Evaluation of Nerve Injuries by Evoked Potentials and Electromyography*

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Axons must not only reach muscle but reinnervate it before current clinical methods of measuring peripheral nerve regeneration become useful. Thus there is a delay between the appearance of axons in muscle and measurable function of that muscle. The electromyogram (EMG) may provide earlier evidence that motor fibers of regenerating neuron have reached muscle. A decrease in the number of fibrillations and denervation potentials, and the appearance of nascent motor units, herald the onset of clinical function. Recent evidence suggests that in vivo evoked nerve action potentials (NAP) can be used to evaluate early axon regeneration through an injury. Ability to record NAP's depends on the presence of some moderate- or large-sized axons and provides a certain insight as to axon population at the recording site. The purpose of these studies was to compare evoked NAP's recorded from the distal stump of injured but regenerating nerves with EMG information recorded from musculature innervated by the injured nerve.

Method

Twelve Macaca mulatta monkeys of both sexes were inspected to insure normal function and muscle bulk in the lower extremities. The animals were anesthetized with intravenous phenobarbital. The resting activity of the proximal and distal lower extremity musculature was recorded by concentric needle electrodes. Either the tibial or peroneal nerves in both lower extremities were exposed from their sciatic nerve origin to the point where the nerve entered the proximal lower extremity musculature. The nerve was suspended on bipolar platinum electrodes proximally and distally and on a ground electrode between the proximal stimulating and distal recording montages. Stimulation was increased until a threshold voltage was established; a supramaximal stimulus was then used to obtain the most complete nerve action potential (NAP) possible. The NAP was recorded by dual beam oscilloscope with a differential amplifier and Polaroid camera. Concentric needle electrodes were placed in the proximal and distal musculature and muscle potentials were recorded simultaneously with the evoked NAP's on the oscilloscope. The nerve on one side was then severely crushed so that neither an evoked NAP nor EMG potential could be recorded distal to the injury. The opposite nerve was severed and primarily repaired, using a standardized technique.

At intervals of 3 to 16 weeks, animals were re-examined clinically and electrically. A resting record of EMG activity was also made. Attention was paid to fibrillations, denervation potentials, and nascent motor units. Nerve injuries were exposed and NAP and EMG studies were repeated. Electrode placements for NAP recordings were measured from the injury site by a graduated caliper. EMG tracings from proximal and distal musculature were once again recorded along with the NAP. Recordings were made of the anterior tibial, peroneus, and extensor digitorum longus muscles for the peroneal nerve and the gastrocnemius and flexor hallucis longus muscles for the tibial nerve.

Injured nerves were then removed, pinned on tongue blades, and fixed in 10% buffered formalin. After paraffin embedding, multiple cross sections were made at measured points from the injury, corresponding with the positions of the recording electrodes. Successive sections were stained with Masson, Bodian, and Luxol-fast blue techniques. Longitudinal sections of the injury site were also cut and stained.

A sampling method was used for axon counts which were done on Bodian stained

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sections. At random, 10 of the 100 squares in an ocular square grid were counted and averaged. The square grid covered a field 970 μ in diameter when under oil immersion. The total number of squares containing axons were counted for each field and multiplied by the average count per square for that field, giving the total axon count per field. This was repeated for successive fields until the entire cross section was covered. Field counts were then added to give total fibers per cross section. Previous experience with this technique has shown that it provides a reliable estimate of axon population.

Axons in the initial fields of each cross section examined were measured by an ocular vernier and divided into fibers with large diameters (greater than 9 μ), medium diameters (4 to 9 μ), and small diameters (less than 4 μ). Axons in subsequent fields were categorized by eye while those of questionable diameter were once again measured with the vernier. Longitudinal sections were graded as to neuroma size, axon carry-

**TABLE 1**

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<th>Interval (wks)</th>
<th>Animal No.</th>
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* Neuroma, connective tissue proliferation, axon disorganization and myelin graded on a 0 (none) to 4 (maximum) basis.
† Distance = mm from injury to recording site.
‡ NAP amplitude = height of NAP graded from 0 to 4.
§ Axons divided into large (greater than 9μ), medium (4 to 9μ), small (less than 4μ).
TABLE 2
Sutured nerves

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* Neuroma, connective tissue proliferation, axon disorganization and myelin graded on a 0 (none) to (maximum) basis.
† Distance = mm from injury to recording site.
‡ NAP amplitude = height of NAP graded from 0 to 4.
§ Axons divided into large (greater than 9μ), medium (4 to 9μ), small (less than 9μ).

through, connective tissue proliferation, and axon disorganization. Distal sections were graded for myelin. Histologic information, including axon counts, was correlated with the NAP and EMG data.

**Results**

Conduction velocity, as measured prior to injury in the 24 nerves, averaged 88.4 m/sec, and threshold averaged 0.22 V at a duration of 0.2 msec. When potentials were present following injury, their amplitudes decreased and latency increased as the electrodes were moved distally. These electrical changes corresponded with a decrease in the number of medium and large axons, and an increase in the number of small axons (Tables 1 and 2). If the injured nerve was crushed distal to the recording site the amplitude and latency of the evoked NAP did not change. This suggested that the response was not due to muscle artifact. Examples of traces obtained after injury are shown in Figs. 1 through 6.

**Crushed Nerves.** At 3 weeks after crushing, neither evoked NAP's nor EMG evidence of innervation could be recorded. Instead, fibrillations and deinnervation potentials
Evaluation of Nerve Injuries

Fig. 1. Animal 848. Oscilloscope tracings. The upper trace in each composite is the evoked muscle potential (EMG) recorded from the tibialis muscle; the lower tracing is the in vivo evoked nerve action potential (NAP).  

a. Pre-injury traces. Oscilloscope set at 0.2 V and 5 msec per division for the NAP and 2 mV and 2 msec per division for the EMG.  

b, c, and d. Post-injury traces at varied distances from stimulus. Oscilloscope was set at 0.1 V and 1 msec for the NAP's and 2 mV and 5 msec for the EMG's. Note the absence of EMG potentials in the post-injury recordings while an evoked NAP was present at 27 and 38 mm, but not 44 mm.

were present in proximal and distal musculature. Small axons had regenerated through the injury into the distal stump, as shown by counts made at 15 mm.

At 6 weeks small evoked NAP's were recorded well distal to the crush site, and at 8 weeks they were also seen in two of the three nerves even though fibrillations and deinnervation motor units were present in the proximal portions of the anterior tibial and peroneus muscles. Proximal and distal muscle action potentials could not be evoked. Axons were moderate and small in size with only a few large fibers.

By 12 weeks, large evoked NAP's could be recorded along most of the distal stump and could be correlated with increased numbers of medium axons. The EMG showed proximal but not distal evidence of reinnervation in one animal while there was no EMG evidence of reinnervation in the other animal. At 16 weeks, NAP's were also recorded along most of the distal nerve stump and there was definite EMG evidence of reinnervation. Large- as well as medium-sized fibers were present several centimeters distal to the crush site.

Microscopic evaluation of the injuries gave less specific information than the recordings. Neuromas were small, and axons seemed to grow through the injured area well. Connective tissue proliferation and axon disorganization were minimal in most of the crushed nerves. There was, however, one nerve from which NAP's could not be recorded at 8 weeks. This was probably due to reduced numbers of medium-sized axons in the distal stump, for there were only 700
Fig. 2. Animal 845. At 12 weeks after nerve was crushed, NAP's (*upper traces a and b*) recorded at 48 and 57 mm but muscle potentials absent in gastrocnemius. The EMG's (*lower trace c*) demonstrated fibrillations and denervation potentials. Oscilloscope was set at 0.1 V and 2.0 msec per division for the NAP and 2 mV and 5 msec for the EMG.

at 15 mm and 94 at 25 mm distal to the crush. This injury site, when compared to others in the crush series, had increased connective tissue proliferation and axon disorganization.

*Sutured Nerves.* Evoked potentials were recorded from two of the three nerves studied 8 weeks after suture even though EMG evidence of distal reinnervation was lacking. Potentials were also recorded from one of the two nerves studied at 12 weeks despite EMG evidence of total distal deinnervation. There was EMG evidence of proximal but not distal muscular reinnervation in one of the two 16-week specimens where NAP's could be recorded, but no EMG evidence of distal return in the other 16-week specimen where NAP's could not be recorded.

Small fibers predominated at 3, 6, and 8 weeks, and some medium axons were present at 12 weeks. Large axons appeared at 16 weeks but not in the numbers seen in the crush series. Connective tissue proliferation and axon disorganization were marked in one 16-week sutured nerve where neither evoked NAP's or EMG evidence of reinnervation could be recorded. These injury site changes were reflected in the axon distribution for there were only a few medium fibers and a large number of small fibers 9 mm from the injury. Similar histologic changes were seen at 8 weeks where NAP's were absent, but not at 12 weeks where connective tissue proliferation and axon disorganization were comparatively less than that seen in the nerve with a recordable potential.

Myelinization of the distal stump in both the crush and suture series was related to moderate or large axons. When distal myelin was significant (Grade 2 or higher) an NAP could usually be evoked (Tables 1 and 2).

**Discussion**

Previous histologic and clinical studies have suggested that there is a delay between the time of return of axons to musculature
and the onset of muscle contraction. In 1944, Gutmann and Young\(^2\) demonstrated this terminal delay in rabbits, whose nerves regenerate exceptionally fast. When a nerve was crushed close to muscle, a delay of 11 days resulted. If the nerve was crushed well proximal to the muscle, a 22-day delay occurred between the return of axons to muscle and the onset of reflex function. For severance and suture of a nerve close to muscle, a 25-day delay was found. If suture of the severed nerve was secondary, the delay stretched to 55 days. Sunderland\(^6\) has discussed terminal delay in relation to human injuries although data comparable to that observed in the laboratory were difficult to obtain. He emphasized three factors that might be responsible for terminal delay: 1)
units. This directly substantiates the work of Gutmann and Young\textsuperscript{1,2} as well as that of Sunderland\textsuperscript{6} and others who have inferred this from histological and functional observations.

The delay is greater in severed and sutured nerves than in a less severe injury such as crushing. As can be seen from Table 1, EMG evidence of reinnervation followed closely the presence of evoked nerve action potentials in the crush series. There was, however, still a hiatus of from 2 to 6 weeks between the onset of NAP's and EMG evidence of distal reinnervation. In the severed and repaired nerves the temporal difference

Our studies have defined this terminal delay by neurophysiologic techniques. Whenever in vivo NAP could not be recorded, EMG evidence of reinnervation was also lacking. On the other hand, the NAP could be recorded in many instances even though EMG evidence of distal function was absent. Although the EMG may give evidence of reinnervation weeks before clinical evidence is available, there is also a significant delay between the appearance of relatively mature axons in the distal stump and their ability to reinnervate muscle in a manner that can be recorded by EMG. Axons of a caliber sufficient to conduct an evoked NAP had penetrated well into the distal stump weeks before the motor end-plates were reinnervated sufficiently to record a muscle action potential or even diminished numbers of fibrillations or nascent motor

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\textbf{Fig. 5.} Animal 111. Tracing made from Nerve 111-L, severed and sutured 8 weeks previously. Small NAP recorded up to 31 mm distal to the neuroma. Fibrillations and deinnervation potentials were recorded from proximal and distal musculature. Oscilloscope was set at 0.2 V and 5 msec per division for the NAP.

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\textbf{Fig. 6.} Animal 59. Tracings 16 weeks after severance and suture. \textit{a.} The EMG was recorded from proximal musculature and shows no activity (\textit{upper trace}). NAP of moderate amplitude but slow velocity was recorded 31 mm from the injury (\textit{lower trace}). \textit{b.} The NAP has increased latency and lower amplitude 47 mm from the injury. Oscilloscope was set at 0.1 V and 2 msec per division for the NAP's and 5 mV and 5 msec for the EMG's.
between the onset of recordable NAP's and EMG evidence of reinnervation was even longer than in the crush series. Axons of sufficient maturity to produce function span the site of a crush injury faster and in greater numbers than in the severed and sutured nerve. These medium and large axons probably tend to keep pace with one another and arrive at their distal input in waves rather than in staggered numbers.

Since evoked potentials can be recorded before there is evidence of distal reinnervation by EMG, they provide a technique for the relatively early evaluation of nerve injuries in continuity. The technique must be used, however, only in conjunction with the traditional clinical and EMG studies. During the early weeks after injury, classic techniques such as palpation and inspection of the lesion yield sparse information, and the situation is analogous to assessing blood flow by palpation of the vessel instead of using a flowmeter. Some technique of evoking potentials through the area of injury may be helpful to the surgeon trying to make a decision about whether to resect the injury or leave it alone.

**Summary**

*In vivo* evoked nerve action potentials (NAP) were recorded from the lower limb nerves of primates. Baseline electromyograms (EMG) and evoked muscle potentials were also recorded. Exposed nerves were then injured by crushing on one side, and severance and suture on the other. At intervals of 3 to 16 weeks, evoked NAP's and EMG were repeated and the nerves were removed for histological study, including axon counts.

NAP's could be recorded weeks before EMG evidence of distal reinnervation was available. Whenever there was EMG evidence of distal reinnervation, an NAP could
also be recorded over the entire length of the distal nerve stump. Ability to record an evoked NAP from the distal stump depended on the presence of moderate or large diameter axons and myelinization. Amplitude of the potential could be related to the axon population beneath the recording electrode. Axons of sufficient maturity to conduct NAP's penetrate well into the distal stump weeks before motor end-plate reconstruction can be recorded by EMG.

Evoked potentials provide useful objective information about the early period of peripheral nerve regeneration.

References