ORDERLY RECRUITMENT OF MOTOR UNITS BY OPTICAL STIMULATION IN TRANSGENIC MICE

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INTRODUCTION

The principle of orderly recruitment states that small fatigue-resistant motor units are recruited before large fatigable motor units [1]. Here we show that it is possible to control motor activity directly with optical stimulation by using a light-sensitive cation channel (channelrhodopsin-2) genetically inserted into the membranes of motor axons that open with millisecond precision and high fidelity in response to blue light (470 nm) [2]. By measuring several indices of motor unit recruitment order, we also show evidence that optical stimulation recruits motor units in their normal physiologic order in transgenic mice. This technology could become the first viable alternative to electrical stimulation in limb re-animation projects, and may allow the possibility of fine motor control and fatigue resistance with optically-based prosthetics.

METHODS

We implanted LED-based optical or bipolar electrical cuffs around the exposed sciatic nerves of anesthetized transgenic mice (Thy1-ChR2). The mouse's body temperature was maintained with an isothermal pad and heat lamp. The sciatic nerve was severed proximal to the cuff prior to experimentation and kept moist with mammalian Ringer's solution. We simultaneously recorded stimulation intensities, measured by rectified integrated EMG, in both lateral gastrocnemius and soleus during single muscle twitches elicited by both electrical and optical stimulation. Optical power at the surface of the peripheral nerve was estimated with the Kubelka-Munk model [3]. The number and size of myelinated motor axons innervating each individual muscle were measured using confocal microscopy in a cross-section of sciatic nerve by use of a retrograde dye (fast blue) injected into each muscle.

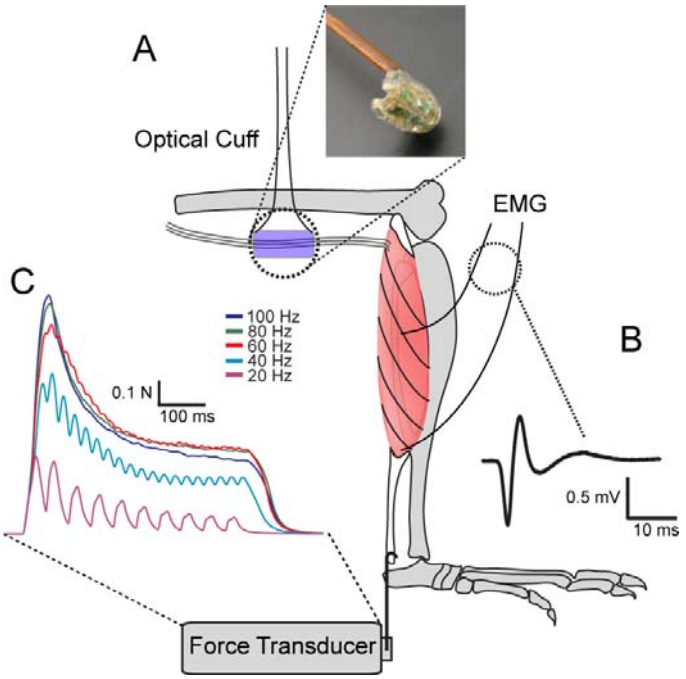

Figure 1: Optical stimulation setup. **A.** An LED-based optical or electrical cuff is implanted around the sciatic nerve of an anesthetized transgenic (Thy1-ChR2) mouse. The optical cuff is 4x2 mm and contains 16 LEDs (468 nm) arranged in a concentric perimeter facing the peripheral nerve in the center, an example is shown. **B.** Fine wire differential EMG are inserted into the muscle belly and near the muscle-tendon junction to record the electrical response of the subject muscle(s). A sample EMG induced by optical stimulation is shown; note the absence of an electrical stimulation artifact. **C.** A force transducer attached to the Achilles tendon by a thin steel hook records the contractile response of the muscle(s). Muscles not being recorded are cut from their attachment to the Achilles tendon. Samples of contractile responses from the medial gastrocnemius using varying frequencies of optical stimulation are displayed.

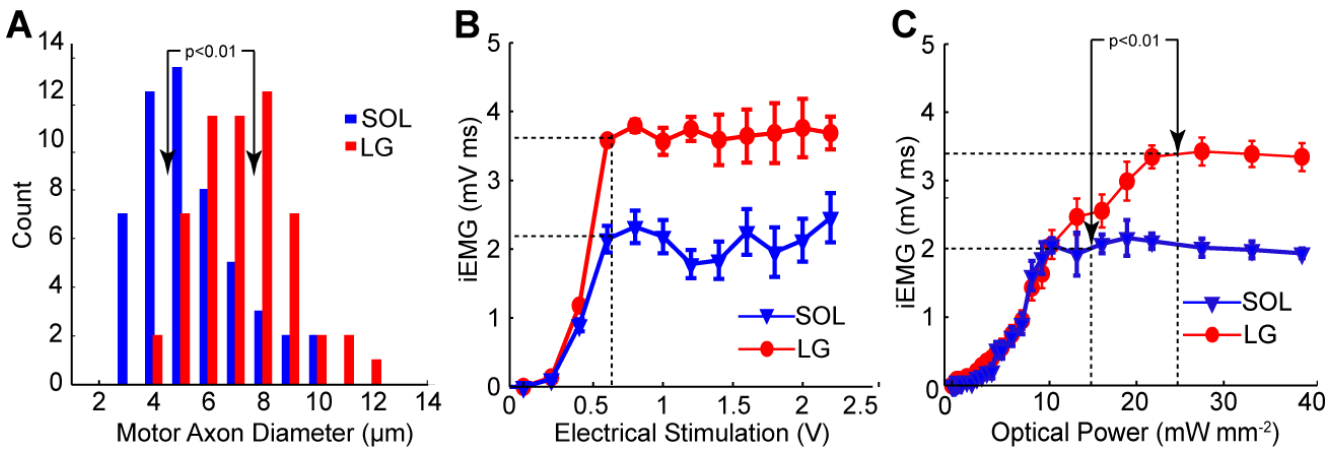

Figure 2: Evidence of orderly recruitment. **A.** Soleus muscle contains an equivalent number of motor units, but has on average significantly smaller motor axons than lateral gastrocnemius. This implies that, on average, soleus contains smaller motor units than lateral gastrocnemius. **B.** Using electrical stimulation both lateral gastrocnemius and soleus reach maximal stimulation at the same electrical stimulation intensity (~0.6 V). **C.** Using optical stimulation, however, soleus reaches maximal stimulation at a significantly lower value of optical stimulation intensity (14 mW mm^{-2}) than lateral gastrocnemius (22 mW mm^{-2}). Since soleus has smaller motor units than lateral gastrocnemius; smaller motor units are recruited at a lower optical power, which is consistent with orderly recruitment.

RESULTS AND DISCUSSION

Our experiments demonstrated the feasibility of using optical stimulation to control skeletal muscle activity. We show the ability to elicit single muscle twitches and tetani, without an electrical stimulation artifact on EMG. The peak muscle force evoked by optical stimulation is comparable to that by electrical stimulation; and is not due to heat or electric field effects produced by the optical cuff as demonstrated in non-transgenic control animals (results not shown).

Our results provide evidence of orderly recruitment by optical stimulation in Thy1-ChR2 mice. We have found that mouse soleus is innervated by equivalent numbers of motor units that are significantly smaller than those innervating lateral gastrocnemius (Figure 2A). Also, we have found that soleus reaches maximal excitation at a significantly lower value of optical power (Figure 2C). This evidence suggests that smaller motor units are recruited prior to larger motor units with increasing optical stimulation intensity which is consistent with orderly recruitment. We found no evidence of orderly recruitment using electrical stimulation (Figure 2B).

Electrical stimulation is generally non-optimal for limb re-animation projects because it recruits large volumes of fatigable muscle. For the first time, we demonstrate an alternative to electrical stimulation that possibly recruits motor units in the normal physiological order. Also unlike electrical stimulation, the use of a transgenic channel allows for specific cell-type targeting and alteration of the channel properties (i.e. kinetics, light sensitivity) for unprecedented control of muscle activity.

REFERENCES

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