



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: - The groups being compared, including control groups. If no control group has been used, the rationale should be stated. - The experimental unit (e.g. a single animal, litter, or cage of animals).	Section 2.1 Animal Surgery. Twelve rats were allocated to the TUS group (n = 6) and the Sham group (n = 6). Section 2.1 Animal Surgery. The experimental unit was an individual rat.
Sample size	2 - 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. - Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	Section 2.1 Animal Surgery. A total of 12 rats were used, with 6 rats allocated to the TUS group and 6 rats to the Sham group. Section 2.1 Animal Surgery. No <i>a priori</i> sample size calculation was performed.
Inclusion and exclusion criteria	3 - 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. - 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. - For each analysis, report the exact value of <i>n</i> in each experimental group.	Section 2.1 Animal Surgery. All animals meeting experimental criteria were included. Section 2.1 Animal Surgery. No animals, experimental units, or data points were excluded from the final analysis. TUS group, n = 6; Sham group, n = 6 for each analysis; means $\pm$ SEMs
Randomisation	4 - 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. - 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.	Section 2.1 Animal Surgery. Rats were randomly assigned to the TUS and Sham groups using a random-number table. Section 2.1 Animal Surgery. No specific strategies were applied to control potential confounders.
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).	Section 2.1 Animal Surgery. Outcome assessment and data analysis were performed by investigators blinded to group allocation.
Outcome measures	6 - Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). - For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Sections 2.1 and 2.4–2.6. Outcome measures included anesthesia recovery time, LFP mean absolute power (MAP), relative power (RP), time-frequency decomposition, and sample entropy. Primary outcome was anesthesia recovery time.
Statistical methods	7 - Provide details of the statistical methods used for each analysis, including software used. - 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.	Section 2.8 Statistical Analysis. GraphPad Prism 8.0 and MATLAB R2020b were used for statistical analysis and data visualization. Section 2.8 Statistical Analysis. For recovery-time comparisons, an independent-samples t-test was used when the assumptions of normality and homoscedasticity were satisfied.
Experimental animals	8 - Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. - Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Section 2.1 Animal Surgery. Twelve healthy male Sprague-Dawley rats weighing 240–260 g were used. Section 2.1 Animal Surgery. Healthy Sprague-Dawley rats were obtained from Beijing Life River Experimental Animal Technology Co., Ltd. (Beijing, China).
Experimental procedures	9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: - What was done, how it was done and what was used. - When and how often. - Where (including detail of any acclimatisation periods). - Why (provide rationale for procedures).	Sections 2.1 Animal Surgery, 2.2 TUS Protocol, and 2.3 Data Acquisition. Sections 2.1–2.3. TUS was applied once for 5 min after etomidate anesthesia, followed by continuous LFP monitoring until recovery of consciousness. Section 2.1 Animal Surgery. All rats were acclimated to the laboratory environment for 7 days before any experimental manipulation under consistent temperature. Section 1 Introduction and Sections 2.1–2.3. The PVT was selected because of its established role in regulating transitions of consciousness during anesthesia. The 7-day
Results	10 For each experiment conducted, including independent replications, report: - Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). - If applicable, the effect size with a confidence interval.	Section 3 Results and Figs. 2–5. Summary statistics are reported for both groups, with TUS group, n = 6 and Sham group, n = 6; data are presented as mean $\pm$ SEM where applicable. Effect sizes and confidence intervals were not calculated.

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, Methods. Twelve healthy adult male rats
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.	Section 1 Introduction. Section 1 Introduction. Rats were selected because their brain anatomy, neural circuitry, and skull acoustic properties are well characterised, allowing investigation of the neuromodulatory effects of transcranial
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Section 1 Introduction. This study aimed to investigate
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.	Ethics Approval and Consent to Participate. All experimental procedures and study protocols were approved by the Animal Ethics and Management Committee of Yanshan University (Ethics ID: 2022018).
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Section 2.1 Animal Surgery. Rats were housed
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.	Sections 2.1 and Animal Care and Monitoring. Isoflurane anesthesia was used during surgical procedures. TUS and sham-stimulation procedures were performed using gentle and standardized procedures. No expected or unexpected adverse events were observed during the study. Predelineated humane endpoints were established before experimentation. Animals were to be euthanised immediately if they exhibited >20% body-weight loss relative to baseline, persistent lethargy, hunched
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.	Section 4 Discussion. Section 4 Discussion. The study was limited to male rats, a single anesthetic agent, and a specific brain region. LFP recordings primarily reflect population-level activity of local neuronal populations in the PVT and do
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).	Section 4 Discussion. The findings are limited to male rats under etomidate anesthesia and stimulation of a specific thalamic region. Further studies are required to determine whether these findings can be generalised to other experimental conditions, species, and humans.
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.	A study protocol was prepared before the experiments began but was not registered in a publicly accessible repository.
Data access	20	Provide a statement describing if and where study data are available.	Availability of Data and Materials. The datasets
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. The authors declare no conflicts of interest. Funding. This work was supported by the Central Government Guides Local Science and Technology Development Fund Project (Grant No. 264Z7763G) and the Bureau of Science and Technology of Qinhuangdao