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Journal of Neuroscience Methods 106 (2001) 147–151

JOURNAL OF NEUROSCIENCE METHODS

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A simple cuff electrode for nerve recording and stimulation in acute experiments on small animals

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Received 25 September 2000; received in revised form 30 January 2001; accepted 30 January 2001

Abstract

We describe a cuff-type electrode specifically designed for recording from, and electrical stimulation of, cut nerves in acute experiments on small animals. Unlike existing designs of cuff electrodes, it is simple to manufacture, inexpensive and takes little time to implant. The electrode was tested on the hypoglossal, phrenic, recurrent laryngeal, and superior laryngeal nerves in anesthetized rats. It provides satisfactory signal-to-noise ratios (3.0 ± 0.8 (mean $\pm$ S.D.)) for hypoglossal and 5.4 ± 2.1 for phrenic nerve activity and stable recording over the time course of a typical acute experiment. It eliminates or minimizes the problems with recording stability and space availability associated with conventional hook-type electrodes, and reduces experiment preparation time. This should facilitate neurophysiological experiments on small rodents involving complex protocols that include recording from, and/or stimulation of, multiple nerves.

Keywords: Cuff electrode; Electrical stimulation; Hypoglossal nerve; Laryngeal nerve; Nerve recording; Phrenic nerve

1. Introduction

The increasing popularity of rats and mice as animal models for acute in vivo neurophysiological experiments has been accompanied by a rapid growth in the complexity of experimental designs. Hook electrodes, while suitable for nerve recording and/or stimulation in acute experiments on rodents, have become a limiting factor because of both the space they occupy and the need to form oil pools or use isolating semi-liquid substances around the recording sites (e.g. Vaseline or silicone grease). In addition, since the available volume of the oil pool is relatively small, extracellular fluid often accumulates, leading to a gradual deterioration (shunting) of the recorded signal over the course of a typical experiment (3–12 h).

The design of the cuff-type electrodes described in the literature is often very sophisticated, their manufacturing and implantation are time-consuming, and the materials used expensive (for example, Ninomiya et al., 1976; Jellema and Teepen, 1995; Sahin et al., 1997). These features make their use in acute experiments impractical and/or economically unjustified. Thus, there is a need to develop a recording/stimulation electrode for acute experiments in small rodents devoid of the problems associated with the use of hook electrodes and complexity of the available cuff-type electrodes.

We describe a simple and inexpensive cuff electrode that can be made and placed on cut nerves in minutes, and provides a satisfactory signal-to-noise ratio and recording stability over the time course of a typical acute experiment. The electrode can be used on short nerves and requires no liquid or semi-liquid isolators around the recording site.

2. Materials and methods

2.1. Electrode design

Fig. 1A shows, schematically, a longitudinal section through the electrode assembly implanted on a cut nerve (1). The assembly consists of a polyethylene tube (2) and two Teflon-coated silver wires; one straight (3)

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0165-0270/01/$ - see front matter
PII: S0165-0270(01)00340-5

and the other ending with a loop that holds the cut (distal) end of the nerve (4). These three elements are held together with red utility wax (5) at the distal end of the tube. The nerve is surrounded by extracelluar fluid (6). The internal diameter of the polyethylene tube is selected to accommodate the straight wire (3) and the nerve (1), without compressing the latter (see Table 1 for tube sizes suitable for different nerves in rats). Depending on the length and thickness of the nerve, the tube can be 3–14 mm long. The bare/coated diameters of the wires are 0.075 mm/0.140 mm (Teflon-coated silver wire; A-M Systems, Inc.). The insulation is removed over a length of 0.2–0.4 mm from the end of the straight wire (3), and 0.5–1 mm from the end of the looped wire (4).

2.2. Electrode implantation

After dissection of the nerve, a piece of polyethylene tube having the length appropriate for the freed segment of nerve is cut and both wires are inserted through the tube. Using a dissecting microscope, the cut end of the nerve is placed inside the looped end of wire (4) and the loop gently squeezed with watchmaker forceps. The tube is then slipped along the wires towards and over the nerve until the loop holding the nerve aligns with the distal end of the tube. Then, the straight wire (3) is pulled inside the tube until its bare end is positioned inside the tube at approximately one-fifth of the tube's length (see Fig. 1A). This is the optimal placement; a more proximal position of the straight wire increases the possibility of contaminating the nerve signal with the electrocardiogram, whereas a more distal position decreases the inter-electrode distance, thereby reducing the signal-to-noise ratio.

Once the nerve and both wires are properly positioned, the distal end of the tube is sealed with a drop of melted wax. This fixes the position of the nerve relative to the wires and the tube, and seals one end of the tube, thereby eliminating the possibility of recording the activity from nearby muscles in non-paralyzed animals. We use low melting temperature wax sticks (G-C Dental Industrial Corp., Japan) and a cautery (Cautery-100; Geiger Philadelphia) set at a low temperature so that the wax is heated to about 80°C. Although other substances can be used (e.g. silicone or epoxy glue), we found it efficient to use dental wax melted by cautery tip. When used carefully, it can be applied rapidly and does not harm the nerve (see Section 3). The assembly is then fixed to the surrounding muscles with 6-0 silk sutures in a position that minimizes stretching and/or compression of the nerve when the muscles and skin overlying the exposed area are closed and the position of the animal is changed.

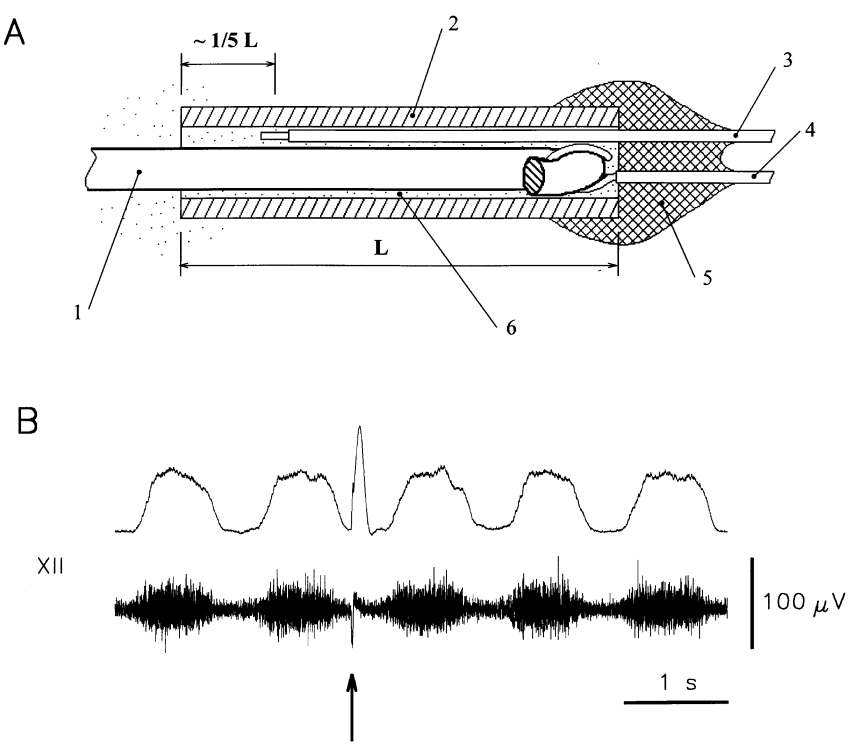

Fig. 1. (A) Sagittal section through the cuff electrode implanted on a peripheral nerve (1); (2) isolating tube (polyethylene); (3) straight wire (Teflon-coated silver); (4) looped wire (Teflon-coated silver); (5) low melting temperature dental wax; (6) extracellular fluid. *L*, Length of the isolating tube. (B) Hypoglossal nerve (XII) activity and its moving average (top trace) recorded in a urethane-anesthetized, vagotomized and artificially ventilated rat at the time when the distal end of the cuff electrode was sealed with a drop of melted dental wax (arrow).

Table 1
Dimensions of the isolating tubes used to manufacture the cuff electrode for different nerves in rats[a]

Nerve	Polyethylene tube actual dimensions		Nominal tube i.d./o.d., manufacturer	Number of experiments
	Length (mm)	I.d. (mm)		
XII	8–14	0.57	0.023/0.038 inch, Intramedic	33
Medial branch of XII	5.5–6.5	0.52	0.50/1.00 mm, Nalume	3
Lateral branch of XII	6–8	0.43	0.015/0.043 inch, A-M Systems	4
Phrenic	5–6	0.34	0.015/0.043 inch, Intramedic	20
Superior laryngeal	3–5.5	0.34	0.015/0.043 inch, Intramedic	6
Recurrent laryngeal	5	0.29	0.011/0.024 inch, Intramedic	2

[a] i.d./o.d., Internal/external diameter.

We used this electrode in anesthetized rats for simultaneous recording from, and stimulation of, at least two of the following nerves: hypoglossal (XII) or its medial (MXII) and lateral (LXII) branch, phrenic (PHR), recurrent laryngeal (RL), and superior laryngeal (SL).

2.3. Electrode cost-effectiveness

At the end of the experiment, the wires can be pulled out without the need to re-expose the implantation site. They can then be cleaned and reused, so that only a short piece of polyethylene tube and a drop of wax are discarded. Therefore, the recurring cost of use of the electrode is less than 10 cents per nerve.

2.4. Animal preparation

The data used in this report were obtained from 38 Sprague–Dawley rats (250–500 g). Four animals were used only to optimize the design and procedures of electrode implantation. The remaining 34 experiments had additional objectives and included recording central neuronal activity, microstimulation within the brainstem and/or microinjections (Fenik et al., 1999a,b). In these experiments, electrode testing was carried out in control conditions before any other manipulations.

The animals were initially anesthetized with isoflurane (about 3%). Anesthesia was continued with urethane (1 g/kg, intraperitoneally (i.p.), supplemented with 50 mg, intravenously (i.v.), as needed), sodium pentobarbital (40 mg/kg, i.p., supplemented with 2.5 mg, i.v., as needed) or isoflurane (1.5–2%). The procedures used to minimize animal discomfort during the induction of the anesthesia and all subsequent procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

During the surgery, an adequate level of anesthesia was indicated by the absence of a withdrawal reflex and the presence of a regular respiratory rhythm. The animal was tracheotomized and catheters placed in the femoral artery for blood pressure recording and femoral vein for injections of fluid, additional doses of anaesthetic or pancuronium bromide. An appropriate cuff electrode was placed on two or three of the six nerves already listed. The animal was then placed in a stereotaxic head holder in either the prone or supine position. Some animals breathed spontaneously, others were paralyzed with pancuronium bromide (1 mg, i.v., supplemented with 0.5 mg, i.v., as needed), vagotomized and artificially ventilated. No movement artifact was observed in the signals recorded with the cuff electrode in spontaneously breathing animals. Following neuromuscular paralysis, an adequate level of anesthesia was judged by the absence of an increase in blood pressure or perturbations of respiratory activity in response to a strong paw pinch.

Ventilation was adjusted to maintain a stable PHR nerve activity similar to that recorded during spontaneous breathing just before artificial ventilation. The end-expiratory CO_2 level was monitored (Micro-Capnometer; Columbus Instruments), and kept constant at 5–7%. Saline (NaCl, 0.9%) or bicarbonate solution (NaCl, 124 mM and $NaHCO_3$, 30 mM) was continuously infused (3 ml/kg/h, i.v.) to maintain circulatory stability (Quintin et al., 1989).

Nerve activities were amplified (Grass, P511; bandwidth, 30–3000 Hz) and the signals fed to analog moving averagers (CWE, MA-821RSP; 100 ms time constant). The SL nerve was stimulated with pulse trains (Grass S88 stimulator and PSIU6 current isolation unit; 0.1–0.2 ms pulses, 50 Hz, 2 s trains). The threshold current for the effect of the train was taken as the lowest current that caused a visible inhibition of PHR nerve activity (Jiang et al., 1991). All recorded signals were monitored on a chart recorder (Gould, TA2000) and stored on a digital tape recorder (Cygnus Technology, CDAT-16). Rectal temperature was maintained at 35–37°C by a heating pad.

The signal-to-noise ratio for the nerve activity was measured as the ratio between the peak-to-peak amplitudes of the signals recorded during the active and silent phase of the respiratory cycle for a given nerve.

3. Results

The possibility of heat damage to the nerve during wax application was assessed by recording XII nerve activity while the distal end of the electrode assembly was sealed with a drop of melted wax, as described in Section 2. The amplitude of the recorded signal was unchanged by this procedure (Fig. 1B, arrow).

The presence of extracellular fluid inside the cuff leads to shunting of the electrical signal generated in the nerve. The longer the tube and the smaller the volume of space filled with fluid, the better the signal-to-noise ratio (Stein et al., 1975). The diameters and the lengths of the tubes that provided satisfactory results with different nerves are presented in Table 1. Our experience with tubes of different sizes led us to the following empirical equation relating the length and the internal diameter of the tube: $L = 8.\text{i.d.} + 2$, where L is the length of the tube (mm) and i.d. the internal diameter of the tube (mm). Examples of respiratory-modulated activities simultaneously recorded with cuff electrodes from selected pairs of nerves are shown in Fig. 2A,B.

In eight isoflurane-anesthetized and paralyzed rats, in which the activities of PHR and XII nerves were recorded using the same size of cuff electrode (5 mm for the PHR, and 8 mm for the XII nerve), the mean signal-to-noise ratios were 5.4 ± 2.1 (mean $\pm$ S.D.) and 3.0 ± 0.8, respectively. There was no obvious degradation of the signal-to-noise ratio while recording over the typical 10-h duration of the experiments (Fig. 2C).

The same electrode assembly was used for electrical stimulation of the SL nerve. The effectiveness of the stimulation was tested by recording the activity of the ipsilateral PHR and XII nerves in six urethane-anesthetized, paralyzed rats. The polyethylene tubes used in the stimulating electrode had an internal diameter of 0.34 mm, and a length of 3–5.5 mm (Table 1). A train of stimuli inhibited phrenic nerve activity (Fig. 2D). The threshold for the minimal visible inhibition of PHR nerve activity varied considerably among the animals, 15–70 μA. There was no obvious decrease in stimulus effectiveness during 4 h of intermittent repetitive train stimulation, and the stimulus artifact was negligible.

4. Discussion

Bipolar cuff electrodes of different designs have been described for acute (Julien and Rossignol, 1982; Juch and Minkels, 1989), or chronic (for example, Ninomiya et al., 1976; Sauter et al., 1983) recording from intact nerves, and for stimulation (Hockman and Butler, 1966; Heusner et al., 1968; Jellema and Teepen, 1995; Warren et al., 1998). A cuff electrode with tripolar configuration, which is preferable for intact nerves, has also been described (Stein et al., 1975; Sahin et al., 1997). All these designs are complex owing to the need to establish at least two points of contact between the nerve and the electrode, and to ensure satisfactory long-term recording/stimulation.

The advantages of the electrode in this study over previous designs are its simplicity, low cost and fast implantation, combined with satisfactory signal-to-noise ratio. Additionally, in contrast to all earlier designs, the leads of this electrode are positioned parallel to the nerve, rather than perpendicular to it. This allows for the simple and reliable implantation of the assembly in a manner that minimizes the potential for nerve compression or electrode displacement. Several

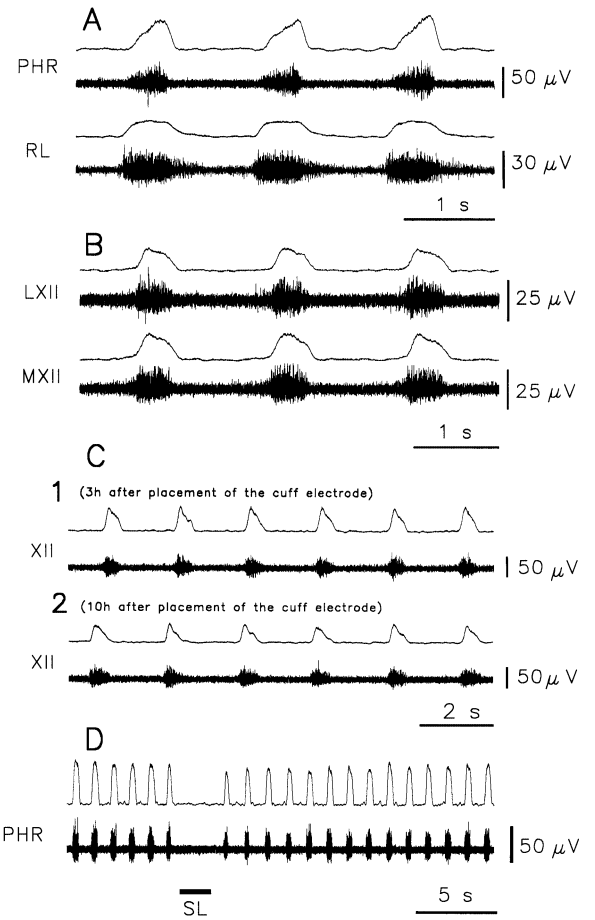

Fig. 2. (A) Typical activities simultaneously recorded from the phrenic (PHR) and recurrent laryngeal (RL) nerves with cuff electrodes in a pentobarbital-anesthetized, spontaneously breathing rat. (B) The activities of the lateral (LXII) and medial (MXII) branches of the hypoglossal (XII) nerve simultaneously recorded with cuff electrodes in a urethane-anesthetized, vagotomized, and artificially ventilated rat. (C) The activity of the XII nerve recorded with a cuff electrode in an isoflurane-anesthetized, vagotomized, and artificially ventilated rat 3 h (1), and 10 h (2) after placement of the cuff electrode on the nerve. (D) Effect of suprathreshold (50 μA) stimulation of the superior laryngeal nerve (SL) on PHR nerve activity in a urethane-anesthetized, paralyzed rat. The period of stimulation (2 s) is shown by a bar under the record.

such electrodes can be quickly placed and used for recording and/or stimulation for many hours. The electrode was specifically designed for use in acute experiments on cut nerves to substitute for conventional bipolar hook electrodes, whose features often present obstacles in designing complex acute experiments in small animals. Although a cuff electrode is a priori inferior to a hook electrode in terms of the signal-to-noise ratio, cuff electrodes are less traumatic to the animal because there is no need for stretching and resection of the skin and muscles to create an oil pool, and the loss of fluid and heat through the widely exposed tissue around the nerve is avoided. Since, in contrast to hook electrodes, no electrode holders or oil pools are needed, the entire space around the animal is available for additional devices. Thus, the described electrode is particularly useful for complex acute experiments on small rodents. Importantly, no semi-liquid fluid is used in the electrode, and the presence of a limited and constant amount of extracellular fluid inside the electrode provides a natural environment around the nerve, thereby helping to maintain the nerve in good condition and providing long-term stability.

Common to all cuff-type electrodes, no adjustment can be made to correct signal problems once the electrode had been implanted. Particular attention should be paid to sealing the cuff to avoid contaminating the recorded electroneurogram with the activity of nearby muscles, which may occur and can be difficult to recognize in non-paralyzed animals. In our experience, practice with the design and implantation of the described cuff electrode over four experiments was sufficient to eliminate problems with either the signal-to-noise ratio or signal contamination with the electrocardiogram or electromyogram. The reliability of the described electrode in subsequent experiments was 100%. During the course of an additional four to eight experiments, we were able to reduce the time needed for electrode implantation to the same or less than that needed to set-up conventional hook electrodes. Since no time and effort were subsequently required for maintenance, the use of this electrode has greatly improved the efficiency of our studies.

Acknowledgements

The authors thank Dr R.O. Davies for critical reading of the manuscript. This study was supported by the National Institutes of Health SCOR grant HL-60287.

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