OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-20-00461
Title: The Future of Adenoassociated Viral Vectors for Optogenetic Peripheral Nerve Interfaces
Reviewer’s Name: Jordan Williams
Reviewer’s country: USA

COMMENTS TO AUTHORS
The majority of my concerns are presented in the comments to the authors. Depending on editorial limits (word count limits, references), the manuscript could be considered short and lightly referenced for a review. The topic is highly relevant and relatively novel in the area of neural interfacing, regeneration, and rehabilitation. In general, the review touches on a majority of relevant factors in viral mediated optogenetic transduction in the peripheral nervous system and does so in a concise format. However, the personal results from the authors' experiments seem weak compared to previously published reports (see [2] in author comments) and could be removed in my opinion.

The submitted manuscript provides a short overview on the use of adeno-associated viruses (AAVs) in the peripheral nervous system to both stimulate and read-out nerve activity for neural prosthetic purposes (i.e. optically stimulating a labeled motor nerve to stimulate paralyzed muscle activity or reading out activity-related fluorescence from a labeled nerve to decode sensory information). The authors discuss a number of topics related to the current state of the art in this field, mainly focusing on the AAV vector including route of AAV injection, cassette and promoter design, viral capsid engineering and tissue tropism for neural tissue, and evaluation of tissue expression. The authors cite several recent relevant studies in the area of viral peripheral optogenetics as well as some of their own experience in the field. Overall, the manuscript is a short, easy to read overview on the topic.

However, while reviewing the manuscript, several concerns emerged:
1) The authors initially focus their motivation and rationale on surface electromyogram control of a prosthesis followed by nerve-implanted electrodes. No articles referencing either technology or control strategy is cited despite their comparatively rich history.
2) In the first paragraph, the authors propose benefits of optogenetic over electrical interfacing with nerves, but do not mention reported benefits of optogenetic stimulation in terms of muscle recruitment qualities over functional electrical stimulation (e.g. natural recruitment order, reduced fatigue, potential muscle-specific activation, etc.) [1,2].
3) Lines 30/31 refer to "Off-target expression can cause toxicity and immunogenicity." However, even targeted expression of opsins such as ChR2 has been shown to elicit immune responses that can damage the nerve, making this a vital avenue for future investigation [3].
4) In the second paragraph, lines 34-42, the rationale for injecting muscles in a partially amputated limb is not clear as the remaining nerves and muscles are still intact and under volitional control of the brain/spinal cord. Would we be trying to control or read-out from the intact muscles/nerves, or trying to interface with a prosthetic device to replace the amputated portion of limb?
5) Lines 46 and 47 state that spinal cord injections such as intrathecal offer less off-target expression. While transduction may be limited to spinal cord tissue with intrathecal injections, they offer little spatial specificity (virus can spread/span the spinal cord) or cell-type specificity (transduction limited by diffusion of virus into the spinal cord) compared to intraparenchymal injections of virus into specific locations/depths of spinal cord.
6) Lines 49-51 posit that expression levels must be high both in the soma as well as the axons of the peripheral nerve. Wouldn't this be highly dependent on the desired application and site of stimulation or visualization? For example, peripheral optical stimulation of ChR2-labeled...
Nerves/muscle activity would require robust expression along neuronal axons (and not necessarily in the soma), while direct optical stimulation of neurons in the spinal cord would benefit from protein expression in the soma and less from peripheral axonal expression.

7) The results presented by the authors in Figure 1B-D appear out of place for this review and not necessarily exciting or groundbreaking. To highlight AAV7 as more efficiently transducing ChAT+ axons than AAVrh10 (implying AAV7 as a potential vector for optogenetic motor stimulation) while only less than 3% of ChAT+ axons are labeled (maybe 2-3 obviously labeled axons in the nerve in Figure 1D) seems misplaced.

[1] Llewellyn M E, Thompson K R, Deisseroth K and Delp S L 2010 Orderly recruitment of motor units under optical control in vivo Nat. Med. 16 1161-5
[2] Towne C, Montgomery K L, Iyer S M, Deisseroth K and Delp S L 2013 Optogenetic Control of Targeted Peripheral Axons in Freely Moving Animals ed E J Kremer PLoS ONE 8 e72691
[3] Maimon B E, Diaz M, Revol E C M, Schneider A M, Leaker B, Varela C E, Srinivasan S, Weber M B and Herr H M 2018 Optogenetic Peripheral Nerve Immunogenicity Scientific Reports 8