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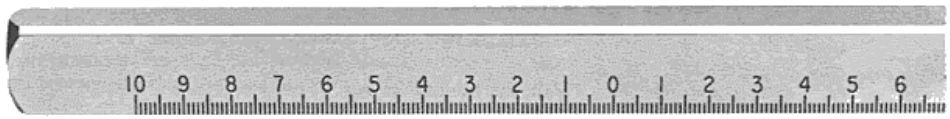

Kopf Carrier #57

Electrophysiological Assessment of Bilateral Spinal Activity in the Rat

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Introduction

The spinal nucleus of the bulbocavernosus (SNB, also known as the dorsomedial nucleus or DM [Schroder, 1980]) of the rat contains motoneurons that innervate the bulbocavernosus (BC) and levator ani (LA) muscles, which control penile reflexes, as well as the anal sphincter (Breedlove and Arnold, 1980; Schroder, 1980). Activity in the BC and LA muscles is highly coordinated, both with each other and with the activity of other perineal muscles (Holmes et al., 1991) and together the muscles produce the characteristic sexual reflexes of the male rat: dorsiflexions of the penis (flips) and flaring of the glans (cups) (Sachs, 1982; Hart and Melese-D'Hospital, 1983). The rat penile reflexes play an essential role in reproduction by dislodging previously deposited seminal plugs from the female's cervix and aiding in the placement of new plugs (Hart and Melese-D'Hospital, 1983).

The SNB is a medially located motor nucleus in the L5-S1 region of the rat spinal cord and its dendritic arbors are extensive: in addition to extending into dorsal portions of the lumbar cord, many SNB dendrites cross the midline to contact their contralateral counterparts. Thus, a large region of dendritic overlap exists at the midline (Kurz et al., 1986). The bilateral nature of dendritic contact in this structure suggests that they mediate communication and synchrony between the two halves of the nucleus (Rose and Collins 1985). For instance, dye coupling in this structure is bilateral and spatially extensive. This posits dendrodendritic loci of direct cell-cell communication across the midline, most probably via gap junctions (Coleman and Sengelaub, 2002).

Although anatomical assessment strongly suggests that dendritic overlap mediates bilateral communication, physiological characterization of bilateral activity will more thoroughly describe the nature of the interaction. We have developed an electrophysiological protocol to assess the respective contributions of each half of the SNB motor nucleus through simultaneous recording of ensemble output at the motor nerves. This method sensitively investigates the normal bilateral interactions in this population of motoneurons and holds great potential for assessing the modes of communication between them. This method's strength lies in its ability to characterize the timing, intensity, and pattern of activity via simultaneous recording of activity from both halves of the nucleus. Pharmacological manipulations can then be used to identify the relative contributions of different communication systems involved in bilateral activity. In the following example we investigate the role of gap junctions in bilateral SNB communication through electrophysiological assessment of motoneuron recruitment before and after pharmacological gap junction blockade in a repeated design.

Methods

Normal adult male rats are anesthetized with chloral hydrate (350 mg/kg ip, with periodic supplements to maintain areflexia to noxious stimuli) and placed on a 37ºC heating pad on a spinal stereotaxic base plate. Rats receive a high thoracic (T2-4) laminectomy and the spinal cord is completely transected, thus preventing descending tonic inhibition of spinal reflexes. The incision is then closed with surgical staples.

The SNB is contained within the lumbar enlargement of the spinal cord, which lies just caudal to the articulation of the spinal column with the final rib. The rib is easily palpable through the skin and serves as a reference along which to create a 2-3 inch longitudinal incision. Underlying fascia and muscle is removed to expose the spinous processes of the vertebrae and the proximal portion of the ribs, with particular care taken to excise soft tissue from between the transverse processes.

A set of custom-made spinal clamps is then affixed to the spinal column just caudal to the final rib. These clamps, mounted on vertical stand posts on both sides of the rat, position two 3-cm rods parallel with the spinal column in the cavity between the transverse spinous processes. This stabilizes the lumbar spinal cord and securely immobilizes the SNB area, thus reducing the potential for movement artifact in the record. A laminectomy is then performed to expose the caudal portion of the lumbar enlargement. The dura mater is cut and the entire region bathed in mineral oil to prevent desiccation. Three dorsal roots (L5, L6, and S1) innervate this portion of the spinal cord and terminate in the dorsal gray commisure (McKenna and Nadelhaft, 1986), the site of a large interneuronal population (Collins et al., 1991). Thus, electrical stimulation of any of these roots will result in activation of the SNB through a polysynaptic, interneuron-mediated pathway. A single dorsal root (typically L6) is selected and carefully separated from nearby structures. A loose knot of fine silk is tied around it, and the root is then draped over a bipolar hook wire electrode (FHC, Model PBCA0750) attached to a vertical stand post. The loose knot encircling the dorsal root is enlarged to include the distal wire and then tightened to ligate and crush the root onto the electrode. The L5-S1 roots are then severed bilaterally, distally to the electrode placement. Severance of the roots prevents any activity in the periphery from introducing artifact into the stimulation pattern.

To expose the site of electrophysiological recording, the proximal portion of the rat's tail is elevated in a tail clamp on a stand post, thus immobilizing the spinal column at a second point. An incision between the tail and anus followed by blunt dissection exposes the BC muscle and the fine motor nerves that innervate it. Mineral oil is applied to the nerves for the duration of the experiment. Both motor nerves are draped over bipolar hook wire electrodes (FHC, as above), crushed onto the distal wire with silk, and severed distally, further isolating the central component of this motor system. A grounding wire is attached to the animal's tail and flexible restraints are attached to the hind legs to minimize movement artifacts.

Stimulus pulses (0.25 ms long, once every 15 seconds) are generated using a Grass S48 stimulator and are passed through a Grass constant current unit (Model PSIU6E) before entering the stimulating electrode. A current probe is attached to the positive lead between the stimulation electrode and the constant current unit to record the actual current received by the animal at the L6 dorsal root. The signal from the recording electrodes at the BC motor nerves is filtered (low: 300 Hz; high: 20 kHz) and amplified 1000X (A-M Systems, Model 1700). Signals from both the current probe and the recording electrodes are digitized and recorded using a computer-based analysis system (SuperScope II, WGI). Stimulation commences at a low intensity and is gradually increased. A total of 200 stimulations are generated to assess the recruitment of motor units. About every 20 stimulations, recording is temporarily suspended to reverse the polarity of current to the stimulating electrode to prevent buildup of voltage between the wires of the bipolar electrode.

To test the effects of gap junction blockade on activity, drugs that block gap junctions (for example, oleamide) are applied directly to the exposed spinal cord. After fifteen minutes for the drug to take effect, the recruitment procedure is repeated. Following completion of electrophysiological recording, animals are sacrificed with an intracardial overdose of urethane (2.5g/kg). Linear distance between the stimulating and recording electrodes is determined using calipers (average of three measurements). The recorded activity is then analyzed in respect to the timing, gain, and pattern of activity in the motor nerves. Calculations of conduction velocity, recruitment curves, maximum peak to peak amplitude, fast Fourier and wavelet transformation, and counts of spikes exceeding ten times background activity are all performed.

The data are examined for differences between ipsilateral and contralateral activity, both before and after gap junction blockade (see Figs. 1 and 2). Differences in the amplitude of activity between ipsilateral and contralateral halves of the nucleus are found both in individual activity traces and in recruitment curves; amplitude of contralateral activation is larger than ipsilateral activation. Furthermore, pharmacological gap junction blockade attenuates recruitment on both halves of the nucleus, but has no effect on the synchrony of activity across the midline (Coleman and Sengelaub, 2001). Thus, our results suggest that while gap junctions potentiate SNB activation, they do not modulate bilateral synchrony. Furthermore, because ipsi- and contralateral activity differs in amplitude, the spinal circuitry underlying the activity in each half of the spinal cord may be anatomically different.

Figure 1. Representative rectified single traces of ipsilateral and contralateral motor nerve activity after stimulation of a single dorsal root. Amplitude of contralateral activity exceeds that of ipsilateral, and gap junction blockade with oleamide attenuates activity bilaterally. Scale bars represent 1 mV and 10 ms.

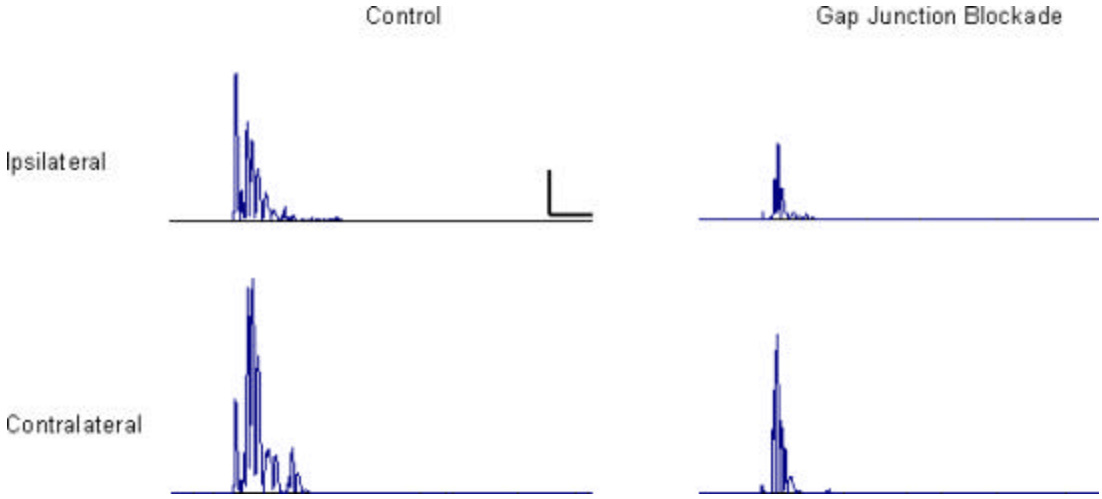
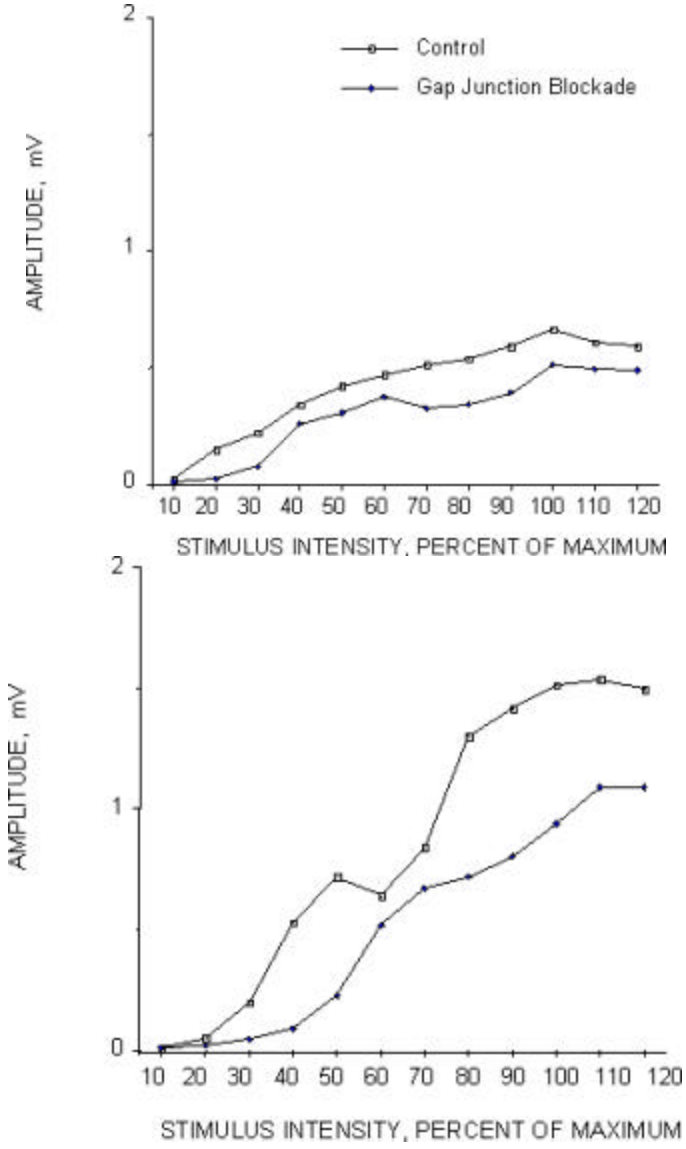


Figure 2. Recruitment curves of ipsilateral and contralateral activity. Contralateral gain exceeds ipsilateral, and both ipsilateral and contralateral activity are attenuated by gap junction blockade with oleamide.



This electrophysiological protocol has proved useful in describing and quantifying ensemble activation in an in vivo spinal motor preparation. Due to the unique midline location and extensive bilateral dendritic arborization of the SNB as well as the need for coordinated ballistic action of the penile muscles they innervate, this method is well suited to analysis of activity in this system. While single-unit or intracellular recordings are valuable tools, this protocol is able to not only characterize the activity within an entire population, but to assess bilateral coordination of the activity through simultaneous, independent recordings of output from each half of the nucleus. Bilateral communication and synchrony within the SNB are likely fundamental sources of the coordinated penile muscle activity; however, the means of the bilateral communication are not fully understood. This technique holds rich potential for describing the mechanisms of bilateral communication in the SNB and its further use will allow more precise descriptions of the underlying central nervous system control of sexual behavior.

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