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Mini-review

Microneurography in rats: A minimally invasive method to record single C-fiber action potentials from peripheral nerves *in vivo*

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ABSTRACT

Microneurography is a method suitable for recording intraneural single or multiunit action potentials in conscious subjects. Microneurography has rarely been applied to animal experiments, where more invasive methods, like the teased fiber recording technique, are widely used. We have tested the feasibility of microneurographic recordings from the peripheral nerves of rats. Tungsten microelectrodes were inserted into the sciatic nerve at mid-thigh level. Single or multiunit action potentials evoked by regular electrical stimulation were recorded, digitized and displayed as a raster plot of latencies. The method allows unambiguous recording and recognition of single C-fiber action potentials from an *in vivo* preparation, with minimal disruption of the nerve being recorded. Multiple C-fibers can be recorded simultaneously for several hours, and if the animal is allowed to recover, repeated recording sessions can be obtained from the same nerve at the same level over a period of weeks or months. Also, single C units can be functionally identified by their changes in latency to natural stimuli, and insensitive units can be recognized as 'silent' nociceptors or sympathetic efferents by their distinctive profiles of activity-dependent slowing during repetitive electrical stimulation, or by the effect on spontaneous efferent activity of a proximal anesthetic block. Moreover, information about the biophysical properties of C axons can be obtained from their latency recovery cycles. Finally, we show that this preparation is potentially suitable for the study of C-fiber behavior in models of neuropathies and nerve lesions, both under resting conditions and in response to drug administration.

1. Introduction

It is often desirable to record from intact axons *in vivo*, using a minimally invasive method that will allow repeated recordings from the same animal. This is particularly true when studying possible mechanisms underlying positive sensory symptoms appearing in neuropathic pain conditions [11,36]. Microneurography has been shown to be particularly useful in this respect. With tungsten microelectrodes percutaneously inserted into human peripheral nerve fascicles, *in vivo* recordings of single-unit action potentials can be obtained from different types of myelinated and unmyelinated nerve fibers (for a review, see [18]). This technique has provided a great deal of information about mechanoreceptive [26], thermoreceptive [8], and nociceptive units, both under physiological [4,7,35,40,44] and pathological conditions [5,29,30,37].

Single-unit recordings from peripheral nerves of rodents have been obtained using a variety of different methods [14,23–25,31], mostly involving permanent damage to the axon itself. This is particularly true for the most commonly used technique in neurobiological studies, i.e. the teased fiber recording technique (for example, see [6,16]). In this method, the end of a cut nerve is successively split into thinner strands that are placed over a pair of recording electrodes, usually fine platinum wires, while the nerve trunk is electrically stimulated at a distance. Splitting of the nerve is pursued until few- or single-unit recordings are obtained. This method has provided valuable information on different aspects of nerve physiology and pathophysiology, but it has the obvious disadvantage that the nerve is destroyed by the procedure.

In recent years, other methods of recording neural activity directly from peripheral nerves have come from the many scientific and technological efforts devoted to develop neuroprostheses. Neuroprostheses involve interfacing the peripheral nervous system by means of appropriate electrodes, which may allow selective nerve stimulation as well as neural signal recording [10,13,38,39]. Electrodes placed inside a peripheral nerve have been proposed in order to enhance selectivity and

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also to increase the signal-to-noise ratio of recordings with respect to extraneural electrodes. Whereas the technology has succeeded in producing a variety of multi-electrode devices, such as longitudinal intrafascicular electrodes, multi-electrode arrays or sieve electrodes (for reviews see [28,33]), little is known of their use for chronic applications and their possible side effects.

In the present study, we describe in detail a method to obtain microneurographic recordings from unmyelinated C-fibers in rats and extend the description of its possibilities that were briefly reported previously [17]. This type of fibers represents an important axonal population involved in several pathological states causing pain and dysautonomia. The method allows prolonged simultaneous recordings from several functionally identified C-fibers and it may be used to evaluate the effects of drug treatments on their physiological or pathophysiological behavior. It also allows recordings over several months from the same nerve, which may be used for longitudinal studies of animal models of neuropathic pain or of axonal regeneration.

2. Experimental procedures

2.1. Animals

Recordings were obtained from the sciatic nerves of Sprague–Dawley adult rats weighing between 200 and 350 g. The experimental procedures followed the recommendations of the European Union for the care and use of laboratory animals and were approved by the CEEAH (Committee for Ethics on Experimental Animal and Human Research) of the Universitat Autònoma de Barcelona. Animals were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) injected i.p. Repeated administrations of one-third of the initial dose were performed as required to maintain the level of anesthesia.

The sciatic nerve was exposed at mid-thigh level, from the sciatic notch to the knee, and carefully freed from surrounding tissues with the aid of a dissecting microscope and microsurgery tools (Fig. 1). Warmed saline was freely applied to the open wound to maintain the exposed tissues hydrated during the whole duration of the experiment, usually between 3 and 5 h. Animals were placed over a warm flat heating pad, controlled by a hot water circulating pump. Skin temperature was monitored with a thermistor probe taped to the skin close to the pair of stimulating electrodes used to electrically activate the units under study, and maintained close to 32 °C with the aid of an isolated infrared lamp placed outside the shielded cage.

After the recording was finished, the rat was killed by i.p. injection of excess anesthetic, or, following some experiments lasting less than 3 h, the wound was closed in layers with sutures and the animal allowed to recover. Animals were rehydrated with a bolus of 10 ml of saline i.p. and preventively treated with wide spectrum antibiotic.

2.2. Chronic experiments: animal models of nerve injury

We studied the applicability of the technique in two peripheral nerve lesion models in the rat, i.e. crushed sciatic nerve model and sutured sciatic nerve model, which represent paradigmatic examples of a clinical situation of neuropathic pain in human patients. Operations were performed under pentobarbital anesthesia (40 mg/kg i.p.) in rats weighing 250–350 g, with the aid of a dissection microscope and microsurgery tools [41]. The sciatic nerve was exposed at mid-thigh level and freed from epineurial adhesions to surrounding tissues. To produce a crush injury, the nerve was crushed three times in succession with a Dumont No. 5 forceps at a constant point, 90 mm from the tip of the third digit. To produce a suture model, the sciatic nerve was cleanly cut with a sharp blade and sutured end-to-end with fascicular 9/0 sutures. The wound was then sutured by layers and disinfected with povidone-iodine. The animals were maintained in a warm environment until full recovery from the anesthetic, and allowed to recover from the nerve injury for at least one month in the case of a crush, or two months in the case of the suture model.

2.3. Microneurographic recordings

Intraneural recordings were performed in a shielded cage made of stainless steel. A lacquer-insulated tungsten microelectrode (FHC, Bowdoinham, ME, USA, nominal impedance 1 MΩ, 200 μm in shaft diameter) was inserted into the nerve trunk with the aid of a micromanipulator. A low impedance reference electrode was inserted outside the nerve trunk into the surrounding tissue. The intraneural recording electrode was carefully advanced and maintained into the nerve with the micromanipulator under a magnifying lens until the characteristic neural signal audio could be heard. The method follows the same principles of the microneurographic technique in humans, a detailed description of which can be found elsewhere [42]. The neural signals were first amplified with an isolated high input impedance amplifier (3+ Cell Isolated Microamplifier, FHC Inc., Bowdoinham, ME, USA), band-pass filtered (maximum range 50–5000 Hz) and fed to a noise eliminator (Hum Bug, Quest Scientific, North Vancouver, Canada). This signal was then fed to an audio-monitor with noise clipper (Grass AM10) and to two PCs running separate software for collecting spontaneous and electrically evoked activity. Spontaneous activity was digitized at 20 kHz and recorded continuously on the hard drive of a PC running Chart software (PowerLab Systems, ADInstruments Ltd., Australia). This instrument was also used to record spontaneous or prolonged discharges occurring during electrical stimulation (e.g. Fig. 8).

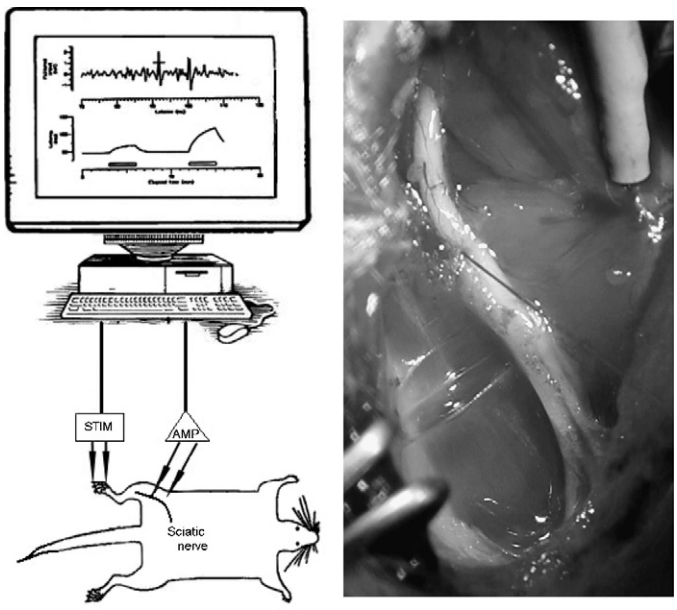

Fig. 1. *Left:* Schematic diagram of recording arrangement, showing stimulation electrodes in cutaneous receptive field of unit recorded by tungsten microelectrode inserted in sciatic nerve. *Right:* Close photography of tungsten microelectrode impaling the right sciatic nerve of a rat. Sciatic notch is situated to the top of the image, and peroneal and posterior tibial nerve divisions can be seen on the bottom right corner. The needle, which is held in a micromanipulator (not shown), produces a slight mechanical distortion of the nerve surface, and epineurial blood vessels are minimally distorted.