

Xue Han, Ph.D.

Peter Paul Career Development Professor

Associate Professor, Biomedical Engineering Department

Joint Professor, Experimental Therapeutics

Member, Photonics Center

Boston University

44 Cummington Street, Boston, MA, 02215

Phone: 617-358-6189  
Email: xuehan@bu.edu  
Web: http://www.bu.edu/hanlab

ATTN: Giuseppi Di Giovanni, Ph.D.

Editor-in-Chief

Journal of Neuroscience Methods

University of Malta, Msida, Malta

Dear Dr. Di Giovanni,

We thank you and the referees for the time and efforts on reviewing our manuscript. Both referees offered addressable, insightful and cogent criticisms. In our revised manuscript, we have addressed each referee and in doing so have added two new figures and have improved our graphical user interfaces. Our response to each referee critique is included below and is written underneath each comment (shown in italics). In addition, major changes to the manuscript in response to reviewers’ comments are identified in red font.

**Referee #1 (Remarks to the Author):**  
*1. In their submitted manuscript, Romano and colleagues offer useful applications of a Teensy 3.2 board as a cheap, reliable and easy to build tool for behavioural research. The authors present here how this board can be used to implement a rotation encoder and a controller for CS-US learning association. Although the data presented serves the purpose, the manuscript could be somewhat improved.*

**Response:** We thank the referee for his/her support, and we have made significant changes in this revision as detailed below.

*2. Figure quality can be improved: Figure 2: connection schema are appreciated, but may in some cases be confusing. The figure could gain in clarity if the connections were color-coded, making them easier to follow (this might also help understand which of the connections in panel B are actually relevant).*

**Response:** We appreciate the referee’s suggestions. We have now color-coded the connections and provided additional labels to the pins that are utilized by the prop shield.

*3.* *Figure 3B: The temporal drift reported in the text might be efficiently represented using a smaller line thickness and inserting a magnification making the divergence more evident (e.g. of the first and the last 30us)*

**Response:** We appreciate the referee’s comment and recognize that our display of temporal drift can be further improved. We have added 3 additional panels (Figure 3Ci-iii) to this figure. The zoomed-in view of Figure 3Ci demonstrates the best-fit line of the measured data versus the theoretical time stamps in red (also shown in Figure 3B), superimposed on a zero-drift line.

*4. Figure 4: Regarding panel A, same observations raised for the panel B of the previous figure applies.*

**Response:** We appreciate the note and have now included 3 subplots here to better demonstrate the time delay as we did in Figure 3C.

*5. About the eye-blink paradig m, it is mentioned that the amplitude of the CS is set to 0 and then increased when needed; does this happen live via the custom GUI mentioned in the Methods? Once acquired, are the time-stamps in 0dB condition distinguishable from those produced in the 75dB? Moreover, once you plug an Arduino board, the script that is loaded on it starts automatically: does the researcher have a way of controlling start and end of session, pausing and restarting the paradigm?*

**Response:** We apologize for the confusion here and have now revised the text to provide a more thorough description of the graphical user interface (GUI). We have clarified the role of the GUI in the *Methods* section. When the Teensy 3.2 board is plugged in, the board automatically initializes the Arduino “setup()” function, which sets up the board to wait for serial input from the computer. Once the user presses “Start” on the GUI, the GUI sends programmed serial inputs to the Teensy 3.2 board. The GUI then saves the Teensy-reported time stamps for each frame.

For the *Motion Tracking Experiment*, the GUI is programmed to save the x and y direction displacements of ADNS-9800 sensors, and the time interval between frames. For the *Trace Conditioning Eye Blink* *Experiments*, the GUI is programmed to save the elapsed within the experiment, within the trial, the trial number, whether the CS is presented, and whether the US is presented.

We appreciate the reviewer’s comment on being able to set parameters related to an experimental session. Accordingly, we have updated our GUIs allow the user to specify the start, end, and duration of a session. Originally, our GUI only had the ability to control the start of an experiment and specify its length either explicitly (for the *Motion Tracking Experiment*) or by specifying the duration and number of trials (for the *Trace Conditioning Eye Blink* *Experiment*). We have now added a “Stop” feature as the Referee suggested to both the GUI code and the Teensy code that allows a user to stop an experiment preemptively and then restart it without having to unplug the Teensy and restart MATLAB. This can function as a preemptive experimental termination, or as a pause, as the user is free to press the “Start” button at any time following usage of the “Stop”. The user does have to specify a new filename after pressing “Stop” and before pressing “Start” again, or the computer will by default assume the user wants to overwrite the stopped experiment.

*6. The manuscript could be strengthened by implementing a third experimental condition in which Teensy is used to play 2 sounds (e.g. having not just a single CS that predicts a US as in the eye-blinking paradigm, but having also a "neutral" CS, not associated with any US).*

**Response:** We think that this is an excellent idea, and have included in the revised program Teensy code that allows the user to control two tones, including their timings and frequency, that can be set in the GUI.

*7. Minor corrections:  
- page 2. The material reference for the Tindie sensors has a link, while other material have not  
- page 5. Typo: "for pre cise image capture"   
- page 5. " eye puff versus the sCMOS camera (Figure 4Bii)."  In this paragraph, Figure 4Biii and 4Biv should also be referred to in the text.  
- page 5. Technical details on the ADNS-9800 sensors would be more suitable for Methods:*

**Response:** We thank the referee for pointing out these errors and have implemented the changes suggested.

**Referee #2 (Remarks to the Author):**

*1. This manuscript proposes to solve technical difficulties inherent to integration of image data acquired via an sCMOS camera with animal behavioural data acquired from other sensors.  
  
I find it difficult to understand which specific problem the presented work seeks to address. It would be useful for the authors to elaborate on how exactly it has been" difficult to easily integrate sCMOS cameras and behavioural experiments". Listing a few specific technical difficulties inherent in this process would have been useful.*

**Response:** We thank the reviewer for this criticism and have added additional background to both the “Introduction” and “Conclusion and Discussion” to describe why the difficulty in integrating high-speed sCMOS cameras into behavioral experiments. The major significance in our view is the flexibility and low cost nature of the Teensy interface in integrating new instruments, such as sCMOS camera, into behavioral experiments.

*2.* *More critically however, while I recognise that this paper is focused upon providing a technical assessment of the teensy microcontroller for neuroscience research applications the authors have performed live animal experiments on a fixed animal. No statement is given regarding the animals genotype, gender, age nor as far as I can see is there any statement regarding ethical approval and licensing for animal experimentation.*

**Response:** We apologize for omitting these technical details. Indeed, we used live animals in the testing. We have now included all the details in Methods, regarding ethical guidelines and approval for animal experimentation, as well as animal genotype, gender, age and other experimental details.

*3. Additionally while the authors frequently refer to a sCMOS camera as part of the experimental apparatus it is not immediately clear whether this was connected during the aforementioned experiments and if so, what it was recordibng*

**Response:**  We thank the reviewer for noting that this was not adequately described. To directly illustrate sCMOS camera image capture by the digital pulses delivered by the Teensy 3.2, we added an animal experimental session, where we showed GCaMP6 fluorescent imaging from neurons in the hippocampus in a mouse performing Trace Conditioning. These results are included in a new Figure 5.