

1 Supplemental Material

2 Examples for study design:

3 **Example 1** A research scientist could distribute both sexes in equal numbers into each treatment
4 and control group keeping the total number of animals in each group and the overall experiment
5 the same. For example, if an investigator typically uses 8 animals on one sex per level of the
6 independent variable, he or she could place 4 of each sex into each level of the independent
7 variable to maintain the group number at 8.

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9 The action serves to address the need to control the variance due to the confounding variable (an
10 identifiable one), which spreads any variance due to sex across the entire experiment. Had the
11 researcher placed all or most of the females in one group and all or most of the males in another
12 group, the variance due to sex could substantially impact the outcome on the dependent variable,
13 rendering a biased statistical outcome and interpretation.

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15 Key points: Includes both sexes; distributes variance across entire experiment; allows for
16 reporting of outcome by sex in the literature; outcomes from including both sexes are
17 generalizable to the entire population. Does not allow for adequately powered test of the sex
18 variable; does not address how sex influences the outcome on the dependent measures.

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20 **Example 2** A research scientist could add additional animals of both sexes to each treatment or
21 control group, increasing the total number of animals in each group and the overall experiment.
22 For example, an investigator may choose to increase the individual group size to 12, and include
23 6 male and 6 female animals, for a total increase in the group numbers and the overall
24 experiment.

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26 This action serves to add additional animals to the experiment that *may* provide sufficient
27 power to detect a statistical difference between males and females, while also spreading the
28 influence of sex on the independent variable across all treatment and control groups.
29 Furthermore, the increase of the overall experiment subject numbers may result in a greater
30 likelihood of finding a statistically significant outcome on the independent variable due to
31 control of variance due to sex and the control of Type II error(when null hypothesis is false, yet
32 accepted)(1, 2).

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34 Key points: Allows for direct control of variance due to sex; allows for statistical test of sex;
35 allows for publication of outcomes by sex in the literature.

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37 **Example 3** A research scientist could incorporate sex as a factor in an experiment by using a
38 factorial design, whereby the independent variable of interest is crossed with both sexes to
39 evaluate statistically the impact of sex on the outcome measures based on each level of the
40 variable of interest. In this case, sex is evaluated in the same manner as all other independent
41 variables in a statistically meaningful manner to demonstrate the extent of influence of sex on
42 outcome measures in relation to the other independent variables. This design may provide clear
43 answers due to the powerful control over variance and systematic testing.

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1 Key points: allows for direct control of variance due to sex; allows for statistical test of sex;
2 allows for publication of outcomes by sex in the literature; allows for interaction of sex with
3 other independent variable of interest.

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1 **References**

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