News Release

Researchers identified "master neurons" for body temperature regulation

Key Points

- **A neuron group that expresses prostaglandin EP3 receptors in brain's thermoregulatory center is activated heat exposure of rats and inhibited by a pyrogenic mediator.**
- **Researchers demonstrated that the EP3 receptor-expressing neurons are "master neurons" that bidirectionally control body temperature via tonic GABAergic transmission.**
- **The present findings will lead to elucidation of the fundamental central circuit mechanism that regulates body temperature and metabolism and also to new technologies for obesity treatment.**

Summary

A research group led by Senior Lecturer Yoshiko Nakamura and Professor Kazuhiro Nakamura at the Department of Integrative Physiology, Nagoya University Graduate School of Medicine, in collaboration with Professor Hioki Hiroyuki at Juntendo University Graduate School of Medicine, has identified a group of neurons in the rat brain that function as a master controller for body temperature regulation.

The body temperature of most mammals, including humans, is strictly maintained at approximately 37°C. When the body temperature is not regulated properly, all regulatory functions in the body are impaired, as seen in patients with heat stroke and hypothermia, leading to death in the worst case. Therefore, the elucidation of the basic mechanism that regulates body temperature is of great medical significance. Although it is known that the thermoregulatory center is located in the preoptic area (POA) of the hypothalamus in the brain, the group of neurons responsible for the command of body temperature regulation has yet to be determined.

The research group focused on a group of POA neurons (POA^{EP3R} neurons) that express the EP3 receptor (EP3R) of prostaglandin E_2 (PGE_2), a pyrogenic mediator, and investigated the function of POA^{EP3R} neurons in the regulation of body temperature. First, they found that exposure of rats to heat (36°C) activated POA^{EP3R} neurons. On the other hand, administration of PGE_2, which elicited fever, inhibited the activation of POA^{EP3R} neurons. Visualization of nerve fibers of POA^{EP3R} neurons revealed that they innervate the dorsomedial hypothalamus (DMH), which controls the sympathetic nervous system. Detailed analyses of more than 80,000 nerve terminals of POA^{EP3R} neurons in the DMH, together with electrophysiological analyses, revealed that many of them release the inhibitory neurotransmitter, GABA. Selective activation of POA^{EP3R} neurons caused dilation of skin blood vessels, promoting heat dissipation, and decreased body temperature. On the other hand, selective inhibition of the POA^{EP3R}→DMH neurotransmission elicited heat production in brown adipose tissue (BAT) and

increased body temperature.

These results indicate that POA^{EP3R} neurons are “master neurons” that send constant (tonic) inhibitory signals to the sympathetic nervous system via the DMH and bidirectionally regulate body temperature by changing the strength of the tonic inhibitory signals. The present identification of the master controller of body temperature will lead to elucidation of the entire central neural circuit mechanism that controls body temperature and whole-body metabolism, and will also lead to the development of new technologies for obesity treatment.

Research Background

The body temperature of most mammals, including humans, is maintained strictly at approximately 37°C. This thermal homeostasis is the most important characteristic of homeothermic animals. Because chemical reactions and functional molecules in the body are optimized to function within the normal range of body temperature, if the strict regulation of body temperature goes wrong and the temperature deviates from the normal range, all regulatory functions in the body are impaired, as seen in heat stroke and hypothermia, and at worst, life cannot be sustained. Therefore, elucidating the basic mechanism of thermoregulation is of great significance not only in basic life science, but also in clinical medicine.

The brain’s thermoregulatory center resides in the POA, an anterior-most region of the hypothalamus. The POA not only monitors body core temperature (brain temperature), but also receives information on environmental temperature via sensory neural pathways from thermoreceptors in the skin. Then, after integrating the temperature information, the POA outputs command signals to the sympathetic nervous system to regulate body temperature, thereby triggering autonomous thermoregulatory responses. These responses include heat production (thermogenesis) in BAT, contraction and relaxation of skin blood vessels (regulating dissipation of body heat), and thermogenesis by skeletal muscle shivering. In addition, the POA commands fever upon receiving PGE_2, a pyrogenic mediator produced during infection.

However, the POA neurons that are responsible for thermoregulation are still unknown, although they have been explored around the world. In particular, the existence of the “master neurons” for thermoregulation, which are the principal controller of body temperature that outputs command signals to bidirectionally regulate body temperature, has yet to be determined.

Research Results

The research group has previously succeeded in producing the sole antibody in the world that can specifically visualize the prostaglandin EP3R. In this study, we focused on a group of neurons in the POA that express the EP3R (POA^{EP3R} neurons). First, to examine whether the activity of POA^{EP3R} neurons is altered in response to changes in environmental temperature, we analyzed the neuronal activity in the POA of rats exposed to heat (36°C), room temperature (24°C), or cold (4°C) for 2 hours, by detecting the expression of Fos protein, a marker for

neuronal activation **(Fig. 1A, B)**. The research group found that the activity of POA^{EP3R} neurons was low in rats exposed to room temperature or cold, which is lower than the comfortable environmental temperature range for rats (approximately 28°C), whereas the activity of POA^{EP3R} neurons was significantly increased in rats exposed to heat **(Fig. 1B)**. This result indicates that the POA^{EP3R} neurons are activated when the body temperature needs to be prevented from rising (or needs to be lowered). Furthermore, injection of PGE_2 into the lateral ventricle of the brain evoked fever and reduced the elevated activity of POA^{EP3R} neurons during heat exposure, indicating that POA^{EP3R} neurons are inhibited when the body temperature needs to be increased **(Fig. 1C–E)**.

Next, to investigate the neurotransmitter used by POA^{EP3R} neurons, the research group generated a genetically modified rat line to express an exogenous gene in POA^{EP3R} neurons and thereby, selectively expressed plasma membrane-targeted green fluorescent protein (palGFP) in POA^{EP3R} neurons **(Fig. 2A)**. Because palGFP labels membrane structures of cells, it clearly visualized axons (nerve fibers) of POA^{EP3R} neurons down to their endings. Their observations revealed that POA^{EP3R} neurons innervate various brain regions; in particular, many axons project to the DMH, which is known to activate the sympathetic nervous system, with synapse-like structures **(Fig. 2B, C)**.

Detailed immunohistochemical analyses of more than 80,000 nerve terminals from POA^{EP3R} neurons in the DMH revealed that many of them contain vesicular GABA transporter (VGAT), which is involved in the release of the inhibitory neurotransmitter GABA **(Fig. 2D, E)**. In contrast, only a few nerve terminals contained vesicular glutamate transporter 2 (VGLUT2), which is involved in the release of the excitatory neurotransmitter glutamate **(Fig. 2D, E)**. Furthermore, electrophysiological analyses confirmed that GABA is released from the DMH nerve terminals of POA^{EP3R} neurons for synaptic transmission. These results indicate that POA^{EP3R} neurons release GABA onto DMH neurons, thereby suppressing the excitatory output from the DMH to the sympathetic nervous system. Interestingly, rats exposed to a hot environment for 2 weeks exhibited a significant increase in VGAT-containing nerve terminals of POA^{EP3R} neurons in the DMH **(Fig. 2E)**. This appears to be a synaptic alteration during long-term heat exposure to efficiently suppress unnecessary sympathetic responses, such as thermogenesis.

To further investigate the role of POA^{EP3R} neurons in thermoregulation, the research group artificially manipulated the activity of POA^{EP3R} neurons using a chemogenetic approach. Selective activation of POA^{EP3R} neurons caused dilation of skin blood vessels, which promoted dissipation of body heat, and lowered body temperature **(Fig. 3A, B)**. On the other hand, inhibition of POA^{EP3R} neurons elicited BAT thermogenesis and increased body temperature **(Fig. 3C)**. Similarly, selective inhibition of POA^{EP3R}→DMH neurotransmission also elicited BAT thermogenesis **(Fig. 3D)**.

Taken together, these results demonstrate that POA^{EP3R} neurons send tonic (continuous) inhibitory signals to DMH neurons via GABAergic transmission, and that the strength of the tonic inhibitory signals from POA^{EP3R} neurons is precisely controlled to adjust body

temperature bidirectionally to maintain it at a set-point level. For example, in a hot environment, the tonic inhibitory signal is augmented to suppress the sympathetic outputs, resulting in skin vasodilation to facilitate heat dissipation to prevent hyperthermia **(Fig. 4, top)**. On the other hand, in a cold environment, the tonic inhibitory signal is reduced to activate the sympathetic outputs, resulting in increased BAT thermogenesis to prevent hypothermia **(Fig. 4, bottom)**. During infection, PGE_2 acts on POA^{EP3R} neurons to suppress their activity, leading to activation of the sympathetic outputs to develop fever **(Fig. 4, bottom)**.

Research Summary and Future Perspective

The finding in this study that POA^{EP3R} neurons function as a master controller in the thermoregulatory center will lead to future elucidation of the entire central circuit mechanism that regulates body temperature and whole-body metabolism. In particular, the present findings will be important in answering fundamental questions in life science, such as "How is our body temperature set at 37°C?" and "What mechanism in neuronal cells determines the body temperature set-point?"

Future development of a technology to artificially manipulate POA^{EP3R} neurons in humans will enable artificial manipulation of body temperature, which is now difficult, but would be applied to a wide range of medical fields, such as treatment of heat stroke and hypothermia, and body temperature control during surgery under general anesthesia. In particular, the development of technologies for humans to adapt to and survive in hot environments is becoming increasingly important because heat waves associated with global warming are becoming a serious worldwide problem.

Furthermore, since body temperature regulation involves energy-consuming responses, such as metabolic heat production in BAT, if POA^{EP3R} neurons can be artificially manipulated to slightly elevate body temperature chronically, it could promote fat combustion, becoming a new technology for obesity treatment. In the future, we expect to see expansion of various studies pivoting on POA^{EP3R} neurons.

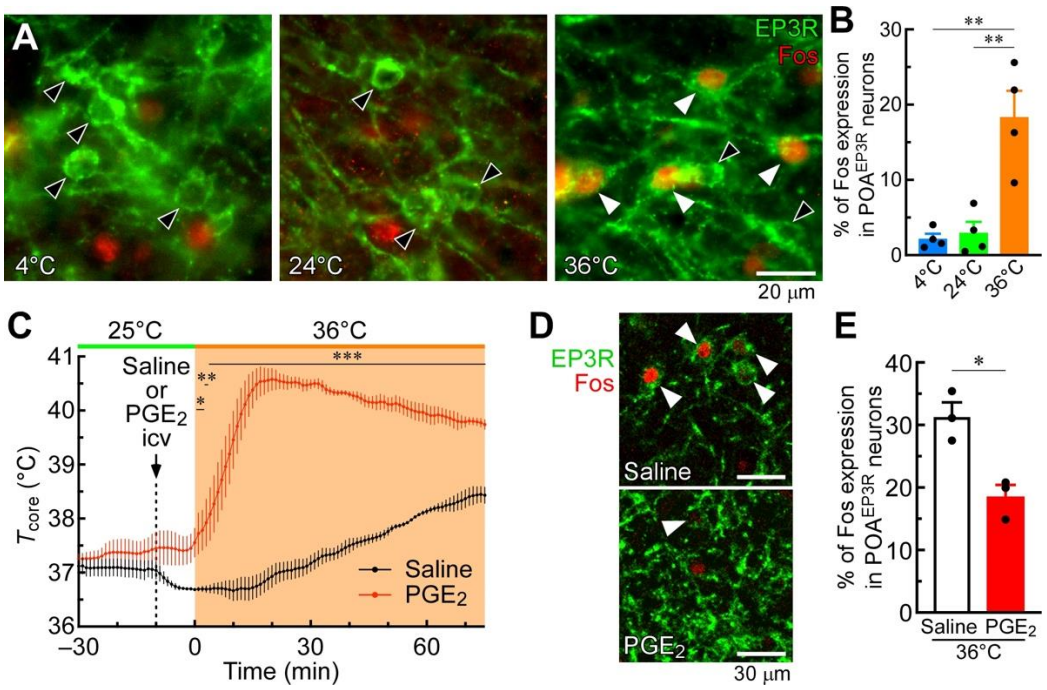

Fig. 1: POA^{EP3R} neurons are activated by heat exposure and inhibited by PGE_2.

A, **B**: Fos expression in POA^{EP3R} neurons of rats exposed to cold (4°C), room temperature (24°C) or heat (36°C). White arrowheads indicate POA^{EP3R} neurons expressing Fos; black arrowheads indicate POA^{EP3R} neurons not expressing Fos. $^{**}P < 0.01$ (Bonferroni's *post-hoc* test following one-way ANOVA).

C: Changes in body core temperature (T_{core}) in rats exposed to heat after an intracerebroventricular (icv) injection of saline or PGE_2. PGE_2 injection evoked fever. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (Bonferroni's *post-hoc* test following two-way ANOVA).

D, **E**: Fos expression in POA^{EP3R} neurons of rats treated as described in **C**. Arrowheads indicate POA^{EP3R} neurons expressing Fos. Fos expression induced by heat exposure was reduced by PGE_2. $^{*}P < 0.05$ (unpaired *t*-test).

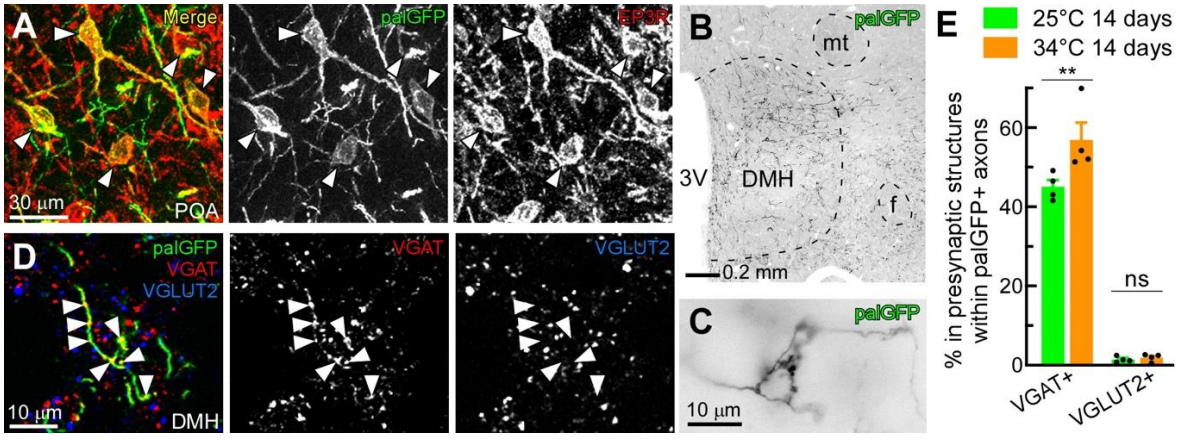

Fig. 2: Nerve endings of POA^{EP3R} neurons in the DMH release GABA.

A–**C**: A virus was injected into the POA of genetically modified rats to selectively express palGFP in POA^{EP3R} neurons (**A**, arrowhead). palGFP-labeled nerve fibers of POA^{EP3R} neurons were distributed in the DMH (**B**)

and exhibited synapse-like structures (**C**). 3V, third ventricle; f, fornix; mt, mammillothalamic tract.

D: Immunostaining for VGAT (a marker for synaptic terminals releasing GABA) and VGLUT2 (a marker for synaptic terminals releasing glutamate) in POA^{EP3R} neuron nerve endings (labeled with palGFP) in the DMH. Arrowheads indicate synaptic terminals of POA^{EP3R} neurons containing VGAT.

E: Synaptic terminals of POA^{EP3R}→DMH axons were predominantly GABAergic (VGAT+) and only a few glutamatergic (VGLUT2+). Rats exposed to heat (34°C) for 2 weeks had more GABAergic terminals than rats kept at room temperature (4 rats per group analyzed). $^{**}P < 0.01$; ns, not significant (Bonferroni's *post-hoc* test following two-way ANOVA).

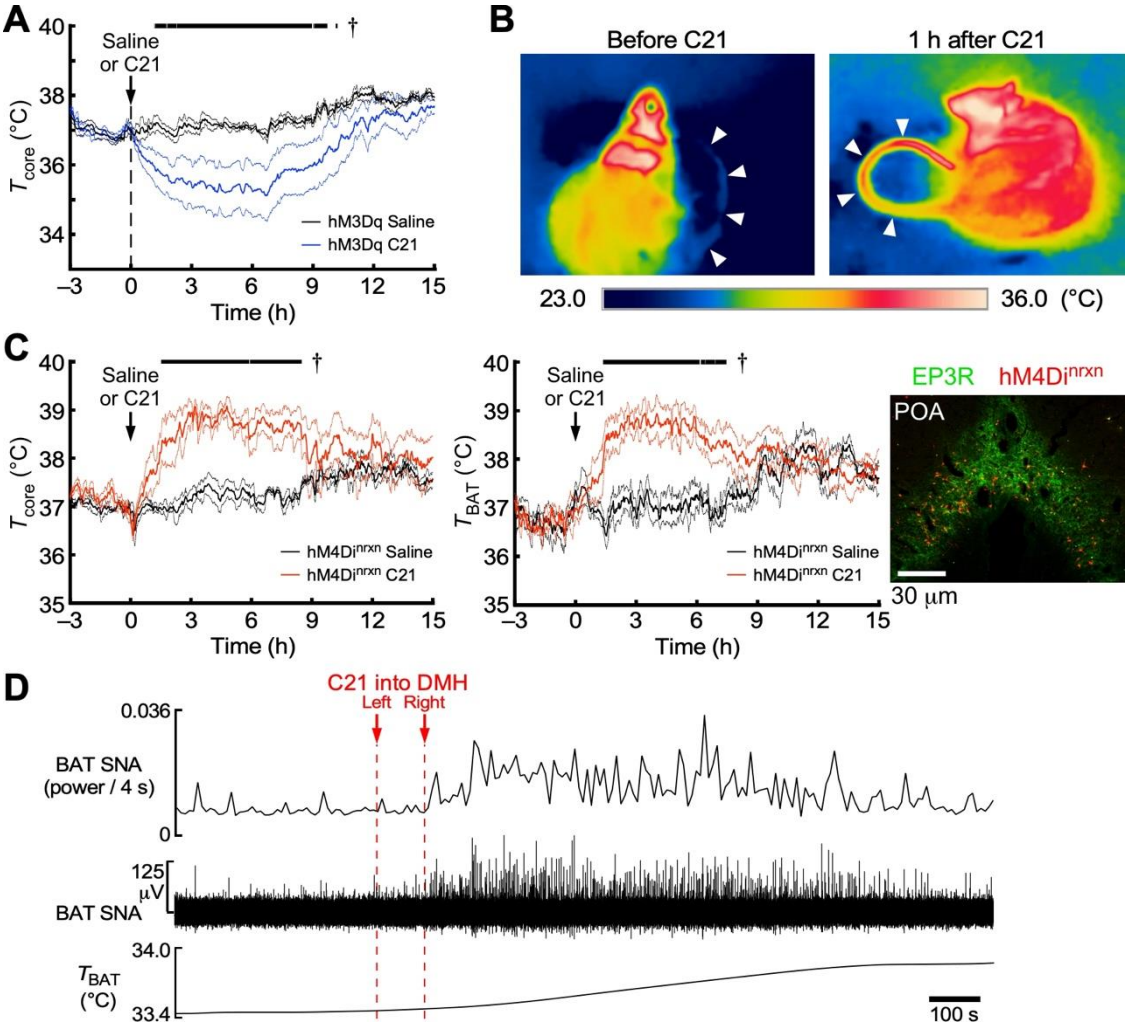

Fig. 3: Changes in the activity of POA^{EP3R} neurons alter body temperature bidirectionally.

A, **B**: Genetically modified rats that selectively expressed hM3Dq, an artificial receptor (DREADD: Designer Receptors Exclusively Activated by Designer Drug) to activate neural activity, in POA^{EP3R} neurons, and then, saline or an agonist (actuator) for the artificial receptor (C21) was injected into the lateral ventricle of the brain. C21 injection reduced body core temperature (**A**). $^{\dagger}P < 0.05$ (Bonferroni's *post-hoc* test following two-way ANOVA). During the decrease in body temperature, a marked increase in tail skin temperature (arrowheads) was observed, indicating that the skin blood vessels dilated to actively dissipate body heat (**B**).

C: Genetically modified rats that selectively expressed hM4Di^{nrxn}, a DREADD receptor to inhibit neural activity, in POA^{EP3R} neurons. Injection of the actuator for the receptor (C21) into the lateral ventricle increased BAT temperature (T_{BAT}) (increasing heat production) and body temperature. $^{\dagger}P < 0.05$ (Bonferroni's *post-hoc* test following two-way ANOVA). The picture on the right shows the distribution of POA^{EP3R} neurons and the expression of hM4Di^{nrxn}.

D: The genetically modified rats in which hM4Di^{nrxn} was selectively expressed in POA^{EP3R} neurons were anesthetized and C21 was microinjected into the left and right DMH to selectively inhibit the release of transmitters from terminals of POA^{EP3R}→DMH axons. C21 injections elicited increases in BAT sympathetic nerve activity (SNA) and BAT temperature, indicating increased BAT thermogenesis.

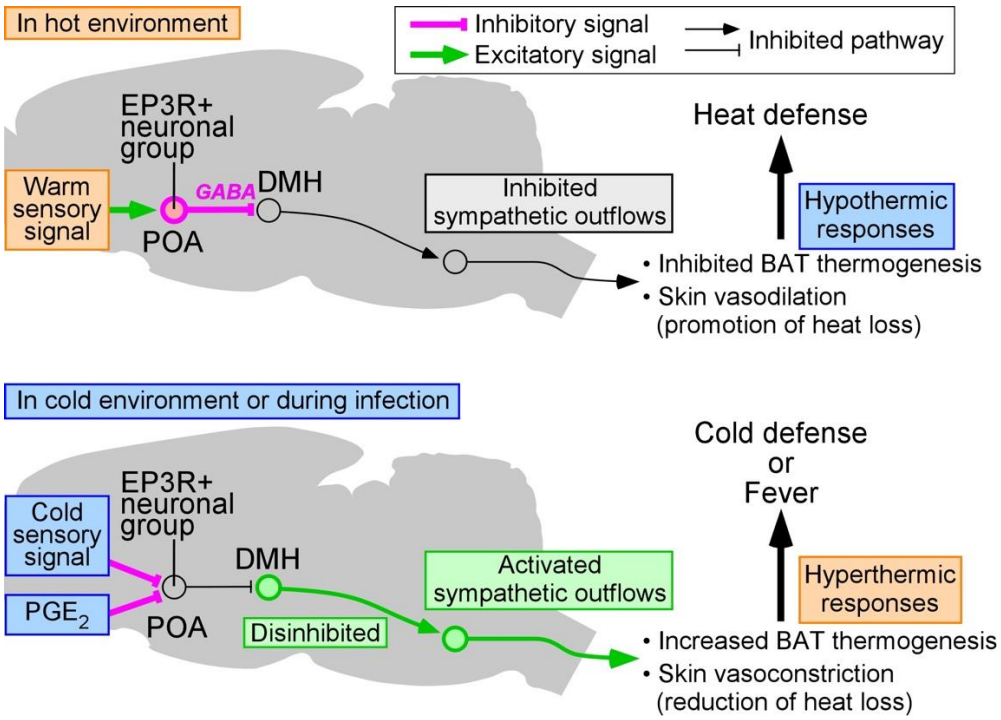

Fig. 4: The neural mechanism by which POA^{EP3R} neurons regulate body temperature.

In a hot environment (top), warm-sensory signals originating from skin thermoreceptors are input to the POA to increase the activity of POA^{EP3R} neurons. This in turn strengthens GABAergic neurotransmission to the DMH, which suppresses the activity of the neural pathways to the sympathetic nervous system, resulting in suppression of thermogenesis and dilation of skin blood vessels (promoting heat dissipation) to avoid hyperthermia. On the other hand, in a cold environment (bottom), cold-sensory signals from the skin are input to the POA to reduce the activity of POA^{EP3R} neurons. When the tonic activity of POA^{EP3R} neurons is reduced, DMH neurons are released from the inhibition (called “disinhibition”) and become excited, resulting in increased sympathetic output to increase BAT thermogenesis and to constrict skin blood vessels (reducing heat dissipation). These sympathetic responses avoid hypothermia. During infection, PGE_2 inhibits POA^{EP3R} neurons to disinhibit DMH neurons to develop fever in the same manner. In most cases, the activity of POA^{EP3R} neurons is somewhere between these two states (top and bottom), and body temperature is probably fine-tuned by precise regulation of the level of the tonic inhibition from POA^{EP3R} neurons.

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