Open peer review report 1

Reviewer: Xavier d'Anglemont de Tassigny, Hospital Universitario Virgen del Rocio, Spain.

Comments to the authors:

In this work, the authors studied the effect of rotenone and 6OHDA stereotaxically injected into the right side of the substantia nigra of adult rats. The damage resulting from rotenone or 6OHDA injection on the nigrostriatal dopaminergic pathway was assessed by two different MRI contrast techniques: diffusion tensor imaging (DTI)-derived parameters for fractional anisotropy (FA) and iron-sensitive parameters T2 at 1, 2, 4 and 6 weeks post-treatment. Histology of the substantia nigra has also been performed after sacrifice at 6 weeks to evaluate the damage of the TH-positive neurons. The authors observed statistical differences between treatments with FA, and little difference with T2. FA values are reduced at 6th week post-rotenone while FA values are reduced in the 1st week post-6OHDA, and then increased. A qualitative post-mortem examination of the SN seems to indicate apoptosis. The authors conclude that their findings indicate that the 6-OHDA-induced model may be more suitable for the study of dopaminergic neurons over short periods, while the RT-induced model may be more appropriate for the long-term study of the pathological and physiological processes of PD as a whole.

General comments:

The paper would benefit from proper scientific English editing since numerous syntactical mistakes, miswording and colloquial phrasing are present throughout the manuscript.

It is not clear to me what exactly the authors want to evaluate primarily. Is this the effect of rotenone and 6OHDA on DA neurons? or the use of DTI to evaluate toxic damage on DA neurons?

The discussion is excessively long and therefore should be presented in a more concise format.

The interest of this work is limited, as many other groups have substantially studied rotenone and 6OHDA neurotoxins. FA and T2 imaging methods do not seem to provide very exciting data to support their use in rat PD models. Furthermore, the conclusions drawn by the authors regarding the effect of rotenone and 6OHDA intranigral administration on DA neurons are to my opinion, little supported by the data presented in the paper.

Please find below my specific comments.

Major comments

1. There is an important caveat in the experimental design. Rotenone was prepared in DMSO while 6-OHDA was dissolved in 0.9% saline solution. However there is only one control group consisting in saline solution. This experiment requires one more group; otherwise the rotenone group cannot be compared with any other group (control and 6OHDA). Control group for rotenone (RT) should receive 2µl of DMSO. DMSO is known to affect cell physiology and it is quite likely that the sole 100% DMSO intra-nigral administraiton impairs neuronal function (for DMSO effect on nerve cells see Hanslick et al. Neurobiol Dis. 2009 Apr; 34(1): 1-10). This is a crucial point to address.

2. Page7, line 1. Dopamine neurons damage was evaluated in a qualitative rather than quantitative manner as mentioned in the text: "reviewed under a microscope by a pathologist with 30 years of experience in neural pathology".

Was the pathologist blinded from treatment? It is strongly suggested to apply other methods of assessment of the nigrostriatal DA pathway damage, especially since the purpose of this work is to compare two models of PD rats.

3. The quality of Figure 2 should be improved as, to my opinion, it is difficult to gauge the effect of the toxic agents on TH positive neurons based on the photos presented. The figure is not sufficiently annotated (scale-bar, arrows to indicate the "piknotic cells"). Contrast should be increased in photos C and D. It is not indicated which photo illustrates the SN of rotenone-treated and which one is 6OHDA-treated (B or D?). Moreover, the TH staining in C does not contain any TH-positive cell body. Finally, I am not certain that lighter stained cytoplasm is a formal criterion to measure cell damage, as

stated in the legend for Figure 2 (page 20, line 14).
4. Since the data presented in figures 3 and 4 are already presented in tables 1 and 2, authors should remove figures 3 and 4, as they do not provide further data than those in the tables and lack clarity.
5. Page 9, line 48. Authors state: "There was obvious apoptosis in tyrosine hydroxylase-positive (TH+) cells in the right SN after RT or 6-OHDA injection, indicating the successful establishment of the models". This is an overstatement since no apoptosis staining has been performed in the study (e.g. cleaved-caspase 3 or TUNEL). If the authors want to draw such conclusions, then they should carry out the appropriate staining.
6. Page 11, line32. Authors state: "At the beginning of the 6-OHDA injection, neuron loss and glial proliferation demyelination leads to a decrease in water diffusion limitation and an increase in FA parameters; (2) This phenomenon may be attributed to the activation of microglial cells induced by the neural inflammatory reaction". Such assumption needs to be demonstrated by a double staining for TH / myelin specific marker (MBP, RIP...) and activated microglia markers (Iba1, CD68, CD86 etc).
7. Page 20, line 1. I do not understand the Figure 1 legend. Are the MRI images from the same animal with different orientation? Or from two different animals (RT in A and 6OHDA in B). Images from control mice is also needed.

Minor comments
1. Page 5, line 24. Please define P, R and I.
2. Page 5, line 28. Please indicate the institution supervising the Ethics Committee.
3. Page 7, line 42. The values indicated into brackets are not precisely the ones described in the preceding sentences. Authors should be more accurate with data description.
4. The same Table 1 and Table 2 are duplicated (pages 18, 19 and 25, 26).
5. Page 12, line 56. In the text: "As Nicolas reported". I believe that this is the first name of the cited author. Authors should change this with the last name.