The ARRIVE guidelines 2.0: author checklist

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| **ITEM**  |  | **RECCOMMENDATION**  |

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| **Section/line** |
| **number, or reason** |
| **for not reporting** |

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| **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. | Figure 1, line 1Figure 2, line 1 |
| b. The experimental unit (e.g. a single animal, litter, or cage of animals). | Line 48 |
| **Sample size** | 2 | a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment | Line 51-52 |
| b. Also indicate the total number of animals used. | Line 43 |
| c. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done | Line 51-52. The sample size (n) was based on a power analysis where a 50% decrease in mean latency for control animals was considered biologically relevant; the standard deviation value was estimated as 35% of the estimated treatment group mean value; the alpha was set to 0.05 and the power to 80%. This gives a group size of 8 animals per group. |
| **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 *a priori.* If no criteria were set, state this explicitly. | Line 47; line 49 regarding males and females  |
| 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. | No exclusions |
| c. For each analysis, report the exact value of *n* in each experimental group. | See figure 1 and 2 legends |
| **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. | Line 49 |
| 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. | Line 49, to control sex biasLine 43 regarding controlled environment |
| **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). | Line 50 |
| **Outcome****measures** | 6 | a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). | Line 56 and 57 |
| b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. | N/A |
| **Statistical****methods** | 7 | a. Provide details of the statistical methods used for each analysis, including software used. | Line 63;Figure 1 and 2 legends |
| 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. | Data were normally distributed |
|  |  |
| **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. | Line 13 |
| b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. | Line 43-47 |
| **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. | Line 54-57 |
| b. When and how often. | Line 54-57 |
| c. Where (including detail of any acclimatisation periods). | Line 43-47 |
| d. Why (provide rationale for procedures). | Line 83-84;Line 91 |
| Results | 10 | For each experiment conducted, including independent replications, report: |  |
| 1. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).
 | Figure 1 and 2 legends |
| 1. If applicable, the effect size with a confidence interval.
 | N/A |
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|  |  | The Recommended Set |  |
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| **Abstract** | 11 | Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions. | See abstract. Line 13-23 |
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| **Background** | 12 | a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. | Line 34-39 |
| b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology. | Line 30-32 |
| **Objectives** | 13 | Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested. | Line 37-39 |
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| **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 (ifapplicable). If ethical approval was not sought or granted, provide a justification. | See section on ethical approval and consent to participate-line 131-137 |
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| **Housing and** | 15 | Provide details of housing and husbandry conditions, including any environmental enrichment. | Line 43-47 |
| **husbandry** |  |
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| **Animal care and****monitoring** | 16 | a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. | -Line 54, according to ref (8), animals were acclimatized to the tail flick and hotplate machines before experiments start-Line 56-57 |
| b. Report any expected or unexpected adverse events. | N/A |
| 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. | Line 56-57 |
| **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 sectionSee limitation section; line 102-104 |
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| **Generalisability/****translation** | 18 | Comment on whether, and how, the findings of this study are likely to generalize to other species or experimental conditions, including any relevance to human biology (where appropriate). | Line 97-100 |
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| **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 study preregistration |
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| **Data access** | 20 | Provide a statement describing if and where study data are available. | Line 128 |
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| **Declaration of** |
| **interests** |

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| a. Declare any potential conflicts of interest, including financial and non-financial. |
| If none exist, this should be stated. |

 | Line 143 |
|  |  | b.List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study | Line 124-125 |
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**PART 2**

**ANIMALS**

LIE 57-58: Each animals was euthanized by cervical dislocation after the experiment.