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Critique of Knockdown Experiment of B-domain of RIBEYE

This proposal is relevant to the Duncan lecture on ribbon synapses as it deals with the structural protein RIBEYE, which was mentioned in class along with CTBP2 as a possible structure comprising tethers in ribbon synapses (the "extra" exon expressed as a CTBP2 piece). Additionally, it was mentioned in class that little is known about effects of mutating CTBP2, making this topic not only relevant but also novel in the study of ribbon synapses. One area in which this proposal might elaborate further is in the significance of this study to humans—how do genetic mutations in CTBP2 affect humans? Is the null form fatal as well, and is there any disorder related to post-developmental mutations of CTBP2 (the intended manipulation of this experiment)? In this experiment, the goal of the morpholino knockdown post-development is to render the protein CTBP2 nonfunctional. The hypothesis is testable in this experiment, as evidence for or against the hypothesis can be measured by density of vesicles around the ribbon between control and experimental mice. However, more information detailing density measurements might be elaborated upon within the paper. In class, it was suggested that CTBP2 may allow for tethering of molecules or comprise the tether itself to the ribbon synapse, but vesicles may also be in cytoplasmic space around the ribbon synapse without tethering—these could be accounted for in better detail. If vesicles still form around the ribbon synapse, for example, but without being tethered, vesicle density "near the ribbon" may still be high, although perhaps more scattered in terms of positioning. The proposal could be more precise in predicting the possible role CTBP2 plays in vesicular density, such as stating that CTBP2 might promote tethering or some other mechanism that would increase density of vesicles at the ribbon, and why or how the density of vesicles at the synapse would decrease as a result of mutations in CTBP2. Additionally, there may be merit in seeing effects of this morpholino knockdown in living mice (if they survive) by measuring their hearing abilities—even though this may not give as detailed information on the actual functioning at the ribbon synapse, it would give insight to the larger-scale effects of the manipulation. If the CTBP2 fatal knockout is only a homozygous recessive phenotype, it may also be helpful to look at heterozygotes for the gene and see if they have a lower concentration of vesicles around the ribbon synapse to test the hypothesis (heterozygotes would only have one copy of the working gene and might show a reduced density of vesicles because of a reduced number of CTBP2 proteins being produced).

As the hypothesis seeks to test how vesicular density is affected by CTBP2 malfunctioning, it seems logical that vesicular density be measured by staining of cryosections and counting vesicles, provided that staining is well-done and very clear. There is room for more detail in describing criteria for counting vesicles, such as how much difference might be expected or if other changes (such as the position of vesicles around the ribbon) might be expected, as cytoplasmic vesicles not tethered to the ribbon could possibly interfere in counting. In the expected results, it is predicted that a ribbon synapse may still form, but without the ability to form tethers. If there is a method to view or test for the presence of tethers instead of counting vesicle presence, this may be a more direct measure of this specific prediction (slightly different from the hypothesis). Additionally, one thing that may need to be accounted for is the 2D nature of slices—precautions would need to be taken to make sure the correct orientation of slices was obtained to account for the 3D aspect of vesicles being counted. On a side note, although less related to our course, the literature cited makes it seem that in practice the visual system and retinal cells are easier to examine with this method (or have been done more often), although this does not reduce the validity of this specific proposal. For the most part, I found the proposal to be clear and understandable—background explanations of RIBEYE and CTBP2 were helpful in understanding the history of research in this area, although I think more information could have been provided on the proposed method of knockdown (more explanation on how it differs from null predevelopmental knockouts of CTBP2, and how it might affect other proteins coded by the gene—I believe it has multiple products? Are the mice still expected to die after the morpholino knockdown due to the loss of gene product? I was unsure about this part, although I think it could be remedied by more explanation within the paper). In general, the hypothesis and expected results might have been more specific and detailed as to how much difference in vesicle density was expected or what other structural differences might occur, including positioning of the vesicles in the ribbon synapse (or if they expect to view any vesicles at all).