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	The effects of perinatal SSRI exposure on neurodevelopmental outcomes: a systematic review and meta-analysis of mammalian animal studies	
2.	Authors (names, affiliations, contributions)	Juliette Rando, BSc, Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, Netherlands: design study, data extraction analysis, meta-analysis, writing manuscript Lisa van de Wijer, MD, Department of Internal Medicine, Radboud University Medical Center: data extraction and analysis, meta-analysis, writing manuscript Judith Homberg, PhD, Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, Netherlands: writing and editing manuscript, third accessor data Arnt Schellekens Department of Psychiatry, Radboud university medical center, Nijmegen, Netherlands : editing manuscript Quirijn de Mast, Department of Internal Medicine, Radboud University Medical Center: editing manuscript Judith van Luijk, MSc, SYRCLE, Central Animal Laboratory, Radboud University Medical Center, Nijmegen, Netherlands: optimizing search strategy, statistics	
3.	Other contributors (names, affiliations, contributions)	Alice Tillema, Rikie Deurenberg-Vos, Medical Library, Radboud University Medical Center, Nijmegen, Netherlands: optimizing search strategy	
4.	Contact person + e-mail address	Juliette Rando, BSc, juliette.rando@gmail.com	

5.	Funding sources/sponsors	Funded by a grant awarded to J. Homberg from the Donders Institute for Brain, Cognition and Behavior.	
6.	Conflicts of interest	None	
7.	Date and location of protocol registration	Nijmegen, Netherlands	
8.	Registration number (if applicable)		
9.	Stage of review at time of registration	Full-text screening	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	The selective serotonin reuptake inhibitor (SSRI) paradox is the phenomenon by which SSRI exposure in adulthood improves symptoms of anxiety and depression, while *in utero* SSRI exposure increases the risk of anxiety, depression, autism, and sensorimotor issues. Most narrative reviews report that the SSRI paradox has been demonstrated in animal models. However, various primary preclinical studies have found no effect or a beneficial effect of perinatal SSRI exposure on neurodevelopment. These results parallel several clinical studies to date, which often do not identify long-lasting neurodevelopmental effects. We aim to systematically review primary preclinical studies in order to determine if there is an overall effect of perinatal SSRI exposure on neurodevelopment in animal models, and if so, under what conditions. The results of this review and meta-analysis may assist in understanding the mixed results of human studies.	
	Research question		
11.	Specify the disease/health problem of interest	Neurodevelopmental outcomes of perinatal SSRI exposure	
12.	Specify the population/species	All mammalian animal species	

	studied		
13.	Specify the intervention/exposure	Exposure to any SSRI starting before the developmental day equivalent to human birth in terms of neurogenesis, GABA cortex development, and axon extension. This period is specific to each species, and was calculated using the Translating Time tool by Workman *et al.,* 2013 [1]. Any administration method is relevant. 1. Workman, A.D., et al., *Modeling transformations of neurodevelopmental sequences across mammalian species.* J Neurosci, 2013. **33**(17): p. 7368-83.	
14.	Specify the control population	Exposure to a control starting before the developmental cut-off day outlined above.	
15.	Specify the outcome measures	Any behavioral outcome, including (but not restricted to) anxiety, depression, locomotor activity, sexual behavior, somatosensation, aggression, grooming behavior, social behavior, sensorimotor integration, cognition, impulsivity.	
16.	State your research question (based on items 11-15)	Does perinatal exposure to SSRIs impair neurodevelopment in animals?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	■ MEDLINE via PubMed ■ Web of Science ☐ SCOPUS ☐ EMBASE ■ Other, namely: PsycInfo ☐ 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])	When available, please add a supplementary file containing your search strategy: search_strategy.docx	
19.	Identify other sources for study	☐ Reference lists of included studies	

	identification	☐Books ■Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	
20.	Define search strategy for these other sources	Screening the reference lists for relevant titles and subsequently the abstracts of these identified studies (using same criteria as initial selection phase)	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1) Title and abstract in relation to research question; 2) Full text based on inclusion/exclusion criteria	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Two investigators (JR,LW) independently screen for phases 1 and 2 using EROS 3.0 (Early Review Organizing Software, Institute of Clinical Effectiveness and Health Policy, Buenos Aires, Argentina); discrepancies that cannot be resolved by discussion will be resolved by an additional investigator (JH)	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: primary, preclinical, controlled (regardless of study quality) Exclusion criteria: review, clinical, not controlled	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: all mammalian animal species, any sex, any age at testing, wildtype Exclusion criteria: humans, non-mammals, background genetic mutation	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: SSRI or control exposure, prenatal/perinatal/early postnatal exposure (starting before the period equivalent to human brain development at birth), any dosage Exclusion criteria: non-SSRI (e.g. Serotonin-norepinephrine reuptake inhibitors, Tricyclic	

		antidepressants, Monoamine oxidase inhibitors), SSRI in combination with another drug, adult or adolescent SSRI exposure, no repeated exposure, exposure from an environmental source, exposure to radioactive-tagged SSRIs	
26.	Outcome measures	Inclusion criteria: any behavioral test Exclusion criteria: no behavioral assessment	
27.	Language restrictions	Inclusion criteria: all Exclusion criteria: none (unless no English translation available)	
28.	Publication date restrictions	Inclusion criteria: all Exclusion criteria: none	
29.	Other	Inclusion criteria: Exclusion criteria:	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase 1 (tiab): 1. Not an original primary study (e.g. review, editorial, conference abstract without full data available or data published in duplicate) or correction to an original primary study 2. Not an *in vivo* mammalian (non-human) study 3. No SSRI treatment Selection phase 2 (full text): 1. Not an original primary study (e.g. review, editorial, conference abstract without full data available or data published in duplicate) or correction to an original primary study 2. Not an *in vivo* mammalian (non-human) study 3. No SSRI treatment 4. No perinatal/ prenatal/ early postnatal exposure (starting before the period equivalent to human brain development at birth) 5. No behavior analyses 6. No control population 7. Animals subjected to other factors (e.g. genetic mutation, exposure to additional drug). However, studies in which animals (or their mothers) were exposed to stress are included, because these studies are likely to be relevant to the translational human case. 8. No repeated exposure 9. No English full text available	

	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	authors, year, title	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	number of groups number of animals per group (total and per test) number of litters per group (total and per test) litter size repeated measures vs. comparison between groups	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	species strain sex age at testing (per test) presence/absence of stress exposure	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	type of control type of SSRI age and duration of exposure administration method dosage (concentration, volume of administration)	
35.	Outcome measures	behavioral test used test outcome	
36.	Other (*e.g.* drop-outs)	number of animals excluded from statistical analysis reason for excluding animals	
	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 (JR, LW) will assess the risk of bias/study quality in each study. Discrepancies that cannot be resolved by discussion will be resolved by an additional investigator (JH).	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and	■ By use of SYRCLE's Risk of Bias tool[4] ☐ By use of SYRCLE's Risk of Bias tool, adapted as follows: ☐ By use of CAMARADES' study quality checklist, e.g. [22]	

	attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	☐By use of CAMARADES' study quality checklist, adapted as follows: ■Other criteria, namely: Was it stated that the experiment was randomized? Was it stated that the experiment was blinded? Is a power/sample size calculation shown?	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	In general, the data will be continuous (generally measured in time, distance, # of times repeating a specific behavior). Units of measurement tend to be seconds/minutes, cm, frequency of performing a certain behavior. In some cases, the data may be categorical/ordinal; for example, in the swimming behavior test, animals are given a score 1-4.	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	1. Extract data from text/tables 2. Extract data from figures using digital image analysis software (ImageJ; http://rsbweb.nih.gov/ij/) by two independent reviewers (JR, LW) 3. Contact authors for missing data	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	One reviewer (JR) will extract data. A second reviewer (LW) will check the extraction process.	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Each behavior (e.g. anxiety, memory or locomotor activity) will be analyzed separately. Separate meta-analyses will be performed for each behavior with enough data (at least 5 studies) including subgroup analyses and sensitivity analyses. A descriptive summary will be written about behaviors for which a meta-analysis is impossible.	
43.	Specify (per	If at least 5 studies are found per behavior, data will	

	outcome measure) how it will be decided whether a meta-analysis will be performed	be pooled for that outcome measure. Heterogeneity will be investigated through subgroup analyses.	
	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)	We will use mean differences if studies use the same experimental test with the same scoring system (e.g. EPM) but standardized mean difference if combining different tests for one behavior (e.g. EPM and open field test for anxiety behavior)	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	animal species sex presence/absence of stress exposure timing of behavioral test (early-life, adolescence, adulthood) type of SSRI test used to study specific behavior (e.g. EPM or open field test for anxiety)	
48.	Any sensitivity analyses you propose to perform	We will perform sensitivity analyses to assess if our underlying assumptions are appropriate and our results are robust.	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	We will perform a Holm-Bonferroni correction to correct for multiple testing. Considering our 6 subgroup analyses, our corrected p value would be 0.006. If we are unable to perform one or more subgroup analyses, we will adjust the p-value accordingly. If there are repeated measurements within the same group, we will pool data separately for the early-life, adolescence, and adult time points. If within one study several doses of an SSRI are	

		compared to one control group, we will divide the number of control animals by the total number of comparisons made with this group in order to correct for repeated use of one control group.	
50.	The method for assessment of publication bias	Funnel plot (if at least 10 studies included in meta-analysis)	
Final approval by (names, affiliations):		Date:	