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Hypothalamic Circuits for Predation and Evasion

Graphical Abstract

Highlights

- PAG-projecting LH neurons are activated during predatory attack
- Activating PAG-projecting LH GABA neurons rapidly drives predatory attack
- Inhibiting PAG-projecting LH GABA neurons reversibly blocks predatory attack
- PAG-projecting LH glutamate neurons control predictive evasion

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In Brief

The inhibitory and excitatory projections from the lateral hypothalamus to the periaqueductal gray drive, respectively, predation and evasion.

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Hypothalamic Circuits for Predation and Evasion

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SUMMARY

The interactions between predator and prey represent some of the most dramatic events in nature and constitute a matter of life and death for both sides. The hypothalamus has been implicated in driving predation and evasion; however, the exact hypothalamic neural circuits underlying these behaviors remain poorly defined. Here, we demonstrate that inhibitory and excitatory projections from the mouse lateral hypothalumus (LH) to the periaqueductal gray (PAG) in the midbrain drive, respectively, predation and evasion. LH GABA neurons were activated during predation. Optogenetically stimulating PAG-projecting LH GABA neurons drove strong predatory attack, and inhibiting these cells reversibly blocked predation. In contrast, LH glutamate neurons were activated during evasion. Stimulating PAG-projecting LH glutamate neurons drove evasion and inhibiting them impeded predictive evasion. Therefore, the seemingly opposite behaviors of predation and evasion are tightly regulated by two dissociable modular command systems within a single neural projection from the LH to the PAG.

INTRODUCTION

Animals in nature typically lack the luxury of eating readily available food at their pleasure. Before feeding, many predators perform a predatory action sequence that includes searching, pursuing, attacking, and consuming (Comoli et al., 2005). Conversely, the prey seeks to evade the predator. Although numerous studies have provided insights into the neural mechanisms underlying feeding behaviors (Morton et al., 2006; Sternson, 2013; Sterber and Wise, 2016), it has been challenging to define the neural circuits for predation. Classical electrical stimulations revealed various hotspots for “quiet biting attack,” including the thalamus, hypothalamus, cerebellum, periaqueductal gray (PAG), and the ventral tegmental area (VTA) (Bandler et al., 1972; Brown et al., 1969; Chi and Flynn, 1971; Jansen et al., 1995; Siegel and Pott, 1988). Viewed together, these early studies suggested that there may be a “predatory attack center” in the brain that coordinates how these multiple brain areas drive predation behavior. The lateral hypothalamus (LH) is a possible candidate for this hypothetical predatory attack center. It connects the ventral tegmental area and the PAG via the medial forebrain bundle (Berthoud and Münzberg, 2011; Nieh et al., 2016; Siegel et al., 1999). The LH receives axon projections from multiple nuclei, including the feeding-related arcuate nucleus, the periventricular hypothalamic nucleus, and the recently identified predatory hotspot in the central amygdala (CeA) that is involved in processing sensorimotor information during predation (Berk and Finkelstein, 1981; Berthoud and Münzberg, 2011; Han et al., 2017; Reppucci and Petrovich, 2016). However, there is controversy about whether or not LH neurons do function in predation behaviors (Comoli et al., 2005). The LH has traditionally been considered to be important for promoting reward seeking, feeding, and arousal (Adamantidis et al., 2007; Harris et al., 2005; Jennings et al., 2013, 2015; Nieh et al., 2015; O’Connor et al., 2015; Sakurai, 2007; Sterber and Wise, 2016; Sutcliffe and de Lecea, 2002). Because electrical microstimulation can activate local neuron somata as well as the pass-by axons of neurons from other brain regions, the precise LH neural circuits underlying predation remain to be dissected precisely. Hypothalamic regions, especially those in the medial aspect, are also involved in evasion behavior (Lammers et al., 1988; Siegel and Pott, 1988), although the role of the LH in evasion remains largely unknown.

The LH contains many genetically distinct cell populations (Berthoud and Münzberg, 2011; Nieh et al., 2016; Sterber and Wise, 2016), and recent advances in neural circuit probing technologies now enable examination of the discrete functions of particular hypothalamic circuits. Using both projection-specific and cell-type-specific optogenetic manipulation, as well as fiber photometry recording, we here show that PAG-projecting LH GABA neurons drive predatory attack upon natural and artificial prey and demonstrate that inhibiting these neurons blocks predatory behavior. Furthermore, we show that PAG-projecting LH glutamate neurons control evasion behavior and demonstrate that inhibiting these neurons impairs the ability to escape from danger.

RESULTS

Activating PAG-Projecting LH Neurons Drives Predatory Attack

Using a dual-virus strategy that selectively expresses genetically encoded calcium indicator GCaMP6m in a projection-specific
Figure 1. Activating PAG-Projecting LH Neurons Drives Predatory Attack

(A) Schematic showing the injection of AAV-retro-Cre into the periaqueductal gray (PAG) and of AAV-DIO-GCaMP6m into the lateral hypothalamus (LH) in a wild-type mouse. An optical fiber was implanted above the LH.

(B) Schematic of fiber photometry of neuronal activity in the GCaMP6-expressing PAG-projecting LH neurons from hungry C57BL/6 mice while hunting crickets. DM, dichroic mirror; PMT, photomultiplier tube.

(C) Heatmap illustration (upper panel) and mean GCaMP signal (lower panel) aligned to the initiation of cricket hunting from all tested animals during the cricket-hunting task (n = 5 GCaMP6).

(D) Schematic for expressing ChR2-mCherry in PAG-projecting LH neurons in C57BL/6 mice.

(E) ChR2-mCherry expression in the LH. 3v, third ventricle; f, fornix.

(F) Cricket-hunting task and the optogenetic stimulation protocol.

(G) Example picture of the hunting arena with dead crickets after 10 trials of optogenetic activation. Out of total of 10 crickets, 9 were killed (red circle), but not consumed. Insets show close-up views of the dead crickets (blue rectangles).

(H) Heatmap illustration of an animal’s predatory action sequence across 10 stimulation trials.

(legend continued on next page)
manner (Chen et al., 2013; Tervo et al., 2016), we first tested whether or not PAG-projecting LH neurons are activated during predatory attack behavior. We injected a retrograde-transport-orientated adeno-associated viral (AAV) vector carrying Cre-recombinase (AAV-retro-Cre) into the PAG and then injected a Cre-dependent vector AAV-DIO-GCaMP6m into the LH to selectively express GCaMP6m in LH neurons projecting to the PAG (Figure 1A) (Atasoy et al., 2008; Tervo et al., 2016; Zhang et al., 2010). We implanted an optical fiber and used photometry to monitor changes in GCaMP fluorescence in the population of PAG-projecting LH neurons (Figure 1B) (Gunaydin et al., 2014; Li et al., 2016; Zhong et al., 2017). After we trained food-deprived mice to hunt crickets, they typically completed the searching, pursuit, and attack actions of the sequence in less than 2 s but spent a long time consuming the prey (~60 s). Aligning the GCaMP signals with video-scored behavioral actions revealed an increase in the activity of PAG-projecting LH neurons starting when starving mice began to hunt crickets (Figure 1C). These results implicated PAG-projecting LH neurons in predatory behavior.

To test whether or not activating PAG-projecting LH neurons would induce predatory attack behavior, we expressed ChR2-mCherry in the PAG-projecting LH neurons using the dual-virus strategy (Figures 1D, 1E, and S1A) (Boyd et al., 2005; Tervo et al., 2016). Whole-cell patch-clamp recordings in brain slices confirmed that brief light pulses reliably evoked spike firing from ChR2-mCherry-expressing neurons in the LH (Figure S1B). In order to assay stimulation-evoked predatory behavior, we used cricket-naive mice that had free access to food and water (Figure 1F). Light pulses were delivered through an optical fiber implanted into the LH (Figure S1C). Without optogenetic stimulation, the nonhungry cricket-naive mice rarely initiated predatory attack against crickets. Immediately following optogenetic stimulation, mice initiated predatory attack, sniffling vigorously around the test chamber, rushing toward a cricket and subduing it with their forepaws, and eventually delivering repeated killing bites with a 91.7% probability (Figures 1G–1I; Movie S1). The stimulated mice did not eat up these crickets within the 30-s stimulation period; rather, they attacked a cricket and left most of its body in the chamber (Figure 1G). As each new round of optogenetic stimulation was delivered, the stimulated mouse would initiate a new predatory attack sequence. The mean attack latency between stimulation and attack (the time from stimulation onset to mouse contact with a cricket) was less than 10 s (Figure S1D). The predatory attack behavior ceased immediately when the light pulses were stopped (Figure 1H; Movie S1, first part). Thus, PAG-projecting neurons in the LH are sufficient to rapidly orchestrate predatory attack without prior training.

The LH neurons labeled with the two-virus strategy projected their axonal terminals in the lateral and ventral lateral region of the PAG (lvPAG) (Figure 1J). Photostimulating the axonal terminals in the PAG produced similarly strong predatory behavior with similarly short latency (Figures 1K and S1E). In patch-clamp recordings of brain slices, stimulating the axonal terminals from retrograde-labeled LH neurons evoked a mixture of GABAergic inhibitory currents and glutamatergic excitatory currents in PAG neurons, indicating that these PAG-projecting LH neurons can release the neurotransmitters GABA and/or glutamate (Figures 1L and 1M). Anatomical analysis of the PAG-projecting LH neurons of sacrificed mice revealed that about 61% were putatively GABAergic (vesicular GABA transporter-positive; Vgat+) and that about 33% are glutamatergic (vesicular glutamate transporter 2-positive; Vglut2+; Figures S1F–S1I).

PAG-projecting LH neurons also project to other brain regions such as the thalamus and the lateral habenular (LHB), and project along the medial forebrain bundle (MFB) to the midbrain ventral tegmental area and the substantia nigra compacta (SNC) as well as the reticular nucleus posterior to the PAG (Figure S1J). Unilateral activating the thalamus projection, the LHb projection, and the reticular projection produced no obvious effects on predatory attack in the cricket-hunting task (Figures S1K, S1L, and S1N). Activating the ventral tegmental area/SNc projection located between the LH and the PAG induced attack behavior similar to what we observed with PAG activation (Figure S1M). However, it is possible that this predatory attack behavior could be caused by some coactivation of PAG-projecting pass-by fibers in the ventral tegmental area/SNc. Our results suggest that PAG-projecting LH neurons may coordinate neuronal activity in multiple brain regions as they orchestrate predatory behavior.

**LH GABA Neurons Are Activated during Predation**

Next, we specifically tested whether the activity of LH GABA neurons encodes particular aspects of the predatory attack behavior sequence. To monitor the activity of the inhibitory projections during predatory attack, we targeted GABA neurons by stereotaxic infusion of AAV-DIO-GCaMP6m vectors into the LH of Vgat-Cre mice (Figure 2A). Using fiber photometry, we measured changes in the GCaMP6 fluorescence of LH GABA neurons during the starvation-induced cricket-hunting task (Figure 2A). Aligning the GCaMP signals with video-scored behavioral actions revealed that neuronal activity increased at the moment that animals started hunting crickets; neuronal activity fell back to the baseline level as the mouse consumed the cricket (Figures 2B–2D and S2A–S2D; Movie S2). We did not detect significant changes in fluorescence signals in LH GABA neurons of
GFP-expressing control mice during the cricket-hunting experiments, confirming that the GCaMP signals genuinely indicate neuronal activity and are not simply reflecting artifacts of motion (Figures S2E and S2F).

In order to more precisely control the timing of predatory attack behaviors, we designed a computer-controlled food-chasing task. We used real-time monitoring and computer-controlled robotic arms to guide the movement of the food dish via remote magnetic force. After a mouse entered the specified trigger zone in an arena for 1 s, a moving dish that contained food pellets (artificial prey) was guided along one of two edges of the chamber (Figure 2E). When a mouse ran after the dish and came within 10 cm of the dish, the dish was stopped so that the mouse could retrieve one small food pellet. After training, mice showed predation-like behavior toward the moving food dish (Figures 2F and S2G). Following triggering of dish entry...
into the arena ("initiation" stage), animals quickly chased after the moving artificial prey dish ("chasing" stage), typically completing a "virtual predation sequence" within 2 s (Figures 2G, S2H, and S2I). Fiber photometry revealed that the activity of LH GABA neurons ramped up during pursuit and reached peak activity during pellet retrieval ("retrieval" stage) (Figures 2H–2J). It should be noted that our fiber photometry reflects the population-level activity of LH GABA neurons. Previous monitoring at single-cell resolution revealed that LH GABA neurons encode either the appetitive or consummatory aspects of reward-related behaviors (Jennings et al., 2015), but it had remained unclear how LH GABA neurons change their activity during hunting.

Inhibition of LH GABA Neurons Suppresses Predatory Behavior

To test whether the endogenous activity of LH GABA neurons is necessary for predation, we used viral-vector mediated, Cre-dependent expression of the *Guillardia theta* anion channel rhodopsin 1 protein (GtACR1) (Govorunova et al., 2015) to optically inhibit GABA neurons in the bilateral LH (Figures 3A, 3B, and S2I). Fiber photometry revealed that the activity of LH GABA neurons ramped up during pursuit and reached peak activity during pellet retrieval ("retrieval" stage) (Figures 2H–2J). It should be noted that our fiber photometry reflects the population-level activity of LH GABA neurons. Previous monitoring at single-cell resolution revealed that LH GABA neurons encode either the appetitive or consummatory aspects of reward-related behaviors (Jennings et al., 2015), but it had remained unclear how LH GABA neurons change their activity during hunting.

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**LH GABA Neurons Drive Predatory Attack**

To determine the role of the LH GABA neurons in predatory attack, we expressed ChR2 in LH GABA neurons by stereotaxic infusion of AAV-DIO-ChR2-mCherry vectors into the LH of Vgat-Cre mice (Figures 4A, 4B, and S4A). Following the expression of ChR2-mCherry in LH GABA neurons, optogenetic stimulation of the ChR2-expressing axonal terminals elicited GABAergic currents in PAG neurons in a brain slice preparation (Figures 4C and 4D). Applying light pulses into the LH rapidly drove strong predatory attack from the ChR2-expressing mice but not from the mGFP-expressing control mice (Figures 4E and S4B; Movie S1, latter part). Stimulation of LH GABA neurons also caused mice to initiate chasing, neck stretching, and biting upon another mouse, albeit with only about half of the attack percentage as for cricket attack (Figures S4C and S4D; Movie S4, first part). The attacks against other mice exhibited typical features of intraspecific predatory attack and differed from defensive attacks, which are characterized by piloerection and back arching (Lin et al., 2011; Siegel et al., 1999). Therefore, LH
Figure 4. LH GABA Neurons Drive Predatory Attack

(A) Schematic of expressing ChR2 in LH GABA neurons for optogenetic activation.

(B) ChR2 expression pattern in the LH following the injection of AAV-DIO-ChR2-mCherry vectors into a Vgat-Cre mouse. The white triangle indicates the optical fiber tip.

(C) ChR2 expression pattern in the PAG (right panel).

(D) In a brain slice preparation, brief optogenetic stimulation (5 ms) evoked outward currents in PAG neurons, and the currents were largely abolished by GABAzine (n = 6 cells tested).

(E) The probability of predatory attack evoked by optogenetic activation of LH GABA neurons (n = 11 ChR2 versus n = 6 mGFP control; two-way repeated-measures ANOVA, virus x stimulation, F(1,15) = 152.8, p < 0.0001, Sidak’s post hoc test).

(F) Artificial prey (wax disk; red) chasing task.

(G) Reduction in the mass of the artificial prey wax disk after 10 trials of LH stimulation (n = 8 ChR2 versus n = 6 mGFP control; two-tailed Wilcoxon rank-sum test).

(H–K) Optogenetic stimulation of LH GABA neurons can switch an animal’s behavior from evasion to predation.

(H) Schematic of the evasion-to-predation transition experiment.

(I) The effects of 15-s optogenetic stimulation on the distance between an approaching disk and a mouse and the locomotor speed of the mouse, illustrating its transition from evasion to predation.

(J) Number of evasion events before and during stimulation (n = 8 ChR2; two-tailed Wilcoxon signed-rank test).

(K) The percentage of light-evoked attack on the approaching disk (n = 8 ChR2 versus n = 6 mGFP control; two-tailed Wilcoxon rank-sum test).

n.s., not significant; **p < 0.01; ***p < 0.001. Data are reported as mean ± SEM. See also Figure S4 and Movies S1, latter part, S4, and S5.
GABA neurons in mice can produce a sufficiently strong motivation to drive attack against both crickets and intraspecific targets.

To test whether the promise of obtaining some calorific value is required for the observed light-evoked predatory attack, we designed an artificial prey item that offered no calorific content by embedding a small magnet in a wax disk, which was controlled by remote magnetic force (similar to the food dish, above). To mimic a natural prey item, the artificial prey was moved away as a mouse approached (Figure 4F). Optogenetic stimulation of LH GABA neurons caused the mice to pursue, attack, and voraciously bite the artificial prey disk (Figures 4G, S4E, and S4F; Movie S4, latter part). A substantial amount of wax had been bitten off the disk by the end of the trial sessions (Figure 4G), suggesting that activating LH GABA neurons can compel vigorous chasing and strong biting attack against a moving object without any calorific value.

To determine whether LH GABA neurons have the potential of switching from evasion to predation, we conducted experiments in which we turned the wax disk into an attacker by guiding it to repetitively strike a mouse. Mice would turn and run away to escape from these strikes in typical evasion behavior (Figures 4H and S4G). However, immediately following stimulation of LH GABA neurons, a mouse would stop running away from disk strikes, instead turning its body around and starting to attack and bite the disk (Figures 4I–4K and S4G–S4K; Movie S5), demonstrating that stimulating LH GABA neurons can produce a sufficiently strong attack drive to switch behavior from evasion to predation.

**The Role of the GABAergic LH-PAG Projection in Predatory Attack**

We carried out several additional experiments to study the role of LH GABA neurons that project specifically to the PAG. First, we carried out optogenetic inhibition specifically in the PAG-projecting LH GABA neurons and their terminals in the PAG during the cricket-hunting task and the food-chasing task. Using the dual-virus strategy, we injected retrogradely transported and Cre-recombinase-dependent AAV-retro-DIO-Fip into the PAG and injected Fip-dependent AAV-fDIO-GtACR1 into the LH of Vgat-Cre mice (Figure 5A). Optogenetic inhibition of both the PAG-projecting LH GABA neurons and their terminals in the PAG significantly suppressed predatory behavior in the cricket-hunting task (Figure 5B). Moreover, optogenetic inhibition of PAG-projecting LH GABA neurons starting from the initiation of food chasing decreased the successful hit rate during the food-chasing task (Figure 5C) but does not affect an animal’s gnawing behavior when such inhibition is started after the animal has begun to consume the food pellet (Figure 5D). These results indicate that the activity of PAG-projecting LH GABA neurons is necessary for predatory behavior.

We then characterized the behavioral effect of activating LH GABA neurons that project to the PAG. We expressed ChR2 in LH GABA neurons by single injection of AAV-DIO-ChR2 into the LH of Vgat-Cre mice. Stimulation of the axonal terminals in the l/vlPAG from LH GABA neurons also produced strong predatory attacks against crickets, intraspecific individuals, and artificial prey (Figures 5E, 5F, and S5A–S5E). We also expressed ChR2 in PAG-projecting LH GABA neurons using the dual-virus strategy of injecting AAV-retro-DIO-Fip vectors into the PAG and Fip-recombinase-dependent AAV-fDIO-ChR2-EYFP vectors into the LH of Vgat-Cre mice (Figure 5G). Optogenetic stimulation of the PAG-projecting LH GABA neurons resulted in attack against crickets, with an attack probability of over 92.5%, and latency of less than 10 s (Figures 5H and S5F–S5I). We observed no attack behavior while stimulating the LH of control mice that had received AAV-fDIO-ChR2-EYFP injection into the LH but no injection of AAV-retro-DIO-Fip into the PAG (Figures 5H, S5H, and S5I). These results demonstrate that activating the PAG-projecting LH GABA neurons is sufficient for driving strong predatory behavior.

In a real-time place preference test, optogenetic activation of PAG-projecting LH GABA neurons induced avoidance behavior (Figure S5J), whereas optogenetic inhibition induced approach behavior (Figure S5K). These results suggest that PAG-projecting LH GABA neurons may transmit a negative-valence signal for predatory behavior, in a manner similar to the feeding drive encoded by AgRP-neurons in the arcuate nucleus (Betley et al., 2015). We also measured the effect of stimulating LH GABA neurons on food intake. Activation of LH GABA neurons for 1 hr (5-ms light pulses at 20 Hz; 30 s ON, 30 s OFF) elicited a significant increase in food pellet intake (Figure S5L) (Jennings et al., 2013, 2015; O’Connor et al., 2015). However, activating PAG-projecting LH GABA neurons using the same parameters did not evoke food intake (Figure S5M). Thus, unlike the entire population of LH GABA neurons, PAG-projecting LH GABA neurons play a more prominent role in predatory attack than in consuming freely available food.

We further investigated the role of PAG neurons in mediating the predation signals from LH GABA neurons. As the activation of Vglut2-expressing glutamate neurons in the PAG suppresses predation (Han et al., 2017), we tested how enhancing the activity of PAG glutamate neurons would affect predatory behavior as elicited by stimulating PAG-projecting LH neurons. Infusing AAV-DIO-hM3Dq-2A-mCherry into the PAG of Vglut2-ires-Cre mice led to the expression of the excitatory designer receptor hM3Dq in PAG glutamate neurons (Figure 5I) (Alexander et al., 2009; Roth, 2016; Sterenson and Roth, 2014). At the same time, we expressed ChR2 in PAG-projecting LH neurons using the dual-virus strategy (Figures 5I and S5N). Administering the designer drug clozapine-n-oxide (CNO) completely abolished both the hunting of crickets by hungry mice (Figures 5J and S5O) and the predatory behavior evoked by optogenetically stimulating PAG-projecting LH neurons of ad libitum mice (Figures 5K and S5P). These results therefore suggest that a reduction in the activity of PAG glutamate neurons might be needed for predatory behavior.

**PAG-Projecting LH Glutamate Neurons Are Necessary for the Prediction of Danger**

After defining the role of PAG-projecting LH GABA neurons in predatory attack, we investigated the functions of PAG-projecting LH glutamate neurons in predation and evasion. Since the Vglut2-ires-Cre mouse line has been successfully used to drive
Gene expression in LH glutamate neurons (Jennings et al., 2013; Nieh et al., 2016; Vong et al., 2011), we used this line to express GCaMP6m and optogenetic tools by infusing Cre-dependent AAV vectors into the LH.

We first tested whether LH glutamate neurons encode specific aspects of evasion behavior. We injected AAV-DIO-GCaMP6m into the LH of Vglut2-ires-Cre mice (Figure S6A). Fiber photometry of GCaMP signals revealed that LH glutamate neurons increased their activity during evasion (Figures 6A–6D), but not during food chasing (Figures S6B–S6D). mGFP-expressing control animals did not show any changes in fluorescence signals during evasion (Figures S6E–S6G). GCaMP signals increased

Figure 5. The Effects of Bidirectionally Manipulating PAG-Projecting LH GABA Neurons on Predatory Behavior
(A) Schematic showing the strategy of expressing GtACR1 in PAG-projecting LH GABA neurons. The experimental group received AAV-retro-DIO-Flp injection into the PAG and received AAV-fDIO-GtACR1 injection into the LH. AAV-retro-DIO-Flp was omitted in the control group.
(B) The number of crickets killed in the 20-min test session by starving animals in both the control and the inhibition of PAG-projecting LH GABA neurons experimental groups of Vgat-Cre mice during the cricket-hunting task. (n = 5 for both groups, two-way repeated-measures ANOVA, virus × session, F(2,16) = 7.922, p = 0.0041, Sidak’s post hoc test.)
(C) Inhibition of PAG-projecting LH glutamate neurons starting from the time of food chasing initiation decreased the successful hit rate during the food-chasing task (n = 5, two-tailed Wilcoxon signed-rank test).
(D) Inhibition of PAG-projecting LH GABA neurons starting after the animal has retrieved the food pellet did not affect an animal’s gnawing behavior during the food-chasing task (n = 5, two-tailed Wilcoxon signed-rank test).
(E) Schematic showing the stimulation of the ChR2-expressing terminals from the LH in the PAG of Vgat-Cre mice.
(F) Predatory attack probability evoked by terminal stimulation in the cricket-hunting task (n = 13 ChR2; two-tailed Wilcoxon signed-rank test).
(G) Schematic showing the strategy of expressing ChR2 in PAG-projecting LH GABA neurons. We injected AAV-retro-DIO-Flp into the PAG and AAV-fDIO-ChR2 into the LH. AAV-retro-DIO-Flp was omitted in the control group.
(H) The effect of optogenetic stimulation of PAG-projecting LH GABA neurons on the predatory attack probability in the cricket-hunting test (n = 4 control and n = 6 ChR2, two-way repeated-measures ANOVA, virus × stimulation, F(1,9) = 28.01, p = 0.0005, Sidak’s post hoc test).
(I) Schematic showing the strategy of expressing ChR2 in PAG-projecting LH neurons and hM3Dq in PAG glutamate neurons.
(J) The effect of applying CNO (1 mg/kg, i.p.) for chemogenetic activation of PAG glutamate neurons on cricket hunting by hungry mice (n = 5 per group; two-way repeated-measures ANOVA, virus × injection, F(1,8) = 9.761, p = 0.0141, Sidak’s post hoc test).
(K) The effect of chemogenetic activation of PAG glutamate neurons on cricket hunting as evoked by optogenetic stimulation of PAG-projecting LH neurons (n = 6 for mGFP group; n = 5 for hM3Dq group, two-way repeated-measures ANOVA, virus × injection, F(1,9) = 91.92, p < 0.0001, Sidak’s post hoc test).

n.s., not significant; *p < 0.05; **p < 0.01; ***p < 0.001. Data are reported as mean ± SEM. See also Figure S5.
before the onset of evasion behavior (Figure 6B), suggesting that the activity of LH glutamate neurons not only reflects evasion behavior but also contributes to predicting the upcoming danger.

To test whether the activity of LH glutamate neurons is needed for successful evasion behavior, we expressed GtACR1 and optically inhibited these neurons (Figure 6E). After training, mice could predict upcoming danger and evade it before the artificial attacker struck them (Movie S6). During optogenetic inhibition of LH glutamate neurons, mice evaded only when the moving attacker contacted their body (Movie S6). Optogenetic inhibition significantly decreased the nearest distance between the attacker and the mouse, which suggested a failure to predict the upcoming danger (Figures 6F and 6G). The inhibition also
decreased the total evasion number during the 15-s evasion bout (Figure S6H) but did not affect the evasion speed (Figure 6H).

Similar observations were made when we inhibited LH glutamate neurons that projected to the PAG. Using the dual-virus strategy of separately injecting AAV-retro-DIO-Fip into the PAG and Flp-dependent AAV-DIO-GtACR1 into the LH of Vglut2-ires-Cre mice, we expressed GtACR1 in PAG-projecting LH glutamate neurons (Figures 6I and S6I). Cell-type- and projection-specific optogenetic inhibition impaired the animal’s ability to predict the upcoming attacker in the artificial attacker evasion task (Figure 6J) and decreased the total evasion number during the 15-s evasion bout (Figure S6J). This inhibition did not have a significant effect on an animal’s peak evasion speed (Figure 6K).

Interestingly, optogenetic inhibition of PAG-projecting LH glutamate neurons induced mild approach behavior in the real-time place preference test (Figure S6K). These results provide strong evidence to show that the activity of PAG-projecting LH glutamate neurons is necessary for making predictive evasion from upcoming danger, but not for evasion movement itself.

PAG-Projecting LH Glutamate Neurons Control Evasion Behavior

We injected AAV-DIO-ChR2 into the LH of Vglut2-ires-Cre mice (Figure S7A). Optogenetic activation of these neurons immediately resulted in high-speed running and occasional jumping in an open-field arena (Figures 7A–7D and S7A–S7C; Movie S7). To determine whether this running and jumping behavior corresponded to evasion, we activated these neurons at the specific moment when the mice captured the food dish in the food-chasing task (Figure 7E). Activating LH glutamate neurons caused the mice to cease the food retrieval and consumption behaviors immediately and to run in the opposite direction of the moving food dish (Figures 7F, 7G, S7D, and S7E; Movie S7). In a brain slice, stimulating ChR2-mCherry-expressing axonal terminals elicited a glutamatergic current in l/vIPAG neurons, demonstrating the presence of a glutamatergic projection from the LH to the PAG (Figures 7H and 7I). Optogenetic stimulation of the l/vIPAG area containing the ChR2-expressing axonal terminals also induced running and jumping behavior (Figures 7J, 7K, and S7F). Utilizing the dual-virus approach on Vglut2-ires-Cre mice, we specifically expressed ChR2 in PAG-projecting LH glutamate neurons (Figure S7L). Stimulating these neurons in the LH produced similar flight-like behavior in Vglut2-ires-Cre mice but not in the control mice (Figures 7M, 7N, S7G, and S7H), further supporting that PAG-projecting LH glutamate neurons drive evasion.

DISCUSSION

Our study defines a hypothalamus-midbrain circuit that contains two dissociable modular command systems that separately control the seemingly opposite behavioral choices of predatory attack and evasion behaviors: PAG-projecting LH GABA neurons drive predatory attack; PAG-projecting LH glutamate neurons control evasion. Consistent with these findings, LH GABA neurons show increased activity during predation and their inhibition blocks predatory attack, whereas LH glutamate neurons show increased activity during evasion and their inhibition impedes the ability to predict danger. Activating the LH-PAG pathway without cell-type specificity produces predatory attack, suggesting that the GABAergic component for predation overrules the glutamatergic component for evasion. This highlights the importance of being able to dissect neural circuits in a cell-type-specific manner.

Rather than using classical electrical stimulation methods (Siegel and Pott, 1988), we here used projection- and cell-type-specific optogenetic manipulations to illustrate the distinct roles of these subpopulations of LH neurons. Other technologies enabled us to make additional important experimental distinctions. For example, rather than simply using stationary targets in the predation experimental model (Siegel and Pott, 1988), we used both natural prey and computer-controlled artificial prey. With the artificial prey, we achieved clear dissection of the predatory behavior into distinct phases: initiation, chasing, retrieval, and consumption. This allowed us to make an important biological discovery. When we optogenetically inhibited LH neurons during these distinct phases of predation, we found that PAG-projecting LH GABA neurons were necessary for initiation, chasing, and retrieval, but not for the consumption phase. Finally, to facilitate our study of evasion behavior, we designed a computer-controlled artificial attacker that could strike the mouse and cause it to flee. This experimental setup effectively simulated the evasion behavior that can occur in the wild and made it possible to apply optogenetic manipulation precisely at pre-defined points. Such manipulation allowed us to demonstrate that PAG-projecting LH glutamate neurons are necessary for predictive evasion, but not for evasion movement itself.

Consistent with the notion that the hypothalamus is essential for various motivational behaviors (Bergquist, 1972; Sterrson, 2013; Stuber and Wise, 2016; Valenstein et al., 1970), we found that activating PAG-projecting LH GABA neurons strongly drives predatory attack, even when mice are not hungry and when their targets offer no caloric value. Although activating the entire population of LH GABA neurons promotes feeding as indicated by our results and previous studies (Jennings et al., 2013, 2015; O’Connor et al., 2015), activating the PAG-projecting LH GABA neurons drives searching, pursuing, attacking, and capturing behaviors toward a moving prey but does not promote feeding. In a real-time place preference test, activation of PAG-projecting LH GABA neurons induced avoidance behavior, whereas inhibition of these neurons induced approach behavior. Interestingly, activation of ventral tegmental area-projecting LH neurons produces appetitive behavior (Nieh et al., 2015, 2016). Our results thus suggest that, unlike ventral tegmental area-projecting LH neurons, PAG-projecting LH GABA neurons that function in predatory behavior may transmit a negative-valence signal, similar to the feeding drive encoded by AgRP-neurons in the arcuate nucleus (Betley et al., 2015); we term this “predation drive.” It should be noted that a comprehensive c-Fos mapping study failed to show high c-Fos expression when rats hunted cockroaches (Comoli et al., 2005). The relatively low-resolution c-Fos mapping experiments were likely unable to resolve the separate populations of LH neurons that are specifically activated during either appetitive or consummatory behaviors (Jennings et al., 2015) and could thus not reveal the selective activation of the GABAergic LH-PAG pathway as a hungry animal hunts and/or consumes insects.
c-Fos mapping study suggested the CeA as an important candidate for predatory behavior (Comoli et al., 2005). Consistent with this, a recent study proposed that CeA neurons relay prey-associated sensory information to brainstem premotor circuits, thus suggesting a role for the CeA in sensorimotor integration (Han et al., 2017). CeA neurons project to the LH directly or indirectly...
via the bed nucleus of the stria terminalis (Reppucci and Petrovich, 2016). We speculate that LH GABA neurons, which we propose to be the neuronal substrate for predation drive, need to integrate sensorimotor information from the CeA to properly direct predatory behavior.

Several lines of evidence support a strong role of the PAG in predatory behavior. Optogenetic activation of the GABA projection from the LH in the PAG evoked predatory attack. Moreover, inhibition of the terminals of PAG-projecting LH GABA neurons in the PAG significantly suppressed predatory behavior, even though its effect was weaker than what was achieved by inhibiting the entire population of LH GABA neurons. We have also observed that direct activation of PAG glutamate neurons blocked the execution of a predatory action sequence. Activating the collateral projections from the PAG-projecting LH neurons to the thalamus, lateral habenula, and pontine reticular nucleus did not produce predatory behavior. Interestingly, activating the collateral projection to the midbrain ventral tegmental area also evoked predation. Consistent with our observations, an elegant study showed that LH-ventral tegmental area projections are involved in compulsive reward seeking (Nieh et al., 2015). Evaluating the role of the LH-ventral tegmental area projection is challenging because the axonal fibers from the LH to the PAG pass through the ventral tegmental area. Data obtained from collateral projection experiments highlighted the complexity of the hunting circuit. As with other behaviors like feeding (Betley et al., 2013) and aggression (Wang et al., 2015), parallel and redundant circuit organization likely exists for predatory behavior. Further studies will be needed to systematically characterize the specific roles played by these collateral projections during predatory behavior.

Classical electrical stimulation of the medial region of hypothalamus induces flight behavior (Lammers et al., 1988; Siegel and Pott, 1988), and multiple studies have dissected the microcircuits in the PAG that control flight behavior (Toyote et al., 2016). However, it is not known how LH neurons function in evasion behaviors. Here, we show that PAG-projecting LH glutamate neurons control evasion behavior. Note that this evasion behavior is different from simple running behavior. The optogenetic activation of LH glutamate neurons induced jumping and fear-like responses and further caused the mice to cease food retrieval and consumption behaviors immediately and to run in the opposite direction of the moving food dish. Moreover, importantly, LH glutamate neurons showed increased activity before the animals actually escaped. Inhibition of these neurons impaired the prediction of upcoming danger, but not the execution of escape behavior upon a strike by the artificial attacker. The prediction of danger would help an animal to prepare to escape and would thus improve its chance of survival. Our study demonstrates that LH glutamate neurons are necessary for the prediction of danger. Viewed alongside previous results showing that LH glutamate neurons are involved in aversive information processing (Jennings et al., 2013; Nieh et al., 2016), we suggest a general role for LH glutamate neurons in the prediction of danger and the coordination of evasion behavior.

Activating the terminals of PAG-projecting LH glutamate neurons in the PAG induced evasion behavior. The important functions that are classically associated with the PAG-defensive reactions—analgesia and autonomic regulation—are integrated by overlapping longitudinal columns of neurons (Bandler and Shipley, 1994). Recent optogenetic studies have shown that activation of vIPAG glutamate neurons induces freezing behavior, while activation of dorsal lateral PAG (dIPAG) glutamate neurons results in flight behavior. This flight behavior is caused by the specific activation of vIPAG local GABA neurons; note that this activation inhibits vIPAG local glutamate neurons (Toyote et al., 2016). This study suggested that vIPAG local GABA neurons might be responsible for the evasion behavior evoked by activation of LH glutamate neurons, an idea that will need to be addressed in future studies.

Considering that activation of the LH is known to be involved in maladaptive behaviors such as compulsive food seeking induced by the LH activation (Nieh et al., 2015) and anorexia caused by lesion of the LH (Teitelbaum and Epstein, 1962), manipulating the neuronal activity in the inhibitory LH-PAG neural circuit may also facilitate therapeutic interventions for eating disorders. Moreover, as carnivores share the instinctive inclination to search, pursue, attack, and capture prey, a mechanistic functional understanding of the neural substrate for predatory behavior should in theory provide an alternative means for animal behavioral training. The role of LH glutamate neurons in predictive evasion represents an important new insight into the neural basis of maladaptive danger prediction. Further identification of molecular and cellular features within the glutamatergic circuit from the LH to the PAG may provide clues leading to interventions for behavioral disorders related to the processing of danger signals.

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

Supplemental Information includes seven figures and seven movies and can be found with this article online at [https://doi.org/10.1016/j.neuron.2018.01.005](https://doi.org/10.1016/j.neuron.2018.01.005#mmc18). A video abstract is available at [https://doi.org/10.1016/j.neuron.2018.01.005#mmc18](https://doi.org/10.1016/j.neuron.2018.01.005#mmc18).
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AUTHOR CONTRIBUTIONS

Y.L., J. Zeng, and M.L. conceived the project, designed experiments, analyzed data, and wrote the manuscript. Y.L. and J. Zeng performed surgery, behavioral experiments, and in vivo activity recordings and analyzed data as guided by M.L. J. Zhang performed and analyzed in vitro slice recordings. W.Z. performed surgery and designed the schematics. C.Y. and Z.L. assisted with histology and microscopy. Q.F. packaged the optogenetic AAV vectors. All authors read and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

### STAR METHODS

#### KEY RESOURCES TABLE

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### CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and programs should be directed to and will be fulfilled by the Lead Contact, Minmin Luo (luominmin@nibs.ac.cn).
EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice
Animal care and use conformed to the institutional guidelines of the National Institute of Biological Sciences, Beijing as well as the governmental regulations of China. Adult (8-16 weeks old) Vgat-Cre mice [STOCK Tg(Slc17a6tm1(cre)tm1(Jm))Vgattm2(cre)Lowl/J] and Vglut2-ires-Cre mice [STOCK Slc17a6tm1(cre)tm1(Jm))Vgattm2(cre)Lowl/J] of either sex were obtained from the Jackson Laboratory (USA). Male C57BL/6N mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (China). All mice were maintained with a 12/12 light/dark cycle (lights off at 8PM) and housed in groups of five for 6-8 weeks. After surgery, mice were housed in groups or individually with an inverted light dark cycle (lights off at 8AM) for at least one week before further experiments. Adult mice of either sex were used. We used simplified genotypes of mouse strains for clarity.

METHOD DETAILS

Viral constructs
Regarding AAV vectors for imaging and optogenetic manipulation: AAV vectors carrying DIO-ChR2-mCherry, DIO-GtACR1-P2A-GFP, DIO-hM3Dq-2A-mCherry, DIO-mGFP, or DIO-GCaMP6m constructs were packaged into AAV2/9 serotype with titers of 1-5 x 10^12 viral particles/ml. We obtained the enhanced membrane GFP (Addgene Plasmid #14757) and GCaMP6m (Addgene Plasmid #40754) from Addgene. The gene encoding the Guillardia theta anion channel rhodopsins 1 (GtACR1) was synthesized according to the sequence from GenBank (KP171708.1). We built a fusion construct that was composed of the sequences for GtACR1, the P2A peptide, and an EGFP reporter(GtACR1-P2A-GFP); this fusion construct, or mGFP, or GCaMP6m was inserted into a pAAV-EF1α-DIO backbone derived from the pAAV-EF1α-DIO-hChR2(H134R)-mCherry plasmid (a gift from K. Deisseroth) in an inverted orientation. These were then packaged into AAV vectors (1-5 x 10^12 v.g./mL). AAV2-retro-hSyn-Cre (2.7 x 10^13 v.g./mL), AAV2-retro-hSyn-Flp (1.75 x 10^13 v.g./mL), AAV2-EF1α-fDIO-hChR2(H134R)-EYFP (1.71 x 10^13 v.g./mL), AAV2-EF1α-fDIO-GtACR1-EGFP (1.57 x 10^13 v.g./mL) and AAV2-retro-CAG-DIO-Flp (1.7 x 10^13 v.g./mL) were purchased from Shanghai Taitool Bioscience Co. (China).

Common surgery and virus injection
Adult mice were anesthetized with pentobarbital (i.p. 80 mg/kg) and mounted on a stereotaxic apparatus (Nanjing ThinkerTech, China). Body temperature was maintained at around 37°C with an electric heating pad, and erythromycin eye ointment (Beijing Shuangji Pharmacy Limited Company, China) was used to maintain eye lubrication. After disinfection with 0.3% hydrogen peroxide, a small incision of the scalp was created to expose the skull. Then, 0.3% hydrogen peroxide was again applied to clean the skull, and craniotomy was conducted. In order to implant two optical fibers bilaterally, we used two stereotactic coordinates to target the lateral hypothalamus (LH). On the left side, we used the following stereotactic coordinates: −0.70 mm posterior to the bregma, 1.0 mm lateral to the midline, and −4.75 mm ventral to the skull surface. On the contralateral (right) side, we targeted the LH at 2.2 mm lateral to the midline with a 14° angle (lateral to middle) with the same anterior-posterior and dorsal-ventral as the left side.

Injections were performed using a microsyringe pump (Nanoliter 2010 Injector, WPI). A Micro4 controller (WPI) was used to deliver the virus at a rate of 46 nL/min. For optogenetic manipulation and fiber photometry of LH, a total volume of 300-500 nL virus was injected into the LH. To specifically manipulate periaqueductal gray (PAG)-projecting LH neurons, we first bilaterally injected AAV-virus at a rate of 46 nL/min. For optogenetic manipulation and fiber photometry of LH, a total volume of 300-500 nL virus was injected into the PAG using the following coordinates: 4.1 mm posterior to the bregma, ± 1.05 mm lateral to the midline, and −2.55 mm ventral to the skull surface with a 14° angle (lateral to middle). Cre-dependent or Flp-dependent AAV viruses were then injected to the LH in the same surgery. After completion of the injection, two minutes were allowed to pass before withdrawing the glass needle by a distance of 50 μm; the glass pipette was left in place for five additional minutes and then slowly withdrawn completely. Optical fiber implantation was carried out immediately after virus injection. A piece of optical fiber (230 μm) was fit into an LC-sized ceramic fiber ferrule. The optical fiber (230 μm O.D., 0.37 NA; Shanghai Fiblaser, China) was implanted in specific brain regions (LH and PAG). The ceramic ferrule was supported with a skull-penetrating M1 screw and dental acrylic. Following surgery, animals were allowed to recover from anesthesia under a heat lamp. Lincomycin hydrochloride and lidocaine hydrochloride gel (Shandong Fangming Pharmaceutical Group, China) was applied to the sterilized incision site as an analgesic and anti-inflammatory drug. Animals were allowed to recover for two to three weeks after surgery.

Fiber photometry
To record fluorescence signals, a beam from a 488 nm laser (OBIS 488LS; Coherent, USA) was reflected via a dichroic mirror (MD498; Thorlabs, USA), focused by a 10× objective lens (NA = 0.3; Olympus, Japan), and then coupled to a rotary joint (FRU_1x1_FC-FC, Doric Lenses, Canada). An optical fiber (230 μm O.D., NA = 0.37; 2-4 m long) guided the light between the rotary joint and the implanted optical fiber. The laser power was adjusted at the tip of the optical fiber to a low level (0.01 - 0.02 mW) to minimize bleaching. The GCaMP fluorescence was bandpass filtered (MF525-39, Thorlabs) and collected by a photomultiplier tube (R3896; Hamamatsu, Japan). An amplifier (C7319; Hamamatsu) was used to convert the PMT current output to voltage signals, which was further filtered through a low-pass filter (40 Hz cut-off). The analog voltage signals were digitalized at 200 Hz and recorded with custom software developed in house using MATLAB or LabView.
**Optogenetic manipulations**

For activation experiments, 5-ms blue laser pulses were triggered from a 473-nm blue solid state laser (MBL-III-473, Changchun New Industries Optoelectronics Technology Co., Ltd., China) using a Master-8 pulse stimulator (A.M.P.I., Israel). The intensity power for activation at the fiber tip was about 15-20 mW. The stimulation frequency and duration varied for the different behavioral tasks (see below). The inhibition experiments used continuous 515-nm green light (MGL-F-515, Changchun New Industries Optoelectronics Technology Co., Ltd., China) or 473-nm blue light. The power at the fiber tip was 3-5 mW. A fiber-optic rotary joint (FRJ_1x1_FC-FC, Doric Lenses) was used to avoid winding of the fiber-optic cable in freely behaving animals. Bilateral stimulation was achieved by dividing light from one optical fiber to two fibers through fusion splicing.

**Chemogenetic manipulations**

The designer drug clozapine-n-oxide (CNO, 1 mg/kg, i.p.; Sigma, USA) was administrated 20 min before a behavioral test.

**Behavioral tasks**

**Cricket hunting task**

We conducted cricket predation experiments in a cylindrical arena with a transparent bottom (diameter 400 mm, height 300 mm). Behaviorally, latency to attack was represented as the time taken from light stimulation onset until a mouse actually attacked a cricket. We also calculated the attack probability which was quantified as the number of attack trials divided by the total light-on or light-off trials in a behavior secession. In the optogenetic activation experiments, *ad libitum* mice were introduced into the arena with ten crickets of medium size (12-20 mm body long). One optogenetic stimulation session of 15 min consisted of 10 stimulation blocks (5-ms pulses of 473-nm blue light at 20 Hz were delivered over a period of 30 s; 20 mW) with random inter-block intervals (40-80 s). In the optogenetic inhibition and activity monitoring experiments, mice were first deprived of standard chow for 12 hours. During fasting, 3-5 crickets were introduced into the home cage of the animal. For inhibition, chow-restricted mice were placed in the cylinder with ten crickets of small size (8-12 mm body long), and 2 s long pulses of 515-nm green laser or 473-nm blue laser light were delivered at 0.25 Hz for 20 min. The intensity at the tip of optical fiber was about 1-5 mW. For neuronal activity monitoring, animals were placed in the arena, and small crickets were delivered manually (one-by-one) to the field. The behaviors of mice and crickets were videotaped via a camera placed ~1 m below the bottom of the cylinder. The onset of camera recording, laser delivery, and neuronal activity were controlled and logged using custom MATLAB programs.

**Computer-controlled food-chasing task**

Mice were food restricted for 12 hours and then habituated to consume dustless precision rodent pellets (14 mg per pellet; Product# F05684, Lot # 200271, BioServ, USA) in their food dish. In the food-chasing task experiments, a total of 10-30 pellets were placed in a dish (diameter 30 mm; height 15 mm). The dish and food-restricted animals were placed in an arena (560 × 560 mm) for food chasing. A magnet (diameter 20 mm; height 5 mm) was glued to the bottom of the dish. We used a motor-driven, two-dimensional motion platform to control the movement of the food dish. The food dish was connected to the two-dimensional motion platform by magnetic force. We developed a program in LabView to control the motorized movement of the food dish. The positions of the animal and the food dish were calculated from the real-time video recorded via an overhead camera.

The food-chasing task encompassed 4 phases: initiation, chase, retrieval, consumption. In the initiation phase, a defined trigger zone in one of the corners of the platform was used to activate a given trial: when a mouse remained in the trigger zone for 1 s, the food dish was introduced to the platform at the corner diagonal to the trigger zone corner. Note that the positions of both the mouse and the food dish were monitored with the overhead camera. In the chase phase, the food dish was moved along the wall (“border”) of the arena. The border along which the food dish was moved in each trial was specified in a pseudo-random order. We progressively increased the speed of the moving food dish from low to high (speed 10 and 20 cm/s) as the animals learned the paradigm. In the retrieval phase, the movement of the food dish was stopped for 1 s and the animal would reach to get a food pellet. The real-time position information for the mouse and the food dish was used to select the moment when the food dish was stopped: for each trial, the food dish was stopped the first time that the distance between the centroid of mouse body and the center of food dish was less than 10 cm. Although the food dish was moved again after 1 s, the consumption phase of the experiment refers to the period when the mouse remains stationary and eats a pellet. Fiber photometry of GCaMP signals were performed throughout all four phases. Optogenetic inhibition was conducted at three separate stages. For each of these, the inhibition manipulation consisted of the delivery of 515 nm or 473 nm laser light for 5 s. The two stages at which the 5 s inhibition laser light could be applied were as follows: when a mouse entered the trigger zone (initiation), or after animals was consuming a food pellet (consumption).

**Artificial prey-chasing task**

We used a cylindrical disk made of red wax as an artificial prey (diameter 30 mm; height 15 mm). A magnet (diameter 20 mm; height 5 mm) was embedded in the prey disk. The movement of the prey disk was controlled by a motor driven two-dimensional motion platform as in the food-chasing task. The prey disk and *ad libitum* mice were placed into an arena (560 × 560 mm). The prey disk was connected to the two-dimensional motion platform by magnetic force. Whenever a mouse came within 100 mm of the prey disk (between the centroid of the mouse body and the center of the disk), the prey disk was moved away (speed ~20 cm/s) from the mouse; note that the evasive maneuvering of the prey disk could occur continuously if the mouse repeatedly approached the prey disk. Optogenetic activation was achieved by delivering 20 Hz pulses of blue laser light to the mouse for 30 s, starting the first
time that a mouse came within 100 cm of the prey disk. The weight loss of the wax disk was determined by the weight difference before and after each artificial prey-chasing session.

**Artificial attacker evasion task**

These experiments used the same red wax disk as the artificial prey-chasing experiments, but the disk was used as an artificial attacker. The disk was moved toward the mouse (chasing speed ~30 cm/s) for 15 s. The mouse would evade from the approaching artificial predator disk several times during the 15 s period. We also used 5 s chasing for simplicity during fiber photometry recording. We tested how optogenetic inhibition of LH glutamate neurons affected the behavior of mice exposed to the chasing of the artificial disk. The inhibition light was turned on 5 s before the artificial disk started chasing the mouse and during the entire chasing phase. The total evasion distance, median evasion number, and median peak evasion speed were analyzed during the 15 s evasion bout. We also analyzed the minimum distance between the escaping mouse and the artificial disk to represent the predictive evasion behavior.

**Evasion-to-predation transition task**

The red wax disk was also used in experiments that tested whether optogenetics stimulation of GABA neurons could switch a mouse’s behavior from evasion to predation. A trial period of 30 s was split into two 15 s halves. The disk was controlled exactly as in the artificial predator evasion task for the whole 30 s. For the first 15 s of predation, mice were not stimulated. However, throughout the second 15 s of the trial, the LH GABA neurons of the mice were activated with 20 Hz blue laser pulses. Note that the computer program controlling the disk movement was not changed throughout the 30 s trial.

**Predation-to-evasion transition task**

We first trained hungry mice that expressed ChR2 in LH glutamate neurons to perform the food-scavenging task. A test session consisted of 30 control trials (“Stim. OFF”) and 30 stimulation trials (“Stim. ON”) that were intermixed in the pseudorandom order. In the stimulation trials, we optogenetically activated LH glutamate neurons (5 ms at 50 Hz for 1 s) at the specific moment when a mouse captured the food dish.

**Intraspecific aggression task**

Before the intraspecific aggression tests, mice were housed in groups with food and water ad libitum. On the test day, the experimental animal was introduced into a cylindrical arena (diameter 400 mm, height 300 mm) and habituated for 1 min. Then, a stranger male mouse (6-8 weeks old, housed in a different group than the subject mouse) was placed into the arena as a target. For the activation experiments, in a single block of stimulation, 5-ms pulses of 473-nm blue light (20 Hz) were delivered for over a period of 30 s. The intensity at the tip of optical fiber was around 20 mW. A 15-min experiment session included ten blocks with random inter-block intervals (40-80 s). The behavior of animals was recorded while a mouse entered the optogenetic-stimulation chamber received 20 Hz blue laser pulses. In the inhibition experiments, the mice received constant 2 s delivery of blue laser light per 4 s when they crossed into the optogenetic-inhibition chamber. Each session lasted for 15 min, and the locations of the mouse were assessed from the video recording data using a custom MATLAB program.

**Real-time place preference task**

These experiments were conducted using the real-time place preference methods with a 3-chamber apparatus as previous study used (Lammel et al., 2012). The 3-chamber apparatus contains a left chamber (28 cm × 24 cm × 30 cm), a center chamber (11.5 cm × 24 cm × 30 cm) with white walls and a smooth metal floor, and a right chamber (28 cm × 24 cm × 30 cm) with a punched metal floor. The walls of the left and right chamber alongside the center chamber were half-open for the freely behaving mouse connected with optical patch cable. We conducted baseline and stimulation sessions, and examined the effect of activation or inhibition of both PAG-projecting LH GABA and glutamate neurons. In the activation experiments, animals that entered the optogenetic-stimulation chamber received 20 Hz blue laser pulses. In the inhibition experiments, the mice received constant 2 s delivery of blue laser light per 4 s when they crossed into the optogenetic-inhibition chamber. Each session lasted for 15 min, and the locations of the mouse were assessed from the video recording data using a custom MATLAB program.

**Food intake test**

Mice with food and water ad libitum were habituated to consume dustless precision rodent pellets (14 mg per pellet; Product# F05684, Lot # 200271, BioServ, USA) in a food dish (10 cm diameter). On the test day, the food intake was measured before, during, and after optogenetic stimulation. Each block lasted for one hour. Stimulation light was delivered (30 s on, 30 s off @ 20 Hz) only during the stimulation block.

**Slice recording**

Slice preparation and whole-cell recordings were performed as described previously (Hu et al., 2012; Ren et al., 2011; Zhang et al., 2016). Animals were deeply anesthetized with pentobarbital (100 mg/kg i.p.) and intracardially perfused with ~5 mL ice-cold oxygenated perfusion solution. The mouse brain was quickly placed in ice-cold oxygenated slicing solution. Coronal sections (250 or 300 µm thick) were prepared with a Leica VT1200S vibratome. The slices were incubated for 1 h at 34°C before patch clamp recording. The perfusion solution consisted of (in mM) 1.25 glucose, 225 sucrose, 119 NaCl, 2.5 KCl, 0.1 CaCl2, 4.9 MgCl2, 1.0 NaH2PO4, 26.2 NaHCO3, 3 kynurenic acid, and 1 Na L-ascorbate. The slicing solution consisted of (in mM) 110 choline chloride, 2.5 KCL, 0.5 CaCl2, 7 MgCl2, 1.3 NaH2PO4, 25 NaHCO3, 20 glucose, 1.3 Na ascorbate, and 0.6 Na pyruvate. The ringer solution consisted of (in mM) 125 NaCl, 2.5 KCl, 2 CaCl2, 1.3 MgCl2, 1.3 NaH2PO4, 25 NaHCO3, 10 glucose, 1.3 Na ascorbate, and 0.6 Na pyruvate. For the whole-cell recording, the pipettes were filled with internal solution that contained (in mM) 130 K-gluconate, 10 HEPES, 0.6 EGTA, 5 KCl, 3 Na2ATP, 0.3 Na3GTP, 4 MgCl2, and 10 Na2-phosphocreatine (pH 7.2–7.4). These chemicals for
brain slicing recording were from Sigma (USA). Whole-cell voltage-clamp recordings were performed with a MultiClamp 700B amplifier (Molecular Devices; USA). The traces were low-pass filtered at 3 kHz and digitized at 10 kHz (Dig)Data 1440, Molecular Devices). For optogenetic stimulation, 5 ms blue laser pulses were applied. The maximal light intensity reaching the brain tissue was ~14 mW. Neurons were held at −10 mV in the voltage clamp mode to study the inhibitory postsynaptic current (IPSC). GABAergic IPSCs were blocked by Gabazine (5 μM) application. Neurons were held at −65 mV in the voltage clamp mode to study the excitatory postsynaptic current (EPSCs). Glutamatergic EPSC was blocked by DNQX (5 μM) application. CNO (5 μM) was puffed onto hM3Dq-expressing cells for the verification of chemogenetic activation in the current clamp mode. TTX (1 μM) and 4-AP (1 mM) were applied to block multisynaptic transmission.

**Histology and fluorescent microscopy**

Mice were anesthetized with an overdose of pentobarbital and perfused intracardially with phosphate buffer saline, followed by 4% paraformaldehyde (PFA, wt/vol in PBS). Brains were removed and postfixed in 4% PFA for 4 hours at room temperature or 1 day at 4°C. The samples were cryoprotected in 30% sucrose solution until they sank. Coronal sections (50 μm) were prepared on a Cryostat microtome (Leica CM1950). Fluorescent images were collected using a confocal microscope (DigitalEclipse A1, Nikon) or an Olympus VS120 virtual microscopy slide scanning system with a 10× objective. Whole brain slices were scanned with an automated slider scanner (VS120 virtual Slide, Olympus), and further processed using ImageJ (NIH). For clarity, large images presented in figures were composites and stitched from scans of multiple small regions automatically.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Fiber photometry data analysis**

We calculated changes in fluorescence using methods similar to those reported in previous studies (Eban-Rothschild et al., 2016; Gunaydin et al., 2014; Li et al., 2016; Zhong et al., 2017). Briefly, we derived the fluorescence change values (dF/F) by calculating (F/F0) − F0/F0, where F0 is the median fluorescence signal intensity value of the whole session. In a minority of sessions, the photometry signal showed a short decay at the beginning of the recording. To address this in a general way across all sessions, the dF/F signal from each session was fitted with a decreasing exponential of the form a × e^bx, where a > 0 and b < 0. We then subtracted the exponential fit from the original dF/F signal. This procedure corrected the signal decrease when it occurred, and left the signal unchanged when no decrease was observed. Then, we segmented the data based on behavioral events within individual trials or bouts. dF/F values are presented with heatmaps or as peri-event plots with a shaded area indicating the SEM.

**Statistics**

We used softwares including MATLAB 2014a and GraphPad Prism 6 to do statistical analysis. We did not predetermine the sample size. In our study, sample sizes (n) usually denote the experimental replications as reported in the figure legends. In all optogenetic activation experiments and fiber photometry recordings, sample sizes (n) denote the number of stimulation or recording sites. In the inhibition experiments, sample sizes denote the number of mice used. In slice recording experiments, sample sizes denote the cell numbers recorded. The exact number of mice, stimulation sites, and recorded cells is described in figure legends. Data were reported as mean ± SEM in all Figures. Individual data are also reported in Figures. Statistical method, statistic value and corresponding p values are reported in the figure legends.

**DATA AND SOFTWARE AVAILABILITY**

Data and custom programs are available upon request.