ARRIVE The ARRIVE Guidelines Checklist

Item	ltem No	RECOMMENDATION	Reported on Page Number/Line Number	Reported on Section/Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	Page 1/line 2-4	Title
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	Page 2/line 29-51	Abstract
INTRODUCTIO	N			
Background	3	 a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology. 	Page 2-4/line 56-94	Introduction/P 1-3
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	Page 4/line 95-97	Introduction/P 4
METHODS				
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	Page 4/line 103 Page 7/li ne 187	Materials and Methods/P1 and P12
Study design	6	 For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out. 	Page 7/line 187	Materials and Methods/P1 2
Experimental procedures	7	 For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used). 	Page 4/line108-112 Page 7/line 188-189	Materials and Methods/P1 and P12

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8	 a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc. 	Page 7/line 185-186	Materials and Methods/P 2
9	 Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment. 	Page 7/line 186-187	Materials and Methods/P1 2
10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group.b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.c. Indicate the number of independent replications of each experiment, if relevant.	Page 7/line 188-189	Materials and Methods/P1 2
11	 a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed. 	Page 7/line 188-189	Materials and Methods/P1 2
12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	Page 5 and 6/line 135-167	Materials and Methods/P5 -9
13	a. Provide details of the statistical methods used for each analysis.b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron).c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	Page 7/line 194-197	Materials and Methods/P1 3
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14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	Page 11/line 306-308	Results/P6
15	 a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%²). b. If any animals or data were not included in the analysis, explain why. 	Page 11/line 306-308	Results/P6
16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	Page 7-12/line 199-395	Results/P1-P6
17	a. Give details of all important adverse events in each experimental group.b. Describe any modifications to the experimental protocols made to reduce adverse events.	Page 7-12/line 199-395	Results/P1-P6
	9 10 11 12 13 14 15 16	plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc. 9 Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of focd, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experimental group. b. Explain how the number of animals used in each experiment, and the number of animals in each experimental group. c. Indicate the number of independent replications of each experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed. 11 a. Give full details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to reach analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether t	plus age range) and weight (e.g. mean or median weight plus weight range). Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g., knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc. Page 7/line 186-187 9 Provide details of: a. Housing (type of facility e.g., specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). P. Lusbandry conditions (e.g., breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food and water, environmental anrichment). Page 7/line 186-187 10 a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. Page 7/line 188-189 11 a. Give full details of how animals were allocated to experiment, if relevant. Page 7/line 188-189 12 Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes). Page 7/line 188-189 13 a. Broycify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). Page 7/line 194-197 15 a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%?), b. If any animals or data were not included in the analysis, expliain why. Page 7.12/line 199-395 14 Report the results or each analysis carried out, with

DISCUSSION				
Interpretation/ scientific implications	18	 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 any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results². c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research. 		Discussion/P1, Discussion /P2, Discussion/P3, Discus sion/P4
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	Page 14/line 392	Discussion/P5
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	NO	NO

From:

Animal Research: Reporting In Vivo Experiments

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References:

- 1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412
- 2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ 340:c332.

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*As the checklist was provided upon initial submission, the page number/line number reported may be changed due to copy editing and may not be referable in the published version. In this case, the section/paragraph may be used as an alternative reference.