JoVE58455R1 POINT–BY-POINT RESPONSE TO EDITOR:

**Editorial comments**

GENERAL COMMENTS (e-mail dated July 2nd, 2018):

1. The editor has formatted the manuscript to match the journal's style, please retain the same.

*Now we maintained the journal's style.*

2. Please address all specific comments marked in the manuscript.

*These were addressed and the text changes evidenced in yellow and further indicated at the end of this document.*

3. All optional steps, caution, and notes need to start from a new line number.

*Thank you, the formatting of all optional steps, caution, and notes was corrected.*

4. Please describe exactly how you did the experiment rather than generalizing.

*The generalizing terms and descriptions were now limited.*

5. Presently the protocol is more than 10 pages. Please reduce the same.

*The protocol is now 9 pages.*

6. Once rewritten, please ensure that the highlight is no more than 2.75 pages including heading and spacings. Also, this should form a cohesive story and logically flow from one step to another. Presently, it is disconnected.

*The part of the document to be filmed is now of about 1.5 pages. To have a cohesive story we decided to further cut the protocol and only the 1 day microdialysis under EEG recording was maintained because it is core of our work.*

*Importantly, we decided to add a flow chart (Figure 3) to the work for better comprehension of experimental design (lines 268, 707-717) hoping that this can help.*

SPECIFIC COMMENTS (manuscript track changes):

7. You are not filming the chromatographic determination of the glutamate and aspartate. Either reword the title or recheck the highlights to ensure it is in line with the title of the manuscript. Additionally, highlights should form a cohesive story and should not be more than 2.75 pages including heading and spacings.

*We agree. Now, the chromatographic determination does not appear in the title. We added the detail on animals used in protocol instead.*

8. line 58 - Please be more crisp.

*We used the term monitored in the phrase (line 58).*

9. line 72 - Not clear, please check the grammar.

*The phrase was substantially modified (lines 72-74).*

10. line 98 - Provide citations to show how the technique has been used for the purpose mentioned here.

*Relative citations are now provided (page 2, 2nd paragraph, lines 101, 103, 104, 107, 109, 116, 118 and lines 914-934) and the numbers of following citations were updated, the reference list was update equally (within lines 935-1098).*

11. line 131 - Behaving animals? Please reword. Animal behavior studies maybe?

*We replaced the term for awake animals (line 131).*

12. lines 135-136 - Is the usage of to correct here?

*Thank you, this was not correct. To was deleted (lines 135-136).*

13. line 160 - Please do not generalize the protocol. Please explain how to perform your experiments with specifics. Please ensure that all the steps have required details, volume, concentration, time, how specifics etc. Please use imperative tense throughout as if directing someone how to do the experiments.

*We reformulated the text according to suggestions of the editor.*

14. line 171 - Please provide step by step figure highlighting important steps to show how the assembly of probe-electrode device is happening to bring out more clarity. Label this as figure 1 and refer to different panels wherever possible in the steps.

*Thank you. We added Figure 1 showing how to assemble the device to the manuscript and we refer to it (lines 175, 177, 181-183, 185, 187-189).*

15. line 178 - So you remove the electrode while performing microdialysis?

*No, only the guide cannula is removed and substituted for microdialysis probe 24h before the dialysis session. Electrode is firmly attached to the guide cannula sleeve and once the device is cemented onto the animal’s skull it cannot be removed. We hope that with Fig. 1 the shift information is more clear.*

16.  line 191 - Please reword: Sterilize the device under germicidal UV light…. What is meant by grilling it on its 4 sides?

*Thank you, indeed it is not precise. We reworded the point (lines 191-192).*

17.  line 203 - Please write this in the order of how you performed the steps. First you anesthetize the rats, fix it on the stereotaxic frame and then implant the device (where is it being implanted- in the brain, which part), etc. Just exactly write the order of the steps in imperative tense as if directing someone to do the experiment.

*Stereotaxic surgery is now described in more details (lines 206-210, lines 212-218, lines 220-223).*

18. line 213 - Where is the device being implanted.

*As above (lines 214-215).*

19. line 207 - What is meant by adult handled? Also, how do you implant. If it is done using standard techniques, please provide a reference.

*The term “handled” was deleted as superfluous. How we implant: as above (point 2.2). References are present.*

20. line 216 and followings - We cannot have two caution statements together. Hence merged. Please check.

*We checked the text, it is fine, thank you (lines 216-218).*

21.  line 220 – Where and how?

*The point 2.3 was rephrased and the information regarding the screw positions was provided (lines 220-223).*

22. line 227 - Please reference the videos here instead of making optional statement.

*We referenced to videos in point 2.4. (lines 229-230).*

23. line 236 - This is not written in imperative tense. Hence converted to a note. If this needs to be a part of the step above, please write in imperative tense and provide the accurate dosage of what antibiotic was given in your experiment.

*The imperative was used and the note comprising the names of used drugs and relative doses is now part of the point 2.6. (lines 237-239). The table of Materials was updated to refer the used drug products.*

24. line 249 - Reworded please check. Also please be crisp while writing steps. Please also provide the needle and syringe size for subcutaneous/ i.p injection

*We checked the text, it is fine, thank you (lines 249-250). The needle and syringe size for i.p injection is now provided (lines 251-252) and Table of Materials updated for these.*

25. line 250 - How do you visually check the induction of SE? include a note stating this.

*The note was added to the point 3.2. (lines 252-254).*

26.  line 259 - Aint the first and the last sentence same? Please write exactly as to how you performed your experiment. How and when did you interrupt/arrest the SE. This will make it easier for someone to follow the protocol. Presently the difference between interrupting and arresting is not coming out well.

*Thank you for your suggestion. The point was reorganized (lines 256-257).*

27.  line 261 - Is this through injection or orally. Please reword. Only 1 ml of the solution is given every day? Don’t you decide the amount as per the body weight?

*The information regarding the type of administration is now included (lines 261-262). Yes, only 1 ml of saline and 1 ml of sucrose solution is given/animal/day and the animals recover well.*

28. line 279 - Please explain how to perform this? Also please remove the redundancy. Looks like step 4.1.1 is just a combined step of 4.1.2-4.1.7. please exactly write how you did the experiment. So you divided the rats into different groups? Did you process the groups differently or all the group undergo same monitoring and analysis?

*The chapter 4.1. would not be part of the video, thus we did not evidence points 4.1.4. and 4.1.5. The precedent point 4.1.1 was deleted because redundant and the following points rearranged (lines 289, 292-293, 297-299, 304-307). We also included Figure 3 (experiments flow) into the manuscript.*

29. line 297 – How?

*Previous option is now part of the point 4.1.5. explaining how to connect the animal (lines 297-299).*

30. line 305 - So EEG and microdialysis is not done together? Please clarify? How do you know when to do what? Again it be nice if you write the specifics of your protocol as to how you did your experiment rather than writing a general protocol.

*The decision is depending on the experimental group investigated. We included the Figure 3 with flow chart to bring more clarity into all story.*

31.  line 309 – We cannot have paragraph of text in the protocol section. Please consider moving some to the discussion section.

*The part of the precedent note text was now integrated in protocol point 4.1. (lines 304-307).*

32. line 331 - So many substeps are not necessary.

*This chapter was substantially rearranged (lines 334-335, lines 344-346, lines 348-350) to be shorter.*

33. line 342 - How?

*The description is provided (lines 342-343).*

34.  line 348 and 349 – Recorded file to one for analysis? What does that mean. Also, how do you copy or transform. Please ensure that you answer how question how is this step performed.

*The was rearranged (lines 348-350).*

35. line 355 - We cannot have paragraph of text in the protocol section. Please only include what is relevant to the current protocol described here and please consider moving some parts to the discussion section.

*We eliminated the in vitro recovery testing form video. The chapter 5.1. was substantially rearranged (lines 361-363, 397-398).*

36. line 368 - This is commercial. Please use generic term instead. Maybe paraffin film.

*The term was changed according editor’s suggestion (lines 368, 371-373).*

37. line 361 - How do you do so? (run the experiment in triplicate).

*The information is now provided (lines 361-363).*

38.  line 390 - What are the testing conditions in your case?

*The text was rearranged and point 5.1.9. added to 5.1. section to be more comprehensive (lines 397-398).*

39. line 394 - Optional, notes and caution steps cannot be filmed. Please remove the highlight from these. Also start all these three from new lines leaving a blank space between step and optional step.

*Option was avoided, the precedent optional text is now part of the point 5.1.8. (lines 394-395).*

40. line 400 - This is not an option. This is what you do. Removed the option word. Please check.

*You are right, thank you. The step is part of point 5.1.10.*

41.  line 405 – Step number please.

*The number was indicated (line 405).*

42. line 423 - What amount of isoflurane is used?

*The information is now provided (line 423).*

43. line 428 - Caution, notes and options cannot be filmed.

*Yes, thank you. This caution is usually reported on manufacturer user's guide of dialyzing probe if these are endowed with protective sleeve thus may not be filmed.*

44.  line 430 – Ambient or ambience? Please check the grammar throughout.

*Yes, thank you, the ambience is better (line 430). The phrase was anticipated because of work logic flow.*

45. line 436 - What is the low pressure used. How much ringer solution and how is it pushed in?

*We added the information, but now we rather refer to velocity of pushing the syringe piston than pressure, because we do not know this precisely (lines 436-437). Also the previous point 5.2.1.8. is part of point 5.2.1.6. now (lines 441-442).*

46. line 484 – What is the right moment?

*The step was reformulated to indicate the moment (lines 484-485, 487).*

47. line 496 - As above. (Option).

*The previous option was included into step 5.2.2.10. and will be a part of the film (lines 496-498).*

48.  line 514 - So the microdialysis is performed in live animals?

*Yes, this was now addressed in the title of section 5.2. (line 410).*

49. line 530 - Provide citation.

*The citation was now provided (line 530), even if we do not know if it is sufficient. The embedding is automatically done by commercially available machines and protocols are very similar between the brands; we use 48 h of 10% formalin.*

50. line 682 - Please ensure that all figures and tables have title for the figure

*We added the missing titles (lines 718, 731).* *Moreover, we added 2 new figures and respective legends: Figure 1, as you suggested, showing how to assemble the dialysis-EEG device (lines 684-695). Figure 3 and respective legend should bring more clarity to entire manuscript (lines 706-716). Previous Figure 1 became Figure 2, and previous Figures 2-6 became numbers 4-8 (lines 697, 718, 726, 731, 738, 749).*

51. line 767 - Please do not make subsections. I have removed it.

*Thank you, we did not know.*