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Physical Rehabilitation of Paralysed Facial Muscles: Functional and Morphological Correlates

With 22 figures

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In memory of my long-standing friend and neuroanatomy teacher Prof. Dr. Kamen G. Usunoff (Department of Anatomy, Medical University Sofia, Bulgaria) who died suddenly on March 1, 2009 during a scientific stay in Rostock, Germany. His sound knowledge, catching enthusiasm, and endless energy for neuroscience research inspired young scientists worldwide for decades.

Abstract

Using a combined morphofunctional approach, we recently found that polyinnervation of the neuromuscular junction (NMJ) is the critical factor for recovery of function after transection and suture of the facial nerve. Since polyinnervation is activity-dependent and can be manipulated, we tried to design a clinically feasible therapy by electrical stimulation or by soft tissue massage. First, electrical stimulation was applied to the transected facial nerve or to paralyzed facial muscles. Both procedures did not improve vibrissal motor performance (video-based motion analysis of whisking), failed to diminish polyinnervation, and even reduced the number of innervated NMJ to one-fifth of normal values. In contrast, gentle stroking of the paralyzed vibrissal muscles by hand resulted in full recovery of whisking. Manual stimulation depended on the intact sensory supply of the denervated muscle targets and was also effective after hypoglossal–facial anastomosis, after interpositional nerve grafting, when applied to the orbicularis oculi muscle and after transection and suture of the hypoglossal nerve. From these results, we conclude that manual stimulation is a noninvasive procedure with immediate potential for clinical rehabilitation following facial nerve reconstruction.

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Contents

Chapter 1
Factors Limiting Motor Recovery After Facial Nerve Injury

The facial nerve is the most frequently damaged nerve in head and neck traumata. Apart from traffic-accident injuries (temporal bone fractures or lacerations of the face), most facial nerve lesions are postoperative (removal of cerebellopontine-angle tumors, parotid resections because of malignancy). Despite the use of fine microsurgical techniques for repair of interrupted nerves in man, the recovery of voluntary movements of all 42 facial muscles and emotional expression of the face remain poor (Vaughan and Richardson 1993; Ferreira et al. 1994; Anonsen et al. 1986; Goodmurphy and Ovalle 1999), and the occurrence of a "postparalytic syndrome" (pareses, abnormally associated movements, and altered reflexes) is inevitable (Kimura et al. 1975; Bento and Miniti 1993; Kerrebijn and Freeman 1998). This insufficient recovery has been attributed to:

1. Persisting posttraumatic rearrangements of motor cortical representation areas (Sanes et al. 1990; Franchi 2000; Taylor et al. 2009). This, in turn, is associated with an acute deafferentation of the axotomy-lesioned motor nuclei ("synaptic stripping", Blinzinger and Kreutzberg 1968) which causes alterations in the synaptic input to the hyperexcitable facial motoneurons (Graeber et al. 1993; Moran and Neely 1996; Brännström and Kellerth 1999)
2. Extensive collateral branching of axons at the lesion site causing "misdirected" or "aberrant" reinnervation of the targets (Montserrat and Benito 1988)
3. Exchange of nerve impulses between adjacent axons (Sadjadpour 1975)
4. Vigorous intramuscular sprouting of axons in the facial muscles

1.1
Altered Synaptic Input to the Axotomized Hyperexcitable Facial Motoneurons

After transection of the facial nerve, the resident microglia show a dramatic increase in mitotic activity, rapidly migrate toward the neuronal cell surface (Rotter et al. 1979), and displace the afferent axo-somatic synaptic terminals (Blinzinger and Kreutzberg 1968). This "synaptic stripping" leads to a deafferentation mainly of proximal but not of peripheral dendrites (Bratzlavsky and

vander Eecken 1977; Titmus and Faber 1990; Nacimiento et al. 1992). The axotomized motoneurons "respond" to their deafferentation with a decrease in the synthesis of transmitter-related compounds, for example, muscarinic and glycine receptors (Senba et al. 1990) and a decrease in activity of enzymes involved in the biosynthesis of transmitters, for example, dopamine-β-hydroxylase, tyrosine-hydroxylase, cholineacetyltransferase, cytochromeoxidase, and acetylcholinesterase (Engel and Kreutzberg 1986; Engel et al. 1988). These changes correspond to the electrophysiological status of regenerating neurons: increased excitability (Eccles et al. 1958; Kuno and Llinas 1970) with preserved integrity of the dendritic input (Lux and Schubert 1975; Kreutzberg et al. 1975; Borgens 1988).

1.2 Excessive Collateral Branching of Axons at the Lesion Site

Injury to the peripheral nerve sets initiates a complex series of changes distal to the site of injury, collectively known as Wallerian degeneration. Within 24 h after lesion, the axonal content begins to necrotize and axonal debris is phagocytosed by blood-borne macrophages and proliferated Schwann cells (Perry and Brown 1992; Hirata and Kawabuchi 2002; McPhail et al. 2004a). When resorption is complete, the Schwann cells form long chains of cells (bands of Büngner), which bridge the interfragmentary gap and form guiding channels for the regenerating branches on their way to the target(s). The architectural pattern of the Büngner's bands of the peripheral stump remains unchanged for 3 months, after which progressive distorsion by proliferating connective tissue occurs. The process of Wallerian degeneration creates an environment that is highly supportive for axonal growth. The preference for axonal growth into a degenerating nerve ensures that the vast majority of axons will regrow into the distal stump, if it remains in continuity with the proximal stump (Bisby 1995).

In spite of that, the regenerating axons do not merely elongate toward the distal stump, but respond with axonal branching (sprouting) by lateral budding mainly at the nodes of Ranvier, up to 6 mm proximal to the injury site. As regeneration proceeds, some of these supernumerary branches are pruned off over a period of up to 12 months (Bray and Aguayo 1974). There are, however, persistently higher numbers of myelinated and unmyelinated axons in regenerated segments of peripheral nerves than in intact nerves. Axonal branching begins from the end-bulb within 3 h after injury (Sjoberg and Kanje 1990). The regenerating branches initially lie on the surface of the Schwann cells. Later, these branches increase in diameter and get surrounded by Schwann cell processes.

Observations in vitro show that axonal branching begins from the end-bulb within 3 h after injury (Sjoberg and Kanje 1990). The regenerating branches initially lie on the surface of the Schwann cells. Later, these branches increase in diameter and get surrounded by Schwann cell processes. The guidance of these immature axons to their final destination can be considered as a series of

short-range projections to intermediate targets under the influence of local guidance cues (see below). Neurons respond to these cues by means of motile sensory apparatus at the tip of the advancing axon termed the *"growth cone,"* which very often does not emerge from the axon at the precise site of injury, but proximal to it (Ziv and Spira 1997). The initial formation of growth cones occurs before the necessary newly synthesized proteins would have time to arrive at the site of axon injury, that is, too rapidly to be dependent on metabolic changes in the cell body (Smith and Skene 1997).

The growth cone borne by neurites is shaped like a webbed foot (Fawcett and Keynes 1990). There is a swollen central core from which flattened processes called *lamellipodia* and numerous stiff fine processes called *filopodia* extend. Current studies have identified three major intracellular cytoskeletal components responsible for the cytomechanical forces in the leading edge of elongating axons: actin microfilaments, myosin, and microtubules (Challacombe et al. 1996). The growth cone formation begins with a restructuring of the neurofilaments and microtubules to form an altered cytoskeletal region proximal to the tip of the transected axon in which vesicles accumulate. This rearrangement of the cytoskeleton forms a transient cellular compartment that traps the transported vesicles and serves as a locus for microtubule polymerization. Microtubuli, in turn, facilitate the fusion of vesicles with the plasma membrane, promoting the extension of growth cone lamellipodia (Spira et al. 2003).

The recognition of specific guiding cues is performed by the actin-rich filopodia, which have a guidance and/or sensory role, sniffing out gradients of trophic or adhesive factors (Lin and Forscher 1993). Isolated filopodia can respond to alterations in their environment by changes in internal calcium concentrations, and filopodia on different parts of the growth cone respond independently (Bixby and Harris 1991; Letourneau and Cypher 1991; Gordon-Weeks 1997).

1.3 Role of Cytoskeleton Reorganization During Axonal Regrowth

In response to axotomy, the synthesis of cytoskeletal proteins in the perikarya is increased (Hoffman and Lasek 1980). A postaxotomy increase in overall tubulin synthesis has been documented (Oblinger and Lasek 1988), and it is thought that upregulated levels of tubulin in the perikarya and increased delivery of mictotubules to regrowing axon tips are essential for effective regeneration after injury (Tetzlaff et al. 1988a, 1991, 1996).

The rate of elongation of an axon is determined by the rate at which the growth cone can advance over the substrate. In rat sciatic nerve, both large and small diameter sensory axons elongate at nearly the same rate as do somatic motor axons (about 4 mm/day; Fawcett and Keynes 1990). In the regenerating (crushed) facial nerve of rats, the rate of axonal elongation is 4.3 mm/day measured from the transport of radiolabeled protein (Tetzlaff and Bisby 1989).

Axonal elongation depends on the advance of microtubules that provide structural support and serve as tracks for axonal transport of membraneous organelles. Stable microtubule bundles project from the axon into the central region (C-domain) of the growth cone, whereas the ends of dynamic microtubules expand and stretch into the actin-rich P-domain (Gordon-Weeks 1991). Goldberg and Burmeister (1986) and Aletta and Greene (1988) have described three phases of axonal elongation. First, lamellipodia and filopodia are extended from the tip of the axon (*protrusion*). Second, microtubules enter the recently protruded regions of the growth cone (*engorgement*). Third, the portions of the growth cones lateral to the engorged regions become quiescent and coalesce to form a new portion of the axon (*consolidation*).

The net protrusion of lamellae and filopodia is largely determined by the rates of F-actin polymerization and retrograde flow (Lin et al. 1994). If actin polymerization is blocked, leading edge protrusion does not occur and F-actin is removed from the peripheral (P) domain by retrograde transport. On the other hand, if F-actin retrograde flow is inhibited, then the rate of protrusion of the leading edge will be determined primarily by the polymerization of F-actin. Rho-family GTPases (Rho, Rac, Cdc42) have been found to mediate the formation of filopodia and lamellipodia, that is, to be involved in axon guidance (see Gallo and Letourneau 1998 for review) and also in growth cone responses to collapsing guidance cues (Jin and Strittmatter 1997).

Results from some additional experiments have suggested that axonal growth requires microtubules (both addition of tubulin to polymer and transport of preestablished polymer) at the growth cone (Yu and Baas 1995; Baas 1997, 1999). Tanaka and Kirschner (1991, 1995) report that microtubules in growth cones appear to be transported by "pushing" toward the leading edge of the P-domain. Consistent with this interpretation, Challacombe et al. (1997) report that looped microtubules in growth cones stain with a marker for stable microtubule polymer (i.e., detyrosinated α-tubulin). Therefore, both microtubule polymerization and transport contribute to axonal elongation by advancing microtubules into the P-domain of the growth cones.

Still, the exact nature of F-actin–microtubule interactions in the axon growth cone is not well understood. Growth cones at the tips of rapidly extending axons are small and highly active. However, in preparation for branching, they may pause for many hours, greatly enlarge, and maintain motility without a forward advance. Subsequently, a new growth cone develops from the tip of the large pausing growth cone and forms a new leading axon. Remnants of the large pausing growth cone remain on the axon shaft as filopodial and lamellar expansions that subsequently give rise to axon collaterals (Halloran and Kalil 1994; Szebenyi et al. 1998).

Microtubules in the central region of advancing growth cones get stretched out. In slowly growing axons, microtubules become bundled and in pausing growth cones – they form prominent loops (Tanaka and Kirschner 1991). Transition to new axonal growth and branch formation is accompanied by splaying of looped