

## Peer Review File

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### **Reviewer A**

The manuscript provides a methodological framework for using fNIRS of frontal cortex to potentially investigate brain-bladder signaling in overactive bladder (OAB)  
Following concerns were noted

**Comment #1:** The experimental readout appears complicated and authors need to establish the baseline data with empty bladder first, to clearly discern signal from noise since frontal cortex keeps consuming oxyhemoglobin regardless of bladder filling status.

**Reply #1:** Thank you for your comment and taking the time to review our work, lines 41-42 in methods section indicated that participants void before they start the study, and post residual volumes were measured using ultrasound; moreover, we have added a phrase to emphasize this would serve as a baseline measurement. Additionally, we have added a table to include the initial ultrasound post residual volumes for the participants. Please refer to results section lines 111-115.

**Comment #2:** Instead of head tilt and verbal communication with subject during experimentation, perhaps pressing a voting device using hand would have reduced noise and motion artifacts from head motion in fNIRS

**Reply #2:** Reporting sensation wasn't the primary source of introducing movement artifacts during participation. Rather, movement artifacts were caused by multiple random events. We have added an explanation statement in the manuscript. Please refer to lines: 173-176 in the discussion.

**Comment #3:** Was bladder filling time of 47-60min measured purely by brain sensation? Perhaps ultrasound measurement of bladder volume would have generated confirmatory evidence as authors are relying on natural filling of bladder

**Reply #3:** Yes, total filling times were calculated based on participants reporting 100% sensation and urge to void using the sensation meter. We have now added a table in the results reporting initial bladder volumes (prior to the hydration) and total voided volumes (post hydration). Please refer to lines: 108-115 in the results section.

**Comment #4:** Looks like short battery life of 1.5h will generate cleaner readout in OAB patients with smaller bladder capacity than healthy adults

**Reply #4:** The power bank was always connected to the NIRS system during all data

collection. A statement was added in the method section to clarify this point. Please refer to lines 93-9.

**Comment #5:** Figure 4 appears to show that oxyhemoglobin levels decline with time in low sensation and increase with time during high sensation within same scale of y-axis. What explains the change in scale of y-axis for fig. 4 and 5

**Reply #5:** Figure 4 represents the methodological steps on one individual channel, and figure 5 shows the averaged channels (3 regions for 24 channels). Therefore, as these are different data sets, different scales were required. After implementing the steps shown in figure 4, an extra phrase was added to the figure legends to explain the differences in scale.

**Comment #6:** Description for panel D &E of Fig.2 is missing from legend.

**Reply #6:** Figure only has panels A, B, C. This has been confirmed in the figure legend.

**Comment #7:** Yellow box for motion artifacts listed in legend are missing in Fig.3 and y-axis legend is clipped in fig.4

**Reply #7:** Figures have now been corrected.

### **Reviewer B**

This is a very brief paper with some small amount of data but not clearly defined outcomes or summary data. Fig 5 seems to be the focus of summary data but lacks statistics - and no obvious method that could be used for statistical differentiations.

Several "findings" are process or methodological and not supported by presented data - perhaps understandable and suspected by the authors but not part of the data outcomes.

**Comment#1:** My suggestion would be to present this as an abstract at a meeting for review and feedback... or perhaps present fuller dataset and possible stats to share in a paper if available.

**Reply #1:** Thank you for taking the time to review our work. We have already published more than 5 abstracts on this data in local, regional, and international conferences. During conference presentations the data was well-received with positive feedback and recommendations to publish with more details in a journal for other researchers to benefit from the proposed methodology.

**Comment #2:** Fig 5 "Hight" misspelled?

**Reply #2:** This has been fixed, and a new figure is re-attached.

**Comment #3:** Lines 195-210 are methods and should be moved to methods...

**Reply #3:** The paragraph has been moved to the method section, please refer to lines: 89-103

**Comment #4:** beginning on line 209 ..."using an iterative process, signal partitioning in two phases (Low Sensation vs. High Sensation) was determined to be more physiologically relevant and methodologically efficient (Figure 4)".\_How did the authors determine this was more physiologically relevant? What test was used?

**Reply #4:** Segmenting the signal into two phases was adopted following the ICS guidelines for reporting of verbal sensory thresholds. The manuscript has been updated to reflect this added information. Please refer to lines 136-140.

**Comment #5:** Also, I don't see any mention of brain areas measured as controls or comparators... this would be useful or essential - unless they think this is a global signal.

**Reply #5:** The firs system comes with a pre-defined template for optode configuration which enables measurement of frontal cortex regions that are recommended by the manufacturer. We have now added a statement to clarify. Please refer to lines64-66.

### **Reviewer C**

I believe the method you present here has quite some potential once further developed, and can give useful new insights into the central control of the lower urinary tract. I have read your manuscript with great interest and have provided some comments and suggestions to improve the manuscript below. Once these comments and suggestions have been implemented I would like to see your work published in Translational Andrology and Urology.

**Comment #1:** Title: 1. Since NIRS is also frequently used to assess detrusor overactivity or bladder fullness it would be useful to indicate that you're studying central nervous system activity in the title of your manuscript (e.g. "A Stepwise Approach for cortex activity measurement using Functional Near Infrared Spectroscopy (fNIRS) during Natural Bladder Filling").

**Reply #1:** Thank you for taking the time reviewing our work. We agree with your suggestion, and the word "cortical" has now been added to the title of the manuscript.

**Comment #2:** Key findings: 2. On line 61 "fNIRS" should be placed between

parentheses ()).

**Reply #2:** The requested change has been made. In addition, please refer to the new Highlight Box file.

**Comment #3:** 3. I would suggest to add one sentence regarding the (positive) impact of your work before the limitation your mention under “implication”.

**Reply #3:**

The requested change has been made before the limitation section. Also, please refer to the new highlight box file text for key findings.

**Comment #4:** Abstract: 4. One line 78 the word “potentially” is redundant.

**Reply #4:** The word has been deleted. Please refer to the updated attached abstract.

**Comment #5:** 5. On line 99 you mention your method is reproducible, but it is unclear what this claim is based on.

**Reply #5:** Thank you for making this point. The word “reproducible” has now been removed to avoid confusion and replaced by the phrase “methodological guide.” Please refer to the updated abstract file.

**Comment #6:** Introduction: 6. Please provide a reference after “voluntary voiding” on line 110.

**Reply #6:** Reference one (1) is now included in the manuscript. Please refer to line 6 in updated manuscript.

**Comment #7:** 7. On line 114 you mention supine position is less ideal for investigating bladder sensations. Please also mention bladder control (voiding specifically) here as well. Under normal circumstances bladder sensations are often evaluated in supine position, the main limitation of fMRI is during voiding - which is not done in supine position under normal circumstances in healthy adults.

**Reply #7:**

The term “voiding” has now been added. Please refer to line 10 in updated manuscript.

**Comment #8:** 8. On line 118 it is unclear what is meant by “regions associated with bladder pressure over short time intervals”. Please elaborate.

**Reply #8:** We meant cortex regions. The word “cortical” has now been added to elaborate. Please refer to line 13 in the updated manuscript.

**Comment #9:** 9. Suggestion to just mention “the cortex” on line 120 and leave out brain.

**Reply #9:** This has been adjusted. Please refer to line 16 in updated manuscript.

**Comment #10:** 10. On line 123: Why only focus on voiding dysfunction and not storage dysfunction?

**Reply #10:** Thank you. We have now changed the term “voiding dysfunction” to “lower urinary tract dysfunction” which encompasses both storage and voiding abnormalities. Please refer to line 26 in updated manuscript.

**Comment #11:** 11. Sentence on line 131 regarding MDAR checklist should be incorporated into the manuscript text. I would suggest to move this to the methods section.

**Reply #11:** Thank you. We have now moved the MDAR checklist statement to the beginning of the methods section as requested. Please refer to line 29 in the updated manuscript.

**Comment #12:** Methods and Materials: 12. Please provide more details regarding the used sensation meter. Is this just one VAS-scale with bladder fullness from 0% to 100% - How do you determine first sensation, first desire and strong desire from this data?

**Reply #12:** We have published multiple studies validating the sensation meter. More details have now been added to explain the sensation meter and its functionality. Please refer to lines 43-49 in method section in the updated manuscript.

**Comment #13:** 13. Why do you not use “first sensation” in your analyses?

**Reply #13:** We used the term “first desire” as this was more consistently reported by participants. A phrase justifying the use of this terms has now been added to the manuscript. Please refer to line 47-48.

**Comment #14:** 14. On line 160 it is mentioned that “after time “0”, participant head circumferences were measured”. You previously (line 153) indicate that the sensation meter was started at time “0”. Does this mean that you only connect the fNIRS setup after drinking and sensation measurement has begun? Why did you decide to not record cortical activity during the first part of the filling cycle? In this case does the data you collected differ between the first and second filling cycle since you already have the fNIRS system ready before the second cycle?

**Reply #14:** Headcap fitting started immediately after time “0”, and fNIRS signal recording started at the same time point. However, this initial portion of the study had very low signal to quality due to head movement as we fit the cap and check the optodes

for signal quality. Initial signal recording and testing for optode saturation is part of the head cap fitting and both are recommended to be done at the same time. Therefore, use of this initial data was not interpretable and explains our decision to focus on the later part of the filling phase. This explanation has been added to the manuscript. Please refer to lines 68-70 in methods section.

**Comment #15:** 15. What parts of the brain do your averaged left, middle and right channels cover?

**Reply #15:** The regions are now highlighted in fig1, and more details have been added to the figure legend. The new figure and legend are now attached.

**Comment #16:** 16. On line 182 you mention “wide variations in filling times”. Is this between or within subjects? At the within subject level I expect that your bladder becomes more sensitive after the first filling-emptying cycle so the second run should be faster. Please elaborate on this.

**Reply #16:** The term was used to denote variation between participants. The manuscript has now been edited to address this point. Please refer to lines 86-88.

**Comment #17:** 17. How did you decide which of 4 cycles to include for your analyses? Please mention this in the methods section.

**Reply #17:** IMU data for each fill was used to assess participant head motion. The fill with the least amount of motion artifacts (least total IMU fluctuations) was therefore used for analysis. More details about this methodology have now been added to the manuscript please refer to lines 80-83.

**Comment #18:** Results: 18. On line 191 you use ounces as unit of measurement while in the methods section you used liters. Please be consistent.

**Reply #18:** Thank you. We now report the values in metric liters. Please refer to Table (1) in the results section.

**Comment #19:** 19. How much fNIRS data do you have per sensory state? Does this differ between participants?

**Reply19:** The fNIRS signal was divided into two phases: low sensation and high sensation, defined as before “first desire” and after “first desire.” Further segmentation was not done. Additional explanation has now been added. Please refer to line 135-142 in updated manuscript.

**Comment #20:** 20. On line 182 you say 1.5 hours while on line 171 you say 90 minutes. Be consistent.

**Reply20:** This has been fixed. Please refer to line 73 in updated manuscript.

**Comment #21:** 21. On line 197: Is “corrected” the best word to use here? Perhaps consider “avoided” instead.

**Reply 21:** The word “corrected” has been changed to “avoided” per your recommendation. Please refer to line 91.

**Comment #22:** 22. In the methods section on line 142 you mention you measured post-void residual volumes with ultrasound. Please also show your data in the results section.

**Reply22:** The results section has been updated, and a table has been added which reports the requested data. Please refer to lines: 110-115.

**Comment #23:** 23. On line 213 it is unclear what “low sensation” and “high sensation” actually means. How does this relate to first desire to void and strong desire to void?

**Reply23:** The manuscript has been updated to add more details on that comment, please refer to lines: 47-50.

**Comment #24:** 24. On line 212 and 213 it is stated that “fNIRS signal decreases during the low sensation”, but it is not stated compared to what state/condition this decreases? The same applies to the high sensation segment.

**Reply24:** fNIRS measures “relative” changes in tissue oxygenation. So, it is decreasing compared to its own baseline. We changed the words “segment” into “phases” to elaborate that high and low sensation are continuous time phases. we also added the word “relative” to the updated manuscript. Please refer to lines 142-146 in updated manuscript.

**Comment #25:** 25. Is there a difference between regions or in between-region communication?

**Reply #25:** We used the manufacturer recommended optode template to capture the entire prefrontal cortex and then grouped (by average) channels to reflect the left, middle, and right. This information is now explained in the updated figure 1. Moreover, a statistical analysis section has been added. Please refer to line 103 in updated manuscript.

**Comment #26:** 26. Why was first sensation not included in analyses?

**Reply26:** First desire was more consistently reported by participants, please refer to your comment #13 and its reply.

**Comment #27:** 27. How do periods of motion artefacts compare between sensory states? Is there more motion in full bladder? - Would episodes of motion during high sensation (strong desire to void) coincide with highest presence of motion artefacts?

**Reply27:** Motion artifacts stem from random head movements during the study. We don't believe there is any correlation between urgency stages and motion artifacts. Please refer to lines 175-178.

**Comment #28:** 28. On line 243 after "data collection." Please add a reference and/or elaborate further.

**Reply28:** The verbiage has been edited to clarify that these are random events we observed during our data collection. Please refer to lines 174-178.

**Comment #29:** 29. It is unclear why your approach is not dependent on a 3D brain model, please elaborate.

**Reply29:** The 3D head models rely only on averaging of optical density signals from channels within a specific region. Moreover, the 3D model doesn't account or correct for any changes in signal due to non-physiologically related events such as random head movements, coughs, and sneezing. The manuscript has been updated clarify this point. Please refer to lines 169-177.

**Comment #30:** 30. In line 282 you mention for the first time that you only analyse one fill per participant. Please explain this a lot earlier in your paper and a lot more thoroughly (methods section). How did you determine which fill to analyse and how did you make sure to select the "best" fill in an objective manner?

**Reply 30:** Choosing the best fill based on IMU quality is now described earlier in the manuscript. Please refer to lines 81-84. Moreover, we have added further details on selecting the best IMU, please refer to your comment number 17 and our reply.

#### **Reviewer D**

Great work. Congratulations. I suggest only in the results to report medians and IQRs instead of mean and SD. I suggest reporting these data in Tables.

**Reply:** Thank you for taking the time to review our paper. A new table has been created with individual participant data and also includes median and IQR values.