NERVE REGENERATION: BASIC AND APPLIED ASPECTS

R. Bruce Donoff
Dean, Professor of Oral and Maxillofacial Surgery, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, Massachusetts 02115

ABSTRACT: Increased knowledge is shedding new light on our understanding of central and peripheral nerve anatomy and molecular biology and function. New tools and methods provide important methods for the study of the behavior of cells, axons, and receptors. This review discusses the current state of that knowledge, with particular regard to the efficacy of the Seddon classification of nerve injury. The correlation of that new information to damage and repair of the peripheral sensory nerve, especially the inferior alveolar and lingual nerves, serves to highlight the progress and problems that exist.

Key words. Inferior alveolar nerve, lingual nerve, injury, repair.

Introduction

The clinically sound and logical management of nerve injuries is based on the classification of the injury. Several classification schemes exist, the most common of these being the Seddon and Sunderland classifications. This paper will review current understanding of nerve regeneration and attempt to correlate this knowledge with clinical management of injured peripheral sensory nerves. The inferior alveolar nerve (IAN) and lingual nerve will be highlighted. The most controversial issues facing the patient and the surgeon are whether an injury requires treatment and, if so, when. This review will examine these issues through animal models, long-term clinical data for untreated injuries, and the current state of electrophysiologic measurements. It is well to remember that these classification schemes were devised for other peripheral nerves, and so application to a sensory nerve like the inferior alveolar or lingual may involve some inaccuracies. More general discussions regarding injury to the trigeminal nerve offer a better overview of the topic (Donoff and Colin, 1990).

Seddon described three types of nerve injury—neurapraxia, axonotmesis, and neurotmesis—based upon the severity of tissue injury, prognosis for recovery, and time for recovery (Seddon, 1943). Neurapraxia is a conduction block resulting from a mild insult to the nerve trunk. There is no axonal degeneration, and sensory recovery is complete and occurs in a matter of hours to several days. The sensory deficit is usually mild and characterized by a paresthesia, with some stimulus detection but poor discrimination and disturbed stimulus interpretation. Axonotmesis is a more severe injury. Afferent fibers undergo degeneration, but the nerve trunk is grossly intact with variable degrees of tissue injury. Sensory recovery is good but incomplete. The time course for sensory recovery depends on the rate of axonal regeneration and usually takes several months. The sensory deficit is characterized by a severe paresthesia. Neurotmesis is a complete disruption of the nerve, the most severe injury in the Seddon classification. Sensory recovery is not expected except when the nerve courses through a canal like the mandibular canal. The sensory deficit is characterized by anesthesia.

The Sunderland classification is based on the degree of tissue injury (Sunderland, 1978). There is similarity between the two systems, with Sunderland’s offering greater detail of description. For the purposes of this review, the reader is referred to good descriptions of Sunderland’s classification. The purpose of this review is to assess critically our knowledge of peripheral trigeminal nerve injury and regeneration in light of Seddon’s
Terms such as "hypoalgesia" and "hypesthesia" serve while anesthesia is the complete absence of touch. The definitions of each are the sensory loss and detection of discrimination usually less in the more severe injury. Both may exhibit dysesthetic symptoms in patients. The definitions of each are often muddled. For this discussion, paresthesia means partial loss of sensation, but still some sense of touch, while anesthesia is the complete absence of touch. Dysesthesia is a partial loss of sensation with a painful or uncomfortable component.

Animal models of nerve injury have used compression, stretching, or sectioning of nerves under controlled experimental conditions to examine the anatomic, physiologic, and neurochemical consequences of peripheral nerve injury. Results show a spectrum of injury that further complicates direct application to humans. Compression or stretching of a nerve may damage the nerve so that it degenerates, either completely or partially. It may occur acutely or may evolve over time due to progressive irritation or compression. Nerves either partially or completely severed may give rise to pathology from disordered central zones, from hyperactive ganglion cells, or from the formation of a neuroma (Devor and Wall, 1990). Nerves injured but continuous maintain conduction, but pathology may arise from disordered function in the zone of injury. Compression or stretching of nerves may produce a complex of acutely severed axons, axons that are injured but functional, or axons that have formed a neuroma-in-continuity.

Even mild injuries may cause segmental demyelination, progressive demyelination at the site of damage which can by itself lead to pathology. These sites may act as ectopic impulse generation areas (Nordin et al., 1984). At the other end of the spectrum, neuromas resulting from complete nerve transection have elevated mechanical and electrical sensitivity (Wall and Gutnick, 1974). This spectrum of consequences resulting from different levels of injury does not make the task of recognition of the type of injury straightforward. What do we know that permits more accurate categorization of injuries into those that will heal without intervention and those that might benefit from treatment?

**Normal Response to Nerve Injury**

Injury to the peripheral trigeminal nerve results in degeneration the degree of which depends in part upon the magnitude of the injury, the age of the patient, and the location of the injury (Lieberman, 1974). Thus, for a given age range and site of injury, the type of injury is often the dependent variable that predicts outcome. Transection of a peripheral nerve branch (axotomy) results in a greater degeneration than with compression or crush injuries. The latter are often reversible. The Seddon and Sunderland classifications are based on histologic findings and do not represent functional outcome analysis. We have already discussed the variability of pathology even with identical injuries. Can we add a degree of conditional outcome analysis to our current state of knowledge?

The normal anatomy deserves some review for further appreciation of the complexity of the subject. The normal nerve trunk is made up of organized collections of axons that are the peripheral extensions of the cell bodies located in the trigeminal ganglion. Schwann cells envelop axons in a predetermined fashion and produce various degrees of myelin. In the peripheral nervous system, a single Schwann cell envelops one axon with a myelin sheath. Unmyelinated axons differ from myelinated axons in that several are ensheathed by one Schwann cell. The endoneurium surrounds either type of axon and consists of organized collagen fibers. The outermost layer of these fibers is the basal lamina of the Schwann cell, called the band of Büngner, and is a basal lamina tube running the entire length of the axon. An outer region of fine collagen fibers is further organized into the sheaths referred to as the endoneurium. Many axons with their endoneurial sheaths are surrounded by a second organization of collagen fibers called the perineurium, which form the fascicle. Fascicular patterns are quite variable (Svane et al., 1980; Girod et al., 1989). The nerve trunk is completed by the internal epineurium, external epineurium, and the mesoneurium, which contains the blood supply.

There is an enormous amount of literature on degeneration and regeneration and the role of the micro-environment (Seckel, 1990). Direct comparison of axotomy and crush injuries will serve to highlight differences. Axon degeneration in both directions from the injury site characterizes complete transection of a nerve. Originally termed "Wallerian degeneration", marked morphological, biochemical, and physiologic changes in the nerve cell body and its processes occur (Gordon, 1983; Barron, 1989; Seil, 1989). If death of the nerve cell does not occur, regenerative activity in the form of nerve sprouts coming from the proximal stump may begin as early as 24 hours.
after injury.

Unlike cutaneous wound healing, the healing of nerve tissues is unique, because the process is greatly dependent upon cellular rather than tissue repair. Successful nerve regeneration requires neuronal growth. The key histologic structure during axonal regeneration appears to be the Schwann cell and its basal lamina (i.e., bands of Büngner). Schwann cells multiply in the distal nerve segment and when contacted by an axon sprout, undergo a cascade of changes that trigger the production of myelin (Pelligrino and Spencer, 1985). Some signal code likely determines if an invading axon will be myelinated or unmyelinated. The early regeneration of unmyelinated axons may explain the early appearance of pain and temperature sensation in patients’ initial recovery (Lundborg, 1988b). Despite this strong basic knowledge, the usual growth rate in humans (Seddon et al., 1943; Buchthal and Kuhl, 1979) of 1 to 2 mm/day makes little sense when applied to the human inferior alveolar nerve.

Using sequential double-fluorescence labeling techniques, Zuniga and O’Conner (1987) demonstrated that, following mental nerve axotomy and immediate repair in adult rats, mental sensory cells regenerate from and maintain an organized somatotopic area within the trigeminal ganglion; regeneration of axotomized cells is a gradual process that is enhanced by immediate surgical intervention; and, although enhanced, surgical repair did not result in complete recovery of all transected cells (Zuniga and O’Conner, 1987). Delayed repair showed similar results except that the time-dependent dynamic increase in the number of regenerating cells was less than if the nerves were repaired immediately (Zuniga et al., 1989). Finally, morphologic quantification of the outcomes of mental axotomy showed that axotomy resulted in 47% loss of cells, which was unaltered by the immediate or delayed repair of the nerves; repair did not affect the size range of surviving cells; and diminished volume of the ganglion and its associated nerve trunk was restored following repair, presumably the result of axonal branching and Schwann cell proliferation (Zuniga et al., 1990).

The well-known limitations of horseradish peroxidase in nerve studies—such as diffusion out of cell bodies and tissue necrosis at the site of injection—led to the use of fluorescent latex microspheres as a retrograde tracer in the sensory peripheral nervous system. When microspheres were used in a crushed or intact rabbit inferior alveolar nerve model, results showed that they were taken up only by damaged axons, they remained in the trigeminal cell bodies for up to three months without degradation or diffusion to extracellular structure, and cells containing them were capable of regenerating axons, as evidenced by the return of evoked sensory action potentials and the retrograde axonal transport of True blue (Colin et al., 1989). This work used a crush injury as a model and provided some of the only existing electrophysiologic data for that type of damage (Colin et al., 1986). It is interesting, at this point, to look at current knowledge of crush injuries and then compare and contrast that information with that for axotomy. The rate of axonal outgrowth is always faster and more complete following crush injuries (Lundborg, 1988a). Minimal changes in cell size and cell death compared with axotomy are described and axons appear to regenerate to original receptor sites because Schwann cell columns are mostly undisturbed and serve as guides (Horch, 1979). This propensity for better regeneration is shown by the finding that even taste sensations are completely recovered in animals whose chorda tympani nerve was crushed and compared with a normal nerve (Robinson, 1988). It is important to appreciate that a relatively simple crush injury may be exaggerated by the effects of compression. As a separate entity, compression may produce a sensory deficit. The acute response of compression is inflammation and edema. Secondary effects owing to fibrosis may include localized nerve fiber changes including segmental demyelination and even Wallerian degeneration if the compression persists. The mechanisms of these injuries include mechanical deforming forces and ischemic factors (Dellon, 1980; Lundborg, 1988a).

The early work of Merrill is of interest despite its technical limitations (Merrill, 1966). He showed that crush injuries in dogs complicated by bony compression healed more poorly than crush injuries alone. The methods used were histologic cell counts and a physiologic pain response. In addition, results strongly supported the importance of decompression by four weeks for reversal of degeneration to take place. Machida and Omori (1979) studied recovery of nerve action potentials after inferior alveolar nerve sectioning in the rabbit. Some animals had the injury compressed by re-application of the bony lateral plate, and others did not. There were no real differences in the recovery of action potentials after the first few weeks, leading the authors to conclude that compression had no lasting effect. It should be observed, however, that compression by replacement of the lateral cortex of bone is quite different and distinct from compression due to a bony spicule placed into the mandibular canal, as performed by Merrill. The fact that Merrill’s work used axon counts and the Machida and Omori study used action potentials in a volume conductor highlights differences in techniques but does not detract from the stronger conclusions of the older work.

Electrophysiologic data of crush injury were also reported by Colin et al. (1986). Longitudinal sensory-evoked potentials of inferior alveolar nerves whose parent cell bodies contained microspheres showed no statistically significant decrements of conduction velocities.
or amplitudes by eight weeks after crush injury when compared with conduction velocities and amplitudes prior to injury and microsphere injection. This return of action potential is identical to results obtained by others after nerve sectioning and repair (Yamazaki and Noma, 1983). The exact relationship of the number of axons to electrical activity and function is unclear. The inferior alveolar nerve and the trigeminal system have an ultrastructural propensity to compressive injury, because the trigeminal system has the greatest proportion of myelinated axons in the entire somatosensory system.

**Clinical Outcome Analysis**

Results correlating clinical findings to resolution of neurosensory disturbance are important to our understanding of the Seddon classification. Recall that neurapraxia results in complete recovery, while in axonotmesis, incomplete recovery is described. Both retrospective and prospective studies have been reported. Kipp et al. (1980) examined outcomes following odontectomy and found that most injuries resolved by six months. This study was limited by loss of patients. Osborn et al. (1985), in a prospective study, described similar results but grouped lingual and inferior alveolar nerve injuries together. In both of these studies, attention was drawn to the fact that most deficits improved or disappeared before the six-month mark. This time period is a traditional benchmark for sensory return. These results suggest that neurapraxies are most common. In a retrospective survey-based paper, Alling (1986) described persistent deficits in 13% of lingual nerve injuries which had an incidence of 0.06%. Others have shown 11% incidence and 0.05% persistence rates (Blackburn and Bramley, 1989). Other data (Carmichael and McGowan, 1992) showed a lingual nerve injury incidence of 15% at 6 to 24 hours, 10.7% at 7 to 10 days, and 0.6% after 1 year. A recent paper (Schultze-Mosgau and Reich, 1993) showed no persistent neurosensory deficits in patients found to be anesthetic at first examination. Other data for the IAN showed an incidence of 5.5% at 6 to 24 hours, 3.9% at 7 to 10 days, and 0.9% after 1 year (Carmichael and McGowan, 1992), and 0.4% incidence and 3.5% persistence at one year (Alling, 1986). In general, recovery of IAN injuries is better than that of lingual nerve injuries, probably owing to the guiding provided by the mandibular canal.

The results suggest that a certain number of IAN and lingual nerve injuries do not resolve by themselves. The task then is how to ascertain which early lesions these represent. Clinical exam with a finding of anesthesia alone or in concert with a positive Tinel's-like sign is one potential guide. This sign is elicited more commonly upon palpation over the lingual alveolus. A tingling or shooting sensation to the tip of the tongue is experienced. For the IAN, a directed abnormal sensation to the lip may be seen in a few situations upon palpation over the third molar socket area. The sensation need not be painful as with a trigger point. A neuroma usually explains these findings. Other methods of testing would be of great usefulness.

**Electrophysiologic Testing**

Electrophysiologic testing can determine the induced action potentials of sensory nerves and conduction velocity (Yamazaki and Noma, 1983). Some of these electrophysiologic methods are applicable in humans, but most are not as yet useful for the peripheral trigeminal system. Nerve conduction velocity testing can probe the peripheral trigeminal complex, and perhaps trigeminal somatosensory evoked potentials (TSEP) may cast light on the more central effects of injury and repair.

The trigeminal system has been clinically evaluated by a variety of electrophysiological techniques; however, each method has inherent shortcomings and limitations. Electrical threshold testing is a technique whereby an electrical current is passed in increasing increments until a sensation is barely elicited. Using this technique, researchers have found electrical sensory thresholds to be elevated when placed on a paresthetic lip, but to diminish with time as the paresthesia resolves (Lavant, 1967). This test is effectively an electrical version of the many sensory reflex examinations that are available. It is limited by the patient's level of cooperation and pain tolerance, and certainly does not localize a neural lesion.

A variety of reflex studies have been developed, like the blink reflex, that test the trigeminal sensory pathways and the motor branches of the facial nerve (Kimura, 1984a,b), but they involve one or more synapses. This test fails to localize the neural lesion in the reflex arc and is a relatively insensitive test unlikely to discern subtle afferent injuries (Leandri and Favale, 1991). The jaw reflex and evoked masseteric silent period, which involve both afferent and efferent loops of the trigeminal nerve throughout the mesencephalic trigeminal nucleus in the brainstem, have the same shortcomings as described. The TSEP has been advocated as an electrophysiologic method for the evaluation of peripheral and central trigeminal pathways in health and disease (Prevac, 1970; Stohr and Petruc, 1979; Bennett and Jannetta, 1980; Findler and Feinsod, 1982; Barker et al., 1987; Godfrey and Mitchell, 1987; Larson and Pogrel, 1992). Electrical stimulation of the trigeminal branches will evoke volleys of peripheral, ganglionic, spinal, and cortical potentials. There is much disagreement as to the normal latencies of these signals because of variability (Pogrel, 1992). A meaningful correlation of the waveforms and the underlying neural generators is still lacking (Leandri and Campbell, 1986). Therefore, the TSEP has not had routine clinical use.

Some progress has been made in the study of animal and human nerve injuries electrophysiologically, as dis-
cussed previously. A recent study in human volunteers (Colin, 1993) found a conduction velocity of 67 m/s at the onset of the signal and a conduction velocity of 50 m/s at the peak of the signal. Others (Matsuda, 1980b) found a mean conduction velocity of 55.8 ± 2.95 m/s for the human IAN by stimulating the mental nerve and recording from the mandibular foramen. In this study, the investigator averaged the onset latency to be halfway between the first positive and the first negative peak, so this did not represent either the conduction velocity at the onset of the signal or the conduction velocity at the peak of the signal, but rather an average of the two. Also, only one side was studied in each of the 20 male and female volunteers, so it is unclear if there is any side-to-side variation or gender difference. Sasaki et al. (1986) found a normal IAN maximal conduction velocity of 61.4 ± 7.8 m/s, ranging from 51 to 77 m/s, but only studied the right IAN in nine volunteers of both genders. Recently, a report claimed successful IAN recording (Jones and Thrash, 1992) but obtained good signals in only six of 10 subjects and estimated the conduction distance.

IAN conduction studies have appeared in the literature. Control rabbit IAN signals have had a similar range of conduction velocities of 54 m/s in our studies (Colin and Donoff, 1990) and in others, 58 m/s (Matsuda, 1980a), 40 m/s (Edinger and Luhr, 1986), and 22.7 m/s (Eppl ey et al., 1989). In these investigations, the conduction distance was estimated from surface measurements, resulting in erroneous calculations of the conduction velocity.

Before electrophysiologic tests like conduction velocity become useful in human patient care, the inaccuracy caused by measurement of the conduction distance at the skin surface must be overcome. Determination of this small distance by a skin measurement is especially inaccurate. Radiographs have been used to try to minimize the inaccuracy of distance determination (Matsuda, 1980b; Colin, 1993). Other aspects of nerve conduction velocity determination have inherent problems. The stimulating circuit, recording system, and inadvertent stimulation of nerves and muscles are just some of the concerns.

The long-term goal of such electrophysiologic studies would be to predict the outcome of injury and provide substantiation of current clinical methods of testing as guides to decision-making. At this time, the evaluation of these sensory disturbances is still best done by history and physical examination.

**Merging Science and Application**

Most clinicians agree that early treatment of severe nerve injuries is indicated, but proof is lacking. Animal studies like that of Zuniga (Zuniga and O’Conner, 1987) support this view, but few hard data exist in humans to support this. Most importantly, while an enormous body of knowledge exists on the neurophysiology of the trigeminal system, little of this is pertinent to clinical considerations in trigeminal nerve injuries. Problems arise in comparing results of outcome studies because of the inconsistent use of terms like paresthesia, dysesthesia, and anesthesia. Exclusion and inclusion criteria do not exist, and there certainly have not been any randomized, double-blinded trials of surgical intervention.

The scientific core of knowledge on the subject does strongly suggest that early intervention will capture the “regenerative” power of the nerve cell. Axonal recovery, cellular recovery, and even receptor recovery appear to be time-dependent. Some clinical results support this contention (Donoff and Colin, 1990), but others have not differentiated among patients with regard to demographics of age, gender, race, and mechanism or type of injury. A multicenter retrospective study grouped patients into four categories: patients with lingual and inferior alveolar nerve injuries, and patients who displayed either hypoesthesia or hyperesthesia stimulus testing, with or without pain (Labanc and Gregg, 1992). The major finding of this study of 521 patients was that there was a high predictability of results for the hypoesthetic patients. Thus, clinical findings of anesthesia, Seddon’s neurotmesis, particularly when combined with a Tinel’s-like sign upon palpation in the lingual area for lingual nerve deficits, permit surgical treatment to be carried out with confidence. It is unlikely that this type of injury falls into any of the categories of nerve damage which may show spontaneous resolution of the sensory deficit.

**Summary and Conclusion**

The healing of a cutaneous wound is characterized by tissue repair with the purpose of regenerating dermis and epithelium. Osseous healing seeks to restore osteocyte and an osteoid matrix capable of calcification. Both of these responses take place within reasonable boundaries of space. Surely there are influences other than local factors, but for the most part, the machinery of repair, the cells involved, the molecular biologic events of DNA synthesis, cell division, etc., take place close to the area of injury.

The healing of a damaged nerve is unique. Cellular events occur in a site, the ganglion, located “miles” from the site of injury. Cells around the body of nerve cell extensions—called axons, Schwann cells—have a crucial role in axonal recovery. A body of knowledge regarding so-called neurotrophic factors exists which hasn’t even been mentioned (Seckel, 1990). That nerve tissue is precious goes without saying. The management of nerve injuries of the IAN and lingual nerves in particular is still embryonic. Much has been learned, but much more needs to be learned about the normal and abnormal behavior of injured sensory nerves.
If this review has emphasized anything, it is that the original Seddon classification, if followed literally, remains an excellent guide to the management of nerve injuries. Consistency of description in clinical examination with careful and close follow-up offers the patient and the clinician the best chance of reaching a successful outcome.

REFERENCES


