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Motor neuron target selectivity and survival after prolonged axotomy

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Abstract

Purpose—After a cut peripheral nerve is repaired, motor neurons usually regenerate across the lesion site, however they often enter an inappropriate Schwann cell tube and may be directed to an inappropriate target organ such as skin, resulting in continued loss of function. In fact, only about 10% of adults who receive a peripheral nerve repair display full functional recovery. The reasons for this are many and complex, however one aspect is whether the motor neuron has undergone a prolonged period of axotomy prior to nerve repair. Previous studies have suggested a deleterious effect of prolonged axotomy.

Methods—We examined the influence of prolonged axotomy on target selectivity using a cross-reinnervation model of rat obturator motor neurons regrowing into the distal femoral nerve, with its normal bifurcating pathways to muscle and skin.

Results—Surprisingly, we found that a prolonged period of axotomy resulted in an increase in motor neuron regeneration accuracy. In addition, we found that regeneration accuracy could be increased even further by a simple surgical manipulation of the distal terminal nerve pathway to skin.

Conclusions—These results suggest that under certain conditions prolonged axotomy may not be detrimental to the final accuracy of motor neuron regeneration and highlight that a simple manipulation of terminal nerve pathways may be one approach to increase such regeneration accuracy.

Keywords

Pathway choice; obturator nerve; femoral nerve; regeneration; delayed repair

1. Introduction

Clinically, many types of traumatic peripheral nerve injury can be successfully repaired surgically, but these repairs often involve a considerable delay between the time of insult and the repair. For the injured motor system in the meantime, motor neuron axons in the proximal portion of the nerve are blocked from growing back to their target end-organs, and the axons in the distal portion of the nerve begin to undergo Wallerian degeneration. The

concept of *prolonged axotomy* refers to the neuronal cell bodies that maintain projections to the portion of the nerve proximal to the injury, and *prolonged denervation* refers to the portion of the nerve and its target organs distal to the injury, e.g. muscle and skin (see Gordon et al., 2011 for review). *Delayed repair* bridges these two concepts and refers to the period of delay prior to the surgical rejoining of the proximal and distal nerve stumps.

Previous studies in the rat have suggested that there is a deleterious influence of prolonged axotomy on motor neurons both in terms of survival and their ability to functionally innervate muscle. Such an effect is in addition to the observation that some motor neurons may degenerate even if an immediate nerve repair is carried out. For instance, an elegant study by Boyd and Gordon (2002) suggested that there was a 43% drop out of tibial motor neurons after axotomy and immediate repair, which then increased to 62% if there was a two month prolonged axotomy. Numerous other studies have also suggested a loss of motor neurons and functional motor units ranging from 16% to even 90% following prolonged axotomy (Snider & Thanedar, 1989; Fu & Gordon, 1995; Sulaiman et al., 2002; Brenner et al., 2004; Furey et al., 2007; Gordon et al., 2011). However, there is an important caveat to all of this previous work. Xu et al. (2010) recently highlighted the distinction between motor neuron drop out due to neuronal death, versus the apparent drop out due to a motor neuron's failure to regenerate into the distal nerve stump. Their study addressed this issue by labeling femoral motor neurons both before and after axotomy, and demonstrated that all of the motor neurons survived two months after prolonged axotomy, but only half of them regenerated axons into the distal nerve stump.

None of this previous work has addressed the important question of the effect of prolonged axotomy on the accuracy of those motor neurons that do regenerate. We and others have used the rodent femoral nerve as a model to examine the regeneration accuracy of motor neurons at the level of a mixed peripheral nerve (for review, see Madison et al., 2007). In this model, both motor and cutaneous axons are represented randomly in the proximal trunk of the parent nerve, but each modality becomes anatomically segregated distally into a muscle branch to the quadriceps muscle, and a cutaneous branch (the saphenous nerve) that innervates skin. Following a lesion to the proximal nerve trunk, regenerating motor neuron axons must select between terminal nerve pathways to muscle or cutaneous end-organ targets. Several different laboratories have shown a preference for regenerating femoral motor neurons to project to the terminal nerve branch to muscle as opposed to skin (Madison et al., 1996; Al-Majed et al., 2000; Robinson & Madison, 2005; Eberhardt et al., 2006; Uschold et al., 2007). However, there has been some question as to whether this tendency is limited to the original femoral motor neuron pool or is a general preference for other foreign motor neuron pools as well, for instance obturator motor neurons (see Franz et al., 2005; 2008 and Robinson & Madison, 2012). In addition, there have been no previous studies that have examined the influence, if any, of prolonged axotomy on the ability of a foreign motor neuron pool to differentiate between a terminal nerve branch to muscle as opposed to skin. The experiments here address both of these unanswered questions, and show that under certain conditions prolonged axotomy may not be detrimental to the final accuracy of motor neuron regeneration and highlight the finding that simple manipulations of terminal nerve pathways may be one approach to increase such regeneration accuracy for both the original motor neuron pool as well as a foreign motor neuron pool.

2. Materials and methods

All rodent procedures were approved by the Veterans Affairs Medical Center animal use committee. Adult female Sprague-Dawley rats (100–150 g, Charles River) were used. Rats were housed on a standard 12 hr light/dark cycle and fed *ad libitum*. Surgical procedures were carried out under deep anesthesia using intraperitoneal administration of ketamine,

xylazine and acepromazine (as a combined solution containing 100, 6 and 1 mg/kg respectively) in normal (0.9%) saline. The fibrin sealant used in the present work for nerve repair was prepared according to the manufacturer's instructions (Tisseel, Baxter Healthcare Products, Glendale, CA).

2.1. Surgical manipulations (Fig. 1)

For the “*Obturator to Femoral Nerve*” preparation ($N=8$, Fig. 1A), the inguinal area skin was clipped and cleaned with 70% ethyl alcohol and 5% povidoneiodine. The parent femoral nerve was exposed by blunt dissection and transected with microscissors above the level of the iliacus branch, a region where axons to the two terminal nerve branches are randomly distributed (Brushart, 1988). The small terminal branches of the iliacus and pectineus nerves were located where they exited from the parent femoral nerve. A permanent ligation was placed around each of these tributary nerves and their proximal segments were then trimmed to be flush with the parent femoral nerve. The distal femoral nerve was then gently translocated approximately 1 cm away from the ligated proximal femoral nerve in order to be aligned with the obturator nerve. The proximal portion of the femoral nerve was then ligated with 7-O silk suture (Ethicon, Somerville, NJ) to prevent regeneration. The obturator nerve, just medial to the parent femoral nerve, was also exposed by blunt dissection and transected with microscissors. The distal portion of the obturator nerve was also ligated. The proximal portion of the obturator nerve was then connected to the distal portion of the parent femoral nerve. After a small amount of fibrin sealer was placed at the apposition site, activator was applied to complete the sealant repair. The site was then closed, the skin sutured with 4-0 Vicryl (Ethicon Inc, Somerville, NJ), and the animal allowed to recover.

For the “*Delayed Obturator to Femoral Nerve*” preparation ($N=7$, Fig. 1B), the obturator nerve was exposed by blunt dissection via an inguinal approach and transected with microscissors. Both the proximal and distal portions of the nerve were then ligated with 7-O silk suture. The site was then closed, sutured and the rat allowed to recover. Eight weeks later, the ligature on the proximal end of the obturator nerve was removed, and the nerve end refreshed with microscissors in anticipation of the cross-repair. The parent femoral nerve was also exposed and transected with microscissors. After ligating the proximal portion of the femoral nerve, the cross-repair was made between the proximal obturator and the distal femoral nerves using fibrin glue. The site was then closed, the skin sutured, and the rat allowed to recover.

For the “*Obturator to Femoral + Shortened Cutaneous*” preparation ($N=8$, Fig. 1C), the proximal obturator nerve was attached to the distal femoral nerve as above, but in addition, the terminal cutaneous branch of the femoral nerve (normally much longer than the muscle branch) was transected at a point where its length was equal to that of the intact muscle branch to the quadriceps muscle. Thus, both terminal branches became equal in length. Both the proximal and distal ends of the cut cutaneous branch were then ligated with 7-O silk suture. The site was then closed, sutured, and the rat allowed to recover.

For the “*Delayed Obturator to Femoral + Shortened Cutaneous*” preparation ($N=6$, Fig. 1D), the obturator nerve was transected and ligated as above, but in addition, during the cross-repair procedure with the femoral nerve eight weeks later, the femoral cutaneous branch was transected at a point where its length was equal to that of the intact muscle branch to the quadriceps muscle. Both the proximal and distal ends of the cut cutaneous branch were then ligated with silk suture. The site was then closed, sutured, and the rat allowed to recover.

2.2. Total motor neuron counts and determination of terminal nerve pathway preference

The number of obturator motor neurons projecting into either the terminal muscle or cutaneous branch of the cross-repaired femoral nerve was determined eight weeks after the cross-repair in the four surgical groups (Fig. 1). The terminal branches of the femoral nerve were re-exposed and separated from each other by food-grade silicone grease dams. They were then trimmed to ~3 mm distal to the normal femoral nerve bifurcation, and randomly assigned to receive crystals of either Alexafluor 488 dextran (D-22910, Molecular Probes, Eugene, OR, USA) or Alexafluor 594 dextran (D-22913, Molecular Probes). After crystal application, each branch was blotted and sealed with silicone grease. The surgical site was closed, sutured, and the rat allowed to recover. Three days later, each rat received an overdose of anesthetic and was perfused through the heart with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in PBS. The lumbar spinal cord was removed, post-fixed for several hours, and sucrose protected overnight. The spinal cord was frozen on dry ice and sectioned with a cryostat. Serial 25- μ m frozen sections were thawed in PBS, mounted onto glass slides, air dried and coverslipped using Prolong (P-36934, Molecular Probes). Retrogradely labeled obturator motor neurons containing a nucleus were identified using a composite filter set that allowed simultaneous visualization of both labels (#51006, Chroma Technology, Brattleboro, VT, USA) in a fluorescence-equipped Zeiss Axiophot microscope, at 250 $\times$ magnification. Motor neuron counts were carried out by blinded independent observers and scored as either single-labeled (488 or 594 dextran only) or double-labeled (both 488 and 594 dextrans). Motor neurons labeled only from the muscle branch, only from the cutaneous branch or from both branches were tabulated and their counts corrected for split cells (Abercrombie, 1946). In seven control rats the obturator nerve was exposed as described above and transected. After creating a small well around the cut proximal nerve using silicone grease, crystals of either Alexafluor 488 dextran or Alexafluor 594 dextran were applied as described above. The surgical site was closed in layers, sutured, and the rat allowed to recover. Three days later the rats were perfused as above and the spinal cords processed for visualization and counting of the labeled obturator motor neurons. Because our data involve quantifying retrogradely labeled motor neurons we consistently refer to regeneration choices made by the motor neurons themselves rather than their axons which we have not attempted to quantify.

A regeneration pathway preference index for each animal was determined by dividing the number of retrogradely labeled motor neurons reinnervating only one terminal nerve pathway by the total number of labeled motor neurons and expressing the quotient as a percentage. Thus, % *muscle pathway preference* was determined by dividing the number of motor neurons labeled from only the muscle pathway by the total number of labeled motor neurons; % *cutaneous pathway preference* was determined in an identical fashion using the number of motor neurons labeled from only the cutaneous pathway as the numerator. Combining these two percentages for each animal is always less than 100% because the number of double-labeled neurons was included in the denominator for each pathway preference (i.e., as part of the total number of labeled cells) but not in the numerator.

2.3. Statistics

Analysis of variance (ANOVA) was used to determine differences among groups. Student-Newman-Keuls *post-hoc* comparisons for paired data were used within groups to compare the number of motor neurons labeled from the muscle and cutaneous branches and pathway preferences. Differences were considered statistically significant when $F < 0.05$ and $p < 0.05$.

3. Results

3.1. Obturator motor neuron target selectivity

Eight weeks after the surgical cross-repairs shown in Fig. 1, terminal nerve pathway preferences for obturator motor neurons were determined by retrogradely labeling and then counting the regenerated motor neurons that projected into the terminal nerve branch to muscle or the terminal nerve branch to skin or into both. The *Obturator to Femoral* group, representing an immediate cross-repair (Fig. 2, first panel), showed a trend to preferentially project to the terminal muscle branch, but was not statistically significant (147 ± 34 for muscle versus 106 ± 57 for cutaneous; mean $\pm$ SD; $p = 0.09$). However, the same cross-repair preparation preceded by 2 months of prolonged axotomy (Fig. 2, second panel) showed a significant preference for the muscle pathway (188 ± 53 for muscle versus 111 ± 45 for cutaneous; mean $\pm$ SD, $p = 0.02$). In both the additional cross-repair groups with a shortened cutaneous pathway obturator motor neurons showed a significant preference for the terminal pathway to muscle in the immediate cross-repair group (Fig. 2, third panel, 228 ± 60 versus 77 ± 31 , mean $\pm$ SD, $p = 0.0001$) as well as after prolonged axotomy (Fig. 2, fourth panel, 235 ± 53 versus 100 ± 22 , mean $\pm$ SD, $p = 0.0007$).

3.2. Terminal nerve pathway preferences

We further examined regenerated motor neurons by analyzing the normalized pathway preference index as described in Materials and Methods. Within-group analyses indicated that there was no difference between the muscle and cutaneous pathway preferences for the *Obturator to Femoral* group (Fig. 3, left-most pair; $p = 0.08$), whereas all three of the other surgical groups showed significant preferences for the muscle pathway ($p = 0.04$, 0.0001 , and 0.001 respectively). Comparisons across surgical groups indicated that the cutaneous pathway preferences were not significantly different from each other ($F = 1.69$, $p = 0.19$). Conversely, muscle pathway preference did show significant differences across groups ($F = 8.82$, $p = 0.0004$). *Posthoc* comparisons indicated that the shortened cutaneous groups, either with or without prolonged axotomy, significantly increased preferential projections to the terminal muscle branch compared to the first two groups with intact cutaneous terminal nerve branches ($p < 0.02$).

3.3. Number of retrogradely labeled motor neurons

Total obturator motor neuron counts for the four experimental groups and for the naive control group ($N = 7$) are presented in Table 1. These counts represent totals of retrogradely labeled motor neurons after two months of cross-repair. There were no significant differences in the number of retrogradely labeled obturator motor neurons among the experimental groups, or when compared to control counts ($F = 0.96$; $p = 0.44$).

4. Discussion

The experiments reported here are the first to examine the effect of *prolonged axotomy* on the ability of a foreign motor neuron pool to preferentially project to a terminal nerve branch to muscle as opposed to skin. We also examined the role of a simple surgical manipulation of the terminal nerve pathways in this choice paradigm to assess any influence on such motor neuron regeneration accuracy.

4.1. Conditioning lesion versus prolonged axotomy

The ability of a prior nerve lesion to significantly enhance axon regeneration after a second lesion of the same nerve some time later is a phenomenon that has been known for some time. The first lesion is referred to as the conditioning lesion and the second lesion is known as the test lesion. Measurements of axon regeneration are then made after the test lesion.

Enhanced regeneration due to a conditioning lesion is well established (McQuarrie, 1978) and has been shown to be effective when the conditioning lesion is given as long as 21 days prior to the test lesion (Jacob & McQuarrie, 1993). With regard to the femoral nerve model of regeneration accuracy, Brushart et al. (1998) reported that conditioning lesions of the terminal nerve pathways did not increase regeneration accuracy of a later test lesion of the parent femoral nerve when measured either three weeks or three months later. Both conditioned and non-conditioned femoral motor neurons preferentially projected to the terminal muscle pathway to the same extent. Interestingly however, if the conditioning lesion was made just proximal to the subsequent test lesion of the parent femoral nerve there was a significant increase in preferential projections to the terminal muscle branch at three weeks that then plateaued to become similar to that seen with an immediate femoral nerve repair. It was also noted in that comprehensive study that a conditioning lesion of the proximal parent femoral nerve, but not the distal terminal nerve branches, resulted in an increase in the number of regenerating axon collaterals at the repair site. Brushart interpreted these findings as suggesting that increasing axon collateral formation increased subsequent motor neuron regeneration accuracy, but simply conditioning the motor neurons by crushes of the terminal nerve branches did not increase such accuracy.

In the present experiments after immediate cross-repair of the proximal obturator nerve to the distal femoral nerve we found no terminal nerve pathway preference, although there was a slight trend for obturator motor neurons to project to the terminal muscle branch (Fig. 2, left panel). This lack of a regeneration preference is the same result as was first observed by Franz et al. (2008) for the obturator motor neuron pool in the mouse. This lack of preferential projections by obturator motor neurons to the terminal muscle branch is different from the robust terminal muscle branch preference that is seen with femoral motor neurons after a similar repair (e.g., Brushart, 1988; 1990), and suggests that possible inherent molecular differences between motor neuron pools may modulate their ability to distinguish terminal nerve branches.

We next investigated the effect of a two month period of axotomy/ligation before the obturator to femoral cross-repair (*Delayed Obturator to Femoral*, Fig. 2) and found that this intervention now resulted in a significant preference for obturator motor neurons to project to the terminal nerve branch to muscle ($p = 0.02$). It is interesting to speculate that the two month prolonged axotomy acted like a conditioning lesion that enhanced the ability of the foreign obturator motor neurons to preferentially project to the terminal muscle branch. Thus there may be interplay between the well-known negative consequences of prolonged axotomy and the beneficial effects of a conditioning lesion. When preferential projections are already robust, such as that seen for the femoral motor neuron pool following simple femoral nerve repair, the effect of adding a conditioning lesion may not be obvious (such as the lack of effect seen by Brushart, 1998). However when preferential projections are just on the borderline, such as that seen by a foreign motor neuron pool projecting to the distal femoral nerve, then a conditioning lesion may enhance the ability of the foreign motor neuron pool to correctly discriminate the terminal nerve pathways to muscle versus skin.

4.2. Manipulations to terminal nerve pathways enhance regeneration accuracy

We next examined the effect of surgically altering the cutaneous terminal nerve pathway such that it would be an equivalent length as that of the terminal muscle branch; a procedure that we have previously shown increases the regeneration accuracy of femoral motor neurons (Robinson & Madison, 2004; 2005; Uschold et al., 2007; Madison et al., 2009). We found that such a manipulation also increased the ability of foreign motor neurons (obturator motor pool) to preferentially project to the terminal muscle branch whether or not there was also a prolonged axotomy period (*Obturator to Femoral with Shortened Cutaneous* data in Figs. 2 and 3). These results suggest that simple manipulations to the distal environment into

which obturator motor neurons regenerate their axons can by itself enhance the ability of a foreign motor neuron to discriminate between terminal nerve pathways.

4.3. Total number of regenerated motor neurons

Two months of cross-repair with or without prolonged axotomy had no apparent influence on the total number of retrogradely labeled obturator motor neurons (Table 1). Previous rat studies using prolonged axotomy models have reported 24% and 38% survival of tibial nerve motor neurons with a two month delay (Boyd & Gordon, 2002; Sulaiman et al., 2002), 56% survival of tibial nerve motor neurons with a one week delay (Brenner et al., 2004), and up to 14% survival of femoral motor neurons with a two month delay (Furey et al., 2007). Recent work has also highlighted the potential disparity between motor neurons that may survive prolonged axotomy, but fail to regenerate or be retrogradely labeled. Xu et al. (2010) prelabeled rat femoral motor neurons with Fast Blue in combination with two months of prolonged axotomy. Six weeks after cross-repair and labeling of regenerating axons, neuron counts revealed that all of the femoral motor neurons survived, but only half of them were retrogradely labeled from the new nerve. The disparity between the present study of obturator motor neurons and these other reports could be due to intrinsic differences between the motor neuron pools contributing to these different nerves. However we think it is more likely to be due to use of fibrin glue versus suture repair, an approach we have previously shown to be beneficial in terms of being able to demonstrate preferential projections in the mouse with its smaller and more difficult-to-repair nerves (Robinson & Madison, 2003).

4.4. Evidence for trophomorphism

The general conclusion from all these studies is that through their regrowing axons, motor neurons show a regeneration preference for the nerve pathway that provides the greatest relative level of trophic support rather than responding to one or more pathway-specific molecular markers. This extension of trophomorphism (Crutcher & Saffran, 1990; Saffran & Crutcher, 1990) and sibling neurite bias (Smalheiser & Crain, 1984) is a simple and intuitive way to understand the complex dynamics of the nerve repair environment where degenerative, regenerative and inflammatory influences intersect. Trophic support in this context is interpreted very broadly to include anything that impacts neuronal survival such as growth factors, structural/metabolic precursors and blood supply. While there may be multiple cellular and circulation-based sources of such trophic support, within the context of the current work we interpret the evidence to indicate that the primary sources for such support are muscle as an end-organ, Schwann cells, and possible end-organ/Schwann cell interactions. As such (see Robinson and Madison, 2009 and Madison et al., 2009 for review) these general concepts can both explain and predict the outcome of selective targeting experiments by understanding the generalized trophic requirements for axonal regeneration after a peripheral nerve is cut and repaired. The present experiments suggest that obturator motor neurons are also subject to trophomorphism and sibling neurite bias based on intraneuronal competition for limited amounts of trophic support. This trophomorphic understanding of neuronal form also predicts that manipulations that increase the disparity between competing pathways will result in more robust selectivity. Conversely, the greatest degree of selective uncertainty for a regenerating motor axon would be predicted under circumstances where the pathways are most similar. For foreign obturator motor neurons regrowing axons into the distal femoral nerve after a simple cross-repair, we interpret the lack of selective targeting to be a reflection of an insufficient distinction between the two terminal pathways, compared to results with the original femoral motor neuron pool. However, the disparity between the two terminal pathways can be increased by shortening the longer cutaneous pathway (the saphenous nerve) to the same length as the quadriceps muscle branch (thereby reducing the number of Schwann cells in the cutaneous pathway and

any influence of skin), and then obturator motor neurons display a strong preference to reinnervate the pathway to muscle.

In conclusion, the data suggest that the target selectivity by motor neurons can be enhanced both by prolonged axotomy and by reducing the influence of pathway competition. We suggest that a sustained conditioning influence on the motor neuron combined with the relative trophic support from muscle and Schwann cells can account for this selective targeting; a mechanism that is distinctly different from the suggestion that inherent molecular differences between Schwann cells residing in the muscle and cutaneous branches (Brushart, 1993; Brushart et al., 1998; Höke et al., 2006), or inherent molecular differences of the motor neuron pools themselves govern motor neuron regeneration accuracy (Franz et al., 2005; 2008). We further suggest that if future therapeutic interventions to increase motor neuron target selectivity are to be successful they will have to take into account the idea of trophomorphism and the intraneuronal competition for limited amounts of trophic support.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Surgical overview of the experiments. In the *Obturator to Femoral Nerve* preparation (**A**), obturator motor neuron axons were given a choice to regrow into the muscle or cutaneous pathway of the femoral nerve (a cross-repair) for two months before retrograde labeling was used to determine pathway preference. Examples of such single (green or red) and double-labeled (green and red appear yellow) motor neurons are shown (photomicrograph at right, scale bar = 50 μ m). In the *Delayed Obturator to Femoral* nerve preparation (**B**), obturator motor neurons were axotomized/ligated for two months prior to the cross-repair to the femoral nerve. In the *Obturator to Femoral Nerve with Shortened Cutaneous* preparation (**C**), obturator motor neuron axons were given the same pathway choices for two months as in (**A**), but the additional trophic influences of the longer cutaneous pathway connected to skin were removed during that time. In the *Delayed Obturator to Femoral with Shortened Cutaneous* preparation (**D**), the cross-repair to the femoral nerve was delayed using axotomy/ligation as in (**B**), but in addition the cutaneous pathway of the femoral nerve was shortened during the two months of obturator motor axon regeneration.

Fig. 2.

Counts of retrogradely labeled obturator motor neurons that projected into the two terminal femoral nerve branches 8 weeks after cross-repair. The *Obturator to Femoral Nerve* group motor neurons showed no significant difference in the number of neurons labeled from the muscle or cutaneous branches of the femoral nerve (no muscle target selectivity). In contrast, all other groups showed significantly more motor neurons projected to the muscle branch than to the cutaneous branch. Bars = mean $\pm$ SD, * = $p < 0.05$, ** = $p < 0.001$. Clock face = delay, M = muscle, S = skin in lower nerve model diagrams.

Fig. 3.

Pathway preference indices for obturator motor neurons with regenerating axons in the femoral nerve 8 weeks after cross repair. For comparisons within each group, the *Obturator to Femoral* group showed no pathway preference (first pair on left). In contrast, a significant increase in the preference for the muscle pathway was observed after prolonged axotomy alone (second pair from left), after reducing the length of the cutaneous pathway alone (third pair from left), and after the combination of prolonged axotomy and the shortened cutaneous pathway (fourth pair from left). Comparing across groups, the cutaneous pathway preferences were not significantly different from each other. However, the muscle pathway preferences in both shortened-cutaneous groups (third and fourth groups from the left) were significantly higher than the intact femoral nerve groups (first and second groups from the left). Circles and squares = mean $\pm$ SD, * = $p < 0.05$, ** = $p < 0.001$, and # = $p < 0.05$. Clock face = delay, M = muscle, S = skin in lower nerve model diagrams.

Table 1

Total obturator motor neuron counts

	Mean $\pm$ SD
Control Obturator	332 $\pm$ 70
Obturator to Femoral	311 $\pm$ 53
Delayed Obturator to Femoral	379 $\pm$ 85
Obturator to Femoral + Shortened Cutaneous	333 $\pm$ 89
Delayed Obturator to Femoral + Shortened Cutaneous	366 $\pm$ 56