Biotelligences Fortnight

Issue 3 (July 9 2014): Buard and Pfrieger, Molecular and Cellular Neuroscience

Another neuro-glial collaboration

Biotelligences Fortnight aims to showcase a recently published high impact article chosen by us, based mostly on the quality of experimental design, statistical analysis and presentation. Biotelligences Fortnight is released every two weeks or so in the form of a summary that highlights the major points that have appealed to us in the article as well as possible points that we think could have been improved. It is not an all-inclusive collection of statistical details but rather a guide for your own research. We are happy to receive suggestions from your recent reading.

This week, we selected a short article by Buard and Pfrieger published in *Molecular and Cellular Neuroscience* in June 2014 (PMID: 24910948). In this short study the authors use co-cultures of cerebellar Purkinje cells and glia from wild-type or Niemann-Pick C1 protein (NCP1)-null mice and suggest that the loss of normal neuronal activity requires the simultaneous absence of NCP1 in both cell types. Although this study does not shed light on the exact mechanisms behind this neuro-glial cooperation, it is well conducted and well presented. In particular, the biostatistics are sound. We liked: **(1)** the statistical paragraph, which contains information about the alpha threshold (0.05), the statistical software used and a description of data presentation (box plots), although disclosures of the policies regarding experimental blinding, randomization and outliers are missing; **(2)** the appropriate use of non-parametric tests (Mann-Whitney test, Wilcoxon matched paired test and Kruskal-Wallis test) throughout the manuscript because the assumptions of normality is not met; **(3)** the effort made to correct for multiple comparisons in Fig 3 and 4 using Kruskal-Wallis test; **(4)** the high statistical power implemented (apart from Fig. 3B); **(5)** the correct (yet unusual) use of box plots and column scatter plots, which both ensure that a maximal amount of information (range, distribution, outliers, median...) is shared with the reader; **(6)** the use of medians instead of means in graphs, as this latter should be avoided in the case of non-parametric tests.

It is worth mentioning that there is a lack of consensus among statisticians regarding whether the Kruskal-Wallis test can be used in cases of unequal variances (heteroscedasticity), with some arguing that sampling populations must have "similar shapes". For many statisticians (perhaps the majority), the marked inequality of variances in Fig. 3 would prevent the use of the Kruskal-Wallis test. The problem however, is that... alternative solutions are neither simple nor consensual, if there are any at all! Our opinion is that resigning yourself to using Kruskal-Wallis is not a flaw in the strict sense, but it should be disclosed in the discussion that there are chances of erroneously finding significant results.

Finally, the nature of post-hoc analysis performed after Kruskal-Wallis is not given. One solution could be a non-parametric Mann-Whitney test corrected with a Bonferroni adjustment. Whatever the test used it must corrected for multiple comparisons and be insensitive to parametric assumptions.

In conclusion, despite several shortcomings, the biostatistics in this article are of high quality.

The Biotelligences team