Reviewer's report

Title: Glucocorticoid receptor mRNA and protein isoform alterations in the orbitofrontal cortex in schizophrenia and bipolar disorder

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Reviewer: Claude P. Muller

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Review of Sinclair D et al.
Glucocorticoid receptor mRNA and protein isoforms alterations in the orbitofrontal cortex in schizophrenia and bipolar disorder.

Recommendation: Major Compulsory Revisions

Sinclair et al present a manuscript investigating the link between bipolar disorder and schizophrenia and GR transcript and isoforms levels in the orbitofrontal cortex of the human brain. This is an important topic, with clear implications for our understanding of both bipolar disorder and schizophrenia. The authors have analysed a large and appropriate series of postmortem brain samples. Their underlying hypotheses are valid, and they have produced a clear, concise, well written manuscript. The statistical tests chosen are entirely appropriate, and the conclusions drawn appear to be valid from the data presented.

There are nevertheless several points that need to be addressed in the manuscript before this article can be accepted for publication

Major Compulsory Revisions

1. The Materials and Methods Section gives no details of diagnostic criteria used (DSM-IV? ICD-10?), and how donors were selected. Was a post-mortem review of the donor’s medical history performed to confirm the diagnosis? This is essential information that should be included. It is important to reassure the reader that in order to have such an impressively large number of post-mortem samples (35 schizophrenia, 34 bipolar disorder, and 35 controls) that there was no unnecessary loosening of the diagnostic criteria.

2. The authors observe a decrease in transcripts containing exons 1B and 1H. However, total GR levels remain unchanged. Is there an explanation for this, given that the other first exons, including 1D, 1E, and 1F were present, but below the minimum detectable level (MDL) of their PCR assay? Was any attempt made to see if, for example, exon 1F increased to levels above the MDL, especially since Yau et al have shown that the rodent orthologue of exon 1F, exon 1-7, is upregulated by antidepressants.

3. Similarly, since it has been previously reported that anti-depressant medication, especially fluoxetine affects GR transcript levels more detailed information on anti-depressants taken would be appreciated in Table 1. This is
important since the regulation of GR was specific to fluoxetine. If there are enough donors, an analysis of the effect of fluoxetine on GR transcript levels is warranted. Similarly, if in any donors exon 1F levels are above the MDL, the effect of fluoxetine intake on GR 1F levels should be investigated.

4. Given the significance of the isoform specific upregulation of GRalpha-D1, the authors should include an extra lane in Figure 2B where the antibody has been pre-incubated with its cognate (blocking) peptide to demonstrate the specificity of the GR immunoreactivity.

5. The importance of the 1H transcript needs to be discussed. Previous reports suggest that in the 5 brain regions so far studied exon 1H represents only 1-3% of the total GR transcripts (Alt et al 2010). The proportion of the total GR represented by exon 1H should be measured, and if the levels are as low as previously reported, then the relevance of the down regulation of such a small percentage of the total GR should be discussed.

6. The authors observe significant down regulation of transcripts containing exons 1B and 1H. The promoters of both exons were genotyped for rs 5871845, and rs10482614 respectively. However the functional promoter 1B SNPs rs3806854 and -5 were not investigated, neither were the additional SNPs in promoter 1H (rs11244544 and rs112794517). These should be investigated and included in the manuscript.

Minor essential revisions

1. Please use the name of the antibody clone used for the Western Blot, rather than the catalogue number, as the P20 clone is a well known clone (p9 line 9).

2. Previously the authors have reported differences in the intensity of the different molecular weight GR immunoreactive band with different batches of the P20 antibody. The authors need to confirm that all their western blots were performed using the same batch of antibody. (Sinclair et al 2011)

3. The legend to Figure 2 needs to include all the abbreviations used, especially since Bp (in this case I assume Bipolar disorder) is often used in western blots to indicate that the antibody has been pre-included with a blocking peptide.


Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests