Aging Accelerates Degradation of Human Neuromuscular Junction Following Peripheral Nerve Injury

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Introduction: While it is widely recognized that there is a difference in how adult and pediatric patients respond and recover after traumatic nerve injuries, the etiology of this variability in outcomes between young adults and elderly adults remains unclear. Although previous studies have emphasized the detrimental impact of increased age in neural regeneration, no study has focused on the effects of aging on human motor endplate (MEP) stability and target end organ innervation following peripheral nerve injury (PNI). In this study, we present the analysis of human MEPs from PNI patients ranging from 22 to 77 years old.

Methods: After receiving IRB approval, uninjured and denervated muscle biopsies were collected during standard of care surgical procedures for patients post-PNI. The muscle samples underwent tissue clearing using the reagents and protocol described by Muniterfering et al, 2018.[1] Samples were fixed in 4% PFA and washed with PBS before immersion in CUBIC R1 solution (urea, quadrol, triton-x) at 37°C on a rotating mixer for 1.5-2 weeks. CUBIC solution was changed every 2 days. After incubation in the CUBIC solution, the tissues turned clear or light yellow. The tissues were then washed overnight in a CUBIC IHC buffer (bovine serum albumin, triton-x, sodium azide) before the immunostaining process began. Samples were stained for acetylcholine receptor-α (1:500;Novus Biologicals), neurofilament (1:750;Biolegend), and synaptophysin (1:300;Abcam). Z-stack images of motor endplates were collected using the Keyence BZ-X810 inverted fluorescence phase contrast microscope at 20x magnification. The data was divided into young (< 60 years old) and elderly (> 60 years old) groups. MEP morphology (pretzel, intermediate, plaque) was assessed according to architecture complexity, compactness, and number of perforations. Innervation status (innervated, retracted nerve, and denervated) of the MEPs were also assessed.

Results: Preliminary analysis of the MEPs revealed no significant difference in the percentage of healthy (pretzel) and unhealthy (intermediate and plaque) morphology between the two groups. The samples obtained from the young group showed an average of 13.50% healthy MEPs and 86.50% unhealthy MEPs while the samples from the elderly group revealed an average of 18.33% healthy MEPs and 81.67% unhealthy MEPs. Regarding MEP innervation, the young group muscle samples revealed an average of 57.75% innervated MEPs, while the elderly group muscle samples revealed an average of 29.70% innervated MEPs.

Discussion: Data from our study revealed MEP survival in all samples from both age groups, even in samples obtained from patients who were biopsied greater than 6 months from injury. Remarkably, there was an observed two-fold increase in the percentage of innervated MEPs in the young group (57.75%) compared to the elderly group (29.17%). This maintenance of MEP innervation indicates an increased likelihood of prolonged MEP survivability in the young group, therefore, suggesting improved functional outcomes from reinnervation surgery.

Significance/Clinical Relevance: Aging has been linked to the decreased rate of neural regeneration; however, our study suggests that it also plays a role in accelerating the rate of neuromuscular junction degradation following injury. By increasing our understanding of the mechanisms that contribute to increased degeneration of MEPs as we age, we can potentially find therapeutic targets that can lead to prolonged MEP survival and improved prognosis for patients with PNI.

References:

1. Muntifering, M., Castranova, D., Gibson, G. A., Meyer, E., Kofron, M., & Watson, A. M. (2018). Clearing for deep tissue imaging. Current Protocols in Cytometry, 86, e38. doi: 10.1002/cpcy.38

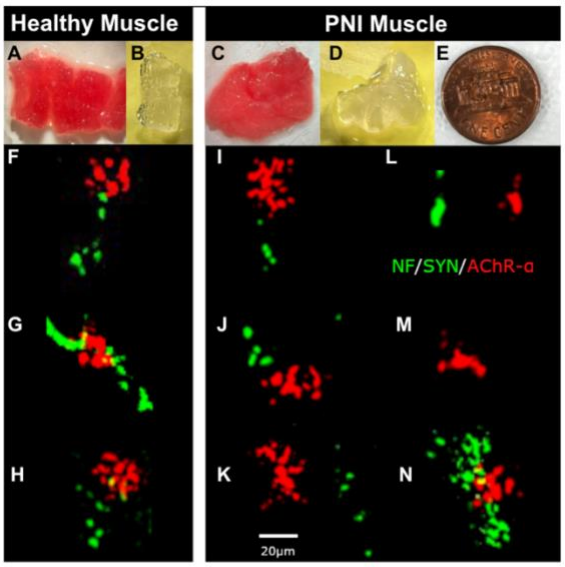

Figure 1. (A) gross appearance of healthy muscle before treatment (B) healthy muscle after cubic treatment (C) gross appearance of PNI muscle (D) PNI muscle after cubic treatment (E) cubic treated muscle placed over a penny (F-H) innervated pretzel MEPs in healthy muscle (I-K) intermediate MEPs in PNI muscle (L-M) denervated plaque MEPs in PNI muscle (N) pretzel MEP in PNI muscle.

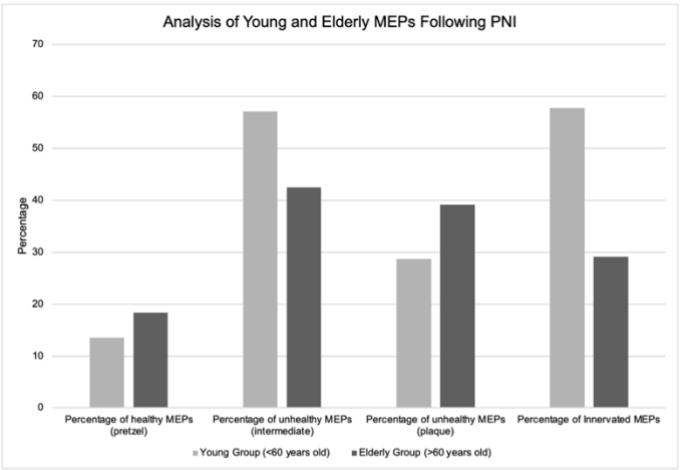

Figure 2. Average percentage of healthy, unhealthy, and innervated MEPs in young and elderly PNI patients. No significant differences were found in the average percentage of healthy and unhealthy MEPs between the two groups. The average percentage of innervated MEPs in the young group is double the average percentage of innervated MEPs found in the elderly group.