Response to reviewers - JoVE56569

**Changes recommended by the JoVE Scientific Review Editor:**  
  
• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

*We have done this.*  
• Some sections of the representative results show significant overlap with previously published work. Please re-write the text highlighted in red font in the attached document to avoid this overlap.

*We have rewritten the indicated section. The overlap with previously published work is probably work on the PainCart (J.L. Hay et al.), also developed by the Centre for Human Drug Research: the exact similar method of analyzing is applicable to the NeuroCart and the PainCart data.*  
• **Abstracts:**  
1) Please revise the Short Abstract so that it clearly states the goal of the protocol within 50 words.

*The short abstract has been re-written to meet the suggestions.*  
  
• Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the guidelines of your institutions human research ethics committee.

*The statement regarding ethical approval for this protocol is updated according to the reviewer’s comments.*  
  
• **Protocol Language:** The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief “Note” at the end of the step (please limit notes).  
1) Please move the descriptive sections of the protocol to introduction, Representative Results or Discussion. The JoVE protocol should be a set of instructions rather a report of a study. Any reporting should be moved into the representative results.

*The descriptive section in the protocol section have been moved to the introduction section.*2) Line 143-164: Please merge these and condense into a single note of no more than ~10 lines.

*This section has been rewritten in the introduction section.*3) Please edit the protocol so that ALL steps are in the imperative tense. Example not in imperative tense: “The subject is instructed to score how they are currently feeling.” This can be rewritten as “Instruct the subject to score how they are currently feeling.”

*The steps have been rewritten zo that they are now stated in the imperative sense.*  
• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more details to the following protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure that all additional details in the protocol section are written in the imperative tense, as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.).  
1) 1.2.5: Are there 3 electrodes in total? Please mention where the electrodes are applied.

*There are indeed 3 electrodes in total, 2 eye electrodes and 1 ground electrodes. The sites for the application of the sites is provided in the preceding steps. We have clarified that there are 3 electrodes in this step.*2) 1.2.12: How? Do you simply push a button?

*A computer program (script) must be started by the research assistant. This has been clarified and added in the protocol steps.*3) 1.3.1: It is unclear what is done here. What is the score scale used here?

*In the introduction we have added information on this test with examples of the visual analogue scale lines, so that it is more clear what is happening. Also, the steps in the protocol have been updated.*  
4) 1.3.3: How? What is happening in this test? Does something happen on the screen? Please add a note to describe what the subject sees.

*In the introduction we have added information on this test with examples of the visual analogue scale lines, so that it is more clear what is happening. Also, the steps in the protocol have been updated.*  
5) 1.4.4: How? What is happening in this test? Does something happen on the screen? Please add a note to describe what the subject sees.

*In the introduction we have added information on this test with examples of the visual analogue scale lines, so that it is more clear what is happening. Also, the steps in the protocol have been updated.*  
6) 1.5.1: This is not clear enough.

*In the introduction we have added information on this test with examples of the visual analogue scale lines, so that it is more clear what is happening. Also, the steps in the protocol have been updated.*  
7) 1.5.2: How? What is happening in this test? Does something happen on the screen? Please add a note to describe what the subject sees.

*In the introduction we have added information on this test with examples of the visual analogue scale lines, so that it is more clear what is happening. Also, the steps in the protocol have been updated.*  
8) 1.6.14: How?

*In general: in the introduction we have added some explanation on the different tests, including references so that it is more clear what is happening to the subject and on the screen. Via the references additional information can be accessed, allowing for replication of the protocol. In addition, the above indicated steps have been reviewed and updated with more detailed information.*  
  
• **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.  
1) Please highlight 1.2.5 for continuity.  
2) **Please ensure that the manuscript title best reflects the filmable content (i.e. the portions you highlight).**

*The above suggestions have been implemented.*  
• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

*Where possible, this has been implemented.*

*A section about troubleshooting is in our view not feasible to describe, the methods described in this paper comprise a rather complex computer set-up and several different tests, for which no short troubleshooting section can be written that would fit the paper. Therefore, troubleshooting has not been described.*  
• **Figures:**:  
1) Fig 1,2 : Please define error bars, mention sample sizes.

*The definition of the error bars has been incorporated in the figures (i.e. 95% confidence interval bars, top left of the figure), as well as in the results section text. We have added the sample size in the legend of the figures, not in the figures themselves.*  
2) Please provide each figure as an individual PDF, TIFF, JPEG or PNG files.

*The figures are provided in TIFF or PNG.*  
• **Figure/Table Legends:**: Please expand the legends to adequately describe the figures/tables. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.

*The legends of the figures have been expanded and updated. The legend of the tables were erroneously not included in the initial submission and have been added.*  
  
• **Commercial Language:**JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are NeuroCart, Cantab, Cogstate, skinPure, AMBU Blue Sensor N, Grass,  
1) Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript (including the title) with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.

*The commercial language has been removed and where necessary references have been added in the text.*  
  
• **Table of Materials:**Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as software.

*The table of Materials has been re-organized and updated (now hard and software per test and for the whole of the NeuroCart is separated for easier reading and understanding).*  
• Please define all abbreviations at first use.

*This has been done.*  
• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.

*This has been done.*  
• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

*The figures have been modified from a previous publication by the authors from the current JoVE paper. According to the guideline for permission for reuse from the British Journal for Clinical Pharmacology (where the figures are published) original authors can reuse their materials without explicit permission from the Journal.*

**Comments from Peer-Reviewers:**   
  
**Reviewer #1:**  
*Manuscript Summary:*  
Hart et al./Groeneveld present the computerized test battery NeuroCart that their group at CHDR has developed over the last 20 years. NeuroCart is designed to characterize pharmacodynamic effects of CNS-targeting drugs, as well as to monitor CNS side effects of peripheral drugs. Compared to other existing test batteries, e.g. Cantab or Cogstate, the NeuroCart battery offers multimodality by combining neurocognitive tests with neurophysiological measurements.The authors' test battery has been validated and published in several peer-reviewed articles. Overall, this is a timely article that will be useful to the clinical Neuroscience community interested in practical applications of early pharmacodynamic readouts and computerized drug-testing batteries.  
  
*Major Concerns:*  
I do not have any major concerns.  
  
*Minor Concerns:*  
- I am confused about the author's interpretation of the data shown in Fig.1 and Fig.2. In lines 331-333 the authors state that "the effects of 10 mg of mecamylamine  
administration is comparable to placebo Administration of 20 mg of mecamylamine led to worse performance on the adaptive tracker test in comparison to placebo". By inspecting the plotted mean+/-error values, one would get an impression that both 10 mg and 20 mg mecamylamine are comparable to placebo and indistinguishable from each other; in contrast, scopolamine appears clearly different from placebo (at least at the 3.5h and 4.5h time points in Fig.2, possibly also at 6h and 8h in Fig.1). Since the authors do not provide any p values, it is difficult to understand what their conclusion about the difference between the effects of these two doses of mecamylamine is based on.

*We would like to thank the reviewer for their comments and suggestions. We agree that the way it was written it was not easy to interpret the data. The text has been updated to include the statistical data (95% confidence intervals and p-values etc).*  
- Please spell out PD in the abstract (line 59); the word "pharmacodynamics (PD)" appears in Introduction (line 71), but the reader of the Abstract alone is left to guess whether PD means Parkinson's disease, pharmacodynamics or something else.

*This was corrected.*  
- In line 139, please refer to either the names of the Ethics Committees, or to the publications where this information can be found (presumably, ref.3-8 and ref.11?)

*The specific ECs were added.*  
- Line 319: when mentioning the ANOVA test, please indicate whether the data were tested for normality of their distribution prior to applying this test.

*Information on checking for normality has been added.*  
- Line 337: please provide the p vaule next to the claim that the the results were "small, albeit statistically significant".

*The text has been rewritten, also based on other comments, so this particular sentence has been dropped from the text. However, we agree with the reviewer that without information on statistical ooutcome the results are difficult to interpret on figures alone. Therefore, in the results text the mean differences between treatments, including 95% confidence intervals and p-values have been added.*  
- Some typos that I spotted:  
line 203 - consider replacing "rests of gel" with "the remaining gel";

*Your suggestion has been implemented.*

line 276 - replace "do not rub to softly" with "do not rub too softly".

*This has been corrected.*

**Reviewer #2:**  
*Manuscript Summary:*  
The authors describe use of Neurocart, a computerized battery of behavioral and electrophysiological tests, as a means to evaluate whether a drug acts on CNS targets and provide insight into potential mechanisms of action. Specifically, healthy subjects were subjected to temporary, reversible mimicry of disease by application of the anticholinergic drug mecamylamine.  
  
*Major Concerns:*  
Neurocart provides a useful preclinical tool that was developed by the authors and has been used to test several drugs. The title and abstract are appropriate (the abbreviation "PD" is included in the abstract but not defined). For the most part, the text clearly describes Neurocart and its use, although the writing style is heavy on jargon and there are many cases where clarity is an issue (see below). The descriptions of the utility / value of Neurocart are vague.  
*We would like to thank the reviewer for their comments and suggestions. We agree that the text is heavy in jargon and have implemented the suggestions by the reviewer for specific parts of the introduction and discussion. In addition we have reviewed the whole text and have rewritten parts to avoid jargon and vague use of language.*  
  
*Minor Concerns:*  
1. The Figures could be more aesthetic (e.g., larger symbols) and the legends are inadequate. There are several abbreviations that indicate experimental groups but these are not defined anywhere.  
*We have updated the figures to be more clear. Symbols and abbreviations used are defined in the legend.*2. It is never clearly stated which "electrophysiology" is included in the battery of tests (a short list is provided near the end of the Discussion (lines 377-380). This would be more useful in the introduction).  
*Specification on electrophysiology tests has been included in the introduction text.*3. There are several vague phrases used without adequate explanation. For example, "domain of CNS", "to be used as a challenge model", "adaptive in nature", "densely measure", and "multimodal in design". In all of these cases, it would be much clearer to just say what is meant rather than invent a phrase that is unclear. So instead of saying "multimodal in design" (lines 103-104) just say the battery includes both behavioral tests and electrophysiological measurements. I am not surewhat "densely measure" means. Rather than "adaptive in nature", just say that you can vary which tests are used to fit each project.  
*We agree with the reviewer that some jargon has been used. We have re-written large sections of the introduction and discussion with this in mind to avoid unclear phrasing.*   
4. In many places, references are not included but are needed (e.g., lines 83-94, lines 111-113, lines 390-395, lines 414-416). There is not enough information provided to actually do any of the individual tests. While it would be inappropriate to include all of the methodology here, a few key references that would allow the reader to perform the tests would be helpful.  
*We agree with the reviewer that there was not enough reference to existing literature. We have added references throughout the manuscript, linking the text more to the published literature.  
In addition, we have added a section to the introduction where more explanation on the individual tests is provided, as well as more detailed instruction in the protocol section. Also, references for the tests have been added, so that it more elaborate descriptions on how to perform can be found in the literature as the current paper does not allow for all detail that is needed to perform the tests.*5. Lines 374-382: It would be useful to list all of the tests in Neurocart and then show how the battery can be tailored to specific projects (rather than just say it can be done).  
*This suggestion has been implemented by describing an example.*6. "PD" is used several times but not defined (line 130, line 323). Is this "post drug"? If so, it does not seem necessary - just say what happens in the presence of the drug.  
*We have removed the abbreviations from the manuscript and have either stated ‘pharmacodynamic’ or just ‘effect’ where appropriate.*  
7. "MoA" is not a useful abbreviation (lines 381,390). It is only used 3-4 times so it would be better to just write out "mechanism of action".  
*This suggestion has been implemented.*8. Line 122: Does "Here" refer to this paper, or are the authors defining the pharmacological challenge model (it needs a clear definition)?  
*This has been rewritten.*9. Lines 402-412: No data are presented in this manuscript to test the reproducibility of Neurocart tests.  
*In the introduction and in the discussion it is stated several times that the tests have been used in multiple studies with different drug types (including references to these studies), thereby providing evidence of the reproducibility of the test battery.*