The future of adenoassociated viral vectors for optogenetic peripheral nerve interfaces

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Prostheses have a several thousand year history for treating limb loss. With time, these prostheses have become more sophisticated and closer to replicating the natural limb. These advances have culminated in the myoelectrically controlled prosthesis, which employs the surface electromyogram to decode the user's intent. But the surface electromyogram lacks fidelity and though convenient, suffers from several problems. The skin-electrode interface undergoes impedance changes throughout the day and electrode liftoff can cause signal loss. A better solution would be to interface directly with the residual nerves which still carry the descending and ascending neural impulses. Signals can be recorded through electrodes implanted within peripheral nerves or the spinal cord, however current electrode technologies generally trade specificity for longevity and reliability. Electrodes that have high specificity use penetrating approaches that often irritate, damage and become encapsulated with fibrotic tissue limiting their long term viability (see (Navarro et al., 2005) for a review of surface electromyogram and electrode based interfaces). Optical approaches may obviate this problem and provide high specificity with limited invasiveness. Through the use of optogenetic actuator and reporter proteins, an optogenetic peripheral nerve interface can circumvent these problems by using light to manipulate and detect neural activity. This interface could be deployed for both prosthesis control following limb loss or limb reanimation following spinal cord injury (for example, see optogenetic actuation in a nonhuman primate model by (Williams et al., 2019)). An additional advantage of using optogenetic actuators in limb reanimation is that they may have a more physiologic motor unit recruitment pattern, recruiting slow oxidative fibers at lower stimulus than fast glycolytic fibers, reducing fatigue compared to electrical stimulation which recruits larger fibers at lower stimulus (Llewellyn et al., 2010). As transgenes, these optogenetic proteins require a method of delivery to the nerve, which can be achieved using viral vectors, which offer a high transduction efficiency. Of these vectors, adenoassociated viral vectors (AAVs) are particularly interesting because they are relatively nonimmunogenic and have been approved by the United States Food and Drug Administration to treat several genetic diseases. Furthermore, unlike lentiviruses and their wild-type progenitors, AAVs rarely integrate into the host genome, yet still provide extended expression. Targeting of these vectors to the motor and sensory neurons, and ultimately the axons of the dorsal root ganglia and ventral horn of the spinal cord is critical. Off-target expression can cause toxicity and immunogenicity. Selectivity is achieved through route of administration, expression cassette design, and serotype selection and capsid engineering (**Figure 1A**).

Unlike treatment of peripheral disease, where disseminated transgene expression is desired to correct a problem affecting many neurons, in an optogenetic peripheral nerve interface, ideally only neurons in a select distribution are transduced. Following transradial amputations, intramuscular injections can allow for restricted expression in neurons innervating the muscles of the residual limb, permitting read-out from nerve fibers innervating extrinsic hand and wrist flexors and extensors. These signals in turn, would be used to a control a prosthetic hand. However, in this situation intrinsic muscles of the hand as well as the skin overlying them are lost, and expression outside of the forearm muscles would provide additional control signals and sensory information. This can be achieved either with intravascular (IV), intranerve or intra-spinal cord injections (for review see (Anderson and Weir, 2019)). IV injections have the benefit of ease of administration. Intranerve injections, while more invasive and have an increased risk of damage to the nerve, benefit from requiring lower viral titers, and lower risk of off target effects, while potentially permitting transduction of neurons innervating that nerve's anatomical distribution. Intrathecal and intraparenchymal injections are possible in the spinal cord. Intrathecal injections offer less off-target expression in organs outside of the CNS, but are much more invasive than IV or intranerve injections, and can result in labeling in undesired regions of the brain and spinal cord. Intraparechymal injections of the spinal cord, offer better spatial specificity, but are also invasive.

The expression cassette plays an important role in controlling the amount and intracellular location of expression of the optogenetic protein. Ubiquitous promoters like the cytomegalovirus promoter allow for strong expression in a variety of tissues such as motor neurons, but also carry the risk of silencing (for review see (Haery et al., 2019)). In our experience and the experience of others, the ubiquitous CMV early enhancer/Chincken beta-actin promoter, was not effective in driving expression in motoneurons, although it can permit strong expression in sensory axons without silencing. Numerous promoters exist to limit expression to neurons (e.g. synapsin). Norante et al. (2017) have demonstrated subcellular targeting in motoneurons using modified homeobox promoters to drive expression of calcium sensitive optogenetic reporters. Further understanding of axonal transport and axonal translation of transgenes will help devise strategies to increase optogenetic protein levels in the axon, potentially reducing off-target effects in the soma. Other elements can be added into the viral genome to enhance expression, such as the woodchuck hepatitis posttranscriptional regulatory element (WPRE) and the polyadenylation signal sequence. These elements can be modified and shortened – for example, Choi et al. (2014) created a shortened version of WPRE, WPRE3, and a modified polyadenylation sequence signal, SL. These changes increased the payload capacity by 399bp over a cassette using WPRE and the bovine growth hormone polyadenylation signal, while retaining the approximately the same level of expression. Such modifications, alongside the development of shortened promoter sequences, allow for larger payloads, permitting the use of more complicated transgene designs. A dual injection of AAVs can also be used to limit expression to specific populations and allow the use of more specific, potentially larger promoters. Gompf et al. (2015) used an AAV driving Cre under a tissue specific promoter to permit expression of channelrhodpsin-2, an optogenetic actuator in tyrosine hydroxylase positive neurons in rat brain. Such a method could be extended to driving the often-high levels of expression required for optogenetic interrogation and modulation in neurons innervating the periphery.

The viral capsid provides a final level of control for the cell tropism. While many AAV serotypes are capable of transducing neurons, they vary in their ability to cross the blood brain barrier (limiting IV delivery) or to undergo retrograde transport from the periphery. Such limitations would then require more invasive routes of administration for successful transduction. Not all serotypes favor neurons or favor neurons selectively. For transduction of motor neurons – AAV serotypes 1, 6, 7, rh10, rh39, and rh43 (reviewed in (Anderson and Weir, 2019)) have been demonstrated to work, and serotypes 1, 5, 6, and 8 have shown tropism for sensory neurons in the dorsal root ganglia (Mason et al., 2010). In our experience, IV injection of AAVrh10 provides a strong candidate for labeling of peripheral choline acetyltransferase (ChAT; expressed in motoneurons) positive axons, while AAVPHP.B provides an alternative for labeling large diameter ChAT- axons. Following intramuscular injection, we saw considerable labeling with AAV6 of both nerve and muscle at the injection site. Recently, with a low viral titer of 2×10^{10} vector genomes, we performed a similar

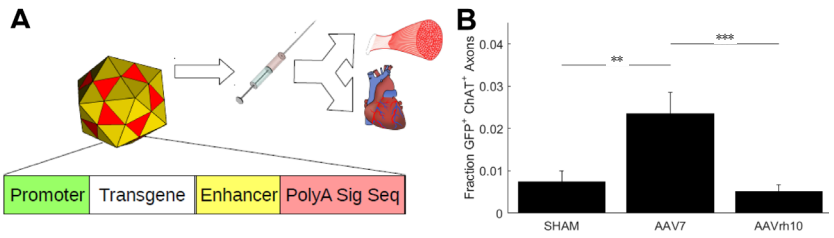

Figure 1 | **Adenoassociated viral vectors for optogenetic peripheral nerve interfaces.**
(A) Factors that can be used to target expression to sensory and motor axons. These are the promoter, enhancer and polyadenylation signal sequence of the expression cassette, the capsid, and the route of administration (e.g. intramuscular or intravenous injection). (B) IV injections at titers of 2×10^{10} vector genomes of AAV7- CMV-GFP label choline acetyltransferase positive axons of the adult murine sciatic nerve, while AAVrh10 did not. AAV7 showed off-target expression in 4/5 livers, AAVrh10 in 5/5 livers. Fraction $ChAT^+$ GFP^+ axons of total $ChAT^+$ axons following injection (data are sourced from our laboratory). AAV7 labeled 2.35 ± 0.512% of $ChAT^+$ axons. $^{**}P < 0.01$. $^{***}P < 0.001$. AAV: Adenoassociated virus; ChAT: choline acetyltransferase; CMV: cytomegalovirus; GFP: green fluorescent protein.

experiment looking at AAV7 and AAVrh10 serotypes following IV injection using a similar method to that outlined in (Anderson et al., 2019), which demonstrated labeling of $ChAT^+$ axons in the murine sciatic nerve with AAV7 and some off target labeling of livers using either vector (**Figure 1B**). This result suggests that AAV7 may also be a viable vector in the optogenetic peripheral nerve interface toolkit, potentially requiring lower viral titers with less off-target expression than AAVrh10, an established vector for peripheral nerve axon labeling (Anderson and Weir, 2019; Anderson et al., 2019). Further work will consider higher viral titers.

Capsid engineering has provided even more opportunities for the development of serotypes with tropism for neurons. Cre recombination–based AAV targeted evolution was used to engineer the PHP.B capsid, which has improved neuronal tropism over AAV9 following IV injection (Deverman et al., 2016). Cre recombination–based AAV targeted evolution could be used to find capsids for transduction of either motor or sensory neurons using a transgenic mouse expressing Cre. While AAVs have been very effective, it is important to note that antibodies against wild type AAVs are common in the population and may impact the effectiveness of vector delivery.

Through careful design of the vector and payload, expression can be tailored to reach certain neurons in the peripheral nerve; however, it is not sufficient that these cells be transduced. Protein levels have to be sufficiently high in the axons of the peripheral nerve for a peripheral nerve interface; therefore it is paramount to evaluate the nerve itself, and not only the soma. Manual, semiautomated, and automated (e.g. as employed in (Anderson et al., 2019)) methods exist for determining the proportion of axons labeled by the transgene in the peripheral nerve. These methods can be combined with retrograde tracers (e.g. FluroGold or cholera toxin B subunit) to assess the transduction efficiency of an injection for a given muscle. Ultimately, a sufficient number and type of axons are required to be labeled for fine control of force.

There still remain many questions beyond vector selection for the development of an optical peripheral nerve interface, particularly around the development of fiber optic and miniaturized microscopy and optogenetic reporters and actuators, especially given that Channelrhodopsin-2, a commonly used optogenetic actuator, is neurotoxic (Maimon et al., 2018). As developments in AAV targeting of peripheral nerves continue to improve, not only is the potential for gene therapy and post-injury nerve neuromodulation advanced, but the possibility of a less intrusive, more specific peripheral nerve interface comes closer.

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References

Anderson HE, Schaller KL, Caldwell JH, Weir RFF (2019) Intravascular injections of adenoassociated viral vector serotypes rh10 and PHP.B transduce murine sciatic nerve axons. Neurosci Lett 706:51-55.
Anderson HE, Weir RFF (2019) On the development of optical peripheral nerve interfaces. Neural Regen Res 14:425-436.
Choi JH, Yu NK, Baek GC, Bakes J, Seo D, Nam HJ, Baek SH, Lim CS, Lee YS, Kaang BK (2014) Optimization of AAV expression cassettes to improve packaging capacity and transgene expression in neurons. Mol Brain 7:17.
Deverman BE, Pravdo PL, Simpson BP, Kumar SR, Chan KY, Banerjee A, Wu WL, Yang B, Huber N, Pasca SP, Gradinaru V (2016) Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. Nat Biotechnol 34:204-209.
Gompf HS, Budygin EA, Fuller PM, Bass CE (2015) Targeted genetic manipulations of neuronal subtypes using promoter-specific combinatorial AAVs in wild-type animals. Front Behav Neurosci 9:152.
Haery L, Deverman BE, Matho KS, Cetin A, Woodard K, Cepko C, Guerin KI, Rego MA, Ersing I, Bachle SM, Kamens J, Fan M (2019) Adeno-associated virus technologies and methods for targeted neuronal manipulation. Front Neuroanat 13:93.
Llewellyn ME, Thompson KR, Deisseroth K, Delp SL (2010) Orderly recruitment of motor units under optical control in vivo. Nat Med 16:1161-1165.
Maimon BE, Diaz M, Revol ECM, Schneider AM, Leaker B, Varela CE, Srinivasan S, Weber MB, Herr HM (2018) Optogenetic peripheral nerve immunogenicity. Sci Rep 8:14076.
Mason MR, Ehlert EM, Eggers R, Pool CW, Hermening S, Huseinovic A, Timmermans E, Blits B, Verhaagen J (2010) Comparison of AAV serotypes for gene delivery to dorsal root ganglion neurons. Mol Ther 18:715-724.
Navarro X, Krueger TB, Lago N, Micera S, Stieglitz T, Dario P (2005) A critical review of interfaces with the peripheral nervous system for the control of neuroprostheses and hybrid bionic systems. J Peripher Nerv Syst 10:229-258.
Norante RP, Massimino ML, Lorenzon P, De Mario A, Peggion C, Vicario M, Albiero M, Sorgato MC, Lopreiato R, Bertoli A (2017) Generation and validation of novel adeno-associated viral vectors for the analysis of Ca2+ homeostasis in motor neurons. Sci Rep 7:6521.
Williams JJ, Watson AM, Vazquez AL, Schwartz AB (2019) Viral-mediated optogenetic stimulation of peripheral motor nerves in non-human primates. Front Neurosci 13:759.

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