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ADMINISTRATIVE INFORMATION

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Registration

Insert registration/publication when applicable.
This protocol will be reported in accordance with the 2015 PRISMA-P guidelines (Moher D et al., 2015).

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Author's Contributions

Dexter Merenick: Development of protocol (introduction, methods), abstract and full text screening, data extraction, writing of review manuscript.
Anisha Jessel: Development of protocol (administrative information, methods), abstract and full text screening, data extraction, writing of review manuscript.
Heather Ganshorn: Development of search strategy, development and editing of protocol, writing of search methodology section of review.
Daniel S.J. Pang: Supervising graduate and summer student work, development and editing of protocol, editing of manuscript.

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INTRODUCTION

Rationale

A common method used as a proxy to assess loss of consciousness (LOC) in rodents is the loss of righting reflex (LORR). When an animal is placed on its back, the righting reflex activates vestibular organs that first initiate head and eye movements. Next, the rest of the body is turned into normal positioning in respect to gravity (Simpson & Finch, 1988). By definition, an animal has successfully lost its righting reflex when the animal is in dorsal recumbency with all limbs up in the air and fails to orient itself onto all four paws into ventral recumbency or a standing position (Shirasaka *et al.*, 2011). Failure to return to ventral recumbency or a standing position is known as a positive LORR test. By contrast, a negative LORR test is defined as the presence of a righting reflex, and therefore, the animal actively rights itself (Chisholm & Pang, 2016).

There is a strong positive correlation between LOC in humans and LORR in rats and mice for various anesthetic drugs (Franks, 2008). Many studies have used LORR as a behavioral outcome to identify LOC in rodents; however, there are many inconsistencies on how the method is performed across studies.

Reported measurements from LORR assessment include the behavioral outcome, test duration, and postural assessment. Behavior is reported as a positive or negative righting attempt. Studies have defined a negative LORR test as the ability for the animal to right itself onto all four paws (ventral recumbency; Hwang, 2010; Shirasaka, 2011; Guidera, 2017), while others define it as the ability for the animal to turn onto its side (lateral recumbency; Mesbah *et al.*, 2021). The LORR test duration is calculated from the moment the animal is placed in dorsal recumbency. Studies have reported waiting 10, 15 or 30 seconds and upwards to 2 minutes (Tung *et al.*, 2005; Pang *et al.,* 2009; McCarren *et al.*, 2013; Baker *et al.*, 2014; Chisholm & Pang, 2016; Katayama *et al.*, 2007; Wang *et al.*, 2016; Mesbah *et al.*, 2021;). Finally, postural assessment is based on the methods used to place the animal onto its back. Described methods include manual positioning (by hand), tilting a chamber containing the animal, or using a motor-driven rotating cylinder (Katayama *et al.,* 2007; Thomas, 2012; Baker *et al.*, 2014; Gelegen *et al.*, 2014). The inconsistencies in LORR methodology increases the likelihood of inconsistent results, and reduces the ability to compare results between studies; therefore, a systematic review will outline and summarize the methodology of LORR reported in the literature.

Objective

This systematic review protocol will evaluate the following question: How is the loss of righting reflex performed across studies to assess unconsciousness during induction of general anesthesia in rats and mice? The overall aim of this paper is to critically assess and evaluate the current published knowledge surrounding the LORR methodology and how it is performed.

METHODS

Eligibility Criteria

The systematic review question was constructed using the PICO framework (i.e. Population, Intervention, Comparison, and Outcome; Shamseer *et al.*, 2015). Articles will be excluded if they are not an experimental or observational study. In addition, studies that use methods to assess LOC other than LORR will be excluded. Finally, articles that do not use general anesthesia to induce LOC will be ineligible.

Study characteristic	**Description**
Population	Rats, Mice
Intervention	Loss of righting reflex (LORR)
Comparison	N/A
Outcome	Assessing loss of consciousness (LOC) during induction of general anesthesia

Population: Studies will focus on both rats and mice. All other animals will be excluded from the analysis.

Intervention: Selected studies must contain LORR as a method for identifying LOC. Articles containing more than one method of assessing LOC, including LORR, will be eligible.

Outcome: Studies selected must use LORR as a method for identifying LOC. Studies will also be considered eligible if euthanasia is an endpoint of the study.

Study designs and language: Peer reviewed articles published in English. No date restrictions were put in place. Eligible studies include that of experimental or observational design.

Information Sources

Literature searches will be conducted using subject headings and keyword searches related to the loss of righting reflex, anesthesia, and mice and rats. The searches will be conducted using the following electronic databases and onset dates: MEDLINE (OVID interface), CAB abstracts (EBSCO interface), and Web of Science (Clarivate) from inception to June 2022. A research librarian from the University of Calgary (HG) will be conduct the search strategy. The MEDLINE search strategy below will be translated to the other databases

Search Strategy

Database(s): **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily** 1946 to June 09, 2022
Search Strategy:

#	Searches	Results
1	exp Rats/	1676871
2	exp Mice/	1728890
3	(rat or rats or mouse or mice).kf,tw.	2708761
4	1 or 2 or 3	3549703
5	exp Reflex, Righting/	244
6	((loss adj5 "right* reflex") or LORR).kf,tw.	1284
7	5 or 6	1423
8	exp Anesthesia/	201754
9	an?esthe*.kf,tw.	413639
10	exp Unconsciousness/	43524
11	(conscious* or unconscious* or LOC).kf,tw.	101653
12	8 or 9 or 10 or 11	597431
13	4 and 7 and 12	435

Data Management

Literature searches will all be recorded and managed using Covidence (Veritas Health Innovation, Melbourne, Australia). Title, abstract and full text screening will be recorded in Covidence and duplicates will be removed by the software

Selection Process

Primary reviewers (DM, AJ) are PhD and undergraduate students, respectively, of the University of Calgary conducting research in the Faculty of Veterinary Medicine. For phase one, both reviewers will independently screen titles and abstracts of the database searches. Both individuals will come to a consensus on the eligibility of independently selected articles based on inclusion and exclusion criteria. Duplicate references will be removed by the Covidence software. In phase two, each selected full-text reference from phase one will be assessed by both reviewers (DM, AJ) using Covidence. Ineligible articles will be removed on communal consensus based on inclusion and exclusion criteria. Studies are to be removed if they do not include one or more inclusion criteria or if they are not relevant to measuring LOC using LORR methodology.

Data Collection Process

Two reviewers (DM, AJ) will conduct the data collection independently. The collected data will include the following: publication details (title, author(s), publication date, language, geographic location), study design, species/animal type (mice, rats), population characteristics (age, production group), intervention type (loss of righting reflex), and outcome type (loss of consciousness during induction of general anesthesia).

Outcomes and Prioritization

The major outcome will be loss of consciousness in the animal through induction of general anesthesia, assessed through a loss of righting reflex inclusive methodology. A triad of general anesthesia induction, loss of consciousness as a result, and assessment based on the loss of righting reflex must be identified within the study.

Risk of Bias in Individual Studies

In each individual study reviewed, the *Risk of Bias 2 Tool* (*RoB 2*) from the Cochrane Review Handbook (Higgins et al., 2022) will be used to assess risk of bias.

The tool consists of 5 domains: (1) bias from the randomization process; (2) bias due to deviations from initial stated interventional; (3) bias from missing data in outcomes; (4) bias from outcome measurement; and (5) bias in selection of reported results. Each assessment made regarding a study will focus on a particular result from a randomized trial, and the results analyzed must have relation to the main outcomes of the review (each result included in the review's findings). Each domain contains "signaling questions" designed to extract relevant information, with the following possible answers: "yes"; "probably yes"; "probably no"; "no"; and "no information". From each domain, a qualitative risk of bias judgment is suggested by a provided algorithm based on answers to the signaling questions, with the following options: "low", "high", or "some concerns". Answers to signaling questions and final domain judgments will have written justifications. Lastly, the overall risk of bias (for each result) will correspond to the least favourable assessment made across all 5 domains. If any of the judgments made are ruled out or changed, written justification will be provided.

The risk of bias assessment will be conducted by two reviewers (DM, AJ) independently, and any disagreement on such will be discussed and resolved collaboratively.

The risk of bias determined for each result/study will be recorded and taken into account when selecting final studies to include as well as when analyzing and interpreting data extracted.

Data Synthesis

The data and information collected during the production of this review will be synthesized in a narrative manner.

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