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A systematic review and meta-analysis of Baihui (GV20)-based scalp acupuncture in experimental ischemic stroke

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Acupuncture for stroke has been used in China for over 2,000 years and nowadays is increasingly practiced elsewhere in the world. However, previous studies had conflicting findings on the results of acupuncture. Here, we conducted a systematic review and meta-analysis to assess the current evidence for the effect of Baihui (GV20)-based scalp acupuncture in animal models of focal cerebral ischemia. Six databases from the inception of each database up to June 2013 were electronically searched. Primary outcomes were infarct size and neurobehavioral outcome. Ultimately, 54 studies involving 1816 animals were identified describing procedures. Meta-analysis results showed that twelve studies reported significant effects of Baihui (GV20)-based scalp acupuncture for improving infarct volume compared with middle cerebral artery occlusion group ($P < 0.01$), and thirty-two studies reported significant effects of Baihui (GV20)-based scalp acupuncture for improving the neurological function score when compared with the control group ($P < 0.01$). In conclusion, Baihui (GV20)-based scalp acupuncture could improve infarct volume and neurological function score and exert potential neuroprotective role in experimental ischemic stroke.

Acupuncture is a therapeutic form of traditional Chinese medicine (TCM) that involves the insertion of fine needles or sometimes laser on the defined points, and usually follows by stimulation using related manual or electrical techniques[1]. Acupuncture has been used for healthcare in China and elsewhere for over 2000 years and now is still a useful medical modality for the treatment of various health problems such as stroke rehabilitation, as recommended by National Institutes of Health consensus panel[2]. Since ancient times, stroke has been very common and is a serious neurological disorder in China. Huangdineijing (*Huangdi's Internal Classic*), the oldest and greatest extant classic TCM literature written by various unknown authors from the Warring States Period to the Han Dynasty (475 BC-220 AD), first recorded different stroke-related symptoms[3] and established the theoretical basis for TCM acupuncture therapy. In modern time, acupuncture continued to be widely used for stroke[4] because stroke remains one of the leading causes of mortality and disability worldwide[5] and the relative poverty of effective conventional treatments, except intravenous recombinant-tissue plasminogen activator (rt-PA) within 4.5 hours after stroke onset[6].

Baihui (GV20) is an acupoint of the Du meridian (the government vessel), which locates at the intersection of the line connecting the apexes of the two auricles and the median line of the head, 7 cun directly above the posterior hairline and 5 cun behind the anterior hairline according to the TCM theory of acupuncture and the WHO definition[7]. Based on the TCM theory, because Baihui is located on the highest place of the head where all the yang meridians meet[8], acupuncture on Baihui (GV 20) could clear the mind, lift the spirits, tonify yang, strengthen the ascending function of the spleen, eliminate interior wind, and promote resuscitation[9]. Thus, the acupoint Baihui (GV 20) is specifically used in neurological and psychiatric diseases such as stroke, headache, dizziness, and anxiety. In fact, Baihui (GV 20) is a principle acupoint which is often selected for stroke patients. From a historical perspective, the long history of acupuncture on Baihui (GV 20) for stroke treatment can be traced back to ancient China. During Tang Dynasty, Beiji Qianjin Yaofang (Essential Prescriptions Worth a Thousand Gold for Emergencies), written by Sun Simiao in 652, recorded moxibustion on Baihui (GV 20) for the stroke patients who presented with paralysis and aphasia. During Ming Dynasty, Puji Fang (*Prescriptions for Universal Relief*), compiled by Zhu Su in 1406, used Baihui (GV 20) acupoint for various symptoms, signs and on different stages of stroke. Especially, scalp acupuncture was set up and separated from traditional acupuncture system under the influence of neuroanatomy, neurophysiology, and bioholographic principle of modern medicine in the early 1970's[10]. Scalp acupuncture is one of the several specialized acupuncture techniques in which a filiform needle is used to penetrate specific stimulation areas on the scalp mainly for the treatment of brain

diseases[10]. There are various nomenclature systems for scalp acupoint, for example, some divided the scalp into zones or regions, while others focused on points or lines. Baihui (GV20)-based scalp penetration needling, such as needling through Baihui (GV 20) to Taiyang (EX-HN 5)[11] and needling through Baihui (GV 20) to Qubin (GB7)[12], is one of most important school of scalp acupuncture for stroke. In modern time, various studies have revealed that Baihui (GV20)-based scalp acupuncture has neuroprotective effects on multi-aspects of the pathophysiology in animal models of ischemic stroke. The effects are listed as follows: (1) Scalp acupuncture at Baihui (GV20) and Qubin (GB7) has rapid and powerful effects on removing limb paralyses caused either by cerebral infarct or by cerebral haemorrhage in stroke-prone spontaneously hypertensive rat[13]. (2) Electroacupuncture (EA) preconditioning at Baihui (GV20) could attenuate brain edema and blood brain barrier (BBB) disruption caused by cerebral ischemia and was mainly through decreasing matrix metalloproteinase (MMP)- 9 expression and activity[14]. (3) EA at Baihui (GV20) and Dazhui (GV14) could increase cerebral blood flow and improve tissue and function recovery through acetylcholine/endothelial nitric oxide synthase (eNOS)-mediated perfusion augmentation in acute moderate focal cerebral ischemia[15]. (4) EA pretreatment at Baihui (GV 20) strongly protects the brain against transient cerebral ischemic injury through the anti-inflammatory effects of α7 nicotinic acetylcholine receptors (α7nAChR) activation[16]. (5) EA at Baihui(GV20) and Qubin(GB7) could activate the cerebral structures related to motor function on the bilateral hemispheres by assessment of Positron emission tomography (PET) in stroke patients, suggesting that EA was very helpful for the cerebral motor plasticity after the ischemic stroke[17]. (6) EA at Zusanli(ST36) and Baihui(GV20) enhanced cell proliferation and neuroblast differentiation in the rat dentate gyrus via phosphorylated cyclic AMP response element-binding protein (pCREB) and brain-derived neurotrophic factor (BDNF) activation[18].

In the past decade, several systematic reviews[19–25] assessing the effects of both acupuncture and scalp acupuncture on patients suffering from stroke have been published; however, the results are inconclusive. Park J and Sze FK et al[19,20] reported that acupuncture had no additional effect on motor recovery but had a small positive effect on disability; Kong et al[22] pointed out that acupuncture did not show a positive effect as a treatment for functional recovery after stroke from rigorous randomized sham-controlled trials (RCT). On the contrary, Wu et al[23] reported that acupuncture was effective in the treatment of post-stroke rehabilitation based on fifty-six RCTs. By comparison, systematic reviews of pre-clinical animal data could inform the planning and improve the likelihood of success of future clinical trials, identify where there is a need for further 'basic' research, preclude unnecessary study replication, and contribute to both 'reduction' and 'refinement' in animal experimentation[26]. Therefore, we conducted a systematic review and meta-analysis of Baihui(GV20)-based Scalp acupuncture (BBA) in animal models of acute focal ischemic stroke.

Results

Study inclusion. We identified 5383 potentially relevant articles from six databases. After removal of duplicates, 2527 records remained. After going through the titles and abstracts, we excluded 2319 papers with at least one of following reasons: (1) case report or review; (2) not an animal research; and (3) not the researches about stroke or ischemia. By reading the full text of the remaining 208 articles which reported the efficacy of BBA in animal models of focal cerebral ischemia, 54 studies were excluded because the outcome measure was neither neurological function score (NFS) nor infarct volume (IV); 48 studies were excluded because other forms of acupuncture or TCM were used in control group; 34 were excluded because of combination with body acupunctures; 18 studies were removed due to the deficiency of useful data and the problem of duplicate publication. Ultimately, 54 eligible studies were identified (Figure 1)[34–79]; Li JA, unpublished Master's thesis, 2005; Wang C, unpublished Master's thesis, 2005; Jin JF, unpublished PhD thesis, 2005; Luo T, unpublished Master's thesis, 2006; Chen X, unpublished Master's thesis, 2007; Ding J, unpublished Master's thesis, 2007; Wang EL, unpublished PhD thesis, 2008; Wu HY, unpublished PhD thesis, 2010.

Study characteristics. The 54 included studies with 1816 rats described 73 groups of comparisons based on two different outcome measures: 24 groups of comparisons including 478 rats reported data as IV, and 49 groups of comparisons using 1736 animals reported data as NFS. The rats species included Sprague-Dawley (SD) rats, Wistar rats and stroke-prone spontaneously hypertensive (SHR-SP) rats. The weight of rats varied between 180–340 g (median 240 g). Sixteen out of the 54 studies (29.6%, n = 681) were permanent middle cerebral artery occlusion (MCAO) models, while the remaining studies (n = 1135) utilized temporary MCAO models. The time of ischemia varied from 30 minutes to 3 hours. Chloral hydrate were used in 32 studies (n = 59.2%) to induce anesthesia, isoflurane in 7 studies[34–36,42,45,57,71], Pentobarbital in 9 studies[38–41,43,44,46,47,60], urethane[59] and thiopental[69] in one study respectively, while no report of anesthetics in the remaining 4 studies[66,67,78,79].

Eight studies performed manual acupuncture (MA)[61,64–67,70,77]; Wang EL, unpublished PhD thesis, 2008 and the rest of studies utilized EA. The selection of acupoints were as follows: 12 studies used Baihui mono-therapy[34–45]; 12 studies selected Baihui plus Shuigou[58–63]; Li JA, unpublished Master's thesis, 2005; Wang C, unpublished Master's thesis, 2005; Jin JF, unpublished PhD thesis, 2005; Luo T, unpublished Master's thesis, 2006; Chen X, unpublished Master's thesis, 2007; Ding J, unpublished Master's thesis, 2007; 12 studies selected Baihui plus Dazhui[46–57]; 7 studies used scalp penetration needling through Baihui to other acupoints[64–70]; the rest of the 11 studies selected Baihui plus other acupoints. Meanwhile, IV was used as the outcome measure in 24 groups (44.4%), and NFS was used in forty-nine groups (90.7%). But the standards of NFS were different, as 21 studies adopted Zea long criterion[30]; 13 studies used Bederson criterion[29]; 10 studies adopted Garcia criterion[31]; Kuluz[80], Ludmia[81], Cai[82] and Sun criteria[83] were cited in 1 study respectively. Sixteen studies adopted both two outcome measures. The basic characteristics of the 54 studies are shown in Table 1.

Study quality and publication bias. The median number of study quality checklist items scored was ranged from 3 to 7 out of a total 10 points. Of whom, seventeen studies got 3 points; sixteen studies got 4; ten studies got 5; six studies got 6; and five studies got 7 points (Table 2). Eight studies were online Master's thesis or PhD thesis and not formally published; Li JA, unpublished Master's thesis, 2005; Wang C, unpublished Master's thesis, 2005; Jin JF, unpublished PhD thesis, 2005; Luo T, unpublished Master's thesis, 2006; Chen X, unpublished Master's thesis, 2007; Ding J, unpublished Master's thesis, 2007; Wang EL, unpublished PhD thesis, 2008; Wu HY, unpublished PhD thesis, 2010. Twenty-eight studies described control of temperature, including control of the room and rats anal temperature. No study described the sample size calculation. SHR-SP rats were used in one study. Random allocation to treatment group and blinded assessment of outcome were described in 49 and 15 studies respectively. No study reported cerebral ischemia was induced by an investigator who was blinded to treatment allocation. Twenty studies mentioned statement of potential conflict of interests. Twenty-four studies reported compliance with animal welfare regulations. No study used anesthetic with significant intrinsic neuroprotective activity.

Effectiveness. Twelve studies reported significant effects of BBA for improving IV compared with MCAO group (n = 172, SMD −1.95, 95% CI: −2.80 ~ −1.10, $P < 0.00001$; heterogeneity $\chi^2 = 43.79$, $P <$

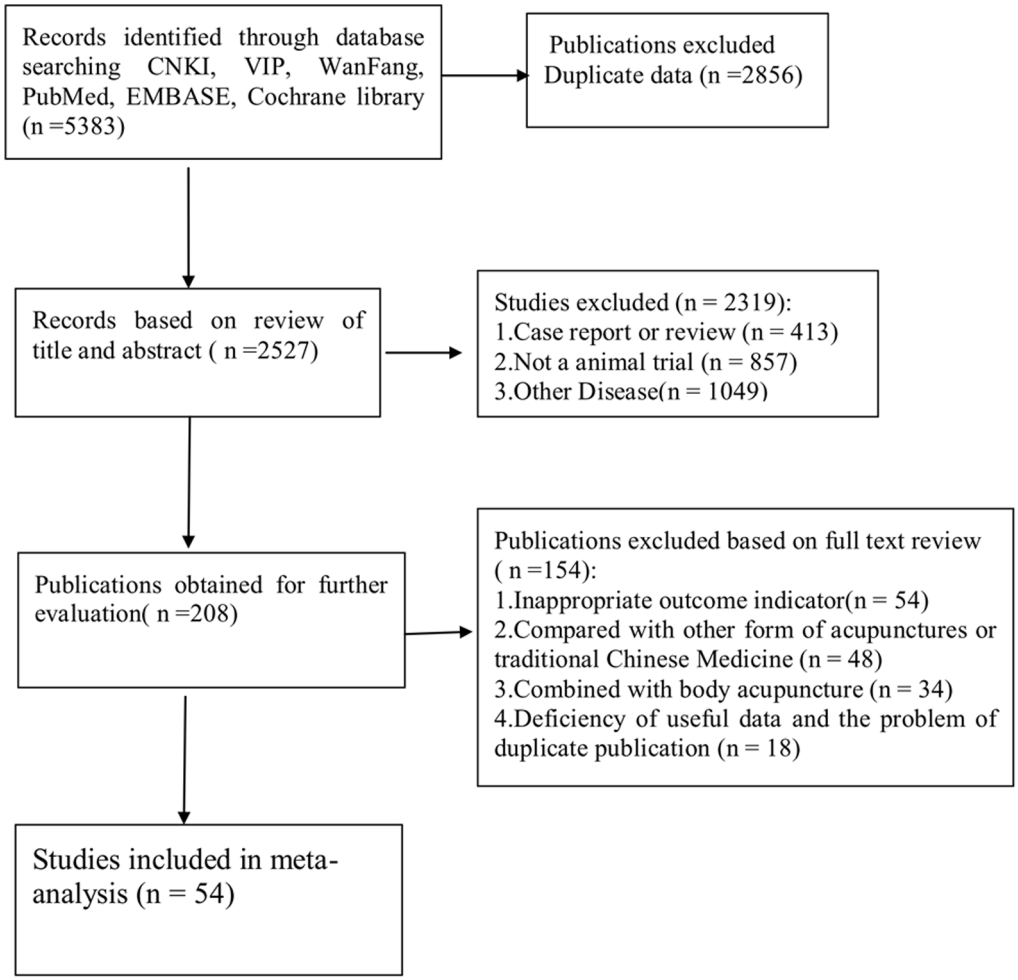

Figure 1 | The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flow Diagram.

0.00001, $I^2 = 75\%$, Figure 2); the remaining twelve studies failed to pool analysis due to the data demonstrated in the form of the TTC staining or IV percentage (%), but all of them reported the significant effects of BBA for ameliorating the IV compared with the control group ($p < 0.05$ or $p < 0.01$).

Fourteen studies showed significant effects of BBA for improving the NFS according to Zea longa criterion (n = 425, SMD −3.93, 95% CI: −5.11 ~ −2.75, $P < 0.00001$; heterogeneity $\chi 2 = 179.39$, $p < 0.00001$, $I^2 = 93\%$, Figure 3), but the remaining seven studies did not provide raw data and thus failed for meta-analysis. NFS was significantly improved in fourteen studies in BBA group compared with control group according to Bederson criterion (n = 384, SMD −1.51, 95% CI: −1.97 ~ −1.05, $P < 0.00001$; heterogeneity $\chi 2 = 44.90$, $p < 0.00001$, $I^2 = 71\%$, Figure 4). Four studies reported significant effects of BBA for improving the NFS according to Garcia criterion compared with control group (n = 58, SMD 3.98, 95% CI: 2.98 ~ 4.98, $P < 0.00001$; heterogeneity $\chi 2 = 0.51$, $p = 0.92$, $I^2 = 0\%$, Figure 5). Six studies reported significant effects of BBA for improving NFS based on the Garcia criterion ($p < 0.05$ or $p < 0.01$), but the data were represented in the graphical form and meta-analysis was unable to be done because we failed to contact the authors. Four studies also reported the significant effects of BBA for ameliorating the NFS according to the Kuluz criteria, Ludmia criteria, Cai criteria, and Sun criteria, respectively ($p < 0.05$ or $p < 0.01$).

Assessment of bias. The funnel plot was roughly symmetric for the effect of BBA on IV and NFS. Thus, funnel plots did not suggest an obvious publication bias (Figure 6).

Factors affecting the outcome measures. In the subgroup analysis for the outcome measure according to NFS, the efficacy of acupuncture at MCAO 30 min/perfusion was better than other longer perfusion time (n = 111, SMD −3.08, 95% CI: −3.65 ~ −2.51, $P < 0.00001$; heterogeneity $\chi^2 = 2.10$, $p = 0.35$, $I^2 = 5\%$, Figure 7A). On the other hand, BBA on MCAO 3 hours and permanent model were less effective than on MCAO 1 and 2 hours ($p < 0.01$). Experiments using chloral hydrate as anesthetics showed better improvement of NFS when compared with other anesthetics such as isoflurane and unreported (n = 291, SMD −5.02, 95% CI: −6.77 ~ −3.28, $P < 0.00001$; heterogeneity $\chi^2 = 142.99$, $p < 0.00001$, $I^2 = 94\%$, Figure 7B). Compared with Wistar rats, SD rats were more sensitive to BBA treatment for improving NFS (n = 214, SMD −1.90, 95% CI: −2.24 ~ −1.57, $P < 0.00001$; heterogeneity $\chi^2 = 9.02$, $p = 0.25$, $I^2 = 22\%$, Figure 7C). BBA treatment in the published studies was more effective than that in un-published studies ($p < 0.00001$, SMD −3.51, 95% CI: −4.07 ~ −2.94; $p < 0.00001$, SMD −2.40, 95% CI: −3.02 ~ −1.78; respectively, Figure 7D). In the subgroup analysis for the outcome measure according to IV, efficacy of BBA at permanent model was better than at other MCAO models (SMD −2.86, 95% CI: −4.12 ~ −1.61, $P < 0.00001$; heterogeneity $\chi 2 = 0.95$, $p = 0.62$, $I^2 = 0\%$, Figure 8A). Experiments using chloral hydrate as anesthetics showed more reduction in IV when compared with those using isoflurane and pentobarbital anesthesia (SMD −2.59, 95% CI: −3.95 ~ −1.21, $P = 0.0002$; heterogeneity $\chi 2 = 21.95$, $p = 0.0005$, $I^2 = 77\%$, Figure 8B). Meanwhile, experiments on SD rats gave a higher estimate of effect size in IV than that using Wistar rats (SMD −2.14, 95% CI: −3.36 ~ −0.93, $P = 0.0005$;

Table 1 | Characteristics of the included studies

Study (years)	Species (Sex,n)	Weight	Model (method)	Anesthetic	Method to acupuncture	Outcome Index (time#)	Intergroup Differences
Lu 2002[34]	SD Rat (male,10/10)	280–320 g	MCAO/2 h (tatlisumak)	4% Isoflurane induce, 2% Isoflurane, maintain	Acupuncture 30 min/d for 5 d before operation; disperse-dense waves of 15 Hz of frequency and current density of 1 mA.	1.NFS (ZL, 24 h) 2.IV	1.$p < 0.05$ 2.$p < 0.05$
Lao 2003[35]	SD Rat (male,10/10)	280–320 g	MCAO/2 h	4% Isoflurane induce, 2% Isoflurane, maintain	Acupuncture 30 min/d for 5 d before operation; disperse-dense waves of 2/15 Hz of frequency and current density of 1 mA.	1.Weight. temperature, operating time 2. NFS (ZL, 24 h)	1.$p > 0.05$ 2.$p < 0.05$
Xiong 2003[36]	SD Rat (male,10/10)	280–320 g	MCAO/2 h (tatlisumak)	4% Isoflurane induce, 2% Isoflurane, maintain	Acupuncture 30 min/d for 5days before operation; stimulated with 1 mA of density and frequency of 15 Hz.	1.NFS(ZL, 24 h) 2.IV	1.$p < 0.05$ 2.$p < 0.05$
Tian 2004[37]	SD Rat (male,12/12)	250–300 g	MCAO/2 h	10% Chloralhydrate (300 mg/kg)	Acupuncture 30 min/d for 5days before operation; disperse-dense waves of 4–16 Hz of frequency and current density of 1 mA.	1.IV (6 h) 2.BCL-2 3.Tunel stain	1.$p < 0.05$ 2.$p < 0.05$ 3. $p< 0.05$
Xiong 2007[38]	SD Rats (Male,6/6)	280–320 g	MCAO/2 h	1%sodium pemobarbital (40 mg/kg)	Acupuncture 30 min/d for 5days before operation; the intensity of 1 mA and frequency of 2/15 Hz	1. IV (24 h) 2.Immunohistochemistry	1.$P = 0.000$ 2.NR
Dong 2009[39]	SD Rats (Male,40/40)	280–300 g	MCAO/2 h (tatlisumak)	Pentobarbital sodium (40 mg/kg)	Acupuncture 30 min/d for 5days before operation; Stimulated with 1 mA of density and frequency of 15 Hz.	1.physio parameters 2.NFS (ZL,24 h) and IV 3.Brain water content 4. MMP-9	1.$p > 0.05$ 2.$p < 05$ 3. $p< 0.01$ 4. $p< 0.01$
Du 2010[40]	SD Rats (Male,10/10)	280–320 g	MCAO/2 h	Sodium pentobarbital (40 mg/kg)	Acupuncture preconditioning for 5 days, with the intensity of 1 mA and frequency of 2/15 Hz for 30 min	1.P-ERK1/2 2.NFS (Garcia, 1 d) 3.IV	1.$p < 0.05$ 2.$p < 0.01$ 3. $p< 0.01$
Wang 2011[41]	SD Rats (Male,8/8)	280–320 g	MCAO/2 h	Sodium pentobarbital (40 mg/kg)	Acupuncture pretreatment for 3 d, with the intensity of 1 mA and frequency of 2/15 Hz for 30 min	1.NFS (Garcia, 3 d) and IV 2. PKC, BCL-2,BAX	1.$p < 0.05$ 2.$p < 0.01$
Kim 2011[42]	SD Rats (Male,6/6)	270–350 g	MCAO/2 h	5% isoflurane	Acupuncture on days 4, 6, 8, 11 and 13 following MCAO,with the intensity of 3–3.5 v and frequency of 23 Hz for 5 min	1.NFS (Garcia, 2 d,7 d,14 d 2.VEGF	1.$p < 0.05$ 2.$p < 0.01$
Wang 2012[43]	SD Rats (Male,10/10)	280–320 g	MCAO/2 h	Sodium pentobarbital (40 mg/kg)	Acupuncture pretreatment for 3 d, with the intensity of 1 mA and frequency of 2/15 Hz for 30 min	1.NFS (Garcia, 3 d) and IV 2. physio parameters 3. a7nAChR and HMGB1	1. $p< 0.01$ 2. $1.p > 0.05$ 3. $p< 0.05$
Zhao 2012[44]	SD Rats (Male,6/6)	280–320 g	MCAO/2 h	Sodium pentobarbital (40 mg/kg)	Acupuncture pretreatment for 5 d, with the intensity of 1 mA and frequency of 2/15 Hz for 30 min	1.Notch1, Notc4 h, Jag1 2.NFS(Garcia,1 d,2 d, 3 d) and IV 3. Hes1	1. $p< 0.05$ 2. $p< 0.05$ 3. $p > 0.05$
Kim 2012[45]	SD Rats (Male,6/6)	NA	MCAO/2 h	5% isoflurane	Acupuncture 5 m every 2 d for 2 weeks with bipolar waveform, 3 Hz pulses was applied for bursts of 5 s, with 2 s intervals	1.NFS (Garcia, 2,7, 14 d) 2.BDNF	1.$p < 0.05$ 2.$p < 0.05$
Ding 2004[46]	SD Rats (Male,5/5)	280–340 g	Permanent MCAO	20 g/LPentobarbital (36–40 mg/kg)	Acupuncture 120 min before operation; disperse-dense waves of 14 r/min of frequency and the intensity of stimulus was based on the slightly visible muscle twitch.	1. IV(6 h) 2.Pathomorphism	1. $p< 0.05$ 2.NR
Liu 2006[47]	SD Rats (Male,36/36)	250–280 g	Permanent MCAO (Bederson),	1% Pentobarbital sodium (50 mg/kg)	Acupuncture Immediately after occlusion for 30 min until sacrifice, disperse-dense waves of 5–10 Hz of frequency and the intensity of stimulus was based on the slightly visible muscle twitch.	1. NFS (Bederson,3 d, 7 d,14 d,21 d) 2. IV	1. $p< 0.01$ 2. $p< 0.01$

Table 1 | Continued

Study (years)	Species (Sex,n)	Weight	Model (method)	Anesthetic	Method to acupuncture	Outcome Index (time#)	Intergroup Differences
Shen 2007[48]	SD Rats (Male,6/6)	360 ± 20 g	MCAO/2 h (ZL),	3%chloralhydrate (1 ml/100 g)	Acupuncture 60 min after reperfusion, disperse dense waves of 80–100 Hz of frequency and current density of 1–3 mA.	1. NFS (Julio,24 h) 2.ERK express 3.EPK cell count	1. $p < 0.01$ 2.NR 3. $p < 0.01$
Li 2008[49]	SD Rats (Male,8/8)	300 ± 50 g	MCAO/1 h (ZL)	10% Chloralhydrate (350 mg/kg)	Acupuncture 30 min after reperfusion 24 h, continuous wave of 3 Hz of frequency and current density of 1–3 mA.	1. NFS and MT count (Julio,24 h) 2. Bax-2,bcl-2	1. $p < 0.01$ 2. $p < 0.001$
Xu 2009[50]	Wistar rats (Male, 18/9)	210–290 g	Permanent MCAO (Bederson),	100 g/LChloralhydrate (400 mg/kg)	Acupuncture after occlusion 30 min (10:00 AM) qd for 2 w, disperse-dense waves of 5–10 Hz of frequency and the intensity of stimulus was based on rats keeping quiet Acupuncture immediately after occlusion 30 min (10:00AM and 15:00PM) bid for 2 w, disperse-dense waves of 5–10 Hz of frequency and the intensity of stimulus was based on rats keep quiet	1. NFS (Bederson, 3 h,14 d) 2.EPSP 3.PS	1. $p < 0.01$ 2. $p < 0.01$ 3. $p < 0.01$ 1. $p < 0.01$ 2. $p < 0.01$ 3. $p < 0.01$
Mu 2009[51]	SD Rats (Male,10/10)	360 ± 20 g	MCAO/2 h (ZL)	3% chloralhydrate (1 m/100 g)	Acupuncture after occlusion 60 min, first rotating manually right and left 0.5 min then disperse-dense waves of 80–100 Hz of frequency and current density of 1–2 mA.	1. NFS (Julio,24 h) 2. ATP,Ca^{2+},Mg^{2+} activity	1. $p < 0.01$ 2. $p < 0.01$
Chen 2009[52]	SD Rats (Male,18/18)	200–250 g	MCAO/3 h (ZL)	10% Chloral hydrate	Acupuncture 15 min before occlusion 12 h and 30 min, and after occlusion every time interval of 12 h until sacrifice, first twirling, lifting and thrusting 1 min, then disperse-dense waves of 10 Hz of frequency and intensity of 2 V	1. NFS (ZL,12 h,24 h, 48 h) 2. TUNEL 3. P13-K and Akt	1. $p < 0.05$ 2. $p < 0.01$ 3. $p < 0.01$
Yang 2010[53]	SD Rats (Male,30/30)	180–240 g	Permanent MCAO (Tamura)	10% Chloral hydrate (400 mg/kg)	Acupuncture 30 min/d after occlusion until sacrifice, disperse-dense waves of 5–10 r/s of frequency and the intensity of stimulus was based on rats keep quiet (3–5 V)	1.NFS (Bederson,2 h,1 d,3 d) 2. Y-Maze test 3. p-ERK	1. $p < 0.01$ 2. $p < 0.01$ 3. $p < 0.01$
Cheng 2011[54]	SD Rats (Male,5/5)	300 ± 20 g	MCAO/2 h (ZL)	10% Chloralhydrate (3.5 ml/kg)	Acupuncture 30 min after reperfusion, disperse-dense waves of 2–4 mA of intensity which needle handle mild shake (1–3 V)	1. NFS (Julio,24 h) 2. Calmodulin	1. $p < 0.01$ 2. $p < 0.01$
Luo 2011[55]	Wistar rats (Male, 30/30)	180–240 g	Permanent MCAO	10% chloralhydrate (400 mg/kg)	Acupuncture 30 min/d after occlusion until sacrifice, stimulated with 1–3 mA of density and frequency of 4/20 Hz	1. NFS (Bederson,2 h, 1 d,3 d,7 d,21 d) 2.SSP, CX43 3. GFAP.,EAAT2	1. $p < 0.01$ 2. $p < 0.01$ 3. $p > 0.05$
Liu 2012[56]	SD Rats (Male,50/50)	180–240 g	Permanent MCAO	10% chloralhydrate (330 mg/kg)	Acupuncture 30 min/d until sacrifice, with 1–2 mA of density and frequency of 4/20 Hz	1.NFS (ZL,2 h,1 d,3 d,7 d, 21 d) 2.Jak2 mRNA, P-JAK2 protein	1. $p < 0.05$ 2. $p < 0.05$
Kim 2013[57]	C57BL mice (male, 10/10)	20–25 g	MCAO/1 h	face mask delivered isoflurane	Acupuncture 20 min/d after occlusion until sacrifice, stimulated with 1 mA of density and frequency of 2 Hz	1.Physiological parameters 2.NFS(ZL,24 h) and IV 3.Cerebral perfusion 4. Ach, mAChR M3, eNOS	1. $p > 0.05$ 2. $p < 0.05$ 3. $p < 0.05$ 4.P < 0.05

Table 1 | Continued

Study (years)	Species (Sex,n)	Weight	Model (method)	Anesthetic	Method to acupuncture	Outcome Index (time#)	Intergroup Differences
Chen 2000[58]	Wistar rats (Male, 6/6)	230–260 g	MCAO/1.5 h (ZL),	10%chloralhydrate (400 mg/kg)	Acupuncture 1 h after occlusion for 7 days, stimulated with 3.5 mA of density and frequency of 100 Hz,	1.BDNF 2.IV 3. NFS (Kuluz,7 d)	1. $p < 0.05$ 2. $p < 0.05$ 3. $p < 0.01$
Zhou 2003[59]	SD Rats (Male,8/8)	250–270 g	MCAO/1 h (ZL),	1 g/kg Urethane	Acupuncture 30 min immediately after occlusion and reperfusion respectively, disperse-dense waves of 1–1.2 mA of intensity	1.CBF 2. NFS (Cai,24 h) 3. IV	1. $p < 0.05$ 2. $p < 0.01$ 3. $p < 0.01$
Li JA (Unpublished Master's thesis, 2005)	SD Rats (Male/ female, 28/28)	200 ± 20 g	MCAO/30 m (ZL),	10%chloralhydrate (300 mg/kg)	Acupuncture 30 min after occlusion for 2days, disperse-dense waves of 4/16 Hz of frequency and intensity of 1–3 V	1. NFS (ZL,4 d) 2.GFAP 3.No	1. $p < 0.01$ 2. $p < 0.01$ 3. $p < 0.01$
Wang C (Unpublished Master's thesis, 2005)	SD Rats (Male,6/6)	200–220 g	MCAO/1.5 h (ZL),	7%chloralhydrate (1 ml/200 g)	Acupuncture 30 min after occlusion, 15 min interval and continuing to 30 min, disperse-dense waves of 5/20 Hz of frequency and current density of 1–4 mA.	1. NFS (Zl, 24 h) 2. IV	1. $p < 0.05$ 2. 2.NR
Jin JF (Unpublished PhD thesis, 2005)	SD Rats (Male/ femal, 30/30)	200 ± 20 g	MCAO/30 m (ZL),	10%chloralhydrate (300 mg/kg)	Acupuncture 30 min after reperfusion 1 h and before sacrificed, disperse-dense waves of 4/16 Hz of frequency and intensity of 1–3 V.	1 NFS (ZL,24 h) 2. Pathomorphism 3. VECA	1. $p < 0.05$ 2. NR 3. $p < 0.05$
Luo T (Unpublished Master's thesis, 2006)	SD Rats (Male,15/15)	230–250 g	MCAO/30 m (ZL)	10% chloralhydrate (300 mg/kg)	Acupuncture 30 min before occlusion and immediately after reperfusion, disperse-dense waves of 4/16 Hz of frequency and intensity of 1–3 V.	1. NFS (ZL,24 h)	1. $p < 0.01$
Chen X (Unpublished Master's thesis, 2007)	SD Rats (Male/ female,10/10)	180–230 g	Permanent MCAO	10%chloralhydrate (300 mg/kg)	Acupuncture 30 min after occlusion for 5 days, continuous wave of 100 r/min of frequency and current density of 1 mA.,	1.NFS (Bederson,5 d) 2.NE,DA 3.5-HT	1. $p < 0.01$ 2.p < 0.01 3. $p < 0.01$
Ding J (Unpublished Master's thesis, 2007)	SD Rats (Male/ female, 10/10)	180–240 g	Permanent MCAO	10%chloralhydrate (300 mg/kg)	Acupuncture 30 min after occlusion for 5 days, continuous wave of 100 r/min of frequency and current density of 1 mA.,	1.NFS (Bederson,5 d) 2.t-PA,PAI-1 3.D-dimer,Flb	1. $p < 0.01$ 2.p < 0.01 3. $p < 0.01$
Zhang 2007[60]	SD Rats (Male,15/5)	230–280 g	MCAO/2 h (ZL)	1.5%Pentobarbital sodium (30 mg/kg)	Acupuncture 60 min before reperfusion, continuous wave of 2 Hz of frequency and current density of 3.5 mA.	1.Infarct volume/total volume(2 h) 2. Pathomorphism	1. $p < 0.01$ 2. NR
Ma 2009[61]	SD Rats (Male,10/10)	200 ± 20 g	permanent MCAO (Tamura),	100 mg/L chloralhydrate (0.35 ml/kg)	Acupuncture 20 min after occlusion 24 h, for 7 days: Baihui rotating needle 1 min, retention 20 min, Shuigou: rotating needle 1 min, unretention	1.NFS (Bederson, 1 h,7 d) 2. Calbind	1. $p < 0.01$ 2. $p < 0.01$
Zhou 2011[62]	SD Rats (Male,NA)	250 ± 10 g	MCAO/1 h (ZL)	chloralhydrate 400 mg/kg	Acupuncture 30 min after occlusion, 5–20 Hz of frequency and current density of 1–1.2 mA.	1.Blood flow 2.NFS and IV	1. $p < 0.01$ 2. $p < 0.01$
Fu 2011[63]	SD Rats (Male,30/30)	180–200 g	MCAO/1 h (ZL),	10%Chloralhydrate (0.3 mg/100 g)	Acupuncture 30 min after occlusion for 15 days, continuous wave of 2 Hz of frequency and current density of 1 mA.,	1.NFS(Bederson, 1 h, 15 d) 2.ERK	1. $p < 0.05$ 2. $p < 0.01$
Zhang 2006[64]	Wistar rats (male 32/32,)	300 ± 20 g	Permanent MCAO (Koizumi)	10% Chloralhydrate (0.3 ml/100 g)	Acupuncture 30 min after occlusion 6 h for 10days, rotating needles 1 min (200 times/min), repeat twice, retention 30 min(PTP taiyang)	1. NFS (Bederson, 1 d,3 d,5 d,10 d) 2. pathomorphism 3. ICAM-1, ET-1	1.p > 0.05 2.p < 0.05 3.p < 0.05
Li 2007[65]	Wistar rats (male 33/33)	200–280 g	Permanent MCAO	10% Chloralhydrate (35 mg/kg)	Acupuncture 30 min after occlusion 6 h for 28 days (6 days a course of treatment, and 1 day interval),then continue to the next period of treatment; rotating needles quickly 1 min, retention 30 min (PTP qubin)	1. NFS (Bederson, 24 h,7 d, 14 d,28 d) 2.NFmRNA	1.p < 0.05 2.p < 0.05

Table 1 | Continued

Study (years)	Species (Sex,n)	Weight	Model (method)	Anesthetic	Method to acupuncture	Outcome Index (time#)	Intergroup Differences
Sun 2009[66]	SD Rats (Male,16/16)	270–300 g	MCAO/2 h (ZL)	Intubation anesthesia (no mention drug)	Acupuncture 30 min after reperfusion for 7days, rotating needles 5 min, repeated every 5 minutes total 30 minutes, retention 30 min (PTP qubin)	1.physio parameters 2.cerebr blood flow 3. NFS (Ludmila,1 h,7 d) 4.infrarct volume	1.$p > 0.05$ 2.$p > 0.05$ 3. $p < 0.05$ 4. $p < 0.05$
Wang 2009[67]	SD Rats (Male,30/30)	280–300 g	MCAO/2 h (ZL),	NR	Acupuncture 30 min after reperfusion 3 h for twice a day, rotating needles 3 times (200 times per min), 5 min each time, retention 30 min (PTP qubin)	1.NFS (4 h,12 h,1 d,2 d,3 d) 2. NF-kB	1.$p < 0.05$ 2.$p < 0.05$
Zhang 2009[68]	SD Rats (Male,30/30)	200 ± 20 g	MCAO/1 h (ZL)	10% Chloralhydrate (30 mg/kg)	Acupuncture 30 min after reperfusion for 3 day, disperse-dense waves and alternated frequency of 2 Hz and 100 Hz at an intensity of 2 mA (PTP qubin)	1. NFS (ZL,24 h,48 h,72 h) 2. pathomorphism 3.COX-2,NF-kBmRNA 4.COX-2,NF-kB,TGF-β1	1. $p < 0.01$ 2. $p < 0.01$ 3. $p < 0.01$ 4. $p < 0.01$
Inoue 2010[69]	SD Rats (Male,12/11)	NA	MCAO/2 h	thiopental (42 mg/kg)	Acupuncture 10 min after stroke onset, 2.5 Hz the voltage was 3–3.5 V	1.NFS(bederson,1 d, 2 d,3 d) 2. MRI observations,IV	1. $p > 0.05$ 2. NA
	SHR-SP Rats (Male,18/15)				Acupuncture 10 min after referfusion, 2.5 Hz the voltage was 3–3.5 V		1. $p < 0.05$ 2. NA
Zhang 2011[70]	Wistar rats (male 32/32)	300 ± 20 g	Permanent MCAO (Koizumi)	10% Chloralhydrate (30 mg/kg)	Acupuncture 30 min after reperfusion 6 h for 10 day; rotating needles 1 min (200 times/min), repeat twice, retention 30 min, (PTP qubin)	1. NFS (Bederson, 1 d,3 d,5 d,10 d) 2. pathomorphism 3.MMP-9	1.$P < 0.05$ 2.NR 3. $p < 0.05$ (3 d,5 d,10 d)
Wang 2003[71]	Wistar rats (Male, 13/13)	250–280 g	MCAO/1.5 h (Abe),	Diethyl ether induce, oxygen/nitrous oxide/isoflurane (30%/69%/1%) maintain	Acupuncture 30 min after reperfusion interval 1 day, total 30 days, disperse-dense waves of 3/20 Hz of frequency and current density of 3 mA (renzhong,baihui)	1.NFS (ZL,30 d) 2.IV	1. $p < 0.05$ 2.NR
Wang 2005[72]	SD Rats (Male,12/12)	200–220 g	MCAO/1.5 h (Koizumi)	7%Chloralhydrate (1 ml/200 g)	Acupuncture 60 min after occlusion. waves of 5/20 Hz of frequency and current density of 1–4 mA (Renzhong and Baihui)	1. NFS (ZL,24 h) 2. IV 3. SOD.GSH-Px and MDA	1. $p < 0.05$ 2. $p < 0.01$ 3. $p < 0.01$
Zheng 2006[73]	SD Rats (femial,25/25)	230–270 g	MCAO/2 h (Koizumi),	100 g/Lchloralhydrate (300 mg/Kg)	Acupuncture 30 min after occlusion 10 min; stimulated with 6 mA of density and frequency of 7 Hz (fengfu,baihui)	1. IV (2 h TTC) 2. IL-1β	1. $p < 0.05$ 2. $p < 0.05$
Li 2007[74]	SD Rats (Male, 6/6)	270–31 g	Permanent MCAO	10%Chloralhydrate (0.4–0.5 ml/10 g)	Acupuncture 30 min after occlusion interval 12 h, total 6 times, disperse-dense waves of 2/30 Hz of frequency and current density of 2 mA (renzhong,baihui)	1.NFS (SJF,12 h,24 h,48 h, 72 h) 2. IV	1. $p < 0.01$ 2. $p < 0.01$
Wang EL (Unpublished PhD thesis, 2005)	SD Rats (Male, 8/8)	270–320 g	MCAO/2 h (ZL),	10%Chloralhydrate (330 mg/kg)	Acupuncture 21 min before operation and after reperfusion for 3 days and 30 min before sacrifice, rotating needles 3 min retention 15 min, rotating again (baihui, qubin)	1.NFS (Bederson,3 h, 1 d,3 d) 2. IV 3. pathomorphism	1. $p < 0.01$ 2. $p < 0.01$ 3.NR
Peng 2009[75]	SD Rats (Male, 18/9)	215–230 g	MCAO/1 h (ZL)	10%Chloralhydrate (0.36 ml/100 g)	Acupuncture 30 min after occlusion 5 min for 3 days, disperse-dense waves of 3.85 Hz/6.25 Hz (1.28 s/2.08 s) of frequency and current density of 0.8–1.0 mA (renzhong,baihui)	1.NFS (ZL,6 h,12 d,1 d, 2 d,3 d,) 2.AQP4 3.AQP4mRNA	1. $p < 0.01$ 2. NR 3. $p < 0.01$

Table 1 | Continued

Study (years)	Species (Sex,n)	Weight	Model (method)	Anesthetic	Method to acupuncture	Outcome Index (time#)	Intergroup Differences
Peng 2010[76]	SD Rats (Male, 6/6)	215–230 g	MCAO/1 h (ZL)	10%Chloralhydrate (3.6 ml/kg)	Acupuncture 30 min after occlusion 5 min for 3 days, disperse-dense waves of 3.85 Hz/6.25 Hz (1.28 s/2.08 s) of frequency and current density of 0.8–1.0 mA (renzhong,baihui)	1. NFS (ZL,24 h) 2. IV	1. $p < 0.01$ 2. $p < 0.01$
Wu HY (Unpublished PhD thesis, 2010)	SD Rats (Male,16/16)	200–250 g	Permanent MCAO	10% Chloralhydrate (300 mg/kg)	Acupuncture 30 min after occlusion 1 h for 7 days, disperse-dense waves of 2–15 Hz of frequency and current density of 1 mA (baihui, qianding, shuaigu, xuanli)	1. NFS (ZL,1 h,7 d,) 2. Nestin 3. GFAP mRNA 4. NSEmRNA	1. $p < 0.01$ 2. $p < 0.01$ 3. $p < 0.01$ 4. $p < 0.01$
Chen 2011[77]	SD Rats (Male,10/10)	230–250 g	Permanent MCAO	10% Chloral hydrate (30 mg/100 g)	Acupuncture 30 min after occlusion 5 h repeated in every 12 h interval, total 6 times, rotating needles 1 min, retention 30 min (dazhui,renzhong,baihui)	1. NFS (ZL,2 h,1 d,3 d) 2. IV	1. $p < 0.01$ 2. $p < 0.05$
Ma 2011[78]	SD Rats (Male,25/25)	180–250 g	MCAO/2 h (ZL)	NR	Acupuncture 30 min after reperfusion 90 min for 21days, disperse dense waves of 20–100 Hz of frequency and the intensity of stimulus was localized muscle contractions were observed (renzhong,baihui)	1.NFS (ZL,1 d,3 d,7 d,14 d, 21 d,) 2.GAP-43 3.GAP-43mRNA	1. $p < 0.05$ 2. $p < 0.01$ 3. $p < 0.01$
Tang 2011[79]	SD Rats (Male/ femial, 12/12)	220 ± 20 g	Permanent MCAO	NR	Acupuncture 30 min after occlusion for 2 weeks, disperse dense waves of 2 Hz of frequency and the intensity of 3–5 mA (fengfu,baihui)	1. NFS (ZL,2 w) 2.CPG15	1. $p < 0.01$ 2. $p < 0.01$

Note: 5-HT: 5- serotonin; a7nAChR: a 7 nicotinic acetylcholine receptors; Ach: acetylcholine; AQP4: aquaporin4; ATP:Adenosine Triphosphate; Bcl-2: B cell lymphoma-2; BDNF: brain derived neurotrophic factor; Ca^{2+}: calcium ion; CBF: cerebral blood flow; COX-2: cyclooxygenase 2; CPG15: candidate plasticity gene 15; CX43; connexin-43; DA: dopamine; d: day; åPKC: epsilon protein kinase C; EAAT2:Excitatory amino acid transporters-2; eNOS:endothelial nitric oxide synthase; EPSP: excitatory postsynaptic potential; ERK: extracellular signal-regulated kinases; ET-1: Endothelin-1; GAP-43: Growth associate protein-43; GFAP; Glial fibrillary acidic protein; GSH-Px: glutathione peroxidase; h: hour; HMGB1: high mobility group box 1; ICAM-1: intercellular adhesion molecule-1; IL-1β: intedeukin-Iβ; IV: infarct volume; m: minute; mAChR: muscarinic acetylcholine receptor; MCAO: middle cerebral artery occlusion; MDA: Malondialdehyd; Mg^{2+}: magnesium ion; MMP-9: Matrix etallopeptidase 9; MT:melatonin; NE: Norepinephrine; NF: neurofilament; NFS: neurological function score; NO: Nitric oxide; NR: no report; PAL-1: plasminogen activator inhibitor- 1; PTP: point to point; PS:population spike; TGF: transforming growth factor; rt-PA: recombinant tissue plasminogen activator; SD: Sprague-Dawley; SOD: supemxide dismutas; SSP: Synaptic Structural Parameters; time#: indicate the time to evaluate the outcomes; VECA: Vascular endothelial cell apoptosis; VEGF: Vascular endothelial growth factor; ZL: Zea Longa.

Table 2 | Risk of bias of included studies

Study	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	Total
Lu2002[34]	√	√	√		√	√					5
Lao2003[35]	√	√	√		√	√					5
Xiong2003[36]	√	√	√		√	√					5
Tian2004[37]	√		√			√					3
Xiong2007[38]	√		√		√	√			√	√	6
Dong2009[39]	√	√	√		√	√				√	6
Du 2010[40]	√	√	√		√	√			√	√	7
Wang2011[41]	√	√	√		√	√			√	√	7
Kim2011[42]	√	√	√		√	√			√	√	7
Wang2012[43]	√	√	√		√	√			√	√	7
Zhao2012[44]	√	√	√		√	√			√	√	7
Kim 2012[45]	√	√	√			√			√	√	6
Ding2004[46]	√		√			√					3
Liu2006[47]	√		√			√					3
Shen2007[48]	√		√			√					3
Li2008[49]	√	√	√			√					4
Xu2009[50]	√	√	√			√			√		5
Mu2009[51]	√		√			√					3
Chen2009[52]	√		√			√					3
Yang2010[53]	√		√			√					3
Cheng2011[54]	√	√	√			√			√		5
Luo2011[55]	√		√			√			√		4
Liu2012[56]	√		√			√					3
Kim 2013[57]	√					√			√	√	4
Chen2000[58]	√		√			√					3
Zhou2003[59]	√	√				√					3
Li JA (Unpublished Master's thesis, 2005)		√	√			√				√	4
Wang C (Unpublished Master's thesis, 2005)		√	√			√				√	4
Jin JF (Unpublished PhD thesis, 2005)		√	√			√				√	4
Luo T (Unpublished Master's thesis, 2006)		√	√			√				√	4
Chen X (Unpublished Master's thesis, 2007)		√	√			√				√	4
Ding J (Unpublished Master's thesis, 2007)			√			√				√	3
Zhang2007[60]	√		√			√					3
Ma2009[61]	√		√			√			√		4
Zhou2011[62]	√	√			√	√				√	5
Fu2011[63]	√		√			√			√		4
Zhang2006[64]	√		√			√					3
Li2007[65]	√		√			√					3
Sun2009[66]	√	√	√			√			√		5
Wang2009[67]	√		√			√					3
Zhang2009[68]	√		√			√			√		4
Inoue 2010[69]	√	√				√	√		√	√	6
Zhang2011[70]	√	√	√		√	√			√		6
Wang2003[71]	√	√	√			√					4
Wang2005[72]	√	√	√			√			√	√	6
Zheng2006[73]	√		√			√			√		4
Li2007[74]	√				√	√			√		4
Wang EL (Unpublished PhD thesis, 2005)			√			√				√	3
Peng2009[75]	√		√		√	√					4
Peng2010[76]	√		√		√	√					4
Wu HY (Unpublished PhD thesis, 2010)		√	√			√			√	√	5
Chen2011[77]	√	√	√			√			√		5
Ma2011[78]	√		√			√					3
Tang2011[79]	√	√	√			√			√		5

Note: Studies fulfilling the criteria of: (1) peer reviewed publication; (2) control of temperature; (3) random allocation to treatment or control; (4) blinded induction of ischemia; (5) blinded assessment of outcome; (6) use of anesthetic without significant intrinsic neuroprotective activity; (7) animal model (aged, diabetic, or hypertensive); (8) sample size calculation; (9) compliance with animal welfare regulations; and (10) statement of potential conflict of interests.

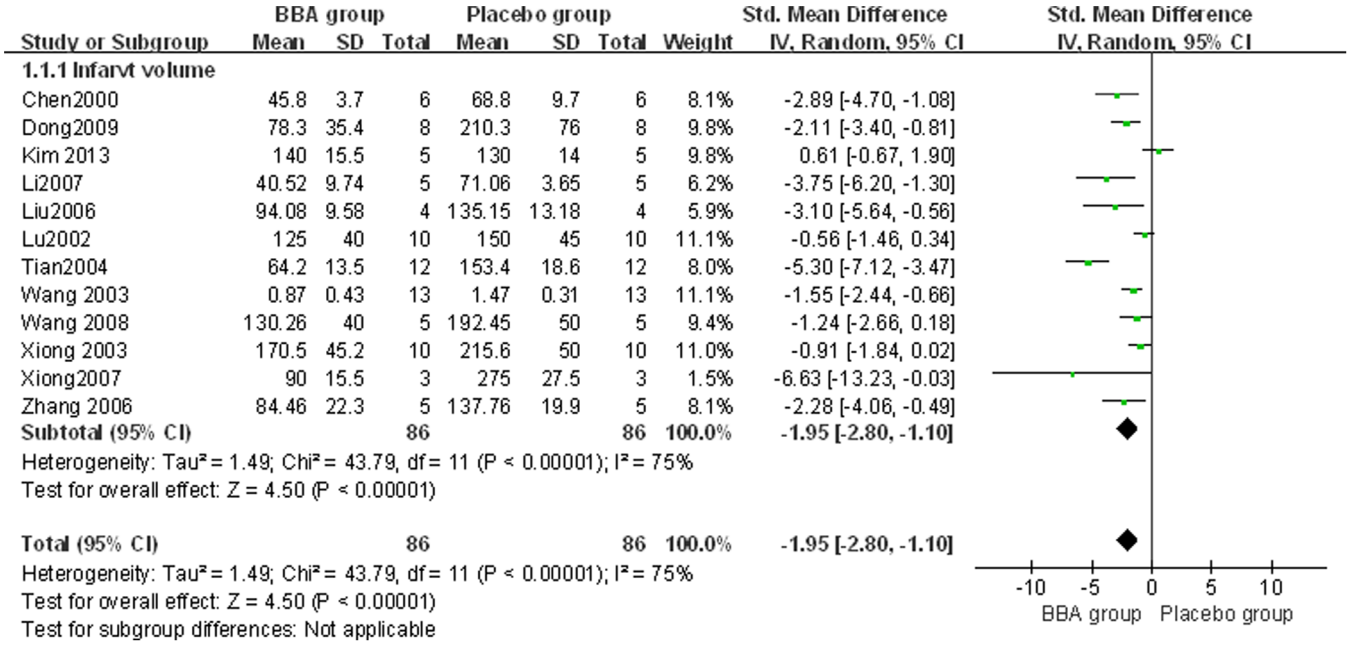

Figure 2 | **Pooled estimate of decrement in infarct volume with Baihui(GV20)-based Scalp acupuncture.**

heterogeneity $\chi 2 = 27.21$, $p = 0.0001$, $I2 = 78\%$, Figure 8C). Treatment in the published studies also was more effective of BBA in the reduction of IV than that in un-published studies (SMD −2.05, 95% CI: −2.99 ~ −1.12, $p < 0.0001$; heterogeneity $\chi 2 = 43.69$, $p < 0.00001$, $I^2 = 77\%$, Figure 8D). These results were consistent with previous subgroup analysis of NFS except time interval from the onset of ischemia.

Discussion

Efficacy of BBA. To our knowledge, this is the first systematic review and meta-analysis of English and Chinese literatures to determine the efficacy of BBA for animal model of acute ischemic stroke with IV and NFS as the outcome measures. The present study indicated that BBA could substantially improve neurobehavioral deficits and reduce infarct size in animal model of focal cerebral ischemia, suggesting that BBA have potential neuroprotective role in acute ischemic stroke.

Methodological considerations. This systematic review has a number of weaknesses. First, our search did not include data in other languages except Chinese and English, which may result in certain degree of selective bias. Second, negative studies are less likely to be published, and some negative results could not be obtained. In the present study, treatment in the published studies was more effective than that in un-published studies. Thus, the effect may be overestimated. Third, methodological quality of the included

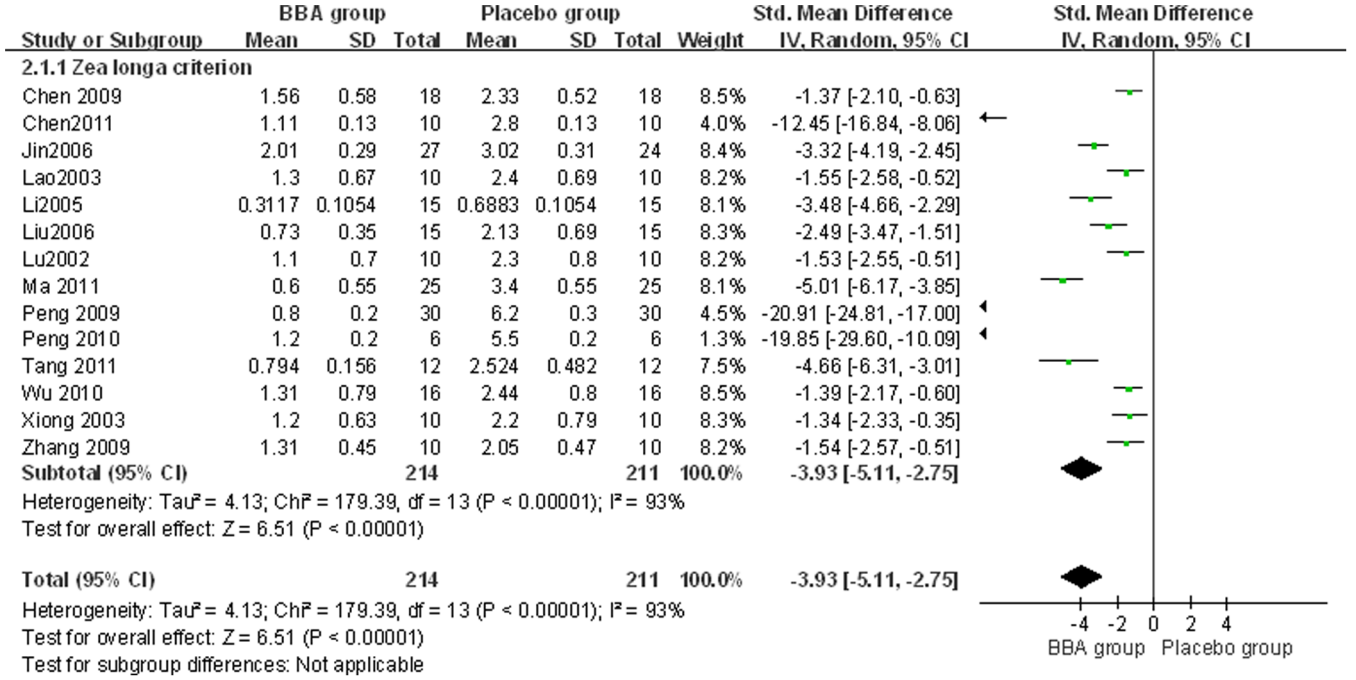

Figure 3 | **Pooled estimate of improvement in neurological function score with Baihui(GV20)-based Scalp acupuncture according to Zea longa criteria.**

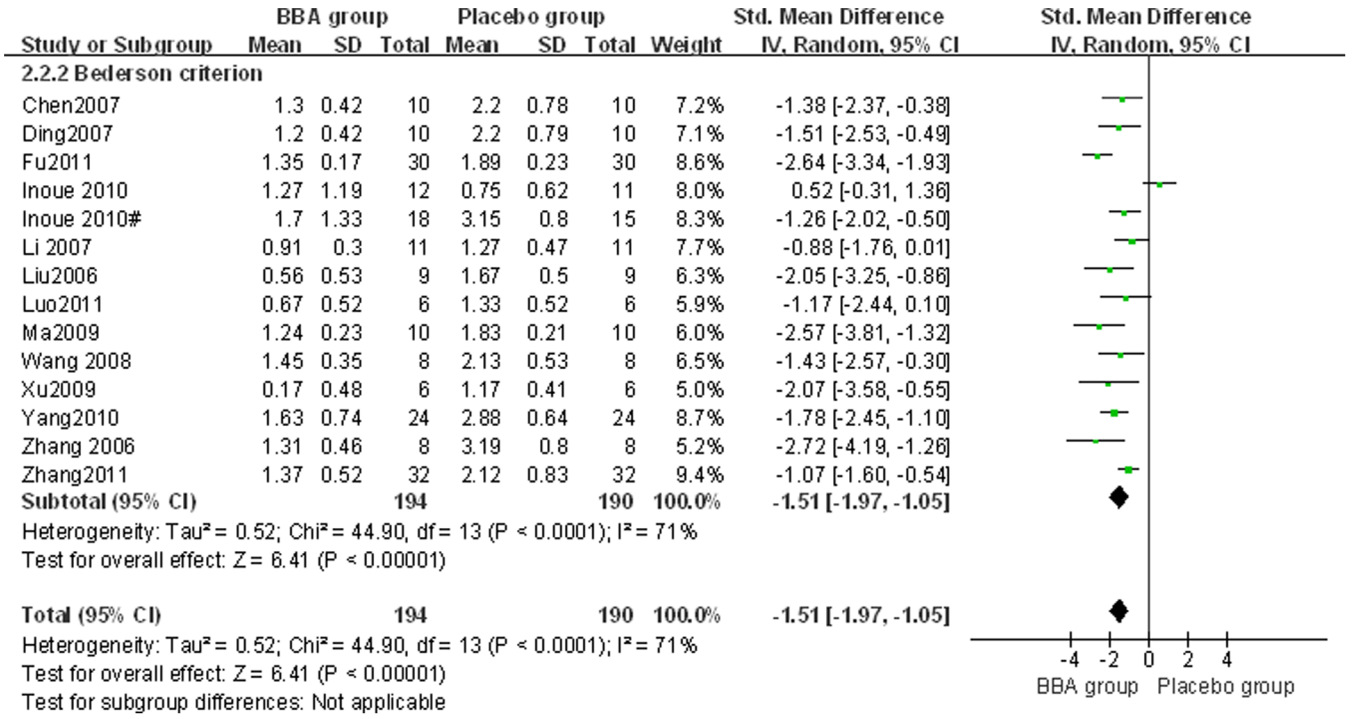

Figure 4 | **Pooled estimate of improvement in neurological function score with Baihui(GV20)-based Scalp acupuncture based on Bederson criteria.**

studies was generally low, which is an inherent limitation in the primary study. Quality of a study has significant impacts on the reported outcome. For example, reporting randomization and blinding are less likely to report positive findings than those neither[84]. Low quality of the included studies suggested that the results should be interpreted with caution.

Implication for further studies. There are various advantages of EA as it is more readily controlled, standardized and objectively measurable than manual acupuncture. In some situations EA was more effective than traditional acupuncture, particularly when strong, continued stimulation is required, as when treating paralysis[85]. EA was also recommended for clinical trials and mechanism researches on acupuncture[86]. In the present study, 46 out of 54 studies performed EA. Thus, EA have priority over Manual acupuncture on acupuncture research for the animal models of stroke.

In animal model of focal cerebral ischemia, the multitudinous pathophysiological processes which are involved in their deleterious effects over different time courses extending from the first hours to several days after vessel occlusion[87]. In the present study, efficacy of BBA was lower in MCAO 3 hours and permanent groups compared with other temporary ischemia groups, thus it might be inferred that BBA could effectively inhibit those pathophysiological pathways preferentially activated by reperfusion. Further studies would be required to evaluate when the optimum time window for BBA treatment would close and to determine the duration of time to achieve maximum efficacy.

According to the effect size, this study indicated that SD rats recovered better than Wistar rats. A hypothesis may arise that individual genetic differences have different neuroprotective role in ischemic stroke. A comparison among anesthetics showed more effectiveness in NFS improvement in studies using chloral hydrate than studies using other two anesthetics. Thus, future study design for animal research need to select suitable anesthetics.

In the present study, most animal models of stroke are established on normotensive animals with occlusion of cerebral artery to artificially induce infarction in brain. The relevance of animal models

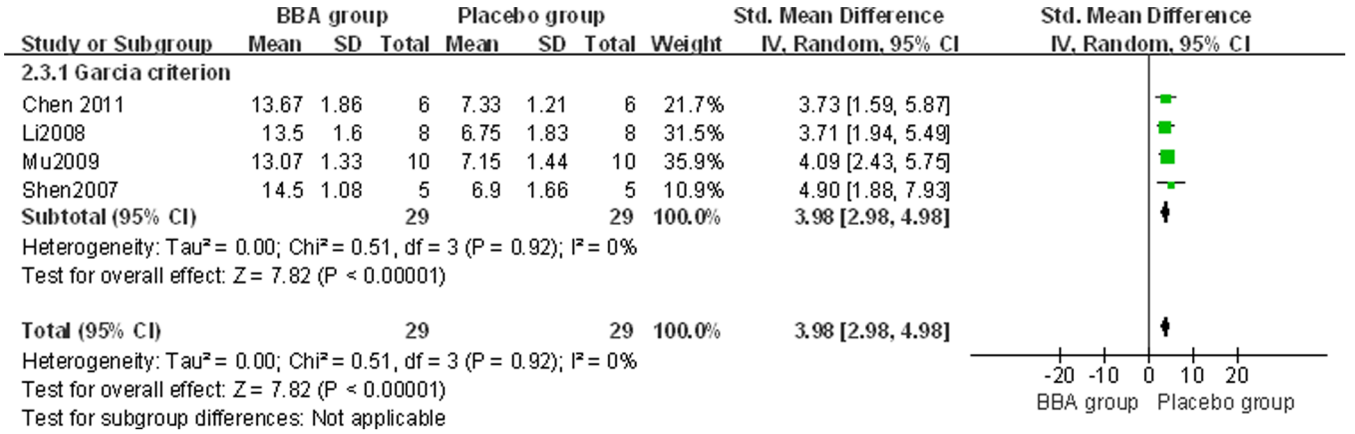

Figure 5 | **Pooled estimate of improvement in neurological function score with Baihui(GV20)-based Scalp acupuncture based on Garcia criteria.**

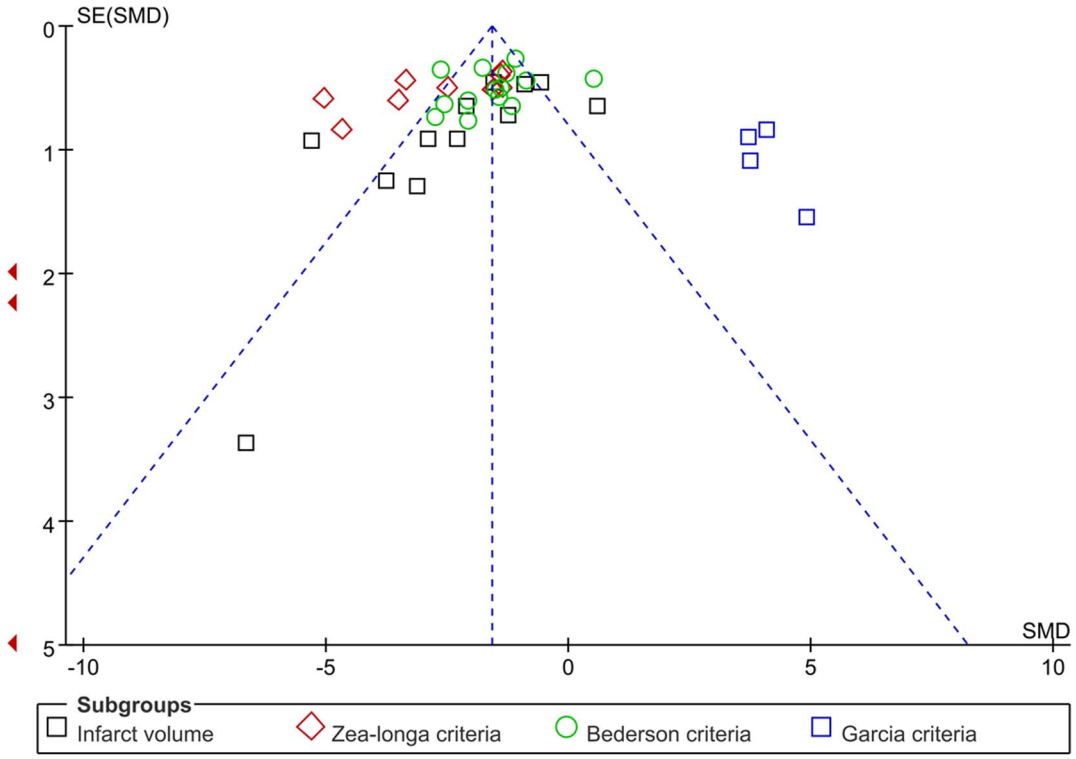

Figure 6 | **Bias assessment plot for the effect of Baihui(GV20)-based Scalp acupuncture on infarct volume and neurological function score.**

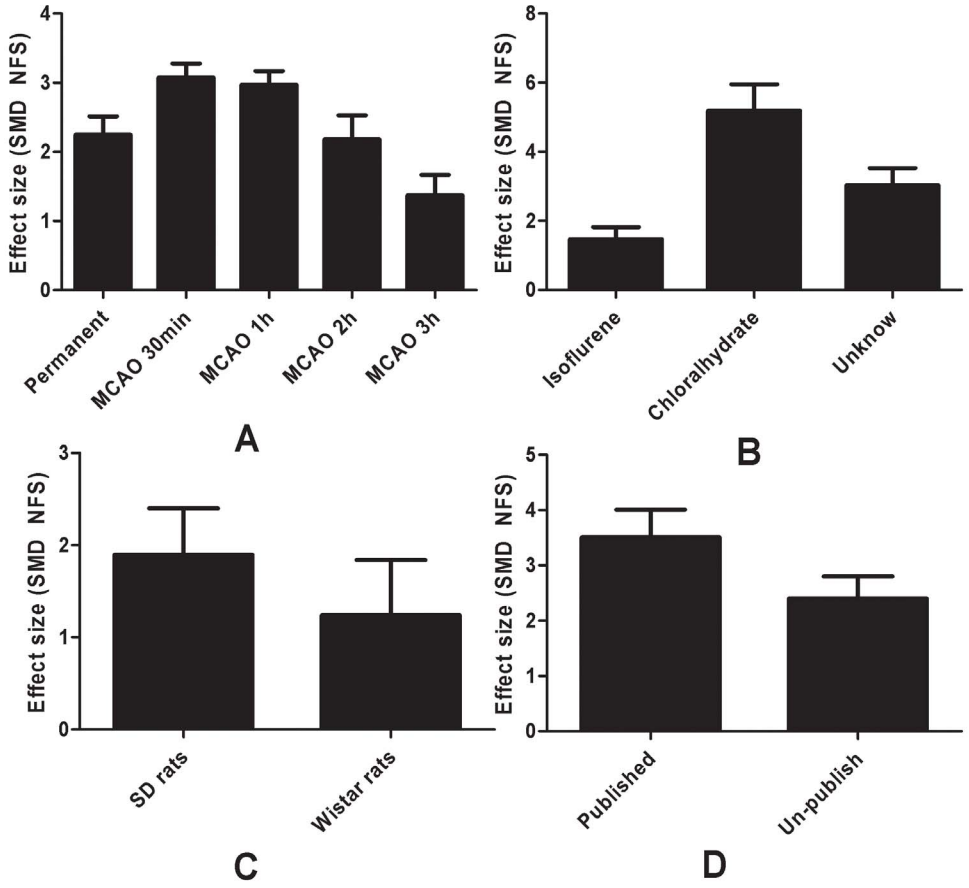

Figure 7 | **Subgroup analysis according to neurological function score (NFS).** (A) The effect of the use of model on the estimate of improvement in NFS outcome; (B) The type of anesthetic on the estimate of improvement in NFS; (C) The type of strain used on the estimate of improvement in NFS; (D)The impact of published studies comparing with un-published studies on the estimate of improvement in NFS outcome. The vertical error bars represent the effect size for the individual estimates.

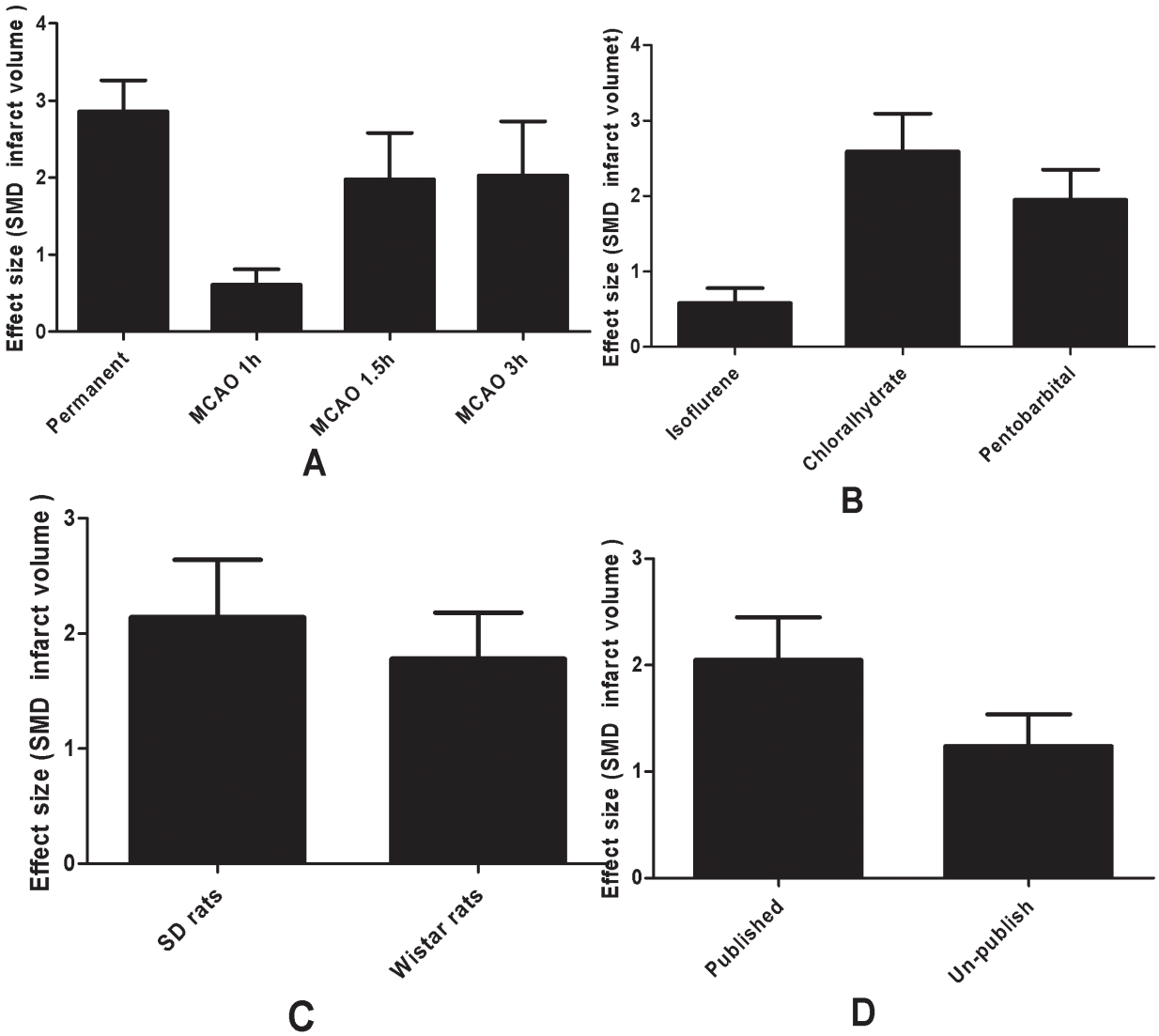

Figure 8 | Subgroup analysis according to infarct volume (IV). (A) The effect of the use of model on the estimate of improvement in IV outcome; (B) The type of anesthetic on the estimate of improvement in IV outcome; (C) The type of strain used on the estimate of improvement in IV outcome; (D)The impact of published studies comparing with un-published studies on the estimate of improvement in IV outcome. The vertical error bars represent the effect size for the individual estimates.

with normal cerebrovascular structure to human conditions remains dubious[88]. Impressively, Scalp acupuncture had a rapid and strong effect on neurological dysfunction only in the hypertensive stroke-model by reducing the vasogenic oedema, but had no significant effects on the cytotoxic oedema, vasogenic oedema or neurological dysfunction of the MCAO rats within the time span examined[89]. Hence, future studies need to investigate EA efficacy in animals with a co-morbidity such as hypertension, diabetes or advanced age.

Conclusion

In animal model of focal cerebral ischemia, BBA could improve IV and NFS. Although some factors such as study quality and possible publication bias may undermine the validity of positive findings, BBA may have potential neuroprotective role in experimental stroke.

Methods

Search strategy. We identified studies of BBA in animal models of acute ischemic stroke from Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, EMBASE, Chinese National Knowledge Infrastructure (CNKI), VIP information database, and Wanfang data Information Site. The publication time is from the inception of each database up to June 2013. The search term used was "Baihui (GV20)" in English or in Chinese. All searches were limited to studies on animals. Reference lists from the included literature were used to identify further relevant publications.

Eligibility. We included all controlled studies of the effect of BBA in animal models of focal cerebral ischaemia, where the outcome was measured as infarct volume (IV) or/and neurological function score (NFS)[27]. IV is an essential indicator of the severity of brain ischemic injury. 2,3,5-triphenyltetrazolium chloride (TTC) staining is an excellent research method that can be used to confirm the size and location of areas of infarction induced by focal cerebral ischemia in rats[28]. Briefly, brain IV was assessed by TTC staining for 10 minutes at 37°C, followed by overnight immersion in 4% paraformaldehyd; unstained areas were defined as IV. NFS can also be useful in animal studies that evaluate the effect of new therapeutic methods; moreover, physical testing of the animals can be repeated over time and thus provide data on the evolution of the neurological deficit. However, measuring methods of NFS were inconsistent in different studies. Three neurological grading systems were most commonly used. The first grading system was published by Bederson et al[29]. in 1986. This system consists of a scale from 0 to 3: (0) no observable deficit; (1) decreased forelimb resistance to a lateral push; (2) forelimb flexion; (3) circling behavior in addition to the former symptoms. The second system was reported by Zea Longa et al[30]. in 1989 . The scale rates the presence or absence of neurological signs in rats, and the details are as follows: 0′-no neurological deficit; 1′-retracts left forepaw when lifted by the tail; 2′-circles to the left; 3′-falls while walking; 4′-does not walk spontaneously; 5′-dead after surgery. The third system was introduced by Garcia et al[31]. in 1995. It consists of 6 different criteria, including spontaneous activity, symmetry in the movement of the 4 limbs, forepaw outstretching, climbing, body proprioception, and response to vibrissae touch. The individual performance in each

test was rated on a 0 to 3 point subscore. The sum of all 6 individual subscores was then calculated to give a range of 3–18. Thus, the score in healthy rats would be 18.

To prevent bias, inclusion criteria were pre-specified as follows: (1) the effect of BBA was tested on an animal model of focal cerebral ischemia induced by temporary middle cerebral artery occlusion (MCAO) or permanent MCAO; (2) IV and/or NFS were compared with control animals receiving vehicle or no treatment. Pre-specified exclusion criteria were: (1) non-focal cerebral ischemia model (such as global, traumatic models, or hypoxic-ischemic models); (2) combined use of BBA and body acupuncture or ear acupuncture or any other agent with potentially neuroprotective effects; (3) no control group; (4) duplicate publications.

Data extraction. The following details were extracted from each study: (1) publication year and the first author's name, model of ischemic stroke (transient, or permanent), and ischemic time; (2) individual data were obtained for each study, including aimal number, species, sex, weight, motor impairment and scale; (3) information on treatment was obtained, including timing for initial treatment, types and method of treatment procedure, and duration of treatment; (4) outcome measures and timing for outcomes assessments were also included. IV and/or NFS were especially extracted separately. If outcomes were presented from the studies of animals at different time points, we extracted data from the last time point. If the data for meta-analysis were missing or only expressed graphically, we tried to contact the authors for further information, or calculate by ourselves if the information needed were available, or excluded the study which we could not get enough information. For each comparison, we extracted data of mean value and standard deviation from each treatment and control group of every study.

Quality assessment. We evaluated the methodological quality of the included studies by applying a ten-item modified scale[32]: (1) publication in a peer-reviewed journal; (2) statements describing control of temperature; (3) randomization to treatment group; (4) allocation concealment; (5)blinded assessment of outcome; (6) avoidance of anesthetics with known marked intrinsic neuroprotective properties; (7) use of animals with relevant comorbidities; (8) sample size calculation; (9) compliance with animal welfare regulations; (10) declared any potential conflict of interest. For the calculation of an aggregate quality score, each item of the ten-item modified scale was attributed one point. Two authors (WWW, XCL) independently extracted data and assessed study quality. Disagreements were solved after discussion on the details of the studies.

Statistical analysis. We considered all NFS and IV as continuous data, and then an estimate of the combined effect sizes utilizing standard mean difference (SMD) with the random effects model was given. We used the random effects model rather than the fixed effects model because heterogeneity between multi-studies has to be taken into account. Publication bias was assessed with a funnel plot[33]. For the assessment of heterogeneity, the I^2 statistic was used.

Furthermore, to explore the impact of factors modifying on the outcome measures, we performed a stratified meta-analysis with experiments grouped according to the following: duration from onset of ischemia to treatment, anaesthetic method used, the experimental data formally published or not, and species of animals used. Difference between n groups was assessed by partitioning heterogeneity and using the $\chi 2$ distribution with n-1 degrees of freedom (df), where n equals the number of groups. All analysises were performed with Revman version 5.1. Probability value $p < 0.05$ were considered significant.

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Author contributions

All authors have contributed to this article. W.W.W., C.L.X. and G.Q.Z. searched the databases, extracted the data, and screened trials. W.W.W., C.L.X. and L.L. reformed the tables. G.Q.Z., W.W.W. and C.L.X. appraised the quality of included trials and drafted the full text. G.Q.Z. and L.L. were responsible for editing. G.Q.Z. also acted as an arbitrator and conceived the article. All authors reviewed the manuscript.

Additional information

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