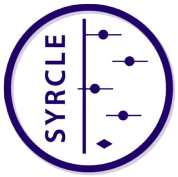

SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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VERSION 2.0 (DECEMBER 2014)

Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	A meta-analysis of ethanol withdrawal effects on anxiety-like behavior in zebrafish	
2.	Authors (names, affiliations, contributions)	Suianny Nayara Chaves da Silva[1], Monica Gomes Lima[2], Caio Maximino de Oliveira[1] [1] Laboratório de Neurociências e Comportamento, UNIFESSPA [2] Laboratório de Neurociências e Comportamento, UEPA	
3.	Other contributors (names, affiliations, contributions)	-	
4.	Contact person + e-mail address	Dr. Caio Maximino de Oliveira Laboratório de Neurociências e Comportamento, Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará, Marabá – PA – Brazil. Phone: +55 94 21017161, E-mail: cmaximino@unifesspa.edu.br OR lanec.unifesspa@gmail.com	
5.	Funding sources/sponsors	This research group received funds from CNPq (Brazilian public agency for research)	
6.	Conflicts of interest	None known	
7.	Date and location of protocol registration		
8.	Registration number (if applicable)		
9.	Stage of review at time of registration	Database complete. Preliminary data from PILOT STUDY 1 (based on the SYRCLE risk of bias tool). PILOT STUDY 2 is in progress.	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Ethanol withdrawal syndrome is one of the main complications of ethanol (EtOH) abuse, and a major drive of dependence by negative reinforcement (for a review, see [1]). Despite the difficulties of producing suitable models that mimic all the subjective, behavioural,	

		and neurobiological aspects of EtOH withdrawal syndrome, there are some animal models with good construct validity, mainly in rodents [1]. Most of these models are based on the anxiogenic effects of EtOH withdrawal, considering the inherent difficulties in modelling hallucinatory states such as delirium tremens [1]. In zebrafish, the anxiogenic effect of drug withdrawal has been demonstrated for different substances [2], including EtOH [3–5]; however, there are many inconsistencies in the effects of EtOH withdrawal, with some studies presenting non-standard group comparisons (e.g., [6]), and others failing to find a significant effect on primary outcomes (e.g., [7]). Besides differences between models, many different reasons may contribute for this low reproducibility, including methodological factors such as poor experimental design and low power analysis, as well as confirmation and publication biases. Therefore, the MAIN OBJECTIVE in this project is to estimate the influence of methodological variables on the outcomes of EtOH withdrawal. A PILOT STUDY 1 was performed to create a database and to evaluate the methodological quality of the published studies, using the SYRCLE Risk of Bias tool for animal studies [8]. Results of this PILOT STUDY indicated that reports on effects of EtOH withdrawal on anxiety-like behavior in zebrafish may neglect some of the actions to avoid bias, including random allocation to a treatment, concealment of treatment allocation, and non-selective outcome reporting. These preliminary data suggest the existence of publication bias, since more than XX% of the experiments evaluated rejected the null hypothesis for the primary outcome. Differences in methods to induce withdrawal syndrome – including differences in EtOH concentration during exposure, total exposure duration, and withdrawal duration – can represent an important source of heterogeneity. Therefore, an additional objective, proposed for PILOT STUDY 2, is to determine the effect sizes for primary outcomes according to EtOH concentration during exposure, total exposure duration, and withdrawal duration in protocols to induce EtOH withdrawal syndrome-like symptoms in zebrafish. This meta-analysis could help to create rational guidelines for preclinical studies using zebrafish to assess EtOH withdrawal syndrome. This protocol was created based on procedures available in [9]. References:	

		1. Gatch, M.B., and Lal, H. (2001). Animal models of the anxiogenic effects of ethanol withdrawal. Drug Dev. Res. 54, 95–115. 2. Stewart, A., Wong, K., Cachat, J., Gaikwad, S., Kyzar, E., Wu, N., Piet, V., Utterback, E., Elegante, M., Tien, D., et al. (2011). Zebrafish models to study drug abuse-related phenotypes. Rev. Neurosci. 22, 95–105. 3. Pittman, J.T., and Ichikawa, K.M. (2013). iPhone® applications as versatile video tracking tools to analyze behavior in zebrafish (Danio rerio). Pharmacol. Biochem. Behav. 106, 137–142. 4. Tran, S., Chatterjee, D., and Gerlai, R. (2015). An integrative analysis of ethanol tolerance and withdrawal in zebrafish (Danio rerio). Behav. Brain Res. 276, 161–170. 5. Holcombe, A., Howorko, A., Powell, R.A., Schalomon, M., and Hamilton, T.J. (2013). Reversed scototaxis during withdrawal after daily-moderate, but not weekly-binge, administration of ethanol in zebrafish. PLoS One 8, e63319. 6. Mathur, P., and Guo, S. (2011). Differences of acute versus chronic ethanol exposure on anxiety-like behavioral responses in zebrafish. Behav. Brain Res. 219, 234–239. 7. Cachat, J., Canavello, P., Elegante, M., Bartels, B., Hart, P., Bergner, C., and Kalueff, A. V (2010). Modeling withdrawal syndrome in zebrafish. Behav. Brain Res. 208, 371–376. 8. Hooijmans, C.R., Rovers, M.M., de Vries, R.B.M., Leenaars, M., Ritskes-Hoitinga, M., and Langendam, M.W. (2014). SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 14, 43. 9. de Vries, R.B.M., Hooijmans, C.R., Langendam, M.W., van Luijk, J., Leenaars, M., Ritskes-Hoitinga, M., and Wever, K.E. (2015). A protocol format for the preparation, registration and publication of systematic reviews of animal intervention studies. Evidence-based Preclin. Med. 1, e00007.	
	Research question		
11.	Specify the disease/health problem of interest	Animal model for ethanol withdrawal syndrome-associated anxiogenesis	
12.	Specify the population/species studied	Zebrafish	
13.	Specify the intervention/exposure	Chronic ethanol exposure and withdrawal	
14.	Specify the control population	Fish exposed to pure water	
15.	Specify the outcome measures	Primary outcomes: time on white compartment or preference index	

		(light/dark test); time on bottom (novel tank test); increase in distance from stimulus (antipredator response); decrease in distance from stimulus, or inter-fish distance (shoaling response)	
16.	State your research question (based on items 11-15)	1) assess the quality of reporting, with a descriptive summary of the relevant items; 2) look at the impact of items know to affect animal research (e.g., randomisation, blinding, etc.); 3) describe the impact of procedural variables (EtOH concentration during exposure, total exposure duration, and withdrawal duration) on primary outcomes of withdrawal.	
	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: ☐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])	A PubMed filter, designed to increase search efficiency for studies on animal experimentation (Hoojimans et al., 2010; doi: 10.1258/la.2010.009117), will be applied	
19.	Identify other sources for study identification	☐Reference lists of included studies ☐Books ☐Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	
20.	Define search strategy for these other sources	N/A	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	Publications returned from the searches will be downloaded, and its informations will be tabulated, with entries identified with DOI, publication date, title, and abstract. Two investigators will independently evaluate the titles and abstracts obtained to assess if they meet the inclusion criteria. Discrepancies between investigators will be resolved by discussion with a third investigator.	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	(A) Two per stage (B) Resolved by discussion with third investigator	
	Define all inclusion and exclusion criteria based on:		

23.	Type of study (design)	Inclusion criteria: Experimental studies involving behavioural effects Exclusion criteria: Studies in which no behavioural effect is reported	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: Adult zebrafish, from any strain or phenotype Exclusion criteria: Embryo, larvae, or juvenile zebrafish	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Studies in which behaviour is assessed in the absence of ethanol for at least 1 h Exclusion criteria: Studies in which animal behaviour is assessed in the presence of ethanol	
26.	Outcome measures	Inclusion criteria: Primary outcomes represented numerically or graphically, with at least, sample sizes, means and standard errors or deviations being reported. Exclusion criteria: Outcomes without appropriate summary statistics	
27.	Language restrictions	Any language	
28.	Publication date restrictions	No restriction	
29.	Other	Exclusion criteria: Experiments reporting co-treatments will be kept only if they also report experiments with single treatments.	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: Stage 1 (Title and abstract) 1. Studies in which no behavioural effect is reported 2. Studies not using adult zebrafish Selection phase: Stage 2 (Full text) 1. Studies in which animal behaviour is assessed in the presence of ethanol 2. Outcomes reported without appropriate summary statistics 3. Studies reporting only the results of co-treatments	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, year, DOI, full title	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	1. Experimental groups 2. Number of animals in each group 3. Statistical test used to compare groups 4. Whether a sequential design was applied (i.e., assessing the effects of chronic EtOH on behaviour and then assessing the effects of withdrawal on the same animal).	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	For each experimental cohort, strain and/or phenotype	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	1. Concentration of EtOH during exposure 2. Exposure duration 3. Withdrawal duration	

35.	Outcome measures	Primary outcomes: time on white compartment or preference index (light/dark test); time on bottom (novel tank test); increase in distance from stimulus (antipredator response); decrease in distance from stimulus, or inter-fish distance (shoaling response)	
36.	Other (*e.g.* drop-outs)	Whether the study reports attrition rates	
	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	(A) Two reviewers (B) Resolved by discussion with third investigator	
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] □By use of SYRCLE's Risk of Bias tool, adapted as follows: □By use of CAMARADES' study quality checklist, e.g [22] □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)	Mean and standard deviations or standard errors for each study will be extracted. These outcomes are expected to be continuous. For "time spent" outcomes, units are expected to be in s or % of trial. Preference indexes are dimensionless units.	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	1. From text 2. From graphs, using PlotDigitizer	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	(A) Two reviewers (B) Resolved by discussion with third investigator	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	1. Quality scores, based on the Risk of Bias tool, will be described for every publication 2. For the primary outcomes, effect sizes for every relevant experiment will be calculated as standardized mean differences (SMDs), with unbiased estimates of sampling variance. These SMDs will be subjected to a mixed-model meta-regression meta-analysis. 3. Based on effect sizes, sample sizes, and standard deviations, the	

		observed power will be calculated. Power analysis will help to determine the rate of “false negative” experiments. Correlations between SMDs and power will be estimated by curve fitting.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	Summary estimates will be provided when 3 or more experimental comparisons are available.	
	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 differences will be used to calculate effect sizes. Unbiased estimates of sampling variances and confidence intervals at the 95% level will be used.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Mixed-effects model	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	Heterogeneity will be assessed using I^2 and τ^2 heterogeneity values	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	1. Concentration of EtOH during exposure 2. Exposure duration 3. Withdrawal duration	
48.	Any sensitivity analyses you propose to perform	Influential case diagnostics will be made by inspecting plots for externally standardized residues, DFFITS values, Cook’s distances, covariance ratios, estimates of τ^2 and test statistics for residual heterogeneity when each study is removed in turn, hat values, and weights for each study included in the analysis.	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	1. A correction for multiple use of control groups will be used, with effective sample sizes for control groups will be calculated as $n'_c = \frac{n_c}{Treatment\ groups\ served\ by\ one\ control}$ 2. When opposite directions of an outcome are provided (e.g., increased time spent on bottom indicates increased anxiety, while increased time spent on the white compartment indicates decreased anxiety), effect sizes will be multiplied by -1 to indicate the worse outcome (increased anxiety) in the treatment group.	
50.	The method for assessment of publication bias	Publication bias will be assessed by inspection of a contour-enhanced funnel plot, with contours at the 90%, 95%, and 99% confidence intervals. Moreover, funnel plot asymmetry will be analysed using a meta-regression test, with total samples size as predictor	

Final approval by (names, affiliations):	1 - Caio Maximino de Oliveira, LaNeC, UNIFESSPA 2 – Monica Gomes Lima, LaNeC, UEPA 3 - Suianny Nayara Chaves da Silva, LaNeC, UNIFESSPA	Date: 29 Jun 2017

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