Pre-selection and Multiple Testing Contaminates Neural Response Analysis

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Recent advances in single unit neurophysiology allow experimenters to record from a large number of neurons with the intent of analyzing that data offline. In order to understand the responses of large populations of neurons, one needs screening tools to select those neurons which are interesting in terms of a particular experiment. One strategy is to pre-select cells which respond to any of the experimental manipulations and then further test those cells for particular experimental effects. For example, choosing neurons which have a response to one or more images, and then testing for differential responses between images, thus, detecting an effect of the images on the response.

This sequential application of two tests to the same data carries the risk that random variations of the data causing pre-selection may cause the second test to show an exaggerated effect. The interaction between these two tests can be subtle and difficult to ascertain. To determine the extent to which this interaction may be important for typical neurophysiological recordings, we simulated the responses of 32 neurons to a varying number of stimuli, each presented for 10 repetitions. The responses were randomly chosen from a Poisson distribution with varying rate, the same for all neurons and stimuli. The neurons were first pre-selected for a response to any of the stimuli (Wilcox rank-sum, p-values 0.1, 0.05, 0.01, 0.001). All neurons having one or more responses to any stimuli were then subjected to an ANOVA for an effect of stimulus on the response (p-values 0.05, 0.01).

For these simulated conditions, the fraction of neurons with a significant effect of stimulus was compared to the fraction expected by chance (p-value ratio, PVR). This ratio ranged as high as 4.5, and depended on p-value of the pre-selection (lower p-value increases PVR) as well as the number of stimuli (fewer stimuli increases PVR). Across these parameter variations, the fraction of cells excluded by the pre-selection (exclusion fraction, EF) correlated with the PVR. In general, EFs > 10% materially increase the PVR and give rise to artifactually large number of neurons with an apparent significant effect of stimulus on the response.

These increases in PVR arise because the random variations in the data used to perform the pre-selection are the same as the random variations assessed by the second test for an effect of the stimuli. The increase can be avoided by performing the pre-selection on a set of data which is independent of the second test, either part of a separate selection experiment or a separate subset of the trials within an experiment. The interactions between two tests on the same data can be difficult to ascertain: a preliminary search of neurophysiological research papers in recent years suggests that 25% of papers apply a pre-selection to reduce the amount of data and may be subject to this increase in p-value ratio.

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