Author’s response to reviews

Title: Structural brain changes associated with antipsychotic treatment in schizophrenia as revealed by voxel-based morphometric MRI: an activation likelihood estimation meta-analysis

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Author’s response to reviews: see over
Response Letter

To Prof. Basant Puri
Editor
BMC Psychiatry

Dear Prof. Puri,

We would like to thank you for sending the manuscript entitled “Structural brain changes associated with antipsychotic treatment in schizophrenia as revealed by voxel-based morphometric MRI: an activation likelihood estimation meta-analysis” for peer review and giving us the opportunity to respond to the reviewers. We would like also to thank the reviewers for their highly valuable comments and willingness to review this manuscript. All comments were addressed and we have provided point-by-point responses to each of them below. We have also uploaded a revised version of the manuscript (changes are highlighted in red).

We believe the manuscript is greatly improved and hope you agree that it is now ready for publication. We appreciate your consideration of our manuscript.

Sincerely,

Ulysses S. Torres, MD

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Editor’s requests

1. Requesting copy editing:

After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further. We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

Answer: Thank you for the advice. We sent the manuscript to a professional English-language editing service.

2. Competing interests:

Manuscripts should include a 'Competing interests' section. This should be placed after the Conclusions/Abbreviations.

Answer: We have added such a section.

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Reviewer 1

Major Compulsory Revisions

1. The authors investigated whether brain structural changes in schizophrenia are associated with antipsychotic use. They applied the meta-analytic technique of Activation Likelihood Estimation (ALE) to voxel-based morphometry (VBM) studies. They identified 11 studies meeting their inclusion criteria (including 360 patients on antipsychotic drugs and 344 controls). The main findings were brain structural changes associated with antipsychotic use: areas of relative volumetric increase in the left anterior cingulate cortex and right putamen; areas of relative volumetric decrease in the left temporal cortex, left inferior frontal gyrus, and superior frontal gyrus. Although potentially an interesting result, there are some methodological issues which need to be clarified.

Answer: We hope that the revised version of our manuscript satisfactorily addresses all points raised by the reviewer, and we are grateful for the constructive criticisms that helped us to improve the quality of the paper significantly.

2. It is not entirely clear in the paper how the primary studies investigated the effect of antipsychotics. For example, the Schaufelberger study appears to compare patients treated with antipsychotics versus patients untreated with antipsychotics. It reported ‘Exploratory analysis across the entire brain revealed no areas of grey matter reduction in those currently treated with antipsychotics (n=84) relative to those 38 who were untreated (P>0.05, corrected).’ Only ‘Small volume-corrected analyses demonstrated significant grey matter reductions in participants treated with antipsychotics relative to the untreated
subgroup in the right insular cortex and in the right superior temporal gyrus’. A small volume-corrected result may introduce bias into the meta-analysis so should probably be excluded.

Answer: We agree with the reviewer and excluded the Schaulfelberger’s study (which reported two foci of gray-matter deficits). Because all the retrieved foci associated with volumetric increases were maintained (i.e., were unaffected by such exclusion), a new analysis was performed using only the subset of peak coordinates associated with volumetric decreases (from 73 to 71 foci). This new analysis showed the same three results previously described (i.e., a cluster of 408 mm$^3$ located in the left lateral temporal cortex, BA 20; a cluster of 192 mm$^3$ located in the left inferior frontal gyrus, BA 44; and a cluster of 120 mm$^3$ located in the left superior frontal gyrus, extending to the left middle frontal gyrus, BA 6), and a new additional cluster was found: a cluster of 104 mm$^3$ located in the right rectal gyrus, BA 11. We updated the whole text, tables and maps with these new results following the Schaulfelberger study exclusion. Figure 1 (PRISMA flowchart) was also uploaded, with the inclusion of said study as “found brain volumetric changes only on small volume-corrected analysis”.

3. It appears that some primary studies were longitudinal (i.e. comparing brain structure in patients before and after antipsychotic use) whereas others were cross-sectional (i.e. comparing brain structure in patients taking antipsychotics versus medication-free). There are also references to healthy controls although it is not clear how these featured in the primary analyses. It would be helpful if Torres et al mapped out the primary analyses used in each of the papers to clarify in each how the effect of antipsychotics was being evaluated. Without this information it is difficult to know whether it is reasonable to combine longitudinal with cross-sectional studies in a single meta-analysis, or whether this is like combining apples with oranges, simply to achieve a large enough sample size. Otherwise it may be necessary to meta-analyse the two sorts of studies separately and then compare the results to see if they are similar.

Answer: In our original Table 1, we identified the cross-sectional studies comparing patients with healthy controls or medication-free subjects (symbols * and γ) and the longitudinal studies (symbol Ω). In addition, we also specified in the Results section (second paragraph) which studies were cross-sectional and which were longitudinal. However, we agree with the reviewer and recognize that these original delineations might be confusing. Accordingly, we excluded the symbols from Table 1 and added a new table (Table 2), mapping out the primary analyses in each study, as requested. We now include the type of study design (specifying how the comparisons were made), the stereotactic space used in each study, and data on statistical thresholds, full-width half-maximum kernel, type of analysis, p values, etc. It should be noted that all remaining studies performed whole-brain analysis and were unchanged, thus justifying only the exclusion of Schaulfelberger’s study.

Regarding the question of combining cross-sectional and longitudinal studies: actually, this is not a problem in an ALE meta-analysis. This type of meta-analysis has the advantage of including only VBM studies that, independent of their designs, report peak coordinates of brain areas with changes that might be associated with antipsychotics (in our case). Thus, the main objective of employing an ALE meta-analysis on this topic (as the name ALE implies) is to establish whether there is a consistent anatomical pattern across the reported changes; it is, after all, an anatomical question. We think that
our comprehensive ALE meta-analysis, unlike a typical meta-analysis or a structured literature review, is strengthened by considering the findings of all the scarce VBM studies on this topic (either cross-sectional or longitudinal), quantitatively integrating different foci across these different studies, and determining the clusters of significant topographic convergence, thus providing a neuroanatomical basis for these changes. It is, tentatively, a first objective panoramic view of this complex question.

Finally, combining cross-sectional and longitudinal studies in an ALE meta-analysis has been performed previously. To cite just one example, please refer to the recent article by Zhao et al., recently published in “Schizophrenia Bulletin” (Zhao Q, Li Z, Huang J, Yan C, Dazzan P, Pantelis C, Cheung EF, Lui SS, Chan RC. Neurological Soft Signs Are Not “Soft” in Brain Structure and Functional Networks: Evidence from ALE Meta-analysis. Schizophr Bull. 2013. Of the six structural meta-analyzed studies, five were cross-sectional and one was longitudinal (all of them were meta-analyzed together).

5. Another difficulty with this analysis is differentiating between the effects of antipsychotics and the effects of potential confounding factors. Although this does not make the analysis invalid, it does need to be more clearly considered in the Discussion.

Answer: We agree with the reviewer and have added new comments on this topic in the Discussion (please see Limitations). This topic is further addressed in the responses to reviewer 2.

6. The authors combine gray and white matter coordinates changes in a single analysis: is this a standard ALE technique, if so the authors should justify it, otherwise should the gray and white matter changes be evaluated separately?

Answer: We agree with the reviewer that this is a relevant point. We would like to clarify that the permutation analyses conducted in ALE meta-analyses (using GingerALE, for example) are anatomically unconstrained and thus they include not only the predominant foci within the gray matter but also the foci within the deep white matter. So, it is a whole-brain analysis, without the need for evaluating the gray and white matter separately. To make it clearer, we have added this topic to the revised manuscript (please see Methods).

Minor Essential Revisions

7. In general, some of the sentences are rather long and could be shorter and simpler to aid the reader.

Answer: We tried to identify all long sentences and reduce them. We also shortened some paragraphs and excluded some lines throughout the text to facilitate understanding by the reader (e.g., the introduction was shortened from 1552 to 1272 words). For example, long paragraphs such as “In the last decades, since the advent of more sophisticated neuroimaging techniques, such as magnetic resonance imaging (MRI), which allowed in vivo studies of the brains of individuals with schizophrenia, structural brain changes in schizophrenia have been extensively characterized [8-10]. The large body of well established evidence provided by initial MRI studies confirmed some post-mortem findings earlier
described [11-14], enabling the general assumption that schizophrenia is importantly linked to structural brain abnormalities, of which the most consistently identified are lateral and third ventricles enlargement, as well as volumetric reduction of medial temporal and prefrontal lobes in comparison to healthy controls [8-10, 15, 16]. A meta-analysis encompassing 58 studies [15] found a smaller mean cerebral volume and greater mean total ventricular volume in patients with schizophrenia, with significant decreases both in gray and white matter.” were shortened to “In the last decades, with the advent of more sophisticated neuroimaging techniques such as magnetic resonance imaging (MRI), which allows in vivo studies of the brains of individuals with schizophrenia, structural brain changes in schizophrenia have been extensively characterized [8-10]. Some of these findings include smaller mean cerebral volumes and greater mean total ventricular volume in patients with schizophrenia, with significant decreases in both gray and white matter [11].”

8. Tables 2 and 3 appear very long – could the information be displayed more concisely?

Answer: Because a significant amount of data on antipsychotic usage and on the foci of brain structural changes are often displayed in a disorganized manner in the selected articles (e.g., some data in tables, some data in texts, sometimes in different sections of the manuscript), we consider important the task of gathering all these data and presenting them for the reader in an easier way. However, we agree with the reviewer that these are long tables and thus may impair the readability of the article. Thus, we now submit these tables as “additional files”; they will not appear in the main article but will be easily accessible to the reader who wants to find more complete data on specific coordinate for foci, for example. We hope the reviewer finds this strategy satisfactory.

9. Page 3: ‘However, as these structural brain changes are often subtle and their course is difficult to appreciate in an evolutive manner, only after robust and longitudinal MRI studies the possibility of progressive brain structural changes over time (favoring the addition of a “neurodegenerative” hypothesis to the dominant “neurodevelopmental” model) has been strengthen’ – The last word should read ‘strengthened’.

Answer: The correction was made.

10. Page 4: ‘Although a neurodevelopmental insult does not preclude an associated neurodegenerative process [34], the idea of progressive structural changes in the brain over time, which could denote neurodegeneration, have been a controversial issue [35-37]’ – ‘have’ should read ‘has’.

Answer: The correction was made.

11. Page 4: ‘as the findings of different studies seems sometimes inconsistent’ – ‘seems’ should read ‘seem’.

Answer: We changed this excerpt to: “particularly because the findings of different studies have at times seemed inconsistent”.

"particular because the findings of different studies have at times seemed inconsistent"
12. Page 4: ‘For many years, albeit a significant part of individuals involved in neuroimaging studies had used antipsychotic drugs, the role of drug treatment as a cause of these changes has been scarcely investigated [49].’ Would read better as: ‘For many years, although a significant number of individuals involved in neuroimaging studies had used antipsychotic drugs, the role of drug treatment as a cause of these changes has been scarcely investigated [49].’

Answer: The correction was made.

13. Page 5: ‘All these aforementioned inhomogeneities make data interpretation difficult, reinforcing the need for literature reviews and meta-analyses of studies comprehending homogeneous morphometric methodologies and samples of patients on this subject.’ Would read better as: ‘All these aforementioned factors make data interpretation difficult, reinforcing the need for meta-analyses of studies of this area using homogeneous morphometric methodologies and including multiple samples of patients.’

Answer: We changed this excerpt to: “All these aforementioned factors make data interpretation difficult, reinforcing the need for meta-analytic studies in this area using homogeneous morphometric methodologies and including multiple samples of patients”.

14. Page 9/10: ‘Among the several variables that possibly could determine or contribute in some extent to the brain structural changes observed in patients with schizophrenia in the numerous neuroimaging studies performed in these recent years, including those specifically related to the illness (age of onset, duration, severity) and the individual (age, gender, scholarity), it is precisely the role of antipsychotics, however, that remains a current and critical question, maybe still far beyond of a definitive answer.’ Would read better as: ‘Among the several variables that could possibly determine or contribute to the brain structural changes observed in patients with schizophrenia in the numerous neuroimaging studies performed in recent years, including those specifically related to the illness (age of onset, duration, severity) and the individual (age, gender, scholarity), it is the role of antipsychotics that remains a critical question, although possibly still beyond a definitive answer.’

Answer: We changed this excerpt to: “Among the several variables that could possibly determine or contribute to the brain structural changes observed in patients with schizophrenia in the numerous neuroimaging studies performed in recent years -- including those specifically related to the illness (age of onset, duration, severity) and the individual (age, gender, scholarity) -- it is the role of antipsychotics that remains a critical question, although possibly still beyond a definitive answer”.

15. Page 10: ‘A relatively low number of studies have been addressing this issue, which is difficult, furthermore, by the complex task of harmonizing or balancing the effects of all the other possible variables’ would read better as: ‘A relatively low number of studies have addressed this issue, which is made more difficult by the complex task of harmonizing or balancing the effects of all the other possible variables’
Answer: The correction was made.

16. Page 10: ‘Indeed, these concerns previously pointed out, besides the additional difficult of gathering hard-to-recruit subjects with psychiatric diseases of low prevalence rates [101], it all have been led to studies with a relatively small number of patients.’ Would read better as: ‘Indeed, the additional difficulty of recruiting subjects with a psychiatric disease of low prevalence rate [101], has led to studies with relatively small numbers of patients.’

Answer: We changed this excerpt to: “Indeed, the additional difficulty of recruiting subjects with a psychiatric disease that has a low prevalence rate [88] has led to studies with relatively small numbers of patients”.

17. Page 10: ‘Evidence from larger studies encompassing more expressive samples of subjects’ – it is unclear what expressive means – do the authors mean ‘chronic’?

Answer: We used this term to mean “bigger”, although this would be redundant. We now use “Evidence from studies including larger samples of subjects” (…)

18. Page 11: ‘Studies with relatively considerable samples,’ – it is unclear what considerable means – do the authors mean ‘large’ – the samples mentioned do not seem particularly large?

Answer: We used this term to mean “large”. We now use “Studies with large samples (…)”.

19. Page 11: ‘as about half of the longitudinal studies did not found or reported progressive brain changes’ should read: ‘as about half of the longitudinal studies did not find or report progressive brain changes’

Answer: The correction was made.

20. Page 11: ‘and that the distinct patterns of effects determined by typicals or atypicals are inconsistent as well’ would read better as: ‘and the distinct patterns of effects determined by typicals or atypicals were inconsistent as well’

Answer: The correction was made.

21. I would recommend omitting the references to primate studies (112-115) as the effect of antipsychotics in primates may not reflect the effect in humans and experimentation on non-human primates should be avoided.

Answer: We excluded these references.
Reviewer 2

1. The question is well defined, and the area of interest is important. However, I do not know that the results are indicative of antipsychotic usage. There are several scientific and statistical issues that I have with this manuscript. First, if there are known prodromal structural deficits, how can you conclude that the results you are seeing in the meta-analysis (of which only a handful of studies are pre-post treatment effects) are not the result of pre-morbid structural deficits? Secondly, the mixing of typical and atypical could be problematic as they act on different neurotransmitter systems (which of course innervate different brain regions). Third, I don’t think there was a compelling argument for the surprising finding of volumetric excesses.

Answer: Regarding the first question, also alluded to by Reviewer 1 (“How can you conclude that the results you are seeing in the meta-analysis are not the result of pre-morbid structural deficits?”), this is a limitation that affects this whole field of research. The results of various studies on this topic allow us currently only to make hypotheses, without reaching definitive conclusions. Indeed, several literature reviews have been published on this topic (all them are cited in our text), and all the conclusions are elusive, for the most part, because disentangling the effects of drug treatment and underlying pathology is difficult. By studying the effects of these drugs in healthy individuals, it might be at least clarified whether they are due to an interaction with an underlying pathological substrate or whether they are a direct effect of the drug on the brain. However, it is not possible to study the effects of antipsychotics in a healthy population, where there is no therapeutic benefit to justify the exposure. Thus, ethical issues preclude a more definitive study design involving, for example, placebo-treated individuals with schizophrenia and antipsychotic-exposed healthy comparison individuals. In summary, our meta-analysis is not able to respond to this question by the reviewer, nor can any study in the current literature. We have at our disposal only statistical tools to approach this question. However, we agree with most of the authors of recent literature reviews that “until we have more reliable studies from non-medicated
patients, the potential impact of the confounding effect of medication must be borne in mind” (Borgwardt et al; Psychological Medicine (2009), 39, 1781–1782) and that “following the Hippocratic mandate to ‘first do not harm’, it should be assumed that the drugs rather than the disorder are causing the effects, until proven otherwise. Similarly, although it is not impossible that antipsychotic-induced brain alterations are beneficial, it seems more prudent to assume that they might be harmful, and to direct research into assessing this possibility” (Moncrieff and Leo, Psychological Medicine, 40 (2010). doi:10.1017/S0033291710001698). Independent of such facts, please note, however, that with this work, we do not intend primarily to question the internal validity of each published study reporting that these changes might be associated with antipsychotics; despite such concerns, they are already published and point to a possible association. Methodologically, they are the best that the current literature has to offer, so we are meta-analyzing them. We agree with the reviewer, however, that such limitations in the literature restricted our findings as well, and, in response to her comments, we are adding these concerns to the Limitations section.

Regarding the second question (“the mixing of typical and atypical could be problematic”), there is no sufficient evidence in the available literature to support the notion of differential effects of typicality on the brain changes associated with antipsychotics. The results of different studies are controversial, and, to cite the conclusions of a recent literature review: “The evidence on typicals and atypicals from the present review was inconsistent and overall it was not possible to conclude that they have differential effects” (Moncrieff & Leo, Psychological Medicine (2010), 40, 1409–1422). In addition, in the largest longitudinal study on antipsychotic usage and brain volumes in schizophrenia published to date, involving 211 patients, Ho et al. found that changes in brain volume were similar for all classes of antipsychotic medications (Ho et al. Arch Gen Psychiatry 2011;68(2):128-137). Thus, we do not think that combining different classes of antipsychotics in our meta-analysis is problematic. However, in response to these concerns, we conducted additional sub-analyses on the effects of typicals vs. atypicals. Although we did not find significant clusters of brain changes with typicals (a low number of foci), we found significant clusters with atypicals, the results (volumetric increases in the putamen and thalamus, for example) being consistent with the literature findings. Please note the updates made to Results and Discussion.

Regarding the third question (“absence of compelling argument for the finding of volumetric excesses”), the findings of volumetric excesses in the meta-analysis are a consequence of such statistically significant findings across multiple different enrolled studies. In response to these concerns, we now discuss these findings in more detail.

2. The data are as best as they can be with the current state of the literature, however, I think the conclusions are overstated given the heterogeneity of the search space. While one of the strengths of meta-analysis is to overcome this, that relies on having an adequate sample size (i.e., a large number of studies with similar contrasts). This study has a small number of studies, and within studies, there is significant variability both in type of antipsychotic examined, and groups studies (either pre-post within subject design or between subject designs).

Answer: We agree with the reviewer that heterogeneity is indeed a concern. However, as we responded to Reviewer 1, an ALE meta-analysis is less likely to be affected by such limitations in that it
quantitatively integrates different foci across studies, determining clusters of significant topographic convergence. In this sense, by combining foci of only two studies and finding significant clusters of anatomical convergence between them, one would have a valid analysis and strengthened results. In fields such as this, in which VBM studies are scarce, gathering 10 studies, as was performed in our case, is very significant. As an example, ALE meta-analyses have been performed with as few as six studies (Ferreira et al. Neurobiology of Aging 2011; 32:1733–1741), seven studies (Hattingh et al. Front Hum Neurosci. 2013; 6: 347), and so forth. Recently, BMC Psychiatry published an ALE meta-analysis that included nine studies (Titova et al., BMC Psychiatry 2013;13:110). Finally, regarding heterogeneity within studies, as ALE aims at pooling data from different experiments that investigate similar questions but employ variations on the experimental design, it is important to emphasize that this revised version of the ALE algorithm models the spatial uncertainty – and thus probability distribution – of each focus using an estimation of the inter-subject and inter-laboratory variability typically observed in neuroimaging experiments. This differs from using a pre-specified full-width half maximum for all experiments, as was originally proposed. In addition, the ALE approach includes a new method of inference that calculates the above-chance clustering between experiments (i.e., random-effects analysis), rather than between foci (i.e., fixed-effects analysis). Thus, this algorithm employs a null distribution of spatial independence across studies rather than a null distribution of random cluster locations, thus minimizing the impact of heterogeneity across studies. We also agree that, despite this, the conclusions may be overstated and, in response to the reviewer’s comments, we have reformulated them (please note the changes in the Conclusion section of the abstract and in the Discussion).

3. As stated above, I think there is an adequate discussion regarding the volumetric decreases found in the studies, but hardly any discussion regarding the excesses, which warrants a greater discussion given that it goes against what the most popular theories are.

Answer: As previously addressed, such results are a consequence of such statistically significant findings of volumetric excesses across multiple different enrolled studies. We added excerpts to the Discussion to better analyze the question of volumetric excesses. Please note that in the Abstract, we now invert the order of appearance of brain changes: first, volumetric decreases, and second, volumetric increases, to validate those findings that are less counter-intuitive.

4. However, I think there needs to be more discussion regarding the heterogeneity of the results. I would like to see additional ALEs run (maybe comparing atypical vs. typical, where possible; or comparing within vs. between designs), to minimize the concerns I have with the search space.

Answer: We added more discussion to the Limitations sections regarding heterogeneity. In addition, as detailed above, we conducted additional sub-analyses on the effects of typicals vs. atypicals. Please note the updates made to Results and Discussion. As also detailed in the response to Reviewer 1, it should be noted that the differences between cross-sectional and longitudinal studies are not a fundamental concern in ALE meta-analyses, because the design of each study does not influence how each retrieved coordinate will affect the ALE results.
5. The content of the writing is acceptable, however, the manuscript could use a read through for minor grammatical/spelling errors.

Answer: Thank you for the advice. We submitted the manuscript to an external English-language professional editing service.

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