Research paper

# In vitro thermosensitivity of rat lateral parabrachial neurons 

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## H I G H L I G H T S

- 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".


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## 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
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 warmingactivated 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/cPOA 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, multilevel 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 $(80-150 \mathrm{~g})$. 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 $0.5-\mathrm{cm}$ section of the brainstem that contained the LPB was prepared and was then sliced into $350-\mu \mathrm{m}$ thick transverse slices with a Vibratome VT1200 tissue slicer (Leica, Germany). After the slices had incubated for at least 1 h at $32^{\circ} \mathrm{C}$, coronal brainstem slices containing the LPB were transferred to a recording chamber and were constantly perfused at $1.2 \mathrm{ml} / \mathrm{min}$ with $300 \mathrm{mOsm} / \mathrm{kgH}_{2} \mathrm{O}$ ACSF consisting of (in mM ) $124 \mathrm{NaCl}, 26 \mathrm{NaHCO}_{3}, 5 \mathrm{KCl}, 2.4 \mathrm{CaCl}_{2}$, $1.3 \mathrm{MgSO}_{4}, 1.24 \mathrm{KH}_{2} \mathrm{PO}_{4}$, and 10 glucose ( pH 7.4 ). The ACSF was gas saturated with $95 \% \mathrm{O}_{2}-5 \% \mathrm{CO}_{2}$ and heated to $35-37^{\circ} \mathrm{C}$ 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 ( $4-7 \mathrm{M} \Omega$ ) were pulled from borosilicate glass and filled with an internal solution consisting of (in mM ) 130 potassium gluconate, 10 EGTA, 10 HEPES, $2 \mathrm{MgATP}, 2 \mathrm{Na}_{2} \mathrm{GTP}, 1 \mathrm{CaCl}_{2}$. This internal solution was adjusted to $295 \mathrm{mOsm} / \mathrm{kgH}_{2} \mathrm{O}$ and pH


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 $200 \mu \mathrm{~m}$.
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 .

### 2.3. Evaluation of thermosensitivity of LPB neurons

The thermoelectric assembly allowed the tissue slice temperature to be periodically varied $3-5^{\circ} \mathrm{C}$ above and below the neutral temperature $\left(35-37^{\circ} \mathrm{C}\right)$ 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/ ${ }^{\circ} \mathrm{C}$ ) 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 $3^{\circ} \mathrm{C}$ ) 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 $/ \mathrm{s} /{ }^{\circ} \mathrm{C}\left(\mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}\right.$ ) or greater, and coldsensitive neurons had thermal coefficients of $-0.6 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$ or less. All other neurons were considered temperature insensitive, and these were further divided into two subpopulations. Lowslope temperature-insensitive neurons were almost completely unresponsive to changes in temperature, and the absolute values of their thermal coefficients were $<0.2 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$. Moderate-slope temperature-insensitive neurons exhibited modest changes in their firing rates during changes in temperature, and their thermal

Warm sensitive neuron


Cold sensitive neuron


Temperature-insensitive neuron


Moderate-slope
Temperature-insensitive neuron





Fig. 2. Effect of temperature on different types of spontaneously firing LPB neurons. Thermal coefficient: warm-sensitive neuron, $1.4 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$; cold-sensitive neuron, $-0.7 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$; low-slope temperature-insensitive neuron, $0.1 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$; moderate-slope temperature-insensitive neuron, $0.4 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$. All four cells exhibited depolarizing prepotential (arrows) that preceded the action potentials.
coefficients were $\geq 0.2 \mathrm{but}<0.8 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$. In addition, silent neurons were classified as cells that did not generate spontaneous action potentials, although spike activity could be evoked by application of depolarizing current pulses.

### 2.4. Data analysis

All data are expressed as means $\pm$ SE. One-way ANOVA followed by LSD test was used to compare differences in firing rates and maximal thermal coefficients of the neurons in the three LPB subnuclei. Chi-square analysis was used to compare their proportions of thermosensitive and temperature-insensitive neurons.

## 3. Results

### 3.1. Spontaneous firing rate

A total of 124 neurons were recorded intracellularly (72) or extracellularly (52) from the tissue slices of 60 rats and characterized according to their firing activity and thermosensitivity. Of these neurons, 32 were found in LPBel, 51 in LPBc, and 41 in LPBd. The majority ( $74 \%$ ) of all LPB neurons had spontaneous activity. 24 of the spontaneous neurons were located in LPBel, 34 were located in LPBC, and 34 were located in LPBd. There were no significant differences in the mean spontaneous discharge rate of neurons located in the three subnuclei. Most spontaneously firing neurons displayed the depolarizing prepotential, which is the slow depolarization that occurs prior to the membrane potential reaching threshold (Fig. 2). The remaining $26 \%$ of the neurons were silent after impalement, action potentials of which were elicited by injection of a small amount of depolarizing current.

Comparisons were made of the spontaneous activity of warm-sensitive, cold-sensitive, low-slope and moderate-slope
temperature-insensitive neurons in the LPB. The warm-sensitive neurons had a significantly higher spontaneous firing rate than the other three cell types at $37^{\circ} \mathrm{C}$ (warm-sensitive neurons $12.29 \pm 1.53 \mathrm{imp} / \mathrm{s} \quad(\mathrm{n}=25)$; cold-sensitive neurons $5.76 \pm 1.86 \mathrm{imp} / \mathrm{s}(\mathrm{n}=9)$; low-slope temperature-insensitive neurons $5.73 \pm 0.83 \mathrm{imp} / \mathrm{s}(\mathrm{n}=18)$, moderate-slope temperatureinsensitive neurons $8.03 \pm 0.57 \mathrm{imp} / \mathrm{s}(\mathrm{n}=35)$ ), while the mean spontaneous discharge rate of the other three cell types did not differ at $37^{\circ} \mathrm{C}$. When comparisons were made among the three different LPB subnuclei, the mean spontaneous rate of the warm-sensitive, cold-sensitive, low-slope temperature-insensitive or moderate-slope temperature-insensitive neurons was found not to be significantly different. The examples shown in Fig. 2 illustrate the effect of temperature on the different types of spontaneously firing LPB neurons. Both warm- and cold-sensitive neurons were very responsive to changes in temperature; while the low-slope temperature-insensitive neuron remained nearly constant during changes in temperature.

### 3.2. Temperature sensitivity

When $0.8 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$ is used as the criterion for warm sensitivity, of 92 spontaneously firing LPB neurons in the present study, 27\% were warm sensitive, $10 \%$ were cold sensitive, and the remaining $63 \%$ were temperature insensitive and there were no differences in these percentages of LPBel, LPBc and LPBd. The numbers of warm-, cold- and temperature-insensitive neurons in the three subnuclei are shown in Table 1. When comparisons were made of the mean maximal thermal coefficients of warm- or cold-sensitive neurons in the three regions, there was no significant difference. We also evaluated the thermosensitivity of induced-firing LPB neurons, of 32 these neurons, $38 \%$ were thermosensitive (warm-sensitive $19 \%$, cold-sensitive $19 \%$ ), and $62 \%$ were temperature-insensitive.

Table 1
Thermosensitivity of neurons located in the LPB.

| Locaion | SFN( $\mathrm{n}=92$ ) |  |  | $\operatorname{IFN}(\mathrm{n}=32)$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Warm-sensitive | Cold-sensitive | Temperature-insensitive | Warm-sensitive | Cold-sensitive | Temperature-insensitive |
| LPBel | 7 | 1 | 16 | 0 | 3 | 5 |
| LPBC | 10 | 2 | 22 | 4 | 3 | 10 |
| LPBd | 8 | 6 | 20 | 2 | 0 | 5 |
| Total | 25 | 9 | 58 | 6 | 6 | 20 |

SFN, spontaneously firing neurons; IFN, induced-firing neurons.

Although there appeared to be a tendency for a lower proportion of warm-sensitive and a greater proportion of cold-sensitive neurons vs. that of spontaneously firing LPB neurons, there was, in fact, no significant difference in the proportions of the two types of neurons (Table 1).

Many warm-sensitive neurons (65\%) showed a linear thermal response over the entire temperature range tested, while other neurons were most sensitive over a smaller thermal range, with threshold temperatures of $36.0 \pm 0.2{ }^{\circ} \mathrm{C}(\mathrm{n}=11)$. As shown in Fig. 3A, this warm-sensitive neuron showed a linear increase in firing rate during successive increases in tissue temperature. In the resulting thermosensitive curve, this warm-sensitive neuron's $m$ over the $33-39^{\circ} \mathrm{C}$ range was $0.9 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$. While the warm-sensitive neuron in Fig. 3B was maximally sensitive over the $36-39^{\circ} \mathrm{C}$ range ( $\mathrm{m}=1.1 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$ ) and showed little response to a temperature change below $36^{\circ} \mathrm{C}$. Contrary to warm-sensitive neurons, many cold-sensitive neurons (87\%) showed a nonlinear thermal response and their cold sensitivity only expressed at a relatively narrow band of temperature. As shown in Fig. 3C, the firing activity of this neuron decreased when the tissue temperature was warmed and decreased steeply to $0 \mathrm{imp} / \mathrm{s}$ around $37^{\circ} \mathrm{C}$, and the firing rate began to increase when cooling back to $36^{\circ} \mathrm{C}$ in the falling phase. The neuron's m over the $31-36{ }^{\circ} \mathrm{C}$ range was $-0.7 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$. Fig. 3D shows another cold-sensitive neuron, which was evoked by continuous injection of depolarizing current ( 45 pA ). The firing activity of this neuron decreased when the tissue temperature was warmed to $36^{\circ} \mathrm{C}$ and decreased steeply to $0 \mathrm{imp} / \mathrm{s}$ when temperature was increased to $37{ }^{\circ} \mathrm{C}$, and the neuron became silent until temperature was cooled back to $36^{\circ} \mathrm{C}$; subsequently, the firing rate increased steeply to a plateau firing level of about $3 \mathrm{imp} / \mathrm{s}$ at $35^{\circ} \mathrm{C}$. The neuron's m over the $35-38^{\circ} \mathrm{C}$ range was $-0.8 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$. Out of 15 cold-sensitive neurons, nine showed such thermoresponsiveness that the firing rate suddenly decreased when the tissue temperature was raised near the threshold temperatures, and fired only below their own threshold temperatures (five were between $36-37.5^{\circ} \mathrm{C}$, four were between $39-40^{\circ} \mathrm{C}$ ).

## 4. Discussion

This study was the first in which the local thermosensitivity of LPB neurons was investigated using the methodology that has frequently been used to characterize thermosensitive neurons in the POA of various homeothermic species. The data showed that neurons in the LPB are, indeed, as thermosensitive as neurons in the POA and spinal cord, and functionally, these neurons may represent the cellular basis for the temperature sensory function of the LPB, which enhance thermoregulatory responses by a negative feedback system in situations of extreme thermal environments when the feedforward thermoregulatory responses driven by changes in skin temperature have proven inadequate to prevent changes in brain, and respond to challenges to thermal homeostasis involving changes in temperature within the body, such as exercise.

The spontaneous activities of warm-sensitive and temperatureinsensitive neurons found in the LPB were not different from those recorded in the POA and spinal cord neurons under iden-
tical conditions. For example, the spontaneous firing rate of the warm-sensitive neurons was significantly greater than that of the temperature-insensitive neurons and most spontaneously firing neurons displayed the depolarizing prepotential. Comparing the numbers of temperature-sensitive neurons from slices of the LPB, POA and spinal cord in vitro in the same studies revealed similar percentages of warm-sensitive and cold-sensitive neurons and is well within the average number of warm-sensitive (20-60\%) and cold-sensitive neurons ( $0-15 \%$ ) generally described in studies on POA or spinal cord slices [6-8,11-14]. The similarity in the spontaneous activities and the local thermosensitivity of neurons in the LPB, POA and spinal cord may result from common functional characteristics. The POA plays a pivotal role in the regulation of body temperature by integrating information on peripheral and central temperatures and providing appropriate command signals to peripheral thermoregulatory effectors. The importance of the spinal cord for the regulation of body temperature has, like that of POA, been demonstrated in various homeothermic species, including the rat $[7,8]$. LPB thermosensory relay neurons are also activated by peripheral thermal input [1-5]. A POA thermosensitivity of at least $0.8 \mathrm{imp} / \mathrm{s} /{ }^{\circ} \mathrm{C}$ is to identify thermal integrative neurons that receive afferent information [14]. With the same criteria as POA, in the present study, $27 \%$ spontaneously firing LPB neurons were identified as warm-sensitive neurons. Thus, cutaneous thermal sensory information is presumably integrated, in an as yet unknown manner, at synapses in the spinal cord and subsequently within the LPB, where cool and warm afferent signals are processed within anatomically distinct regions and integrated with local thermal signals and then transmitted to the POA. In addition to thermosensory signals coming from the skin through the spinal cord, the LPB receives massive visceral afferent information [15], including temperature, which converges at the level of the nucleus tractus solitarius before passing to the LPB, so the LPB may integrate both skin and visceral thermal information [4]. Further studies are needed to determine whether LPB thermosensory relay neurons integrate information on peripheral (cutaneous and likely visceral) and local, parabrachial temperature.

Unlike warm-sensitive neurons in the LPB, the thermoresponsiveness of which was similar to that studied previously in the central nervous system in vitro, including the POA and spinal cord [6-8,11-14,16-18], many cold-sensitive neurons in the LPB displayed different responses to temperature changes from those in the POA. Cold-sensitive neurons in the POA were reported to have thermoresponse curves that appear to "peak" near thermoneutrality, which have their maximum firing rates between 36 and $38^{\circ} \mathrm{C}$ [13]. By contrast, we observed that some cold-sensitive neurons in the LPB were silent between 36 and $38^{\circ} \mathrm{C}$, the firing rate of which decreased immediately to $0 \mathrm{imp} / \mathrm{s}$ when temperature was increased to $37{ }^{\circ} \mathrm{C}$ around and their cold sensitivity expressed at a narrow thermal range below $37{ }^{\circ} \mathrm{C}$. The similar thermoresponsiveness was also observed in some cold-sensitive neurons in rat midline thalamus [16] and posterior hypothalamus [17], and most cold-sensitive neurons in rat dorsal medulla oblongata in vitro, although these medullary cold-sensitive neurons only fired at a relatively lower level ( $2-6{ }^{\circ} \mathrm{C}$ below normal) [18].


Fig. 3. Digitized polygraph recordings and thermoresponse curves of warm- and cold-sensitive LPB neurons. A: a warm-sensitive neuron with a linear thermal response over the entire temperature range tested; B: a warm-sensitive neuron maximally sensitive over the $36-39{ }^{\circ} \mathrm{C}$ range; C : a cold-sensitive neuron maximally sensitive over the $31-36^{\circ} \mathrm{C}$ range; D: a cold-sensitive neuron maximally sensitive over the $35-37^{\circ} \mathrm{C}$ range, which was evoked by continuous injection of depolarizing current ( 45 pA ). FR, firing rate; Temp, temperature.
$65 \%$ of warm-sensitive neurons in the LPB showed a linear thermal response over the entire temperature range tested, and $35 \%$ were most sensitive over a smaller thermal range. On the contrary, $87 \%$ of cold-sensitive neurons were most sensitive only over a narrow thermal range. Therefore, we presume that specific subpopulations of temperature-sensitive neurons discriminate local cold or warm stimuli with overlap between the stimulatory temperatures. Some warm-sensitive and most cold-sensitive neurons had a relatively narrow band of temperature activation, indicating that these neurons have different sensitivities over different temperature ranges and may work at the narrow temperature zone.

In the present experiments, induced-firing neurons by injection of a small amount of depolarizing current are as thermosensitive as spontaneously firing neurons. Accordingly, it is possible to hypothesize that excitatory synaptically driven neurons, such as LPB thermosensory relay neurons glutamatergically activated by cutaneous thermosensory signals, may also be involved in response to local temperature changes.

The percentages of thermosensitive and insensitive neurons within the three subnuclei were not significantly different, nor were the mean maximal thermal coefficients of the thermosensitive neurons. This suggests that the three LPB subnuclei share some thermoresponse characteristics and exert common thermosensory function, although they transmit different cutaneous thermal information to the POA.

The data from this study together with that from the studies just discussed suggest that the LPB receive peripheral thermoreceptive information and respond to local temperature changes just as the POA and spinal cord. In light of the anatomical and functional considerations of the spinal cord, LPB and POA in the spinal-LPBPOA afferent pathway, it is likely that all of these structures are associated with physiological or behavioral responses to peripheral and central thermal stimulation. The discovery of thermoreceptive elements in the LPB not only indicates that the LPB might be an important extrahypothalamic "thermoregulator", but also adds further evidence in support of the multi-input, multi-level concept of thermoregulation.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' contributions

Y.W.X. carried out the experiment and the data analysis. Y.L.Y., Y.T. and J.H.X. participated in designing the research study and constructed the experimental room. M.P.Y. participated in the experiment. Y.X.Z. participated in data analysis. J.ZH. had full access to all of the data in the study, took responsibility for the integrity of the data, designed the study and wrote the manuscript.

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[^0]:    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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