UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
Instituto de Fisiología Celular
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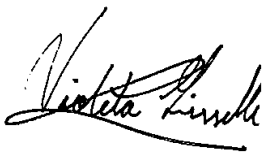
Dear Editor,

Please receive the revised manuscript entitled 'In vivo wireless optogenetic control of skilled motor behavior' by Diana L. Rodriguez-Munoz, Omar Jaidar, Marcela Palomero-Rivero, Mario A. Arias-Garcia, Gordon W. Arbuthnott and Violeta G. Lopez-Huerta.

We appreciate very much your work on the manuscript to improve the writing and attach to the journal guidelines. We have revised the manuscript and done the pertinent changes. We also reply to the reviewer concerns and added a few lines in the manuscript to address the comments.

We thank for your and reviewers' time and comments that have substantially improved the paper and we hope that after the revision the manuscript is now suitable for publication.

Sincerely



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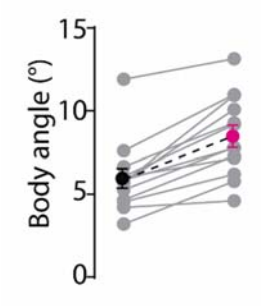
Minor Concerns:
The data in figure 2G&H (formerly 2D&F) is comparing differences in successful execution of complex behavior and should be between animals. A paired comparison of data from the 28 individual mice is suitable, but comparison of individual reaches pooled across all mice is not appropriate.

Thank you for your accurate observation. We have done the analysis as paired comparisons with a subset of animals chosen randomly and we get a very similar result and figure (shown below).



The definition of trials and reaching success need to be defined in the protocol, not just in the response to reviewers.

We have included this information in lines 325-328.

Are cameras position on the same side regardless of which paw is used for reaching, or are cameras repositioned based on paw preference? If so, this should be indicated in the protocol.

Thank you for noting this point. Cameras are positioned left or right of the chamber depending on sidedness of the animal. We have included this information in lines 267 and 268 (step 2.12).

In the optogenetics experiments shown, was the stimulation started as the mouse approached the front of the chamber, or as the reach was initiated? The movement is so quick that it seems that manual triggering of the stimulus must be done prior to reach onset and may therefore affect the stance and movements prior to reach initiation. Do you have any quantification of results that would inform the reader of inter-user reliability with manual triggering?

The stimulation was triggered when the mouse took the stance to initiate the movement. Only one experimenter performed all the experiments. As the reviewer points out we also thought the posture of the animal could change with our manipulations and we performed different analysis of the stance as step width and length and body angle. We found differences in body angle but not in the other parameters. Additionally, kinematic analysis shows that not only the posture is affected but also the trajectory of the movement changes with optogenetic stimulation. We are aware of the inconvenience and downsides of manual stimulation and we are currently working to automatize the system. At the

moment, we do not have any data to inform the reader of inter-user reliability. We have added a recommendation in line 280.

Is the difference in distance between ipsi and contra stimulation (Fig 3B) indicative of a larger effect of contralateral stimulation? In E2, the duration and distance in control mice appears significantly different from controls in D2 and the sample traces in Fig 2E.

Thank you, we do observe a larger effect with contralateral manipulations. We also observe some differences between the control condition of animals implanted in contralateral and ipsilateral hemispheres in relation to the paw but the overall performance is not significantly different among controls. For example, we noticed that the distance in control of ipsi and contralateral conditions is different but the performance of the animals is not significantly different and the proportion of errors is also similar in both controls. It is possible that the cannula is damaging some involved regions and that is the reason we observe differences in the controls.

What is the distance from the inside of the front chamber wall to the pellet? Is this distance similar across trials?

The distance from the inside of the front chamber wall to the pellet is 10 mm, additionally the mice took the stance to perform the reaching movement at around 15-20mm from the inside of the chamber. The distances are very similar across trials and individuals.