Why Do Infants and Children Recover from Nerve Injury better than Adults? Differential Expression of Acetylcholine Receptor Subunits at the Motor Endplates

Vivian Y Chen, Luigi Gonzales, Tyler Johnston, Oswald Steward[1], Ranjan Gupta[2]
[1]University of California Irvine, [2]Univ of California Med Ctr

INTRODUCTION: It is widely recognized that there is a difference in how adult and pediatric patients respond and recover after a brachial plexus injury or traumatic nerve injuries. To date, there is a limited understanding as to the etiology for this age-related differential neural response to injury. In children, increased neural plasticity and shorter regeneration distances have been hypothesized as the cause of their superior recovery after nerve repair; however, since no mechanistic studies have been conducted, these reasons remain conjecture. Most of our current understanding of age-related changes have demonstrated that there is an early, more robust up-regulation of pro-regenerative gene pathways as well as a greater ability to clear myelin debris with more efficient Wallerian degeneration. With increasing age, this response changes as well as the ensuing Schwann cell dysfunction that creates a less favorable environment for nerve regeneration. Yet, there is currently no data as to whether there is an age related differential response by the target end-organ motor endplates (MEPs) after nerve injury. It is our hypothesis that there is a differential expression in MEP morphology, subunit composition and distribution between adult and children that may account for their differential response to injury.

METHODS: A murine model (C57BL/6J) was used to evaluate age-related differences in MEPs from the early postnatal period through adulthood. Whole leg and the tibialis anterior (TA) muscles were harvested from sacrificed neonatal and adult mice at weekly intervals following birth through 4 weeks, then at monthly intervals through 4 months (n=24, 3 animal specimens at each time point). Unsectioned muscle tissues were fixed overnight in PFA, followed by immunostaining with alpha-bungarotoxin (α-BTX) and acetylcholine receptor-gamma (AChR-γ). All tissues were imaged with Keyence BZ-X810, an inverted fluorescence microscope. In addition, Western blot analysis with protein from whole leg fetal tissue and adult TA was extracted with a lysis buffer, quantified with BCA assay, and separated with gel electrophoresis. Samples were stained for AChR-γ and compared against Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) control.

RESULTS:

MEPs found in adult and fetal mice tissue differed profoundly in terms of subunit composition and morphology. Rigorous analysis of MEP morphometry revealed that both mature (α-BTX +) and immature endplates (α-BTX +, AChR-γ +) were present in fetal tissue whereas only mature endplates were present in adults.

Adult tissue had no immature endplates as confirmed with immunohistochemistry and western blot analysis (Fig. 2). Western blot analysis also revealed that 0 day old mice contained higher levels of AChR-γ than 1-7 day old mice (Fig. 2).

Mature endplates in 0 day old mice also differed drastically from the morphology of mature endplates in > 2 month old mice TA. Complex perforations with pretzel morphology are not seen until around the 2 month mark (Fig. 3). Moreover, immature endplates were distributed throughout fetal muscle in two patterns: 1) individual endplates adjacent to mature MEPs and 2) clusters of endplates not adjacent to mature MEPs. Importantly, there is also a differential expression and spatial localization of AChR subunit γ between early postnatal and adult animals.

DISCUSSION AND CONCLUSION: Despite the universal recognition that infants and children have a different response than adults to nerve injury and most often recover faster and more effectively, there remains a limited understanding as to why this difference exists. Impediments to functional recovery after nerve injury include slow rates of regeneration, poor specificity of reinnervation, glial scar formation, segmental nerve defects, and degeneration of the target end organs including the MEP.

To date, most studies have focused on evaluating age-related differences in neuronal cell and Schwann cell function; however, our study aimed to investigate a different aspect of differential functional recovery—degeneration of the target-end organ MEPs. Our study provides novel data that details fetal MEPs differ in subunit composition and morphology from adult MEPs. Fetal tissue contained a mixture of immature MEPs containing AChR-γ and mature MEPs with very basic morphology. Adult MEPs had fully developed complex morphology with no immature endplates.

These novel data provide insight into potential pathways that may account for the well-documented differential response to nerve injury across the human lifespan. Accordingly, further investigation is warranted as these findings open new possibilities for adjunct treatment modalities following nerve injury.

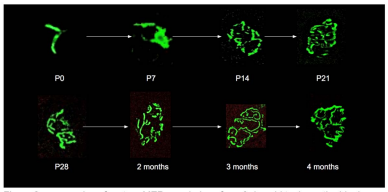

Figure 3: progression of mature MEP morphology from 0 day old to 4 month old mice

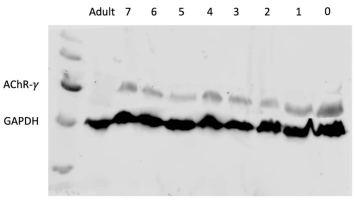

Figure 2: Western Blot with AChR-γ and GAPDH (control) levels in adult and 0-7 day old mice

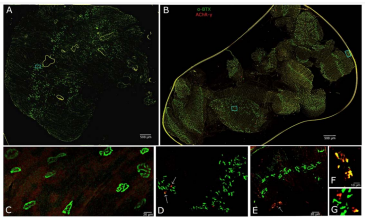

Figure 1: (A) TA from adult mice. (B) 0 day old mice whole leg muscle. (C) magnification of blue box in adult mice. (D-E) magnification of blue boxes in 0 day old mice. (F-G) magnification of immature MEPs (white arrows)