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Brain Research 1045 (2005) 72 – 79

BRAIN RESEARCH

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Research report

Dorsal horn neuron response patterns to graded heat stimuli in the rat

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Accepted 15 March 2005
Available online 26 April 2005

Abstract

Sensory input from various receptors in the periphery first becomes integrated in the spinal cord dorsal horn. The response of the spinal cord dorsal horn neurons to mechanical stimuli are classified as low threshold, high threshold, and wide dynamic range neurons. However, the response pattern of deep dorsal horn cells to heat has not been well described. In this study, the response of the spinal cord dorsal horn neurons to graded heat stimuli were characterized in 147 neurons in rats by extracellular single cell recording. After a differentiable cell was identified, the Peltier heat stimulator was applied to the receptive field and the base temperature was set at 30 °C. The heat stimulus was delivered for 10 s from 37–51 °C in 2 °C increments, with an inter-stimulus interval of 30 s. Out of the 147 neurons, five statistically distinguishable response patterns were identified by latent class cluster analysis. It is concluded that variation of temperature may account for the observed results and indicate functionally different subsets of heat-responsive cells in the deep dorsal horn.

Theme: Sensory systems
Topic: Pain modulation: anatomy and physiology

Keywords: Dorsal horn neuron; Spinal cord; Primary afferent; Synapse

1. Introduction

In the rat, the response of the spinal cord dorsal horn neurons are typically classified as low threshold (LT), high threshold (HT), and wide dynamic range (WDR) neurons according to their response to mechanical stimuli [9,10,36]. Spinal cord dorsal horn neurons have been studied extensively for their response to thermal stimuli, ranging from noxious cold, cool, warm, to noxious heat [4,6–8,13,16,22,24,27,30,33,34,37,39–41]. Typically, dorsal horn neurons respond proportionally to graded heat stimuli. An inhibition of background discharge as well as to noxious mechanical stimulation was demonstrated in dorsal horn neurons in laminae III–V when warm temperatures (32–42 °C) were applied [26]. However, there is little information about the response patterns of dorsal horn neurons to heat stimuli, particularly in deeper laminae. Although much is known about the properties of primary afferent warm and cool fibers in various mammalian species, much less is known about central mechanisms of thermoreception [1]. Spinothalamic tract (STT) neurons in lamina I of the dorsal horn in cat and monkey can be classified according to their response patterns to a range of mechanical and thermal stimuli. More specifically, in addition to the distinction between cold and warm cells, Craig and colleagues also differentiate between two other types of lamina I projection neurons: nociceptive specific (respond solely to noxious mechanical and thermal stimulation) and HPC cells (respond to noxious heat, cold, and pinch) [15]. Centrally, dorsal horn neurons responsive to thermal stimulation are generally thought to be located only in laminae I and II of the spinal cord although they have been reported in deeper laminae as well [21]. Consequently, there is a lack of classification pertaining to the properties of neurons in the

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doi:10.1016/j.brainres.2005.03.030

deeper laminae of the rat dorsal horn in response to thermal stimulation.

Interestingly, we noticed distinct patterns of firing for different dorsal horn neurons in the deep lamina of normal animals in response to graded thermal stimulation. Therefore, the purpose of this study was to further explore the possibility that these cells can be categorized according to their response to thermal stimulation. Preliminary results have been presented previously in abstract form [5].

2. Materials and methods

Twelve male Sprague–Dawley rats served as the subjects. All surgical procedures were approved by the University of Texas at Arlington Institutional Animal Care and Use Committee. The procedures were in accordance with the guidelines published by the Committee for Research and Ethical Issues of IASP [48].

2.1. Animal preparation

Animals were anesthetized using sodium pentobarbital (50 mg/kg, i.p.). As described previously [37], the spinal cord was exposed by performing a 3- to 4-cm laminectomy over the lumbosacral enlargement. A cannula was inserted in the trachea for artificial respiration. The anesthesia and paralysis of musculature was maintained by intravenous administration of a mixture of 50 mg of sodium pentobarbital and 5 mg of pancuronium bromide in 9 ml of 0.9% saline at a rate of 0.02 ml/min. The spinal cord was immobilized in a stereotaxic frame and covered with mineral oil. The end tidal CO_2 was maintained at around 30 mm Hg and body temperature was maintained at 37 °C using a feedback controlled heating pad and rectal thermal sensor probe.

2.2. Data acquisition

A 10–12 MΩ tungsten microelectrode (FHC, Bowdoinham, ME 04008, USA. http://www.fh-co.com) was used for electrophysiological recordings in the L5 and L6 region of the spinal cord dorsal horn. Single unit extracellular recordings of dorsal horn neurons were searched by mechanical stimulation of the receptive field in the plantar region of the hind paw. Responses to intensity-coded mechanical (brush, pressure, and pinch) and thermal (37–51 °C in 2° increments) stimulation was recorded using SPIKE2 computer software program. Multiple sites were recorded in the spinal cord of each rat.

2.2.1. Measurement of mechanical stimulation responses

Following the identification of a differentiable cell, three mechanical stimuli of increasing intensity (brush, pressure, and pinch) were applied to the receptive field. Each stimulus was applied once for 10 s with an inter-stimulus interval of 20 s. The response to each mechanical stimulus was measured as the number of action potentials per second. By using the ratio between responses to brush and pinch, dorsal horn neurons can be classified as LT (best response to brushing, =100%), WDR (<100% and >10%), and HT (=10%) neurons [10].

2.2.2. Measurement of heat stimulation responses

After the mechanical stimulation, a Peltier heat stimulator was applied to the receptive field and the base temperature was set at 30 °C. The heat stimulus was delivered for 10 s from 37 to 51 °C in 2 °C increments, with an inter-stimulus interval of 30 s. The heat-evoked response was calculated as the response to heat stimulus minus the background activity (spikes/s).

2.3. Data analysis

The stored digital record of unit activity was retrieved and analyzed off-line. For single neuron recordings, responses to the heat stimuli applied to the receptive field for 10 s were calculated by subtracting the preceding 10 s of background activity to yield a net change in discharge rate, where a negative number indicated an inhibition. This change in discharge rate was defined as heat-evoked response.

A latent class cluster analysis (LCCA) was performed using the Latent Gold software [45]. Briefly, LCCA allows one to estimate (or test hypotheses about) the number of clusters in a multivariate response data set, and provides methods for estimating the fraction of the cell population in each cluster, as well as the cluster membership of each cell.

The primary justification for using LCCA in this study is that LCCA has the capability to provide an estimate of the number of clusters in a population, with specified statistical confidence. This formal statistical inference capability of LCCA is in contrast to standard clustering methods (e.g., *k*-means), which do not allow statistical confidence statements to be ascribed to the clusters they produce. However, the formal inference capability of LCCA comes at a price: requiring parametric assumptions about the distribution of the eight temperature responses (37–51 °C) within each extant cluster. The standard clustering procedures (e.g., *k*-means) require no such assumptions, but generally do not provide formal inference capabilities. An exception is the so-called “valley hunting” procedure implemented in PROC MODECLUS of the SAS package, which does provide some formal inference capability without parametric distributional assumptions. However, owing to its being based on nonparametric multivariate density estimation, and due to the high dimension (eight) of the response vectors in the present study, the validity of the “valley hunting” method in the present context would require data from a prohibitively-large number of cells (the so-called curse of dimension-