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Meta Analysis

Systematic review and Meta-analysis of brain plasticity associated with electroacupuncture in experimental ischemic stroke

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Abstract

OBJECTIVE: To systematically evaluate the role of electroacupuncture in maintaining brain plasticity in ischemic stroke mediated brain damage.

METHODS: We searched for all relevant trials published through Oct 7, 2022 from seven databases. Methodological quality was assessed using the CAMARADES Risk of Bias Tool. A Meta-analysis of comparative effects was performed using Review Manager v.5.3 software.

RESULTS: A total of 101 studies involving 2148 animals were included. For most studies, primary outcomes results of the Meta-analysis indicate that EA significantly improved ischemic stroke rat's postsynaptic density thickness [Standardized Mean Difference (*SMD*) = 1.41, 95% confidence interval (*CI*) (0.59, 2.23), *P* = 0.0008], numerical density of synapses [*SMD* = 1.55, 95% *CI* (0.48, 2.63), *P* = 0.005] compared with non-EA-treated. Similarly, EA could improve parts of biomarkers of synapses, neurogenesis, angiogenesis and neurotrophin activity than the control group (*P* < 0.05).

CONCLUSION: The existing evidence suggests EA regulating ischemic stroke may be through brain plasticity. More rigorous and high quality studies should be conducted in the future.

Keywords: electroacupuncture; brain plasticity; experimental ischemic stroke; animal; systematic review; Meta-analysis

1. INTRODUCTION

After the onset of stroke, the infarction area exhibits excitotoxicity, depolarization, and generation of reactive oxygen species; these factors eventually lead to neuronal apoptosis and necrosis.[1] The process of neuronal apoptosis in the ischemic penumbra can be salvageable.[2] Therefore, detecting injured neurons is a prerequisite for defining the degree of focal ischemic brain injury, and the apoptosis mechanisms of neurons may be key determinants of ischemic stroke disease processes.[3] The past decade has seen a rebirth of interest in cerebral ischemia, catalyzed in large part by studies of brain plasticity.[4,5] Brain plasticity involves neurogenesis, regeneration and rearrangement of synaptic structure and function, leading to the activation of new neurons and the integration of these cells into neuronal networks.[6]
Electroacupuncture (EA) can mitigate the neurological symptoms of ischemic stroke both clinically and in animal models, and it can promote the recovery of motor and sensory functions after stroke. However, the mechanism by which EA repairs ischemic stroke is unknown. Some studies have indicated that EA treats ischemic stroke by activating the dormant nerve structures, increasing neurotrophic factors, preventing apoptosis, inhibiting inflammation, changing the microenvironment and suppressing oxidative stress.[7,8] Recent studies have suggested that the therapeutic mechanism of EA against ischemic stroke is related to the improvement of brain plasticity. However, there are

still controversies regarding how these regulatory effects occur and whether the regulation is effective. A previous Meta-analysis was conducted to evaluate whether EA could effectively repair ischemic stroke by regulating the mechanism of nerve regeneration.[9] The results showed that EA could effectively improve nerve regeneration in ischemic stroke, repair ischemic brain tissue and improve the symptoms of nerve defects. However, the study evaluated the effect of EA on ischemic stroke only from the perspective of brain plasticity. No study to date has comprehensively examined the regulatory role of EA in ischemic stroke recovery from the overall perspective of brain plasticity (neurogenesis, synaptic plasticity, angiogenesis and neurotrophic factors).

Therefore, in this study, we searched for recent studies on the mechanism of EA in ischemic stroke recovery. First, we summarized the literature as comprehensively as possible to characterize the repair of ischemic stroke by EA through various aspects of brain plasticity. Second, we performed a Meta-analysis to determine whether EA could effectively repair ischemic stroke through brain plasticity. This study provides an evidentiary basis for further research on the mechanism of EA in ischemic stroke recovery.

2. METHODS

2.1. Search strategy

We searched all relevant citations published through Oct 7, 2022 from the Medline, EMBASE, Cochrane Library, China National Knowledge Infrastructure (CNKI), Chinese Biomedical Literature Database (CBM), Wanfang Database and China Science and Technology Journal Database (VIP). The main search terms from the Medline were as follows: "(cerebral infarction OR cerebral ischemia OR ischemic stroke OR ischemic infarction OR ischaemic stroke OR ischaemic infarction OR cerebral ischaemia OR Carotid Artery Diseases OR stroke OR middle cerebral artery OR MCA OR middle cerebral artery occlusion OR MCAO OR anterior cerebral artery OR ACA OR anterior cerebral artery occlusion OR ACAO) AND (acupuncture or acupuncture therapy or acupuncture analgesia or acupuncture electro acupuncture or electro-acupuncture or meridians or acupuncture points) AND (animal or experimental model OR rats OR rat OR mice OR mouse) NOT (human)". The specific search strategy for each database is listed in supplementary File 1.

2.2. Inclusion and exclusion criteria

Two investigators reviewed publications based on their titles and abstracts. The investigators included every available randomized controlled trial (RCT) that met the following criteria: (a) the study used an animal model of focal cerebral ischemia induced by occlusion of the middle or anterior cerebral artery with no comorbid diseases; (b) the intervention was EA, and the comparison group received no treatment; and (c) the study outcomes included brain plasticity indicators within the scope of this review, as listed in the Introduction. Studies presenting insufficient data or irrelevant outcomes were excluded. Conference abstracts, animal experimental protocols, review articles, and clinical studies were also excluded.

2.3. Data extraction and quality assessment

Data and relevant information were extracted from the eligible studies by two independent investigators (YU Wei and GUO Hongji). Basic information about the included studies, consisting of the sample size, intervention, comparison, and outcome, was extracted and compiled in supplementary Table 1. The quality of the included studies was evaluated by two reviewers (YU Wei and GUO Hongji) based on the CAMARADES Risk of Bias Tool.[10] If any discrepancies existed, the third reviewer (WU Chunxiao) was consulted to reach an agreement. The quality assessments contained the following factors: peer-reviewed publication; statement of control of temperature; random allocation to treatment or control; blinded induction of ischemia; blinded assessment of outcome; use of anesthetic without significant intrinsic neuroprotective activity; specialized animal model (aged, diabetic, or hypertensive); sample-size calculation; compliance with animal welfare regulations; and statement of potential conflict of interests. Each study was given a quality score out of a possible total of 10 points, and the group median was calculated.

2.4. Outcome

The primary outcome of this Meta-analysis was postsynaptic density (PSD) thickness and synaptic numerical density (Nv). We also examined improvements in synaptic structure [synaptic cleft width and synaptic surface density (Sv)] and in the expression of synaptic plasticity-related marker proteins [growth-associated protein-43 (GAP-43), synaptophysin (SYN) and postsynaptic density-95 (PSD-95)].Other aspects of brain plasticity, namely, neurogenesis [indicated by glial fibrillary acidic protein (GFAP), Nestin and Nestin mRNA], angiogenesis [indicated by angiopoietin-1 (ANG-1), vascular endothelial growth factor (VEGF) and VEGF mRNA], and neurotrophins [indicated by nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF) and BDNF mRNA], were used as secondary outcomes. The higher the expression levels of the above outcome indicators, the greater the improvement in brain plasticity.

2.5. Data analysis

We analyzed continuous outcomes after calculating the mean difference (*MD*) or standardized mean difference (*SMD*) with 95% confidence intervals (*CI*s). For the pooled results of different detection methods, the *SMD* was calculated. We measured heterogeneity using I^2

(higher values indicate higher heterogeneity). If we obtained $I^2 > 50\%$ and a χ^2 P-value of less than 0.1, we used a random-effects model; otherwise, we selected a fixed-effects model. We conducted subgroup analyses to explore sources of high heterogeneity and performed a sensitivity analysis on the main outcomes. We examined publication bias in published studies with Egger's test. We performed all statistical analyses using Review Manager 5.3 (Cochrane Collaboration, Copenhagen, Denmark) and Stata 14.0 software (StataCorp LLC, Austin, TX, USA).

3. RESULTS

3.1. Study selection

A total of 2135 citations were searched from seven databases according to the search strategies, and we removed 906 duplicate articles. A total of 1229 publications were retained for screening based on their abstracts and titles. After screening, 357 remaining articles were further examined for potential eligibility by full-text review. We excluded 255 studies because 2 were conference abstracts, 13 were non-RCTs, 29 were data duplications, and 218 had irrelevant outcomes or unrelated interventions. Finally, 101 studies met the inclusion criteria and were included in a Meta-analysis. The specific screening procedure is summarized in Figure 1.

3.2. Characteristics of the included studies

In total, the 101 studies in our analysis reported data from 2148 animals. These data were divided into 16 different results and underwent 2 comparisons between the EA group and the control group:[11-111] 5 studies reported synaptic structure (including PSD thickness, Nv, synaptic cleft width, Sv),[11-15] 18 studies reported synaptic plasticity-related marker proteins (including GAP-43, SYN, PSD-95),[16-33] 13 studies reported Nestin and Nestin mRNA,[24,36,38,41-50] 7 studies reported the exp-ression of GFAP,[34-40] 4 studies reported Ang-1,[51-54] 21 studies reported VEGF and VEGF mRNA,[22,25,48,54-69] 18 studies reported NGF,[40,70-86] 7 studies reported bFGF,[80,87,88,90-93] 24 studies reported BDNF and BDNF mRNA,[20,30,33,36,51,72,94-111] and 3 studies reported GDNF.[88,89]

3.3. Reported study quality

The quality scores of the included studies ranged from 2 to 7 out of a total of 10 points. Among the 101 studies, 8 studies (7.9%) received 2 points[20,23,24,55,56,61-63], 56 studies (55.4%) received 3 points,[11-14,18,19,27-29,31,32,38,43,47-54,57-60,64-66,69,70,72,74,77,78,80-85,87,89,92-94,98,100-105,109-111] 30 studies (29.7%) received 4 points,[15-17,21,22,25,26,30,34,35,41,42,46,67,68,71,

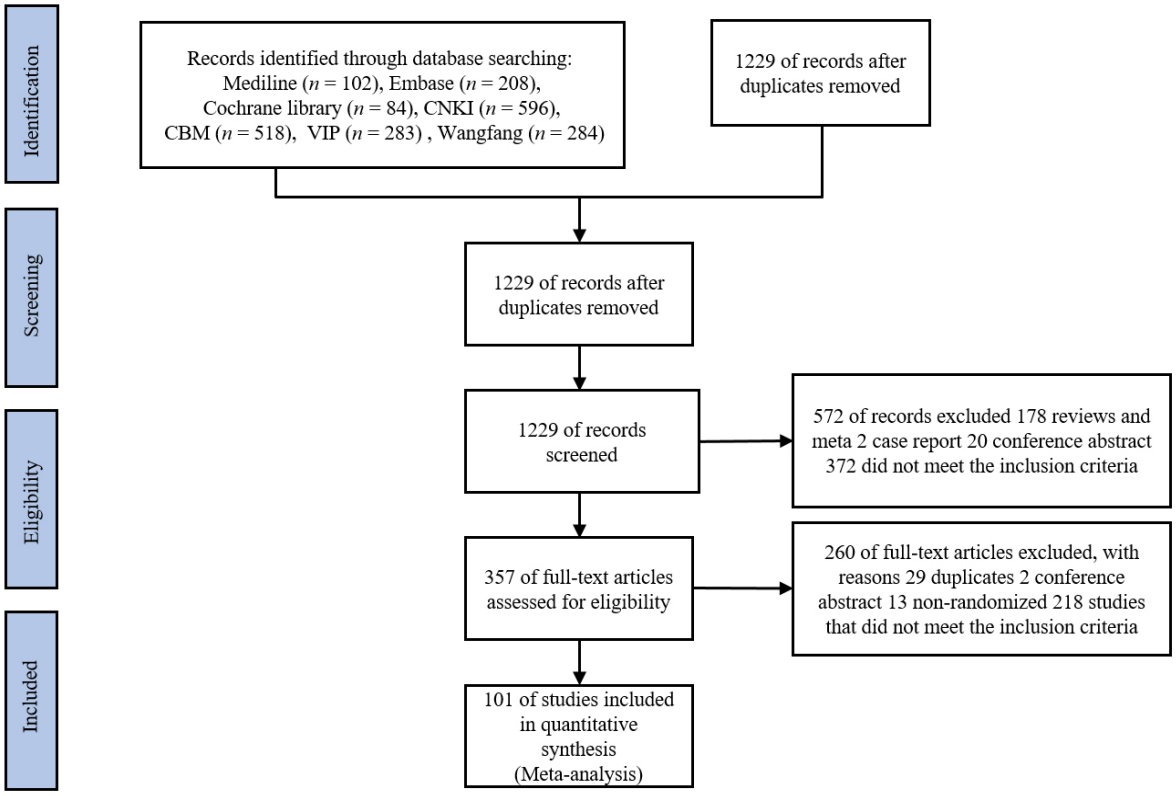

Figure 1 Study flow diagram: a PRISMA flow diagram of the study
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; CNKI: China National Knowledge Infrastructure Database; CBM: Chinese Biomedical Literature Database; VIP: China Science and Technology Journal Database.

[75,76,79,86,88,90,91,95,97,99,106-108] 5 studies (5%) received 5 points,[33,37,40,73,96] 2 studies (2%) received 6 points,[36,39] and 2 studies (2%) received 7 points.[44,45] Eighty studies (79.2%)[15-19,22-23,25,26,29,30,33-42,44-46,48,49,52,56,59,60,64-71,73-80,82,83,85-91,93,95-111] included in our analysis were published in peer-reviewed journals. Seventy-one studies (70.3%)[11-17,21,22,25-28,30-41,43-47,50,51,53,54,57,58,62,65,67-69,71,72,75,76,79,81,83,84,86,87,88,90-92,94,95,97,99,104,106-108,111] reported regulating the temperature of the animals during the induction of ischemia. All studies (100%)[11-111] reported random allocation to the treatment and control groups. Five studies (5%)[36,39,40,44,45] reported blinded assessments. Only seven studies (7%)[23,56,62,65,83,104,111] left it uncertain as to whether the anesthetics they used had significant intrinsic neuroprotective activity. None of the studies used a specialized animal model (aged, diabetic, or hypertensive) because we included only studies that used cerebral ischemia models with no comorbidities. Seven studies (7%)[33,37,39,44,45,73,96] mentioned compliance with animal welfare regulations. Six studies (5.9%)[36,42,44,45,73,96] provided statements of potential conflicts of interest. None of the studies described masked induction of the stroke model or sample-size calculation. The median reported study quality score was 3 (interquartile range, 2–7) for the 101 studies. The specific quality score of each study included in our analysis is summarized in Table 1.

3.4. Analysis of outcomes

3.4.1. Primary outcomes

3.4.1.1. PSD thickness

Five studies assessed the effect of EA on PSD thickness, showing that EA could significantly increase the thickness of the PSD compared with that of the control group [*SMD* = 1.41; 95% *CI* (0.59, 2.23), *P* = 0.0008] (Figure 2A).

To identify the source of heterogeneity, we performed a sensitivity analysis by serially removing individual studies. The results of the sensitivity analysis were stable, and the removal of any given study did not change the pooled results (supplementary Figure 1).

3.4.1.2. Nv

Three studies showed that EA could significantly increase Nvcompared with that of the control group [*SMD* = 1.55; 95% *CI* (0.48, 2.63), *P* = 0.005] (Figure 2B).

3.4.2. Secondary outcomes

3.4.2.1. Synaptic plasticity

We performed a Meta-analysis to assess synaptic plasticity, indicated by markers including Synaptic cleft width, Sv, GAP-43, SYN and PSD-95, as an aspect of brain plasticity.

Three studies revealed that EA could increase the width of the synaptic cleft compared with that of the control group [*SMD* = 1.26; 95% *CI* (0.09, 2.43), *P* = 0.03] (supplementary Figure 2A).

Two studies examined the effect of EA on Sv and demonstrated a significant difference between EA and the control group [*SMD* = 2.26; 95% *CI* (0.57, 3.95), *P* = 0.009] (supplementary Figure 2B).

Eight studies assessed the effect of EA in terms of GAP-43 and showed that EA significantly increased GAP-43 expression compared with that of the control group [*SMD* = 18.45; 95% *CI* (17.17, 19.74), *P* < 0.000 01] (supplementary Figure 2C).

Eight studies examined the effect of EA on SYN expression and demonstrated a significant difference between the EA group and the control group [*SMD* = 0.07; 95% *CI* (0.07, 0.08), *P* < 0.000 01] (supplementary Figure 2D).

Four studies showed that EA significantly increased PSD-95 expression between the EA and control groups [*SMD* = 0.05; 95% *CI* (0.05, 0.06), *P* < 0.000 01] (supplementary Figure 2E).

3.4.2.2. Neurogenesis

We performed a Meta-analysis to assess neurogenesis, indicated by markers including GFAP, Nestin and Nestin mRNA, as an aspect of brain plasticity.

Seven studies reported the expression of GFAP. A random-effects model was selected due to the high statistical heterogeneity of the pooled results (*P* < 0.000 01; I^2 = 90%). The results revealed that the effect of EA had no significant difference on GFAP expression compared with that of the control group [*SMD* = 0.54; 95% *CI* (−1.95, 3.04), *P* = 0.67] (supplementary Figure 3A).

The pooled results of EA showed significant improvements in Nestin and Nestin mRNA levels in the EA group compared to the control group [*SMD* = 3.74; 95% *CI* (2.68, 4.79), *P* < 0.000 01]. EA significantly improved both Nestin and Nestin mRNA expression compared with the levels in the control group (*P* < 0.00001 for both), and there were no subgroup differences between studies that examined Nestin and Nestin mRNA (*P* = 0.28; I^2 = 15.3%) (supplementary Figure 3B).

3.4.2.3. Angiogenesis

We performed a Meta-analysis to assess angiogenesis, indicated by markers including ANG-1, VEGF and VEGF mRNA, as an aspect of brain plasticity.

The level of ANG-1 was significantly different between the EA group and the control group [*SMD* = 1.85; 95% *CI* (1.27, 2.44), *P* < 0.000 01] (supplementary Figure 4A).

Due to the heterogeneity of the pooled results (*P* = 0.40; I^2 = 0%), a fixed-effects model was used for the analysis. The overall results revealed that the EA group had significantly higher VEGF and VEGF mRNA levels than the control group [*SMD* = 2.28; 95% *CI* (1.54, 3.01), *P* < 0.000 01]. There were no subgroup differences between

Table 1 Quality assessment of studies included in the Meta-analysis using the CAMARADES risk of bias tool

Author	Year	1	2	3	4	5	6	7	8	9	10	Score
Bai MH[87]	2005	√	√	√			√					4
Bao DP[49]	2007		√	√			√					3
Cao XL *et al* [16]	2018	√	√	√			√					4
Chen B *et al* [41]	2014	√	√	√			√					4
Chen C *et al* [70]	2015	√	√	√			√					4
Chen JJ[24]	2004			√			√					2
Chen LR[11]	2008		√	√			√					3
Chen WS[71]	2011		√	√			√					3
Chen YH，Huang XF[72]	2000	√		√			√			√	√	5
Cheng CY *et al* [94]	2014	√	√	√			√					4
Ding J *et al* [73]	2017	√	√	√			√					4
Ding J *et al* [74]	2018	√		√			√					3
Ding L *et al* [90]	2012	√	√	√			√					4
Du YX[12]	2008		√	√			√					3
Duan XD *et al* [75]	2018	√	√	√			√					4
Fan YS, Luo Y[76]	2008	√		√			√					3
Fan YS[91]	2006		√	√			√					3
Han XH *et al* [92]	2006	√		√			√					3
Han YS[17]	2013	√	√	√			√					4
Huang GX[13]	2009		√	√			√					3
Huang LJ *et al* [55]	2013	√		√								2
Huang XF，Chen YH[18]	2002	√		√			√					3
Ji Z[77]	2005	√		√			√					3
Jiang C[50]	2006		√	√			√					3
Jiang HZ *et al* [63]	2006	√		√			√					3
Kim Won-Seok *et al* [97]	2009	√	√	√			√					4
Li HL[31]	2015		√	√			√					3
Li J *et al* [64]	2014	√	√	√								3
Li M *et al* [109]	2009	√		√			√					3
Li QL *et al* [56]	2018		√	√			√					3
Li QP *et al* [25]	2015	√	√	√			√					4
Li T[32]	2016		√	√			√					3
Li XJ *et al* [99]	2017	√		√			√					3
Li XJ *et al* [98]	2015	√	√	√			√					4
Lin R *et al* [33]	2017	√	√	√			√			√		5
Lin T[57]	2009		√	√			√					3
Lin XM *et al* [26]	2017	√	√	√			√					4
Liu B[27]	2004		√	√			√					3
Liu D *et al* [100]	2014	√		√			√					3
Liu L, Zhang XQ[78]	2018	√	√	√			√					4
Liu Y *et al* [101]	2014	√		√			√					3
Liu YN *et al* [102]	2010	√		√			√					3
Liu D *et al* [88]	2011	√		√			√					3
Luo D *et al* [42]	2014	√		√			√				√	4
Luo Y[14]	2009		√	√			√					3
Luo ZD *et al* [103]	2002	√		√			√					3
Luo ZD *et al* [79]	2004	√		√			√					3
Lyu H[43]	2016		√	√			√					3
Ma JX *et al* [51]	2013	√		√			√					3
Ma JX[65]	2008	√		√			√					3
Ma RR(1) *et al* [19]	2011	√		√			√					3
Ma XM[80]	2009		√	√			√					3
Ma Y *et al* [81]	2018	√		√			√					3
Mao QJ, Chen BG[66]	2012	√	√	√			√					4
Mi XJ[20]	2010			√			√					2
Pan J[58]	2011	√		√			√					3
Pan J *et al* [82]	2017	√	√	√								3
Qin B *et al* [21]	2014	√	√	√			√					4
Shen F[83]	2006		√	√			√					3
Shen F *et al* [89]	2012	√	√	√			√					4

Table 1 Quality assessment of studies included in the Meta-analysis using the CAMARADES risk of bias tool (continued)

Author	Year	1	2	3	4	5	6	7	8	9	10	Score
Shen GY[52]	2009		√	√			√					3
Song CM[28]	2018		√	√			√					3
Song YS, Zhou MF[34]	2008	√	√	√			√					4
Tan F *et al* [35]	2017	√	√	√			√					4
Tan F *et al* [44]	2018	√	√	√		√	√			√	√	7
Tao J *et al* [36]	2016	√	√	√		√	√				√	6
Tao J[96]	2014	√	√	√		√	√			√	√	7
Wang GB *et al* [15]	2005	√	√	√			√					4
Wang J *et al* [59]	2015	√		√			√					3
Wang L[60]	2015			√			√					2
Wang MP *et al* [67]	2018	√	√	√			√					4
Wang Q *et al* [84]	2010	√		√			√					3
Wang S *et al* [104]	2018	√	√	√								3
Wang YC *et al* [105]	2012	√		√			√					3
Wei XY[93]	2013		√	√			√					3
Xiang F[61]	2015		√	√								2
Xiao YC *et al* [37]	2013	√	√	√			√			√		5
Xie CC[68]	2014		√	√			√					3
Xu L *et al* [22]	2013	√	√	√			√					4
Xu NG *et al* [106]	2004	√	√	√			√					4
Yang MG *et al* [29]	2018	√		√			√					3
Yao G *et al* [23]	2007	√		√								2
Ye F *et al* [45]	2012	√	√	√			√					4
Ye XQ *et al* [107]	2014	√	√	√			√					4
Yi W *et al* [85]	2006	√	√	√			√					4
Yin TR[86]	2005		√	√			√					3
You HL[46]	2012		√	√			√					3
Yu JH *et al* [110]	2015	√		√			√					3
Zhang HX *et al* [47]	2011	√		√			√					3
Zhang SL[53]	2016		√	√			√					3
Zhang XM[54]	2012			√			√					2
Zhang YG *et al* [38]	2014	√		√			√					3
Zhang YK, Tao LF[48]	2013	√		√			√					3
Zhao HJ *et al* [39]	2018	√	√	√		√	√			√		6
Zhao JH *et al* [40]	2015	√	√	√		√	√					5
Zhao JP *et al* [30]	2017	√	√	√			√					4
Zhao JX *et al* [111]	2013	√	√	√								3
Zheng CX[108]	2017	√	√	√			√					4
Zhou CL *et al* [62]	2007			√			√					2
Zhu YH *et al* [69]	2015	√		√			√					3

Notes: the quality assessments contained the following factors: 1: peer-reviewed publication; 2: control of temperature; 3: random allocation to treatment or control; 4: blinded induction of model; 5: blinded assessment of outcome; 6: use of anesthetic without significant intrinsic neuroprotective activity; 7: appropriate animal model (aged, diabetic, or hypertensive); 8: sample-size calculation; 9: compliance with animal welfare regulations; 10: statement of potential conflict of interests. Meaning of the Quality Scores: 1-2 points: Very low, extremely low credibility and reproducibility; 3-4 points: low, compromised credibility and reproducibility; 5-6 points: moderate, reasonable credibility and reproducibility; 7-8 points: high, highly credible and reproducible; 9-10 points: very high, extremely credible and reproducible.

VEGF and VEGF mRNA (P = 0.05; I^2 = 75.1%). The VEGF subgroup and the VEGF mRNA subgroup were both significantly different from the control group [*SMD* = 1.66; 95% *CI* (0.83, 2.49), P < 0.000 01; *SMD* = 3.38; 95% *CI* (1.93, 4.83), P < 0.000 01, respectively] (supplementary Figure 4B). Because the heterogeneity of the overall results was I^2 = 86%, P < 0.000 01, a random-effects model was selected.

3.4.2.4. Neurotrophins

We performed a Meta-analysis to assess neurotrophins, including NGF, GDNF, bFGF, BDNF and BDNF mRNA, as an aspect of neurogenesis.

The pooled results of 18 RCTs revealed that the EA group had significantly higher NGF than the control group [*SMD* = 3.94; 95% *CI* (2.65, 5.23), P < 0.000 01] (supplementary Figure 5A). Due to the existence of substantial heterogeneity (P < 0.000 01; I^2 = 88%), we calculated the pooled results using a random-effects model.

Heterogeneity was high for GDNF (3 studies), with I^2 equal to 90%. Overall, there was no significant difference in GDNF between the EA group and the control group [*SMD* = 0.65; 95% *CI* (−1.68, 2.97), P = 0.59] (supplementary Figure 5B).

The EA group was found to have higher bFGF than the

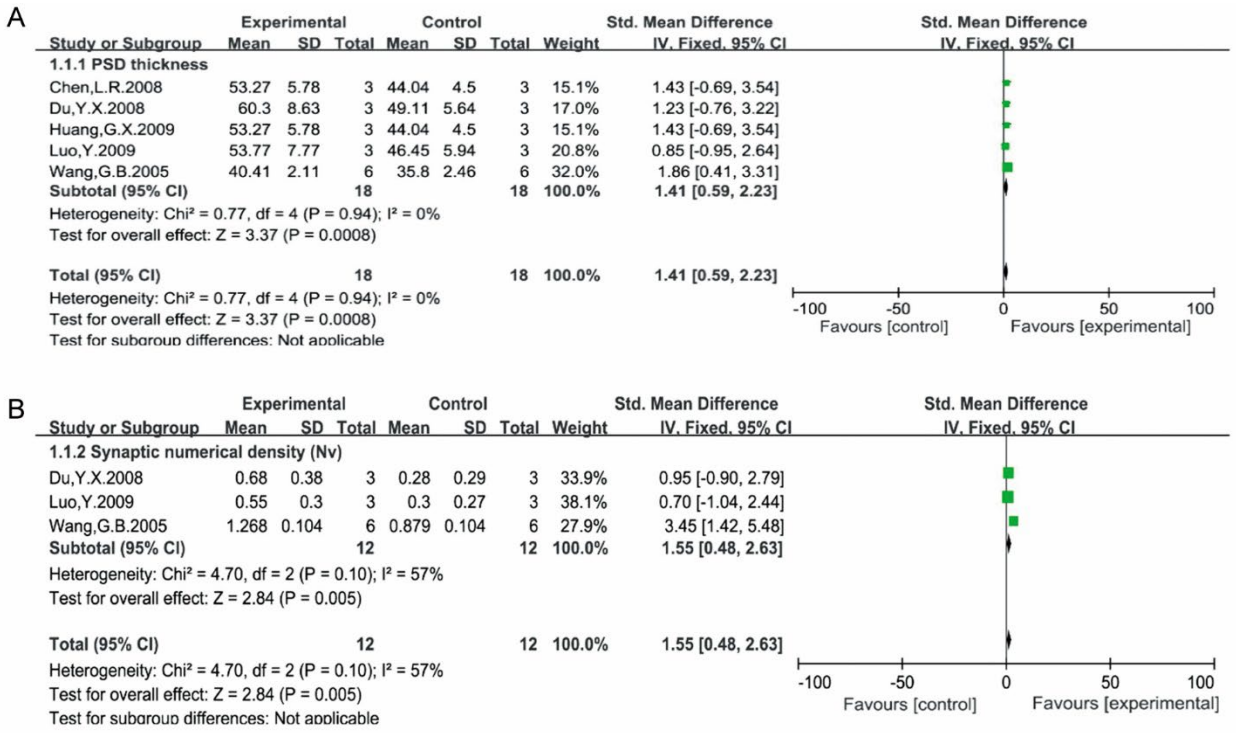

Figure 2 Forest polot of the effects of EA on synaptic structure
A: forest plot of the effects of EA on PSD thickness; B: forest plot of the effects of EA on Nv; PSD: postsynaptic density; Nv: synaptic numerical density; EA: electroacupuncture; *CI*: confidence interval; IV: inverse variance; *SD*: standard deviation.

control group [*SMD* = 2.61; 95% *CI* (0.95, 4.28), *P* = 0.002] (supplementary Figure 5C). Due to the heterogeneity of the pooled results ($P < 0.000\ 01$; $I^2 = 88\%$), a random-effects model was used for the analysis. The pooled results revealed that the EA group had significantly higher BDNF and BDNF mRNA than the control group [*SMD* = 3.82; 95% *CI* (2.96, 4.67), $P < 0.000\ 01$]. Because of the high heterogeneity ($P < 0.000\ 01$; $I^2 = 83\%$) the pooled data from twenty-eight studies were analyzed using a random-effects model. However, no significant difference between the subgroups was found ($P = 0.09$; $I^2 = 66.1\%$). Additionally, the BDNF and BDNF mRNA subgroups showed significant improvements in the expression of BDNF and BDNF mRNA compared with that of the control group [*SMD* = 3.46; 95% *CI* (2.59, 4.34; $P < 0.000\ 01$; *SMD* = 6.06; 95% *CI* (3.23, 8.88), $P < 0.000\ 01$, respectively] (supplementary Figure 5D).

4. DISCUSSION

4.1. Summary of findings

In this study, four mechanisms of EA therapy can promote neural repair after cerebral ischemia. This analysis included 101 randomized animal studies with 2148 animals. We assessed the effect of EA on brain plasticity, including synaptic plasticity, neurogenesis, angiogenesis and neurotrophin, in ischemic stroke rats. To the best of our knowledge, this is the first comprehensive Meta-analysis of the effect of EA on brain plasticity after ischemic stroke. We drew several conclusions, as follow.

The repair process induced by EA therapy involves promoting the recovery of nerve function in the injured area, including nerve regeneration and angiogenesis. Initially, EA therapy improves neuronal function by repairing the transmission between synapses and helps to establish new neural circuits. These damaged neurons can repair function in time and integrate into the neural network.[112] Our aggregate results concerning EA and synaptic plasticity showed that markers of synaptic plasticity were more abundant in the EA group than in the control group, and the results were sustained and consistent when sensitivity analyses were conducted. These results were consistent with a previous review showing that EA therapy promoted the formation of synaptic plasticity.[113] Several studies performed worldwide have noted comparable findings, reporting that EA promoted synaptic structure, including synaptic numerical density and synaptic surface density,[114] in addition to generating a distinct synaptic gap, increasing synaptic curvature and reducing the synapse cleft width in the peri-infarct area.[115] EA upregulated SYN, GAP-43 and PSD-95 in the peri-ischemic region.[116,117]

Moreover, neuronal plasticity after cerebral ischemia represents the repair ability of the brain, and its strength has relation to the changes of their supporting system (such as glial cells and particularly astrocytes).[118] An analysis of neurogenesis, mainly including GFAP, Nestin and Nestin mRNA, was conducted. Our findings suggest that EA in general can improve the expression of Nestin and Nestin mRNA. However, the effects of EA on GFAP were less clear. Nestin and Nestin mRNA are involved in neuronal regeneration and tissue repair under

pathological conditions.[119] Our results demonstrated that EA could promote neurogenesis in injured areas, which was beneficial for repairing ischemic cerebral tissue.

Additionally, neurotrophins are also crucial factors for brain plasticity. Neurotrophins can promote the growth of neurons and the formation and stability of synapses.[120,121] Our results indicated that EA was advantageous for increasing neurotrophins (mainly NGF, bFGF, and BDNF). However, the effects of EA on GDNF was less conclusive. Previous studies have also proposed that EA has positive effects neurotrophin activity,[122] which would be consistent with our results.

Finally, microangiogenesis affects cerebral blood flow and nutrient supply in injured areas. Angiogenesis and maturation are regulated by VEGF and ANG-1.[123] Our results indicated that EA was advantageous for increasing angiogenesis markers, including ANG-1 and VEGF, Previous studies have also proposed that EA has positive effects on angiogenesis,[124] which would be consistent with our results. Induction of angiogenesis can improve neural repair, neuronal plasticity and brain inflammation after stroke, etc. In conclusion, functional recovery after ischemic stroke is associated with brain plasticity. Brain plasticity alters the structure and function of the brain, enabling it to adapt to memory, cognition, movement and rehabilitation after stroke. EA can promote the brain function remodeling to adapt to different functional needs in dynamic process.[125]

The quality scores of the included studies ranged from 2 to 7 points, and the median score was 3. Despite the large number of included articles (101 papers), the overall quality of the articles was low. Because our study included only cerebral ischemia models with no comorbidities, the item for appropriate animal models (aged, diabetic, or hypertensive) was not given points.

4.2. Findings in relation to previous reviews

To our knowledge, our review is the first to analyze the major types of brain plasticity and comprehensively evaluate the regulatory effects of EA on various types of brain plasticity in cerebral ischemia models. Previous Meta-analyses concentrated on one type of brain plasticity, such as neurogenesis.[9] Most other studies included multiple types of acupuncture, such as hand, scalp and ear acupuncture, which could introduce confounding factors and might not provide a comprehensive, precise evaluation of the effect of EA on brain plasticity. Therefore, our research focused only on the effect of one type of acupuncture (EA) and extensively assessed various types of brain plasticity in cerebral ischemia rats, in contrast to previous studies. Nonetheless, our results were in line with those of previous studies that associated one type of brain plasticity with acupuncture intervention.

4.3. Limitations

Our Meta-analysis has several limitations. First of all, while we searched the worldwide database, most of the researches were mainly concentrated in China. One of the main reasons might be is that EA is not widely studied for ischemic stroke in other parts of the world. Secondly, the qualitative scores ranged from 2 to 7 points and the median score was 3, indicating that the included studies had poor methodological quality. Although all the studies described random allocation to the treatment groups, none of them reported details on how the animals were randomized. Thirdly, In the included studies, the mean experimental methods are immunohistochemistry and western blotting without electrophysiological observation. Additionally, none of the included studies reported its sample-size calculation, and only six studies reported that the investigators were blind to the outcomes. Moreover, heterogeneity was high among the included studies. Although we conducted a subgroup analysis and sensitivity analysis to identify the source of the heterogeneity, some heterogeneity still existed. Second, blinding of outcome assessment should be fully described because blinding can prevent measurement bias and subjective bias on the part of the researcher. However, only five studies described the blinded assessment of outcomes.

4.4. Implications for animal experiments

Our pooled results present some potential implications for animal experiments. First, this study provides preclinical evidence as a reference for research related to EA treatment of cerebral ischemia. Second, EA can promote synaptic structural repair by regulating synaptic numerical density, synaptic surface density, PSD thickness and synaptic cleft width. Third, synaptic plasticity-related marker proteins including GAP-43 and SYN are significantly increased by EA. Fourth, EA helps improve neurogenesis, angiogenesis and neurotrophin activity, which promote cerebral ischemia repair.

Finally, this Meta-analysis provides evidence of the benefits of EA intervention for brain plasticity, including synaptic plasticity, neurogenesis, angiogenesis and neurotrophin activity, in cerebral stroke rats. Future studies on the mechanism of EA in the regulation of brain plasticity after ischemic stroke could use this evidence-based study for reference.

5. SUPPORTING INFORMATION

Supporting data to this article can be found online at http://journaltcm.cn.

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