

Explanatory Accompaniment to the Checklist for Reporting Design and Methodological Features of Studies Involving Experimental Models of Stroke

Experimental Design	
Experimental groups and study timeline	<p>A clear definition of experimental groups would entail reporting the total number of groups in which animals were divided, along with the treatment, placebo/vehicle, or no-treatment used for each intervention. Different iterations of the intervention (eg, doses and time points of administration) are to be described as well.</p> <p>Controls groups may either be placebo concurrent, no-treatment concurrent, active-treatment concurrent, or dose/timing comparison concurrent. This needs to be clearly stated. The number of animals in each group is also to be reported. It is required that investigators use a concurrent control for each intervention group. Using singular control data across several experiments conducted over a course of time is similar to using historical controls in clinical research—a practice that does not hold its merit in evaluation of evidence for clinical trials.¹³</p> <p>A timeline of experimentation (ie, duration/period for completion of each experimental phase) is desirable. The objective is to inform whether all animals within each experimental group were analyzed together or were they composite of more than one experiment conducted at different time. It is possible that certain experiments take long time to complete; however, the inclusion of animals and experimental phases need to be concurrent. Timeline may include experimental phases such as: animal selection, randomization, intervention, and follow-up, for each experimental and control arm. If multiple interventions are being reported, please clearly state if the interventions are concurrent or a collection of experiments over a longer period of time. Authors are encouraged to provide a schematic or graphical representation of the timeline with either the main article or as supplementary material.</p>
Inclusion and exclusion criteria	<p>A priori defined criteria to include and exclude animals for experimentation are to be determined and described. These may be various parameters like animals' species, sex, strain, weight, developmental state, source nomenclature, genotype, drug, or test naive, and so on. Any criteria used after induction of ischemic or hemorrhagic lesion for inclusion/exclusion (eg, baseline deficit) should also be reported.</p>
Randomization	<p>Randomization is considered the gold standard for studies intended to test hypotheses. The use of randomization for assignment of animals to control or experimental groups is required to be reported. Certain studies such as those with only a single arm (intervention only) or those with exploratory or mechanistic aims may not use randomization. Authors are requested to provide clear explanations of such exceptions.</p> <p>The type and methods used to randomize animals to various study arms are to be reported clearly.</p> <ol style="list-style-type: none"> Type of randomization, for example, simple or stratified is to be stated. If stratified, please describe which variables/factors were used for randomization and why. It is also recommended that investigators report measures of successful randomization, by providing baseline characteristics of animals in various comparison groups. Process of randomization: please provide details of the process used for randomization. These may include (but are not limited to) use of electronically generated lists, sealed envelopes, coin toss, dice roll, etc. Other procedural details of randomization include describing the independence of team members generating randomization sequences, performing randomization and group allocation, and conducting experimentation. <p>Allocation concealment is an important aspect of randomization.¹⁴ If the study team member(s) performing randomization have knowledge of treatment assignment groups before selection of animals, then the allocation is not concealed, and a selection bias can be introduced. Allocation concealment can be achieved if deidentified codes are generated and provided to team members performing randomization. This usually entails an independent team member producing randomization sequences and lists. The journal encourages use and reporting of such procedures.</p>
Blinding	<p>Blinding or masking refers to:</p> <ol style="list-style-type: none"> Inability of the investigator administering the study intervention to discern treatment from the placebo/vehicle. This can be achieved by providing the investigator with animals having codes for group allocation, as well as study drug/product and placebo using a concealment procedure. The journal strongly recommends using and reporting blinding procedures in administration of treatment/placebo, wherever possible. For experimentation that in itself is surgical in nature, masking of the investigator performing study intervention may not be possible. We encourage appropriately reporting these aspects in the article. Besides the masking of intervention, it is also important that all outcomes be assessed in a blinded manner. Although blinding the investigator administering the treatment may not be possible in all instances, blinded assessment of behavioral and histological outcomes is almost universally possible. This is achieved by an effective tracking mechanism for the experimental animals and independence of the study team member performing outcome ascertainment from administration of the study intervention. The contributors to the journal are expected to provide an account of the experimental phases that were blinded to various investigator(s) and the steps undertaken to maintain blinding while transitioning from one experimental phase to the other.
Sample size and power calculations	<p>The contributors to <i>Stroke</i> are encouraged to include an explanation of power and sample size calculations for experimentation involving formal hypothesis testing. Rationale for purported effect size of the intervention on a priori determined primary outcome(s) should be presented, along with the anticipated parameter of variability. The sources that form the bases of power and sample size calculations should be cited. We do recognize the exploratory nature of certain experiments where a formal power and sample size calculation may not be possible or meaningful. These exploratory analyses should be recognized as such, and the authors are encouraged to present befitting interpretations of these analyses, along with an explanation of why power calculations were not formally performed.</p>
Data reporting and statistical methods	<p>Numbers of animals included in experiments and analyzed: For comprehensive reporting on number of animals in intervention and control groups, consider using a flow diagram showing: number of animals available for randomization, number of animals randomized to various treatment/control groups, number of animals for which baseline data were collected, number of animals included in the intervention, number of animals on whom follow-up/outcomes were assessed, and any animals that were lost at any stage during the experimental process.</p>

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Experimental Design	
	<p>Baseline data: For each experimental group, report-relevant characteristics of animals and baseline data on measured functional and behavioral outcomes. This may include animal characteristics such as age, developmental stage, sex, species, genetic strain, comorbidities, and baseline deficit.</p> <p>Adverse events/deaths: Please provide details on all important adverse events including death in all experimental groups. If none, please state so.</p> <p>Statistical methods: Authors are required to provide details of statistical methods used for each analysis and hypothesis tested. Descriptive analyses should use use of measures of central tendency and spread suitable to the distribution of the data. Measures of spread (eg, SD or SE) should be suitably used, reported, and interpreted.¹⁵ Report levels of significance used for hypothesis testing and if any adjustments were done for multiple or repeated testing.</p> <p>Reporting results: Investigators are encouraged to report summary data as tables (ie, not limit data to figures). Figures in themselves, at times, do not allow for precise reporting of measures of central tendency and spread for different time points. Space limitations can be overcome by providing tabulated results in supplementary materials.</p>
Experimental details, ethics, and funding statements	<p>It is important to provide as many experimental details as possible and necessary for interpretation, comparison, and possible replication of the intervention. These details include (but are not limited to) stroke model, formulation, and dosage of therapeutic agent, site and route of administration, use of anesthesia and analgesia, temperature control during experimentation, and postprocedural monitoring. Further details on primary and secondary outcomes, the timeline for their assessment, and methods used for ascertainment of outcomes should also be provided or clearly referenced.</p> <p>The importance of using sex-specific models is well documented.¹⁶ Although the use of both sexes is not required, the authors are requested to give due consideration to the use of animals with both sexes during experimentation. Assuming only males or females are used, reasons need to be provided in the article.</p> <p>The journal requires all studies reporting animal experimentation to abide by respective institutional animal welfare and ethics regulations and reporting of approvals.</p> <p>Funding and conflict of interest statements should also be provided. The funding of the study by a drug development or technology development industry should be clearly stated.</p>

Although a collective effort for scientific rigor in all facets of preclinical and clinical stroke research is required for successful translation, we hereby provide important metrics for the conduct and reporting of preclinical treatment studies related to cerebral vascular pathologies. We also recognize that certain standards communicated above may not apply to exploratory, hypothesis-generating, or mechanistic animal models. We promote and welcome such contributions, however, encourage the authors to address various areas and describe why adherence to particular design elements was not practical or meaningful. These guidelines are complementary to the Journal's earlier communications and other cited literature. The recommendations provided herewith are intended to facilitate the Journal's overall mission of promoting robust scientific inquiry and experimentation, along with clearly communicating the desired standards to contributors and investigators. We encourage all facets of exploratory and confirmatory preclinical research and recognize that a large number of investigators adhere to robust experimental guidelines, however, such may not be clearly reported. We promote the use of online supplements for providing detailed protocols on experimentation and communicating any secondary results not reported in the published article.