Reviewer's report

Title: Proteomic profiling of the phosphoproteins in the rat thalamus, hippocampus and frontal lobe after propofol anesthesia

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Reviewer: Stuart Forman

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There are two major issues that must be resolved before this manuscript can be considered for publication.

First, the authors state that animal procedures were performed in accordance with ethics guidelines of their University, but also indicate that mature animals were sacrificed by decapitation. It seems that at least some of these animals (the control animals in the first experiments or the P2 group in the second set of experiments) were awake during decapitation. This is not allowed by most US research oversight authorities. The authors must clarify if 1) the animals were awake or sedated during decapitation; 2) if awake, why it was scientifically necessary; and 3) if awake, specifically state that their institutional oversight committee approved the procedure.

Second, the methods state that 24 animals were used in each initial set of proteonomics experiments. However, it is unclear whether proteonomic analyses were performed on tissues from individual animals or pooled tissue from multiple animals. The methods and figure 1 legend must indicate if the gels are from individual animals or pooled animals. The figure 2 legend indicates that densitometry analyses were “calculated from 6 different gels.” Why weren’t the data from 24 gels, given that the study used 24 animals in each group? If the 24 animals were divided into sub-groups, this must be stated and justified in the methods. Without clarification of these issues, the primary analyses may not represent the actual variance among individual animals in the studied groups, and this would negate any inferences drawn from the experiments.

Additional issues that need attention:

Given that the hypothesis appears to be that thalamus, hippocampus, and cortex are specifically important in anesthesia, the paper would be strengthened by comparison to other parts of the CNS that are less important in anesthesia (e.g. cerebellum). Comparison to other excitable tissue (muscle) would also be of value to test the relevance of the data to the overall hypothesis.

Figure 1 identifies different phosphoproteins in each of the three CNS tissues studied. However, the results do not compare phosphoprotein expression patterns and propofol effects among the these three tissues, which is of potentially
great interest. Examination of Figure 1 shows that many of the same phosphoproteins are present in each of the three tissues studied, so the paper should report and compare which phosphoproteins were affected by propofol in each of the tissues. If they differ, is this unexpected? Could the variations be simply random?

How many distinct phosphoproteins did you identify in each tissue displayed in Figure 1? We learn about the 21 differentially expressed proteins, but what percentage of all phosphoproteins does this represent?

Figure 2 legend states that densitometry data is from 6 different gels. Again, are these gels for individual animals? If so, why weren’t data for all 24 animals included in the analysis? Or are they 6 gels of pooled protein for the three tissues x 2 conditions? If the latter, then what measurements led to the estimate of variances for statistical analysis?

The discussion is mostly a catalog of entirely speculative associations and correlations that for the most part fail to convincingly connect the data to potential mechanisms of propofol anesthesia. A few of these connections, particularly to phosphoproteins and Alzheimer’s Disease, seem relevant to anesthetic neurotoxicity rather than to anesthetic-induced hypnosis. The authors should familiarize themselves with the work of Stuart Hameroff and Roger Penrose, who proposed a “quantum” theory of consciousness based on microtubular assemblies. It’s not a mainstream theory, but it links their results to their hypothesis with a bit more than pure speculation.