Response to editor comments

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
    a. **We have gone back through the manuscript and attempted to correct any and all copy-edit issues.**

2. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage, doi: DOI (YEAR).] For more than 6 authors, list only the first author then et al.
3. Please abbreviate all journal titles.
4. Please include volume, issue numbers, and DOIs for all references.
    a. **We went through all 24 references and made sure formatting was followed except for the following:**
        i. **1 does not have a volume or issue**
        ii. **3, 13, and 20 are books are thus have no volume or issue or doi**
        iii. **15 cites a manual and all information available is provided**
        iv. **18 I could not locate a doi**
        v. **19 I could not locate an issue or volume, I assume this journal does not follow that nomenclature**
        vi. **23 I could not locate a doi**

5. Please define all abbreviations before use.
    a. **The entire document was reviewed and ensured that all abbreviations were defined before use.**

6. Please use focused images of uniform size/resolution (at least 300 dpi).
    a. **All figures were exported as TIFF files with uniform resolution of 600 dpi.**

7. 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 in separate columns in an xls/xlsx file.
    a. **The table has been revised to be more complete.**

8. Unfortunately, there are a few sections of the manuscript that show significant overlap with previously published work. Please rewrite the text to avoid plagiarism (including self-plagiarism). Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. These sections are lines: 10-19, 27-36, 50-53.
    a. **Although we had attempted to vary sentence construction and word choice from sources in describing the techniques of microdialysis (10-19), voltammetry (27-36), and biosensing (50-53), we went back through each of these sections and made additional changes to structure and verbage.**

9. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products

should be sufficiently referenced in the Table of Materials and Reagents.
For example: Pinnacle sensors in Figure 1, Pinnacle in the manuscript text, Sirenia, etc.

a. **Because this experimental technique relies so heavily on commercially available sensors from Pinnacle, we initially included commercial language with regards to products obtained from this company. The document has been reviewed and any reference to specific products or companies have been removed.**

10. Please include a Short Abstract to clearly describes the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …"
11. Please include a Long Abstract between 150-300 words.
12. Please provide at least 6 keywords or phrases.
13. Please provide all author affiliations in the manuscript text.
    a. **Our apologies. We initially misunderstood that if these sections were submitted in the different submission boxes through the submission portal that they were also required to be included in the manuscript document. We have added these sections into the manuscript document proper.**

14. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.
    a. **We have gone through the protocol section and ensured that no "could be," "should be," or "would be" phrases were used. We took any text within the protocol that was not in the imperative tense and added them as separate notes as instructed.**

15. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.
    a. **Notes to the reader that were not direct imperative commands were separated as notes. In particular, if the editor is referencing the paragraph about the use of hamsters at the beginning of the protocol, and the description of a copulatory bout in section 4, these sections were moved to the introduction so that the protocol only contains action items (aside from separated notes).**

16. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.
    a. **Additional details were provided where necessary, and reference to the original source was made more apparent (e.g. in the case of ovariectomies and stereotaxic surgeries, which are well-established techniques, and we do not want to be stepwise filmed).**

17. Please use SI abbreviations for time: h, min, s, (Figure 2, etc).
    a. **The document was reviewed for time abbreviations, and changes were made where necessary (hr to h; wk to week since there no SI abbreviation for week or days and the SI website instructs to spell these words out in their entirety).**

18. 1.3: How is the anesthesia done? How are the ovariectomies and cannulations done? If this is to be filmed, we need explicit step wise details.
    a. **Details regarding anesthetic were provided. Reference to sources re: ovariectomies and cannulations were made apparent, and additional details regarding cannulations were given. We are not intending to have these filmed as they are previously established techniques.**

19. 1.3.1: Implant how? What is the area of interest?
    a. **Additional details regarding implantation and determination of coordinates for area of interest were added.**

20. 1.3.2: Please ensure you are using the correct abbreviation for microns (lower-case m).
    a. **We thank you bringing out attention to this oversight; we have since corrected the incidences of using a capitalized M for microns. The two incidences of the use of microns in the manuscript were changed to lower case m.**

21. 3.1: What is used and how much?
    a. **A sentence detailing euthanizing agent, dose, and method of administration was included.**

22. Please revise step 4 to be in the imperative tense throughout.
    a. **All stepwise instruction portions for step 4 were made imperative tense.**

23. Please note that we can only film step 4 if there are stepwise details at each step and there is a graphical user interface with all user input commands: File | Save | etc.
    a. **The data analysis portion of the protocol is not intended to be stepwise, but rather an overall explanation of how this lab utilizes one method for analyzing the output data from this technique. We have attempted to break it down into more stepwise detail, utilizing the use of non-imperative "notes" as instructed. We hope that these changes will be sufficient, but are willing to make additional accomodations as the editor sees fit.**

24. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The highlighted steps should form a cohesive narrative with a logical flow from one highlighted step to the next. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.
    a. **We have highlighted key portions of the protocol to be filmed.**

25. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.
    a. **We have ensured that the highlighted steps form a cohesive narrative with logical flow and complete sentences.**

26. Please define the error bar and asterisks in Figure 5.
    a. **A sentence explaining that error bars indicate SEM and asterisk indicates significance of $p < 0.05$ has been added to the figure legend.**

Response to reviewers' comments

Reviewer #1:

1. Line 82: "Trivially" is out of place here. Movement artifacts are not trivial. They are actually a major reason for using hamsters instead of other rodents.
    a. **Thank you for this observation. This word is definitely not the right fit. Indeed, these artifacts are one of the main reasons we use hamsters instead of other rodents for these experiments. "First" has been substituted for "Trivially" as the first word to that sentence.**

2. Lines 94-96: This sentence does not make sense.
    a. **In the editing process the words "is ideal for chemical recordings" was somehow removed in this sentence and have been replaced. We hope the sentence: *"The copulatory sequence in Syrian hamsters (Mesocricetus auratus) is ideal for neurochemical recordings due to the lack of solicitation behaviors typically seen in rats and mice[11]."* now makes sense.**

3. Line 99: Why is it "necessarily" associated? Seems like too strong of a claim unless additional justification is included.
    a. **"can be" has been substituted for "necessarily" in this sentence.**

4. Lines 152-153: What dose/type of anesthesia?
    a. **This sentence lists that isoflurane in an induction chamber is used to lightly anesthetize the animals.**

5. Lines 162-164: Is bedding really added after the animal is placed in the arena?
    a. **This section of the protocol has been rearranged to appear before the section listing the animal being added to the arena.**

6. Line 166: Is recording really performed during the 2-4 equilibration? Or is this line out of place?
    a. **Recording is performed during the equilibration period to ensure proper sensor function and equilibration.**

7. Lines 256-258: Better pictures of the various behaviors related to intromission would be useful. It is difficult to see many of these in the picture provided in Figure 4.
    a. **We have made a new figure that shows more detail and have added further clarification in the figure legend.**

8. Figure 2 legend: Missing. It would be useful to provide details of the skull cap construction.
    a. **The figure 2 legend was expanded, and reference to the explicit stepwise manual was made.**

9. Figure 1: Has the company given permission to use their figure? If available, a better figure may be one that focuses solely on the neurotransmitters described in the article.
    a. **This image was provided by the company and we have obtained permission to use it in this publication. The generalized image is representative of other sensors that readers may be interested in (e.g. glucose, ethanol, etc), but we have expanded the figure to include the enzymatic layer for glutamate, and added additional description in the legend.**

10. Figure 4: It is very difficult to see the differences between mounting and intromission. One way of fixing this would be to provide multiple pictures of intromission. Another would be to label the pictures with the specific details of each behavior (i.e., what to look for). A non-specialist would be hard pressed to describe the differences between these behaviors based solely upon these images. Perhaps this can also be directly addressed in the movie clips associated with this article?
    a. **We have made a new figure with a more zoomed in image that we hope is more clear in the distinction between these behaviors. In addition, this protocol is meant to detail the use of enzymatic and carbon fiber recording for utilization in multiple paradigms, sexual behavior is presented as an example that our lab uses.**

11. Figure 5A: Missing axis numbers and units.
    a. **This image is intended to be a stylized representative example so the reader can see the overall tonic increases in dopamine during a mating bout.**

Reviewer #2:

1. In the abstract, the investigators claim the biosensors permit recording of multiple neurotransmitters simultaneously. In the introduction, they state that the ability to measure both electroactive and non-electroactive molecules provides the opportunity to examine converging neurotransmitter release (line 63-65). Nevertheless, it is difficult to understand whether dopamine and glutamate levels are measured simultaneously in this manuscript. If the authors are not measuring multiple neurotransmitters in the same animal in this manuscript I suggest they not make these claims in the abstract and introduction. Rather, they might discuss future directions in the discussion section. Below are a few cases where the measurement of multiple neurotransmitters should be further clarified.
    - Line 121-123. More detail is needed on probe implantation. Are both dopamine and glutamate probes inserted in the same animal?
        - **A note explaining that the data presented are from single cannula implants, but that the system allows up to 4 sensors to be implanted and recorded from simultaneously has been added.**
    - Figure 2 needs a caption that explains what probes, cannulas, and/or reference electrodes are implanted. It appears that the rostral probe in the BASi guide cannula, although the caudal probe in unclear and should be identified.
        - **We have attempted to clarify this figure by adding additional description.**
    - Line 126-127. More detail is needed about implanting a reference electrode. Is the reference electrode needed only for the dopamine probe? Does it require an additional stereotaxic implant? Can it be included on the skull if both dopamine and glutamate probes are implanted?
        - **A note providing further clarification on each of these points was added.**
    - The methods appear to be written for the measurement of a single neurotransmitter per animal. How would the multiple stereotaxic implants and differential equilibrium times affect the simultaneous measurement of both dopamine and glutamate?
        - **A sentence describing what to do in this situation has been added.**
    - Line 386-391. Again, the investigators mention dual-probe recordings and the convergence of neurotransmission in multiple brain regions. Were dual-probe recordings used in this study?
        - **No, single probe recordings were used in this study and we have attempted to clarify that in the text.**

2. The authors use the term sex behavior and sexual behavior interchangeably. I think they should use one term consistently and believe sexual behavior is the more appropriate term.
    a. **We agree and have changed all instances of "sex behavior" to "sexual behavior."**

3. Line 94-96. This is an incomplete sentence.
    a. **In the editing process the words "is ideal for chemical recordings" was somehow removed in this sentence and have been replaced. We hope the sentence: *"The copulatory sequence in Syrian hamsters (Mesocricetus auratus) is ideal for neurochemical recordings due to the lack of solicitation behaviors typically seen in rats and mice[11]."* now makes sense.**

4. Line 113-115. The authors should briefly explain that hamsters are housed in a 14:10 light/dark cycle to maintain seasonal reproductive condition.
    a. **This is an important clarifying point, thank you for the suggestion. We have added a note that explains this.**

5. The table of materials and equipment is not formatted properly in the PDF file. The excel file is okay, but the table needs to be reformatted for publication.
    a. **We are unsure how the documents are compiled into the single PDF file through the submission portal; we are only able to control the formatting of the excel file that we upload.**

6. Line 142-143. In the manuscript the authors explain that the enzymatic biosensor should be calibrated before use. However, the table of materials says that it is necessary to calibrate the biosensor at the conclusion of an experiment. Should biosensors be calibrated at both the start and end of an experiment? Please clarify.
    a. **Thank you for pointing out this discrepancy. Although the company suggests both pre- and postcalibration, our lab precalibrates only because we prefer to have exact anatomical placement of our sensors confirmed and thus perfuse with the sensor implanted as removal prior to perfusion makes it more difficult to locate the very small 1mm track of the probe from the bottom of the cannula.**

7. Line 145-150. The sentence about carbon fiber electrodes is confusing and should be clarified. Is damage to the thin carbon fiber a problem for calibration performed by the company?
    a. **Due to the delicate nature of the carbon fibers, we limit the amount of handling that occurs in the laboratory. Since the company does calibrate the sensors before shipping them and because the carbon fibers sensors do not undergo any degradation, by using the company's calibrations, we can reduce the risk of breaking the carbon fiber.**

8. Line 181. Delete 'is' in "a stimulus male is into the testing chamber".
    a. **Deleted.**

9. Line 210. Insert space between lines.
    a. **A space has been inserted.**

10. Line 321-322. The investigators mention that there are negligible dopaminergic responses to other copulatory behaviors such as anogenital investigation. It would be useful to create an additional figure showing that AI start and AI end do not overlap with the dopaminergic peaks.
    a. **We removed this sentence as not to induce confusion.**

11. Figure 1. The legend describes an additional enzymatic layer of AA oxidase that converts the interferent to non-electroactive water. It appears to me this layer of AA oxidase is not shown in the figure and should be identified as 'not shown' in the figure legend.
    a. **We have added an additional image to this figure that demonstrates how the glutamate sensor functions specifically, including demonstrating the AA oxidase present.**

12. Figure 3. I do not understand how glutamate, dopamine, and ascorbic acid are applied to the biosensor. Is this performed in vitro? More detail is needed here.
    a. **In vitro was added for clarification as well as elaboration and stepwise detail of this calibration procedure.**

13. Figures 6 and 7. Figure legends that describe the data shown should be provided. The investigators should indicate that these plots are from representative animals. Also, they should indicate if the dopamine and glutamate data were obtained from the same animal (i.e. the issue of dual-probe recordings discussed in #1 above).
    a. **These changes have been made to provide clarification.**

Reviewer #3:

1. Grammar: lns 40-43, 57-61, 80, 94-96, 218, 293, 368-371
    a. **40-43: A dash was substituted for the semicolon since these were not two complete sentences**
    b. **57-61: The extra common was removed**
    c. **94-96: “is ideal for” was added**
    d. **218: “is” was removed**
    e. **293: “verses” was changed to “versus” and “lordosis” was also added for clarification**
    f. **368-371: This sentence was reworded for clarification**

2. Surgery: Why was this age chosen (ln 111), and what does L:10 hr D mean (ln 113)?
    a. **A note explaining the decision for the age chosen as well as spelling out “light” and “dark” instead of “L” and “D” was added.**

3. Biosensor testing: Please be specific about what insertion means. It is penile insertion. ln 183.
    a. **“Penile” was added for clarification.**

4. Behavioral coding: define intromission (ln 228), and remove the word "gently" on ln 240.
    a. **“Penile” was added and “gently” was removed.**

5. Legends: for figure 4 please include more information, as there are 3 panels.
    a. **More information was included, including the addition of panel labels for clarification.**

6. Figures:
    a. 5: the time is missing.
        i. **This image is intended to be a stylized representative example so the reader can see the overall tonic increases in dopamine during a mating bout.**

    b. 6-7: please include the mount and ejaculatory behaviors on this too. My understanding is that these behaviors will not be associated with the peaks. Is this only one animal?
        i. **Because this is a methods paper describing the technique of enzymatic biosensing/carbon fiber recording and not meant to be a detailed report discussing scientific findings from sexual behavior in hamsters, we chose to present data with one specific behavior (intromission) that is locked to dopamine and glutamate as an easy-to-read example. When this data is published for scientific dissemination information on other copulatory behaviors will also be included.**

Reviewer #4:
This reviewer had no comments on the manuscript.