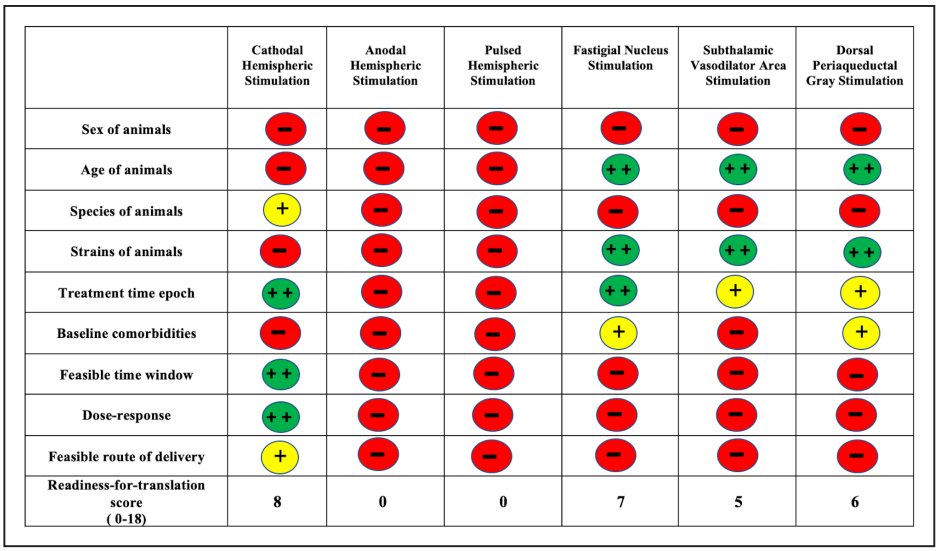

Figure 4. Intervention-level evidence quality ratings and readiness-for-translation scoring. Quality items based on Stroke Therapy Academic Industry Roundtable recommendations.[32] Green indicates high evidence quality; Yellow indicates intermediate evidence quality; Red indicates low evidence quality. Note that for the 2 stimulation subtypes of anodal and electrical hemispheric stimulations, red was allotted to all the quality items due to lack of benefit of the 2 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.

recommended content items advanced by the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (general preclinical science) and Stroke Therapy Academic Industry Roundtable (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 articles 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 articles 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 9-item score was developed, based on Stroke Therapy Academic Industry Roundtable 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 (preconditioning 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 the 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 placed on the analyses of each strategy individually, rather than overall summary effect. Third, some of the individual experiments were performed with stimulation before or immediately after the start of cerebral ischemia, which would lead to overestimation 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]

Conclusions

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

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Disclosures

The City University of New York has intellectual property on neurostimulation system and methods with Dr Bikson as inventor. Dr Bikson has equity in Soterix Medical Inc and serves as a consultant for Boston Scientific, GSK, and Mecta. Dr Saver has received contracted hourly payments and travel reimbursement from BrainsGate for services as a scientific consultant advising on rigorous trial design and conduct. The other authors report no conflicts.

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