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A hypothalamic circuit that controls body temperature

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The homeostatic control of body temperature is essential for survival in mammals and is known to be regulated in part by temperature-sensitive neurons in the hypothalamus. However, the specific neural pathways and corresponding neural populations have not been fully elucidated. To identify these pathways, we used cFos staining to identify neurons that are activated by a thermal challenge and found induced expression in subsets of neurons within the ventral part of the lateral preoptic nucleus (vLPO) and the dorsal part of the dorsomedial hypothalamus (DMH). Activation of GABAergic neurons in the vLPO using optogenetics reduced body temperature, along with a decrease in physical activity. Optogenetic inhibition of these neurons resulted in fever-level hyperthermia. These GABAergic neurons project from the vLPO to the DMH and optogenetic stimulation of the nerve terminals in the DMH reduced body temperature and activity. Electrophysiological recording revealed that the vLPO GABAergic neurons suppressed neural activity in DMD neurons, and fiber photometry of calcium transients revealed that DMD neurons were activated by cold. Accordingly, activation of DMD neurons using designer receptors exclusively activated by designer drugs (DREADDs) or optogenetics increased body temperature with a strong increase in energy expenditure and activity. Finally, optogenetic inhibition of DMD neurons triggered hypothermia, similar to stimulation of the GABAergic neurons in the vLPO. Thus, vLPO GABAergic neurons suppressed the thermogenic effect of DMD neurons. In aggregate, our data identify vLPO→DMD neural pathways that reduce core temperature in response to a thermal challenge, and we show that outputs from the DMD can induce activity-induced thermogenesis.

A

Significance

Thermal homeostasis is essential for survival in mammals. Although it is known that temperature-sensitive neurons in the hypothalamus can control body temperature, the precise neural types and dynamics of neurons responding to temperature changes have not been well defined. In this study, we identified subsets of temperature-activated neurons in two hypothalamic nuclei, the preoptic area (POA) and the dorsomedial hypothalamus (DMH), and showed that modulating their activity can lead to alterations in core temperature. The data further suggest that heat-activated GABAergic neurons in the POA reduce the activity of cold-activated neurons in the DMH, which function to increase thermogenesis and physical activity. These data identify a neural circuit that controls core temperature and thermogenesis.


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See Commentary on page 1765.

1Sufficient To Drive Hypothermia.

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fully elucidated. For example, there are conflicting data concerning the potential role of GABAergic neurons in the POA (2, 5, 6). To identify these key neural populations, we characterized the pattern of cFos activation following a thermal challenge (SI Appendix, Fig. S1). We found heat-induced cFos expression in the medial preoptic area (MPO) and the vLPO, and it colocalized with the GABAergic marker GAD67 (Fig. 1A and SI Appendix, Fig. S5A). Both the MPO and the vLPO have been suggested to play important roles in thermoregulation (1, 2, 9–11). To test the function of the vLPO neuronal population, we targeted expression of channelrhodopsin-2 (ChR2) fused with eYFP to GABAergic neurons within the vLPO by injecting Cre-dependent adenovirus-associated virus (AAV) 5 viruses into the vLPO of Vgat-IRES-Cre driver mice (Vgat stands for vesicular GABA transporter) (Fig. 1B). In slice recordings, we confirmed that the delivery of blue light to ChR2-expressing vLPOGat neurons resulted in neural excitation (Fig. 1C). In vivo, we found that light-induced activation of vLPOGat neurons triggered a rapid reduction in Tcore with a decrease in physical activity (ΔTcore = −1.8 ± 0.9 °C at t = 30 min, mean ± SEM, Fig. 1D and E) in freely behaving mice. This effect was specific to ChR2, because injection of control AAV5 viruses (expressing eYFP) did not significantly affect Tcore or activity.

Next, we asked whether inhibiting these neurons was sufficient to drive hyperthermia. Using the Guillain–Barré theta ani channelrhodopsin 1 (hGtACR1), which robustly silences neural activity in response to blue-yellow light (12), we found that blue light delivery was sufficient to silence neurons in slice recordings (Fig. 1C). Remarkably, light stimulation of mice expressing hGtACR1 in vLPOGat neurons caused severe hyperthermia with elevated activity levels (maximal Tcore = 40.6 °C, Fig. 1D and E). Indeed, we needed to minimize the time in which animals received light illumination to prevent hyperthermia-induced death.

We also tested the function of MPO GABAergic neurons by targeted expression of ChR2 to GABAergic neurons within the MPO of Vgat-IRES-Cre driver mice (SI Appendix, Fig. S5B). The GABAergic neurons in the MPO have been suggested to play important roles in thermoregulation (1, 2). Surprisingly, we found that optogenetic activation of these MPOGat neurons did not significantly affect Tcore or activity (SI Appendix, Fig. S5C and D). Similar to our results, DREADD activation of these neurons has a minimal effect on Tcore (5).

Thus, our results establish that activation of GABAergic neurons within a preoptic subregion (vLPO) can inhibit thermogenesis, whereas inhibition of these neurons dramatically raises core temperature. We next explored the functional targets of these neurons.

Critical Role of the POA–DMD Connection in Reducing Tcore. We thought that vLPOGat neurons might project to the DMH because the DMH is known to participate in thermoregulation, and because we observed that thermal stimuli induced strong cFos staining within the DMD (SI Appendix, Fig. S1). To test whether vLPOGat neurons directly innervate DMD neurons, we first performed anterograde tracing from these neurons by injecting ChR2 into vLPOGat neurons (Fig. 2A) and found staining of nerve terminals in the DMD (Fig. 2B). We then performed retrograde labeling by injecting the retrograde protein, cholera toxin B subunit (CTb; ref. 13) into the DMD and found that CTb labeled many neurons in the POA, including heat-activated neurons (as indicated by induction of cFos) in the vLPO (Fig. 2C).

We tested the function of this vLPO–DMD projection by stimulating vLPOGat terminals in the DMD after viral injection of ChR2 into the vLPO (Fig. 2D). We found that stimulation with blue light triggered a significant reduction of Tcore (ΔTcore = −2.3 ± 0.6 °C at t = 60 min, mean ± SEM, Fig. 2E) along with a decrease in activity (Fig. 2F). The magnitude of the effect was similar to that observed after direct stimulation of Vgat neurons in the vLPO. Thus, stimulating vLPOGat nerve terminals in the DMD recapitulated the phenotype observed when vLPOGat cell bodies were stimulated (Fig. 1D and E, Upper).

These functional data suggest that vLPOGat neurons inhibit DMD neurons and reduce core temperature and thermogenesis. We next set out to confirm this inhibition directly by recording inhibitory postsynaptic currents (IPSCs) in DMD neurons after vLPOGat terminals were stimulated. This recording is important because our data could also be explained by inhibition of axon fibers that pass through the DMD without directly innervating DMD neurons. In slice preparations, we confirmed that stimulation of vLPOGat terminals expressing ChR2 by blue light-induced IPSCs in the DMD neurons and that these currents were blocked by a GABA_A receptor antagonist, bicuculline (Fig. 2G). The onset of these currents was <3 ms after light delivery, which is within the time range of monosynaptic transmission (14). Furthermore, to identify which types of DMD neurons are synaptically connected to the vLPOGat neurons, we used the recording pipette to isolate mRNA from individual, light-responsive DMD neurons immediately after recordings and analyzed gene expression by single-cell reverse transcription PCR (RT-PCR) (15). These single-cell RT-PCR data showed that there were both Vgat+ and Vgat−/nociceptive post-synaptic neurons in the DMD, with the majority being Vgat+ (Fig. 2H and SI Appendix, Fig. S6).

Several reports (16–21) have suggested that DMD neurons can regulate thermogenesis, but the inputs to these neurons have not been elucidated. Our studies in anterograde and retrograde...
tracing, optogenetic activation of terminals from vLPO\textsuperscript{Vglut} neurons in the DMD, slice recordings, and single-cell RT-PCR suggest the possibility that there are functional connections between the vLPO\textsuperscript{Vglut} neurons and DMD\textsuperscript{Vglut} or DMD\textsuperscript{Vgat} neurons to regulate core temperature. We next tested these connections directly by monitoring and modulating the activity of neurons in the DMD.

**Fiber Photometry Reveals That DMD Neurons Are Cold Activated.** The observation that inhibitory neurons that project to the DMD cause hypothermia suggests that neurons within the DMD may be cold sensitive (via either direct or indirect inputs) and act to cause hypothermia suggests that neurons within the DMD may yield stable recordings from the dorsal raphe (23) (Fig. 4). We also observed an even larger Ca\textsuperscript{2+} increase in response to cooling in DMD\textsuperscript{Vglut} neurons (25–13 °C) (ΔF/F\textsubscript{0,max} = 22.7 ± 2.8%, mean ± SEM; Fig. 3E, Right). This Ca\textsuperscript{2+} signal increased rapidly (within seconds) after the temperature shift and diminished more slowly than in DMD\textsuperscript{Vgat} neurons. ΔF/F\textsubscript{1/2(max)} was significantly larger than baselines (Fig. 3E, Right). We fitted the response curve by using a sigmoidal function and found the full width at half maximum (FWFM or T\textsubscript{1/2(max)} of the DMD\textsuperscript{Vglut} neurons was larger than that of DMD\textsuperscript{Vgat} neurons (T\textsubscript{1/2(max)} = 22.6 s and 16.5 s, respectively; SI Appendix, Fig. S8A and B).

Taken together, these results indicate that both GABA\textsuperscript{ergic} and glutamatergic neurons in the DMD are activated by cooling. The response of DMD\textsuperscript{Vgat} neurons lasted longer than that of DMD\textsuperscript{Vgat} neurons. All of our temperature stimuli-evoked calcium responses appeared to result from periphery sensory input, rather than from a slow change in body temperature, based on the observation that inhibitory neurons that project to the DMD cause hypothermia suggests that neurons within the DMD may be cold sensitive (via either direct or indirect inputs) and act to cause hypothermia suggests that neurons within the DMD may yield stable recordings from the dorsal raphe (23) (Fig. 4). We also observed an even larger Ca\textsuperscript{2+} increase in response to cooling in DMD\textsuperscript{Vglut} neurons (25–13 °C) (ΔF/F\textsubscript{0,max} = 22.7 ± 2.8%, mean ± SEM; Fig. 3E, Right). This Ca\textsuperscript{2+} signal increased rapidly (within seconds) after the temperature shift and diminished more slowly than in DMD\textsuperscript{Vgat} neurons. ΔF/F\textsubscript{1/2(max)} was significantly larger than baselines (Fig. 3E, Right). We fitted the response curve by using a sigmoidal function and found the full width at half maximum (FWFM or T\textsubscript{1/2(max)} of the DMD\textsuperscript{Vglut} neurons was larger than that of DMD\textsuperscript{Vgat} neurons (T\textsubscript{1/2(max)} = 22.6 s and 16.5 s, respectively; SI Appendix, Fig. S8A and B).

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the fast-rising kinetics. The calcium responses occurred within seconds after the temperature shift (Fig. 3 D and E), whereas the changes in $T_{\text{core}}$ happened minutes after the temperature shift (SI Appendix, Fig. S2).

**DMD Neurons Promote Thermogenesis.** The finding that cooling leads to abrupt Ca$^{2+}$ transients in DMD$^{\text{Vglut2}}$ neurons suggests that these neurons might play a functional role in cold-induced thermogenesis. We next tested this by using DREADDs to activate DMD$^{\text{Vglut2}}$ neurons remotely, allowing us to measure energy expenditure (EE) without being impeded by optical fibers required for optogenetics. As predicted, we found that injection of the ligand clozapine-N-oxide (CNO) into mice expressing designed human M3 muscarinic receptor coupled to Gq (bM3D) (8) in DMD$^{\text{Vglut2}}$ neurons strongly increased $T_{\text{core}}$, along with an increase in EE and physical activity ($\Delta T = 1.4 \pm 0.3^\circ C$, $\Delta EE = 47.3 \pm 5.1\%$ at $t = 100$ min, mean ± SEM; Fig. 4 A–D). These increases are direct evidence that activation of DMD$^{\text{Vglut2}}$ neurons promotes thermogenesis, which is consistent with previous studies showing that the DMD may send excitatory (or glutamatergic) projections to promote cold- or febrile-induced thermogenesis (16, 17, 24, 25).

The findings that cooling induced calcium transients in DMD$^{\text{Vglut2}}$ neurons (Fig. 3E) and that cold-induced cFos colocalized with GAD67 in the DMD (SI Appendix, Fig. S7A) suggest that these neurons could also be important for regulating cold-induced thermogenesis. We tested this by using both DREADDs and optogenetics to activate DMD$^{\text{Vglut2}}$ neurons. We found that injection of CNO, but not saline, into mice expressing hM3D in DMD$^{\text{Vglut2}}$ neurons resulted in a slow, yet long-lasting increase in $T_{\text{core}}$, with an increase in EE and activity ($\Delta T = 1.3 \pm 0.2^\circ C$, $\Delta EE = 47.4 \pm 7.5\%$ at $t = 100$ min, mean ± SEM; Fig. 4 E–H). We fitted the response curve with a sigmoidal model and calculated maximum change rate ($k$) and full width at half maximum (FWFM) (SI Appendix, Fig. S8 C and D). For the same dose of CNO, the $k$ for the T$_{\text{core}}$, EE, and activity response curves were smaller for DMD$^{\text{Vglut2}}$ neuronal activation than was observed after DMD$^{\text{Vglut2}}$ neuronal activation. However, the FWFWs were longer after DMD$^{\text{Vglut2}}$ neural activation versus activation of DMD$^{\text{Vglut2}}$ (SI Appendix, Fig. S8 C and D). The kinetics of these biological responses matched that seen by recording from these neurons, which indicates that cold-induced Ca$^{2+}$ transients of DMD$^{\text{Vglut2}}$ neurons diminished more slowly than that of DMD$^{\text{Vglut2}}$ neurons (Fig. 3 D and E and SI Appendix, Fig. S8 A and B). Also, we observed an increase in $T_{\text{core}}$ and activity after optogenetic stimulation of mice expressing ChR2 in DMD$^{\text{Vglut2}}$ neurons (SI Appendix, Fig. S8 B–D). In aggregate, these data show that activation of both glutamatergic and GABAergic neurons in the DMD can increase $T_{\text{core}}$, EE, and activity, although the kinetics of the biological responses to activation of these neurons were different.

**Inhibition of DMD Neurons Is Sufficient To Drive Hypothermia.** The finding that vLPO$^{\text{Vglut}}$ inputs to the DMD lower core temperature led us to predict that optogenetic inhibition of DMD neurons would have a similar effect. This prediction was tested by injecting AAV9 viruses expressing hGtACR1 into the DMD of Vglut2-IRES-Cre or Vgat-IRES-Cre driver mice (thereby driving hGtACR1 expression in DMD$^{\text{Vglut2}}$ or DMD$^{\text{Vgat}}$ neurons, respectively). We found that blue light stimulation of mice expressing hGtACR1 in DMD$^{\text{Vglut2}}$ neurons resulted in significant reductions in $T_{\text{core}}$, along with a decrease in activity ($\Delta T = -1.3 \pm 0.2^\circ C$ at $t = 60$ min, mean ± SEM; Fig. 5 A and B), similar to optogenetic activation of the vLPO$^{\text{Vglut}}$ neurons (Fig. 1 D and E). Similarly, blue light stimulation of mice expressing hGtACR1 in DMD$^{\text{Vgat}}$ neurons resulted in significant reductions in $T_{\text{core}}$, along with a decrease in activity ($\Delta T = -2.5 \pm 0.5^\circ C$ at $t = 60$ min, mean ± SEM; Fig. 5 C and D). Taken together, these results define elements of a POA→DMH neural circuit in the hypothalamus (including the vLPO$^{\text{Vglut}}$→DMD$^{\text{Vglut2}}$ and vLPO$^{\text{Vgat}}$→DMD$^{\text{Vgat}}$ connections) that regulate thermogenesis.

**Discussion**

The maintenance of a stable core temperature is essential for survival. Our study elucidates a central mechanism through which changes in core temperature (in response to alternations in ambient temperature or other stimuli) elicit a set of adaptive thermogenic responses that defend $T_{\text{core}}$. In aggregate, we find that in response to a heat challenge, heat-activated GABAergic neurons in the vLPO directly inhibit the activity of cold-activated glutamatergic and GABAergic neurons in the DMD to lower $T_{\text{core}}$, whereas the DMD neurons do so (in part) by suppressing EE and activity. Thus, we have elucidated pathways for controlling body temperature and activity-induced thermogenesis.

In connection with our study, previous studies have suggested that the vLPO is an important site for thermoregulation. Local warming of the vLPO causes paw vasodilation in rats (9), suggesting the existence of WSNs in this area that can drive hypothermia. Also, the vLPO is labeled when a retrograde tracer (pseudo rabies virus) is injected into the interscapular brown adipose tissue (BAT) of rats (26), suggesting its involvement in thermoregulation. Interestingly, a recent study discovered that a heat-sensitive channel, TRPM2, in the POA (including the vLPO) may be part of the WSN heat sensor to limit fever (5). Thus, it will be interesting to see whether TRPM2 is important for vLPO neurons to detect brain warmth and lower $T_{\text{core}}$.

We found that heat activated a subset of GABAergic neurons in the vLPO, which then lowered $T_{\text{core}}$. Although a similar (or stronger) effect was observed after activation of glutamatergic neurons in several preoptic subregions [MPA (6), and MPO (5)], activation of GABAergic neurons in these areas has a small effect on $T_{\text{core}}$ and the role of GABAergic neurons in the vLPO was not studied previously. We found that activation of GABAergic neurons in the vLPO is sufficient to induce hypothermia (Fig. 1 B and D). However, this activation does not include the ventrolateral preoptic nucleus (vLPO), which is important for sleep regulation (27, 28) but may be dispensable for thermoregulation, because its lesion has a minimal effect on $T_{\text{core}}$. (27)

The vLPO GABAergic neurons comprise a functionally confirmed GABAergic population in the POA. In addition, we found that inhibiting vLPO GABAergic neurons induced fever-level hyperthermia, suggesting that these neurons can control $T_{\text{core}}$ in either direction (Fig. 1D), and that these neurons provide a key entry point for studying the neural mechanisms controlling thermogenesis. Furthermore, we found heat can also activate a subset of glutamatergic neurons in the vLPO, which can drive severe hyperthermia and hypoactivity (SI Appendix, Table S1). **$*P < 0.01$; ***$P < 0.001$.**
Thermogenesis in DMD neurons via hGtACR1 resulted in significant decreases in core temperature (ΔTcore) changes from the mean level before light delivery (t = 30 to 10 min). The baseline (b.s.) (average of t = 30 to 10 min) and t = 60 min are shown in the bar graph. The average of activity in 30-min intervals between t = 40 and 10 min (baseline, b.s.) and between t = 30 and 60 min are shown in the bar graph. Stimulation protocol: light on for 30 s (473 nm, 10 mW) followed by a 90-s break, with the sequence repeating for 1 h. (C and D) Bilateral inhibition of DMD neurons (n = 4) via hGtACR1 resulted in significant decreases in Tcore, (C) and activity. Stimulation protocol is the same as in A. (E) Model of heat-induced suppression of thermogenesis. Solid line indicates the connection verified in the current study. Dash lines represent proposed connections based on our data and other reports. (+), activation; (-), inhibition. All data are plotted as mean ± SEM. The P values, compared with baseline (b.s.), are calculated based on statistical tests listed in SI Appendix, Table S1.

**Fig. S3**. Thus, vLPO GABAergic neurons might receive local inputs from vLPO glutamatergic neurons or other preoptic glutamatergic neurons as proposed (1, 2) (such as the MnPO, because the MnPO receives inputs from the periphery (3) and sends glutamatergic outputs specifically to the vLPO) (37). vLPO neurons preferentially innervate DMD neurons and reduced Tcore in response to heat by inhibiting DMD neurons. To test this hypothesis, we combined anterograde and retrograde tracing, terminal opsinogenetic stimulation, IPSC recording, and single-cell RT-PCR (2) to show that vLPOGAT neurons preferentially innervated DMD neurons, compared with DMDVGlut neurons (Fig. 2H).

Previous studies have shown that DMD neurons, especially glutamatergic neurons, play an important role in promoting thermogenesis (16–18, 29, 30). However, the response of these neurons to thermal stimuli was unknown. Here, we recorded calcium dynamics in the DMD by using fiber photometry and found, surprisingly, that both glutamatergic and GABAergic neurons were activated by cooling (25–13 °C; Fig. 3D and E), but that these neurons responded with different dynamics. The glutamatergic neurons responded more abruptly and their response decayed more quickly compared with the GABAergic neurons (SI Appendix, Fig. S5). Consistent with these kinetics, we found that increases in Tcore, EE, and activity levels resulting from CNO-mediated activation of DMD glutamatergic neurons arose more rapidly and decayed more quickly than those elicited by CNO-mediated activation of DMD GABAergic neurons (Fig. 4 and SI Appendix, Fig. S8).

The role played by GABAergic neurons in the DMD in thermoregulation has not been studied previously, although the DMD contains more GABAergic than glutamatergic neurons (31). Here, we have shown unequivocally that these GABAergic neurons are essential for controlling thermogenesis. Their activation strongly promoted increases in Tcore, EE, and physical activity (Fig. 4 E–H and SI Appendix, Fig. S5), whereas their suppression reduced Tcore and activity (Fig. 5 C and D). We therefore have uncovered a type of thermogenic neuron.

Several reports have suggested that neurons expressing the leptin receptor (Lepr) in the DMD are important for thermogenesis (19–21). Cold-induced cFos expression colocalizes with Lepr (21), and activation of these Lepr neurons increases Tcore and EE. Our immunohistochemistry results suggest that DMDVGlut neurons contain both glutamatergic and GABAergic types, with the majority being glutamatergic (SI Appendix, Fig. S9). Thus, DMDVGlut neurons might be a downstream target of the vLPOVGlut neurons.

It remains unclear how DMD neurons are connected with premotor neurons to direct thermogenesis. Early observations suggested that glutamatergic neurons may project directly to premotor neurons within the rostral medullary region (rMR) to promote thermogenesis (16–18). It has also been reported that premotor neurons receive inhibitory inputs from other medullary regions (32, 33). Thus, DMDVGlut neurons may directly innervate rMR premotor neurons to promote thermogenesis, and DMDVGlut neurons might disinhibit rMR neurons by suppressing their inhibitory inputs.

Delineating the specific neural cell types involved in thermoregulation is a key step toward understanding these critical neural circuits. Using the PhosphoTRAP approach (34), which enables the immunoprecipitation of translational ribosomes via phosphorilated ribosomal protein S6 (a marker of neural activity), we (SI Appendix, Fig. S10) and Tan et al. (35) independently discovered that brain-derived neurotrophic factor (BDNF), a classic neurotrophic factor (36), is transcriptionally activated following heat challenge and is a novel marker for heat-activated neurons. It is interesting to see that heat affects the expression of a neurotrophic factor, suggesting that long-term heat challenge may affect nerve growth and cause remodeling of thermoregulatory networks. The exact role played by BDNF in this context must be further tested. BDNF-expressing neurons in the ventromedial preoptic nucleus (VMPO) can drive hypothymia without affecting physical activity (35). Thus, the circuits involving VMPOBDNF neurons and vLPOVGlut neurons may act coordinately to regulate EE, BAT thermogenesis, activity, and vasodilation to lower Tcore.

Our results establish a neural circuit (Fig. 5E) for regulating heat loss behaviors, in which environmental heat indirectly activates POA glutamatergic neurons (refs. 1–3, 5, and 6 and SI Appendix, Fig. S3), which may, in turn, activate a population of GABAergic neurons in the vLPO (Fig. 1 and SI Appendix, Fig. S4). The vLPO GABAergic neurons inhibit thermogenic neurons in the DMD to suppress EE and activity, thereby lowering Tcore (Figs. 2, 4, and 5). The DMD neurons include both glutamatergic and GABAergic subtypes and are cold activated (Fig. 2 and 3). DMD glutamatergic neurons may send excitatory input to premotor neurons in the rMR to stimulate thermogenesis (16–18). DMD GABAergic neurons might disinhibit rMR premotor neurons by suppressing their inhibitory inputs (32, 33).
Materials and Methods

Mice. Animal care and use conformed to institutional guidelines of ShanghaiTech University, Shanghai Biomodel Organism Co., and governmental regulations. All experiments were performed on adult mice (8–16 wk old). Mice were housed under controlled temperature (22–25 °C) in a 12-h reverse light/dark cycle (light time, 8 PM to 8 AM) with a standard chow diet (4% (wt/wt) fat SPF Rodent Feed) and ad libitum drinking water. The following male mice strains were from Jackson Laboratory (USA): C57BL/6J (000664); Vglut2-IRES-Cre (016963); Vgat-IRES-Cre (028862); Ai14 (007914); LepR-Cre (008320); Gad67-GFP (006340).

AAV Vectors. We used Cre-inducible AAV vectors (titer 1.26×10^{12}–2×10^{13} PFU/mL) from Vector Company, University of California, San Diego (AAVS-EF1a-DIO-hChR2(H134R)-eYFP, AAVS-EF1a-DIO-eYFP, AAVS-Hsyn-DIO-hM3D-mCherry, and AAVS-Hsyn-DIO-H4-M4d-mCherry), the Vector Core at the University of Pennsylvania (AAVS-Syn-Flex-GCaMP6f), and Shanghai Taitool Bioscience Co. AAVS-Hsyn-GaACTR1-P2A-EGFP. The latter construct was a gift from Minmin Luo, National Institute of Biological Sciences, Beijing, originally described in ref. 12.

Surgeries. A detailed description is provided in SI Appendix, SI Materials and Methods. We delivered 0.2 μL (unless specified) of AAV virus through a pulled-glass pipette and a pressure microinjector (Nanoject II, 3–000-205A, Drummond). The fiber-optic inserts (200 μm i.d., AniLab Co.) were chronically implanted (200 μm above viral injection sites) and secured with dental cement.

Behavioral Assays. A detailed description is provided in SI Appendix, SI Materials and Methods. T_{max}, EE, and activity were monitored by animal monitoring system with temperature telemetry (Columbus, with G2 E-Mitter transponders).


