SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item	Section/Subsection/Item	Description
	A. General	
1.	Title of the review	A systematic review of preclinical studies exploring the role of insulin signalling in executive function and memory
2.	Authors (names, affiliations, contributions)	Martina Presta[1]: data extraction and analysis, writing the first draft of the manuscript Aet O'Leary [2]: data extraction and analysis, revising subsequent drafts, consolidating the manuscript and contributing to its final version Angela Maria Ottomana[1]: data extraction and analysis, writing the first draft of the manuscript Mairéad Sullivan [3]: data extraction and analysis Edoardo Pisa[1]: data extraction and analysis Giovanni Laviola[1]: revising subsequent drafts Geert Poelmans[4]: revising subsequent drafts, consolidating the manuscript Jeffrey Glennon[3]: data extraction and analysis, revising subsequent drafts, consolidating the manuscript and contributing to its final version Francesca Zoratto[1]: revising subsequent drafts, consolidating the manuscript and contributing to its final version David Slattery[2]: revising subsequent drafts, consolidating the manuscript and contributing to its final version Simone Macrì[1]: revising subsequent drafts, consolidating the manuscript and contributing to its final version Affiliations: [1] Center for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Rome Italy [2] Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, University Hospital Frankfurt, Frankfurt, Germany [3] Conway Institute of Biomolecular and Biomedical Sciences, School of Medicine, University College Dublin, Dublin, Ireland [4] Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands
3.	Other contributors (names, affiliations, contributions)	None
4.	Contact person + e-mail address	Francesca Zoratto: francesca.zoratto@iss.it
5.	Funding sources/sponsors	This review is supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 847879 (to JGG, SM, GP and DS) and by Regione Lazio "Progetto LazioInnova", POR FESR LAZIO 2014-2020 (ID code A0375E0169)
6.	Conflicts of interest	None
7.	Date and location of protocol registration	The protocol was submitted to the PROSPERO registry on 16/05/2022
8.	Registration number (if applicable)	Registration number: CRD42022331458 (12/06/2022)

9.	Stage of review at time of registration	Preliminary searches: Completed Piloting of the study selection process: Completed Formal screening of search results against eligibility criteria: Started Data extraction: Not yet started Risk of bias (quality) assessment: Not yet started Data analysis: Not yet started
	B. Objectives	
	Background	
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Beside its involvement in somatic dysfunctions (*e.g.* type 2 diabetes and metabolic syndrome), altered insulin signalling constitutes a risk factor for the development of mental disorders (*e.g.* Alzheimer's disease and obsessive compulsive disorder). While insulin-related somatic and mental disorders are often comorbid, the causal mechanisms and the directionality of this relationship are still elusive. Thus, just as altered insulin signalling may affect neuronal patterning and synaptic transmission, ultimately predisposing towards mental disorders, so also chronic alterations in glucose metabolism may be secondary to mental disease. Rodent models represent a valuable tool to identify cause-effect relationships and deconstruct the fundamental mechanisms involved in insulin-related mental and somatic comorbidities. These models are apt to prospective studies in which causative mechanisms can be manipulated via multiple tools (*e.g.* genetically engineered models, pharmacological studies and environmental interventions) and experimentally dissociated to control for potential confounding factors. Here, we will provide a narrative (qualitative) synthesis of available preclinical studies investigating the association between hyperglycaemia – as a proxy of insulin-related metabolic dysfunctions – and alterations in behavioural phenotypes isomorphic to symptoms of mental disturbances: working and spatial memory and attentional set-shifting. Ultimately, the present review will advance our knowledge on the role of glucose metabolism in the comorbidity between somatic and mental illnesses.
	Research question	
11.	Specify the disease/health problem of interest	Comorbidity among insulin-dependent somatic and mental disorders
12.	Specify the population/species studied	Rats and mice
13.	Specify the intervention/exposure	Rats and mice exhibiting hyperglycaemia as a function of any of the following: individual/strain differences; environmental manipulations (*e.g.* high fat diets, altered food availability); transgenic approaches; pharmacological modulations
14.	Specify the control population	Control strains, inbred or outbred, specific for those models selected for variations in glucose metabolism/insulin signalling; corresponding control condition for the environmental manipulations (*e.g.* subjects exposed to standard diets for models based on dietary interventions); wild-type controls for the transgenic approaches; vehicle-treated animals for the pharmacological modulations

15.	Specify the outcome measures	Behavioural phenotypes isomorphic to working memory, spatial memory and/or attention; if available, glucose metabolism-/insulin signalling-related parameters (obtained after the original induction of hyperglycaemia, for example through glucose tolerance, insulin resistance, etc.)
16.	State your research question (based on items 11-15)	Based on evidence from rodent models (rats and mice), is there a relationship between altered insulin signalling and mental disorders?
	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])	See the supplementary file containing the search strategy: "Search strategy.pdf"
19.	Identify other sources for study identification	☐Reference lists of included studies ☐Books ☐Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/organizations, 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)	First phase: screening based on title and abstract; second phase: full-text screening of the eligible articles
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	First phase: two independent observers per article (MP and AMO) Second phase: two independent observers per article per affiliation site (MP and AMO; AO and DS; MS and JGG); differences will be solved through discussion or by consulting a third investigator (SM or JGG) To ascertain consistency in the full-text screening across sites, an identical subset of articles will be reviewed independently at each site and evaluated jointly in a dedicated meeting
	Define all inclusion and exclusion criteria based on:	
23.	Type of study (design)	Inclusion criteria: No restrictions on the types of study design eligible for inclusion will be applied Exclusion criteria: None
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: Rats and mice, both sexes, all ages Exclusion criteria: Studies in vitro; studies in humans; studies in non-human animals other than rats and mice

25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Direct/specific manipulation of glucose metabolism/insulin signalling (*e.g.* pharmacological modulations, transgenic approaches, etc.) or more indirect/generic manipulation (individual/strain differences, environmental manipulations) that result in hyperglycaemia Exclusion criteria: Experimental manipulations not resulting in hyperglycaemia
26.	Outcome measures	Inclusion criteria: Behavioural phenotypes of interest (working memory, spatial memory and/or attention) Exclusion criteria: Outcome measures other than working memory, spatial memory and/or attention
27.	Language restrictions	Inclusion criteria: English language Exclusion criteria: Language other than English
28.	Publication date restrictions	Inclusion criteria: All publication dates Exclusion criteria: None
29.	Other	Inclusion criteria: Original research; full-text article Exclusion criteria: Non-original research (*e.g.* review, commentary, editorial, book chapter); no full-text article (*e.g.* meeting abstract)
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: screening based on title and abstract 1. Language other than English 2. Non-original research (*e.g.* review, commentary, editorial, book chapter) 3. No full-text article (*e.g.* meeting abstract) 4. Studies in vitro; studies in humans; studies in non-human animals other than rats and mice 5. Outcome measures other than working memory, spatial memory and/or attention Selection phase: full-text screening of the eligible articles 5. Outcome measures other than working memory, spatial memory and/or attention 6. Experimental manipulations not resulting in hyperglycaemia 7. Other control conditions (*e.g.* low-fat diet used as control instead of standard diet, etc.)
	Study characteristics to be extracted (for assessment of external validity, reporting quality)	
31.	Study ID (*e.g.* authors, year)	DOI, title, authors, publication year, journal
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Number of experimental groups, number of subjects per group, type of study design (*i.e.* within- vs. between-subjects)
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Species, strain, sex, age and/or weight at the beginning of the study, type of test used to evaluate spatial memory, working memory and/or attention
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Type of experimental manipulation adopted to induce hyperglycaemia, details regarding the experimental manipulation, type of non-hyperglycaemic control, details on the assessment of hyperglycaemia (*i.e.* higher blood glucose concentrations compared to controls and/or to a predefined threshold; with or without fasting)

35.	Outcome measures	Direction of the variation of the behavioural phenotypes isomorphic to working memory, spatial memory and/or attention in experimental subjects exhibiting hyperglycaemia and in non-hyperglycaemic controls If available, variation of glucose metabolism-/insulin signalling-related parameters (obtained after the original induction of hyperglycaemia, for example through glucose tolerance, insulin resistance, etc.)
36.	Other (*e.g.* drop-outs)	None
	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) The criteria will be independently assessed by two reviewers per affiliation site (MP and EP; AO and DS; MS and JGG) (b) Differences of opinion that cannot be resolved by discussion will be solved by principal investigators of each site (FZ, SM, JGG, DS and GP) during joint meetings To ascertain consistency in the assessment across sites, an identical subset of articles will be reviewed independently at each site and evaluated jointly in a dedicated meeting
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)	Variation of the behavioural phenotypes of interest, usually expressed as percent preferences (*e.g.* preference for a specific stimulus in working memory tests), continuous data (*e.g.* latency to reach the target zone in spatial memory tests), absolute values (*e.g.* number of trials and errors in tests for attention) Variation of glucose metabolism-/insulin signalling-related parameters (if available), usually expressed as differences in absolute values or percent variations over baseline in glucose concentrations in response to glucose/insulin administration, etc. Units of measurement vary depending on the specific test applied and will entail continuous, integer and dichotomous data
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	The direction of the variation of the behavioural phenotypes of interest (*e.g.* memory/attention impairment/improvement) will be retrieved (no quantitative data will be extracted) If available, the variation of glucose metabolism-/insulin signalling-related parameters will be also retrieved
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	(a) The data will be independently extracted by two reviewers per site (MP and AMO; AO and DS; MS and JGG) (b) Discrepancies will be resolved by principal investigators of each site (SM, JGG, DS and GP) during joint meetings To ascertain consistency in data extraction across sites, an identical subset of articles will be reviewed independently at each site and evaluated jointly in a dedicated meeting

	Data analysis/synthesis	
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	A narrative (qualitative) synthesis will be performed
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	We anticipate that there will be limited scope for quantitative synthesis (meta-analysis) because the included studies are expected to be not sufficiently homogenous
	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)	None planned as this is a narrative (qualitative) synthesis
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	None planned as this is a narrative (qualitative) synthesis
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	None planned as this is a narrative (qualitative) synthesis
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	This is a qualitative synthesis and while subgroup analyses may be undertaken it is not possible to specify the groups in advance
48.	Any sensitivity analyses you propose to perform	None planned as this is a narrative (qualitative) synthesis
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	None planned as this is a narrative (qualitative) synthesis
50.	The method for assessment of publication bias	None planned as this is a narrative (qualitative) synthesis

Final approval by (names, affiliations):
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