A META-ANALYSIS OF REMOTE ISCHAEMIC CONDITIONING IN EXPERIMENTAL STROKE

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ABSTRACT

Remote ischaemic conditioning (RIC) is achieved by repeated transient ischaemia of a distant organ/limb and is neuroprotective in experimental ischaemic stroke. However, the optimal time and methods of administration are unclear. Systematic review identified relevant preclinical studies; two authors independently extracted data on infarct volume, neurological deficit, RIC method (administration time, site, cycle number, length of limb occlusion (dose)), species and quality. Data were analysed using random effects models; results expressed as standardised mean difference (SMD). In 57 publications incorporating 99 experiments (1406 rats, 101 mice, 14 monkeys), RIC reduced lesion volume in transient (SMD -2.0; 95%CI -2.38, -1.61; p<0.00001) and permanent (SMD -1.54; 95% CI -2.38, -1.61; p<0.00001) focal models of ischaemia; and improved neurological deficit (SMD -1.63; 95%CI -1.97, -1.29, p<0.00001). In meta-regression, cycle length and number, dose and limb number did not interact with infarct volume, although country and physiological monitoring during anaesthesia did. In all studies, RIC was ineffective if the dose was <10 or ≥50 minutes. Median study quality was 7 (range 4-9/10); Egger's test suggested publication bias (p<0.001). RIC is most effective in experimental stroke using a dose between 10 and 45 minutes. Further studies using repeated dosing in animals with co-morbidities are warranted.

Key words

Ischemic stroke, pre-clinical, meta-analysis, remote ischemic conditioning, stroke

INTRODUCTION

The paradigm of ischaemic conditioning conferring organ protection from a subsequent or ongoing ischaemic insult has been under investigation since the 1980s [1] but its apparent pre-clinical benefit has yet to be translated consistently in randomised controlled trials. The potential to induce ischaemic tolerance in distant tissue beds by remote, transient, non-lethal limb ischaemia (remote ischaemic conditioning, RIC) is an attractive therapeutic strategy in terms of cost and ease of intervention delivery, performed simply by inflating a blood pressure cuff on an arm or leg.

Applying RIC before, during or after an ischaemic event (pre-conditioning [RIPreC], per-conditioning [RIPerC] or post-conditioning [RIPostC]) shows promise in multiple vascular diseases.[2-4] However, although early trials of RIC prior to coronary artery bypass grafting demonstrated a reduction in peri-operative myocardial injury, larger phase III trials were neutral in improving long term outcomes,[5, 6] which is potentially explained by interactions with cardioprotective anaesthetic agents.[7] In the setting of protecting the brain from injury with RIC, multiple neuro-humoral mechanism are implicated (see [8]), but human clinical evidence is limited. In a large meta-analysis of randomised trials of ischaemic conditioning in all conditions, the risk of recurrent stroke was significantly reduced, though the evidence is of low quality.[9] Further, early proof-of-concept human trials assessing RIC in acute stroke (RIPerC and RIPostC),[3, 10, 11] intracranial stenosis (RIPostC) [12, 13] and carotid stenting (RIPreC) have commenced.[14]

Despite the move into human trials, there are a number of unanswered questions regarding the application of RIC, namely optimal method (e.g. one versus two limbs), dose (number and length of cycles of limb ischaemia and reperfusion), and timing of intervention. We therefore systematically reviewed and meta-analysed the accumulating

pre-clinical evidence in acute stroke models of RIPreC, RIPerC and RIPostC to help provide further insight and inform future work.

METHODS

The systematic review was performed in accordance with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[15] The protocol was registered with PROSPERO, reference CRD42018095739. Preclinical (non-human) studies evaluating the effects of RIC in animal models with induced focal ischaemic stroke were searched up to December 2019 in Embase, Medline, Pubmed and Web of Science. Two authors independently performed the search and acquired the data. Search key words included: (*stroke or cerebrovascular disease or brain infarction or brain ischemia or carotid artery disease or cerebral artery disease or cerebrovascular accident or (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or CVA))) AND (remote isch?emic conditioning or (remote adj3 (preconditioning or perconditioning or postconditioning)) or RIC or RIPerC or RIPostC or RIP or RIPC or RPC or IPerC or rIPC).*

The identified abstracts and titles of the studies were checked and removed if they were not relevant to the study. If only the abstract of a study was available, it was excluded. The studies were included if the following criteria were met: (i) there was a control group; (ii) the study was completed in nonhuman subjects; (iii) a focal ischaemic stroke, not global; (iv) treatment was given in acute models (within 7 days), not chronic; (v) RIC was the only treatment administered, not in conjunction with other treatments; (vi) RIC must be administered before, during or after the onset of an ischaemic stroke; (vii) there were measures on infarct size or neurological score; (viii) data was from original articles not review articles.

Risk of selection, performance, detection, attrition and reporting bias was assessed using SYRCLE's risk of bias tool.[16]

Data Acquisition

The number of animals, mean outcome, standard deviation or standard error of the mean were collected for control and treatment groups. Studies providing summary data on the infarct size as a volume or area (mm^3 or as a percentage [%] of the whole brain size) and neurological score were gathered from all included papers along with species, gender, stroke model and quality. If data was not written, published graphs were enlarged and the position of the data points determined using *Grab* software (version 1.10) on Apple Mac. If studies conducted more than one experiment against a single control, the number of animals in the control group was divided by the number of comparison groups (to prevent double counting control animals). Data were independently extracted by three authors (PW, RM and TE).

The time of first dose was recorded relative to the time of ischaemia onset and not the time of reperfusion. For example, if RIC commenced 10 minutes after reperfusion in a transient model of 120 minutes middle cerebral artery occlusion (MCAo), a time of 130 minutes was recorded.[17] Time of treatment was categorised as either RIPreC (treatment started before ischaemia), RIPerC (after ischaemia onset but before reperfusion) or RIPostC (started after reperfusion). It was not possible to consistently separate RIPerC and RIPostC groups and these were combined to form one group.

Study Quality

The quality of the article from included studies was assessed using the scoring system recommended by CAMARADES (range 1-10).[18, 19] A point was awarded to the study if it met the following criteria: (i) peer-reviewed publication (ii) statement of control of

temperature, (iii) random allocation to treatment or control, (iv) blinded induction of ischaemia, (v) blinded assessment of outcome, (vi) use of anaesthetic without significant intrinsic neuroprotective activity, (vii) appropriate animal model (transient, permanent, embolic or photothrombotic models), (viii) sample size calculation, (ix) compliance with animal welfare regulation, (x) statement of potential conflicts of interest. Further, assessment of data quality was determined by the presence or absence of physiological monitoring during anaesthesia, including blood glucose, blood gas, cerebral blood flow and blood pressure.

Data Analysis

The data was analysed using Cochrane Review Manager (version 5.3, Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) according to pre-specified subgroups: species, model of ischaemia (permanent versus transient), time of administration (RIPreC vs RIPerC and RIPostC), dose (number, length and total length of cycles) and study quality. Continuous data is presented as a standardised mean difference with 95% confidence intervals. Statistical significance was set at $p<0.05$. Egger's statistic and meta-regression of subgroups was performed using Stata/SE (version 15.1 for Mac). Data reliability was assessed through sensitivity analyses by re-analysing the dose-response relationship in all studies that provided a statement of physiological monitoring during anaesthesia.

RESULTS

Study characteristics

The primary search for studies on Medline, Pubmed, Embase and Web of Science identified 804 studies (Supplementary Figure I). After the exclusion criteria were applied, 57 studies remained and were used in the meta-analysis. Studies were conducted across seven countries (Canada [n=1], China [43], Italy [1], Japan [1], Russia [2], Slovak Republic [2] and USA [7]) across 41 laboratories. In 99 experimental paradigms including 1521 animals, RIPreC, RIPerC and RIPostC significantly reduced infarct volume, SMD -1.87 (95% CI, -2.18, -1.56), which was equivalent to a 34% reduction (weighted by number of animals per study) compared to control.

Eighty of the 99 experiments used Sprague-Dawley rats (n=1311 animals), five tested Wistar rats (n=50), two examined outbred rats (n=43), eight studied C57BL/6 mice (n=89), one studied CD1 mice (n=12) and only three used primates (n=14) (Supplementary Table I). The majority of publications (n=81 experiments) induced transient focal cerebral ischaemia ranging from 10 to 120 minutes of MCAo. In three studies,[20-22] ischaemia was induced by embolic MCAo and three studies used permanent models of middle cerebral artery occlusion.[23-25] RIC was mostly administered by occlusion of the femoral arteries or hind limbs (Supplementary Table I), however, in one study RIC was achieved through infrarenal aortic occlusion (categorised as bilateral limb occlusion) [26] and another study occluded the unilateral renal artery.[27] The administration and frequency of RIC varied between studies and therefore allowed a comparison between different times of administration and the number and length of cycles. Timing of treatment was not clear in two of the experiments, which were consequently excluded from time-to-treatment analyses.[28, 29]

All studies

RIC was significantly effective in both RIPreC and RIPerC/RIPostC models (Table 1, Figures I and II), the greatest magnitude in the latter, though there was no interaction with infarct volume when the two groups were analysed in meta-regression (SMD [95% CI]: RIPreC -1.54 [-2.07, -1.01] versus -2.0 [-2.38, -1.61,], p=0.368). Notably, there was significant statistical heterogeneity: I^2 = 71% in RIPreC studies and 80% in per/post conditioning experiments. Efficacy was evident in both transient and permanent stroke models though much fewer animals were assessed with permanent ischaemia (n=140). RIC was not effective if the length of each cycle was less than 5 minutes, or if the total length of limb ischaemia was less than 10 minutes (Table 1). RIC also improved neurological function significantly (SMD -1.63 [-1.97, -1.29], p<0.00001) in studies using the Garcia 18-point scale (by 2.5 points, p=0.002), Longa 5-point scale (0.9 points), focal neurological score (9.7 points), the 12-point scale (1.4 points) and the Spetzler motor score in monkeys (1.5 points); but not in studies using the Neurological Severity Score or the 3-point scale (Table 1).

Pre-conditioning (RIPreC)

We assessed protocol variables against infarct volume change in the RIPreC studies using meta-regression. There was a significant interaction caused by species, with RIC effective in rats but not mice (p=0.01, Figure 3A). RIC cycle length (up to 15 minutes) and total length of limb occlusion ($\geq$30 minutes and up to 45 minutes) significantly reduced infarct volume, with longer periods of cycle length and total limb ischaemia leading to greater falls in infarct volume (although there was no significant interaction with meta-regression p=0.115 and 0.102 respectively, Figure 3A). Using either one or two limbs to administer RIPreC reduced infarct volume, but using one limb was not significantly better than two limbs (SMD -2.00 [-2.76, -1.24] versus -0.72 [-1.29, -0.16], p=0.134).

Per- and post-conditioning (RIPerC and RIPostC)

We assessed protocol variables against infarct volume change in the RIPerC and RIPostC studies using meta-regression (Figure 3B). Infarct volume was significantly decreased in all species except Rhesus monkeys; reduced in transient and permanent models; with one, two, three or four cycles of RIC; total length of limb occlusion was effective ≥10minutes but not at 50 minutes; and using one or two limbs. There was no interaction with species, model type, cycle number or length, and total length of limb occlusion. Using two limbs might be more effective than one (SMD -2.53 [-3.07, -1.99] versus -1.33 [-1.78, -0.89]) but the use of four limbs was worse than both meaning there was no significant interaction with limb number (p=0.182).

Study quality and risk of bias

Quality of study median score was 7 (range 4 to 9, Supplementary Table I and II). The study quality score did not impact on infarct volume estimate (meta-regression p=0.495, Figure 4a). Median risk of bias score was 7 and ranged from 5 to 10 (Supplementary Table I and III); the score also did not influence infarct volume (p=0.672). The cohort of studies were of reasonable quality with 68% of publications giving statements on randomisation and blinded assessment of outcome, but only 6% using a sample size calculation and 57% provided a statement on conflicts of interest. Further, statements of physiological monitoring during anaesthesia were limited: blood pressure 25%, blood gas 19%, blood glucose 5% and regional CBF 40%. Monitoring of blood glucose and CBF interacted significantly with the infarct volume outcomes (p=0.047 and p=0.032 respectively), in that those studies which monitored glucose and CBF demonstrated greater reductions in infarct volume (Figure 4b).

In sensitivity analyses (dose-response by studies with statements of physiological monitoring, n=57 of 99 experiments), an effective dose range remained between 15 and 45 minutes of total limb occlusion time using two, three or four cycles of RIC (Supplementary Figure II). Analysing for further sources of statistical heterogeneity determined that there was no interaction by laboratory, but there was by country, with greatest efficacy seen in studies from China (Supplementary Figure III). Begg's funnel plot (Figure 5) indicates an asymmetry in published studies, i.e. the possibility of missing data due to a publication bias (Egger's statistic $p<0.001$).

DISCUSSION

This comprehensive systematic review and meta-analysis of 1521 animals has confirmed the potent effect of remote ischaemic conditioning in improving infarct volume and neurological outcome in pre-clinical stroke models when applied before the insult (RIPreC) or during and after the stroke (RIPerC and RIPostC). In all studies, the average reduction in infarct volume in RIC groups compared to control was 34% and appeared to be efficacious in RIPerC/RIPostC studies more than RIPreC, in both rats and mice, in transient and permanent ischaemia, using one or two limbs and using a total length of limb ischaemia $\geq$ 10 minutes. Total length of limb ischaemia for greater than 50 minutes was ineffective with an optimal period between 15 and 45 minutes.

Both RIPreC and RIPerC/RIPostC groups demonstrated significant statistical heterogeneity and our pre-specified subgroup analyses in both experimental paradigms helped to explore the reasons for this. In RIPreC experiments, there was a significant interaction with species, suggesting that RIPreC was ineffective in mice; RIPerC/RIPostC was equally effective in rats and mice, however. This raises concern of treatment failure when moving into human clinical trials due to inter-species differences. There was only one study assessing larger gyrencephalic species, a recent study assessing the effects of RIPerC on stroke related cardiac dysfunction in rhesus monkeys (5 control, 9 RIC);[22] multi-limb RIPC improved motor neurological scores (in addition to reducing cardiac enzymes, von-Willebrand factor and C-reactive protein) without affecting cerebral infarct volume, suggesting improvements might be mediated through improving endothelial injury and anti-inflammatory mechanisms. This study is confounded by a small sample size and the use of Propofol during anaesthesia, which interferes with RIC efficacy and a factor that may have contributed to the neutral findings in prior cardiac bypass surgery RIC trials.[7] Clinical trials assessing RIC in mechanical thrombectomy in hyperacute stroke (often using general anaesthesia) need to factor this into their design. Other trials

of RIC in cerebrovascular disease are underway and small trials have been completed.[30] Interpreting results will, however, be challenging since they are fraught with heterogeneity in terms of RIC protocols and stroke subtype assessed.

RIPreC studies showed no significant interaction with the total length of limb occlusion (a product of cycle number and length of each cycle, reflecting the 'dose' of RIC), though only doses greater than 25 minutes reduced infarct volume significantly. Doses above 45 minutes were not tested in the RIPreC group but were ineffective in the RIPerC/RIPostC group suggesting the presence of a therapeutic window. A higher dose may also be reflected by the number of limbs used to administer RIC, but here we obtained no statistical interaction with the number of limbs used in either of the subgroups. We did not find any differences in the number of cycles used nor in the length per cycle, though analysis of all studies revealed the therapeutic window of total dose to be between 15 and 45 minutes. Whether repeated dosing provides additional benefit remains largely untested except in two studies, where RIPostC for up to 14 days was more effective at improving outcome than a single per-conditioning dose.[31, 32] Interestingly, delayed daily RIPostC, started at day five, using three cycles of 10 minutes of limb ischaemia, improved neurological function and brain injury (without impacting on early lesion volume) through pleiotropic effects such as angioneurogenesis and modulation of the inflammatory response.[32]

The mechanisms of RIC are still under exploration, appear to be multi-modal, and not fully dependent on achieving reperfusion, which is important since only 50% of strokes achieve early recanalization after iv thrombolysis.[33] Beneficial mechanisms, in addition to attenuation of reperfusion injury, include anti-inflammatory,[34] anti-oedema,[35, 36] angioneurogenic,[31, 32, 37] anti-platelet,[38] and vasodilatory (enhancement of collateral microvascular circulation) effects,[39] mediated through numerous neurohumoral chemical

messengers,[8] including release from endothelial derived exosomes.[40] This meta-analysis confirms that RIC reduces infarct volume in both transient ischaemic models (standardized mean difference, SMD 1.93, $p<0.0001$) and permanent models (SMD 1.59, $p<0.001$), suggesting that reperfusion is not necessary, though desirable, for RIC to achieve beneficial effects.

The majority of papers is this review utilise young male rodents with a notable absence of animals with co-morbidities such as age, hypertension and diabetes, factors which may inhibit the effects of RIC.[41] Of some concern is the absence of effect seen in an aged model of right MCAO occlusion treated with 'direct' ischaemic conditioning (not remote).[42] Studies are present testing RIC efficacy in female rodents (n=46) [40, 43, 44] which is important to examine considering the neuroprotective effects and potential interaction of female hormones.[45] In post-hoc analyses, RIC studies in female rodents reduce infarct volume to a similar extent to that seen in all studies (SMD -1.76 95% CI -3.07 to -0.45, $p=0.009$, excluding Xiao 2017 which contains both male and female rats). Other experimental paradigms important for translation into human trials have also been tested including co-administration of thrombolysis (a synergistic effect),[43, 46], use in large animals,[22] experiments specifically designed to address dose response [47] and the time-window of administration. [32]

The risk of bias in our findings exists considering the presence of significant statistical heterogeneity. This does not appear to be explained by differences in study quality (CAMARADES criteria) or risk of bias (SYRCLE criteria).[48] Indeed, reporting of randomisation and blinding of outcome assessments were moderately high (68%) but, disappointingly, the use of sample size calculations (6%) is lacking despite calls to include these in animal study design.[49] Sources of heterogeneity were significant for the presence/absence of physiological monitoring of CBF and glucose, and also the country

in which the experiment was performed (but not the laboratory). Somewhat reassuringly, in sensitivity analyses, an effective RIC dose range between 15 and 45 minutes remained. The presence of significant publication bias also raises concern, theoretically leading to an under- or over-estimation of effects due to unpublished neutral or negative data. It is also feasible that we missed publications in our literature search but this was comprehensively performed independently by two authors. Overall, however, this is a robust and comprehensive review of the current literature strengthened by pre-registration and pre-specified analyses.

In summary, RIC significantly reduces lesion volume and neurological impairment in experimental models of focal ischaemic stroke. Statistical heterogeneity may be explained by RIPreC cycle length, dose and number of limbs; monitoring CBF and glucose during anaesthesia; and country in which the experiment was conducted. Dose analyses suggests a therapeutic window of between 10 and 45 minutes in RIPerC and RIPostC models. The presence of publication bias raises the possibility that neutral/negative studies have been performed but not published. Pre-clinical studies in animals with co-morbidities using protocols with repeated dosing that would be deemed feasible in humans are warranted.

Conflicts of interests

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Data Sharing

Data will be shared on reasonable request through direct contact with the corresponding author.

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Table 1: The effect of administration time, species, stroke model, and RIC administration method on (A) infarct volume in all preclinical studies of remote ischaemic pre- per- and post-conditioning, compared to control; and (B) the effect of remote ischaemic pre- per- and post-conditioning, compared to control, on neurological score. P-values compare the RIC group to control.

	No. of experiments	No. of animals	SMD [95% CI]	P-value
A. Lesion Volume				
Time of Administration				
Remote Ischaemic Pre-Conditioning	27	361	-1.54 [-2.07, -1.01]	<0.00001
Remote Ischaemic Per/Post-Conditioning	72	1160	-2.00 [-2.38, -1.61]	<0.00001
Species				
Sprague-Dawley Rats	80	1313	-2.13 [-2.50, -1.77]	<0.00001
Wistar Rats	5	50	-1.02 [-1.88, -0.16]	0.02
Outbred Rats	2	43	-0.36 [-1.15, 0.43]	0.38
C57BL/6 Mice	8	89	-1.07 [-1.82, -0.32]	0.005
CD1 Mice	1	12	-4.11 [-6.41, -1.81]	0.0005
Rhesus Monkey	3	14	0.16 [-0.97, 1.30]	0.78
Stroke Model				
Permanent ischaemia	13	140	-1.59 [-2.34, -0.84]	<0.0001
Transient ischaemia - All	81	1339	-1.97 [-2.32, -1.63]	<0.00001
30 minute ischaemic model	4	49	-2.93 [-4.75, -1.11]	0.002
45 minute ischaemic model	4	30	-0.03 [-0.97, 0.90]	0.95
60 minute ischaemic model	7	109	-1.12 [-1.81, -0.43]	0.0003
90 minute ischaemic model	31	505	-2.67 [-3.36, -1.99]	<0.00001
100 minute ischaemic model	11	105	-1.02 [-1.72, -0.32]	0.005
120 minute ischaemic model	24	541	-1.98 [-2.53, -1.44]	<0.00001
Number of RIC cycles				
1 cycle	10	95	-1.29 [1.94, -0.63]	0.0001
2 cycles	8	71	-1.20 [-2.02, -0.37]	0.005
3 cycles	62	1114	-2.33 [-2.75, -1.91]	<0.00001
4 cycles	11	159	-1.20 [-1.94, -0.45]	0.002
More than 4 cycles	3	40	-0.51 [-1.10, 0.08]	0.09
Length of each RIC cycle				
< 5 min cycles	2	18	-1.81 [-4.07, 0.44]	0.11
5 min cycles	29	403	-1.32 [-1.82, -0.83]	<0.00001
10 min cycles	36	777	-2.36 [-2.90, -1.82]	<0.00001
15 min cycles	22	234	-1.64 [-2.22, -1.05]	<0.00001
> 15 min cycles	4	37	-2.40 [3.74, 1.07]	0.0004
Total length of limb ischaemia				
Less than 1 min	2	18	-1.81 [-4.07, 0.44]	0.11
5 mins	2	20	-0.52 [-1.43, 0.39]	0.27
10 mins	5	47	-0.84 [-1.49, -0.20]	0.01
15 mins	16	220	-2.17 [-3.07, -1.27]	<0.00001
20-25 mins	16	205	-1.07 [-1.64, -0.50]	0.0003
30 mins	32	731	-2.39 [-2.96, -1.82]	<0.00001

40-45 mins	18	208	-2.06 [-2.73, -1.38]	<0.00001
50 mins +	2	20	-0.07 [-0.81, 0.68]	0.86
Number of limbs occluded				
One	40	485	-1.53 [-1.92, -1.14]	<0.00001
Two	54	994	-2.15 [-2.60, -1.69]	<0.00001
B. Neurological Score				
Method*				
Garcia 18-point scale	8	200	-2.52 [-4.10, -0.94]	0.002
Longa 5-point scale	27	406	-0.89 [-1.09, -0.69]	<0.00001
Focal neurological score	1	30	-9.70 [-10.57, -8.83]	<0.00001
Neurological severity score	5	194	-3.03 [-6.43, 0.38]	0.08
3-point scale	3	30	-0.36 [-0.76, 0.04]	0.08
12-point scale	10	163	-1.37 [-2.26, -0.47]	0.003
Spetzler motor score	3	14	-1.48 [-2.03, -0.92]	<0.00001

RIC, remote ischemic conditioning; SMD, standardised mean difference; *Neurological score data expressed as weighted mean difference

Figure Legends

Figure 1. Effect of remote ischemic pre-conditioning (RIPreC) compared to control on infarct volume, expressed as a standardised mean difference, by individual publication experiment

Figure 2. Effect of remote ischemic per- and post-conditioning (RIPerC and RIPostC) compared to control on infarct volume, expressed as a standardised mean difference, by individual publication experiment

Figure 3. Subgroup analyses of RIC in experimental stroke. Each point estimate represents the change in infarct volume in treated animals compared to control, divided by subgroups according to animal model and RIC administration (pre-conditioning, RIPreC; per-& post-conditioning, RIPerC & RIPostC). The p values, obtained through meta-regression analyses, indicate whether the respective parameter has a significant interaction with infarct volume.

Figure 4. Impact of study quality on infarct volume by (a) CAMARADES criteria: each point represents one study, the size of the circle is proportional to the study size. The y-axis is infarct volume change expressed as the standardised mean difference (SMD) between RIC treated animals and control. The was no statistical interaction with study quality and infarct volume (meta regression p=0.495); and **(b)** measurement of physiological monitoring (blood glucose, blood gas, cerebral blood flow (CBF), and blood pressure (BP)). The p values indicate whether the respective parameter has a significant interaction with infarct volume.

Figure 5. Begg's funnel plot. An asymmetric funnel indicates a relationship between treatment effect estimate and study precision. Egger's test suggested significant publication bias (p<0.001).

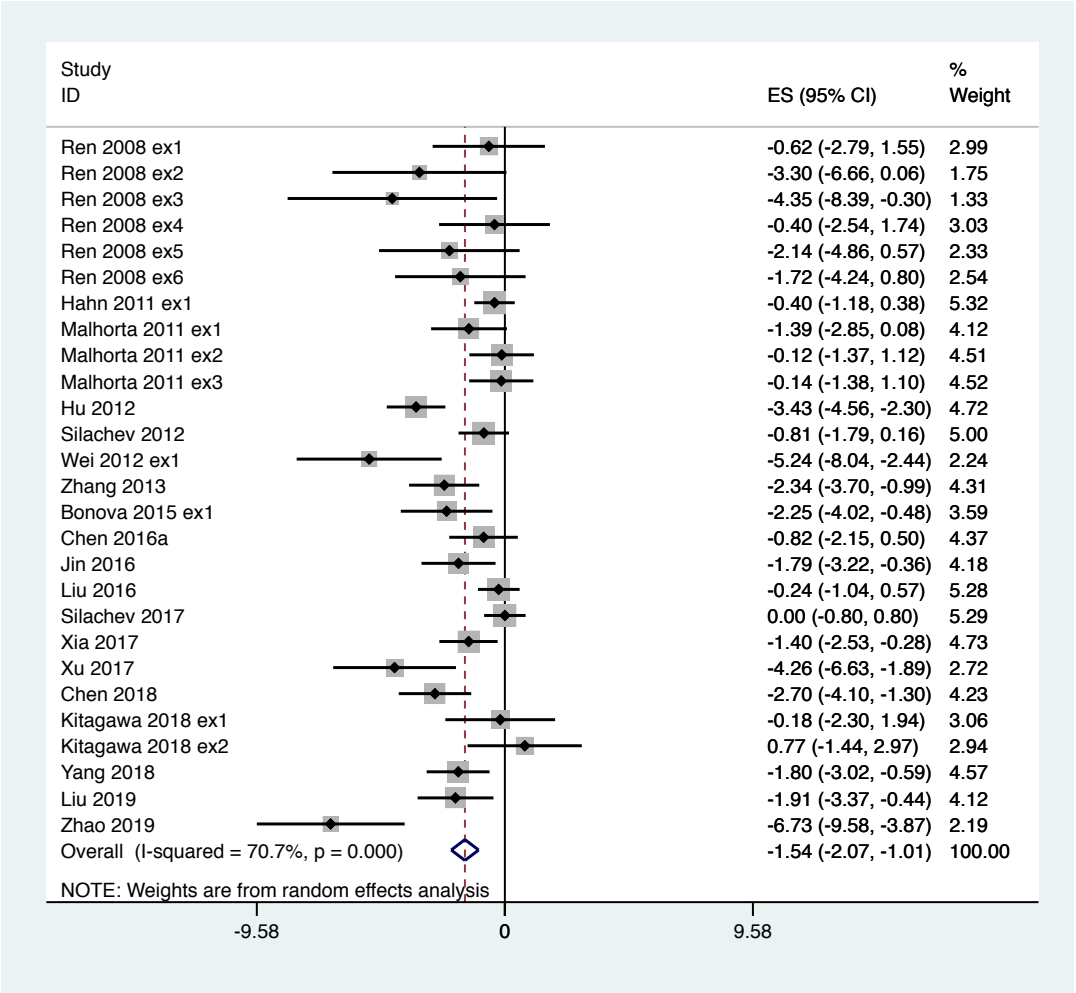

Study
ID
ES (95% CI)
%
Weight
Ren 2008 ex1 -0.62 (-2.79, 1.55) 2.99
Ren 2008 ex2 -3.30 (-6.66, 0.06) 1.75
Ren 2008 ex3 -4.35 (-8.39, -0.30) 1.33
Ren 2008 ex4 -0.40 (-2.54, 1.74) 3.03
Ren 2008 ex5 -2.14 (-4.86, 0.57) 2.33
Ren 2008 ex6 -1.72 (-4.24, 0.80) 2.54
Hahn 2011 ex1 -0.40 (-1.18, 0.38) 5.32
Malhorta 2011 ex1 -1.39 (-2.85, 0.08) 4.12
Malhorta 2011 ex2 -0.12 (-1.37, 1.12) 4.51
Malhorta 2011 ex3 -0.14 (-1.38, 1.10) 4.52
Hu 2012 -3.43 (-4.56, -2.30) 4.72
Silachev 2012 -0.81 (-1.79, 0.16) 5.00
Wei 2012 ex1 -5.24 (-8.04, -2.44) 2.24
Zhang 2013 -2.34 (-3.70, -0.99) 4.31
Bonova 2015 ex1 -2.25 (-4.02, -0.48) 3.59
Chen 2016a -0.82 (-2.15, 0.50) 4.37
Jin 2016 -1.79 (-3.22, -0.36) 4.18
Liu 2016 -0.24 (-1.04, 0.57) 5.28
Silachev 2017 0.00 (-0.80, 0.80) 5.29
Xia 2017 -1.40 (-2.53, -0.28) 4.73
Xu 2017 -4.26 (-6.63, -1.89) 2.72
Chen 2018 -2.70 (-4.10, -1.30) 4.23
Kitagawa 2018 ex1 -0.18 (-2.30, 1.94) 3.06
Kitagawa 2018 ex2 0.77 (-1.44, 2.97) 2.94
Yang 2018 -1.80 (-3.02, -0.59) 4.57
Liu 2019 -1.91 (-3.37, -0.44) 4.12
Zhao 2019 -6.73 (-9.58, -3.87) 2.19
Overall (I-squared = 70.7%, p = 0.000) -1.54 (-2.07, -1.01) 100.00
NOTE: Weights are from random effects analysis
-9.58
0
9.58

Figure 1

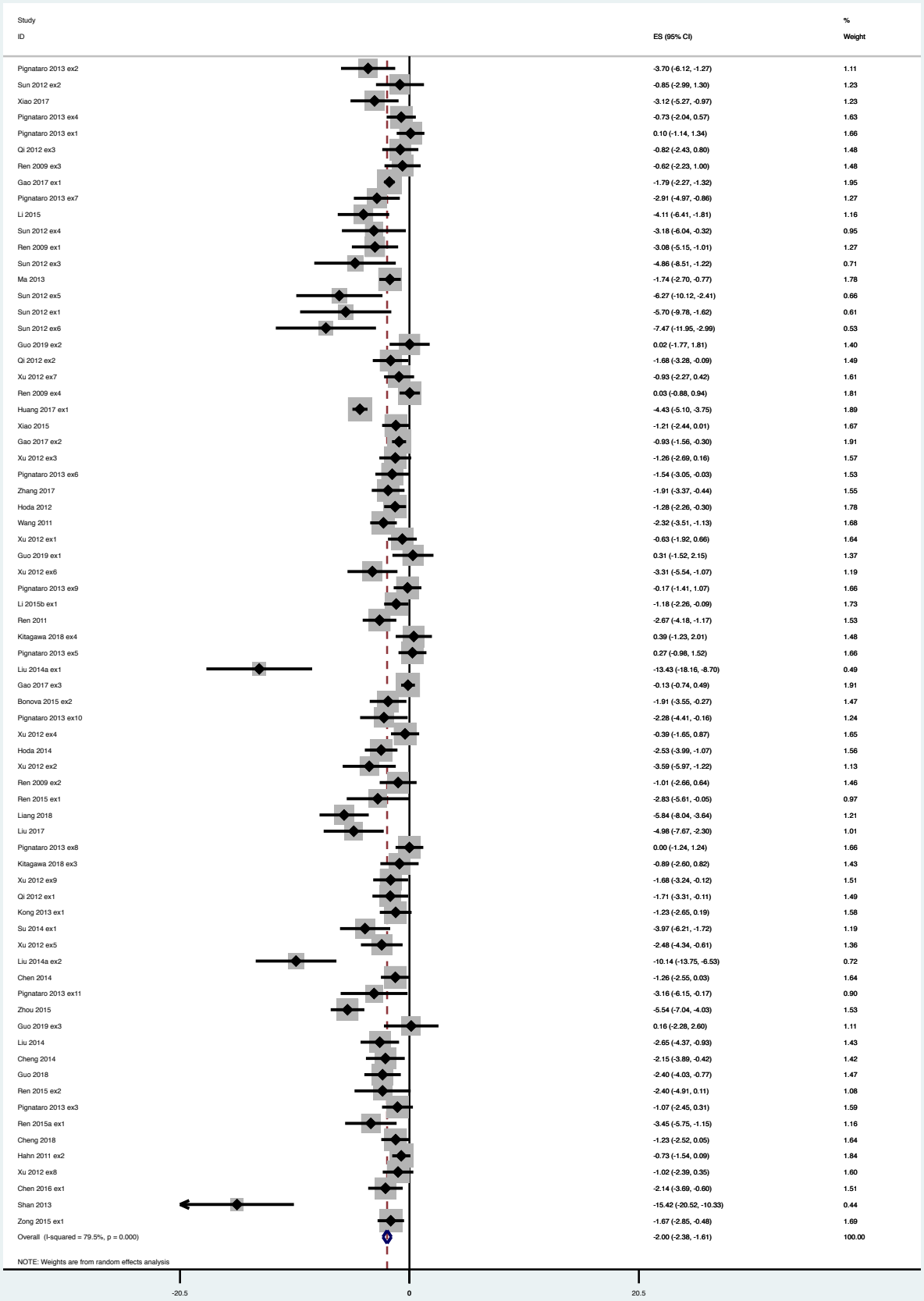

Study
ID
ES (95% CI)
%
Weight
Pignataro 2013 ex2 -3.70 (-6.12, -1.27) 1.11
Sun 2012 ex2 -0.85 (-2.99, 1.30) 1.23
Xiao 2017 -3.12 (-5.27, -0.97) 1.23
Pignataro 2013 ex4 -0.73 (-2.04, 0.57) 1.63
Pignataro 2013 ex1 0.10 (-1.14, 1.34) 1.66
Qi 2012 ex3 -0.82 (-2.43, 0.80) 1.48
Ren 2009 ex3 -0.62 (-2.23, 1.00) 1.48
Gao 2017 ex1 -1.79 (-2.27, -1.32) 1.95
Pignataro 2013 ex7 -2.91 (-4.97, -0.86) 1.27
Li 2015 -4.11 (-6.41, -1.81) 1.16
Sun 2012 ex4 -3.18 (-6.04, -0.32) 0.95
Ren 2009 ex1 -3.08 (-5.15, -1.01) 1.27
Sun 2012 ex3 -4.86 (-8.51, -1.22) 0.71
Ma 2013 -1.74 (-2.70, -0.77) 1.78
Sun 2012 ex5 -6.27 (-10.12, -2.41) 0.66
Sun 2012 ex1 -5.70 (-9.78, -1.62) 0.61
Sun 2012 ex6 -7.47 (-11.95, -2.99) 0.53
Guo 2019 ex2 0.02 (-1.77, 1.81) 1.40
Qi 2012 ex2 -1.68 (-3.28, -0.09) 1.49
Xu 2012 ex7 -0.93 (-2.27, 0.42) 1.61
Ren 2009 ex4 0.03 (-0.88, 0.94) 1.81
Huang 2017 ex1 -4.43 (-5.10, -3.75) 1.89
Xiao 2015 -1.21 (-2.44, 0.01) 1.67
Gao 2017 ex2 -0.93 (-1.56, -0.30) 1.91
Xu 2012 ex3 -1.26 (-2.69, 0.16) 1.57
Pignataro 2013 ex6 -1.54 (-3.05, -0.03) 1.53
Zhang 2017 -1.91 (-3.37, -0.44) 1.55
Hoda 2012 -1.28 (-2.26, -0.30) 1.78
Wang 2011 -2.32 (-3.51, -1.13) 1.68
Xu 2012 ex1 -0.63 (-1.92, 0.66) 1.64
Guo 2019 ex1 0.31 (-1.52, 2.15) 1.37
Xu 2012 ex6 -3.31 (-5.54, -1.07) 1.19
Pignataro 2013 ex9 -0.17 (-1.41, 1.07) 1.66
Li 2015b ex1 -1.18 (-2.26, -0.09) 1.73
Ren 2011 -2.67 (-4.18, -1.17) 1.53
Kitagawa 2018 ex4 0.39 (-1.23, 2.01) 1.48
Pignataro 2013 ex5 0.27 (-0.98, 1.52) 1.66
Liu 2014a ex1 -13.43 (-18.16, -8.70) 0.49
Gao 2017 ex3 -0.13 (-0.74, 0.49) 1.91
Bonova 2015 ex2 -1.91 (-3.55, -0.27) 1.47
Pignataro 2013 ex10 -2.28 (-4.41, -0.16) 1.24
Xu 2012 ex4 -0.39 (-1.65, 0.87) 1.65
Hoda 2014 -2.53 (-3.99, -1.07) 1.56
Xu 2012 ex2 -3.59 (-5.97, -1.22) 1.13
Ren 2009 ex2 -1.01 (-2.66, 0.64) 1.46
Ren 2015 ex1 -2.83 (-5.61, -0.05) 0.97
Liang 2018 -5.84 (-8.04, -3.64) 1.21
Liu 2017 -4.98 (-7.67, -2.30) 1.01
Pignataro 2013 ex8 0.00 (-1.24, 1.24) 1.66
Kitagawa 2018 ex3 -0.89 (-2.60, 0.82) 1.43
Xu 2012 ex9 -1.68 (-3.24, -0.12) 1.51
Qi 2012 ex1 -1.71 (-3.31, -0.11) 1.49
Kong 2013 ex1 -1.23 (-2.65, 0.19) 1.58
Su 2014 ex1 -3.97 (-6.21, -1.72) 1.19
Xu 2012 ex5 -2.48 (-4.34, -0.61) 1.36
Liu 2014a ex2 -10.14 (-13.75, -6.53) 0.72
Chen 2014 -1.26 (-2.55, 0.03) 1.64
Pignataro 2013 ex11 -3.16 (-6.15, -0.17) 0.90
Zhou 2015 -5.54 (-7.04, -4.03) 1.53
Guo 2019 ex3 0.16 (-2.28, 2.60) 1.11
Liu 2014 -2.65 (-4.37, -0.93) 1.43
Cheng 2014 -2.15 (-3.89, -0.42) 1.42
Guo 2018 -2.40 (-4.03, -0.77) 1.47
Ren 2015 ex2 -2.40 (-4.91, 0.11) 1.08
Pignataro 2013 ex3 -1.07 (-2.45, 0.31) 1.59
Ren 2015a ex1 -3.45 (-5.75, -1.15) 1.16
Cheng 2018 -1.23 (-2.52, 0.05) 1.64
Hahn 2011 ex2 -0.73 (-1.54, 0.09) 1.84
Xu 2012 ex8 -1.02 (-2.39, 0.35) 1.60
Chen 2016 ex1 -2.14 (-3.69, -0.60) 1.51
Shan 2013 -15.42 (-20.52, -10.33) 0.44
Zong 2015 ex1 -1.67 (-2.85, -0.48) 1.69
Overall (I-squared = 79.5%, p = 0.000) -2.00 (-2.38, -1.61) 100.00
NOTE: Weights are from random effects analysis
-20.5
0
20.5

Figure 2

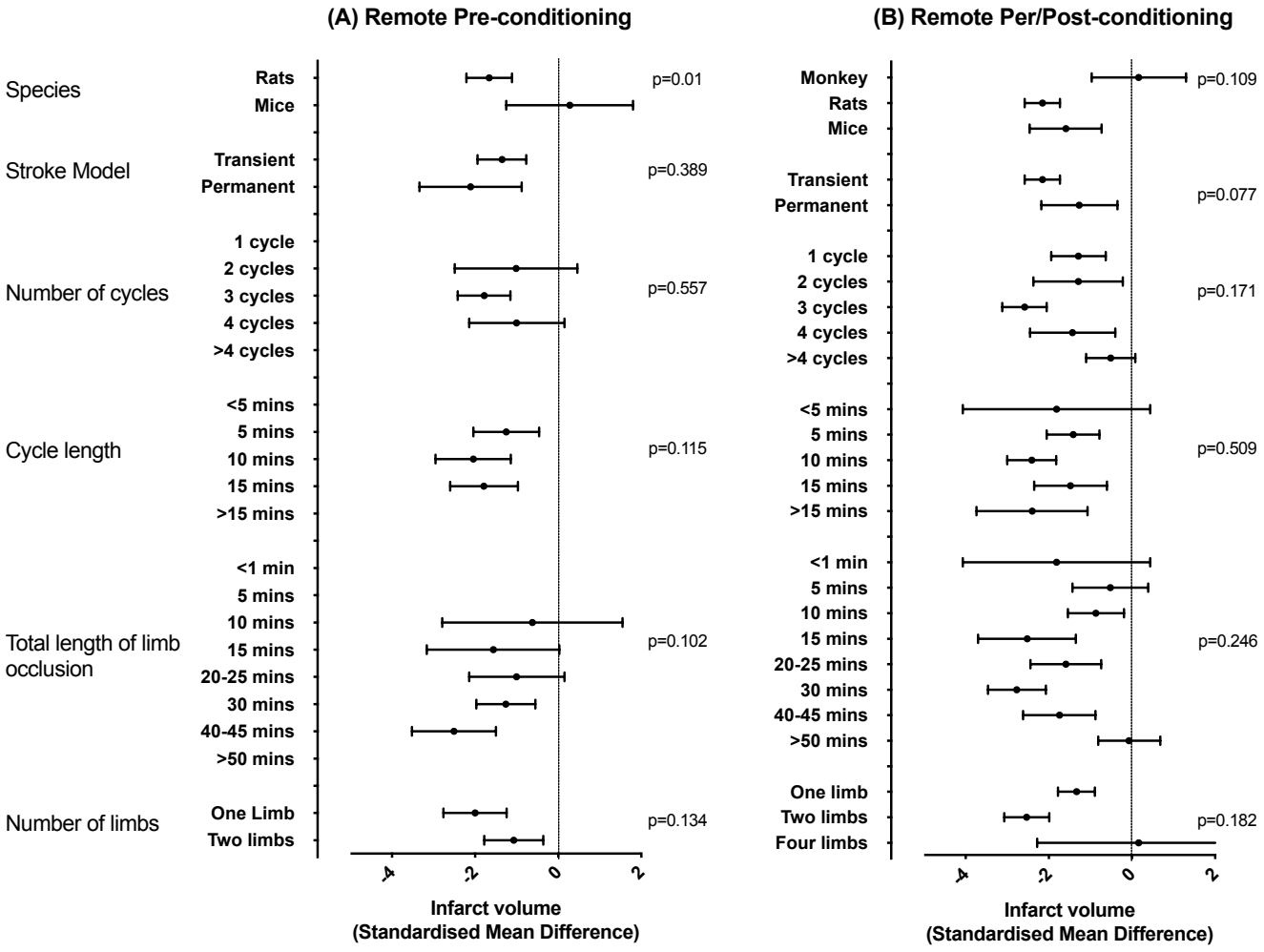
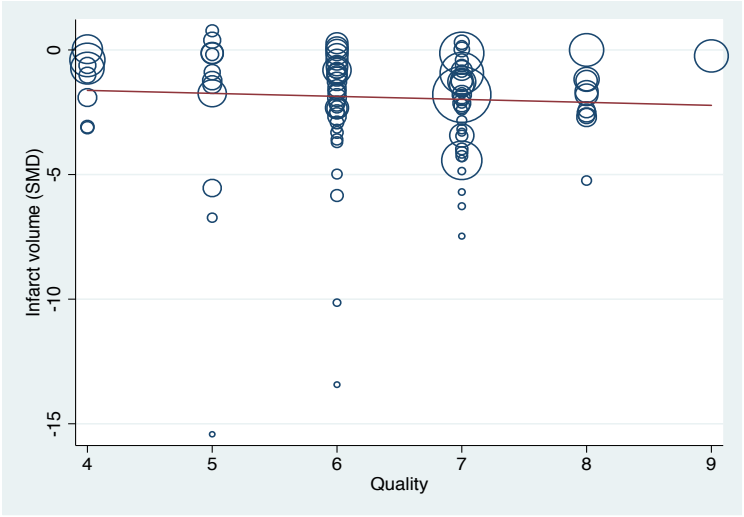
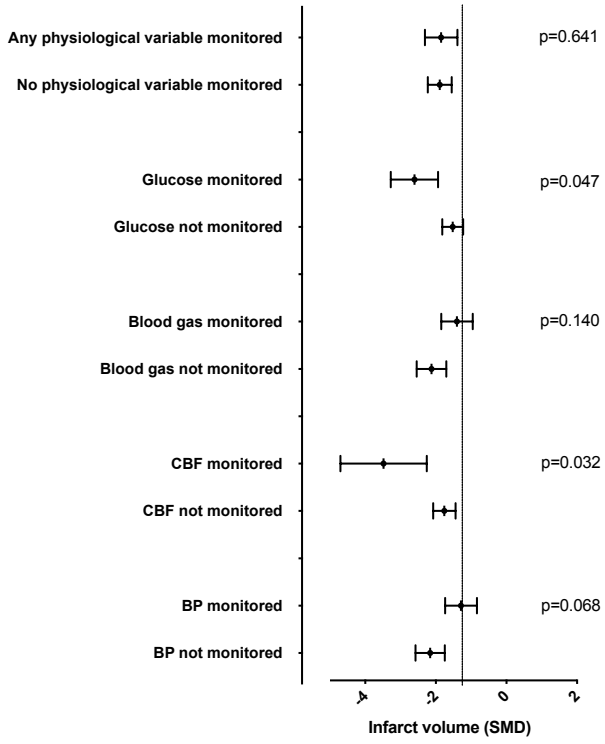

(A) Remote Pre-conditioning
(B) Remote Per/Post-conditioning
Species
Rats
Mice
p=0.01
Stroke Model
Transient
Permanent
p=0.389
Number of cycles
1 cycle
2 cycles
3 cycles
4 cycles
>4 cycles
p=0.557
Cycle length
<5 mins
5 mins
10 mins
15 mins
>15 mins
p=0.115
Total length of limb occlusion
<1 min
5 mins
10 mins
15 mins
20-25 mins
30 mins
40-45 mins
>50 mins
p=0.102
Number of limbs
One Limb
Two limbs
p=0.134
Monkey
Rats
Mice
p=0.109
Transient
Permanent
p=0.077
1 cycle
2 cycles
3 cycles
4 cycles
>4 cycles
p=0.171
<5 mins
5 mins
10 mins
15 mins
>15 mins
p=0.509
<1 min
5 mins
10 mins
15 mins
20-25 mins
30 mins
40-45 mins
>50 mins
p=0.246
One limb
Two limbs
Four limbs
p=0.182
-4
-2
0
2
Infarct volume
(Standardised Mean Difference)
-4
-2
0
2
Infarct volume
(Standardised Mean Difference)

Figure 3


Infarct volume (SMD)
0
-5
-10
-15
4
5
6
7
8
9
Quality

(a)


Any physiological variable monitored
No physiological variable monitored
Glucose monitored
Glucose not monitored
Blood gas monitored
Blood gas not monitored
CBF monitored
CBF not monitored
BP monitored
BP not monitored
p=0.641
p=0.047
p=0.140
p=0.032
p=0.068
-4
-2
0
2
Infarct volume (SMD)

(b)

Figure 4

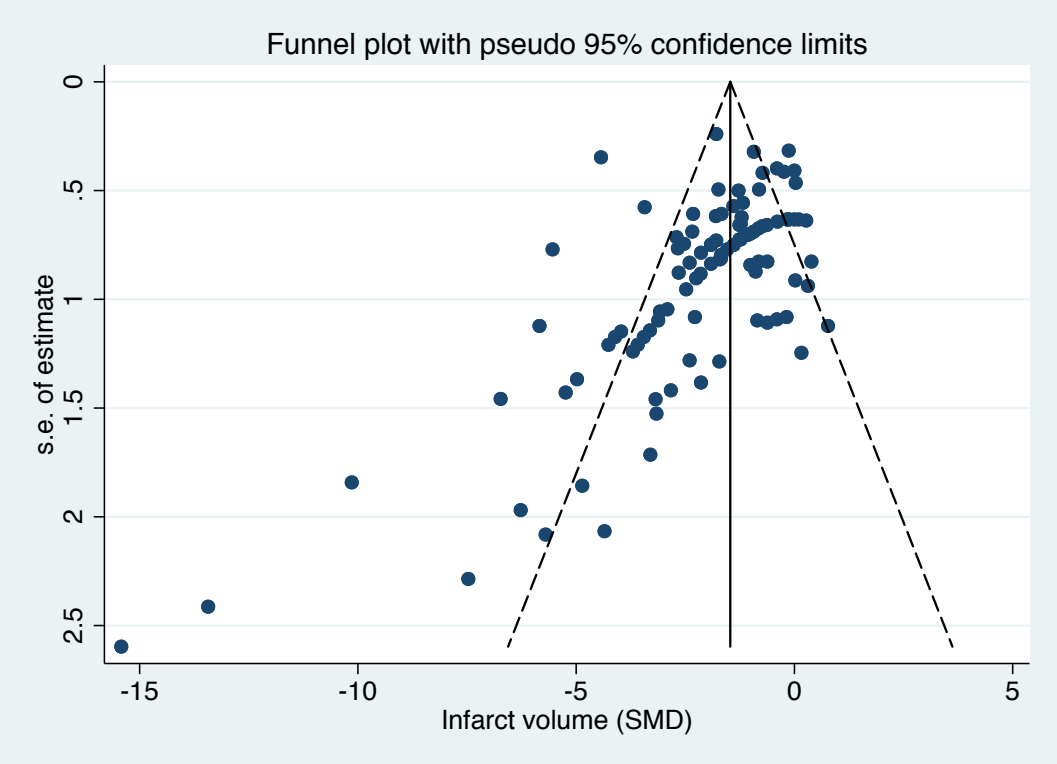

Funnel plot with pseudo 95% confidence limits
s.e. of estimate
0
.5
1
1.5
2
2.5
-15
-10
-5
0
5
Infarct volume (SMD)

Figure 5

Supplementary Data

Supplementary Table I

Study	Species	Model (mins)	Number and length of cycles	Method of RIC	Time of administration	Measurement and units	Time of assessment	Quality of study score	Risk of bias score
Bonova 2014[50]									
Experiment 1 & 2	M, albino Wistar rats	T 90	3 cycles, 5 min phases	Hind limbs	1 h pre-; 0.5 h post-ischaemia	Infarct size -mm^3	24 h	7	5
Experiments 3 & 4					0.5 h post-ischaemia	Longa 5-point scale	24 h		
Burda 2014[51]	M & F, albino Wistar rats	T 10	1 cycle, 20 min phases	Hind limbs	48 h post-ischaemia	Morris water maze test - seconds	Day 6 & 7	7	6
Chen 2014[52]									
Experiment 1	M Sprague-Dawley rats	T 120	3 cycles, 15 min phases	Femoral artery	At reperfusion	Infarct size - %	24 h	7	8
Experiment 2						Postural reflex test			
Experiment 3						Vibrissae-elicited forelimb placing test - %			
Experiment 4						Tail hang test - %			
Chen 2016[53]									
Experiment 1	M Sprague-Dawley rats	T 90	3 cycles, 5 min phases	Femoral artery	At reperfusion	Infarct size - %	24 h	7	8
Experiments 2-4					0; 1 h; 3 h post-reperfusion	Postural reflex test			
Experiments 5-7					0; 1 h; 3 h post-reperfusion	Tail hang test - %			
Chen 2016a[36]									
Experiment 1	M Sprague-Dawley rats	T 90	3 cycles, 15 min phases	Femoral artery	Immediately pre-ischaemia	Infarct size - %	48 h	7	6
Experiment 2						Longa 5-point scale	48 h		
Chen 2018[54]									
Experiment 1	M Sprague-Dawley rats	T 120	3 cycles, 15 min phases	Hind limb	Immediately pre-reperfusion	Infarct size - %	24 h	8	7
Experiment 2						Longa 5-point scale	24 h		
Cheng 2014[55]	M Sprague-Dawley rats	T 90	3 cycles, 5 min phases	Hind limb	Immediately post-ischaemia	Infarct size - %	24 h	6	6
Cheng 2018[56]	M C57BL/6 mice	T 60	3 cycles, 10 min phases	Bilateral femoral arteries	Immediately post-ischaemia	Infarct size - %	3 days	9	9
Gao 2017[57]									
Experiments 1-3	M Sprague-Dawley rats	T 120	3 cycles, 10 min phases	Bilateral femoral arteries	0; 10; 30 mins post-reperfusion	Infarct size - %	24 h	7	8

Experiment 4-6					0; 10; 30 mins post-reperfusion	Garcia 18-point scale	24 h		
Guo 2018[58]									
Experiment 1	M C57BL/6 mice	T 120	3 cycles, 10 min phases	Bilateral femoral arteries	At reperfusion	Infarct size - %	12 h	8	9
Experiment 2						Longa 5-point scale	12 h		
Guo 2019[22] Experiments 1-3	Male Rhesus Monkeys	Thrombo -embolic	10 cycles, 5 min phases	1, 2,or 4 limbs	Immediately post-ischaemia	Infarct size – mm^3	24 hours	7	5
						Spetzler rating scale	3h 1d 30d 60d		
Hahn 2011[59]									
Experiment 1 & 2	M Sprague-Dawley rats	T 120	4 cycles, 5 min phases	Hind limb	40 mins pre-; 80 mins post-ischaemia	Infarct size -mm^3	24 h	4	5
Hoda 2012[20]									
Experiment 1	M C57BL/6 mice	P	5 cycles, 5 min phases	Hind limb	120 mins post-ischaemia	Infarct size - %	48 h	7	10
Experiment 2						Longa 5-point scale	48 h		
Hoda 2014[21]									
Experiment 1	F C57BL/6 mice	P	4 cycles, 10 min phases	Hind limb	120 mins post-ischaemia	Infarct size - %	24 h	8	10
Experiment 2						Longa 5-point scale	24 h		
Hu 2012[60]									
Experiment 1	M Sprague-Dawley rats	T 120	3 cycles, 5 min phases	Hind limb	60 mins pre-ischaemia	Infarct size – mm^3	24 h	7	8
Experiment 2						Longa 5-point scale	24 h		
Hu 2013[61]									
Experiment 1-5	M Sprague-Dawley rats	T 60	3 cycles, 5 min phases	Hind limb	60 mins pre-ischaemia	Morris water maze test - seconds	3, 4, 5, 6 and 7 days	8	8
Huang 2017[62]									
Experiment 1	M Sprague-Dawley rats	T 90	3 cycles, 10 min phases	Bilateral femoral arteries	90 mins post-ischaemia	Infarct size - %	3 days	7	8
Experiment 2-8						Neurological severity score 18-point scale	1, 2, 3, 7, 14, 21 and 28 days		
Jin 2016[63]	M Sprague-Dawley rats	T 30	3 cycles, 15 min phases	Femoral artery	60 mins pre-ischaemia	Infarct size - %	24 h	7	6
Kitagawa 2018[64]									
Experiments 1-4	M C57BL/6 mice	T 45 mins	4 cycles, 5 min phases	Hind limbs	24 h pre-; immediately pre-; immediately post-ischaemia; at reperfusion	Infarct size -mm^3	24 h	5	7
Experiments 5-8					24 h pre-; immediately pre-; immediately post-ischaemia; at reperfusion	Longa 5-point scale	24 h		

Kong 2013[65]									
Experiment 1	M Sprague-Dawley rats	T 120	3 cycles, 5 min phases	Femoral artery	At reperfusion	Infarct size - %	48 h	5	6
Experiments 2-4					At reperfusion	Neurological severity score 18-point scale	24 h, 48 h and 7 days		
Liang 2018[66]	M Sprague-Dawley rats	T 90	3 cycles, 10 min phases	Hind limbs	2 days post-ischaemia and continued for 21 days	Infarct size – mm^3	21 days	6	7
Li 2015[67]									
Experiment 1	M CD1 mice	T 60	3 cycles, 5 min phases	Bilateral femoral arteries	Immediately post-ischaemia	Infarct size - %	24 h	7	8
Experiment 2						Focal neurological score 28-point scale			
Li 2015a[44]	M Sprague-Dawley rats	T 120	3 cycles, 10 min phases	Bilateral femoral arteries	Immediately post-ischaemia	Longa 5-point scale	24 h	7	8
Li 2016[68]									
Experiments 1 & 2	F Sprague-Dawley rats	T 60	3 cycles, 10 min phases	Bilateral femoral arteries	Immediately post-ischaemia	Infarct size - %	1 and 3 days	8	9
Experiments 3 & 4						Garcia 18-point scale	1 and 3 days		
Liu 2014[69]									
Experiment 1	M Sprague-Dawley rats	T 120	3 cycles, 10 min phases	Bilateral femoral arteries	At reperfusion	Infarct size - %	24 h	8	8
Experiment 2						12-point scale			
Liu 2014a[70]									
Experiments 1 & 2	M Sprague-Dawley rats	T 120	3 cycles, 10 min phases	Bilateral femoral arteries	8; 24 h post-ischaemia	Infarct size - %	8 h and 24 h	6	6
Experiments 3 & 4					8; 24 h post-ischaemia	Longa 5-point scale			
Liu 2016[71]									
Experiment 1	M Sprague-Dawley rats	T 90	4 cycles, 5 min phases	Bilateral femoral arteries	60 mins pre-ischaemia	Infarct size – mm^3	3 days	9	8
Experiments 2-5						Longa 5-point scale	0.5, 24, 48 and 72 h		
Liu 2018[28]	M Sprague-Dawley rats	T 30	3 cycles, 15 min phases	Femoral artery	Post-ischaemia	Infarct size - %	48 h	6	5
Liu 2019[72]	M Sprague-Dawley rats	T 120	3 cycles, 10 min phases	Bilateral femoral arteries	1 hr pre-ischaemic	Infarct size Neurological severity score 18-point scale	7 days 1, 3, 7 days	4	3
Ma 2013[73]									
Experiment 1	M Sprague-Dawley rats	T 90	3 cycles, 10 min phases	Bilateral femoral arteries	Immediately post-ischaemia	Infarct size - %	24 h	5	6
Experiment 2					Immediately post-ischaemia	12-point scale			
Malhorta 2011[26]									
Experiments 1-3	M Wistar rats	T 120	3 cycles, 10 min phases	Infrared aortic occlusion	24; 48; 72 h pre-ischaemia	Infarct size – mm^3	24 h	5	8

Experiments 4-6					24; 48; 72 h pre-ischaemia	3-point scale			
Pignataro 2013[74]									
Experiments 1, 4, 5, 6 & 8	M Sprague-Dawley rats	T 100	10 cycles, 10 min phases; 1 cycle, 10 and 20 min phases; 2 cycles, 10 min phases; 1 cycle, 10 min rep & 20 min occ	Femoral artery	10 mins post-reperfusion	Infarct size - %	24 h	6	7
Experiments 2, 3 & 7			2, 3 or 1 cycles, 5 min phases		5 mins post-reperfusion				
Experiment 9-11			1 cycle, 20, 30 or 40 min rep, 20 min occ		20, 30 or 40 mins post-reperfusion				
Qi 2012[75]									
Experiments 1-3	M Sprague-Dawley rats	T 120	3, 4 or 5 cycles, 10 min phases	Bilateral femoral arteries	0; 10; 30 mins post-reperfusion	Infarct size - %	22 h	6	7
Experiments 4-6			3, 4 or 5 cycles, 10 min phases		0; 10; 30 mins post-reperfusion	12-point scale			
Ren 2008[23]									
Experiments 1-3	M Sprague-Dawley rats	P	2 cycles, 5 min phases; 2 or 3 cycles, 15 min phases	Femoral artery	Immediately pre-ischaemia	Infarct size - %	24 h	7	6
Experiments 4 & 5			2 or 3 cycles, 15 min phases		12 h pre-ischaemia				
Experiment 6			3 cycles, 15 min phases		2 days pre-ischaemia				
Ren 2009[24]									
Experiments 1-3	M Sprague-Dawley rats	P	3 cycles, 15 min phases	Femoral artery	Immediately; 3 h; 6 h post-ischaemia	Infarct size - %	2 days	4	9
Experiment 4					Immediately post-ischaemia		60 days		
Experiments 5-11					Immediately post-ischaemia	Vibrissae-elicited forelimb placing test - %	2, 7, 14, 21, 30, 37, 60 days		
Ren 2011[76]	M Sprague-Dawley rats	T 90	3 cycles, 10 min phases	Bilateral femoral arteries	Immediately post-ischaemia	Infarct size - %	24 h	6	5
Ren 2015[31]									
Experiments 1 & 2	M Sprague-Dawley rats	T 90	3 cycles, 10 min phases	Bilateral femoral arteries	Immediately post-ischaemia; immediately post- ischaemia for 14 days	Infarct size - %	7 and 14 days	7	7
Experiments 3, 4 and 6					Immediately post-ischaemia	12-point scale	1, 7 and 14 days		

Experiments 5 & 7					Immediately post-ischaemia for 14 days	12-point scale	7 and 14 days		
Experiments 8, 9 & 11					Immediately post-ischaemia	Tail hang test - %	1, 7 and 14 days		
Experiment 10 & 12					Immediately post-ischaemia for 14 days	Tail hang test - %	7 and 14 days		
Ren 2015a[77]									
Experiment 1	M Sprague-Dawley rats	T 90	3 cycles, 10 min phases	Hind limbs	Immediately post-ischaemia	Infarct size - %	48 h	7	7
Experiments 2 & 3						12-point scale	2 and 48 h		
Ren 2018[37]	Male Sprague-Dawley rats	T 90	3 cycles, 10 mins phases	Hind limbs	Immediately post-ischaemia and continued for 14 days	Tail hang test - % Foot fault	14 days	9	7
Shan 2013[78]	Male Sprague-Dawley rats	T 90	3 cycles, 5 min phases	Hind limbs	60 mins post-ischaemia	Infarct size - %	24 h	5	8
Silachev 2012[27]	M outbred white rats	T 60	3 cycles, 5 min phases	Unilateral renal arteries	24 h pre-ischaemia	Infarct size - %	24 h	6	5
Silachev 2017[79]	M outbred white rats	T 60	3 cycles, 5 min phases	Hind limbs	24 h pre-ischaemia	Infarct size – mm^3	24 h	8	6
Su 2014[80]									
Experiment 1	M Sprague-Dawley rats	T 120	4 cycles, 10 min phases	Bilateral femoral arteries	10 mins post-reperfusion	Infarct size - %	24 h	7	8
Experiments 2-5						Garcia 18-point scale	1, 2, 3 and 7 days		
Sun 2012[47]									
Experiments 1-3	M Sprague-Dawley rats	T 90	3 cycles, 15 seconds, 5 or 8 min phases	Bilateral femoral arteries	3 h post-ischaemia	Infarct size - %	72 h	7	7
Experiments 4-6			3 cycles, 15 seconds, 5 or 8 min phases		6 h post-ischaemia				
Wang 2011[81]									
Experiment 1	M Sprague-Dawley rats	T 120	3 cycles, 10 min phases	Femoral artery	At reperfusion	Infarct size - %	24 h	6	8
Experiment 2						Garcia 18-point scale			
Wei 2012[82]								8	8
Experiment 1	M Sprague-Dawley rats	T 30	3 cycles, 15 min phases	Femoral artery	Immediately pre-ischaemia	Infarct size - %	48 h		
Experiments 2-5						Postural reflect test	1, 2, 7 and 44 days		
Experiment 6-13						Vibrissae-elicited forelimb placing test - %	1, 2, 7, 10, 14, 21, 44 and 60 days		
Xia 2017[83]									

Experiment 1	M Sprague-Dawley rats	T 90	4 cycles, 5 min phases	Hind limbs	60 mins pre-ischaemia	Infarct size - %	24 h	7	7
Experiment 2						Neurological severity score 18-point scale	24 h		
Xiao 2015[84]									
Experiment 1	M Sprague-Dawley rats	T 30	3 cycles, 10 min phases	Bilateral femoral arteries	Immediately post-ischaemia	Infarct size - %	24 h	7	8
Experiment 2						12-point scale	24 h		
Xiao 2017[40]	M & F Sprague-Dawley rats	T 120	3 cycles, unclear length	Femoral artery	2 h post-ischaemia	Infarct size – mm^3	24 h	4	6
Xu 2012[85]									
Experiments 1-3	M Sprague-Dawley rats	T 90	1, 2 or 3 cycles, 15 min phases	Bilateral femoral arteries	At reperfusion	Infarct size - %	24 h	6	9
Experiment 4-6			1, 2 or 3 cycles, 10 min phases			Infarct size - %			
Experiments 7-9			1, 2 or 3 cycles, 5 min phases			Infarct size - %			
Experiments 10-12			1, 2 or 3 cycles, 15 min phases			Longa 5-point scale			
Experiments 13-15			1, 2 or 3 cycles, 10 min phases			Longa 5-point scale			
Experiments 16-18			1, 2 or 3 cycles, 5 min phases			Longa 5-point scale			
Xu 2017[25]									
Experiment 1	M Sprague-Dawley rats	P	3 cycles, 15 min phases	Femoral artery	Immediately pre-ischaemia	Infarct size - %	48 h	7	8
Experiment 2 & 3						Vibrissae-elicited forelimb placing test - %	24 and 48 h		
Yang 2018[86]									
Experiment 1	Sprague-Dawley rats	T 90	3 cycles, 10 min phases	Hind limbs	24 hours pre-ischaemia	Infarct size - %	24 hours	8	9
Experiment 2						Longa 5-point scale	24 hours		
Experiment 3						Postural reflex test	24 hours		
Experiment 4						Tail hang test - %	24 hours		
Zhang 2012[87]									
Experiment 1	M Sprague-Dawley rats	T 120	3 cycles, 10 min phases	Bilateral femoral arteries	Daily for 3 days pre-ischaemia	Infarct size - %	24 h	6	6
Experiment 2						12-point scale			
Zhang 2017[88]									
Experiment 1	M Sprague-Dawley rats	T 120	3 cycles, 10 min phases	Bilateral femoral arteries	At reperfusion	Infarct size - %	24 h	6	7
Experiment 2						Garcia 18-point scale			
Zhao 2019[89]	M Sprague-Dawley rats	T 120	4 cycles, 5 min phases	Bilateral femoral arteries	24 hours pre-ischaemia	Infarct size - % Neurological severity score 18-point scale	24 h	5	5

Zhou 2015[90]									
Experiment 1 Experiment 2	M Sprague-Dawley rats	T 90	3 cycles, 10 min phases	Bilateral femoral arteries	At reperfusion	Infarct size - % Longa 5-point scale	24 h	5	6
Zong 2015[91]									
Experiment 1 Experiment 2-4	Sprague-Dawley rats	T 60	3 cycles, 10 min phases	Hind limbs	Post-ischaemia	Infarct size - % Garcia 18-point scale	3 days 1, 3 and 7 days	8	9

T, transient ischaemia; P, permanent ischaemia; F, female; M, male; occ, occlusion; rep, reperfusion.

Supplementary Table II

Assessment of quality in all studies

Study	Peer-reviewed publication	Statement of control of temperature	Random allocation	Blinded induction of ischaemia	Blinded assessment of outcome	Use of neuroprotective anaesthetic	Appropriate animal model	Sample size calculation	Compliance with animal welfare regulation	Statement of potential conflicts of interest	Overall Score
Bonova 2015	1	1	0	0	1	1	1	0	1	1	7
Burda 2014	1	1	1	0	1	1	1	0	1	0	7
Chen 2014	0	1	1	0	1	1	1	0	1	1	7
Chen 2016	1	0	1	0	1	1	1	0	1	1	7
Chen 2016a	1	1	1	0	0	1	1	0	1	1	7
Chen 2018	1	1	1	0	1	1	1	0	1	1	8
Cheng 2014	1	1	1	0	0	1	1	0	1	0	6
Cheng 2018	1	1	1	1	1	1	1	0	1	1	9
Gao 2017	1	1	1	0	1	1	1	0	1	0	7
Guo 2018	1	1	1	0	1	1	1	0	1	1	8
Guo 2019	**1**	**0**	**0**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	7
Hahn 2011	1	0	1	0	0	1	1	0	0	0	4
Hoda 2012	1	0	1	0	1	1	1	1	1	0	7
Hoda 2014	1	0	1	0	1	1	1	1	1	1	8
Hu 2012	1	1	1	0	1	1	1	0	1	0	7
Hu 2013	1	1	1	0	1	1	1	0	1	1	8
Huang 2017	1	1	1	0	1	1	1	0	1	0	7
Jin 2016	1	1	1	0	0	1	1	0	1	1	7
Kitagawa 2018	1	0	0	0	1	1	1	0	1	0	5
Kong 2013	1	0	1	0	0	1	1	0	1	0	5
Liang 2018	1	1	0	0	1	1	1	0	1	0	6
Li 2015	0	1	1	0	1	1	1	0	1	1	7
Li 2015a	1	0	1	0	1	1	1	0	1	1	7
Li 2015b	1	1	1	0	1	1	1	0	1	1	8
Liu 2014	1	1	1	0	1	1	1	0	1	1	8
Liu 2014a	1	1	1	0	0	1	1	0	1	0	6
Liu 2016	1	1	1	0	1	1	1	1	1	1	9
Liu 2017	1	1	1	0	0	1	1	0	1	0	6
Liu 2019	**0**	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**4**
Ma 2013	0	1	1	0	0	1	1	0	0	1	5
Malhorta 2011	1	1	1	0	1	1	1	0	1	0	7
Pignataro 2013	1	0	0	0	1	1	1	0	1	0	5
Qi 2012	1	0	0	0	1	1	1	0	1	1	6
Ren 2008	1	1	1	0	0	1	1	0	1	0	6
Ren 2009	1	1	1	0	1	1	1	0	1	0	7
Ren 2011	0	1	0	0	0	1	1	0	1	0	4
Ren 2015	0	1	0	0	1	1	1	0	1	1	6
Ren 2015a	1	1	0	0	1	1	1	0	1	1	7
Ren 2018	1	1	0	0	1	1	1	0	1	1	7
Shan 2013	1	1	1	1	1	1	1	0	1	1	9
Silachev 2012	1	1	0	0	0	1	1	0	1	0	5
Silachev 2017	1	1	1	0	0	1	1	0	1	0	6
Su 2014	1	1	1	0	1	1	1	0	1	1	8
Sun 2012	1	1	0	0	1	1	1	0	1	1	7
Wang 2011	0	1	1	0	1	1	1	0	1	0	6
Wei 2012	1	1	1	0	1	1	1	0	1	1	8
Xia 2017	1	1	0	0	1	1	1	0	1	1	7
Xiao 2015	1	0	1	0	1	1	1	0	1	1	7
Xiao 2017	0	1	0	0	0	1	1	0	1	0	4
Xu 2012	0	1	1	0	1	1	1	0	1	0	6
Xu 2017	1	1	1	0	1	1	1	0	1	0	7
Yang 2018	1	1	1	0	1	1	1	0	1	1	8
Zhang 2013	1	1	0	0	0	1	1	0	1	1	6
Zhang 2017	1	0	0	0	1	1	1	0	1	1	6
Zhao 2019	**1**	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**5**
Zhou 2015	0	1	1	0	0	1	1	0	0	1	5
Zong 2015	1	1	1	0	1	1	1	0	1	1	8

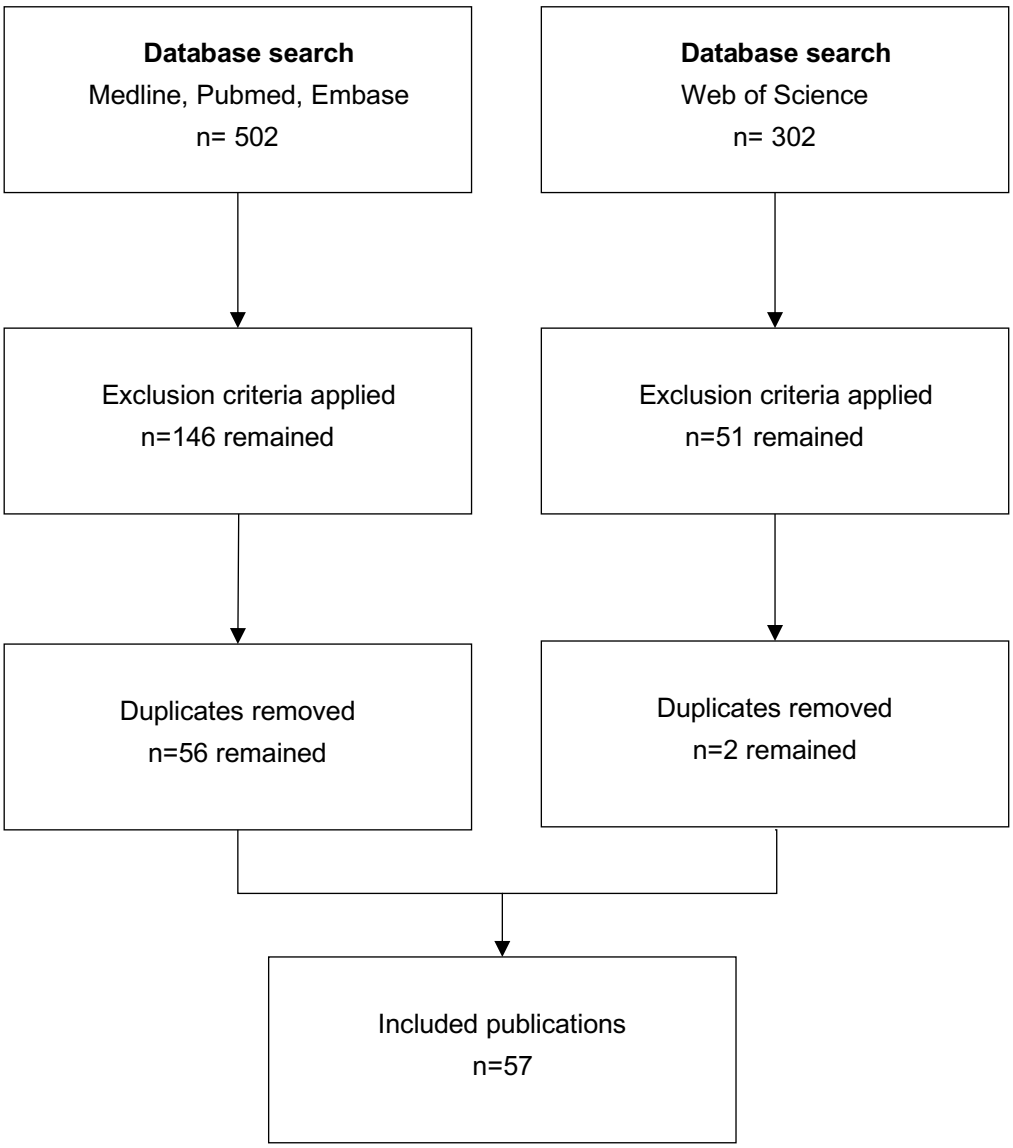
Supplementary Table III

SYRCLE assessment of bias in all studies

Study	Selection Bias			Performance Bias		Detection Bias		Attrition Bias	Reporting Bias	Other	Overall Score
	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome assessment	Blinding	Incomplete outcome data	Selective outcome reporting	Other sources of bias	
Bonova 2015	1	0	0	0	1	0	0	1	1	1	5
Burda 2014	0	1	0	0	1	1	1	1	1	0	6
Chen 2014	1	1	0	0	1	1	1	1	1	1	8
Chen 2016	1	1	0	0	1	1	1	1	1	1	8
Chen 2016a	1	1	0	0	0	1	0	1	1	1	6
Chen 2018	1	1	0	0	1	1	0	1	1	1	7
Cheng 2014	1	1	0	0	0	1	0	1	1	1	6
Cheng 2018	1	1	0	1	1	1	1	1	1	1	9
Gao 2017	1	1	0	0	1	1	1	1	1	1	8
Guo 2018	1	1	1	0	1	1	1	1	1	1	9
Guo 2019	0	0	0	0	1	0	1	1	1	1	5
Hahn 2011	1	0	0	0	1	1	0	1	1	0	5
Hoda 2012	1	1	1	1	1	1	1	1	1	1	10
Hoda 2014	1	1	1	1	1	1	1	1	1	1	10
Hu 2012	1	1	0	0	1	1	1	1	1	1	8
Hu 2013	1	1	0	0	1	1	1	1	1	1	8
Huang 2017	1	1	0	0	1	1	1	1	1	1	8
Jin 2016	1	1	0	0	0	1	0	1	1	1	6
Kitagawa 2018	1	1	0	0	1	0	1	1	1	1	7
Kong 2013	1	1	0	0	0	1	0	1	1	1	6
Liang 2018	1	1	0	0	1	0	1	1	1	1	7
Li 2015	1	1	0	0	1	1	1	1	1	1	8
Li 2015a	1	1	0	0	1	1	1	1	1	1	8
Li 2015b	1	1	1	0	1	1	1	1	1	1	9
Liu 2014	1	1	0	0	1	1	1	1	1	1	8
Liu 2014a	1	1	0	0	0	1	0	1	1	1	6
Liu 2016	1	1	0	0	1	1	1	1	1	1	8
Liu 2017	1	1	0	0	0	0	0	1	1	1	5
Liu 2019	0	0	0	0	0	1	0	1	1	0	3
Ma 2013	1	1	0	0	0	1	0	1	1	1	6
Malhorta 2011	1	1	0	0	1	1	1	1	1	1	8
Pignataro 2013	1	1	0	0	1	0	1	1	1	1	7
Qi 2012	1	1	0	0	1	0	1	1	1	1	7
Ren 2008	1	1	0	0	0	1	0	1	1	1	6
Ren 2009	1	1	1	0	1	1	1	1	1	1	9
Ren 2011	1	1	0	0	0	0	0	1	1	1	5
Ren 2015	1	1	0	0	1	0	1	1	1	1	7
Ren 2015a	1	1	0	0	1	0	1	1	1	1	7
Ren 2018	1	1	0	0	1	0	1	1	1	1	7
Shan 2013	1	1	0	0	1	1	1	1	1	1	8
Silachev 2012	1	1	0	0	0	0	0	1	1	1	5
Silachev 2017	1	1	0	0	0	1	0	1	1	1	6
Su 2014	1	1	0	0	1	1	1	1	1	1	8
Sun 2012	1	1	0	0	1	0	1	1	1	1	7
Wang 2011	1	1	0	0	1	1	1	1	1	1	8
Wei 2012	1	1	0	0	1	1	1	1	1	1	8
Xia 2017	1	1	0	0	1	0	1	1	1	1	7
Xiao 2015	1	1	0	0	1	1	1	1	1	1	8
Xiao 2017	1	1	1	0	0	0	0	1	1	1	6
Xu 2012	1	1	0	1	1	1	1	1	1	1	9
Xu 2017	1	1	0	0	1	1	1	1	1	1	8
Yang 2018	1	1	0	1	1	1	1	1	1	1	9
Zhang 2013	1	1	0	1	0	0	0	1	1	1	6
Zhang 2017	1	1	0	0	1	0	1	1	1	1	7
Zhou 2015	1	1	0	0	0	1	0	1	1	1	6
Zhao 2019	0	0	0	0	1	0	1	1	1	1	5
Zong 2015	1	1	0	1	1	1	1	1	1	1	9

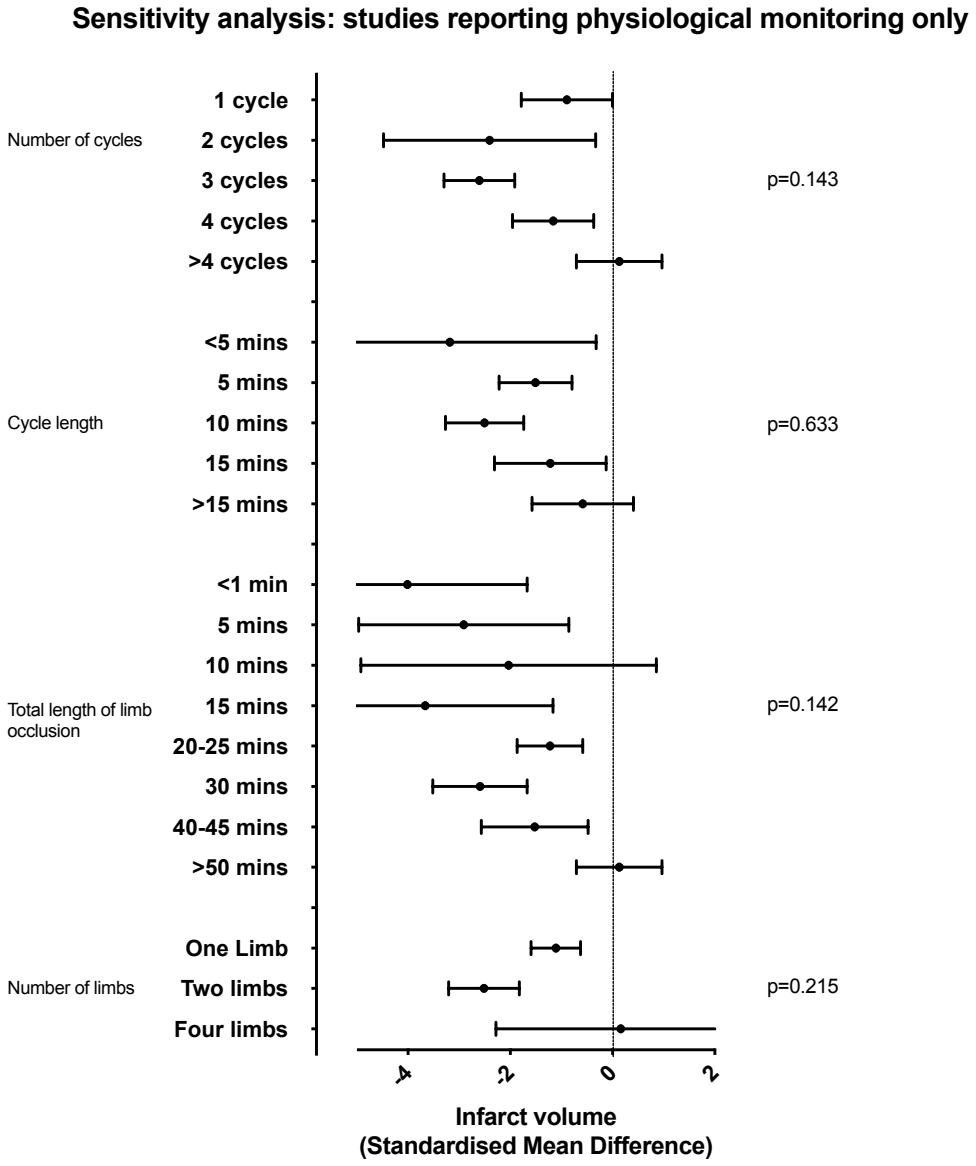
Supplementary Figure I

Database search process



Supplementary Figure II

Subgroup analyses of RIC in experimental stroke by studies reporting measurement of physiology during anesthesia. Each point estimate represents the change in infarct volume in treated animals compared to control, divided by subgroups according to animal model and RIC administration. The p values indicate whether the respective parameter has a significant interaction with infarct volume (meta-regression analyses).



Supplementary Figure III

Subgroup analyses of RIC in experimental stroke by country. Each point estimate represents the change in infarct volume in treated animals compared to control, divided by groups according to animal model and RIC administration ((a) all studies; (b) RIPreC; (c) RIPerC & RIPostC). The p-values indicate a significant interaction by country with infarct volume (meta-regression, p=0.002).