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Re: Decision on submission to *Journal of Visualized Experiments* (JoVE63085)

Dear Dr. Vidhya Iyer,

Thank you for sending us reviewer reports on our manuscript entitled "Implantation and control of wireless, battery free systems for peripheral nerve interfacing", by Hongkai Wang, Dom D'Andrea, Yeon Sik Choi, Yasmine Bouricha, Grace Wickerson, Hak-Young Ahn, Hexia Guo, Yonggang Huang, Milap S. Sandhu, Sumanas W. Jordan, John A. Rogers, and Colin K. Franz.

The reviewers provided helpful suggestions and positive comments, and recommended publication of the manuscript after revisions. In the following, we list these comments, along with our point-by-point responses and associated additional results and modifications to the manuscript. We believe that the revised manuscript is suitable for publication in *Journal of Visualized Experiments*.

Thank you in advance for your time and attention.

Sincerely,

Colin K. Franz

Point by point response to reviewer comments:

Reviewer #1:

Manuscript Summary:

The authors propose a wireless implantable electronic system for electrical stimulation as a peripheral nerve interface. The demonstrated protocal has great potential of being used for various healthcare purposes. Thus, I would like to suggest minor revision before the formal acceptance of this manuscript.

--We thank the reviewer for their positive assessment of this manuscript, which they deem to have "great potential for various healthcare purposes".

Major Concerns:

- Biphasic pulse is essential for electrode and neural tissue in neural stimulation. But, this approach only enables monophasic pulse. Can you comment on this issue? Your reference paper [8] was also used biphasic pulses. How do you handle for heating issue?

--In terms of the ability of monophasic and biphasic stimuli to recruit peripheral axons and induce axonal regeneration, prior studies have reported that the differences in waveform characteristic had a negligible effect[1], and in our hands we have been able to achieve therapeutic electrical stimulation enhancement with the same monophasic current parameters in mice[2] and rats[3]. Furthermore in our prior studies we looked at biocompatibility in vivo and in vitro and did not find any evidence of tissue damage from heating effects or the materials themselves. Given these prior findings, and the limited duration of therapeutic electrical stimulation in the present study, monophasic, rather than biphasic, stimuli were used.

1. *Hingne PM, Sluka KA. Differences in waveform characteristics have no effect on the anti-hyperalgesia produced by transcutaneous electrical nerve stimulation (TENS) in rats with joint inflammation. J. Pain 8, 251–255 (2007).*
2. *Guo H, D'Andrea D, Zhao J, Xu Y, Qiao Z, Janes LE, Murthy NK, Li R, X Zie, Song Z, Meda R, Koo J, Bai W, Choi YS, Jordan SW, Huang Y, Franz CK*, Rogers JA*. Advanced Materials in Wireless, Implantable Electrical Stimulators that Offer Rapid Rates of Bioresorption for Peripheral Axon Regeneration. Adv Functional Materials. Adv Funct Mater. 2021 May (doi.org/10.1002/adfm.202102724)*
3. *Koo J, MacEwan MR, Kang SK, Won SM, Stephen M, Gamble P, Xie Z, Yan Y, Chen YY, Shin J, Birenbaum N, Chung S, Kim SB, Khalifeh J, Harburg DV, Bean K, Paskett M, Kim J, Zohny ZS, Lee SM, Zhang R, Luo K, Ji B, Banks A, Lee HM, Huang Y, Ray WZ, Rogers JA. Wireless bioresorbable electronic system enables sustained nonpharmacological neuroregenerative therapy. Nat Med. 2018 Dec;24(12):1830-1836.*

- Did the author used the same cuff electrode (implanted one and just changed energy source for direct comparison) shown in Fig. 5? The conduction velocities are different.

--The cuff electrodes were not the same. We stimulated at two different points and we demonstrate the experimental setup more clearly with an extra panel added in as Fig 7A showing the relative positions the electrodes are placed on the sciatic nerve. The different on latency was due to the different distance the stimulators to the recording electrode.

- Recorded EMGs shown in Fig 7 were appeared in every 20 ms, which means 50 Hz stimulation was applied. Can you comment on this issue?

--This was a typographical error in labeling the X-axis of the graph. We have made appropriate corrections as 20Hz stimulation was applied.

Minor Concerns:
- Where is the result for the regeneration of sciatic nerve?

--We have added additional data in the new figure 9 that shows examples of retrogradely labeled motor neurons from the distal branches of the sciatic nerve after injury.

- What is the difference compared with Ref [20]?

--Reference 20 (Guo et al. 2021) has several differences to the present study. The cuff was interfaced at a different position (tibial nerve branch rather than more proximally on the sciatic nerve), a mouse rather than a rat model was used, and we have included new data on phrenic nerve implantation and interfacing here.

Reviewer #2:
Manuscript Summary:
The authors describe methods to implant and wirelessly power electrodes for stimulation of peripheral nerves (phrenic and sciatic) in a rat model. This approach is innovative and novel and is relevant to preclinical and potential clinical applications. The methods described and the related imaging/figures are very clear and straightforward.

--We thank the reviewer for commenting on the "innovative" and "novel" nature of this manuscript. We are pleased to learn they found it "very clear and straightforward".

Major Concerns:
One major comment I have is that the technology (the wireless interface/stimulator) is not described in sufficient detail so that another lab could independently apply these approaches. The authors should consider adding additional details so that other groups can learn and apply these innovative approaches.

--Thank you for pointing this out. We have added additional details including a subsection with details on the design and how to make these devices, as well as added an additional figure (now labeled figure 2) to the manuscript. In addition even greater details can be found in our recent publications that are cited[1-3].

1. *Guo H, D'Andrea D, Zhao J, Xu Y, Qiao Z, Janes LE, Murthy NK, Li R, X Zie, Song Z, Meda R, Koo J, Bai W, Choi YS, Jordan SW, Huang Y, Franz CK*, Rogers JA*. Advanced Materials in Wireless, Implantable Electrical Stimulators that Offer Rapid Rates of Bioresorption for Peripheral Axon Regeneration. Adv Functional Materials. Adv Funct Mater. 2021 May (doi.org/10.1002/adfm.202102724)*
2. *Koo J, MacEwan MR, Kang SK, Won SM, Stephen M, Gamble P, Xie Z, Yan Y, Chen YY, Shin J, Birenbaum N, Chung S, Kim SB, Khalifeh J, Harburg DV, Bean K, Paskett M, Kim J, Zohny ZS, Lee SM, Zhang R, Luo K, Ji B, Banks A, Lee HM, Huang Y, Ray WZ, Rogers JA. Wireless bioresorbable electronic system enables sustained nonpharmacological neuroregenerative therapy. Nat Med. 2018 Dec;24(12):1830-1836.*
3. *Choi YS, Hsueh YY, Koo J, Yang Q, Avila R, Hu B, Xie Z, Lee G, Ning Z, Liu C, Xu Y, Lee YJ, Zhao W, Fang J, Deng Y, Lee SM, Vázquez-Guardado A, Stepien I, Yan Y, Song JW, Haney C, Oh YS, Liu W, Yoon HJ, Banks A, MacEwan MR, Ameer GA, Ray WZ, Huang Y, Xie T, Franz CK, Li S, Rogers JA. Stretchable, dynamic covalent polymers for soft, long-lived bioresorbable electronic stimulators designed to facilitate neuromuscular regeneration. Nat Commun. 2020 Nov 25;11(1):5990.*

Minor comments:
"The experiments involve increasing the stimulation voltage until the response magnitude plateaus at the physiological maximum."
Is the word "physiological" necessary?

--No. We deleted this word.