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Research paper

# In vitro thermosensitivity of rat lateral parabrachial neurons

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## HIGHLIGHTS

- We evaluated the local thermosensitivity of rat lateral parabrachial (LPB) neurons.
- Warm- and cold-sensitive neurons were recorded in the LPB in vitro.
- Warm sensitivity in the LPB was similar to that in the preoptic area and spinal cord.
- Cold sensitivity in the LPB was distinct from that in the preoptic area.

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## ABSTRACT

The lateral parabrachial (LPB) neurons play a pivotal role in the thermoregulatory afferent pathway by transmitting cutaneous thermosensory signals received from spinal neurons directly to the thermoregulatory command center, the preoptic area (POA). The present study was conducted to electrophysiologically characterize the local temperature responsiveness of rat LPB neurons in brain slices to evaluate their local thermosensitivity and permit comparison with thermosensitive neurons in POA and spinal cord slices under consistent experimental conditions. In current clamp, warm- and cold-sensitive neurons were recorded in LPBel, LPBc and LPBd, the three LPB subnuclei responsible for the transmission of cutaneous feedforward signals. Of the 92 spontaneously firing LPB neurons, 27% were warm sensitive, 10% were cold sensitive, and 63% were temperature insensitive, and the spontaneous firing rate of the warm-sensitive neurons was significantly greater than that of the temperature-insensitive neurons. These proportions and spontaneous activity are similar to results obtained in the POA and spinal cord. Furthermore, the thermosensitivity was also present in 38% of silent neurons evoked by injection of a small amount of depolarizing current. Warm-sensitive neurons in the LPB were similar in thermoresponsiveness to those in the POA and spinal cord. However, cold sensitivity in the LPB was distinct from that in the POA. The firing rate of most cold-sensitive neurons changed steeply at a relatively narrow band of temperature, and some of them were silent near thermoneutrality. The percentages of thermosensitive and insensitive neurons within the three LPB subnuclei were not significantly different, nor were the mean maximal thermal coefficients of the thermosensitive neurons. These results suggest that LPB have local thermosensory functions as POA and spinal cord, and might be an important extrahypothalamic "thermoregulator".



## 1. Introduction

The lateral parabrachial nucleus (LPB) is located in the dorsolateral pons and is composed of at least seven distinct subnuclei, distinguished by cell morphology and spatial clustering. Recent studies [1–4] have identified LPB as a crucial relay for afferent signals from the skin that promote defensive thermoregulatory responses before changes in environmental temperature affect

*Abbreviations:* ACSF, artificial cerebrospinal fluid; LPB, lateral parabrachial nucleus; LPBc, central subnucleus of LPB; LPBd, dorsal subnucleus of LPB; LPBel, external lateral subnucleus of LPB; POA, preoptic area; SCP, superior cerebellar peduncle.

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body core temperature. The LPB receives spinal input from skin cooling-activated neurons (the external lateral (LPBel) with an extension into the central subnucleus (LPBc)) and skin warming-activated neurons (the dorsal subnucleus (LPBd)). Third-order thermosensory relay neurons arise from these regions, which then project to the preoptic area (POA) [1–5]. Thus, the spinal-LPBel/c-POA pathway and spinal-LPBd-POA pathway are respectively responsible for the transmission of cool and warm feedforward signals from the skin to the POA.

POA thermosensitive neurons are important in generating both physiological and behavioral responses to temperature changes. In vivo studies have indicated that these neurons are sensitive not only to local, hypothalamic temperature, but to changes in skin and spinal cord temperature as well [6]. Sensitivity to both local and peripheral temperature changes appears to be a phenomenon not unique to the POA. The spinal cord contains thermosensitive neurons as well [7], and the spinal thermal signals could be integrated with cutaneous thermal signals at the spinal cord level [8]. The discovery of thermoreceptive elements in the spinal cord is pivotal for the establishment of the currently accepted multi-input, multi-level concept of thermoregulation [7,8]. Recently, single LPB cells in vivo have been shown to be activated by changes in skin temperature [1,2]. However, the local thermosensitivity of LPB neurons has not yet been determined.

The aim of this study was to electrophysiologically characterize the local temperature responsiveness of in vitro LPB neurons in brain slices to evaluate their local thermosensitivity and permit comparison with thermosensitive neurons in POA and spinal cord slices under consistent experimental conditions. Because there are physiological differences in the transmission of cool and warm thermal information from the skin to the POA between LPBel, LPBc and LPBd, we also compared the firing activity and thermosensitivity of neurons in these three LPB subnuclei.

## 2. Materials and methods

### 2.1. Preparation of brain slices for electrophysiological recording

According to procedures reported previously [9], coronal brainstem slices containing the LPB were prepared from male Sprague–Dawley rats (80–150 g). Briefly, each rat was anesthetized with pentobarbital and quickly decapitated in accordance with procedures approved by the NIH and Chengdu Medical College Laboratory Animal Care and Use Committee. The brain was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF). A 0.5-cm section of the brainstem that contained the LPB was prepared and was then sliced into 350-μm thick transverse slices with a Vibratome VT1200 tissue slicer (Leica, Germany). After the slices had incubated for at least 1 h at 32 °C, coronal brainstem slices containing the LPB were transferred to a recording chamber and were constantly perfused at 1.2 ml/min with 300 mOsm/kg$H_2O$ ACSF consisting of (in mM) 124 NaCl, 26 $NaHCO_3$, 5 KCl, 2.4$CaCl_2$, 1.3 $MgSO_4$, 1.24 $KH_2PO_4$, and 10 glucose (pH 7.4). The ACSF was gas saturated with 95% $O_2$–5% $CO_2$ and heated to 35–37 °C using a thermoelectric Peltier assembly (SC-20, Warner Instruments Inc., USA).

### 2.2. Recording firing activity of LPB neurons

The firing activity of neurons located in the LPBel, LPBc or LPBd was recorded in cell-attached or whole-cell current clamp mode. Patch pipettes (4–7 MΩ) were pulled from borosilicate glass and filled with an internal solution consisting of (in mM) 130 potassium gluconate, 10 EGTA, 10 HEPES, 2 MgATP, 2 $Na_2GTP$, 1 $CaCl_2$. This internal solution was adjusted to 295 mOsm/kg$H_2O$ and pH of 7.2. The LPB was identified visually as a crescent-shaped lucent region at the dorsolateral surface of the pons that was bordered dorsally by the ventral spinocerebellar tract and ventrally by the superior cerebellar peduncle (SCP) [9,10]. The three LPB subnuclei were defined by their relationship to the SCP [10], which was visualized and photographed by the infrared differential interference contrast videomicroscopy (Fig. 1). Recordings were usually made from slices at the middle level of the LPB in the rostral-caudal dimension. Not only is the surface area of the LPB largest, but the three subnuclei detected all appeared at this level [10]. The ground electrode was maintained at a constant temperature in an outer bath connected to the inner recording chamber [11,12]. Recordings were carried out using a Multiclamp 700B amplifier (Axon, USA) or an EPC10 (HEKA, Germany). When spontaneous activity was recording, no holding current was applied to neurons. The program package pCLAMP 10.1 or Patchmaster was used for data acquisition and analysis. Recordings were digitized at 10 kHz and filtered with low-pass filter of 2 kHz.

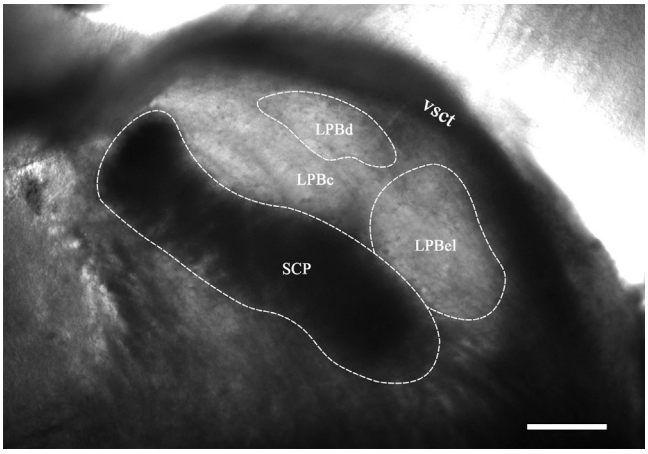


**Fig. 1.** The lateral parabrachial nucleus (LPB) under infrared differential interference contrast videomicroscopy. LPBc, central subnucleus of LPB; LPBd, dorsal subnucleus of LPB; LPBel, external lateral of LPB; SCP, superior cerebellar peduncle; vsct, ventral spinocerebellar tract. Scale bar is 200 μm.

### 2.3. Evaluation of thermosensitivity of LPB neurons

The thermoelectric assembly allowed the tissue slice temperature to be periodically varied 3–5 °C above and below the neutral temperature (35–37 °C) to characterize the thermosensitivity of each recorded neuron. The slice temperature was monitored continuously by a thermocouple placed near the slice. The firing activity of neurons was continuously recorded during cyclic temperature changes. Criteria for classifying LPB neuronal thermosensitivity were similar to numerous investigations of preoptic temperature sensitivity [11,12]. Thermosensitivity (impulses/s/°C) was defined by the linear regression slope (or thermal coefficient, m) of firing rate plotted as a function of temperature. This plot was determined over a (minimal 3 °C) temperature range in which a neuron was most sensitive. With the use of the same criteria as POA, warm-sensitive neurons had thermal coefficients of 0.8 impulses/s/°C (imp/s/°C) or greater, and cold-sensitive neurons had thermal coefficients of −0.6 imp/s/°C or less. All other neurons were considered temperature insensitive, and these were further divided into two subpopulations. Low-slope temperature-insensitive neurons were almost completely unresponsive to changes in temperature, and the absolute values of their thermal coefficients were <0.2 imp/s/°C. Moderate-slope temperature-insensitive neurons exhibited modest changes in their firing rates during changes in temperature, and their thermal