News Release

Researchers discovered two separate brain neural pathways for avoidance of hot and cold environments

Key Points

- **Neurotransmission from the lateral parabrachial nucleus to the thermoregulatory center, preoptic area mediates heat avoidance behavior.**
- **Neurotransmission from the lateral parabrachial nucleus to the emotion center, amygdala mediates cold avoidance behavior.**
- **Both of the two groups of lateral parabrachial nucleus neurons mediating the neurotransmission are required for brown fat thermogenesis in response to skin cooling.**
- **These findings indicate that the brain mechanisms that generate unpleasant emotions in response to heat and cold sensations are different, potentially leading to a better understanding of the onset mechanisms of heat stroke and hypothermia.**
- **The findings also promote a new understanding of the brain's neural circuit mechanisms that appropriately regulate body temperature and metabolism, and may lead to the development of new obesity treatment technologies that promote fat combustion.**

Summary

A research group led by graduate student Takaki Yahiro, Designated Senior Lecturer Naoya Kataoka, and Professor Kazuhiro Nakamura at the Department of Integrative Physiology, Nagoya University Graduate School of Medicine has discovered the neural pathways in the brain that develop thermoregulatory behaviors to avoid hot and cold environments.

Animals, including humans, use thermoregulatory behaviors to maintain body temperature within a range appropriate for their activities. Thermoregulatory behaviors include turtles basking in the sun and humans using air conditioning with light clothing in the summer and heating with heavy clothing in the winter. In 2017, the research group reported that a brain region called the lateral parabrachial nucleus (LPB) is required for thermoregulatory behavior to occur. In the present study, the group investigated how neurotransmission through the LPB develops thermoregulatory behavior in rats.

The researchers found that two different groups of neurons in the LPB transmit thermosensory information from skin thermoreceptors to different

areas of the forebrain, thereby developing behaviors to escape from heat and cold, respectively. Thermoregulatory behavior is thought to be driven by discomfort (unpleasant emotion) caused by heat or cold sensation, and therefore the neural pathways discovered in this study may be involved in the formation of unpleasant emotions caused by temperature sensations. The present findings provide a clue to the cause of heat stroke and hypothermia, which are likely caused by an inability to appropriately form unpleasant emotions in response to heat and cold sensations.

Both groups of neurons in the LPB were also found to be required for the thermogenic response in brown adipose tissue in response to cold stimuli. This finding promotes a new understanding of the neural circuit mechanisms in the brain that maintain health by appropriately regulating body temperature and metabolism. Future advances in this research may lead to the development of technologies for early detection of metabolic diseases at pre-disease stages and the development of new obesity treatment technologies that promote fat combustion.

Research Background

Thermoregulation is one of the most fundamental biological functions of animals. Homeothermic animals, including humans, use two forms of thermoregulation to maintain their body temperature around 37°C. One is called “behavioral thermoregulation,” a mechanism to maintain body temperature through instinctive thermoregulatory behavior based on comfort or discomfort of the temperature sensed. In humans, examples of thermoregulatory behaviors include conscious behaviors such as putting on or taking off clothing based on the ambient temperature and optimizing room temperature by using air conditioning. Behavioral thermoregulation is common in most animals: not only homeotherms (warm-blooded animals), but also poikilotherms (cold-blooded animals), such as snakes and insects, have been observed to flee cold environments and move to warmer places. The other thermoregulatory mechanism is “autonomous thermoregulation,” which involves involuntary (unconscious) responses to regulate body temperature, such as sweating to actively dissipate body heat in a hot environment or shivering in muscles to generate heat in a cold environment. Homeothermic animals efficiently maintain their body temperature through a combination of behavioral and autonomous thermoregulation.

In recent years, due in part to rising air temperatures caused by global warming, tens of thousands of people in Japan have been transported to emergency rooms for heat stroke each year. One of the reasons for this is that many of heat stroke victims are elderly people who are unable to regulate their

body temperature properly, as many of them have difficulty being motivated to turn on the air conditioner when they feel hot. In Japan, where temperatures are rising and the population is aging, there is an urgent social need to find a mechanism for proper thermoregulatory behavior and to elucidate the onset mechanism of heat stroke. However, while research on the neural circuits of autonomous thermoregulation has progressed, those of behavioral thermoregulation have remained a mystery.

In 2017, the research group reported that the thermoregulatory behaviors of escaping from heat and cold requires thermosensory information relayed by the brain region, LPB, but not that by the spinothalamocortical tract, a sensory pathway for consciously "feeling" temperatures. The LPB is a brain region located in the pons and is known to relay various sensory information in the body to a variety of forebrain regions. In the present study, the research group used rats to investigate the neural pathways from the LPB to the forebrain that transmit thermosensory information from the skin to develop thermoregulatory behaviors.

Research Results

First, the research group used adeno-associated virus to express plasma membrane-targeted green fluorescent protein (palGFP) in neurons of the rat LPB and thereby visualized their nerve fibers (axons). They found that axon terminals of LPB neurons are densely distributed in the median preoptic nucleus (MnPO) of the preoptic area, a hypothalamic region that serves as a thermoregulatory center, and in the central nucleus of the amygdala (CeA), a center for emotion and fear memory. Therefore, the research group hypothesized that thermosensory information delivered from the skin to the LPB via the spinal cord is further transmitted from the LPB to the MnPO and CeA to develop thermoregulatory behavior (**Fig. 1A**).

Previous studies of the research group have shown that autonomous thermoregulation requires LPB→MnPO transmission of thermosensory information. However, LPB→CeA transmission of thermosensory information has not been investigated. The research group labeled LPB neurons that extended axons to the CeA by using cholera toxin B subunit, a retrograde neural tracer that is taken up from axon terminals and transported in the opposite direction to label neuronal cell bodies. They found that these LPB neurons innervating the CeA are activated in response to cold exposure of rats and form a group distinct from LPB neurons innervating the MnPO. These results suggested that the two groups of LPB→MnPO and LPB→CeA neurons may have different functions in regulating body temperature.

To analyze thermoregulatory behaviors of rats, the research group performed

two-floor thermal plate preference tests. Two metal floor plates were placed side by side, with one plate set at 28°C (neutral temperature) and the other at 39°C (high temperature) or 15°C (low temperature) (**Fig. 1B**). Normal rats spent more time on the neutral plate than on either the high or low temperature plate, representing a thermoregulatory behavior called heat or cold avoidance.

The scientists then injected the rat brain with two types of adeno-associated viruses, one that infects from axon terminals and the other that infects neuronal cell bodies, to selectively block one of the LPB→MnPO and LPB→CeA neural pathways that were double-infected. First, the rats in which the LPB→MnPO pathway was suppressed with a chemogenetic technique (**Fig. 2A**) were unable to show heat avoidance and rather stayed slightly longer on the 39°C plate (**Fig. 2B**). However, they showed cold avoidance like normal rats (**Fig. 2C**). Next, optogenetic techniques were used to suppress LPB→CeA neurotransmission in another group of rats (**Fig. 3A**). These rats were unable to show cold avoidance, but heat avoidance was intact (**Fig. 3B, C**).

In addition, cooling of the rat trunk skin normally induces heat production in brown adipose tissue as an autonomous thermoregulatory response to prevent hypothermia. Interestingly, however, when either group of LPB→MnPO or LPB→CeA neurons was inhibited by expression of tetanus toxin light chain, skin cooling-evoked brown fat thermogenesis did not occur (**Fig. 4A**). Furthermore, these rats exhibited hyperthermia in a hot environment and became hypothermic in a cold environment (**Fig. 4B**).

These experimental results demonstrate that thermosensory information delivered from the skin to the LPB via the spinal cord is further transmitted to the MnPO and CeA, and that the LPB→MnPO transmission develops heat avoidance behavior, whereas the LPB→CeA transmission develops cold avoidance behavior (**Fig. 5**). These thermoregulatory behaviors are thought to be driven by the discomfort (unpleasant emotion) generated by temperature sensation, and thus the present results suggest that unpleasant emotions for heat and for cold are generated by different neural circuit mechanisms. In addition, both LPB→MnPO and LPB→CeA neural pathways are required for autonomous thermoregulatory responses, such as brown fat thermogenesis. In particular, the involvement of the amygdala in thermoregulation has not been previously reported, and therefore the present results suggest that there is an as-yet-unknown neural circuit mechanism responsible for the regulation of body temperature and metabolism.

Future Perspectives

The present study is the first to show that the brain mechanisms that produce heat avoidance and cold avoidance are different, and revealed the

framework of the neural circuitry for thermoregulatory behavior, which is an instinctive behavior common to animals. In addition to thermoregulatory behavior, animals have various instinctive behaviors, such as feeding, drinking, sexual, and collective behaviors, which are driven by instinctive emotions and desires. However, the brain circuit mechanisms that generate the emotions and desires that drive instinctive behaviors are mostly unknown. The neural pathways discovered in this study involve emotion circuits, including the amygdala, which are probably responsible for the generation of comfort and discomfort (pleasant and unpleasant emotions) caused by temperature sensation. The operating principle of these neural pathways may be common to the mechanism that generates emotions and desires that drive other instinctive behaviors, and further research may lead to the elucidation of the common neural mechanism that determines animal behaviors in general.

In recent years, the number of heat stroke cases has been increasing due to climate change. To prevent heat stroke, it is important to generate appropriate unpleasant emotions in response to heat exposure and thereby develop appropriate thermoregulatory behaviors. In the present study, rats in which LPB→MnPO thermosensory transmission was blocked failed to avoid heat and developed hyperthermia in a hot environment. In humans, especially in the elderly, the generation of heat discomfort via the LPB may be weakened due to reduced temperature sensitivity of skin thermosensors, which would decrease the motivation for thermoregulatory behavior and thus increase the susceptibility to heat stroke. To reduce heat stroke cases, it is necessary to educate the public about the importance of preventive thermoregulatory behavior based on objective air temperature and humidity information, rather than instinctive thermoregulatory behavior based on subjective emotional feelings. If future studies make it possible to measure neural activity in brain regions that generate pleasant and unpleasant emotions in response to environmental temperature, such as the MnPO and CeA, it will contribute to the risk assessment of heat stroke and hypothermia in the elderly and to the development of clothing and room temperature management systems that reduce the physical and mental burden caused by environmental temperatures.

Both groups of LPB→MnPO and LPB→CeA neurons identified in this study were also found to be necessary for brown fat thermogenesis in response to cold stimuli. This finding promotes a new understanding of the neural circuit mechanisms in the brain that maintain health by appropriately regulating body temperature and metabolism, and may lead to the development of technologies for early detection of metabolic diseases, including diabetes, at pre-disease stages, as well as new obesity treatment technologies that promote fat combustion.

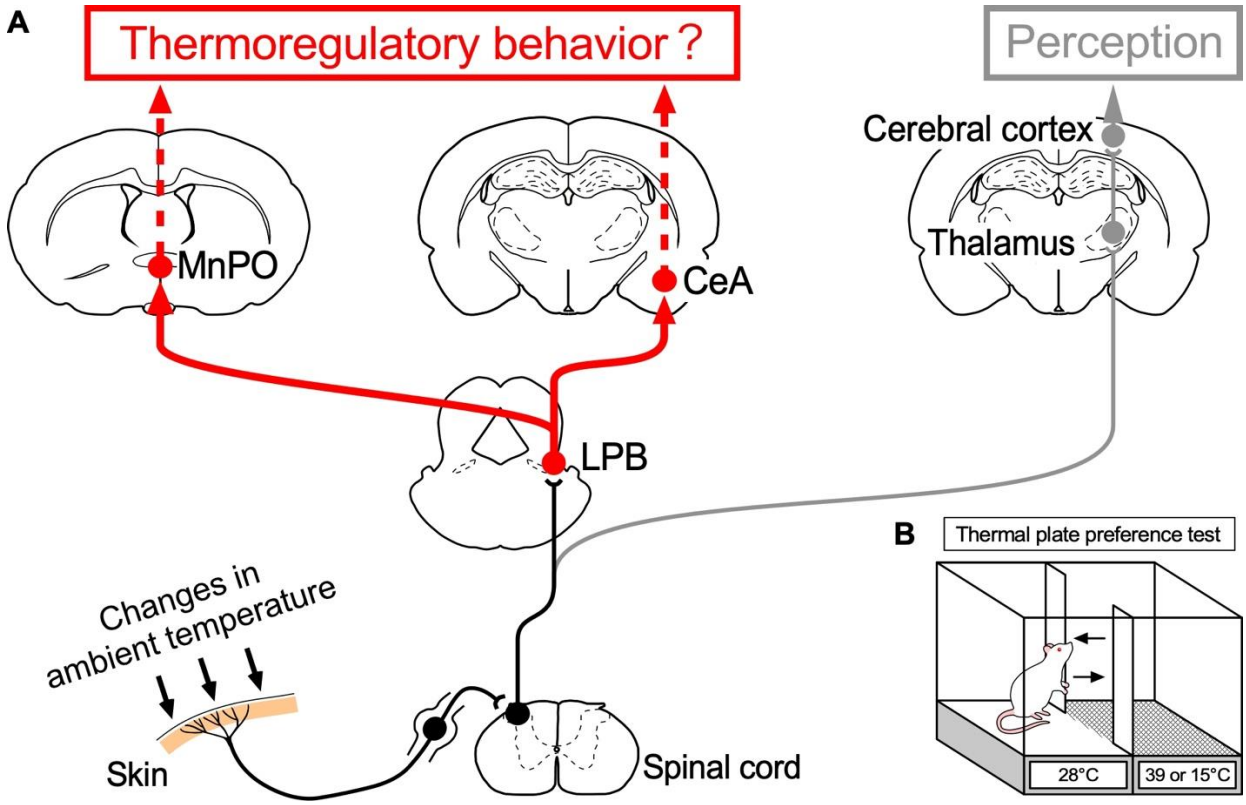

Fig. 1: Working hypothesis tested in this study

A: The hypothesis that information on environmental temperature sensed by skin thermoreceptors is transmitted via the spinal cord and the lateral parabrachial nucleus (LPB) to the median preoptic nucleus (MnPO) and to the central nucleus of the amygdala (CeA) to develop thermoregulatory behavior. Previous studies by this research group have shown that the neural pathway from the spinal cord through the thalamus to the primary somatosensory cortex (spinothalamocortical pathway) is not necessary for thermoregulatory behavior.

B: Two-floor thermal plate preference test. Normal rats spend more time on the 28°C plate than on the 39°C or 15°C plate, representing heat and cold avoidance behaviors.

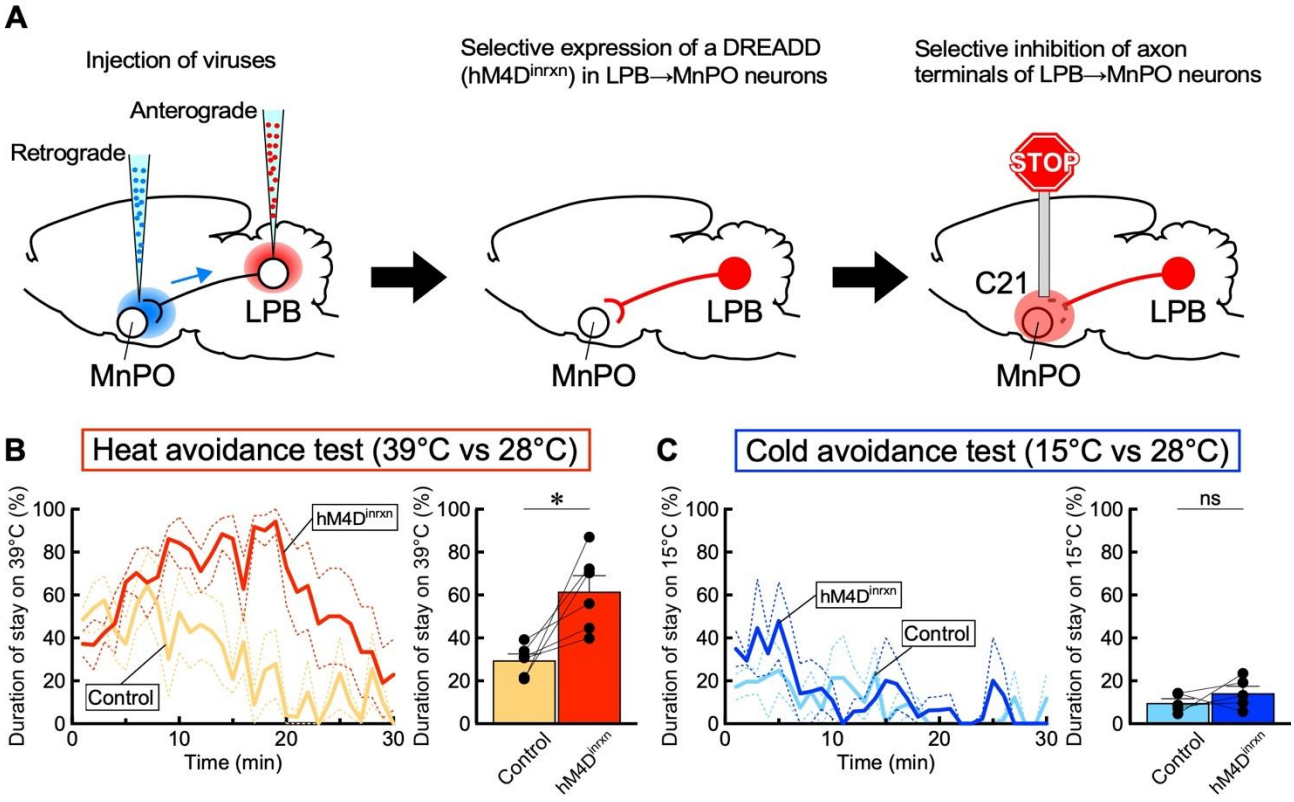

Fig. 2: LPB→MnPO neurotransmission is required for heat avoidance behavior

A: Using a double infection method with two adeno-associated viruses that infect from cell bodies (anterograde) and from axon terminals (retrograde), we selectively expressed hM4Di^{nrxn}, a chemogenetic artificial receptor (DREADD: Designer Receptors Exclusively Activated by Designer Drug) that inhibits neuronal activity, in LPB→MnPO neurons. Injection of the agonist (C21), which stimulates hM4Di^{nrxn}, into the MnPO inhibited neurotransmission from LPB→MnPO axon terminals.

B: In heat avoidance tests, rats in which LPB→MnPO neurotransmission (hM4Di^{nrxn}) was blocked spent significantly longer on the 39°C (hot) plate than control rats ($*P < 0.05$, paired t-test).

C: In cold avoidance tests, there was no difference in the time spent on the 15°C (cold) plate between the control and inhibited (hM4Di^{nrxn}) groups (ns: not significant, paired t-test).

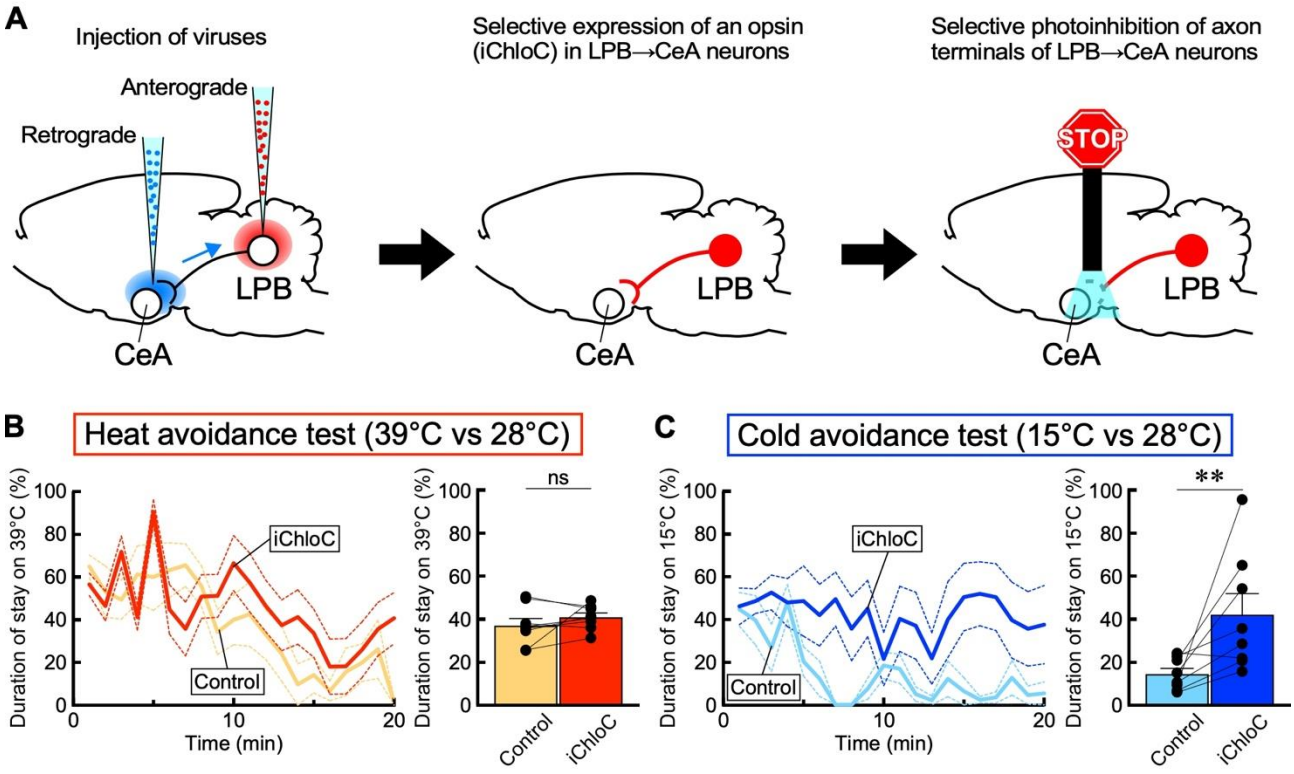

Fig. 3: LPB→CeA neurotransmission is required for cold avoidance behavior

A: Using a double infection method with two adeno-associated viruses that infect from cell bodies (anterograde) and from axon terminals (retrograde), we selectively expressed the light-sensitive ion channel (opsin), iChloC, in LPB→CeA neurons. By inserting an optical fiber into the brain of these rats and illuminating the CeA, we inhibited neurotransmission from LPB→CeA axon terminals.

B: In heat avoidance tests, rats in which LPB→CeA neurotransmission (iChloC) was blocked were able to escape from the 39°C (hot) plate comparable to the control group (ns: not significant, paired t-test).

C: In cold avoidance tests, rats in which LPB→CeA neurotransmission (iChloC) was blocked stayed significantly longer on the 15°C (cold) plate than control rats (**$P < 0.01$, paired t-test).

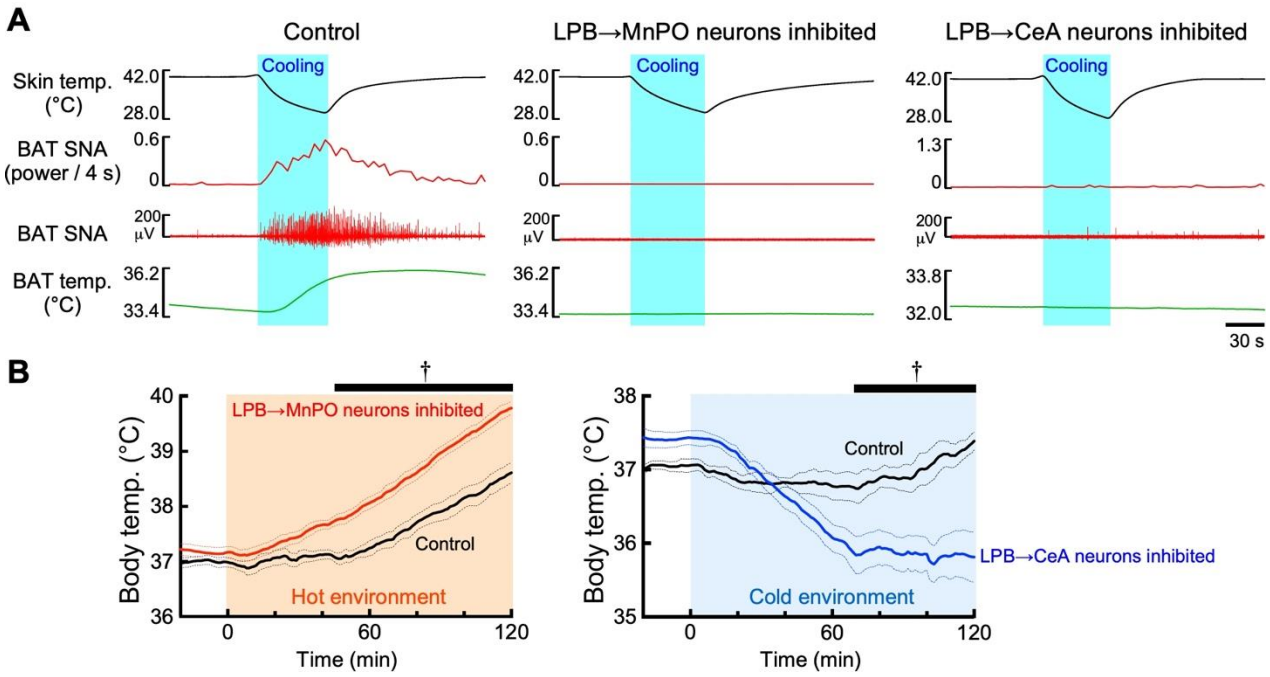

Fig. 4: Both LPB→MnPO and LPB→CeA neurons play important roles in autonomous thermoregulation

A: Cooling of the rat trunk skin (skin temp.) increased sympathetic nerve activity (BAT SNA) and temperature (BAT temp.) in brown adipose tissue, inducing heat production in normal (control) rats. On the other hand, rats in which tetanus toxin light chain was selectively expressed in either LPB→MnPO and LPB→CeA neurons to block their neurotransmission did not show skin cooling-evoked brown fat thermogenesis.

B: Rats in which either LPB→MnPO and LPB→CeA neurons were suppressed by tetanus toxin light chain exhibited hyperthermia in a hot environment (36°C) and became hypothermic in a cold environment (4°C) ($^{\dagger}P < 0.05$, Bonferroni's test after two-way ANOVA).

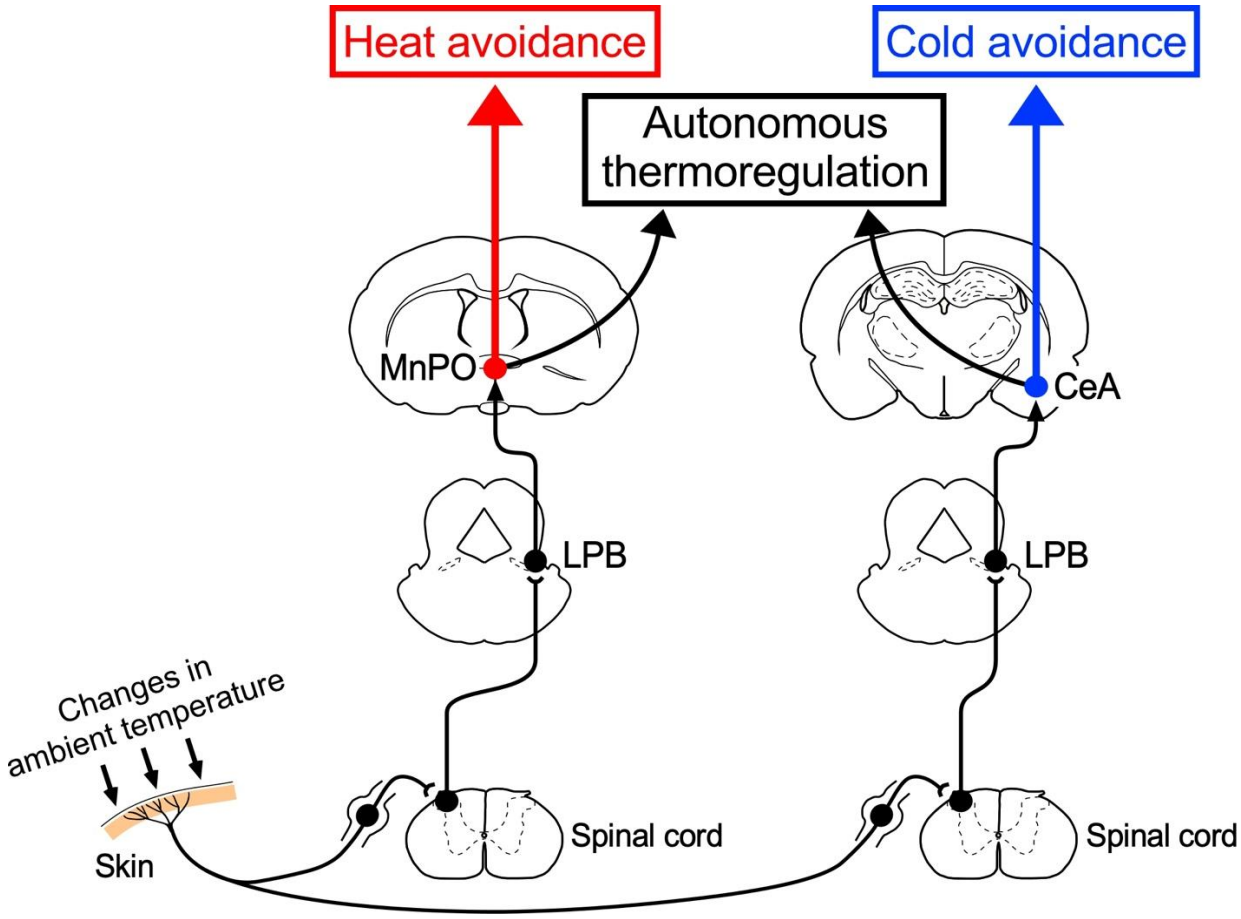

Fig. 5: Two neural pathways for heat avoidance and cold avoidance behaviors revealed by this study

Warm (heat) sensory information from skin thermosensors is transmitted to the MnPO via the spinal cord and the LPB, while cold sensory information is transmitted to both the MnPO and CeA via the spinal cord and the LPB. The present study revealed that the thermosensory information transmitted from the LPB to the MnPO develops heat avoidance behavior, while the thermosensory information transmitted from the LPB to the CeA develops cold avoidance behavior. These pathways are thought to generate unpleasant emotions toward hot and cold sensations, respectively, to develop the thermoregulatory behaviors.

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