Histological Rearrangement in the Facial Nerve and Central Nuclei Following Immediate and Delayed Hypoglossal–Facial Nerve Anastomosis

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Hypoglossal–facial nerve (XII–VII) anastomosis is the treatment of choice when the facial nerve itself cannot be restored.

The best timing of hypoglossal–facial nerve anastomosis remains controversial.
Introduction

- Little is known about the histological changes in peripheral facial nerves and central nuclei after anastomosis at different time intervals.
- We designed this study to determine the influence of the timing of anastomosis on histological changes in the peripheral nerves and the central nuclei.
20 guinea pigs received transection of the L’t labyrinthine segment of the facial nerve.

They were divided into
A (immediate anastomosis)
B (3-month-delayed anastomosis)
The facial nerve trunk was transected peripheral to the stylomastoid foramen. Then hypoglossal nerve was dissected as far as possible beyond the external carotid artery and was transected, then the proximal stump was approximated to the distal stump of the facial nerve.
After 6 months after the anastomosis, the operative wound was reopened. The junction was examined for the presence or absence of a “regenerated facial nerve bundle” (RFNB), a small nerve bundle bridging the junction.
*Fig. 1.* Anastomotic junction at 6 months after hypoglossal–facial anastomosis. Regenerated facial nerve bundle (RFNB), hypoglossal nerve (H), facial nerve (F) (bar = 1 mm).
Horseradish peroxidase (HRP) study

- 6 months after the anastomosis, a 5-mm segment of buccal branch was removed and a 10-mm plastic tube with one closed end was sutured to the proximal stump of the buccal branch, and HRP was injected into the tube.
- After 72 h had been allowed for HRP retrograde transport, the animals were sacrificed.
Horseradish peroxidase (HRP) study

- 40-micron thick brain stem coronal frozen sections were made, extending from the hypoglossal nucleus to the facial nucleus.
- The number of facial motor neurons, hypoglossal neurons on the surgery and control sides and HRP-labelled neurons on the surgery side were counted under a light microscope.
Histology of the regenerating nerve

- The 5-mm segment of the buccal branch was removed and serial sections 0.5 mm thick were cut.
- An axon count was performed.
- The cross-surface area of the axons was counted.
Six months post-anastomosis, 8 guinea pigs in group A and 9 guinea pigs in group B had survived. The incidence of RFNB was 75% (6 out of 8) in group A and 77.7% (7 out of 9) in group B. (not statistically significant)
Table I. *Survival ratio of hypoglossal and facial neurons after XII–VII anastomosis*

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<th>Hypoglossal neurons (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Facial neurons (%)&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>Group A</td>
<td>94.3 ± 2.3 (n = 8)</td>
<td>23.3 ± 2.6 (n = 6)</td>
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<tr>
<td>Group B</td>
<td>93.0 ± 1.9 (n = 9)</td>
<td>24.0 ± 6.5 (n = 7)</td>
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<sup>a</sup>Ratio of the operated side to the unoperated side. Group A: immediate anastomosis; Group B: 3-month-delay anastomosis.
**HRP reaction**

- In the animals with RFNB, the HRP-labelled neurons from the anastomosis side were distributed diffusely in both the hypoglossal and facial nuclei without specific localization.
- If the RFNB was cut (2 animals in each group) prior to application of HRP, no HRP-labelled neurons could be found in the facial nucleus.
- Animals with no RFNB demonstrated a similar finding.
Fig. 2. HRP-labelled neurons in the hypoglossal nucleus. (a) Group A. (b) Group B. HRP-labelled neurons in the facial nucleus. (c) Group A. (d) Group B. The number of HRP-labelled neurons is small in group B (bar = 0.1 mm).
Regeneration of the buccal branch 
and RFNB

- A cross-section of the buccal branch showed well-myelinated axons.
- The average number of axons was 2,9999+-442 in group A
  16559+-405 in group B.
- A significant difference (Student’s t-test, p<0.005).
Fig. 3. Regenerated axons in the buccal branch of the facial nerve 6 months after hypoglossal–facial anastomosis. (a) Group A. (b) Group B (bar = 5 μm). The number of axons is greater in group A.
Regeneration of the buccal branch and RFNB

- The axon size (cross-surface area): Group A are larger than in group B
- Myelinated axons in the RFNB were scanty, and distributed randomly in the connective tissue. Only a few fasciculi could be found.
Fig. 4. Histogram of axon size (cross-surface area). Axons are larger in group A than in group B, similar to the control side.
Fig. 5. Axon regeneration in the RFNB. Axons are scanty and diffusely distributed in the connective tissue. The fascicule number and size are small in both groups A and B, but group A has a better regenerating picture. Fasciculi are indicated by the arrow. (A = group A, B = group B) (bar = 20 μm).
DISCUSSION: axon numbers

- The number of myelinated axons in RFNB and the buccal branch were significantly greater in the early anastomosis group.
- Mechanisms?
Mechanisms 1
Peripheral nerve injury in the proximal portion of the nerve may induce more severe central degenerative changes.
Perhaps 3 months is not long enough for the proximal portion to regenerate.
DISCUSSION: axon numbers

- Mechanisms 2
- Schwann cell tubule has an important role both in supporting rapid growth and in the remyelination.
- If they are not reinnervated after a certain period of time, they are atrophy and are replaced by fibrous tissue.
DISCUSSION : axon numbers

- Mechanisms 3
- Neurotrophic factors such as nerve growth factor (NGF) play a key role in the process of nerve regeneration.
- Axotomy leads to a biphasic increase in NGF, the first phase is within the first 2–6 h and the second phase beginning 2 days after axotomy.
DISCUSSION: neuron survival

- The most important determining factor of neuron survival is the level of the lesion, not the timing of the anastomosis.
- The more proximal lesion leading to more severe neuron degeneration.
DISCUSSION

- HRP-labelled neurons appeared in both the hypoglossal and facial nuclei.
- Indicating that both nuclei contained neurons with axons that sprouted to the buccal division of the facial nerve.
DISCUSSION

- The number of HRP-labelled neurons in the hypoglossal nuclei was greater than in the facial nucleus.
- This indicates that most of the restored mimetic muscle innervation is from the hypoglossal nerve whether anastomosis is performed early or late.
The number of surviving neurons in the facial and hypoglossal nuclei was almost the same.

Further study is needed to determine whether these results would be the same after long-term follow-up.
The physiological function and role of the RFNB in control of mimetic muscle movement are still unknown.

subconscious smiling?
The end