Facial paralysis is a devastating condition which leaves patients with myriad aesthetic and functional consequences. Current treatment options are limited by synkinesis, asymmetric contraction, and the lack of spontaneous movement such as that seen with emotions.

Muscle-Nerve-Muscle (MMN) neurotization is a reinnervation technique which involves implanting an autogenous nerve graft conduit between an innervated “donor” muscle and a denervated “recipient” muscle. Once axons have grown across the graft, a muscle-nerve-muscle “circuit” is created whereby stimulation of the donor muscle results in simultaneous contraction of the recipient muscle. This technique requires no neurorraphy, and targets only one muscle, thereby decreasing the likelihood of synkinesis. As it allows for the coordinated contraction of paired muscle groups, MMN grafting also enables coordinated spontaneous movement congruent with the uninjured side of the face. We also employed electrical stimulation and exogenous gonadal steroids which have been shown to enhance CN VII recovery after injury. More specifically, while electrical stimulation decreases the lag time to axonal sprouting after injury, testosterone propionate actually increases the rate of axonal growth. The net result of this combinatorial therapy is a significantly decreased time to recovery of facial movement after facial nerve injury.

**Methods**

24 M Sprague Dawley rats were randomly assigned to one of 3 treatment groups of 8 rats each: no graft (control), MMN grafting alone (MMN), or MMN grafting + electrical stimulation (ES) and testosterone propionate (TP) (MMN+). All rats then underwent harvest of the buccal and marginal mandibular branches of the right facial nerve which innervate the rat vibrissae (whisker) pad. Those animals randomized to nerve grafting then underwent implantation of the harvested facial nerve grafts across the snout from the nasolabialis muscle of the innervated (left) whisker pad to the muscle of the denervated (right) whisker pad (figure 1).

In the group randomized to undergo an ES + TP protocol, a 10mm silastic capsule containing crystalline testosterone propionate was implanted subdermally in the dorsal neck. For electrical stimulation, the facial nerve was identified at the stylomastoid foramen via an additional postauricular incision on the uninjured (left) side. Ground and stimulating electrodes were placed and stimulation was carried out for 30 minutes.

Assessment of recovery of facial nerve function was carried out on a weekly basis. A 6-point scale was used to assess recovery of vibrissae orientation and movement/coordinated. Video analysis of vibrissae movement was also performed using a video analysis and modeling tool (tracker v4.80) 16 weeks post surgery. Left and right sided vibrissae were analyzed on the basis of movement, coordination, and amplitude; the angle of the vibrissae and the orientation of the snout as compared to the midline axis of the head were also compared.

Electromyography (EMG) was performed on anesthetized animals pre- and post-operatively on the day of anatomy and weekly thereafter. The uninjured (left) vibrissae pad was stimulated with recording of responses on both the uninjured and injured sides in order to obtain the peak amplitude and latency of the evoked response. At the conclusion of the 16 week experiment, all animals were sacrificed with their MMN grafts harvested.

**Objective**

To determine if MMN grafting can improve facial function following facial nerve injury. Our hypotheses were: (1) MMN grafting is a viable means of facial reanimation after facial nerve injury which results in decreased synkinesis and improved return of coordinated movement and (2) the application of electrical stimulation and exogenous gonadal steroids will enhance regeneration across the nerve graft.

**Results**

As shown in figure 2, both MMN and MMN+ groups had faster return of vibrissae mobility and also improved to a more normal level/function of vibrissae mobility as compared to the control animals; the difference amongst groups did reach statistical significance using a 2-way ANOVA (P<0.05). The multiple comparison post-hoc test determined multiple individual data points that were significant compared to the control group (P<0.05). Improvements in snout asymmetry were obtained faster in the experimental groups than the control group; this relationship was statistically significant for the MMN+ animals (P<0.05). As shown in table 1, achievement of a vibration mobility score of 25 (vibrissae movement coordinated with the opposite side) was achieved at 16 weeks by 0% of the control animals, 71% of the MMN animals, and 85% of the MMN+ animals. Electromyography (EMG) demonstrated conductance across the nerve grafts in both the MMN and MMN+ES+TP animals. A two-way ANOVA of relative peak amplitude yielded a statistically significant interaction between group and week (P<0.05). As shown in figure 3, the multiple comparison post-hoc test determined multiple individual data points that were significant compared to the control group (P<0.05). Histologic examination of MMN grafts harvested at the conclusion of the experiment demonstrated growth of myelinated axons across the grafts (figure 4).

**Discussion**

This study demonstrates that MMN grafting achieves statistically significant improvements in vibrissae mobility scores compared to the control animals with a significantly decreased recovery time to coordinated vibrissae movement. These improvements were further enhanced by the application of ES+TP as demonstrated by the significant improvements in both snout asymmetry and recovery of coordinated vibrissae movement. Regrowth of functional, myelinated axons across our nerve grafts was demonstrated via both EMG and histologic section.

However, as demonstrated in figure 2, many of the control animals did regain some degree of vibrissae mobility which was attributed to compensation from a small branch of the zygomatic branch of the facial nerve which was not sectioned. This was confirmed on the final day of the experiment whereby after sectioning of the right (experimental) facial nerve at the stylomastoid foramen, only those animals which had undergone MMN grafting retained vibrissae mobility on that side.

Overall, these results demonstrate that MMN grafting is a viable means of facial reanimation in the rat model. The achievement of movement coordinated with uninjured side also suggests that this technique could enable symmetric, coordinated movement in paired muscle groups.

**Conclusion**

This study demonstrates that reinnervation following MMN grafting can not only improve tone and movement in a selected muscle following a facial nerve injury, but that therapy with electrical stimulation and exogenous gonadal steroids further enhances the recovery of these functional measures. This type of nerve grafting has widespread implications in facial reanimation as it has the capacity to enable spontaneous, coordinated movement congruent with the uninjured side of the face, something that conventional facial reanimation procedures are unable to provide.

**References**