SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Pre- and postconditioning methods in traumatic brain injury – A systematic review of animal studies	
2.	Authors (names, affiliations, contributions)	- H. F. den Boogert† – setting up review protocol, design search strategy, in- and exclusion criteria, data extraction, data-analysis, quality and risk of bias assessment, writing paper - Dr. K. E. Wever‡ - supervising review protocol, design search strategy, in- and exclusion, data-analysis, supervising research, critical revision of manuscript - J. van Luijk‡ – supervising review protocol, design search strategy, in- and exclusion, data-analysis, supervising research, critical revision of manuscript - Prof. dr. van der Hoeven§- critical revision of manuscript - Prof. dr. Rongen* - critical appraisal review protocol, supervising writing paper, critical revision of manuscript - Prof. dr. R.H.M.A. Bartels† – critical appraisal review protocol, article selection, data-analysis, quality and risk of bias assessment, supervising writing paper, critical revision of manuscript † Department of Neurosurgery, RadboudUMC, Nijmegen ‡ Department of Systemic review Centre for Laboratory animal Experimentation (SYRCLE) § Department of Intensive Care Medicine, RadboudUMC, Nijmegen * Department of Internal Medicine and Pharmacology & Toxicology, RadboudUMC, Nijmegen	
3.	Other contributors (names, affiliations, contributions)	A. Tillema, Medical library Radboud University, Nijmegen –design search strategy	
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5.	Funding sources/sponsors	NA	
6.	Conflicts of interest	none	
7.	Date and location of protocol registration	Nijmegen, 22-12-2015	
8.	Registration number (if applicable)	NA	
9.	Stage of review at time of registration	Preliminary searches performed	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention?	Severe traumatic brain injury (TBI) is worldwide a major cause of traumatic disability and death. On average 200 and 103 per 100,000 persons are admitted for TBI annually in Europe and the US,	

	Why is it important to do this review?	respectively, with a mortality incidence rate of 15 and 18 per 100.000 persons.[1-3] Primary traumatic brain injury occurs simultaneously with the trauma as a result of the direct forces of impact. The complex interplay of biochemical, cellular and genomic changes of damaged tissue in minutes to days after the trauma can lead to secondary traumatic brain injury, which is characterized by increased necrosis and apoptosis, cerebral ischaemia, cytotoxic edema and increased intracranial pressure.[4] Currently, treatment strategies aim at early identifying the evolvement of secondary traumatic brain injury and subsequent clinical worsening (neuro-monitoring), and they try to counteract this process by means of directed ICU therapy or surgical procedures. However, these measures remain topics of discussion amongst experts and an effective treatment to reduce or prevent secondary traumatic brain injury is still lacking. Conditioning a target organ by rendering it more resistant to a lethal stressor through applying a sublethal stimulus or stressor before (pre), during (per) or after (post) the lethal event, has showed very promising results in cardiac research,[5,6] kidney,[7] and in ischemic brain disease (e.g. stroke).[8,9] In stroke models often an ischemic sublethal stimulus is used to induce an ischemic tolerance state, but also other stimuli are known to be effective.[10,11] In contrast to the abundancy of ischemic brain injury studies, research to the potential therapeutic merits of these concepts in TBI research has been very limited, and has mainly focussed on the use of preconditioning.[12] This is surprising since the unpredictable nature of TBI makes preparing the brain at forehand impossible. To date, a systematic review on the potentially beneficiary role of both pre- and postconditioning in TBI is lacking. Results so far of preclinical trials are, however, very promising and mandate further (translational) research. This review aims at filling out this gap of a structured, well-designed, systematic review on both pre-, per- and postconditioning in TBI. The goal of this review is to comprehensively aggregate all known stimuli that induce endogenous neuroprotection in experimental TBI research. A meta-analysis will be done if possible to assess the effect of this stimuli on TBI-related outcomes. This review will be used to identify knowledge gaps and 'ethically justify' investigating new stimuli in animal models in the search for an effective treatment of secondary traumatic brain injury. More information on the subject can be found in appendix A. References: 1. Tagliaferri F, Compagnone C, Korsic M, Servadei F, Kraus J. A systematic review of brain injury epidemiology in Europe. Acta neurochirurgica. 2006;148(3):255-68; discussion 68. 2. Corrigan JD, Selassie AW, Orman JA. The epidemiology of traumatic brain injury. J Head Trauma Rehabil. 2010;25(2):72-80. 3. Maas AI, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. The Lancet Neurology. 2008;7(8):728-41. 4. Kunz A, Dirnagl U, Mergenthaler P. Acute pathophysiological processes after ischaemic and	

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	Research question		
11.	Specify the disease/health problem of interest	Traumatic brain injury (TBI), with in particular the occurrence of secondary traumatic brain injury	
12.	Specify the population/species studied	Animals	
13.	Specify the intervention/exposure	Any conditioning stimulus	
14.	Specify the control population	Non-conditioned animals: receiving the same trauma without a conditioning stimulus.	
15.	Specify the outcome measures	Outcomes related to brain damage after trauma	
16.	State your research question (based on items 11-15)	What are the neuroprotective effects of conditioning strategies (regardless of timing) on secondary traumatic brain damage in animal models of TBI, when compared to non-conditioned animals? *Sub-questions:* 1. Which pre-, per- or postconditioning stimuli are currently known in TBI animal models? 2. What are the neuroprotective effects of these pre-, per- and postconditioning strategies on TBI inflicted brain damage in animal models of TBI, when compared to non-conditioned animals? 3. Which knowledge gaps can still be found and offer an opportunity for investigating novel conditioning methods in the field of experimental TBI research?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	X MEDLINE via PubMed X Web of Science ☐SCOPUS X EMBASE X Other, namely: SciELO Citation Index, KCI-Korean Journal Database ☐Specific journal(s), namely:	

18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	(Based on step-by-step guide, Leenaars et al, 2012) From our research question we first determined the most important search components: TBI and pre-, per- and postconditioning. We collected MeSH (PubMed) and Emtree (EMBASE) terms, and identified all possible free-text terms and searched for them in title, abstract and keywords (only EMBASE). In all the databases an animal filter was used. It has been proven difficult to refine the search terms so that they would include only traumatic brain injury models, and exclude ischemic tolerance experimental disease models. Attempts to do so also resulted in the omission of TBI models from the search results. Therefore, no such restriction was applied. The authors are aware that this will decrease the specificity of the search, but increase its sensitivity. In the realization of this protocol an own literature database with suitable articles was made by one of the authors which we used to evaluate the specificity of the search. New free-text terms were determined from the articles that were not found by the current search and were assessed for relevance by screening title and abstract of the articles that appear in the search results due to that specific term. The complete search for all the databases will become available after publication.	
19.	Identify other sources for study identification	X Reference lists of included studies ☐Books X Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	
20.	Define search strategy for these other sources	Included studies and relevant reviews were checked for references that are potentially relevant but were not found by our search in PubMed, EMBASE and Web of Science. Possibly relevant references will be identified based on its citation in the manuscript text and its title in the reference list. Subsequently the reference is assessed as described under 21-22.	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1. Removal of duplicates 2. Screening on title and abstract using EROS (web-based software designed to help organizing the early phases of SR (www.eros-systematic-review.org) 3. Full text evaluation for inclusion, also using EROS	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Screening on title/abstract (phase 2) and full text evaluation for inclusion (phase 3) will be done by two reviewers (HdB, RB). Discrepancies will be dealt with through discussion between the two reviewers until consensus is reached.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: - Primary study with unique data	

		- Presence of a non-conditioned control group Exclusion criteria: - Not a primary study (reviews, editorials, comments, conference abstracts/lectures) - No control group	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: - All animal types, any age and sex - Using (any) TBI model in both the experimental group as the control group (disease model) Exclusion criteria: - In vitro models, humans - Other forms of disease models (e.g. stroke models)	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: - All stimuli used before, during or after inflicting TBI Exclusion criteria: - none	
26.	Outcome measures	Inclusion criteria: All TBI-related outcomes (e.g. neuronal cell loss, lesion volume (histology), biomarkers, behavioural testing). Exclusion criteria: No TBI related-outcomes	
27.	Language restrictions	Inclusion criteria: All languages Exclusion criteria: None	
28.	Publication date restrictions	Inclusion criteria: All years of publication Exclusion criteria: none	
29.	Other	Inclusion criteria: animals undergoing only a head trauma Exclusion criteria: using other non-TBI experimental disease or damage models additionally to inflicted TBI (e.g. besides TBI also using a four-vessel occlusion model)	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase 2 (screening title/abstract): 1. No original data (no primary study, e.g. review, editorial, conference abstract) 2. No in vivo animal study 3. No use of an experimental TBI model Selection phase 3 (full text inclusion): Same as phase 2 with addition of 4. No relevant outcome measures 5. No relevant control group 6. animal models including other diseases or damage besides TBI 7. Full text not retrievable	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Author(s), title, journal, year of publication	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Number of experimental groups, number of control groups, number of animals per group. Brief description of the groups.	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Species, strain, sex, weight, comorbidities, housing conditions, genetically modified, type and duration of anesthesia, type of analgesics. Type and characteristics of TBI model used (e.g. weight drop model, fluid percussion model, controlled cortical impact) (Albert-Weissenberg & Siren, 2010)	
34.	Intervention characteristics	- Type of stimulus	

	(*e.g.* intervention, timing, duration)	- Timing in relation to trauma (pre-, per- or postconditioning) - Duration of stimulus - If intermitted how many cycles, duration per cycle - Time between stimulus and trauma (delay)	
35.	Outcome measures	Histology: lesion volume, cortical or hippocampal (CA1) neuronal cell loss, brain water content, blood-brain barrier integrity. Biochemical: biomarkers Imaging: post trauma MRI, PET, CT Neurobehavioral assessments, memory and sensorimotor evaluation	
36.	Other (*e.g.* drop-outs)	Number, reason of drop-outs.	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	Two reviewers will assess the risk of bias/study quality (HdB, RB). In case of discrepancies a discussion will be conducted until consensus is reached.	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	☐By use of SYRCLE's Risk of Bias tool[4] X By use of SYRCLE's Risk of Bias tool, adapted as follows: - Experimental model well described in detail? Y/N - Reporting on temperature Y/N - Reporting on blinding/randomisation Y/N - Reporting of a power/sample size calculation (Y/N) ☐By use of ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	Continuous - All histological outcome measures: lesion volumes [units: usually mm^3], neuronal cell loss [counts/mm^3]. Blood-brain barrier integrity, brain water content [various methods]. - Imaging: lesion volumes [mm^2], FDG uptake [optical densities in ROI] Both - Biomarkers can be both continuous variables when serum values [mmol/U] are used or dichotomous when only is reported if levels are elevated or reduced based on predetermined cut off values. Other Neurobehavioral, sensorimotor or memory testing: A preliminary search and studies from our own literature database (see also question 18), showed a wide variety of different tests used to assess neurological functioning after trauma. In order to address this variety we first provide an overview of all tests done by the included studies. Second, categories will be made with comparable tests to create more overview. Tests which are encountered as outcome measurements in four or more studies, will be taken into account for further analysis.	
40.	Methods for data extraction/retrieval (*e.g.* first	1. Data extraction from test, tables, and figures 2. In case of graphic data digital image software will be used to obtain	

	extraction from graphs using a digital screen ruler, then contacting authors)	these data 3. Authors will be contacted when data is missing or additional data is needed. In case of no response after at least 3 weeks and two attempts to reach the author, the article will be excluded. All data will be collected as mean and standard deviation (SD).	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	One reviewer will extract the data (HdB), a second reviewer (RB) will check the extracted data for inconsistencies. Disagreements will be discussed until consensus is reached.	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	A descriptive summary of all included studies and their outcomes parameters will be given. A meta-analyses will only be performed if sufficient data is present.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	A meta-analysis will be performed if outcome measurements are reported in the same specific manner in ≥4 studies. For subgroup analysis a minimum of 3 studies is required.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	Standardized mean difference for all continuous variables where baseline or sham data are not available. Normalised mean difference will be used if sham or baseline values are available, or if a conservative estimate can be made. In case of dichotomous values (e.g. biomarkers, certain neurobehavioral testing) we shall use risk ratio.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effect model. We expect high between-trial heterogeneity due to the fact that the type of experimental TBI model used amongst the studies will vary greatly and even if identical models are used, standardisation is often lacking.	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	(residual) I^2 and adjusted R^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	- Animal species - Comorbidity (e.g. age, hypertension) - Trauma related complications (e.g. wound infections, epilepsy, extracranial traumatic lesions) - Type and strength of experimental TBI model (e.g. weight drop model with or without open skull, fluid percussion model, controlled cortical impact) - Type, duration and dosage of anesthesia. - Type, duration and dosage of analgesics - Relation in time between stimulus and trauma (before, during, after) - Type of stimulus - Per stimulus category: frequency of stimuli - Per stimulus category: duration of stimuli - Per stimulus category: delay between stimulus and trauma	
48.	Any sensitivity analyses you	If applicable: the influence of categories made for e.g. duration and	

	propose to perform	delay of the intervention, pooling of neurobehavioural outcome measures, choice of time-point of outcome measure for data extraction.	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	If applicable, we will perform a Bonferroni-Holmes correction for testing multiple subgroups. In case several intervention groups are compared with only one control group, the number of animals in the control group will be divided by the number of comparisons made with this control group.	
50.	The method for assessment of publication bias	Funnel plots to assess publication bias when deemed appropriate If >10 studies are included in the meta-analysis, trim and fill analysis and/or Egger's test for small study effects will be used to determine Funnel plot asymmetry.	

Final approval by (names, affiliations):
Den Boogert, RadboudUMC

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