



The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item	Recommendation		Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods 2.3. The experimental unit was not explicitly reported in the manuscript.
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	Methods 2.3 and figure legends. Exact sample sizes are reported for some experiments; however, several figure legends report sample-size ranges (e.g., $n = 5-6$ or $n = 6-8$ per group), and the total number of animals used in the study is not reported. No <i>a priori</i> sample-size calculation was reported.
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established <i>a priori</i>. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of n in each experimental group.	Methods 2.3. Mice failing to achieve LORR within 5 min after propofol injection were excluded. However, it is not stated whether this criterion was established <i>a priori</i> , and inclusion/exclusion criteria for the other experiments or data points are not reported. Methods 2.3. An exclusion criterion was reported for the LORR/RORR experiment; however, the number of animals actually excluded from each experimental group was not reported. Figure legends. Exact n values are reported for some analyses; however, several figure legends report ranges (e.g., $n = 5-6$ or $n = 6-8$ per group), so the exact n for each experimental group is not reported for all analyses.
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Methods 2.9. Mice were randomly assigned to control and treatment groups; however, the method used to generate the randomisation sequence was not reported. Strategies used to minimise potential confounders, such as treatment order, measurement order, or cage location, were not reported.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Methods 2.9. Behavioral tests were stated to be conducted in a blinded fashion; however, it was not specified who was aware of group allocation during allocation, experimental procedures, outcome assessment, and data analysis.
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Methods 2.3 and 2.6–2.8; Results 3.1–3.6. Primary outcome used for sample-size determination was not reported.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods 2.9. Methods 2.9. Normality is mentioned, but the method used to assess normality and the approach taken when statistical assumptions were not met were not reported.
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Methods 2.2 Methods 2.2; Further information not reported.
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Methods 2.3–2.8 Methods 2.3–2.8. Methods 2.2, 2.4 and 2.6. Housing conditions, postoperative recovery, handling habituation and tethering habituation are described; however, the experimental location is not consistently specified for all procedures. Introduction; Methods 2.3.
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Results 3.1–3.6; Fig. legends report mean $\pm$ SEM and n . Effect sizes with confidence intervals not reported.

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract; animal sex and the strain/background of the wild-type mice are not reported in the Abstract.
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction Introduction; specific rationale for mouse model not explicitly stated.
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Introduction
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Methods 2.2 and Ethics Approval and Consent to Participate. The manuscript states that procedures were approved by the Institutional Animal Care and Use Committee, whereas Approval No. ACE-003-2023 is attributed to the Ethics Committee. The committee name associated with the approval number should be clarified and made consistent.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Methods 2.2. Housing conditions are partially reported; environmental enrichment is not reported.
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Methods 2.4 and 2.7. Anesthesia and supportive measures are described; however, perioperative/postoperative analgesia following stereotaxic surgery is not reported. Adverse events not reported. Not reported. Humane endpoints, monitored clinical signs, and the frequency of animal monitoring were not described.
Interpretation/scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Discussion. Discussion (Limitations). Several study limitations are discussed; however, potential sources of bias, limitations of the animal model, and imprecision are not explicitly addressed.
Generalisability/translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Not reported.
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	No statement was provided regarding whether a study protocol was prepared before the study or whether/where the protocol was registered.
Data access	20	Provide a statement describing if and where study data are available.	Data access was reported in the section of A
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	Conflicts of Interest was reported in the section of Conflicts of Interest. The authors declare no conflicts of interest. Funding sources and the grant number are reported in the Funding section; however, the role of the funder(s) in study design, analysis, and reporting is not stated.