degeneration in animal models is unlikely to be the same as in the human condition, and thus cannot provide precise information that would help inform surgical intervention and the timing for adjuvant therapy. Here, we provide novel data about the morphologic changes at the human motor endplate and ensuing degeneration at the NMJ following traumatic nerve injury.

METHODS: IRB approval was obtained so as to permit biopsies from denervated muscles in patients with BPI ranging from complete pre-ganglionic C5-T1 BPI to less severe traumatic injuries. Specimens were processed for immunohistochemistry and visualized with two-photon excitation and confocal microscopy. Motor endplates were labeled with alpha-bungarotoxin, presynaptic vesicles with synaptophysin, and axons with neurofilament. Human muscle samples from multiple timepoints after injury were analyzed along with control specimens from innervated muscles so as to create a temporal sequence of events for human motor endplate degeneration following traumatic nerve injury.

RESULTS: Denervated muscle samples show distinct differences from innervated muscles, including fragmentation and dispersion of acetylcholine receptors. There is also a noted decrease in NMJ volume as seen in 3D reconstruction, and a trend towards plaque endplate morphology. Moreover, comparison of denervated muscles shows signs of temporal degeneration. NMJs from early denervated muscles still show well preserved circular morphology with definite acetylcholine receptors arranged in distinct folding patterns. By one year status post traumatic brachial injury, NMJs begin to present with greater fragmentation. Moreover, synaptic gutters start to fade, and asymmetry in acetylcholine receptor distribution is noted. Interestingly, even after one year of denervation, NMJs were able to retain their overall circular shape.

CONCLUSIONS: This study details the novel and critically important data about the sequence of events involved in human motor endplate degradation after a clearly defined traumatic nerve injury. Surprisingly, human NMJs persist and retain their structures even after the 6-month window of opportunity for meaningful functional recovery has elapsed, which may indicate a limited utility of animal models for traumatic peripheral nerve injuries. This temporal profile highlights the importance of species-specific findings and provides invaluable data that can answer important questions pertaining to the optimal timing of surgical intervention.

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Acellularized Nerve Allografts Guide Axon Regeneration to a Controlled Termination in a Rat Model

Miles J. Bichanich, BS1, Thomas Hong, MS1, Daniel A. Hunter, RA1, Lauren Schellhardt, BA1, Ying Yan, MD, PhD1, Susan E. Mackinnon, MD1, Thomas Davis, PhD2,3, Scott Tintle, MD2,3, Matthew D. Wood, PhD1, Amy M. Moore, MD1

1Washington University in St. Louis School of Medicine, St. Louis, MO, 2USU Walter Reed Department of Surgery, Uniformed Services University, Bethesda, MD, 3Naval Medical Research Center, Regenerative Medicine Department, Silver Spring, MD

PURPOSE: The myriad of existing management approaches to neuroma highlight the difficulty in treating this clinical entity, and no satisfactory approach has been developed. Evaluation of “off-the-shelf” acellular nerve allografts (ANAs) as a means to bridge nerve gaps has shown an unintentional, controlled termination of axonal regrowth within long (>3cm) ANAs. We hypothesized that long ANAs can be beneficially utilized to “cap” injured nerve and guide regenerating axons to a gradual termination effectively neutralizing neuroma formation.

METHODS: Thy1-GFP and Lewis rats were randomized to eight groups which received: 1) nerve transection alone, 2) traction neurectomy, 3) transection and 0.5 cm closed end silicone conduit, 4) transection and 0.5 cm ANA, 5) transection and 2.5 cm ANA, 6) transection and 5.0 cm ANA, 7) transection and proximal nerve crush, or 8) transection, proximal nerve crush and 5.0 cm ANA. In all groups, the distal nerve stump was ligated and the distal nerve turned from the proximal end to remove any trophic influence. The Thy1-GFP rat nerves were serially imaged at 4, 8, and 20 weeks to provide a visual history of regeneration. Lewis rats were sacrificed at 5 and 20 weeks for quantitative nerve histology and IHC. ANOVA with post hoc analysis were performed to evaluate significance (p<0.05).

RESULTS: GFP animals that received transection alone, traction neurectomy, or transection and crush showed
signs of neuroma with chaotic nerve regeneration (multidirectional axonal regrowth confirmed by histology) extending from the proximal stump as early as 4 weeks. At 5 weeks, axons grew through the entirety of the 0.5 cm ANAs, with neuroma formation extending beyond the grafts. In the 2.5 and 5.0 cm ANAs, robust axonal regeneration was demonstrated in the proximal portions of the grafts with a gradual tapering of regeneration as it moved distally, and axons failed to grow beyond the grafts. At 20 weeks, gross visualization of Thy1-GFP labeled axons demonstrates that regeneration dwindles and terminates within 5.0 cm ANAs without neuroma formation. Further histological analysis is ongoing, as are additional 20 week experiments to evaluate controlled termination with histology and IHC.

CONCLUSION: Following nerve transection, long ANA “caps” can be used to control disorganized axonal regrowth, and therefore prevent the formation of a neuroma. As such, the “capping” of a transected nerve with a long ANA is a potential surgical tool in the future of neuroma management. Based upon these results, further studies are underway in a swine model to evaluate the use of ANAs in neuroma prevention in a neuroma model more similar to the human.

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An Animal Model Of Corneal Neurotisation Using The Thy1-GFP+ Rat To Further Investigate How Reinnervation Influences Corneal Health

Joseph Catapano, MD, Jennifer J. Zhang, MD, PhD, Kira Antonyshyn, BSc, Gregory H. Borschel, MD, FACS

University of Toronto, Toronto, ON, Canada

PURPOSE: Patients with corneal anaesthesia develop neurotrophic keratopathy, causing corneal scarring and vision loss. Corneal neurotisation restores sensation but it remains unknown whether donor nerves reinnervating the cornea contain neuromediators that are required for corneal epithelial maintenance and repair. Further investigation of the effect of neurotisation on corneal health requires an animal model in which tissue can be harvested for analysis. Here we describe a rat model of corneal neurotisation.

METHODS: Thy1-GFP+ rats, which express green fluorescent protein in axons, were used to monitor corneal denervation and reinnervation. Parameters for the stereotactic electrocautery of the ophthalmic branch (V1) of cranial nerve V, which innervates the cornea, was determined using serial imaging of whole mount corneas at 1, 2 and 4 weeks to determine optimal parameters for corneal denervation. Blink reflexes were assessed to confirm corneal denervation. After establishing a method of corneal denervation, corneal neurotisation was accomplished using common peroneal (CP) and sural nerve grafts coapted to the contralateral infraorbital nerve. Four corneas were harvested 4 weeks after neurotisation and denervation to determine corneal nerve density and four corneas were retrograde labelled with 4% Fluorogold to determine whether reinnervating axons derived from the contralateral or ipsilateral trigeminal ganglion (TG). Neurotised corneas were compared with uninjured (normal) and denervated (injured) controls. CP and sural grafts were harvested for histomorphometry.

RESULTS: Optimal corneal denervation was achieved by ablating V1 at the stereotactic coordinates (+ 1.5 mm, + 2.0 mm, 10 mm) with 3 W for 60 s. Stereotactic electrocautery of V1 was well tolerated, however injury to the TG resulted in unacceptable morbidity. Corneal neurotisation using CP and sural nerve grafts was successful resulting in a significant increase in corneal axon density. Denervated corneas demonstrated minimal reinnervation after 4 weeks (2301 μm/mm² ± 1347) and reinnervation was restricted to the peripheral stroma. Neurotised corneas exhibited significantly greater corneal nerve density (62872 μm/mm² ± 12400; p < 0.0001), which extended to the central cornea and subbasal layer and was comparable to uninjured (normal) controls (46165 μm/mm² ± 3965). Histomorphometry demonstrated significant growth of myelinated axons across the grafts. Retrograde-labelling of uninjured cornea controls labeled 478 ± 16 neurons in the ipsilateral TG innervating the cornea with no labeled neurons in the contralateral TG. In contrast, labelling of neurotised corneas demonstrated no labeled neurons in the ipsilateral TG (0 ± 0) with a significant number of labelled sensory neurons in the contralateral TG (353 ± 215), suggesting axons reinnervating the cornea after neurotisation derived from the donor grafts and contralateral face.

CONCLUSION: The described animal model of corneal neurotisation is valuable to further investigate how reinnervation of the cornea using foreign donor nerves influences corneal epithelial health, including epithelial healing and protein expression.