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Switching-on and -off of bistable spontaneous discharges in rat spinal deep dorsal horn neurons

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Abstract

Somatosensory deep dorsal horn spinal neurons were previously shown to present in vitro a bistable state of activity in which a fixed firing rate is maintained over prolonged periods in the absence of stimulation. Those periods of enhanced spinal spontaneous discharge may play a role in the genesis or maintenance of hyperalgesic states, where episodes of durable spontaneous pain are commonly reported. Here we show in vivo that a small percentage of deep spinal neurons (4% of the recorded population) are capable of rapidly shifting between low-frequency and high-frequency levels of spontaneous activity. At least one of the transitions between the two states was induced by stimulation of the receptive field, making this an interesting and unique case in which stable firing rates are switched-on or -off by somatosensory stimuli.

Keywords: Strychnine; Formalin; Pain; Oscillations; Multielectrode extracellular recordings

Central sensitization is a form of prolonged spinal hyperactivity associated with enhanced pain perception [2,12] that may appear after nerve injury, injury-induced inflammation, pharmacological manipulation or low-frequency repetitive stimulation of unmyelinated c-fibers [31,30,10]. Several mechanisms of synaptic plasticity have been associated with the development of central sensitization [34], and correlated with both the expression of spinal long-term potentiation and spinal action potential windup [17,15]. Recent in vitro studies demonstrated a clear correlation between action potential windup and the onset of spinal plateau potentials that depolarize the membrane potential of the neuron to levels in which autoregenerative discharges may be initiated in the absence of of stimulation [20]. These self-sustained discharges are maintained independently of continuous synaptic excitation and permit the cell to switch back and forth between two stable modes of spontaneous firing. Bistability in the levels of spontaneous neuronal activity has been observed in a number of different cortical, thalamic and spinal populations [11,29,8,4], where persistent enhanced activity is thought to play a crucial role in the long-term signalling of brief events by enabling the neurons to switch between two functional regimens according to the instantaneous synaptic drive [32]. This maintenance of persistent activity may be dependent on intrinsic membrane currents [24,19], be based on synaptic reverberation of local recurrent circuits [27], or on a combination of both mechanisms.

Hyperalgesic states are characterized by spontaneous bursts of neuronal activity and prolonged afterdischarges that usually outlast the period of stimulation [20,9,21,28] and as such, spinal and supraspinal autoregenerative responses may play a role in the genesis and maintenance of chronic pain. However, nociceptive-related bistable autoregenerative responses have been only described in in vitro preparations. In intact animals, description of spinal post-discharge activity at constant, non-decaying, firing rates is scarce and indirect [5,14,13]. Here we report the occurrence of a small number of spinal cells with bistable activity modulated by somatosensory stimulation in intact animals, and report that the number of these cells increases in hyperalgesic states.

Adult male (300–450 g) Sprague–Dawley rats ($n = 33$) were used in this study. All experiments were carried out according to the European Communities Council Directive (86/609/EEC) and to the ethical guidelines for investigation of experimental pain in animals [35]. Animals were anaesthetized with ure-

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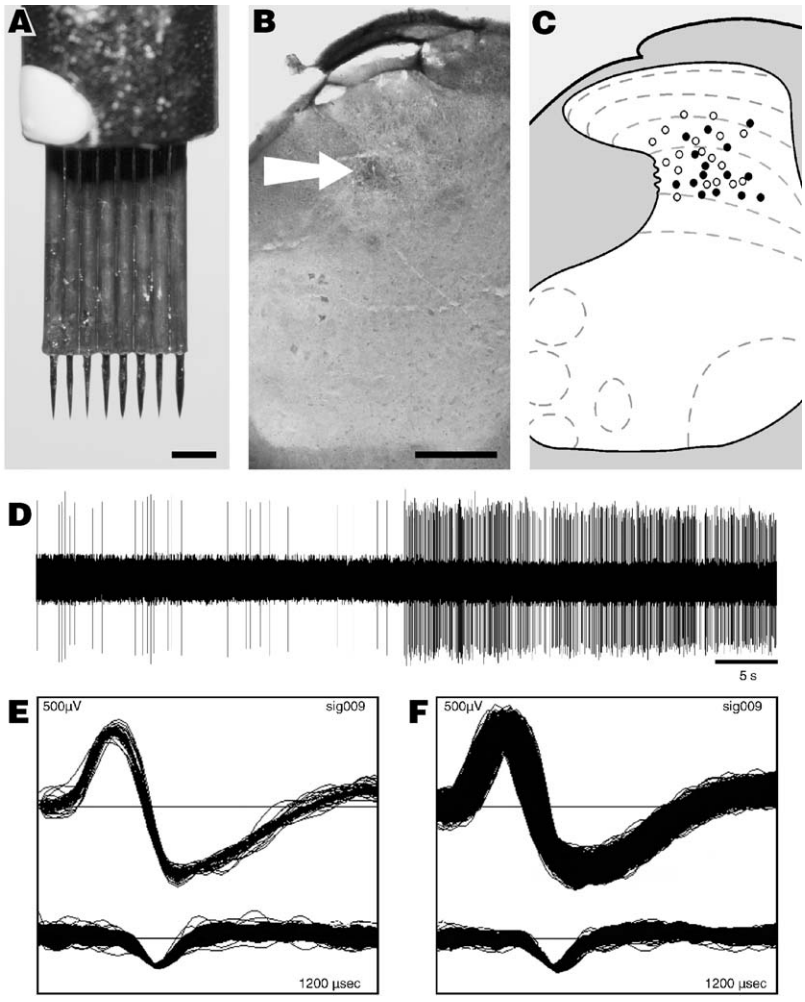

Fig. 1. Multielectrode recording details: (A) photograph of tip of eight-electrode matrix used in the majority of experiments. Scale bar, 500 μm; (B) photograph of spinal cord section depicting electrical lesion used for locating recording sites (arrow). Scale bar, 500 μm; (C) diagram of recording sites of the 33 experiments. Black filled circles mark the sites of experiments in which bistable neurons were recorded; (D) raw data of 60 s of recording showing the large signal to noise ratio obtained from the used electrodes. The selected recording corresponds to the spontaneous switch-on that is also shown in Fig. 2F; (E and F) waveform clustering of the multiunit raw recording of panel D into a large single-unit (top waveform) and a smaller waveform that corresponds to non-isolated multiunit neuronal activity (not considered for neuronal counts). Panel E shows the waveforms obtained from the 30 s before the switched-on period, while panel F shows the waveforms obtained from the 30 s within the switched-on period, demonstrating that it belongs to the same neuron.

thane (1.5 g/kg, i.p.), and depth of anesthesia was assessed by regularly testing the corneal blink, hindpaw withdrawal and tail-pinch reflexes. Body temperature was maintained by means of a homeothermic blanket system.

Animals were mounted in a stereotaxic frame using vertebral clamps, and a laminectomy was performed to expose the lumbar enlargement of the spinal cord. The dura mater was opened and the surface of the cord was covered with warm saline. Neuronal activity was recorded extracellularly in spinal segments L2–L4 using multielectrode matrices of four or eight independent tungsten filaments (3–6 MΩ), spaced at 240 μm (FHC Inc., Bowdoinham, USA; Fig. 1A). Multineuronal signals were amplified and digitized in real time at 40 kHz per channel (MAP16 System, Plexon Inc., Dallas, USA) while separation and identification of individual neurons was made offline using computational methods based on the waveform profiles of the recorded action potentials (Offline Sorter, Plexon Inc., Dallas, USA). Only one recording session was done in each animal.

Spontaneous activity was recorded for 30 min before initiating the periods of stimulation. Cutaneous receptive fields in the glabrous surface of the hindpaw were mapped using the following stimuli: Tap (tapping the skin with a soft-hair brush attached to a robotic servo-motor) and Pinch (using a surgical clamp). Each period of stimulation consisted on 60 s of tapping (non-noxious mechanical stimulation; 1 Hz), 120 s of interval and 30 s of pinch (noxious mechanical stimulation); these stimulations were repeated every 10 min. Neuronal responses to the mechanical stimuli were used to classify the cells as wide dynamic range (WDR neurons—responding to all stimuli), low threshold (LT neurons—had the most vigorous response to tap with little or no response to pinch), nociceptive specific (NS neurons—responding mostly to pinch with little or no response to tap) or non-responsive (NR) neurons.

In 15 animals, after five cycles of stimulation, a subcutaneous injection of diluted formalin (50 μL, 5% formalin) was made in the dorsal hindpaw to induce a persistent pain state, and the neu-