Author’s response to reviews

Title: Test-Retest Variability of High Resolution Positron Emission Tomography (PET) Imaging of Cortical Serotonin (5HT2A) Receptors in Older, Healthy Adults

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Version: 3 Date: 13 January 2009

Author’s response to reviews: see over
We thank you and your reviewers for very helpful and supportive comments. We have revised the manuscript to address the reviewers points, as reflected on the text with changes marked which I have directly email attached to you. The clean revision has been uploaded to the website. Below please find responses to the reviewers in italics.

**Reviewer #1**

**Reviewer's report:**
This is a straightforward study in which test retest reliability for setoperone is assessed in 6 subjects over the age of 60 after intervals of 5-16 weeks. A single method of kinetic analysis was used, the simplified reference tissue method, using time-activity curves obtained from regions of interest. Statements are made about differences between ICCs in different regions but these are not subjected to statistical examination. Sample size may be too small to find significant differences.

**Major Compulsory Revisions**
1. Are the differences between regions statistically significant?
   No. Confidence intervals, as requested below, have been added to the tables and show considerable overlap.

2. Please provide confidence intervals for the ICCs
   See above

3. Please provide greater information on the regions of interest (e.g. volume)
   Volumes have been added to Table 1, with accompanying descriptions in Results and Discussion, as requested by other reviewers as well. We called upon Dr. Graff-Guerrero to help us derive these volumetrics and have therefore added him to the co-author list.

4. Please list time between scans for the subjects
   Done.

5. The authors suggest that partial volume effect may have contributed to low ICC in the cingulated. From the T1 weighted MRI could they provide information on proportion of grey, white and CSF in each of the ROIS.
For this paper, we have provided the volume contained in each ROI. A separate paper on the effects of aging and gender is under revision for another journal and will include the partial volume corrections.

Discretionary Revisions
1. It would be interesting to examine other methods of kinetic analysis and other brain regions.
   Please let us know if you prefer we pursue this. For the number of subjects and relative continuity with test-retest previously reported in the literature, it seems a bit superfluous.

Reviewer #2

Reviewer's report:
Major compulsory revisions:
Page 5: Methods: Were the subjects screened for handedness? If so, that should be reported here. If not, a brief discussion point should be included citing this as a potential confound (see below).
   All subjects were right-handed- we have added this information to characterization of sample and discussion of the asymmetry of the anterior cingulate ROIs, as suggested by this reviewer below.

Page 8: The repeatability coefficient (RC) should be defined at first mention. In addition to the computation method already present (but in the wrong place), the interpretation in terms of confidence intervals should be included, and both be given a reference citation.
   Then RM% can be dealt with. The choice of the name RM should be explained, and if it is not original with this paper, a citation is needed. It should not be called a coefficient of variation, or even related to a coefficient of variation, as this term is universally accepted to mean the standard deviation of something divided by the mean of the same thing. This object expresses twice the standard deviation of the difference between two things divided by the mean of those things, not the mean of the difference. The desire to scale the differences by the magnitude of the things that are differing would be understandable, but the RC is not a difference, but the variability of a difference. This needs interpretation and clarification in both the Methods and Discussion sections. One simple solution would be to delete all reference to RM% as it adds very little meaningful information. Another might be to scale the standard deviation by the mean of the absolute difference.
   We deleted all reference to RM%.

Page 8: The comment about intersubject differences in fND is unjustified and unnecessary. This paper has only one point (at present), that the same measurement performed twice gives the same result. The data have nothing to say about what determined that result. If the revision includes commentary about age dependence, that would be the time to say that the changes in BPND are interpreted as changes in Bmax, if the assumption is made (about which the
current data say nothing) that fND is the same at all ages. 

*Also deleted comment about intersubject differences in BP*$_{ND}$*

Page 9: The result of excluding two subjects from the analysis of the anterior cingulate cortex is hard to follow. Presumably the pairs of end points given are for the left and right sides, but this needs to be said explicitly. Also it was the change in the ICC that was not statistically significant, not the ICC itself, as the sentence currently states. 

*Text has been clarified re left and right sides. We appreciate the opportunity to also clarify the point about change in ICC, as opposed to ICC statistics.*

Page 9: The striking left-right asymmetry in the anterior cingulate cortex cannot be ignored. ICC’s of 0.7 strongly support the presence of an actual asymmetry. This was the basis of the handedness comment above. I don’t believe the asymmetry is real, but it can’t be brushed aside without some comment on the statistical implications of this behavior of the data. 

*This has been added to the Results section.*

Page 10: The discussion of the scale of the measured BP’s is confusing. I didn’t have access to reference 19, but I did access references 13 and 20 (by the way, the citation for reference 20 is garbled), both of which have nearly identical disease-independent demonstrations of a striking age dependence. If anything, the new BP’s are not too small, but too big. The role of resolution is not discussed, except to say that it is different for the various references and this paper. The effect of resolution, however, would be dependent on the ROI sizes and shapes. This entire discussion should be revised. The results (partial volume effects aside) imply that the loss due to aging levels off eventually. This is the point of reference 17, already cited. If the previous data were from another group, the reluctance to combine the results in older subjects with those from younger subjects would be understandable. However, this paper is in a position to say something about the age dependence of receptor density, as the data for other ages was published by the same (or a closely related) group. (Reference 17 makes no mention of an asymmetry in anterior cingulate cortex, of relevance to the previous point.)

*References have been corrected.*

We are not sure that we can agree with this reviewer that our BPs are larger than reported elsewhere (see first paragraph of Discussion)- Hurlemann 2008 showed BPs of 2.3 and 2.25 for the anterior cingulate regions of young subjects with schizophrenia, which is an order of magnitude larger than our measures in Table 1.

We have added a comment to the Discussion on the high resolution of our scan data and the potential effect on our large (lobar) ROIs vs. smaller ones (anterior cingulate).

Summary comment: The reliability in older subjects is clearly and soundly presented. An opportunity exists to extend the results to new or confirmatory conclusions about age-dependent loss. 

*As stated above, we report age effects on setoperone BPs in a separate paper.*
Reviewer’s Report #3

- In the introductions the authors state that radiolabeled metabolites of [11C]MDL enter the brain, however, a recent study (Hinz et al, JCBF 2007) addressed this issue did not find any evidence that these metabolites enter the brain. We thank this reviewer for calling this new information to our attention. We have altered this paragraph in the Introduction section to emphasize instead the choice of setoperone for its less invasive methodology for older subjects.

- The time between scans ranged from 5 – 16 weeks. This seems to be quite a large range. Since this is a small study of 6 subjects, it would be helpful and possible to state the exact time between scans for each subject, this would allow the reader a better idea of the distribution in the time between scans and provide a breakdown of how many subject feel into the ‘shorter’ and ‘longer’ categories used in the statistical test. As above, this information is now available in the Methods section.

- The [18F] setoperone BPnd values obtained in this population are quite low, ranging from .22 to .60. The authors highlight this point in the discussion around the low values obtained in the anterior cingulate and state that a partial volume correction may be necessary for between group comparisons. If available, it would enhance the manuscript if a partial volume correction was applied to these data. This would allow the authors to provide evidence for the utility of such a correction in the study of elderly individuals with [18F]setoperone. As above, partial volume corrected data from this sample are to be reported elsewhere.

- In the discussion it would be interesting if the authors provided a comparison of the BPnd values from the literature in elderly individuals obtained with the other radiotracers for the 5HT2A receptor. If possible, a conclusion/recommendation by the authors of which radiotracer provides the best signal-to-noise ratio (i.e. BPnd) and therefore should be used in future studies in this population. As above, we have complied with the first request in the first paragraph of the Discussion section, but there don’t seem to be enough data readily available to comment on comparative signal-to-noise ratios.

Reviewer #4

Imaging of Cortical Serotonin (5HT2A) Receptors in Older, Healthy Adults” by Chow and colleagues was a pleasure to read. The methodology is sound, and the journal appropriately chosen. There is, however, a number of typos that the authors should correct, e.g., ”risperidone” (page 3) or ”has been obeserved in” (page 10). We have scanned for typos.

When it comes to Altanserin-PET, the authors also should cite
"Hurlemann et al. (2008)" who did a study in young individuals at enhanced risk for psychosis.  
*This has been added and we are thankful for the updated reference.*

Please let us know if you require further revisions to make this paper acceptable for publication in *Medical Imaging*.

Sincerely,

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[corresponding author remains Dr. David C. Mamo]