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14. ABSTRACT
The overall objective of this project was to leverage our lab's expertise in cortical neuroprosthetics with emerging viral optogenetic techniques in peripheral nerves to produce a hybrid, brain-controlled Functional Optical Stimulation (FOS) system in non-human primates, which would offer several advantages over traditional Function Electrical Stimulation (FES) systems to restore volitional motor control. Initial experiments were used to evaluate the efficacy of several viral vectors, including adeno-associated virus (AAV) and non-replicating rabies virus (NRRV) constructs, in transducing light-sensitive opsins into peripheral motor nerve axons in macaques and rats

15. SUBJECT TERMS
Optogenetics, brain computer interface, neural prosthetic, peripheral motor nerve stimulation, peripheral viral transduction, optical nerve cuff

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Report Title

Final Report: Brain Control Optical Stimulation of Muscles

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used opsin, ChR2. Finally, a prototype micro-LED nerve cuff was designed and tested for chronic stimulation of opsins tranduced in peripheral nerves.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper	
02/28/2017	1	J.J. Williams, A.L. Vazquez, C. Wirblich, M.J. Schnell, A.B. Schwartz. Virus, opsin, and immunomodulation approaches for optogenetic control of peripheral motor function, Society for Neuroscience. 11-NOV-16, San Diego, California. : ,
TOTAL:	**1**	

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper
TOTAL:	

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(d) Manuscripts

Received	Paper
TOTAL:	

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Jordan Williams	0.00
Hongwei Mao	0.50
FTE Equivalent:	**0.50**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Andrew Schwartz	0.50	
FTE Equivalent:	**0.50**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

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Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Kathleen Hansell-Prigg	0.20
Emrah Diril	0.20
FTE Equivalent:	**0.40**
Total Number:	**2**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See attachments

Technology Transfer

This project initiated the development of a micro-LED nerve cuff for chronic functional optical stimulation of peripheral nerves transduced with light sensitive ion channels. This LED nerve cuff was designed in conjunction with Draper Laboratories (Cambridge, MA). Researchers at the University of Pittsburgh provided desired design specifications, in vivo testing, and feedback of cuff prototypes, while Draper Labs provided initial device designs, production, and testing. Development and production of these cuffs is still ongoing, but recent progress is discussed in the “Summary of Important Results” section.

Subject Terms: Optogenetics, brain computer interface, neural prosthetic, peripheral motor nerve stimulation, peripheral viral transduction, optical nerve cuff

Abstract: The overall objective of this project was to leverage our lab's expertise in cortical neuroprosthetics with emerging viral optogenetic techniques in peripheral nerves to produce a hybrid, brain-controlled *Functional Optical Stimulation* (FOS) system in non-human primates, which would offer several advantages over traditional *Function Electrical Stimulation* (FES) systems to restore volitional motor control. Initial experiments were used to evaluate the efficacy of several viral vectors, including adeno-associated virus (AAV) and non-replicating rabies virus (NRRV) constructs, in transducing light-sensitive opsins into peripheral motor nerve axons in macaques and rats. The results of these experiments showed successful yet highly variable expression with several AAV constructs, but no detectable expression of optogenetic products when using NRRV-based constructs. Evaluation of several injection techniques suggested that spinal cord injections of virus were the most consistent in eliciting gene expression but the least specific in targeting specific muscles, while muscle injections showed variable expression but demonstrate muscle specific labeling of motor nerve fibers. Next, a new opsin, "Chronos", demonstrated desirable light sensitivity and channel kinetics over the traditionally used opsin, ChR2. Finally, a prototype micro-LED nerve cuff was designed and tested for chronic stimulation of opsins tranduced in peripheral nerves.

Publications, conference proceedings, books, dissertations, Thesis and manuscripts:

J.J. Williams, A.L. Vazquez, C. Wirblich, M.J. Schnell, A.B. Schwartz (2016) Virus, opsin, and immunomodulation approaches for optogenetic control of peripheral motor function. *Society for Neuroscience Abstracts*, November 12-16, San Diego, California.

Honors & Awards: None

Patents: None

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Schwartz Lab Final Report to ARO (DARPA) on the "Brain Control Optical Stimulation of Muscles" Project

February 28, 2017

By: Jordan Williams, MD, PhD, and Andrew Schwartz, PhD

1: Statement of the Problem Studied

Currently, people who are paralyzed by stroke, spinal cord injury and degenerative diseases such as amyotrophic lateral sclerosis and multiple sclerosis face permanent disability. The ultimate goal of this project and its future directions is to reverse upper extremity paralysis. By extracting volitional arm movement signals from motor cortex and using them to control the activation of a set of arm muscles, we can generate an intended movement. This extraction of brain signals for the volitional control of arm movement is now a proven technology (i.e. brain-computer interfaces or BCIs), but has mostly been used with robotic prosthetic arms [1] or in virtual reality [2]. Artificial activation of muscle is also well established, but has been limited by impoverished signals and carried out with electrical current through techniques known as functional electrical stimulation (FES), which pose a number of problems in terms of their efficacy in restoring normal muscle function. In this project, we proposed to use a new technique, peripheral optical activation of motoneuron fibers, which has great promise for elaborate control of muscles. This peripheral optical activation is facilitated by the viral transduction of optogenetic gene products called 'opsins' (i.e. light sensitive ion channels) into peripheral motor nerves, allowing these fibers to be stimulated with light of a specific wavelength. Optical activation of peripheral fibers in this manner offers several advantages over traditional functional electrical stimulation (FES) of muscles for rehabilitation purposes including a more natural recruitment order of muscle fibers and a resulting decrease in muscle fatigability [3], as well as the potential to independently optically stimulate multiple muscles from a proximal nerve location [4].

Although optical activation of motoneurons using opsins such as channel rhodopsin (ChR2) has been achieved in rodents, and recombinant DNA has been inserted into primate motoneurons [5], to date, optical activation of peripheral primate motoneurons using ChR2 has not been demonstrated. By developing and combining this technology with our proven BCI signal-extraction methods, these techniques could one day allow paralyzed individuals to regain control of their native muscles. Thus, a major hurdle to translating this combination of BCI and optogenetic control of peripheral motoneurons to a paralyzed patient population would be the demonstration of these combined techniques in a non-human primate (NHP) model.

With this goal in mind, this project initially sought to advance these techniques toward use in NHPs through three aims. **The first aim of this proposal** was to test the efficacy of two potential viral optogenetic vectors, adeno-associated virus (AAV) and non-replicating rabies virus (NRRV), in transducing NHP peripheral motonerves with opsins, and then optimize these vectors for chronic BCI-optogenetic use. **The second aim of this proposal** was to develop a chronic micro-LED cuff for functional optical stimulation (FOS) of virally transduced peripheral nerves in NHPs. Finally, **the third aim of this proposal** was to demonstrate volitional BCI control of FOS to reanimate temporarily

paralyzed muscles in a NHP model in order to perform a simple reaching task. This final report will summarize our results, progress, shortcomings, and future directions in relation to these goals.

2. Summary of Important Results

Initial work on this project focused on the first aim with preparations and evaluation of acute experiments in non-human primates. Two monkeys were injected into a set of leg muscles with virus constructs designed to confer light sensitivity to the nerves leading to these muscles such that shining light of a specific frequency on the nerve should stimulate it to activate a given muscle. We tested two such viral vectors: a non-replicating strain of rabies virus (NRRV), used for its natural tropism for entering the peripheral nervous system, and AAV, which has shown promise as a gene therapy vector in both non-human primates and humans and also exhibits a relatively low risk profile. Following an incubation period for the virus to transfect the desired nerves, we evaluated the efficacy of each in enabling optical stimulation of peripheral musculature. Unfortunately, neither of our first two acute monkey experiments was successful in exciting the injected muscles with optical stimulation. Though these experiments were useful for gaining technical experience from surgical and hardware perspectives, it soon became obvious that translating past successes of peripheral optogenetics in rodents to non-human primates and eventually humans would not be a trivial exercise. We obtained some histological evidence that one viral construct (AAV6-hSyn-ChR2-eYFP) successfully infected the peripheral nervous system and the spinal cord of a macaque (see Figure 1), but apparently not enough to enable optical stimulation of the injected muscles. Whether this failure was due to factors associated with the virus (low infection rate), expression of its construct (low expression, faulty expression, spatial distribution of expression), or shortcomings of our stimulation techniques was unclear.

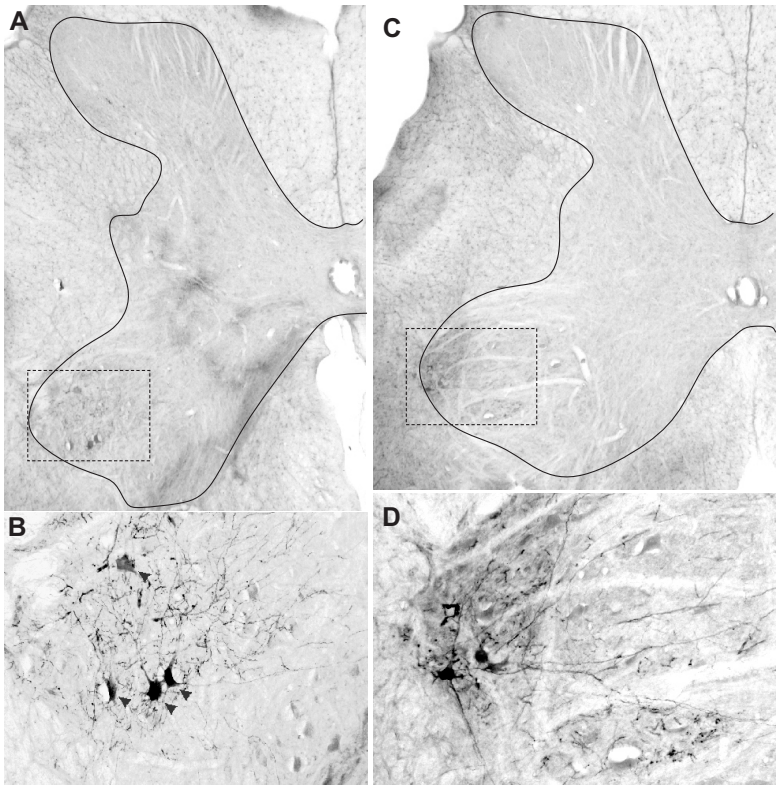


Figure 1. Lumbar spinal cord histology. Panels A and C show two sections of lumbar spinal cord stained with antibodies to green fluorescent protein (GFP) and its variants (e.g. enhance yellow fluorescent protein (EYFP)). The ventral horns of gray matter in the lower left corner exhibit darker staining, consistent with EYFP expression resulting from retrograde transport of the AAV construct from the injected muscles to the corresponding motoneurons in the spinal cord. Panels B and D show magnified views of the boxed insets in A and C, respectively, and demonstrate labeling of several motoneuron cell bodies as well as significant labelling of dendrites.

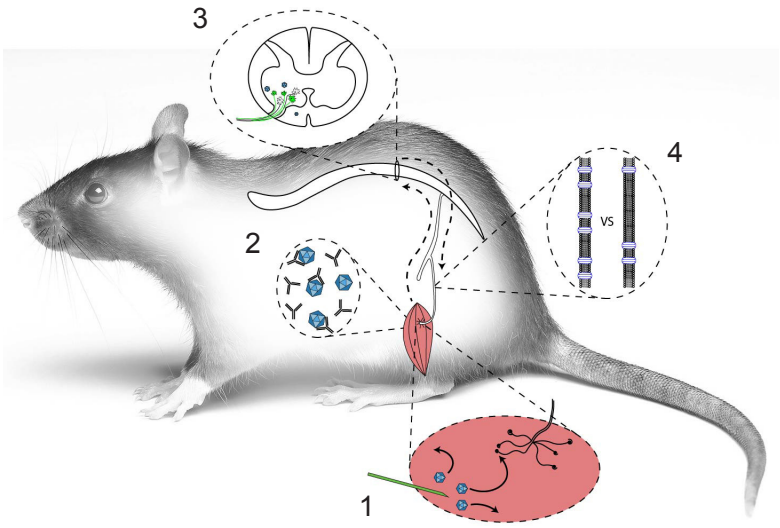


Figure 2. Potential pitfalls of viral-mediated peripheral motor optogenetics. Viral transduction of peripheral motor nerves following muscle injections requires: 1) diffusion of virus from the injection site to the neuromuscular junction for uptake, 2) evading immune response clearance of the virus, 3) retrograde travel along the relatively long nerve axons to the motor neuron cell bodies in the spinal cord and expression, and 4) anterograde transport of expressed opsins back to the nerve with potentially variable trafficking to the axonal membrane.

Due to the lack of success in our initial non-human primate experiments, we decided to suspend our acute NHP experiments for our first aim while pursuing similar experiments in rats, which were intended to refine injection techniques, immunosuppression, approaches and viral constructs prior to returning to non-human primate experiments. These experiments were performed to systematically address or circumvent many of the potential pitfalls in transducing peripheral nerves with light-sensitive opsins using viral injections. These confounding factors of viral peripheral optogenetics are described in Figure 2. In total, we injected a total of 25 rats (male, Sprague Dawley) over approximately 35 injection sites. We examined three injection strategies, each with its own modifications and nuances: muscle injections, direct nerve injections, and spinal cord injections. We utilized two AAV constructs (AAV6-hSyn-ChR2-eYFP and AAV9-hSyn-Chronos-GFP) and two NRRV constructs (SAD-deltaG-ChR2-YFP (a vaccine strain) and N2c-deltaG-ChR2-YFP (a less immunogenic form)). Finally, a subset of these rats were administered Dexamethasone to test its efficacy in blunting the immune response to an AAV or NRRV construct. The injection techniques and summary of these experiments is described below:

Muscle injections: Muscle injections were initially performed as described by Towne et al. [5] for our initial monkey and rat injections. We eventually modified this approach to use electrical stimulation through an injectable stimulating needle to identify the motor endplate as well as drive virus into the muscle via iontophoresis. This process and methods to visualize these injections are depicted and described in Figure 1.

Direct Nerve Injections: To avoid potential complications due to virus diffusion and uptake in muscle, we attempted several procedures in which virus solution was directly injected into the nerve innervating the target tibialis anterior muscle. The injection process and visualization is described in Figure 4.

Stimulating Spinal Cord Injections: To circumvent retrograde trafficking of our viral constructs entirely, we injected virus directly into the ventral horn of the lumbar spinal cord using an injectable monopolar needle. This needle was used to stimulate and verify the depth of motor neuron pools as well as their corresponding muscles. These injections and testing procedures are described in Figure 5.

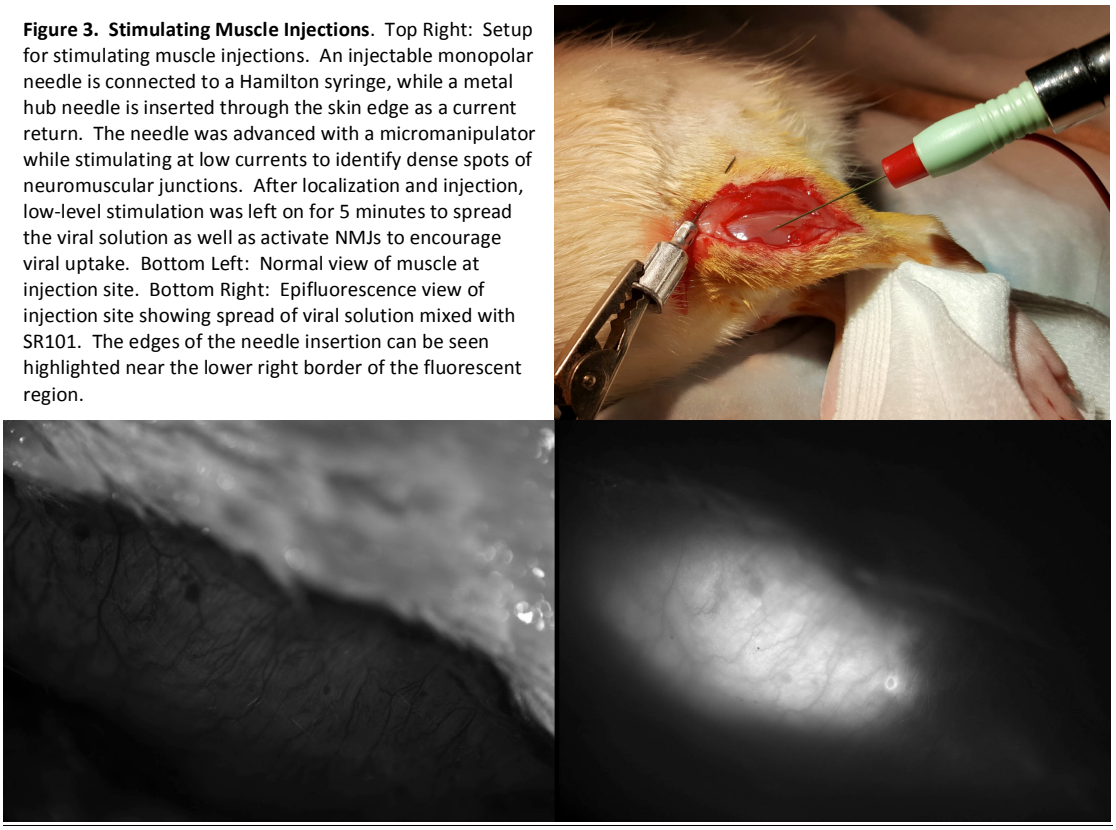
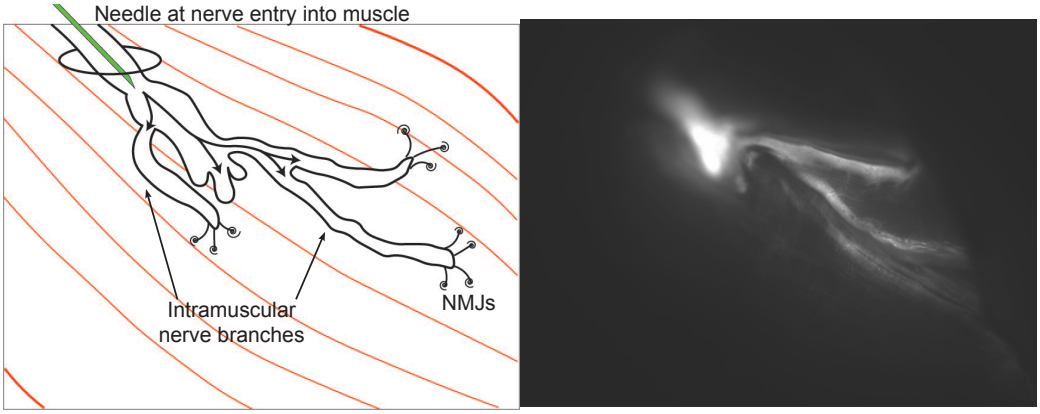


Figure 3. Stimulating Muscle Injections. Top Right: Setup for stimulating muscle injections. An injectable monopolar needle is connected to a Hamilton syringe, while a metal hub needle is inserted through the skin edge as a current return. The needle was advanced with a micromanipulator while stimulating at low currents to identify dense spots of neuromuscular junctions. After localization and injection, low-level stimulation was left on for 5 minutes to spread the viral solution as well as activate NMJs to encourage viral uptake. Bottom Left: Normal view of muscle at injection site. Bottom Right: Epifluorescence view of injection site showing spread of viral solution mixed with SR101. The edges of the needle insertion can be seen highlighted near the lower right border of the fluorescent region.



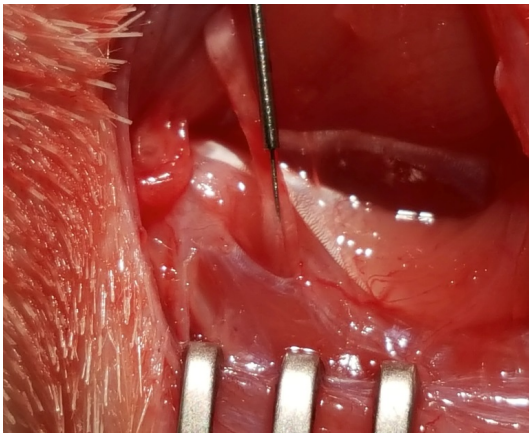


Figure 4. Direct nerve injections. Top Left: Rationale for direct nerve injections. The schematic depicts a needle inserted into the nerve sheath close to the nerve's entry into the muscle. As the viral construct is injected proximally, it flows and fills branches distally in the muscle. Ideally, the virus spills out of the nerve sheath at the termination of the nerve branches in the vicinity of neuromuscular junctions, facilitating virus uptake. Top Right: Epifluorescence image of actual nerve injection corresponding with left panel. SR101 was mixed with a viral construct solution and injected as described for the left panel. The viral solution can be seen to fill distal branches as desired. Bottom Left: Picture of actual nerve injection. A 35 gauge needle is inserted near parallel into the nerve sheath at the nerve's insertion into the tibialis anterior muscle.

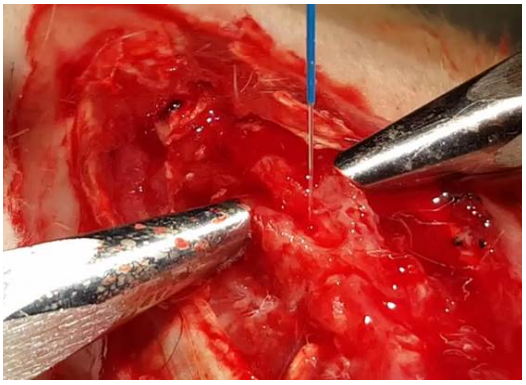
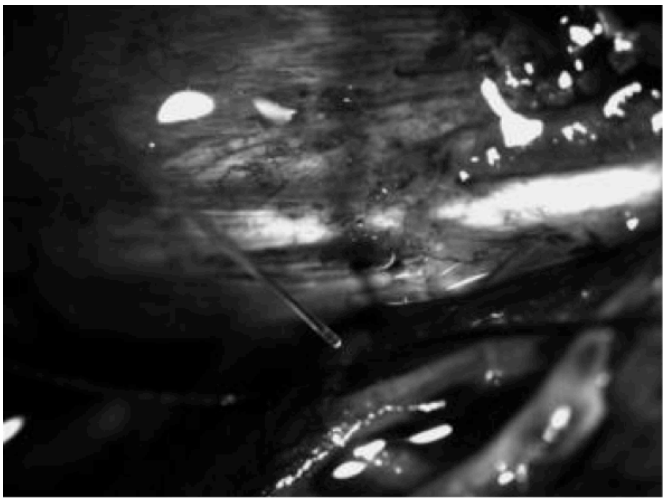


Figure 5. Spinal cord injection setup. Left Panel: A stimulating monopolar injectable needle is advanced via micromanipulator through a laminectomy toward the ventral horn at the target vertebral level. The rat's spine is stabilized via stereotactic ear bars. The needle is advanced while electrically stimulating to observe for maximum muscle contractions. Once a pool of motor neurons has been identified, ~ 2 uL of virus is injected at 0.5 uL/min. Bottom Panel: 1-2 weeks later, viral expression was evaluated by fiber optic stimulation at the injection site.

Rat Injection Results Summary: Example results from an injection experiment with successful transduction of ChR2 and the fluorescent marker (eYFP) is shown in Figure 6. Our rat experiments produced a spectrum of successes and failures with many experiments yielding no overt expression of opsin or fluorescent marker, some with minimal expression resulting in weak muscle contractions, and some with strong expression resulting in brisk contractions and functional movements at the ankle.



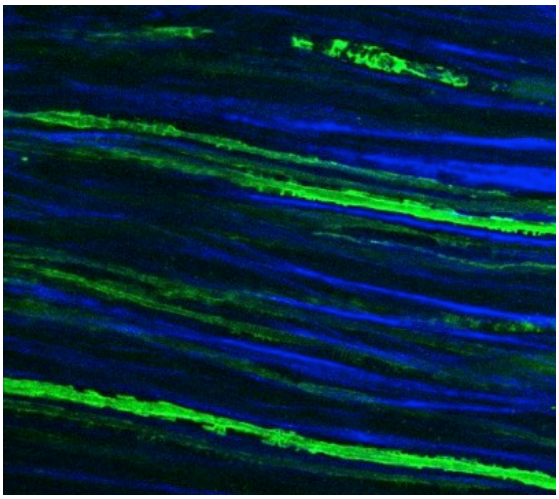


Figure 6. Successful transduction of ChR2-eYFP construct in rat peripheral nerve following AAV-vector injection in rat muscle. (Left). Close-up of fiber-optic nerve stimulation with target muscle in view. For this experiment, inspection at this close distance was necessary to observe muscle contraction with optical stimulation. Later injection experiments demonstrated varying degrees of contraction strength from contractions easily visible on the muscle but not resulting in functional movements to brisk contractions resulting in overt foot movements. (Right) Two-photon image of transfected nerve with second harmonic imaging. Green shows dense eYFP expression along entire length of nerve axon, while second harmonic imaging is used to contrast the structure/outline of individual axons in blue.

The overall transduction results obtained during rat injection experiments are summarized in Tables 1 and 2 below. As can be seen from the tables, we did experience some success with each injection method, but spinal cord injections of AAV seemed to be the most consistent in a limited sample set (1 AAV6-ChR2 and 1 AAV9-Chronos). The success rate with muscle injections was very low compared to expected rates from discussions with an author of previous reports [5,6], Dr. Chris Towne, and his experiences. According to him, if a virus batch works in one rat, it should work in multiple rats similarly given the same injection parameters. Our discussions lead us to believe that the source and quality of our virus production from the UNC Vector Core may have had a large influence on the success of these experiments, and we may want to try other virus vendors for our AAV constructs in the future.

Regarding viral vectors and serotypes, the successful use of AAV9 in the spinal cord is, to the best of our knowledge, the first use of AAV9 in the periphery to transduce ChR2 in the periphery. However, other reports had previously suggested that AAV9 could be efficacious in spinal cord or intrathecal injections [7,8]. Surprisingly, NRRV was not successful in any of our experiments including spinal cord injections. We did not expect this outcome, especially given rabies prevalence as a neuroanatomical tracer for the peripheral nervous system [9]. However, moving forward, the use of AAV (6 or 9) would be desired over any rabies vector due to its safety profile.

Table 1. Viral Constructs and Overall Results

			# Injection Sites/Successes					
			Muscle		Nerve		Spinal Cord	
Construct	**Viral Titer (vg/mL)**	**Source**						
AAV6-hSyn-ChR2-eYFP	$1.4x10^{13}$	UNC Vector Core	8	2	5	1	1	1
AAV9-hSyn-Chronos-GFP	$3.1x10^{13}$	UNC Vector Core	6	0	0	0	1	1
SAD-deltaG-ChR2-YFP	$1x10^{9}$	Schnell Lab	2	0				
N2c-deltaG-ChR2-YFP	10^{8}-10^{10}	Schnell Lab	5	0	5	0		

Yellow boxes highlight instances of success.

The use of dexamethasone as an immunosuppressant is highlighted in Table 2. Again, it did not appear to have a meaningful impact on successful transduction given the low overall number of successes with or without it. This lack of transduction enhancement could be confounded by the previously mentioned possibility that the source/quality of our virus was less than optimal. The short-term transduction and long-term expression effects of immune suppression may not be readily apparent until we use a virus that achieves some significant baseline level of success over what we have seen in these experiments.

Table 2. Success Rates with Dexamethasone Immunosuppression

	# Injection Sites (Successes)					
	Muscle		Nerve		Spinal Cord	
Construct	**Dex**	**No Dex**	**Dex**	**No Dex**	**Dex**	**No Dex**
AAV6-hSyn-ChR2-eYFP	7 (2)	1 (0)	4 (1)	1 (0)		1 (1)
AAV9-hSyn-Chronos-	4 (0)	2 (0)				1 (1)

GFP						
SAD-deltaG-ChR2-YFP	2 (0)					
N2c-deltaG-ChR2-YFP	4 (0)	1 (0)	4 (0)	1 (0)		

Yellow boxes highlight instances of success.

During our rat experiments, we also had the opportunity to observe successful expression of both ChR2, which has been the typical opsin used in similar peripheral motor experiments to date, and Chronos, which has faster kinetics and higher sensitivities that may be beneficial to peripheral motor applications [4]. With a pair of spinal cord injection experiments, we were able to compare the functional sensitivity and frequency responses of these opsins as shown in Figures 7 and 8, respectively. As seen from these figures, Chronos does appear to hold higher sensitivity to low levels of blue light (472 nm) as well as better tracking of higher frequency pulse trains to elicit functional muscle activations than ChR2. Although these experiments contain a low sample from which to draw firm conclusions, they do suggest that Chronos is worth exploring further for use in FOS applications.

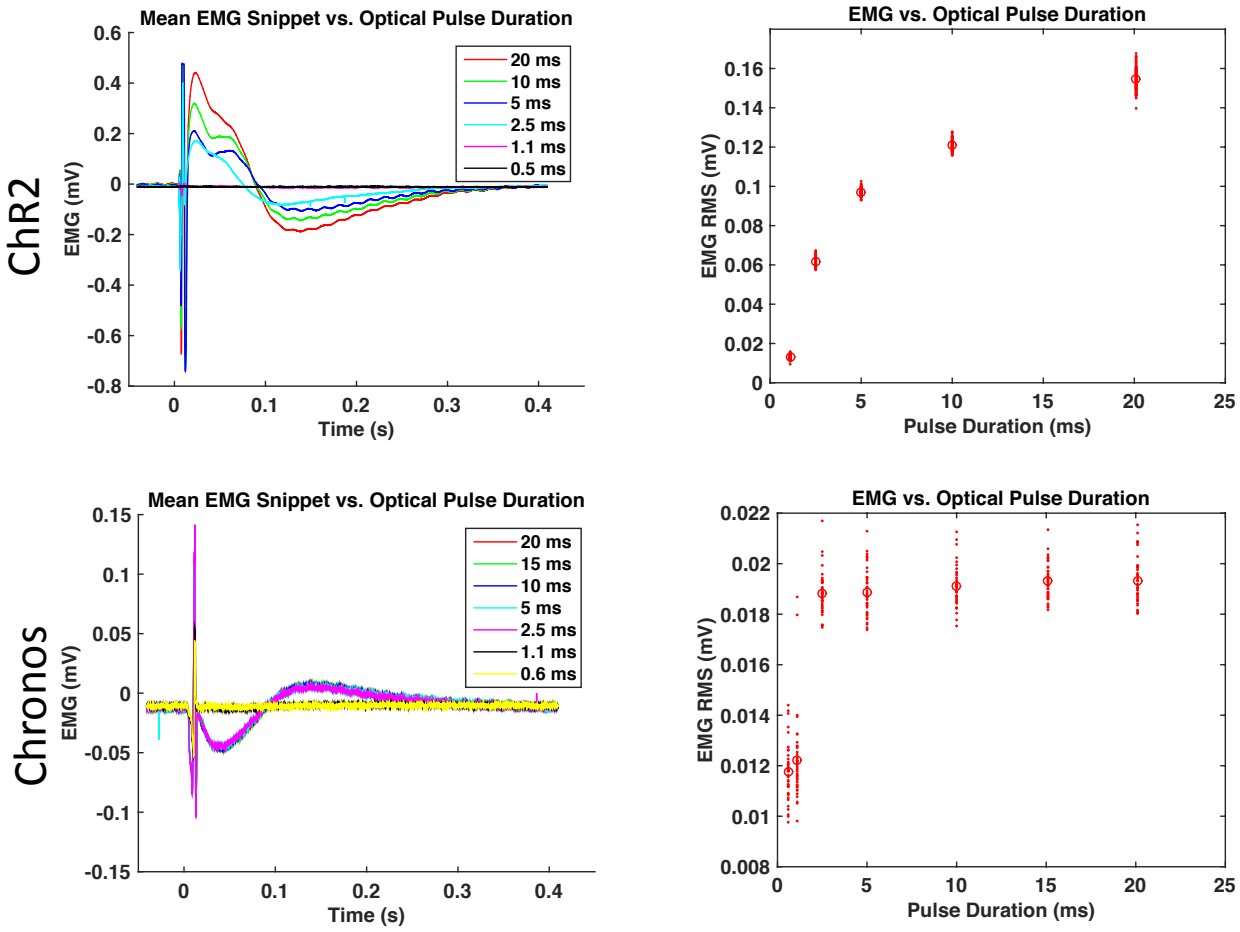


Figure 7. Opsin sensitivity with pulse duration. The optical pulse width of a 472 nm fiber optic laser source was varied from 20 ms to 0.5 ms. The top row depicts the mean EMG waveform and mean EMG rms vs. pulse width for the standard (H134) ChR2 from an AAV6-hSyn-ChR2-eYFP spinal cord injection, while the bottom row depicts the same for the Chronos opsin from a spinal cord injection of AAV9-hSyn-Chronos-GFP. ChR2 demonstrates a gradual increase in elicited EMG amplitude as pulse width increases, while Chronos appears to be much more sensitive to stimulation at lower light levels.

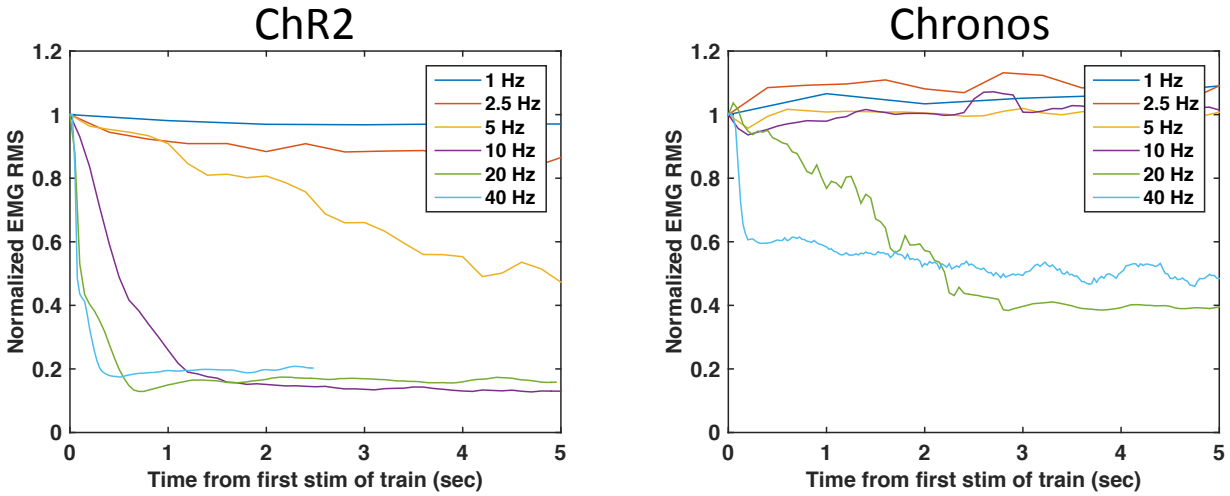


Figure 8. Opsin frequency response. Trains of 10 ms duration pulses were used to stimulate the spinal cord of rats injected with either AAV6-hSyn-ChR2-eYFP (left panel) or AAV9-hSyn-Chronos-GFP (right panel) at pulse frequencies ranging from 1-40 Hz while recording the elicited EMG activity to assess each opsin's functional recovery time constant (i.e. ability to track or "keep up" with stimulation). The amplitude of each stimulus-locked EMG spike was normalized to the amplitude of the first EMG spike in the 10 second train, and the resulting normalized train was plotted as a function of time for each pulse train frequency. ChR2 started to show a decrease in EMG response with pulse-train frequencies above 5 Hz, while Chronos was able to maintain maximal stimulus response up to roughly 20 Hz, while even at higher frequencies strong responses were still present at 40-50%.

Following termination of one of the unsuccessful experimental rats, we sought to test a method to visualize the distribution of neuromuscular junctions (NMJs) in the muscle as these are the likely sites for viral construct entry into the nerve. To visualize NMJs in the tibialis anterior muscle we had previously injected, we first bathed the excised muscle in a solution of alpha-bungarotoxin (α-BTX) conjugated with rhodamine for several minutes, and then washed the muscle with saline. α-BTX irreversibly binds to NMJs while the conjugated rhodamine serves as a fluorescent marker to localize the toxin/NMJ. Following bathing and washing of the muscle, the muscle was imaged under a 2-photon microscope for labelling of the NMJ as shown in Figure 10. The labelling and characteristic structure of the fluorescent objects agreed with past reports of NMJ labelling using this technique [10,11], demonstrating that this would be a viable option for examining spatial density patterns in a given muscle and targeting injections appropriately. In addition, imaging suggested that the most heavily

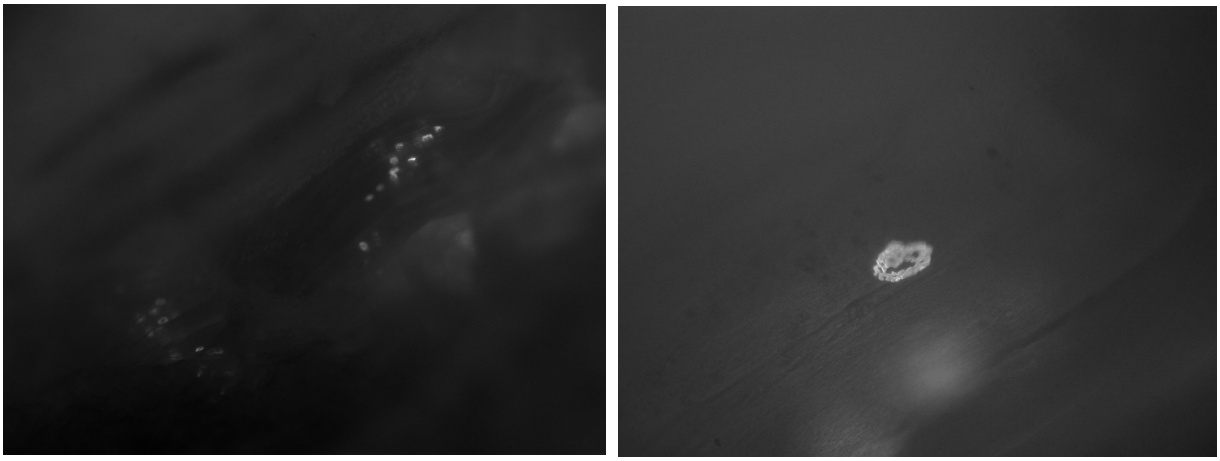


Figure 10. Post-mortem labelling of neuromuscular junction (NMJ) with alpha-bungarotoxin (α-BTX) conjugated with rhodamine. The left panel shows a zoomed out view of the underside of the muscle and the distribution of NMJs, while the right shows a zoomed in view of an individual NMJ with its characteristic structure.

concentrated areas of NMJs were located on the underside of the belly where the nerve could be seen to fan out. This information was used in targeting future injections.

Finally, during the course of these experiments, we designed a micro-LED nerve cuff in conjunction with Draper Laboratories (Cambridge, MA). These cuffs were intended for use in chronic optical stimulation of NHP peripheral nerves transduced with light sensitive opsins. The design is shown in Figure 11. As we did not have any NHP injection experiments that resulted in optically induced muscle contractions, we were unable to test these devices in the targeted animal model. However, we did receive two batches of prototype cuffs that we were able to test for acute stimulation purposes during our rat experiments as shown n Figure 12. During these experiments, the nerve cuffs were shown to be capable of eliciting similar contractions to those stimulated with a fiber optic laser source, but it was obvious that the nerve cuffs were also capable of a larger repertoire of spatial and temporal stimulation patterns. Production of a final batch of nerve cuffs was underway at the time of writing this report.

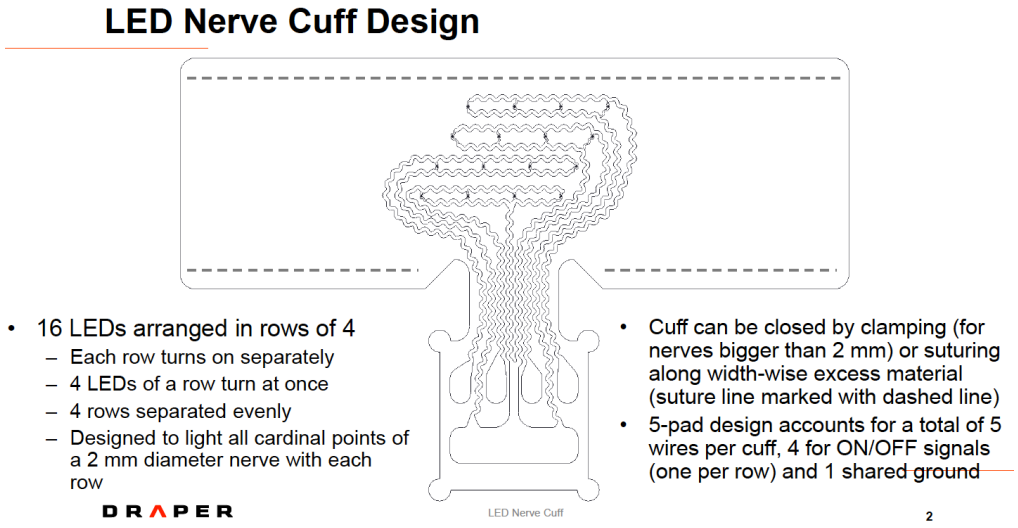


Figure 11. LED Nerve Cuff Design. Schematic with feature descriptions. (Courtesy of Draper)

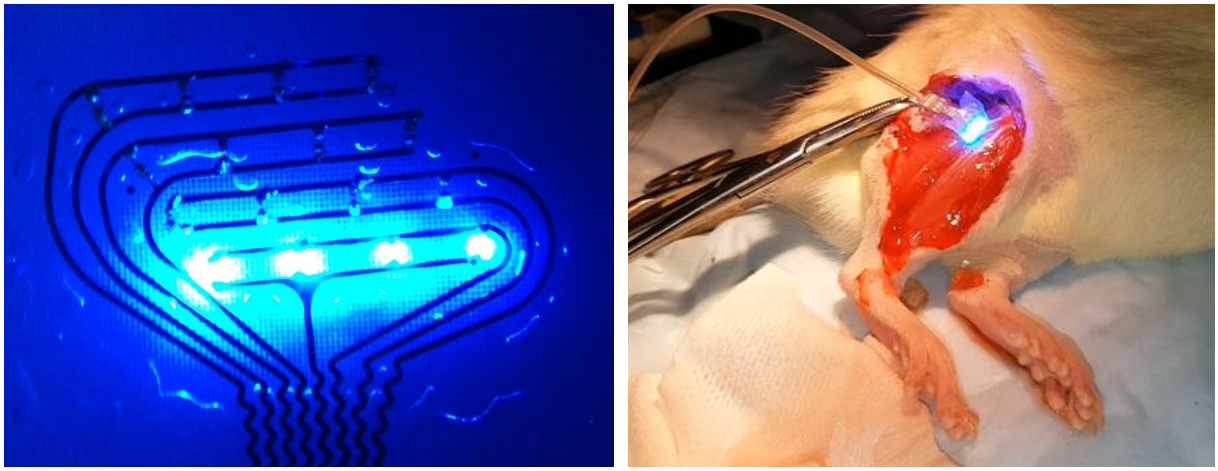


Figure 12. *In vivo* testing of LED nerve cuff. Left: Demonstration of independent lighting of single row of LEDs. Right: The LED nerve cuff (designed for monkey-sized nerves) was placed underneath the exposed rat nerve and rotated such that a row of LEDs was in line with the nerve. Pulsing of this row of LEDs led to brisk leg contractions similar to those elicited by fiber optic stimulation.

Conclusions and Future Directions: Through the course of these experiments, we were unable to address our third aim of incorporating peripheral optogenetic stimulation with our BCI expertise in a NHP model. Early difficulties in obtaining consistent opsin expression in NHPs or even rats made this aim unfeasible for this project period, and forced us to focus on optimizing injection techniques and immunosuppression approaches. However, we do suspect that many of our difficulties may have been beyond our immediate control and instead related to the quality of viral constructs we used. Despite these setbacks, we were able to thoroughly examine and characterize several injection techniques as well as develop chronic stimulation hardware that will likely prove to be very beneficial in future investigations.

We are optimistic that these results and techniques will facilitate the translation of this technology to NHP BCI experiments in the near future. Looking forward, we will first attempt similar experiments in rats with a new virus vendor per recommendations of Dr. Chris Towne. If initial experiments prove to be much more successful, we will proceed to our originally planned NHP experiments to verify the improved virus constructs extend well to NHPs. During these experiments, we may also use our chronic LED cuff designs to monitor the time course of opsin expression during the incubation period by periodically stimulating through an implanted LED nerve cuff. If these NHP experiments prove fruitful, we may then transition to pairing this optical stimulation in upper extremity muscles with brain signals extracted from implanted intracortical arrays to execute basic reaching movements in order to fulfill our originally planned aims.

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