Author's response to reviews

Title: GluN2B protein deficits in the left, but not the right, hippocampus in schizophrenia

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Version: 3 Date: 29 August 2014

Author's response to reviews: see over
August 29th, 2014

Dr Alice Murray  
Executive Editor,  
BMC Psychiatry  

Dear Dr Murray,  

Re: MS: 2566271891302896  

Thank you for the comments on our paper titled, “GluN2B protein deficits in the left, but not the right, hippocampus in schizophrenia”. We are grateful for all comments and suggestions from the reviewers, which we found constructive to the paper. Please find below our point-by-point responses to the reviewers comments.

Yours sincerely,  

Kelly A. Newell, PhD
Response to Reviewer 1 Paul Lombroso

Q1. It appears that the western blots were performed on total homogenates and not on a differential centrifugation of the samples to isolate membrane fractions. This experiment would address more directly a possible disruption to GluN2B trafficking. In addition, a complementary experiment would be to blot using a specific phospho-antibody to Tyr1472, the residue implicated in regulating trafficking of GluN1/GluN2B to and from membranes.

A1. We agree with the reviewer that to specifically examine membrane fractions as well as GluN2B phosphorylation would be a valuable experiment to conduct to provide evidence of a possible disruption to GluN2B trafficking. Unfortunately a lack of tissue availability means that we are unable to perform additional experiments on this region/cohort. Despite this, we have added this important point to the discussion. Please seepage 9, lines 207-210.

Q2. The authors should add further discussion for readers not familiar with [3H]Ifenprodil binding studies whether they are measuring surface GluN2B receptors, or total GluN2B in all compartments including ER, etc.

A2. This has been added to the discussion. Please see page 8, lines 193-196.

Q3. A better discussion of the apparent discrepancy between lower GluN2B levels by western blot, but no change in GluN2B binding: interpretation of this result, how would this work, and how has the literature discussed this type of discrepancy

A3. We have extended on this in the discussion including discussing the work of Beneyto and Meadoor-Woodruff (2008) and Kristiansen et al (2010) who report no change in [3H]ifenprodil binding in the cortex but a reduction in GluN2B protein. Please see pages 8/9, lines 187-210.

Q4. Page 2 lines 49-50, “Altered synaptic connections and changes”... it would be good if the authors elaborate on the kind of synaptic changes that are found

A4. This has now been added. Please see page 2, lines 50-51

Response to Reviewer 2 Kenji Hashimoto

Q1. Introduction: the use of [3H]Ifenprodil binding for GluN2B was mentioned.

A1. This has now been added to the introduction. Please see page 3, lines 67-69 and 83-84.

Q2. It is well known that [3H]Ifenprodil binds to sigma-1 and sigma-2 receptors (Hashimoto et al, Eur. J. Pharmacol. 1993; 1994). These papers should be cited. The reasons for the use of (+)3-PPP should be mentioned in the method section. Ifenprodil also binds to alpha-1 adrenergic receptors. Please comment on this.

A2. The references have now been cited. Please see page 5, line 116. Information on the nonspecific binding of ifenprodil has been added. Please see page 5, lines 114-120 and page 8, lines 188-191.