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Minireview

Magnetic measurements of peripheral nerve function using a neuromagnetic current probe

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Abstract

The progress made during the last three decades in mathematical modeling and technology development for the recording of magnetic fields associated with cellular current flow in biological tissues has provided a means of examining action currents more accurately than that of using traditional electrical recordings. It is well known to the biomedical research community that the room-temperature miniature toroidal pickup coil called the neuromagnetic current probe can be employed to measure biologically generated magnetic fields in nerve and muscle fibers. In contrast to the magnetic resonance imaging technique, which relies on the interaction between an externally applied magnetic field and the magnetic properties of individual atomic nuclei, this device, along with its room-temperature, low-noise amplifier, can detect currents in the nano-Ampere range. The recorded magnetic signals using neuromagnetic current probes are relatively insensitive to muscle movement since these probes are not directly connected to the tissue, and distortions of the recorded data due to changes in the electrochemical interface between the probes and the tissue are minimal. Contrary to the methods used in electric recordings, these probes can be employed to measure action currents of tissues while they are lying in their own natural settings or in saline baths, thereby reducing the risk associated with elevating and drying the tissue in the air during experiments. This review primarily describes the investigations performed on peripheral nerves using the neuromagnetic current probe. Since there are relatively few publications on these topics, a comprehensive review of the field is given. First, magnetic field measurements of isolated nerve axons and muscle fibers are described. One of the important applications of the neuromagnetic current probe to the intraoperative assessment of damaged and reconstructed nerve bundles is summarized. The magnetic signals of crushed nerve axons and the determination of the conduction velocity distribution of nerve bundles are also reviewed. Finally, the capabilities and limitations of the probe and the magnetic recordings are discussed.

Keywords: magnetic measurements, neuromagnetic current probe, peripheral nerve, muscle, nerve conduction velocity distributions, reconstructed nerves

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Introduction and background

The electric signals associated with living tissues have been investigated since the early part of the 18th century. These early investigations have led to several recent innovations in medical diagnostic and treatment techniques. Some examples of such innovations based on electric signals are the electrocardiogram (ECG), the electromyogram (EMG) and the electroencephalogram (EEG). In addition, the original measurements of human magnetocardiogram (MCG),[1] the magnetoencephalogram (MEG)[2] and the magnetomyogram[3] provided clear evidence of the existence of the magnetic fields that are associated with ionic action currents in electrically active tissues. Cohen *et al.*[4] used a point-contact superconducting quantum interference device (SQUID) magnetometer for the first time inside a shielded room to measure the MCG. They reported an order-of-magnitude improvement in sensitivity in the recorded MCG over the previously recorded MCGs. The same investigator[5] repeated the MEG measurements using a more sensitive SQUID magnetometer without noise averaging. He compared the EEG and the MEG of alpha-rhythm recorded simultaneously from normal and abnormal subjects. He showed that the MEG yielded some new and different information that was provided by the EEG. Because the heart can produce a relatively large magnetic field compared with the brain and some other organs, the early research on biomagnetic fields originated with mathematical modeling of MCG. The early experimental studies were also

concentrated largely on the MCG. In addition, those experimental studies suffered from unavoidable poor spatial resolution and low sensitivity because of the lack of sophisticated detecting instruments. With improved technologies, the investigations expanded into brain function, and preliminary studies of the evoked MEG started to emerge in the 1980s. Those studies provided some details about which neuronal populations were contributing to the magnetic signals arising from the brain. However, the signals from single neurons were too weak to be detected, and a group of more than 10,000 dendrites were needed as a group to produce a detectable MEG signal.[6] At the time, abundant physiological, technical and mathematical limitations hampered quantitative comparisons of theory and experiment involving human ECGs as well as other biomagnetic recordings. The lack of accurate microscopic source models made it even harder to agree on what specific physiological factors affected the strength of the MEG and other biomagnetic signals, and which factors dominated the attainable spatial resolutions.[6]

Over the last three decades, a significant number of studies have been conducted to measure and analyze the magnetic fields created by ionic currents flowing in isolated nerve axons and muscle fibers. These measurements have been supported by some sophisticated theoretical investigations and development of an ultrasensitive room-temperature amplifier and a neuromagnetic current probe. Now magnetic recording at the cellular level has been well established as a quantitative measurement technique of action current, and this methodology has been applied to clinical neurophysiology. In this review, we first describe the generation of cellular magnetic field and the instrumentation required to measure these signals. We then present the very first investigations on magnetic fields created by single axons and bundles. We finally discuss non-uniform propagation of action signals, and some clinical applications of this technique to investigate injured and reconstructed nerve bundles.

First measurement of magnetic fields created by a single nerve axon

It is well known that both a propagating action potential and a magnetic field are associated with an electrically active nerve axon. Traditionally, only the electrical potential is measured; however, the magnetic field, which encircles the nerve as shown in Figure 1, represents an additional source of information about the nerve. The typical configuration of the encircling magnetic field is indicated with the wide arrows. According to Ampere's law, the strength of this field is proportional to the total current flowing through the neuromagnetic current probe. The typical strength of the encircling magnetic field 1 mm from a 10-μm diameter nerve axon is about 50 fT,[6] which is nine orders of magnitude smaller than the magnetic field of the Earth.

Wikswo and his colleagues at Vanderbilt investigated these magnetic fields in great detail. They first started these detailed investigations using isolated nerves and

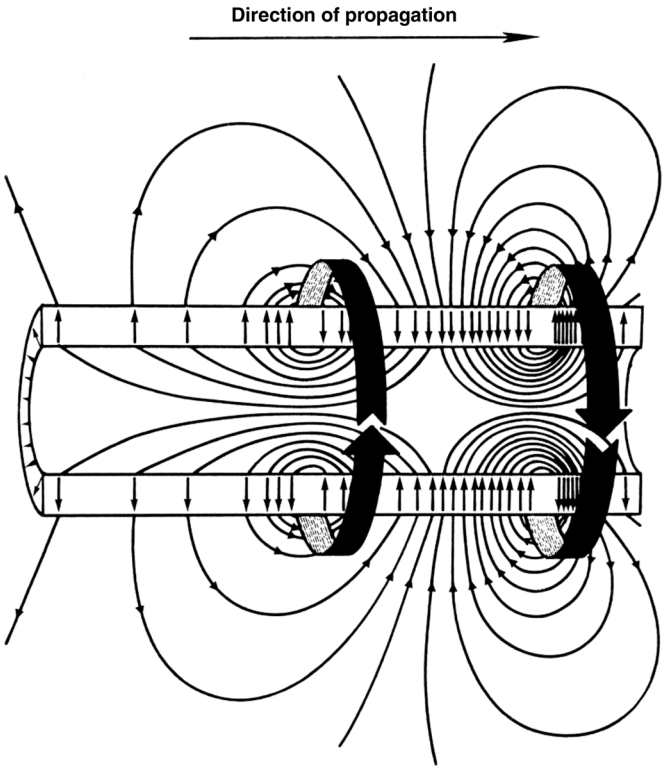

Figure 1 A cross-section of a nerve axon showing the electric current (thin lines) and the magnetic field (wide bands) associated with a propagating nerve action signal. The membrane thickness is greatly exaggerated. (Reprinted from Wikswo[7])

muscles. They explored simultaneously the theoretical aspects, the instrumentation requirements and the experimental techniques required for both basic and applied research into cellular magnetic fields. The first successful measurement of the magnetic field produced by a peripheral nerve was reported by the same group in 1980.[8] They excised the sciatic nerve from a frog and threaded it through a toroid. The original toroidal pickup coil was built using a ferrite-core with 1.2 mm width, 0.6 mm inner radius and 1.3 mm outer radius, and it was wound with just four turns of copper wire. They immersed both the toroidal pickup coil and the nerve in a saline bath and used a very brief electric pulse to stimulate the nerve. They had built the pickup coil such that the ionic current within the nerve passed through the toroid but the return current external to the nerve did not. A SQUID magnetometer detected the current induced in the toroid winding, due to the ionic or action currents in the nerve bundle. The first peak of the observed biphasic magnetic signal had a 70 pT amplitude with 1 ms total duration. They also showed a reversal of polarity when the direction of the propagation was reversed. These investigators subsequently improved their recording techniques and instrumentation[9–12] to a point where a variety of isolated, one-dimensional tissues can be studied.[13–16] One important advance was the development of a room-temperature amplifier that is sensitive enough to measure the current in the miniature toroidal pickup coil windings, thereby eliminating the cost and inconvenience of cryogenic technology.[17,18]

In addition to the very first measurement of the magnetic field created by a nerve bundle, Wikswo's group also went on to carry out one of the most fundamental biomagnetic experiments that could be performed on a nerve: the simultaneous measurements of the magnetic field and transmembrane potential created by just a single axon.[19–21] A schematic drawing of their experiment to measure the transmembrane potential and the magnetic field of an isolated crayfish median giant axon is shown in Figure 2.[20] Roth and Wikswo[20] utilized the very same technique employed during their earlier experiments to measure these signals. The measured magnetic field and the transmembrane potential of the crayfish giant axon are shown in Figure 3. These data, and also other data recordings, were analyzed using their own mathematical models that had been developed specifically for analyzing the magnetic field produced by nerve axons.[22–27] As noted before, by Ampere's law, the magnetic field measured by the toroid is proportional to the net current flowing through it. However, the net current is the sum of the intercellular current and fraction of the extracellular current passing through the neuromagnetic current probe called the return current. For a probe with a small inner radius, the return current is negligible and the recorded magnetic signal is due mainly to the intracellular current, which is equal to the axial derivative of the transmembrane potential divided by the resistance per unit length of the fiber.[26] If the probe is large enough that the return current is significant, it cannot be neglected unless the space between the axon and the toroid is filled with an insulator, for example mineral oil or petroleum jelly. The return current can be evaluated using Ohm's law once the extracellular potential of the tissue sample is identified. The extracellular potential can be obtained by solving the Laplace equation.[27,28] Woosley *et al.*[27] also investigated the inverse problem associated with recorded single axon

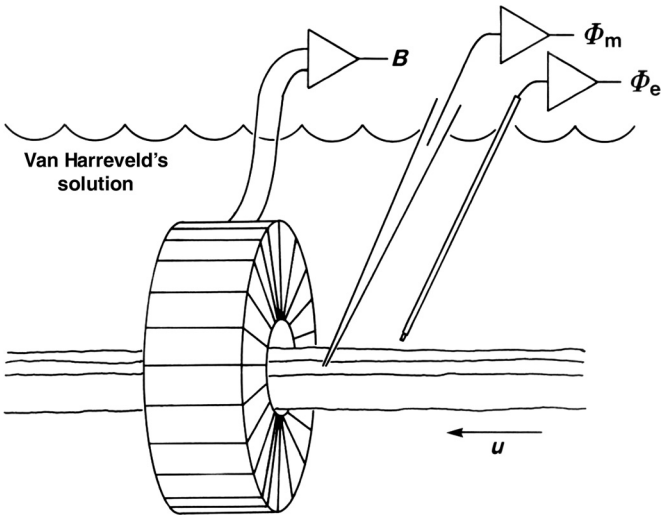

Figure 2 Schematic diagram of an experiment to measure the transmembrane potential Φ_m, the extracellular potential Φ_e and magnetic field B produced by a single crayfish giant axon. A toroid is used to measure the magnetic field, a glass microelectrode is used to measure the transmembrane potential and an extracellular electrode is used to measure the extracellular action potential. (Reprinted from Roth and Wikswo[20] with kind permission from Elsevier)

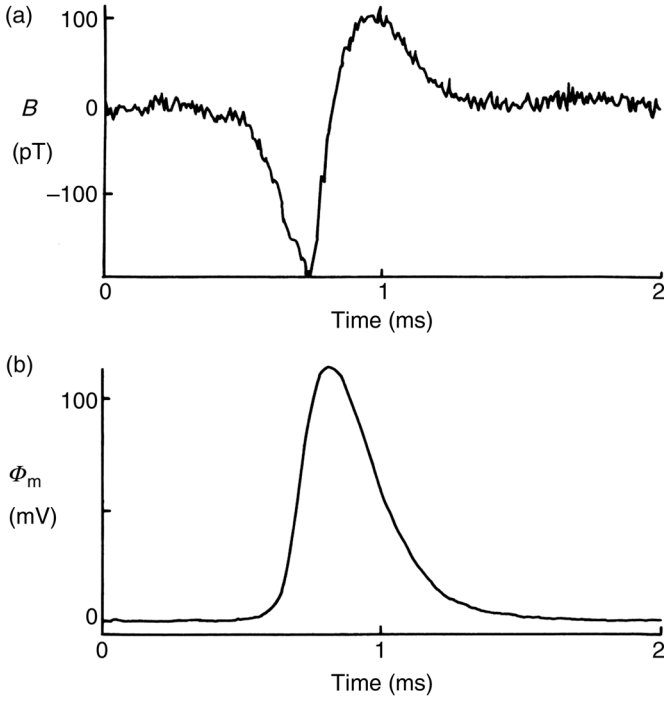

Figure 3 (a) An un-averaged magnetic field B and (b) the transmembrane potential Φ_m, measured from an isolated crayfish giant axon. (Reprinted from Roth and Wikswo[20] with kind permission from Elsevier)

magnetic fields, which is the calculation of the transmembrane potential from the measured magnetic field. Figure 4 shows a comparison of the measured transmembrane potential and the calculated transmembrane potential from the measured magnetic field of an isolated crayfish giant axon. While the shapes of these two traces agree quite well, their amplitudes do not. However, Woosley and his colleagues found that they could adjust just one unknown parameter in the model until the amplitudes of those two traces matched. Using this technique for the intracellular conductivity, they found the intracellular conductivity of a crayfish giant axon was 1.44 S/m with ± 0.33 S/m uncertainty.

Wikswo and colleagues[29,30] also reported the first measurement of compound action current (CAC) associated with a nerve bundle. Performing an experiment on an isolated frog sciatic nerve bundle with the neuromagnetic current probe, they measured the CAC associated with the individual ionic current in each axon in the nerve bundle. During the same experiment, they simultaneously recorded the compound action potentials created collectively by the individual axon in the bundle. Wijesinghe and colleagues[28,29] simultaneously kept developing the mathematical models needed for analyzing the recorded data. In doing so, they used an anisotropic volume conduction model to simulate the single fiber action signals (SFASs) needed for the inverse model[28] which furthermore included the effects of fiber myelination in the anisotropic axial and radial conductivities, σ_z and σ_ρ. For this purpose, they used the expressions derived by Roth *et al.*[30] for calculating the 'effective' axial conductivity, σ_z, and radial conductivity, σ_ρ, of the nerve bundle in terms of other bundle parameters. In addition, these expressions included the effect of inhomogeneities in the area surrounding the active fiber

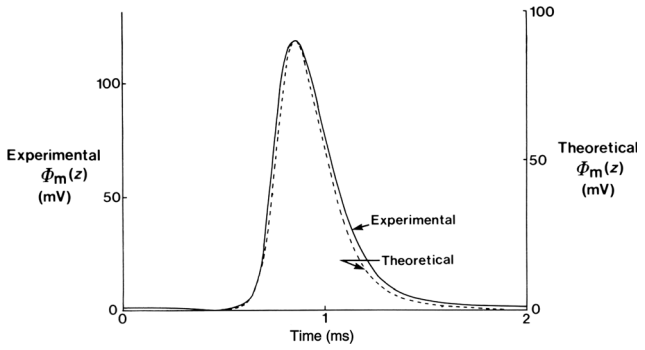

Figure 4 Comparison of the measured transmembrane potential (512 averages) and the transmembrane potential calculated from measured magnetic field. (Reprinted from Roth and Wikswo[20] with kind permission from Elsevier)

which arose from the neighboring fibers. During their investigation, Roth *et al.* assumed that microscopically inhomogeneous fibers could be replaced by a macroscopically homogeneous medium with electrical properties represented by 'effective' electrical conductivities. Variations in the intracellular action potentials with recording temperature and axon conduction velocity were also incorporated into the volume conduction calculation. Figure 5 depicts a block diagram of their forward calculation which simulated the compound action signals (CASs).[2] Using recorded magnetic and electric signals as inputs for their inverse model,[29] they calculated the fiber diameter histograms of the nerve bundle and compared them with the fiber diameter histogram that was obtained from the histologically prepared nerve bundle after the experiment.

The inverse model of the CASs allows the calculation of the conduction velocity distributions (CVDs) from the measured CASs. The CAS is a weighted sum of the delayed SFASs, which are dispersed in time. The CAS can be written as

$$\mathrm{CAS}(t,l) = \sum_{j=1}^{N} \mathrm{SFAS}_j(t-\tau_j; v_j) \qquad (1)$$

where l is the propagation distance, N is the number of active fibers in the nerve bundle, v_j is the conduction velocity of the jth nerve fiber, SFAS_j is the SFAS for the jth nerve fiber and τ_j is the time delay between the stimulus time and the arrival time at the recording electrode of the jth nerve fiber. The above equation can be reduced to a product of two matrices[28]

$$(\mathrm{CAS})_{N\times 1} = (\mathrm{SFAS})_{N\times M} \cdot (\mathrm{ATD})_{M\times 1} \qquad (2)$$

where the dot denotes matrix multiplication; $(\mathrm{CAS})_{N\times 1}$ is the digitized CAS; N is the number of data points in the digitized CAS; M is the number of bins in the arrival time distribution (ATD); $(\mathrm{SFAS})_{N\times M}$ is the matrix with all digitized extracellular SFAS, corresponding to all conduction velocity classes; and $(\mathrm{ATD})_{M\times 1}$ is the arrival time distribution matrix.[26] The ATD can be transformed into CVD using the propagation distance, l.[28]

Figure 6 shows the recorded signals during one of the experiments and the corresponding CVD histograms. The agreement between the three predicted CVDs is best for the faster conduction velocity classes, whereas the slower classes are more susceptible to errors primarily due to the recording noise embedded in the data. This detailed analysis showed that the magnetic technique may have some advantages over the electric technique in determining the fiber diameter histograms. In addition, Wikswo's group[29,31] investigated the effect of recording temperature on the histograms and the conduction velocity of individual action signals.

Action signal propagation that exhibits a variation in the shape of signals along the sample (non-uniform propagation) is difficult to measure using the traditional electric methods because it requires either multiple simultaneous measurements, or sequential recordings made while scanning the sample. The traditional intracellular recording methods cannot be applied for this purpose because of the risk of cell damage inherent from multiple electrode impalements. Also, extracellular electric potential measurements do not provide accurate quantitative information[31] because of the exquisite sensitivity of the measurement to the spacing of the electrodes and the amount of moisture on the exterior surface of the nerve bundle. However, the neuromagnetic current probe benefits from the absence of physical contact

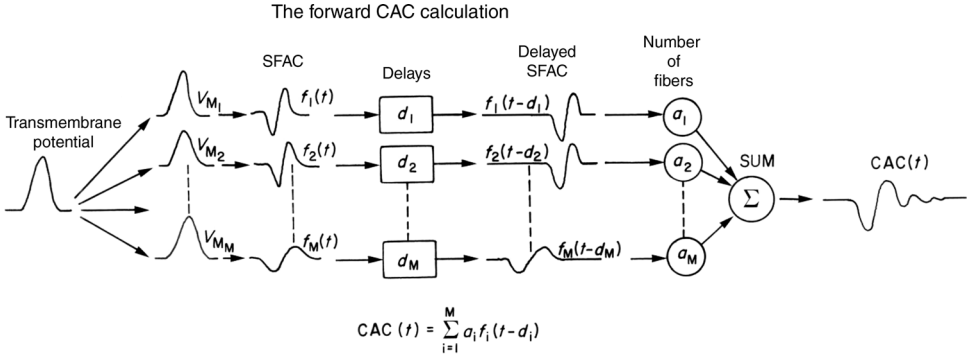

Figure 5 Block diagram of the forward procedure for simulating the compound action current (CAC). The single fiber action currents (SFACs) in each of M conduction velocity classes are calculated from an assumed transmembrane potential using a generalized volume conduction model of an axon in a nerve bundle. Then the SFACs are delayed according to their conduction velocity and the distance between the stimulus and recording site is multiplied by the number of fibers in each class, and are summed to obtain the CAC. (Reprinted from Wijesinghe *et al.*[28] with kind permission of Springer Science and Business Media)

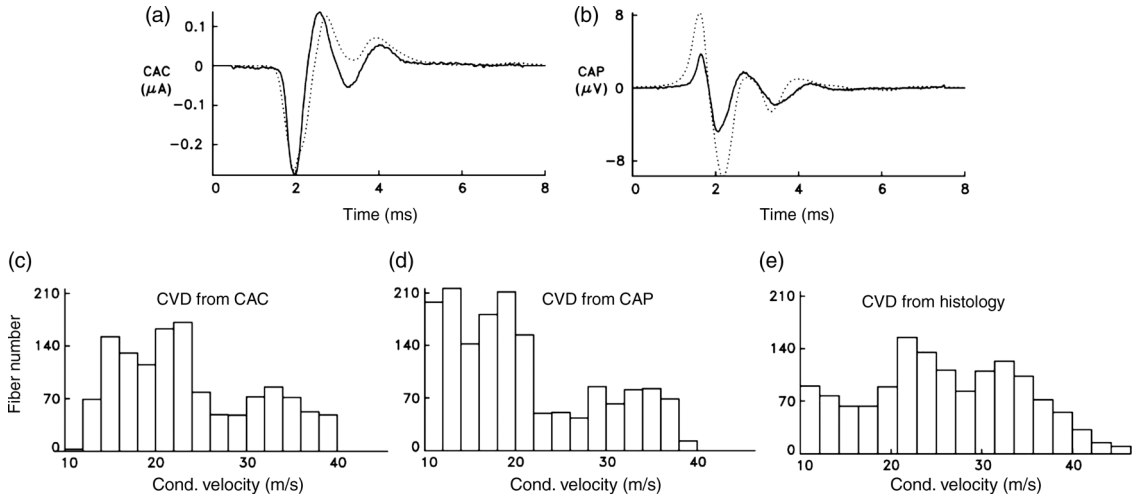

Figure 6 (a) Compound action current (CAC) and (b) compound action potential (CAP) recorded from a bull frog sciatic nerve bundle at 21°C. The propagation distances for the CAC and CAP were 78 and 60 mm, respectively. The conduction velocity distribution (CVD) predicted from the (c) CAC and (d) CAP, and (e) the histologically measured CVD. (Reprinted from Wijesinghe *et al.*[31] with kind permission of Springer Science and Business Media)

between the probe and the sample and allows these measurements to be made on isolated nerve axons. Exploiting this advantage, van Egeraat *et al.*[32] recorded the response of a crayfish medial giant axon to a nerve crush with the help of an improved and much simpler neuromagnetic current probe.[33] The experimental data they recorded, shown in Figure 7, were interpreted with a mathematical model that incorporated both the radial and axial ionic transport and membrane kinetics. Their experiments showed that the effects of the crush were manifested statically as an increase of the resting potential and dynamically as a reduction in the amplitude of the action current and potential, and were observable as far as 10 mm from the crush site. In addition, the normally biphasic magnetic signal became monophasic near the crush. Their mathematical model reflected these observations accurately. Based on the experimental data, their mathematical model predicted that the crush seals with a time constant of 45 s. They also estimated the injury current density entering the axon through the crush to be initially on the order of 0.1 mA/mm^2. That current lasted until the crush sealed or the concentration gradients between the intra- and extracellular spaces came to equilibrium. Later van Egeraat and Wikswo[34] developed an analytical model to investigate the axonal propagation incorporating both the radial and axial transport in an axon. The main goal of that

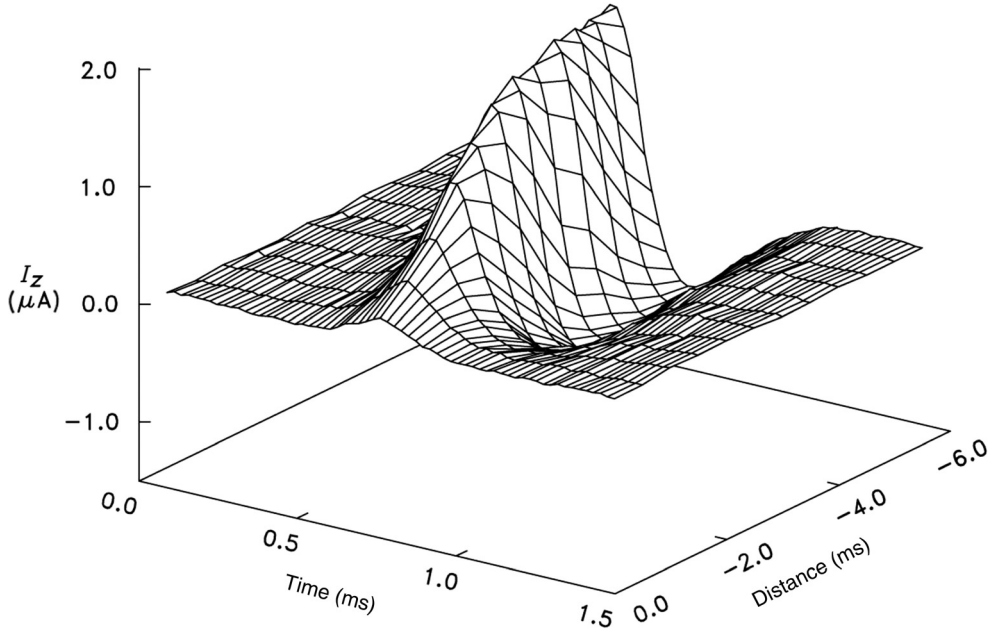

Figure 7 Time course of the measured action current, I_z, at 12 different axial locations along the medial giant axon of an isolated crayfish nerve bundle (0.5 mm spacing). The crush was at 0.0 mm. The data were taken 8 min after crushing. (Reprinted from van Egeraat *et al.*[32] with kind permission from Elsevier)

investigation was to understand the long-term behavior of transected or injured axons.

Taking their research effort to another level, which was facilitated by the neuromagnetic current probe, van Egeraat *et al.*[35] reported the first measurement of the magnetic field recorded from a single muscle fiber. They reported that currents associated with the magnetic field of a single muscle fiber was also biphasic and had peak-to-peak amplitudes ranging between 50 and 100 nA. These amplitudes mostly depended on the fiber diameter. Following the same experimental procedure which Roth and Wikswo employed on the crayfish giant axon and using a core conductor model, they calculated the transmembrane potential of the muscle fiber using the recorded magnetic field. Their estimated values for the intracellular conductivity and the effective membrane capacitance of the muscle fiber were 0.20 ± 0.09 S/m and 0.030 ± 0.011 F/m^2, respectively. They also demonstrated that the anisotropic conductivity of the muscle bundle did not significantly affect these estimated values.

Clinical applications of the magnetic technique

Kline and his colleagues[35–39] first introduced electrophysiological methods to clinical settings hoping to provide both qualitative and quantitative determinations of the permanence of the axons at a site of nerve injury. They performed intraoperative electrophysiological assessments of nerve bundles by stimulating and recording electric signals (compound action potentials) from them. Six years later, using the then-latest developments in microsurgical techniques, Terzis *et al.*[40] refined intraoperative assessment by stimulating and recording electric signals from individual fascicles or groups of fascicles. These recording techniques have all utilized a pair of closely separated electrodes that raise the nerve bundle into the air from its original position, which could lead to drying up the preparation unless it is continuously kept moisturized. In addition, reliable and repeatable measurements of amplitude, conduction velocity and shape of the recorded electric signal require very careful and reproducible electrode placement.[41,42] This may also require significant dissection of the nerve and keeping the nerve suspended in the air for a considerable period, exposing the nerve to the danger of cell death. In addition, small changes in electrode placement or the conductivity of the layer of moisture on the nerve makes it difficult to obtain reproducible data.[36] The magnetic technique is therefore ideally suited to overcome the deficiencies of the traditional electric technique.

The instrumentation and mathematical models developed for the purpose of studying the magnetic field created by an isolated single axon can be likewise applied to clinical research with some modifications. For this purpose, Hentz *et al.*[36] developed a crude openable neuromagnetic current probe with the intention of showing that the magnetic fields created by intact nerve bundles could be measured. Figure 8 shows schematically how such a probe can be used to record human CASs. After the nerve has been surgically exposed, the intact nerve can be enclosed by the openable probe. Then it can be stimulated proximal to the injury, and the probe can be scanned over the nerve bundle to record action signals along the bundle. Wikswo's group achieved the first intraoperative recordings of the CAC from the human median nerve in 1990,[43] from a patient undergoing surgical section of the flexor retinaculum for decompression of the carpal tunnel at the Vanderbilt University Medical Center (see Figure 9). They exposed the median nerve proximal to the flexor retinaculum and followed distally to demonstrate the lateral and medial branches. A 2-mA amplitude pulse with 100-μs duration was used for the nerve stimulation. The openable neuromagnetic current probe was placed in the palm of the hand to encircle the primary branch of the median nerve innervating the index and middle fingers, 6.0 cm distal to the stimulus electrodes. The muscle signal in Figure 9 was produced by return currents going, as anticipated, through the toroid. The nerve response was extracted and is shown in Figure 9b. The peak-to-peak amplitude of the signal was about 0.65 μA. Using their inverse mathematical model,[29,31] they estimated the CVD of the nerve

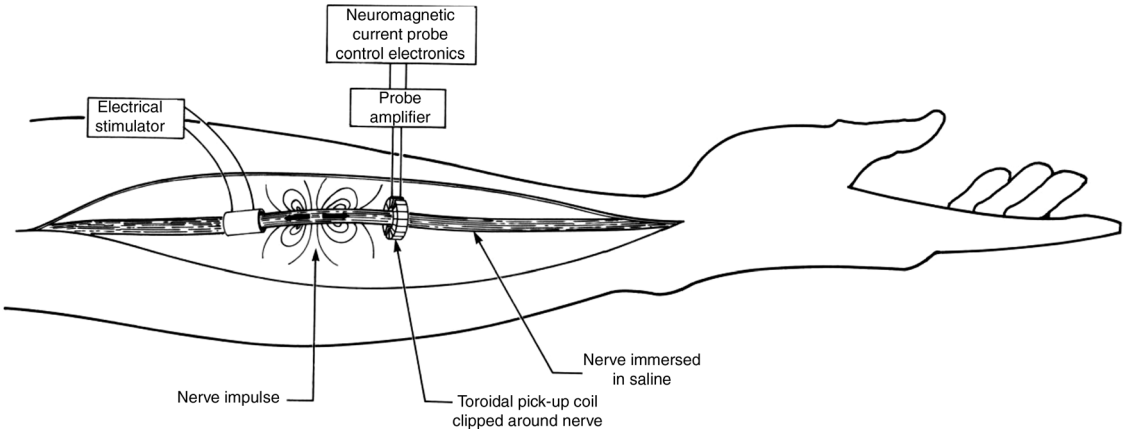

Figure 8 Schematic representation of the neuromagnetic current probe being used in the clinical settings. The surgically exposed nerve is stimulated proximally, and the magnetic signal produced by propagating action currents is recorded distally using the probe. (Reprinted from Wikswo[14] with kind permission)

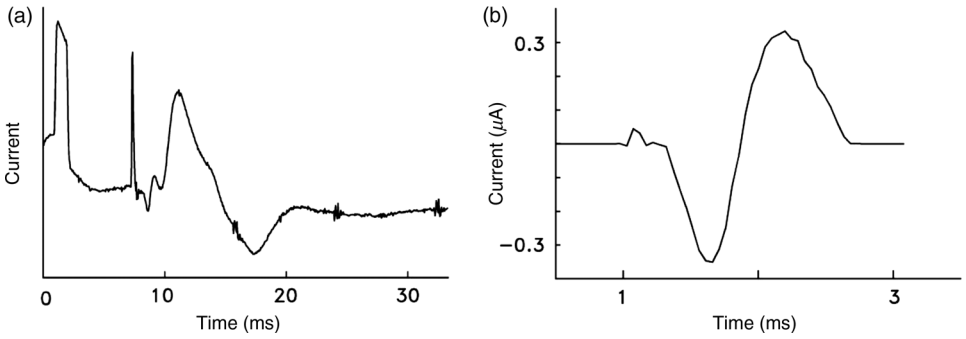

Figure 9 The first intraoperative recording of the magnetic field of a human median nerve. (a) A 2 μA, 1 ms calibration pulse precedes a sharp stimulus artifact followed by a biphasic nerve signal and finally by a large, biphasic muscle signal. This signal has been averaged 64 times with 3 Hz stimulus frequency. (b) The action current isolated from (a) with appropriate signal processing. (Reprinted from Wikswo *et al.*[43] with kind permission from the IEEE)

bundle and it is shown in Figure 10. Similar to previous observations of animal preparations, the slower conduction velocity classes have been overestimated in the CVD mainly due to the recorded noise embedded in the signal and uncertainties in the model parameters. Therefore, in general, the magnetic technique offers the advantages of being made with the nerve immersed in saline, and it appears that the magnetic technique is easier to utilize in clinical settings than the electric technique. Furthermore, magnetic measurements are more accurate, less noisy and less vulnerable to artifacts than those of the traditional electric measurements.[44]

Recently, Wells *et al.*[45,46] reported a novel method for stimulating peripheral nerve bundles *in vivo* preparation called optical stimulation. This technique uses low-level, pulsed infrared laser light to elicit nerve and muscle potentials. These potentials are spatially selective, artifact-free, spatially precise and damage-free since they are initiated by infrared light with energies well below tissue ablation thresholds.[45] Therefore this procedure offers a contact-free *in vivo* neural activation that has major implications for basic and clinical applications. This method will help clinicians and medical technicians to stimulate living tissues without being concerned about the electrode-related tissue contaminations. Given this technology, the magnetic recordings of action currents should be much more attractive than in the past because this technique combined with the neuromagnetic current probe can be utilized to measure nerve and muscle signals invasively without using the traditional stimulating and recording electrodes.

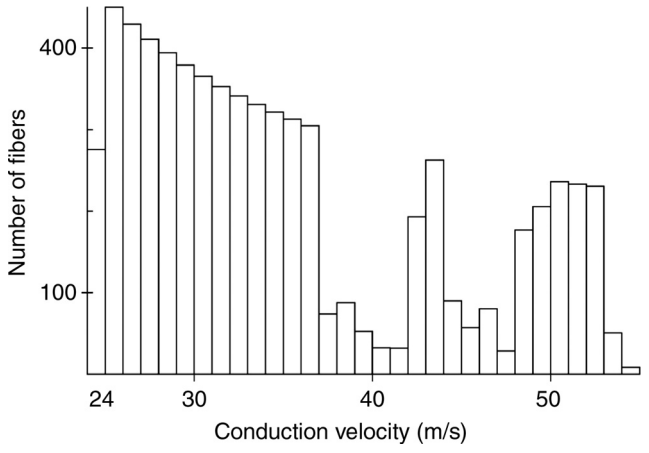

Figure 10 Conduction velocity distribution (CVD) of human nerve median nerve fibers in the palm, immediately distal to the nerve's exit from the carpal tunnel. This distribution was obtained from the intraoperatively recorded magnetic signal shown in Figure 9. (Reprinted from Wikswo *et al.*[43] with kind permission from the IEEE)

Magnetic assessment of nerve regeneration

The quality of a peripheral nerve reconstruction is primarily evaluated based on the functional recovery. In most cases, a connective scar tissue is formed around a nerve bundle following an injury to a peripheral nerve bundle, thus preventing proper regeneration of the nerve axons, and surgical procedures must be explored to restore the nerve function. Results are generally unsatisfactory due to the formation of scars.[44] To investigate this important surgical question, action signal propagation along a nerve graft in a monkey has been studied[44] using the neuromagnetic current probe. The median nerve of a *Macaca fascicularis* primate was exposed at the wrist and a 15-cm long segment was excised. Then the nerve was incised and sutured together. Six months were allowed for the nerve axons to regenerate across the repair site and into the hand, after which the median nerve was exposed surgically once more and the probe was placed around the nerve at the elbow. Then the nerve was stimulated at both the proximal and distal to the grafted segment. Figure 11 shows the recorded action current signals with the stimulus applied to both sides of the graft. The difference in amplitude of the signals reflects the amount of the axons in the nerve that have effectively linked both sides of the graft. The second signal is delayed more than that of the first one with respect to the stimulus *S*. The calculated conduction velocities of these two signals indicate that the conduction velocity is also decreased in the regenerated part of the nerve. In 1993, Kuypers *et al.*[47] further investigated the merits of nerve CASs recorded using the electric and magnetic techniques. They compared the two techniques using the rabbit peroneal nerve after a nerve reconstruction. They recorded signals two, four, six and eight weeks after the nerve reconstruction and also concluded that magnetic

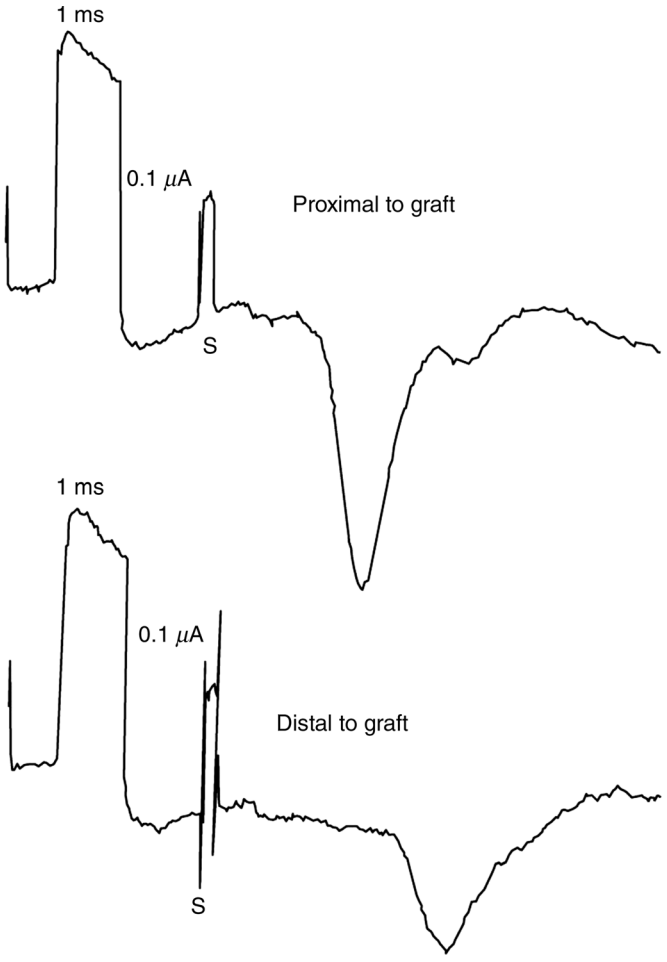

Figure 11 A recorded action current signal in a grafted right median nerve in a *Acaca fascicularis* primate using the neuromagnetic current probe. The recording toroid was left fixed, and the stimulation pulse was applied to each side of the graft (average of 256 recordings). (Reprinted from Wikswo *et al.*[44] with kind permission)

signals were far more reproducible and less prone to artifacts than electric signals. Kuypers' group also reported that the magnetic recording was able to demonstrate that the number of axons that had regenerated increased with time, which could not be achieved using the traditional electric recordings or histological methods. These investigators also found another unique feature coupled with the magnetic technique. They quantified the number of functional myelinated neuronal units in the peroneal nerve of New Zealand White rabbits 20 weeks after the reconstruction. They found that the magnetic technique was the only technique that could quantitatively predict how many regenerated fibers in a nerve bundle could conduct action signals after a reconstruction.[48] The reason behind this finding was that even though histological techniques could quantify the number of axons in a nerve, they were unable to provide information about their ability to conduct an action signal. There was a significant difference in conductive and non-conductive axons in reconstructed nerves. Another important observation which Kuypers *et al.*[49] made was that the magnetic technique could be useful in evaluating the success of peripheral nerve reconstruction shortly after it was performed. The magnetic technique appeared to be capable of predicting the eventual functional recovery of the nerve, based on the quality of the amplitude of the signal recorded during the early stages of recovery. This outcome is a very important decision-making asset for neurosurgeons. These investigations indicate the feasibility of magnetic measurement of nerve regeneration in mammals.

Advantages and limitations of the magnetic technique

The magnetic measurement technique has several remarkable advantages. In most cases, as described in this review, a single magnetic measurement is adequate to decide quantitatively what is the intracellular action current of an axon without making assumptions regarding tissue resistivity. This has not been the case with the traditional electric methods. On the other hand, magnetic and electric measurements can be combined to acquire new information on tissue parameters such as intracellular conductivity[20] and anisotropy.[50] There is no need for physical contact between the probe and the tissue sample during the recording of magnetic signals. Therefore, the sample can be scanned along its axial direction to obtain the spatial variation in the signals and to correct for virtual cathode effects. The virtual cathode effect produces a displacement of the site of real stimulation from the stimulating electrode, thereby reducing the propagation delay. When an extremely high stimulus intensity is used, the apparent activation site, or virtual cathode, may not correspond to the location of the cathodal electrode.[51] For stimulus intensities ranging between 2 and 20 times maximal, excitation of the fastest fibers may occur at 1–2 cm from the electrode. This effect is much less pronounced with lower stimulus levels. Gilliatt *et al.*[52] observed delay variations on the order of 0.05–0.15 ms for stimulus intensities between threshold and 25% above that required for maximal CAS amplitude, as measured at the elbow. It is reasonable to assume that this phenomenon affects fibers of different conduction velocity classes unequally,[53] and therefore a variable correction factor might be included for each fiber class. Since the virtual cathode effect leads to a reduced delay, at least in nerve fibers, it can be treated as a negative delay. A more recent detailed analysis of the virtual cathode effect in cardiac tissue can also be found in the article by Wikswo *et al.*[54] Electric measurements with intracellular microelectrodes would necessitate multiple electrode insertions with great risk of damage to the sample. Because of this advantage associated with the magnetic technique, magnetic measurements create the opportunity for researchers to study non-uniform propagation in systems with an axial inhomogeneity, such as a nerve crush, bifurcations, synapse and tapered fibers or bundles. Although extracellular electric measurements can also be used for scanning, the quantitative interpretation of the results is mired in the uncertainty in experiential parameters and recording noise. The magnetic technique is less sensitive to the model parameters.[29] In addition, the absence of any electrical connection between the probe and the tissue makes the

probe insensitive to changes in the electrochemical interface between the probe and the sample. Thus, magnetic recordings are not subject to the artifacts that take place in electric recordings during superfusion of media that has altered composition or temperature and tissue motion during recording. During the clinical intraoperative application of the neuromagnetic current probe, the probe and the nerve are immersed in saline, thereby reducing the risk associated with elevating and drying the nerve segment in the air, as required for traditional electric recordings.[36,38] Moreover, the current probe is much less sensitive to stimulus artifacts, which is important in cases in which the proximity of the detector from the stimulating electrode is important.[55] This is due to the geometry of the probe and the low input impedance of the room-temperature amplifier, which allows it to recover faster from the nonlinear behavior produced by an overload, such as that caused by the stimulus, than do high-input-impedance electric amplifiers.[10] However, the magnetic technique too has its own limitations. The spatial resolution of the probe is in the order of 1 mm when it is placed in proximity to the tissue sample. Therefore, the spatial extent of the propagating action signals must exceed 1 mm before the configuration of the propagating current pattern can be resolved. However, this resolution prerequisite can usually be met in most measurements on peripheral nerve or muscle.

Conclusions

In this review, the measurements of magnetic fields created by living tissues using the neuromagnetic current probe have been described. This technique has come a long way since it was first introduced to the biomagnetic research community in 1980. It has helped the investigators to make and analyze the first measurements of magnetic fields created by nerve axons and muscle fibers. Since then, crushed nerve axons, nerve and muscle bundles, reconstructed nerve bundles, and non-uniform propagation of signals in nerve axons have been comprehensively investigated. During these studies, this technique showed its superiority over the electric technique. This technique could be an invaluable tool in measuring activities of action signals during non-uniform signal propagation and, most importantly, estimating the quality of reconstructed nerves during the recovery period. With the neuromagnetic current probe, we can map the variations in action signals with position by scanning the probe along the tissue preparation, without the risk of damage intrinsic to the traditional intracellular recordings. As it was revealed in several of the studies reviewed here, extracellular electric recordings are more sensitive to electric parameters and do not have the reproducibility inherent in magnetic recordings. All of these studies have now led to a more comprehensive understanding of the generation of magnetic fields by living tissues and the neuromagnetic current probe for quantifying some potentially very important clinical aspects, such as trauma and non-uniform action signal propagation in nerve axons.

Not to be overlooked, however, are SQUID magnetometers, which are also widely used to measure magnetic fields created by living tissues and have their own advantages. The latest SQUID microscopes can provide a spatial resolution of $\sim$50–100 μm, but these would require liquid helium and would have to be placed in very close proximity of the exposed nerve.[56,57] The neuromagnetic current probes can be positioned very close to the living tissues where the magnetic field is strongest leading to a stronger and better signal-to-noise ratio. However, the SQUID magnetometer can be employed to measure magnetic signals non-invasively from living tissues. Although this may not be significant for basic studies using isolated nerves or muscles, it is very important when considering the clinical applications of this technique and measuring signals that arise from *in situ* experiments.

The neuromagnetic current probe is a very valuable research tool for the biomedical engineering community and its capabilities need to be fully exploited. A recent publication by this author [58] concludes, based on the previous findings involving the neuromagnetic current probe, that the currently available magnetic resonance imaging techniques may not be adequate to detect the neuronal currents in peripheral nerves and muscles. This topic has been the main focus of more than a dozen recently published papers (e.g. see references[59–63]) even though the data required to come to the above conclusion was already in the literature.

There are several important practical applications of the magnetic technique yet to be investigated and applied to clinical medicine. One such example is the application of the magnetic technique to examine peripheral nerve injury and repair. Several studies have been done on this topic as described in this paper. However, these investigations need to be continued until some definitive results are obtained. For that reason, it will be necessary to optimize the size of the pickup coil because large pickup coils will be an impediment to recording signals in the clinical settings. Since the development of the first magnetic current probe and its amplifier in the late 1970s, the technology has been much improved. Therefore, the biomedical research community should be able to develop a better amplifier-probe system, improving its capabilities such as signal-to-noise ratio. This will boost the investigators' ability to compare experimental and theoretical results such as CVDs of nerve bundles. The role that the return current plays in the recorded signals needs to be addressed more thoroughly. This could be a crucial factor when dealing with humans. In general, unlike in the animal preparations, insertion of mineral oil or an air bubble in the pickup coil to block the return current is less likely to be an acceptable procedure when dealing with humans.

With the invention of non-invasive optical nerve stimulation recently, the magnetic current probe could be more attractive to clinical research than ever before. Once both equipments are commercially available, these two techniques could be concurrently used as a non-invasive technique to measure nerve action signals. With its ability to measure magnetic signals in femtotesla range, the

neuromagnetic current probe may even have a place to fill in the field of nanomedicine.

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