Guidance on the planning and reporting of experimental design and analysis

Article

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Guidance on the Planning and Reporting of Experimental Design and Analysis

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Abbreviations

ANOVA, analysis of variance;
BJP, British Journal of Pharmacology;
s.e.mean, standard error of the mean

The aim of this guidance document is to help authors plan experiments, conduct analyses and present the results of work intended for publication in the *British Journal of Pharmacology* (*BJP*). The guidance is structured to minimise the risk of generating and publishing false findings. Below, we explain the key elements of experimental planning (blinding, randomization and adequate group sizes) and how to avoid generating incorrect findings (false positives in particular). We explain how to capture the relevant design and analysis information in your manuscript so it can be examined quickly, efficiently, and fairly in peer review. In accordance with previous modifications to our requirements at BJP, we have eliminated a great deal of the items set forth in the most recent guidance document (Curtis et al., 2022) that were found to be too granular or subject-specific. We also hope to dispel some myths about what BJP will consider, in a clear and helpful manner, and encourage more authors to submit their work confidently to the journal according to the vision of the editorial board (Papapetropoulos et al., 2023).

Experimental planning

The three key elements of experimental planning should be reported in the 'Experimental design and analysis section' of 'Methods'. Compliance with the guidance cannot normally be adjusted after the experiments of a study are complete. However, data generated by experiments not conforming to these three elements may be reported in the paper if such data are a preliminary or minor part of your narrative. There is an easy way to decide if such data are appropriate for BJP: if the data in question can safely be excluded from your abstract and your conclusions without undermining the narrative, then you may include them in your paper. We expect referees to consider such data and not simply reject the manuscript by triage.

Throughout this document three categories of experiments will be referred to: (1) in vitro, (2) ex vivo and (3) in vivo. In vitro experiments will specifically refer to experiments done with cell lines and/or any other material not directly derived from multiple individual animals or humans. For example, a western blot or RT-qPCR measurement conducted on protein or RNA generated by treating the same culture of HEK293T cells with various drugs would be an in vitro experiment. Ex vivo experiments are those that involve work with material derived from animals or humans (excluding cell lines). Typically, this would include animal or human tissue sections or slice as well as whole tissues or organs. As individual animal and human products within an experimental group may have high interindividual variability this has to be accounted for in statistical analysis. In vivo experiments are those done with live animals or human trials. As with ex vivo experiments, interindividual variability again needs to be accounted for.

1. **Group size and replicates.** If you plan to subject data to statistical analysis the minimum group size, 'n', should be equal to at least 5 independent samples. If there are excluded animals or samples that are not replaced, so long as all 'n' values are at least 5 per group then any planned statistical analysis may be undertaken. Group size selection should be explained. Group sizes can become unequal due to loss of animals or samples. If this is the case it should be explained and the variations reported either in the 'Methods' section or in 'Results'. The experimental design should incorporate procedures for replacing lost samples or animals to maintain equality of group size taking into consideration proper randomization as well if applicable. If this is not practicable this should be explained in Methods. In vivo experiments are required to have at least 5 animals per experimental group. If the in vivo experiment uses animals of both sexes data for each sex should be analyzed separately in addition to any pooled analyses performed. If the experiment has not been restricted to one sex, this may require that there are at least 5 animals per sex per experimental group. Further guidance on accounting for animal sex in experimental design can be found in Docherty et al., 2019. Ex vivo experiments are required to use products derived from at least 5 separate animals per experimental group. In vitro experiments should be repeated 5 separate times in order to be considered biological replicates on which it is appropriate to conduct statistical analysis. Each individual in vitro experiment may contain multiple technical replicates per experimental group but these are not considered to be biological replicates and therefore do not contribute towards achieving n = 5; therefore only full repeats of the experiment are sufficient for

statistical analysis (n=5 biological replicates 'in triplicate' is therefore n=5, not 15, for purposes of data analysis). BJP is open to considering papers that contain experiments that do not meet these criteria. However, if the data were deemed to be critical to the conclusions of the paper we will request experiments are repeated and/or group sizes expanded to satisfy the n = 5 criteria.

However, BJP *will* consider papers where data sets are not subjected to statistical analysis. BJP has published at least one paper in the last ten years with no statistical analysis whatsoever (for example a paper with a range of studies done on tissue samples from a family with a rare genetic condition meaning n values were generally less than 5). The value of such a paper will be determined based on its likely scientific impact as well as the provenance and quality of the data set. If BJP's guidelines have not been followed, the statistics should be removed and the author should find alternative means to persuade referees that the data credibly conveys a scientific message.

2. **Randomization.** Randomization should always be part of experimental design. Randomization should be implemented for ex vivo and in vivo studies and this process should be described in the 'Methods' section. If randomization were not applied to such studies this must also be explained in 'Methods.' Some types of experiments, for example those where transgenic animals must be used as and when they become available, cannot be randomized. This must also be explained. For in vitro experiments when randomization is not practicable this should be explained in the methods.

3. **Blinding.** Blinding is required for in vivo and ex vivo studies and where practicable for in vitro experiments. The study must be blinded to reduce bias and minimize the risk of false positive results. The simplest approach is for the investigator to be blinded to the identity of interventions (i.e. drugs, wild type versus transgenic animals, or any other aspect of the study that generates groups of data that you wish to compare to assess differences or effects). If the investigator cannot be blinded, then the data processing must be blinded. For example, one investigator can anonymize the data prior to analysis by another investigator. This requires planning and may require that an independent person create and curate a coding document (e.g., a document that lists the identity of the group and the code used to blind the data analyst - normally the person who did the experiment). The method of blinding should be reported in 'Methods.'

Data analysis, exclusions, and use of statistics

In 'Methods', please explain which P value you have stipulated to denote statistical significance when comparing between groups, time points, etc. This is almost always $P<0.05$.

When ANOVA or related multi-group statistics are employed, **remember the F statistic and the variance homogeneity** are the gatekeepers that determine whether you can justifiably compare individual groups with one another. Please state in 'Methods' that "post hoc tests (such as Tukey's test) were run only if F were significant ($P<0.05$) and there was no variance inhomogeneity". The same requirement applies to more complex multi-group analysis; repeated measures analysis and analysis of covariance, for example. It is particularly important to follow this rubric and make this clear in 'Methods' as some software packages will allow a post hoc test to be run even when these conditions are not met, generating false positive results.

If you planned to perform **parametric analysis** (t test, or multiple comparison tests) but cannot because conditions are not met, you may find that a log transform generates Gaussian data that removes the variance inhomogeneity. The data may then be amenable to parametric testing. If this is not the case, then please use non-parametric statistics.

Individual values or samples **should not be excluded from data analysis unless the exclusion criteria have been defined** in 'Methods' and the number of samples or values excluded per group is reported. The best place for reporting such an occurrence is the figure/table legend.

Summary

If an experiment is worth doing, it is worth planning. If it is not worth planning, it may be not worth doing. Any issues concerning data analysis can be resolved once a study has been completed, but a badly designed study may be unpublishable. Here we explain how to plan your experiments so they incorporate randomization, blinding, and independent group sizes of at least n = 5 into the design; and how to get your paper published if you cannot do this. The key points are summarized in Figure 1. It means that experiments that are not randomized or blinded or adequately powered may be included in the paper, but the author will need to explain the value of the data which must be presented without the statistical analysis that pharmacologists normally use as a pattern recognition aid. You are invited to contact the BJP consulting editor for design and analysis if you would like advice on your experimental planning – before you start your experiments.

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