NEURAL MECHANISMS OF ABNORMAL SENSATIONS AFTER NERVE INJURY

Patients with injured nerves often report that tapping at the site of injury produces tingling sensations in the original territory of the nerve. Recordings from single nerve fibers in primates indicate that a sensitivity to mechanical stimuli develops after a nerve injury at regenerating nerve fibers; this sensitivity likely accounts for the sensations reported by patients. Vibratory stimuli were applied to regenerating fibers to determine their frequency-response properties. Fibers were grouped into three types, based on their responses: (1) fibers most sensitive to frequencies less than 5 Hz; (2) fibers most sensitive to middle frequencies (10–50 Hz); and (3) fibers most sensitive to frequencies greater than 100 Hz. These responses are similar to those of normal mechanoreceptors in the skin responsible for touch sensation. On the basis of these results, we propose a mechanism that explains the development of mechanosensitivity in injured nerve fibers.

INTRODUCTION

Injury to a limb sometimes results in injury to a nerve, which typically leads to abnormal sensations. Immediately after injury, patients report the perception of numbness for nerves that supply skin and muscle weakness or paralysis for nerves that supply muscle. These effects result from the tissue no longer being supplied by the nervous system.

As tissue begins to heal, most patients report that light tapping at the nerve injury site produces a tingling "pins and needles" sensation that projects to the area originally supplied by the nerve. This phenomenon is known clinically as the Tinel sign. As the nerve regenerates toward its original target, the site on the limb at which mechanical stimulation produces these sensations progresses out along the limb; thus, the Tinel sign is used to determine the extent of nerve regeneration.

The initial objective of our research was to characterize the response properties of injured nerves and to identify the neural mechanisms responsible for the Tinel sign. Our experiments led to the discovery that regenerating nerve fibers develop an entrained response to vibratory stimuli, similar to that seen in normal receptors in the skin. On the basis of these observations, we propose a mechanism to explain the development of mechanical sensitivity in injured nerves. The results of this study are described in greater detail elsewhere.1

EXPERIMENTAL TECHNIQUE

A controlled injury was produced in a minor cutaneous nerve of a monkey. Two to six weeks thereafter, the injured nerve was exposed, and recordings were made from single nerve fibers proximal to the injury site. Action potential activity was recorded in response to vibratory mechanical stimuli applied to the injury site. All protocols were approved by the Johns Hopkins Animal Care and Use Committee.

Nerve Injury

In an anesthetized monkey, the superficial radial nerve (which supplies the back of the hand) or the sural nerve (which supplies the side of the foot) was exposed. A fine suture was tied tightly around the nerve, and the nerve was then cut distal to the suture. In this nerve injury model, the regenerating nerve fibers are constrained by the suture to form a neuroma, a compact bulb of nervous tissue.2

Neural Recording Technique

Recordings were performed two to six weeks after the nerve injury. The monkeys were anesthetized with a continuous infusion of pentobarbital (3–6 mg/kg·h). The animals were artificially ventilated to maintain their expired pCO₂ at 32 to 40 torr. Electrocardiogram and heart rate were monitored continuously. Core temperature, measured via a rectal probe, was maintained near 38°C by using circulating-water heating pads under servo control.

A skin incision was made along the course of the nerve, and the edges of the incision were sutured to a metal ring to form a well (Fig. 1A). Warm paraffin oil was placed in the well to prevent drying of the tissue and also to form an electrical insulator.

A standard teased-fiber technique3 was used to record action potential activity from single nerve fibers. At the proximal end of the well, the nerve was dissected from connective tissue and placed in a groove next to a small dissection platform. Under an operating microscope, the tissue surrounding the nerve was opened, and a small
bundle of nerve fibers was cut away from the nerve trunk and rotated onto the dissection platform. The bundle was thus disconnected from the central nervous system while still connected to the neuroma. This bundle was then dissected into fine strands, which were placed on a fine electrode for extracellular recording of action potential activity. Although each strand contained several nerve fibers, the shapes of their extracellularly recorded action potentials differed. An amplitude and time window discriminator was used to differentiate the action potential shape of a single nerve fiber from other action potentials and from noise. At the distal end of the well, the tissue above the neuroma was dissected to allow for mechanical stimulation at the end of the regenerating fibers.

Mechanical Stimulation Techniques

Regenerating nerve fibers responsive to mechanical stimulation were first identified by tapping the neuroma with a cotton swab. Sinusoidal stimuli of varying frequency and amplitude were applied to the neuroma to characterize the frequency-response properties of fibers responsive to tapping.

An APL-developed, feedback-controlled mechanical stimulator was used to deliver displacement stimuli to the neuroma via a 0.8-mm-diameter Plexiglas probe. Stimuli consisted of cosine waves of various amplitudes and frequencies superimposed on a 300-μm step indentation (Fig. 1B). The probe was moved across the neuroma, and a micromanipulator was used to locate the site of maximum sensitivity. Sinusoidal stimuli were delivered to this site at frequencies of 5, 10, 20, 50, 75, and 100 Hz. Peak-to-peak amplitudes at each frequency ranged from 50 to 800 μm. Each stimulus was 1 s in duration, and the interstimulus interval was 14 s. The 300-μm step indentation was applied 1 s before the stimulus and removed at the end of the 1-s stimulus interval (Fig. 1B).

On the basis of the fiber's response to preliminary tests with vibratory stimuli, appropriate amplitude ranges were selected for each fiber so that the minimum amplitude could be determined for entrainment (one action potential for each cycle of stimulation) at each frequency. All subsequent stimuli were presented under computer control. The computer also recorded the time of occurrence of each discriminated action potential.

**RESPONSE OF REGENERATING NERVE FIBERS TO VIBRATORY STIMULI**

Thirty nerve fibers were studied in detail over the course of twelve experiments. We studied only the faster-conducting myelinated fibers; the mean conduction velocity of the fibers in this study was 23 ± 2 m/s. Slower-conducting unmyelinated fibers were not studied.

Figure 2 presents an example of the response of a single nerve fiber to a 20-Hz vibratory stimulus (peak-to-peak amplitude of 150 μm) applied to the neuroma. The cosine stimulus is shown at the top of the figure, and the
response is shown at the bottom of the figure, where each vertical tick corresponds to the time at which an action potential occurred. For this figure, the time for the action potential to propagate from the neuroma to the recording site (4 ms) has been subtracted. The response of the fiber was clearly in phase with the sinusoidal stimulus.

The response of this fiber to a 75-Hz stimulus of different amplitudes is shown in Figure 3. It responded weakly at lower stimulus amplitudes (bottom of Fig. 3A). The duration of response increased with the stimulus amplitude. At higher stimulus amplitudes (top of Fig. 3A), an action potential occurred for each stimulus cycle, and thus complete entrainment was observed. As indicated in Figure 3B, the response during the 1-s stimulus interval increased as the stimulus amplitude was increased to 300 \( \mu \text{m} \), at which point the response reached a plateau that corresponded to complete entrainment.

The periodicity of the response was investigated by looking at the time between action potentials. Figure 4 is a histogram of the time between action potentials for the response of the fiber shown in Figure 3. At lower amplitudes, the histogram shows peaks at integer multiples of the stimulus period, corresponding to Figure 3A, which shows that at low amplitudes a response does not occur for each stimulus cycle. At 300 \( \mu \text{m} \), complete entrainment occurs, and the histogram develops a single peak. At 800 \( \mu \text{m} \), a peak appears in the histogram at a short interval, due to the development of a doublet action potential response during some stimulus cycles.

Figure 5A depicts the response of the fiber to various stimulus frequencies and amplitudes. The order of stimulus presentation was randomized to minimize systematic effects of stimulus interactions.\(^5\) Data were normalized by dividing the response to a given stimulus by the total number of cycles in the sinusoidal stimulus; these normalized stimulus response curves are called entrainment curves. The fiber responded best to the 30-Hz stimulus, as evidenced by the fact that a lower stimulus amplitude was needed to achieve entrainment. At high stimulus frequencies, entrainment required substantially higher stimulus amplitudes.

The frequency-response properties of a regenerating nerve fiber have been represented by a plot, called the tuning curve, of the smallest amplitude required for entrainment as a function of stimulus frequency. The tuning curve for the fiber in Figure 5A is shown in Figure 5B. Because 100% entrainment was not always achieved, we used a 90% entrainment criterion for the tuning curves. This plot graphically illustrates that the fiber was sensitive to a wide range of frequencies.

Complete tuning curves were obtained for nineteen fibers (Fig. 6); four with tuning curves having a positive slope, meaning the fibers were most sensitive to the lowest frequency tested (Fig. 6A); eleven with U-shaped
tuning curves, meaning the fibers were sensitive to a broad range of frequencies (Fig. 6B); four with tuning curves having a negative slope, meaning the fibers were most sensitive to the highest frequency tested (Fig. 6C). For eleven additional fibers, a complete tuning curve was not obtained, but sufficient testing was performed to identify the range over which the fiber was most sensitive. The thirty fibers in this study thus could be grouped into three distinct types on the basis of their frequency-response properties: (1) seven fibers were most sensitive to low frequencies of vibration (≤5 Hz), (2) thirteen fibers were sensitive over a broad range of middle frequencies (10–75 Hz), and (3) ten fibers were most sensitive to high frequencies (≥100 Hz).

RESPONSE OF CUTANEOUS RECEPTORS TO VIBRATORY STIMULI

Three types of response to vibratory stimuli are also seen in low-threshold mechanoreceptors located in the skin. For example, in the glabrous (nonhairy) skin of the hand, three types of low-threshold mechanoreceptors are present (Fig. 7). These receptors are responsible for different aspects of touch sensation. Merkel cells respond best to gentle-pressure stimuli and are most sensitive to low frequencies of vibration. Meissner corpuscles respond well to stroking stimuli and are most sensitive to frequencies of about 50 Hz. Pacinian corpuscles are most responsive to high frequencies of vibration (about 250 Hz). We speculated that the three types of tuning curves seen in the injured nerve might correspond to the three types of receptors in the skin. As a first test of this hypothesis, we obtained tuning curves for the three types of low-threshold mechanoreceptors found on the glabrous skin (Fig. 8). The Merkel cells were most sensitive to low-frequency vibrations and had tuning curves with a positive slope. Meissner corpuscles had U-shaped tuning curves. Pacinian corpuscles were most sensitive to high-frequency vibrations and had tuning curves with a negative slope.

Although the threshold for activation is significantly higher in a neuroma preparation compared with that in receptors in normal skin, tuning curve shapes obtained from regenerating nerve fibers (Fig. 6) are similar to those from cutaneous receptors (Fig. 8). This result suggests that frequency-response properties of cutaneous receptors are determined, at least in part, by the properties of the parent nerve fiber, contradicting current physiology textbook tenets that cite the structure of the cutaneous receptor end organ as the determining factor. For example, the laminated, onion-like structure of the Pacinian corpuscle has been shown to act like a high-pass mechanical filter and is thought to be responsible for the Pacinian corpuscle being most sensitive to high frequencies of vibration. Although no corpuscular structures are present in the neuroma, some regenerating nerve fibers exhibited a selective sensitivity to high frequencies of vibration, indicating that the mechanical transducer itself must have some frequency selectivity.
Our results also suggest that a regenerating nerve fiber adopts the mechanical response characteristics (tuning curve features) that it had when it was intact with its cutaneous receptor.

HYPOTHESIS ON THE DEVELOPMENT OF MECHANICAL SENSITIVITY IN REGENERATING NERVE FIBERS

Our results suggest the following hypothesis to explain why regenerating nerve fibers develop mechanical sensitivity and why patients may develop a Tinel sign: The frequency-response properties of low-threshold cutaneous mechanoreceptors are determined, at least in part, by the frequency-response properties of the mechanical-to-electrical transducers located in the terminal membrane of the nerve fiber. The cellular components necessary for mechanical-to-electrical transduction are manufactured in the cell body and conveyed to the terminal membrane by means of an active transport system within the nerve fiber referred to as axonal transport. When the nerve is cut, these cellular components accumulate at the site of injury and are incorporated into the membrane to impart mechanical sensitivity.

EXPERIMENTS IN PROGRESS

To test the hypothesis that the cellular components necessary for transduction are transported from the cell body to the periphery, we are conducting a series of three experiments to alter axonal transport:16 (1) Axonal transport is known to slow down when the nerve temperature is decreased.17 In preliminary experiments, we found that the rate of development of mechanical sensitivity was significantly reduced by lowering nerve temperature from 38°C to 28°C. (2) A nerve injury causes the cell body to increase the manufacture and transport of certain substances. In preliminary experiments, the rate of development of mechanical sensitivity was significantly increased by making a conditioning lesion on the nerve one week before the acute experiment. (3) Transport can be stopped by cutting the nerve proximal to the recording site; this procedure significantly reduced the rate of development of mechanical sensitivity. These three different experiments provide evidence that axonal transport is involved in the development of mechanosensitivity at a nerve injury site.

To test whether the vibratory properties of the cutaneous mechanoreceptor match those of the parent axon, we have planned an experiment in which first the vibratory properties of a given low-threshold cutaneous mechanoreceptor will be determined; then the receptor end organ will be cut away from the skin, leaving the bare, injured nerve fiber. Within about 10 h, this ending should develop mechanosensitivity, at which time we will again determine the frequency-response properties of the regenerating nerve fiber and compare the tuning curves with those obtained earlier from the intact receptor.

RELATED RESEARCH AND FUTURE DIRECTIONS

Although the mechanosensitivity that develops in the regenerating nerve fibers appears to account for the Tinel sign, other abnormalities in pain sensation have been reported by some patients, in particular that lightly touching the skin in the affected limb is very painful. Pain is thus perceived in reaction to stimuli that normally activate only the low-threshold mechanoreceptors that signal touch sensation.

Possible explanations for this phenomenon include the following: (1) The cutaneous nociceptive receptors that normally respond only to intense, noxious stimuli and signal pain sensation have been sensitized so that they now respond to mild mechanical stimuli. (2) The nerve injury has caused a breakdown in the isolation between nerve fibers so that signals in the nerve fiber connected to low-threshold mechanoreceptors cross talk at
the injury site to the nerve fibers connected to the nociceptive receptors. (3) The processing of signals in the central nervous system has been altered so that input from low-threshold mechanoreceptors gains access to the pain-signaling pathway.

We recently developed a model for this abnormal pain state, on the basis of available clinical and experimental data, proposing that abnormalities occur in both the peripheral and central nervous systems. This model predicts that certain therapeutic manipulations will result in pain relief. Future experiments will focus on verifying this model.

SUMMARY

Regenerating myelinated fibers develop a sensitivity to vibratory stimuli that appears as soon as 4 h after a nerve injury. The fibers were classified into three groups according to the frequency range over which they were most sensitive to vibratory stimuli applied to the regenerating tip: (1) a low-frequency group most sensitive to vibratory frequencies less than Hz, (2) a mid-frequency group most sensitive to a broad range of middle frequencies (20–75 Hz), and (3) a high-frequency group most
sensitive to frequencies greater than 100 Hz. These three response classes are similar to the three classes of response associated with the different cutaneous low-threshold mechanoreceptors: (1) slowly adapting receptors (e.g., Merkel cells) are most sensitive to low frequencies of vibration, (2) rapidly adapting receptors (e.g., Meissner corpuscles) are sensitive to a wide range of middle frequencies, and (3) Pacinian corpuscles are most sensitive to high frequencies. These observations led us to postulate that mechanical sensitivity in regenerating fibers results from the accumulation, at the regenerating tip, of the mechanical-to-electrical transducers that normally transported down the nerve fiber to the cutaneous mechanoreceptors.

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REFERENCES


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