Author’s response to reviews

Title: Haloperidol affects bones while clozapine alters metabolic parameters - sex specific effects in rats perinatally treated with phencyclidine

Authors:

Tatjana Nikolic (tatjana.nikolic@med.bg.ac.rs)
Milan Petronijevic (milanpetronijevic@yahoo.com)
Jelena Sopta (jslabic@yahoo.com)
Milica Velimirovic (drmilicavelimirovic@gmail.com)
Tihomir Stojkovic (stihomir85@yahoo.com)
Gordana Jevtic Dozudic (jevtic.gordana86@gmail.com)
Milan Aksic (milan.aksic@med.bg.ac.rs)
Nevena Radonjic (nevence@yahoo.com)
Natasa Petronijevic (natasapetronijevic@yahoo.com)

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Author’s response to reviews:

Dear Editor,

Thank you very much for your encouraging comments regarding our manuscript that we have found valuable and helpful in revising and improving our paper. We have studied the comments carefully and have made corrections which we hope will be met with approval. Please find below answers to all raised questions.

Responses to Reviewer No 1:

1. I believe that title of the manuscript should be changed to the conclusion type of title. It will attract more readers.
Response: Thank you very much for your kind advice. According to your suggestion, we have modified the title of the manuscript as below:

“Haloperidol affects bones while clozapine alters metabolic parameters - sex specific effects in rats perinatally treated with phencyclidine”.

2. Introduction, pages 5-6, lines 60 and 4. Please, explain what does it mean "positive and negative symptoms"

Response: Thank you for your careful review. We have accepted your suggestion and modified the paragraph (page 6, lines 1-5 ) at the following manner:

“PCP is non-competitive antagonist of glutamatergic N-methyl-D-aspartate (NMDA) receptors, capable to produce a broad spectrum of effects in healthy human volunteers that resemble SCH. PCP may induce positive symptoms including agitation, audiovisual hallucinations and paranoid delusions, negative symptoms represented as blunting of affect and apathy, as well as, cognitive disorders [27, 28].

3. Material and Methods. Page 9, lines 1-18. This paragraph should be moved to subsection 2.1 after sentence: "Food and water were available ad libitum throughout the experiment."

Response: We have moved mentioned paragraph to subsection 2.1 as you suggested (Page 7, lines 6-9).

4. Discussion section. General comment. While I like the direction of the discussion of the side effect problems, but it sounds more like a review article or introduction to the paper. It will be beneficial for the manuscript, if authors will discuss more of their results and how these results have corresponded to already published preclinical and/or clinical observations.

Response: Thank you for your constructive advice. We have modified the “Discussion section” as you suggested. We hope that in this form the discussion is more directed to our results.
5. Lastly, this manuscript needs to be read by English native speaker and edited. For example, the abstract starts from the sentence: "The expression of schizophrenia (SCH) is different between the sexes." Maybe it will be better to say: "The presentation of schizophrenia (SCH) symptoms is different between the sexes". Additionally, second paragraph in the discussion section, page 18. There are so many abbreviations that it is difficult to keep in mind what is what. It will be easy to read this manuscript, if authors will spell out some abbreviation in the discussion section and other places with excessive number of abbreviations.

Response: Thank you for your comment. The manuscript has been edited by an English native speaker. According to your suggestion, we have modified the first sentence in the Abstract. Also, we have checked the text carefully and spelled out some abbreviation in the discussion section, page 18, as well as in other places in the manuscript. We hope that now it will be easier to read.

Responses to Reviewer No 2

1. The number of rats in each group and the routes of administration of the anti-psychotic drugs are not found in the Abstract.

Response: Thank you for your careful review. According to your suggestion, we have added the number of rats in each group and the routes of administration of the antipsychotic drugs in the Abstract.

2. It was better if the authors could prove induction of schizophrenia through studying the psychotic behaviors of the experimental rats.

Response: Thank you for this remark. Behavioral experiments that prove induction of schizophrenia like symptoms in the same animal model have been previously published. Perinatal treatment with PCP in rats has resulted in several alterations that represent equivalents to the behavioral changes observed in schizophrenia. Deficit in the prepulse inhibition of acoustic startle response, a measure used in the assessment of sensorimotor gating deficits in schizophrenia, increased locomotor activity that is accepted as an index of positive symptoms, disturbances in working memory that are a core feature of the cognitive dysfunction, have been repeatedly found in rats perinatally treated with phencyclidine (Wang et al., 2001; reviewed in Mouri et al., 2007; Grayson et al., 2015).

Due to your constructive suggestion we have tried to explain this better in the present form of the manuscript (Page 6, lines 6-13).
Reference


Responses to Reviewer No 3

1. In Figure 1, it is confused when the authors claimed there is significant difference between experimental group and control group because the difference is actually very small. Does this small difference necessarily mean anything?

Response: Thank you for your comment. We have modified y axis marks to improve visibility of the difference at the graphs on Figure 1. Usually the changes of BMD by DXA are not easily visible after short periods of time and in humans control DXA measurements are performed at least after one year of treatment (Kling et al., 2014). Also, several authors have found small, but statistically confirmed, changes of BMD measured by DXA, in rats after different experimental procedures (Sasso et al., 2015; Boudenot et al., 2014). As we have described in Material and methods section, all data were analysed using the one way ANOVA with Fisher's post hoc test and all results were presented as mean values with standard error of the mean (SEM). P value less than 0.05 was considered statistically significant.

To prove the changes observed by DXA we have performed quantitative microscopic analysis.


2. In Figure 1 and 2, it is interesting to see huge difference between male and female rats. But why PCP has almost no effects on female rats if it also targets the NMDA receptor. Compared to the PCP group, why PCP+H or PCP+C is worse?

Response: Thank you for your comment. Sex specific effects of PCP treatment are frequently found in different animals and the exact reason for their appearance is not completely understood. Usually, the observed gender differences are assigned to the protective effects of estradiol and progesterone in female rats (Elsworth et al., 2014). In the following paragraph in the Discussion section of our manuscript we have discussed this matter:

Page 22, lines 11-21 - we described sex specific effects of PCP investigation on primate PCP model of SCH in the juvenile subjects.

Page 22, lines 22-23; Page 23, lines 1-8 - selective sensitivity of PCP perinatally treated females to haloperidol has been discussed related to hypoestrogenism.

Changes in PCP+H or PCP+C groups represent the effects of antipsychotics. Haloperidol or clozapine sometimes do not affect the changes caused by PCP, but in some cases given antipsychotic reverses or worsens changes caused by PCP. Differences in these effects are probably due to their mechanism of action and the specificity of changes caused by PCP. Perinatal PCP treatment produces changes of several neurotransmitters in the brain and
antipsychotics have also influence on neurotransmitter systems. PCP is a noncompetitive antagonist of the NMDA subtype of the glutamate receptor, and it causes the release and inhibits the reuptake of monoaminergic neurotransmitters, including dopamine, serotonin and norepinephrine (Ahmadi et al., 2014). Typical antipsychotics such as haloperidol, primarily inhibit dopaminergic pathways while atypical antipsychotics like clozapine have both anti-dopaminergic and anti-serotonergic activity (Freedman, 2003).

Reference


3. In Figure 3, please point out and label the differences properly to help the readers.

Response: Figures 3 and 4 are original mycrophotographs taken directly from the microscope using morphometry software and image analysis. All dimensions in the photographs are measured and recorded in the image by software. We have labeled Figures 3 and 4 to point out the differences between groups.
Responses to Reviewer No 4

1. The experimental design lack a Haloperidol-treated group and treated with an antiresorptive (e.g., bisphosphonates) or osteoanabolic agent (e.g., calcitonin), this group is important since it provides clues about the mechanistic effect of haloperidol on BMD.

Response: Thank you for this suggestion. Our study was designed to define the effects of haloperidol and clozapine chronic treatment on bones and metabolic parameters in PCP animal model of SCH and observed changes certainly will direct future investigation. We will try to conduct the experiments, according to your suggestion, involving antiresorptive and osteoanabolic agents in the rats treated with haloperidol, although there is a huge problem with the route of administration of these drugs. But, at the present study, we have included this in the section “Limitations of the study” (Page 28, lines 15-22; Page 29, lines 1-2).

2. Also, the authors need to measure the levels of serum calcitonin and PTH 2 hormones mainly involved in bone and minerals metabolism.

Response: This is also an important suggestion. We were unable to perform the measurements of PTH and calcitonin but we have measured the serum concentrations of the calcium and phosphates in the present study. Findings of normal concentration of these electrolytes in our animals indirectly indicate normal levels of the hormones involved in calcium homeostasis.

In the future we are planning to set additional experiments in order to elucidate the mechanism of antipsychotics effects on bones and to measure PTH, vitamin D, calcitonin, biochemical markers of osteosynthesis and osteoresorption as well as RANK (receptor activator of nuclear factor kappa-B)/RANK ligand and osteoprotegerin molecules.

We have also added the statement about calcitonin and PTH in the section “Limitations of the study” (Page 28, lines 15-22; Page 29, lines 1-2).