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Zona incerta GABAergic neurons integrate prey-related sensory signals and induce an appetitive drive to promote hunting

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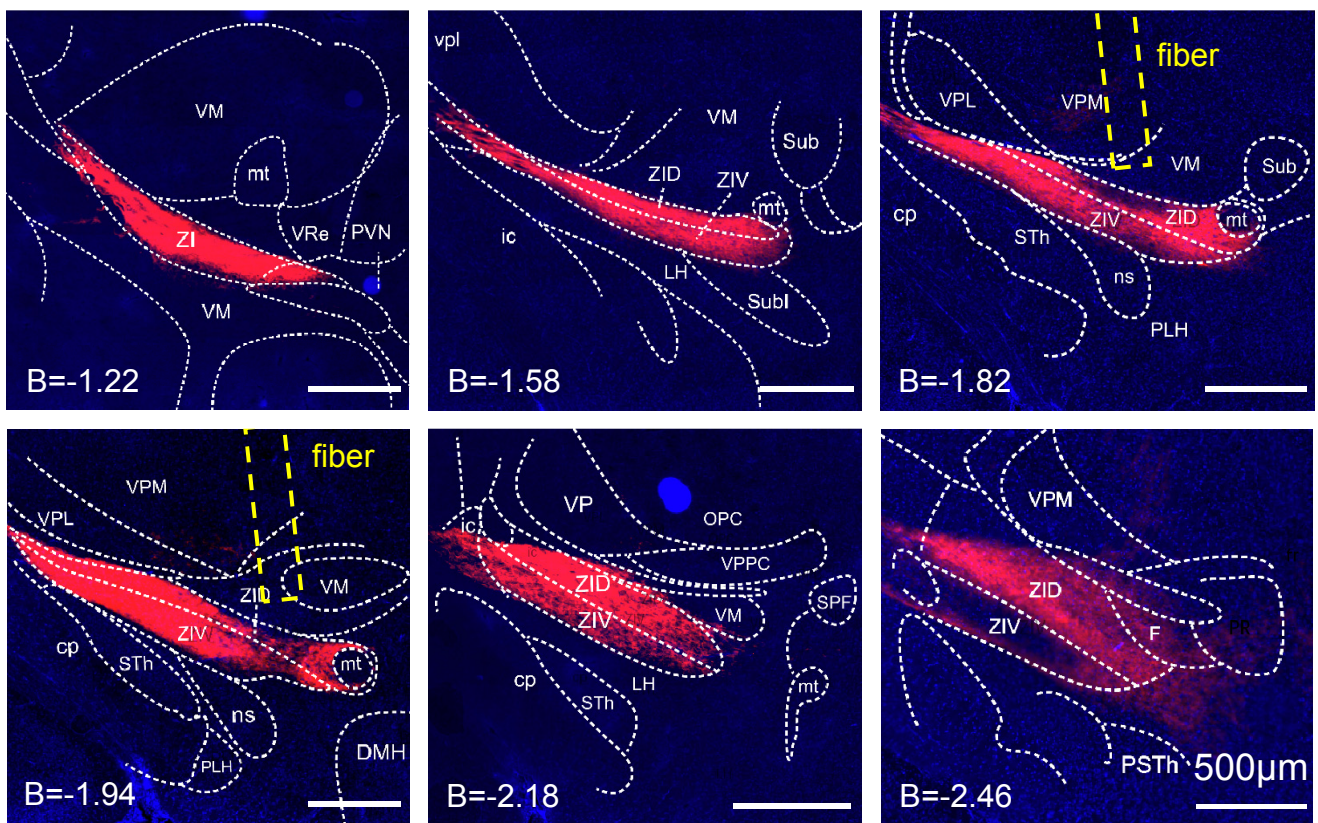
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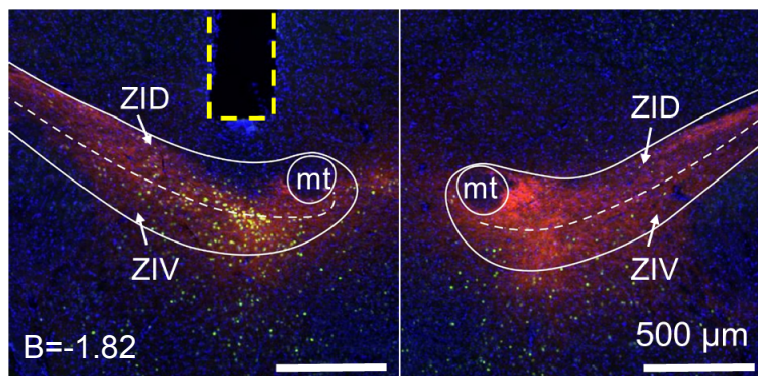
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a

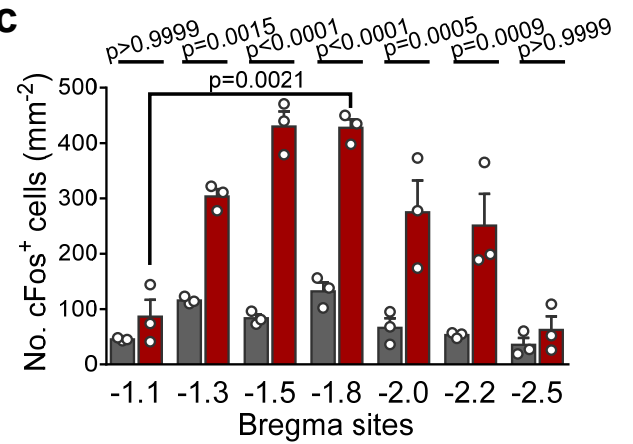


b

ChIEF-tdTomato / cFos / DAPI

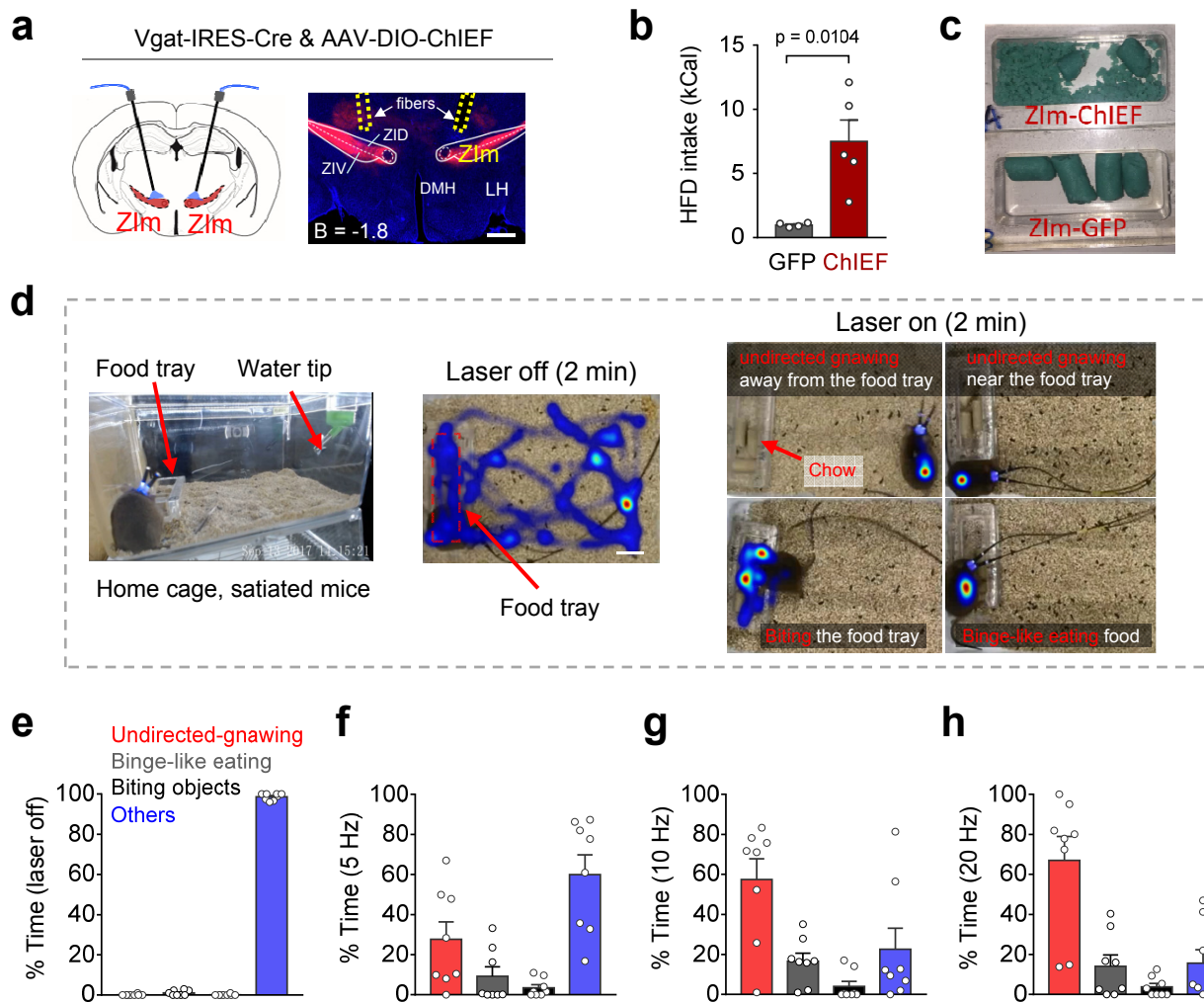


c



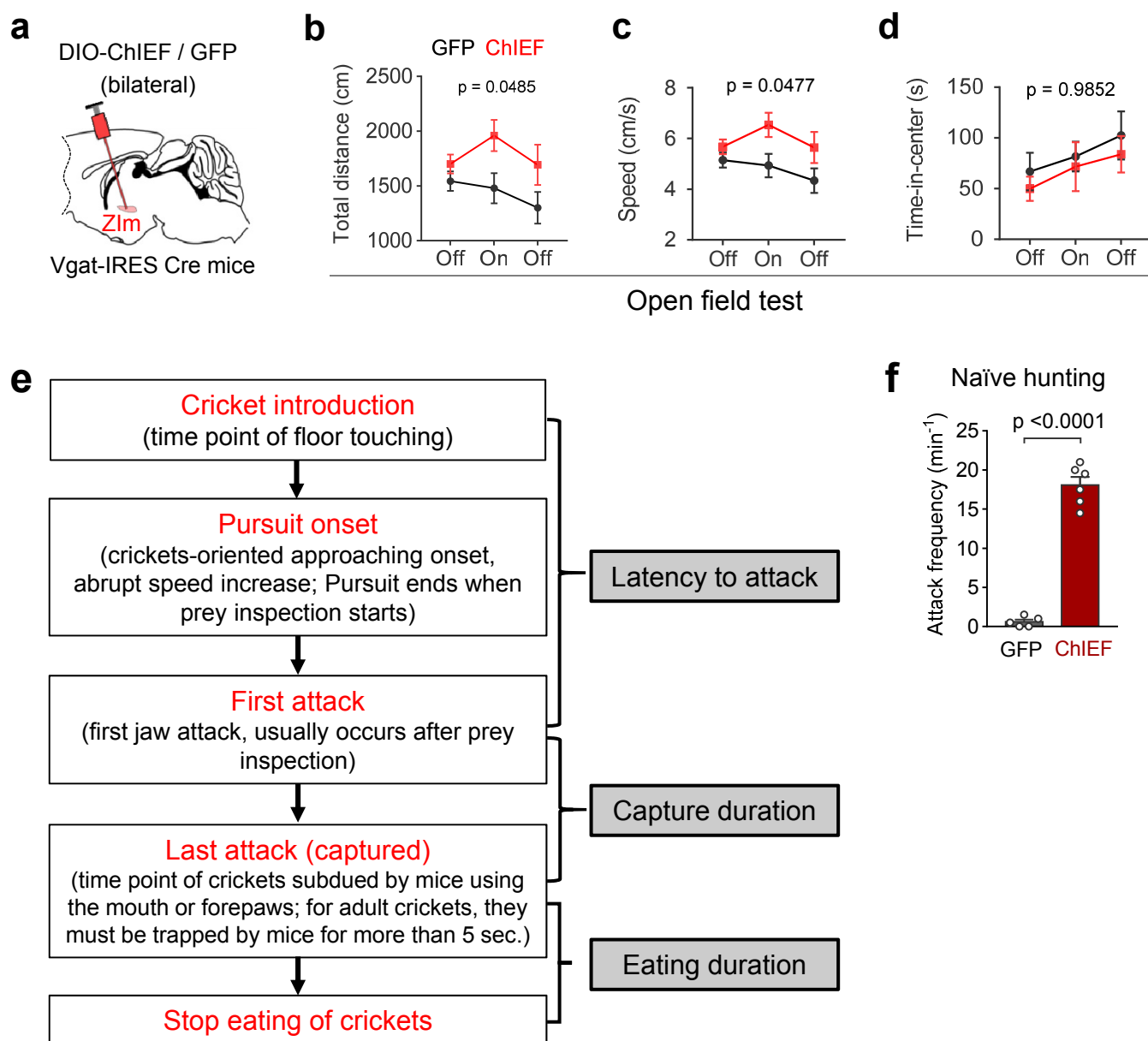
Supplementary Figure 1. Viral expression of DIO-ChIEF: tdTomato in ZI^{Vgat-IRES-Cre} mice.

a, ChIEF: tdTomato expressions at different bregma sites. The dotted yellow lines show the position of an optical insert in the anterior medial sector of ZI (ZIm), which is composed of the ZID and the ZIV. **b**, Light-induced cFos expression in the ZI (bregma = -1.8). Unilateral laser stimulation (left side) induced strong cFos expression and the contralateral side was used as the control. Laser pattern, 473 nm, 10 mW, 20 Hz, 10 ms, 2-min on, followed by 30-min off, 2-min on and 60 min off before sacrifice. **c**, The distribution of cFos⁺ cells in the ZI at different bregma sites. Scale bars are 500 μ m. n = 3 mice. All data are shown as mean $\pm$ s.e.m. The p-value was calculated based on two-way repeated-measures ANOVA with Bonferroni's multiple comparisons test. All p values are indicated in figures. B, bregma; VM, ventromedial thalamic nucleus; mt, mamillothalamic tract; ZI, zona incerta; VRe, ventral reuniens thalamic nu; PVN, paraventricular hypothalamic nu; vpl, ventral posterolateral thalamic; ic, internal capsule; ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; Sub, submedius thalamic nucleus; LH, lateral hypothalamic area; VPM, ventral posteromedial thalamic; ns, nigrostriatal bundle; PLH, posterior lateral hypothalamic; VPPC, ventral posterior nucleus of the thalamus, parvicellular part; SPF, subparafascicular thalamic nu; STh, subthalamic nucleus; PSTh, parasubthalamic nucleus; F, fornix; cp, cerebral peduncle; DMH, dorsomedial hypothalamic nucleus.



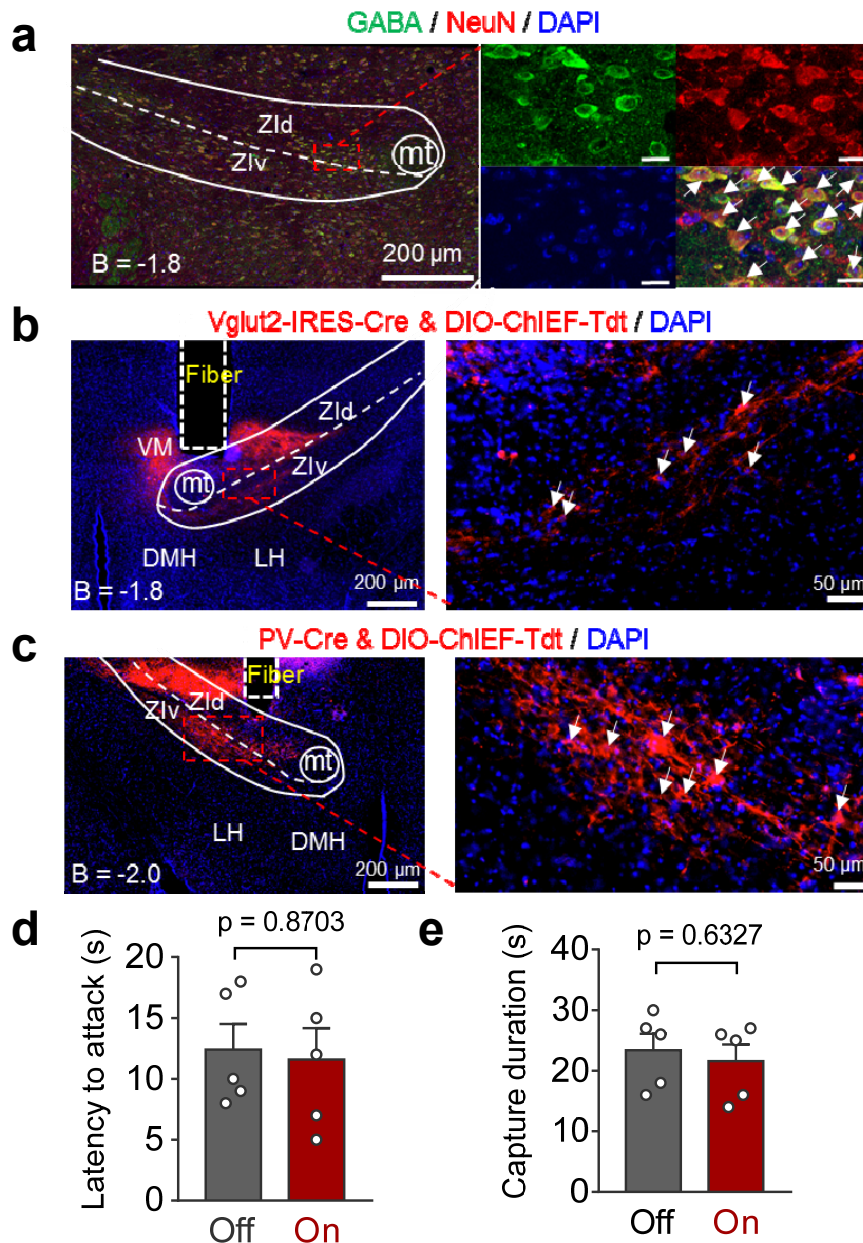
Supplementary Figure 2. Home-cage behaviors after photoactivation of Zlm^{Vgat} neurons.

a, Scheme of optogenetic activation and Cre-dependent viral expression of ChIEF in Zlm^{Vgat} neurons. Dotted yellow lines show the position of optical inserts. Scale bar is 500 μ m. ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; LH, lateral hypothalamic area; DMH, dorsomedial hypothalamic nucleus. **b**, Quantification of home-cage HFD intake during 4×10 min photostimulation. Laser pattern: 473 nm, 10-min on (10 ms, 20 Hz, 10 mW), 30-min off, 4 trials per mouse ($n = 4$ mice for GFP; $n = 5$ mice for ChIEF). **c**, The representative image of gnawed food after HFD intake tests. A big portion of the food was gnawed into pieces without swallowing in the photostimulation group. **d**, Behaviors elicited by photoactivation of Zlm^{Vgat} neurons (473 nm, 10 ms, 20 Hz, 10 mW, 2-min on). The locomotion heat maps (in blue-red) in the middle and the right represent the relative time mice spent. Definitions: Undirected gnawing, mice executed fictive eating or chewing non-eatable bedding materials or feces; Binge-like eating, mice rapidly bit and consumed food pellets; Biting objects, mice bit on the food tray or the water noddle; Others, other undefined behaviors including walking, digging, and grooming. **e-h**, Time proportion of mice spent on different behaviors defined in (**d**) under different laser stimulation (Laser off, 5 Hz, 10 Hz, 20 Hz, respectively; $n = 8$ mice). All data are shown as mean $\pm$ s.e.m. The p-value was calculated based on two-tailed unpaired t test and indicated in the figure.



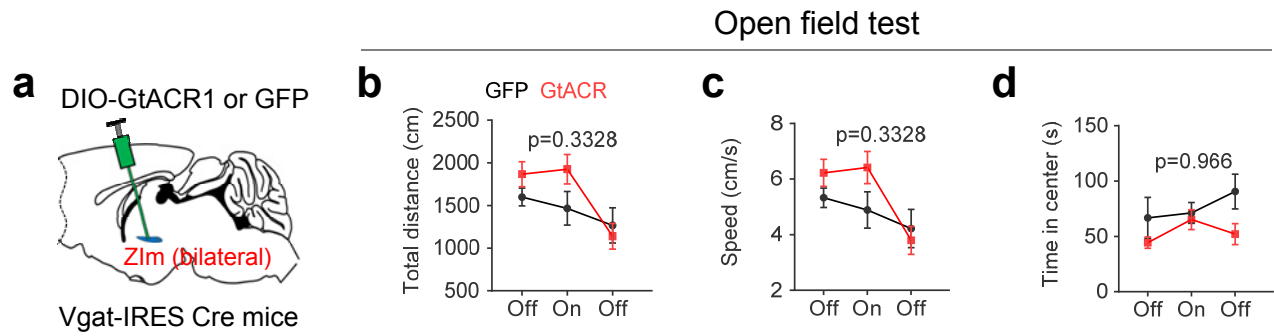
Supplementary Figure 3. Phenotypes of photoactivation of ZIm GABAergic neurons, related to Fig. 1.

a, Diagram of photoactivation of ZIm^{Vgat} neurons. **b-d**, Traveled distance (**b**), average speed (**c**) and time-in-center (**d**) during the open field tests before, during and after photoactivation of ZIm^{Vgat} neurons (5-min interval). No anxiogenic patterns were observed ($n = 7$ mice). **e**. Behavioral definitions of mice hunting behaviors. **f**, Bite-attack frequency for cricket-naïve mice ($n = 5$ mice for GFP; $n = 6$ mice for ChIEF). Cricket-naïve mice typically could not finish all hunting program (Supplemental Video 6) in a given short time (2-min). We only quantified bite-attack frequency in 2-min young cricket hunting task. Laser pattern: 473 nm, 10 ms, 20 Hz, 10 mW, 2-min on. All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on repeated-measures-two-way ANOVA with Bonferroni's multiple comparisons (**b-d**), and two-tailed unpaired t-tests (**f**). All p values are indicated in the figure.



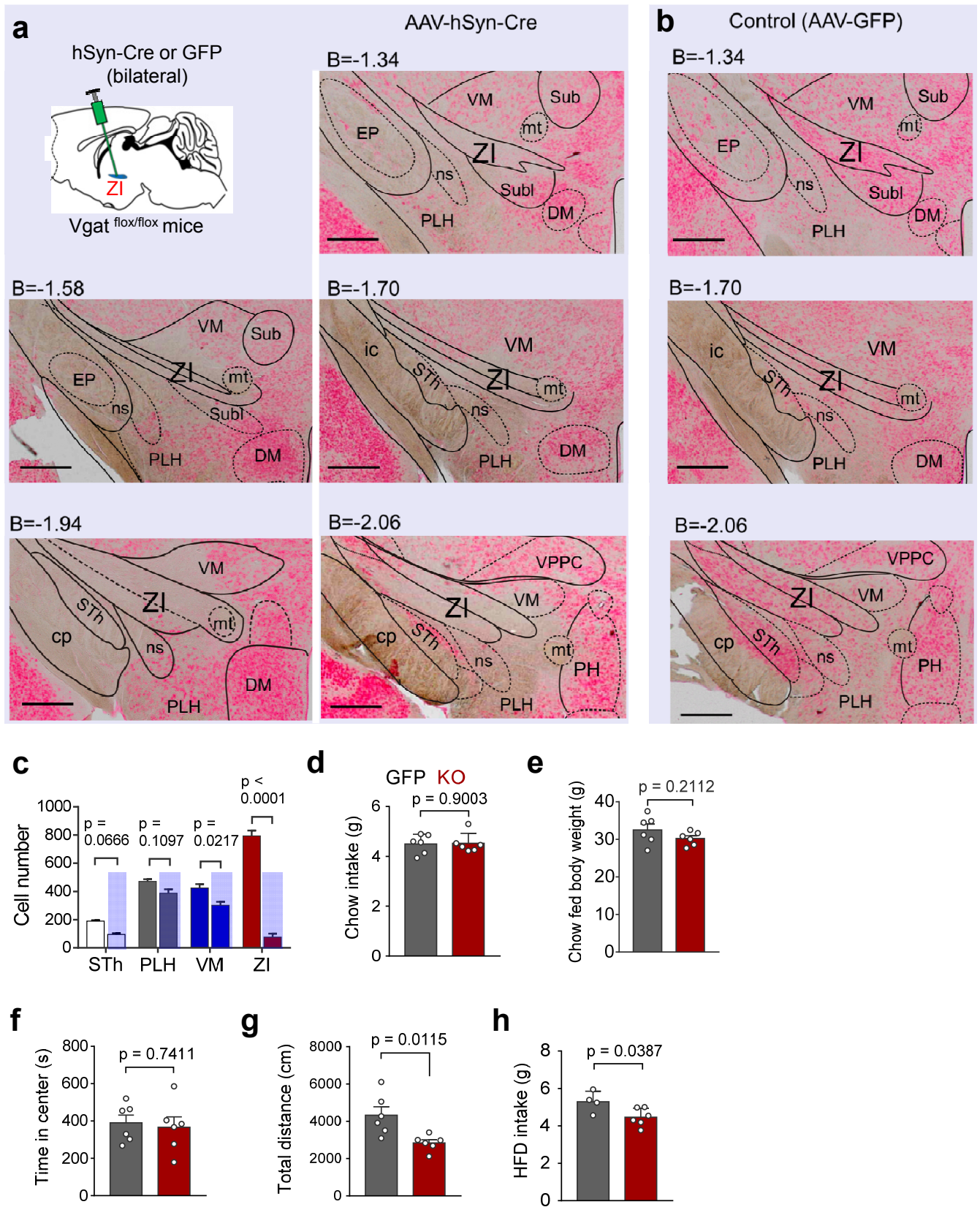
Supplementary Figure 4. Phenotypes of photoactivation of ZI Vglut2 and PV neurons.

a, The overlapping between GABA and NeuN (a pan neuron marker) in the ZI suggests the GABA neuron is the predominant cell type ($209 / 245 = 85\%$). Arrows indicate overlapped cells for GABA and NeuN. Scale bar in the right panel, $20\ \mu\text{m}$. Images from one mouse ($n = 2$ mice in total). **b**, The expression of DIO-ChIEF in the ZIm of Vglut2-IRES-Cre mice. The fiber path is marked in the figure. Photoactivation of these neurons (may also include neurons in the ventromedial thalamic nucleus) did not promote hunting (Supplementary Video 3). Arrows indicates the somas of ChIEF expressing neurons. Representative images are from one mice (4 mice in total). **c**, The expression of DIO-ChIEF in the ZIm of PV-Cre mice (PV stands for parvalbumin). The fiber path is marked in the figure. Arrows indicates the somas of ChIEF expressing neurons. Representative images are from one mice (5 mice in total). **d-e**, Changes in attack latency and capture duration revealed no hunting promotion effect after PV-Cre+ neural activation ($n = 5$ mice). Laser pattern: $473\ \text{nm}$, $10\ \text{ms}$, $20\ \text{Hz}$, $10\ \text{mW}$, 2-min on. All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on two-tailed unpaired t-tests. All p values are indicated in the figure. B, bregma; ZId, zona incerta, dorsal part; ZIv, zona incerta, ventral part; DMH, dorsomedial hypothalamic nucleus; LH, lateral hypothalamic area; VM, ventromedial thalamic nucleus.



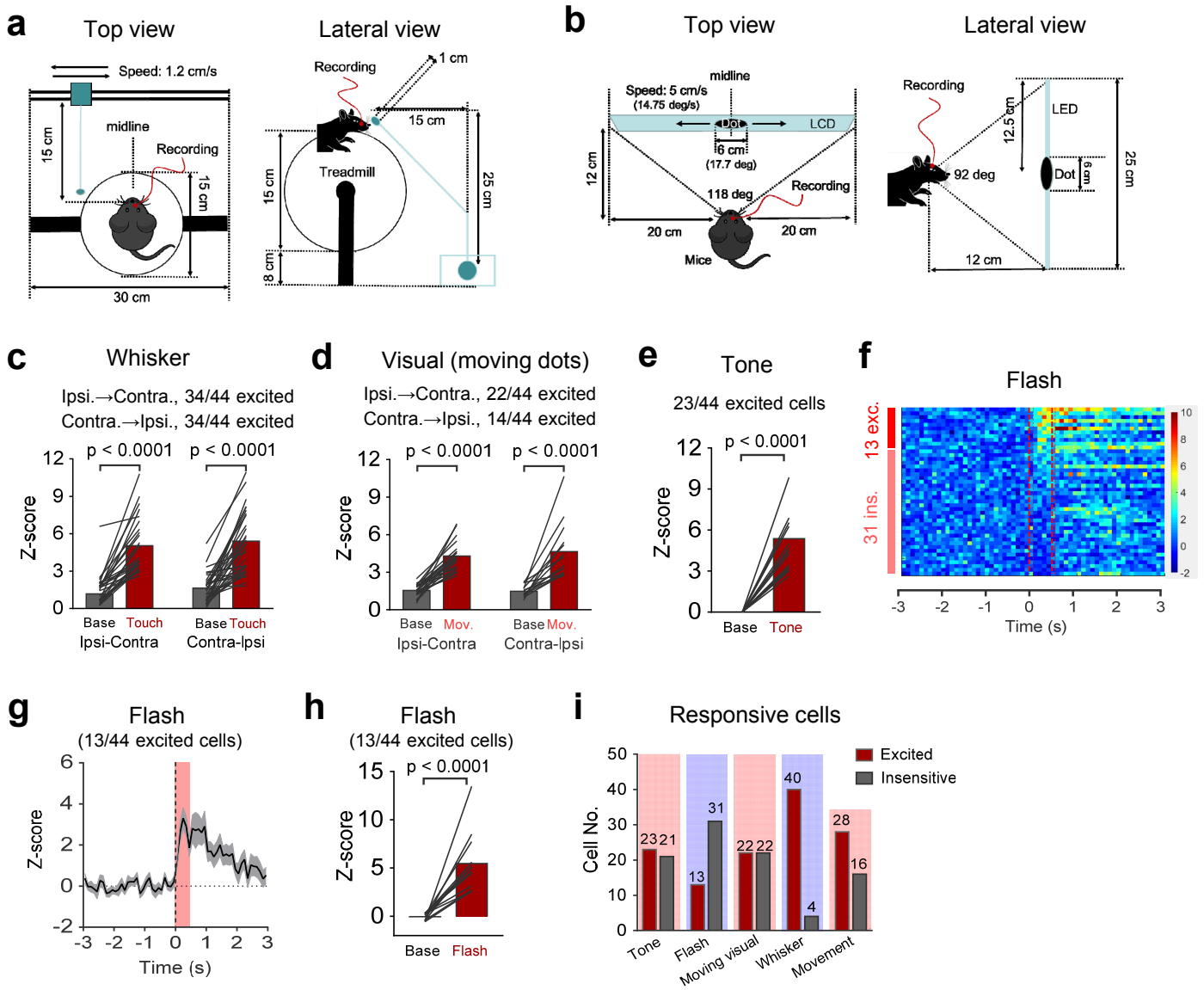
Supplementary Figure 5. Phenotypes of photoinhibition of ZIm GABAergic neurons, related to Fig. 2.

a, Diagram of photoinhibition of ZIm^{Vgat} neurons. **b-d**, Traveled distance (**b**), average speed (**c**), and time-in-center (**d**) during the open field tests before, during and after photoinhibition of ZIm^{Vgat} neurons (5-min interval). No anxiogenic patterns were observed ($n = 5$ mice for both groups). Laser pattern: 473 nm, 3 mW, with constant 15-s laser on, and 15-s off. All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on repeated-measures two-way ANOVA with Bonferroni's multiple comparisons. All p values are indicated in the figure.



Supplementary Figure 6. Phenotypes of knocking-out Vgat in the ZIm, related to Fig. 2.

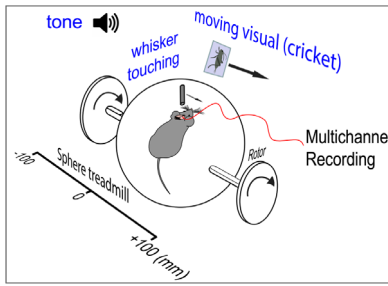
a, Diagram of ZIm Vgat conditional knocking-out and representative expression of Vgat mRNAs after Cre mediated knocking-out in different bregma sites. **b**, The representative expression of Vgat mRNAs in different bregma sites in control mice without Cre expression. **c**, Quantification of Vgat+ neurons in the ZI and nearby regions. Shaded area, Cre+ mice; n = 3 mice for both groups. **d**, No changes found in daily chow food intake after Vgat deletion in the ZI (2 months after surgery, n = 6 mice for both groups). **e**, No changes found in chow fed body weight after Vgat deletion in the ZI (4 months after surgery, n = 6 mice for both groups). **f**, Quantification of daily HFD intake of Vgat KO and GFP control mice (5 months after surgery, KO: n = 6 mice; GFP: n = 4 mice). **g-h**, Time-in-center and traveled distance during the open field tests (15-min interval) after Vgat deletion in the ZI (KO: n = 6 mice; GFP: n = 6 mice). All data are shown as mean $\pm$ s.e.m. For (**c**), the p-values were calculated based on two-way repeated-measures ANOVA with Bonferroni's multiple comparisons. For the rest, the p-values were calculated based on two-tailed unpaired t test. All p values are indicated in the figure. B, Bregma; VM, ventromedial thalamic nucleus; mt, mammillothalamic tract; ZI, zona incerta; EP, entopeduncular nucleus; vpl, ventral posterolateral thalamic nucleus; ic, internal capsule; ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; Sub, submedius thalamic nucleus; Subl, subincertal nucleus; VPM, ventral posteromedial thalamic nucleus; ns, nigrostriatal bundle; PLH, peduncular part lateral hypothalamic area; VPPC, ventral posterior nucleus of the thalamus, parvicellular part; SPF, subparafascicular thalamic nu; PSTh, parasubthalamus; cp, cerebral peduncle; DM, dorsomedial hypothalamic nucleus; PH, posterior hypothalamic nucleus.



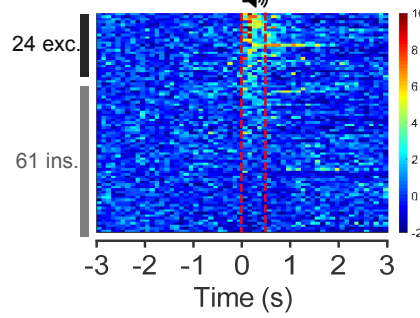
Supplementary Figure 7. Head-fixed multichannel recoding of ZIm neural activity, related to Fig. 3.

a, Design for delivery of the whisker-contact stimulus by moving a small object. **b**, Design for delivery of the visual stimulus by moving a spot cross the LED screen. **c**, Z-score analyses of whisker-contact excited cells. The maximum z-scores (0.5 bin) during stimulation (0 ~ 10-s) are used to compare with the baseline z-scores (mean value of -5-s ~ 0). Contra., contralateral; Ipsi., ipsilateral, relative to recorded ZI side. **d**, Z-score analyses of moving visual (moving spot) excited cells. The maximum z-scores during stimulation (0 ~ 10-s) are used to compare with the baseline z-scores (mean value of -5-s ~ 0). Contra., contralateral; Ipsi., ipsilateral. **e**, Z-score analyses of tone excited cells. The maximum z-scores during stimulation (0 ~ 0.5-s) are used to compare with the baseline z-scores (mean value of -3-s ~ 0). Tone: 5 kHz, 70 db, 0.5 s. **f**, Heat map of flash light responses. Flash light: 0.5 s duration, 5 lumen. **g**, Z-score traces of flash excited cells. **h**, Z-score analyses of flash excited cells. The maximum z-scores during stimulation (0 ~ 0.5-s) are used to compare with the baseline z-scores (mean value of -3-s ~ 0). **i**. Summary of cell responses to indicated stimuli. Numbers are cells. All data are shown as mean $\pm$ s.e.m. except (i). The p-values were calculated based on ordinary two-way ANOVA tests with Tukey's multiple comparisons (c, d) and two-sided paired t-tests (e, h). All p values are indicated in the figure.

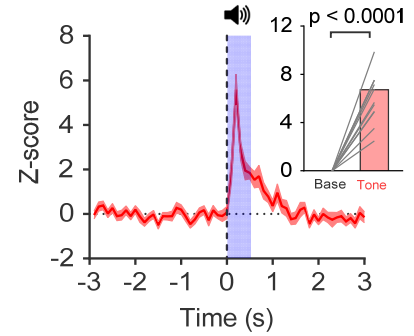
a Head-fixed recording



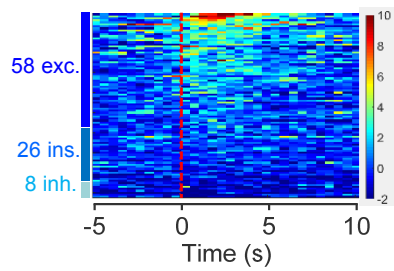
b Tone



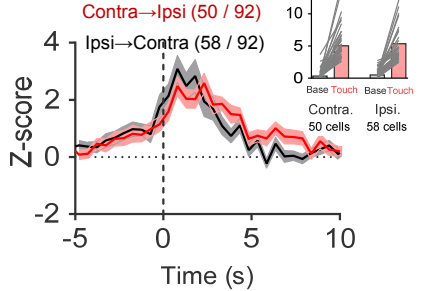
c Tone, 24 exc. cells



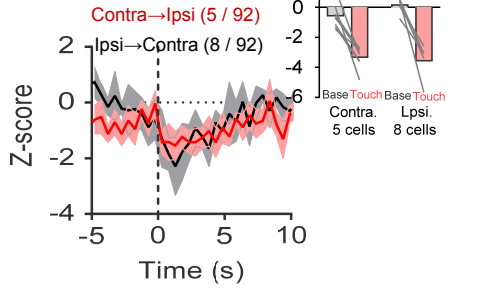
d Whisker-contact (Lpsi.→Contra.)



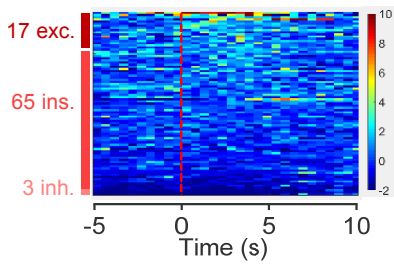
e Whisker-contact excited cells



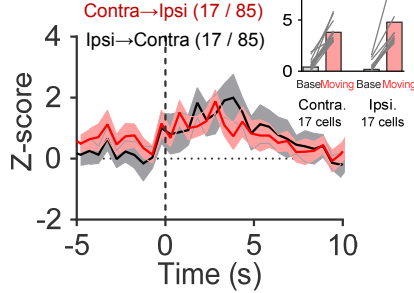
f Whisker-contact inhibited cells



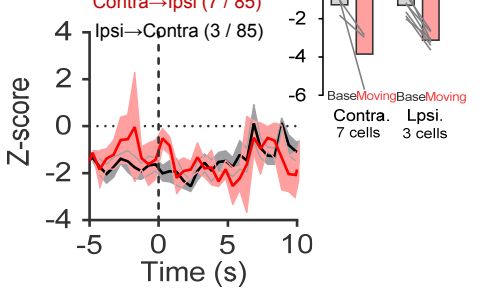
g Visual (moving cricket) (Lpsi.→Contra.)



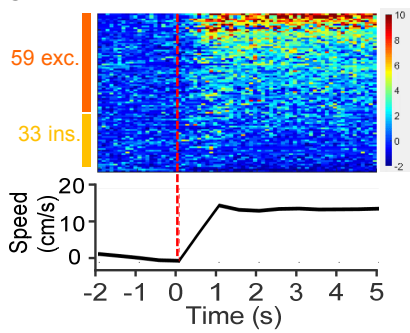
h Cricket Visual excited cells



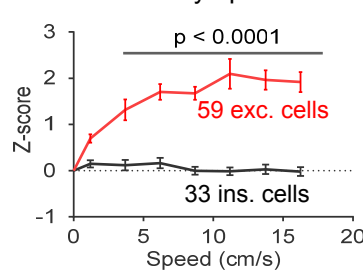
i Cricket Visual inhibited cells



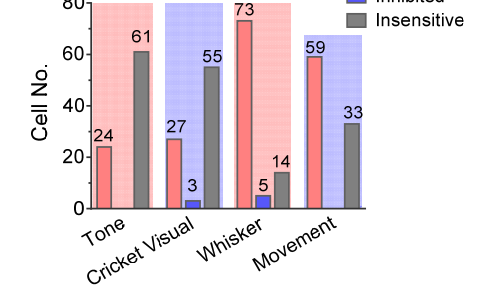
j Movement



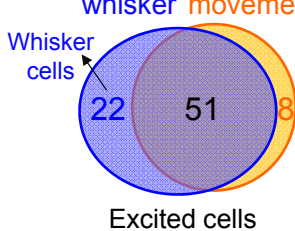
k Activity-speed



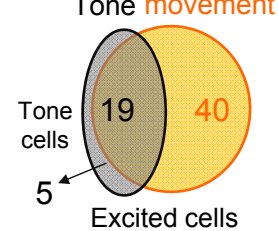
l



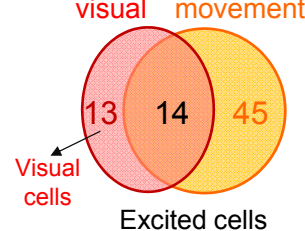
m whisker movement



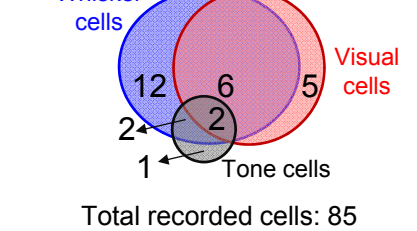
n Tone movement



o visual movement

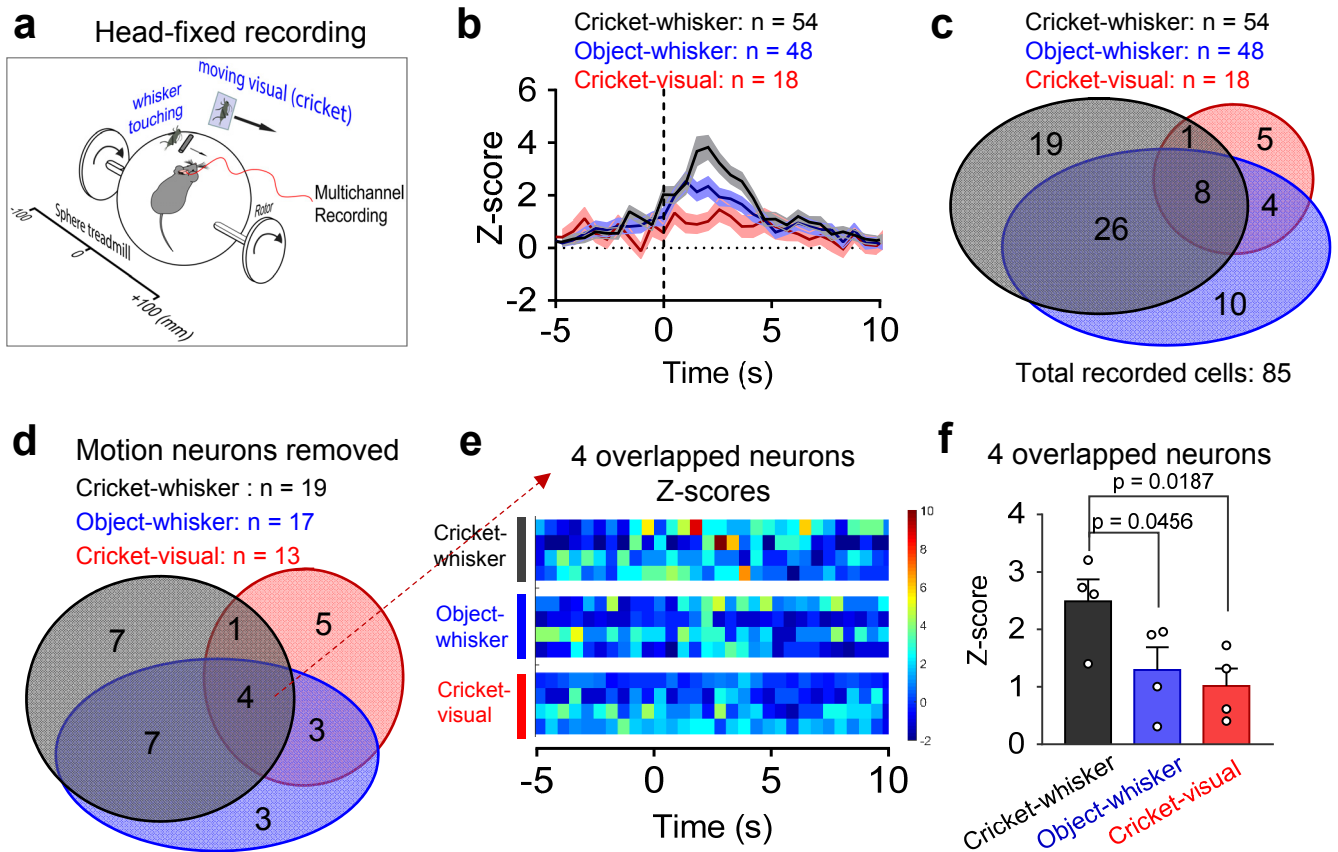


p Whisker cells



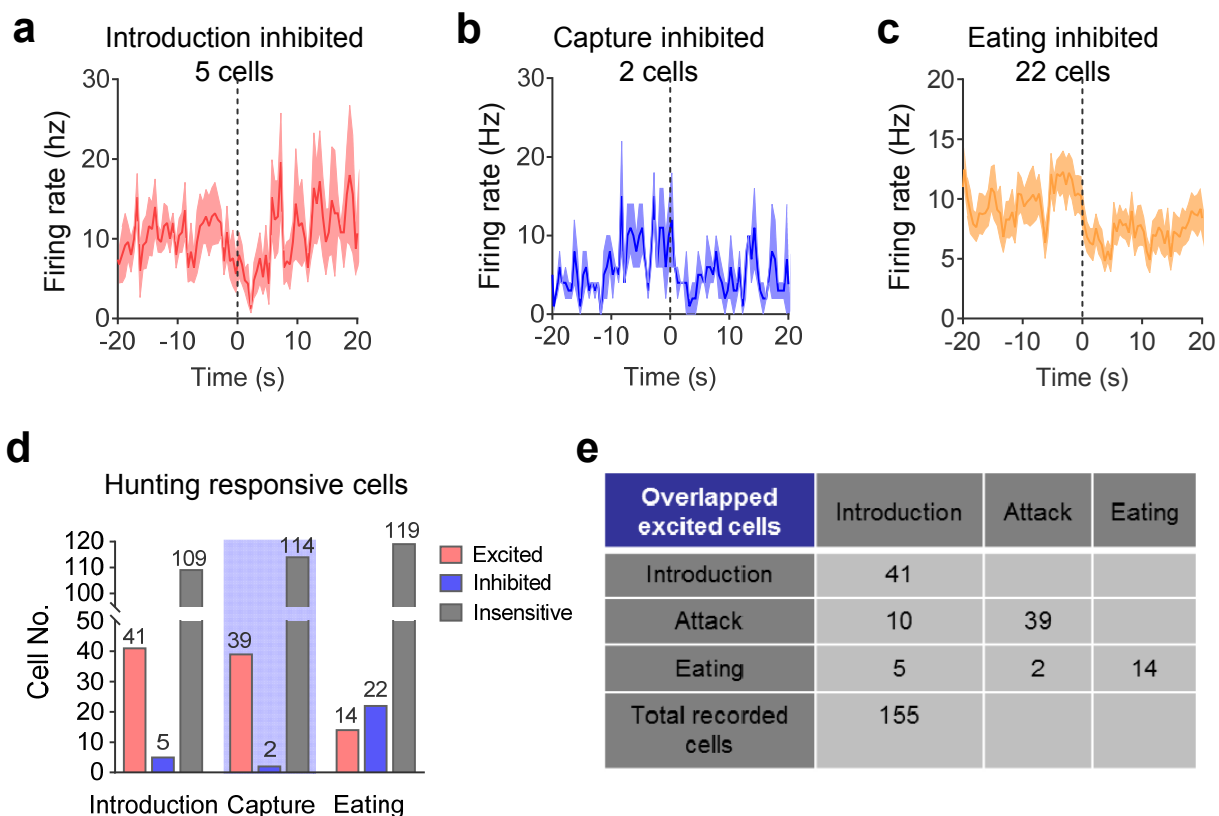
Supplementary Figure 8. ZIm neural activity in response to moving visual of live crickets, related to Fig. 3.

a, Design of head-fixed set-up to record sensory responses. Visual stimuli is delivered directly by moving a live cricket on a motorized track, 10 cm away from the nose tip. The mice and crickets were separated by a transparent glass. **b**, Heat map of tone responses. Tone: 5 kHz, 70 db, 0.5 s. **c**, Normalized firing rate (Z-score) of tone excited cells. The comparison between the maximum z-scores during stimulation (0 ~ 0.5-s) and the baseline z-scores (mean value of -3-s ~ 0) is shown in the small window. **d**, Heat map of object-contact induced whisker responses by a small object moving from ipsilateral (ipsi.) to contralateral (contra.), directions are relative to recorded ZI side. exc, excited; ins, insensitive; inh, inhibited. **e**, Z-score of whisker excited cells by object moving in two directions. The comparison between the maximum z-scores (0.5 s bin) during stimulation (0 ~ 10-s) and the baseline z-scores (mean value of -5-s ~ 0) is shown in the small window. **f**, Z-score of whisker inhibited cells by objects moving in two directions. The comparison between the minimum z-scores during stimulation (0 ~ 10-s) and the baseline z-scores (mean value of -5-s ~ 0) are shown in the small window. **g**, Heat map of moving visual responses induced by moving live cricket from ipsilateral (ipsi.) to contralateral (contra.). exc, excited; ins, insensitive; inh, inhibited. **h**, Z-score of cricket visual excited cells by moving in two directions. The comparison between the maximum z-scores during stimulation (0 ~ 10-s) and the baseline z-scores (mean value of -5-s ~ 0) is shown in the small window. **i**, Z-score of cricket visual inhibited cells by moving in two directions. The comparison between the minimum z-scores during stimulation (0 ~ 10-s) and the baseline z-scores (mean value of -5-s ~ 0) is shown in the small window. **j**, Heat map of movement induced responses recorded during forced treadmill movement. The treadmill speed is plotted in the bottom. exc, excited; ins, insensitive; inh, inhibited. **k**, Activity-speed correlation revealed the existence of movement sensitive neurons. **l**, Summary of cell responses to indicated stimuli. Numbers are cells. **m-o**, Removing of movement-excited cells to obtain whisker cells (**m**), tone cells (**n**) and (cricket) visual cells (**o**) respectively. **p**, The overlapping between whisker cells, (cricket) visual cells and tone cells. The numbers are cells. All data are shown as mean $\pm$ s.e.m. except (**l**). The p-values were calculated based on two-side paired t-tests (**c**), two-way ANOVA tests with Tukey's multiple comparisons (**e, f, h, i, k**). All p values are indicated in the figure.



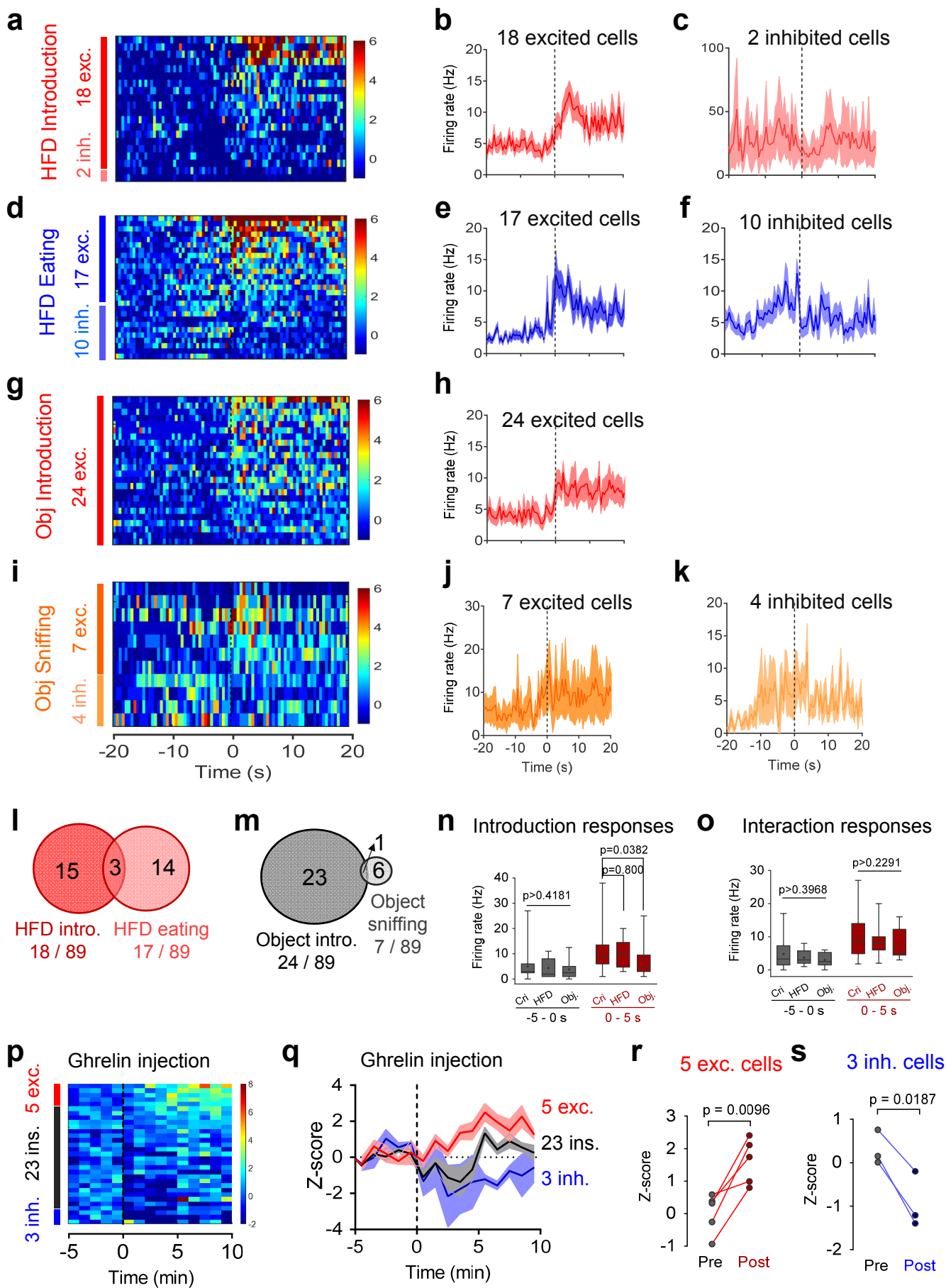
Supplementary Figure 9. ZIm neural activity in response to cricket-whisker touch, related to Fig. 3.

a, Design of head-fixed set-up to record sensory responses. Visual stimuli is delivered directly by moving a live cricket on a motorized track (cricket visual), 10 cm away from the nose tip. The mice and crickets were separated by a transparent glass. Object-whisker and cricket-whisker stimuli were delivered by moving a live cricket or a small object on a motorized track. The familiarized objects and crickets will touch the mouse whisker but not the mouse skin when in close proximity. **b**, Z-scores over time of excited cells by cricket-whisker, object-whisker and cricket-visual. **c**, The overlapping between excited cells by cricket-whisker, object-whisker and cricket-visual stimuli. The numbers are cells. **d**, The overlapping between excited cells by cricket-whisker, object-whisker and cricket-visual stimuli after removing movement sensitive cells. The numbers are cells. **e**, Heat map of the four overlapping cells from (**d**) in response to single stimulus. **f**, Mean Z-scores (0 - 3 s) of the four overlapping cells from (**d**) in response to single stimulus. All data are shown as mean $\pm$ s.e.m.. The p-values were calculated based on one-way ANOVA tests (Uncorrected Fisher's LSD test) (**f**). All p values are indicated in the figure.



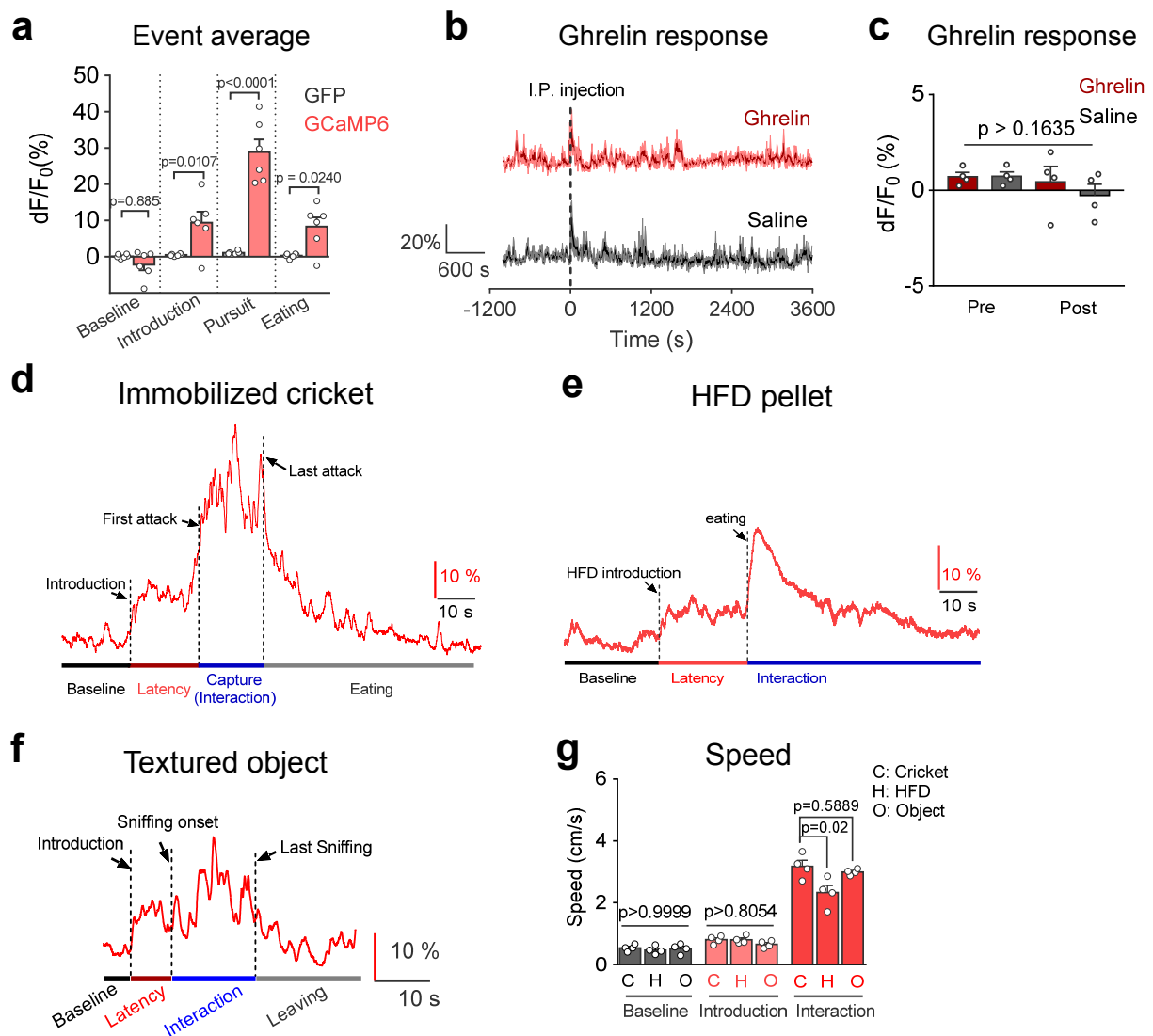
Supplementary Figure 10. Multichannel recoding of Zlm neural activity during free hunting, related to Fig. 3.

a, Firing rates of cricket introduction inhibited cells. **b**, Firing rates of cricket capture inhibited cells. **c**, Firing rates of cricket eating inhibited cells. **d**, Summary of cell responses during different hunting events. Numbers are cells. **e**, Summary of the overlapping between excited cells during different hunting events. Numbers are cells. All data are shown as mean $\pm$ s.e.m except (**d**, **e**).



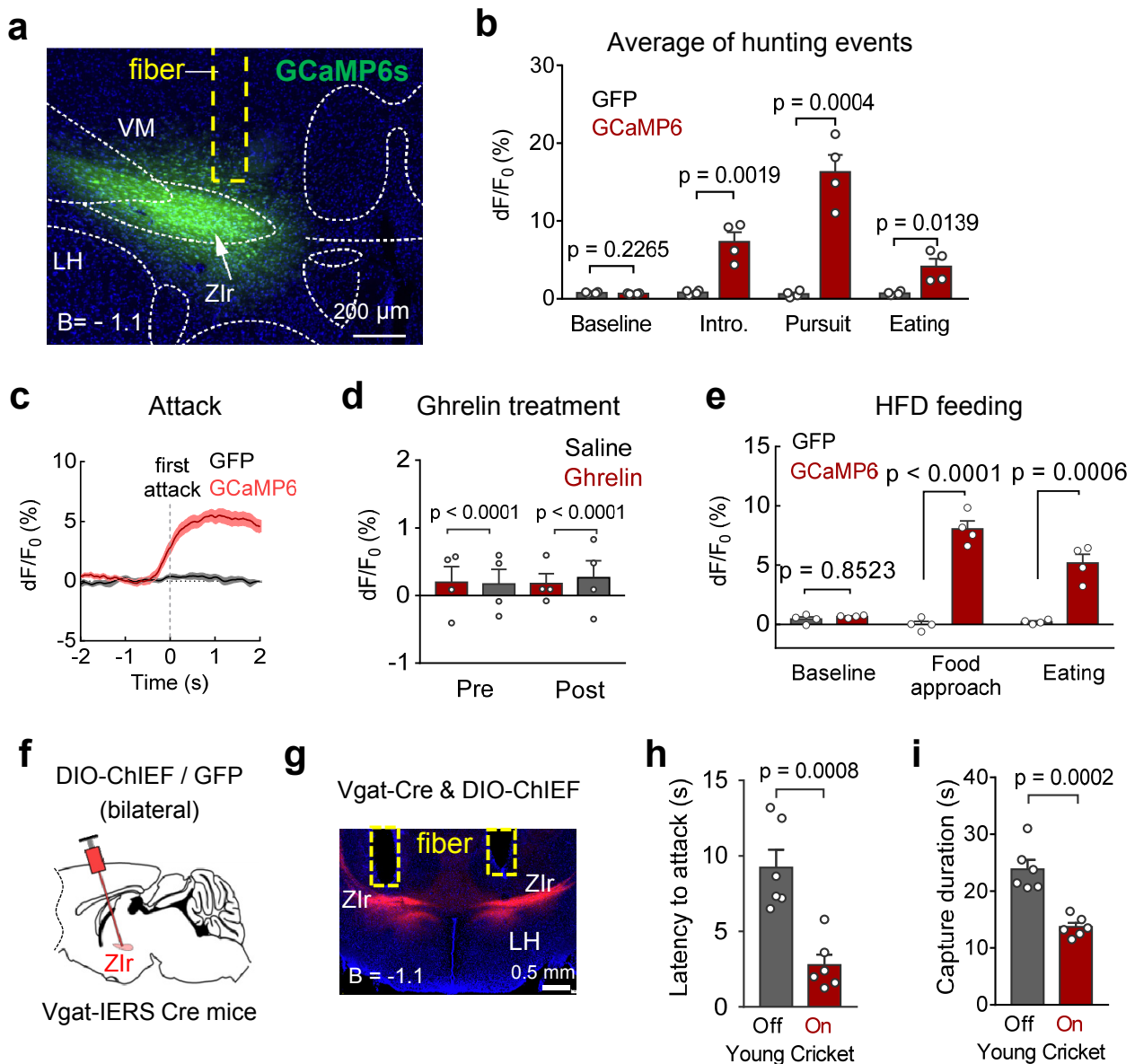
Supplementary Figure 11. Multichannel recoding of Zlm neural activity during interaction with different targets, related to Fig. 3.

a, Heat map of HFD introduction induced responses, aligned to the time point of food floor touching. exc, excited cells; inh, inhibited cells. **b**, Firing rates of HFD introduction excited cells. **c**, Firing rates of HFD introduction inhibited cells. **d**, Heat map of HFD eating induced responses, aligned to the time point of eating onset. **e**, Firing rates of HFD eating excited cells. **f**, Firing rates of HFD eating inhibited cells. **g**, Heat map of small textured object (obj) introduction induced responses, aligned to the time point of object floor touching. **h**, Firing rates of object introduction excited cells. **i**, Heat map of object sniffing induced responses, aligned to the time point of first sniffing of objects. **j**, Firing rates of object sniffing excited cells. **k**, Firing rates of object sniffing inhibited cells. **l**, The overlapping between HFD introduction and eating excited cells. **m**, The overlapping between object introduction and sniffing excited cells. **n**, Mean firing rates of introduction excited cells by crickets (41 trials), HFD (18 trials) and objects (24 trials). **o**, Mean firing rates of interaction excited cells by crickets (39 trials), HFD (17 trials) and objects (7 trials). **p**, Heat map of ghrelin injection induced responses, aligned to the time point of injection (i.p). exc, excited; ins, insensitive; inh, inhibited. **q**, Z-scores over time for ghrelin injection induced responses. **r-s**, Quantification of z-scores before and after ghrelin injection (between 5-10 min) for excited (**r**) and inhibited (**s**) cells. All data are shown as mean $\pm$ s.e.m except in (**n** and **o**); (**n** and **o**) are shown as box and whisker plots (medians, quartiles (boxes), means (+) and ranges min to max(whiskers)). The p-values were calculated based on two-tailed paired t-tests (**r**, **s**), two-way ANOVA tests (Uncorrected Fisher's LSD test) (**n**, **o**). All p values are indicated in the figure.



Supplementary Figure 12. Neural activity changes by fiber photometry, related to Fig. 4.

a, Quantification of the average calcium signals during different hunting stages in young cricket hunting tasks in Zlm^{Vgat} neurons ($n = 6$ mice, 3 trials averaged per mouse). **b-c**, Calcium traces (**b**) and average changes (**c**) after ghrelin ($60 \mu\text{g} / \text{mouse}$, total volume $200 \mu\text{l}$) or saline injection, aligned to the time of injection ($n = 4$ mice). dF/F_0 represents change in GCaMP6 fluorescence from the mean level of before i.p. injection (-600 to -1 s). **d-f**, Representative traces of neural activity during interaction with immobilized cricket(**d**), HFD pellet (**e**), and small textured object (**f**) respectively. dF/F_0 represents a change in GCaMP6 fluorescence from the mean level of before introduction (-10 to -1 s). **g**, Average speed during introduction and interaction with immobilized crickets, HFD pellets and textured objects ($n = 4$ mice, cricket: 24 trials, HFD: 20 trials, object: 16 trials). All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on two-way repeated-measures ANOVA with Bonferroni's multiple comparisons (**a**, **c**, **g**). All p values are indicated in the figure.



Supplementary Figure 13. Zlr GABAergic neurons promote hunting.

a, The expression of GCaMP6 in Zlr^{Vgat} neurons. **b**, The averaged calcium changes during different hunting stages (GCaMP6, $n = 4$ mice; GFP, $n = 4$ mice; 4 trials averaged per mouse). Baseline: average Ca^{2+} signal before hunting (-10 to -1 s). dF/F_0 represents change in GCaMP6 fluorescence from the mean level of the baseline stage before hunting. **c**, Calcium transients induced by jaw-attack, aligned to the jaw opening ($n = 30$ trials from 4 mice). dF/F_0 represents change in GCaMP6 fluorescence from the mean level of before attacking (-2 to -1 s). **d**, The averaged calcium changes after ghrelin (60 μ g / mouse) or saline injection (post injection 20 min), aligned to the time of injection ($n = 4$ mice). dF/F_0 represents change in GCaMP6 fluorescence from the mean level of before i.p. injection (-600 to -1 s). **e**, The averaged calcium changes during HFD feeding, aligned to food delivery ($n = 4$ mice). dF/F_0 represents change in GCaMP6 fluorescence from the mean level of before eating (-100 to -1 s). Food approach is duration between food delivery and the onset of eating. **f**, Diagram for optogenetic activation of Zlr^{Vgat} neurons. **g**, The expression of ChIEF in Zlr^{Vgat} neurons. **h-i**, Shortened attacking latency and capture duration induced by optogenetic activation of Zlr^{Vgat} neurons ($n = 6$ mice). Laser pattern: 473 nm, 10 ms, 20 Hz, 10 mW, 2-min on. All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on two-way repeated-measures ANOVA with Bonferroni's multiple comparisons (**b**, **d**, **e**) and two-tailed paired t tests (**h**, **i**). All p values are indicated in the figure. B, bregma; VM, ventromedial thalamic nucleus; Zlr, zona incerta, rostral part; LH, lateral hypothalamic area.

a

ZI GABA neurons function	Activation		LOF		Ghrelin response
	Hunting	Eating	Cell-killing	Vgat KO	
Zlr[B=-1.1]	Wood gnawing ¹ and + ²	++ ¹	Weight loss ¹		+ (in vitro) ¹ - (in vivo) ²
Zlr[B=-1.1]→PVT	NA	++ ¹			
Zlm[B=-1.8]	++ ²	+ ²		1. No weight loss in chow feeding ² ; 2. Weight loss in HFD feeding ² ; 3. Hunting impaired ² .	- (in vivo) ²
Zlm[B=-1.8]→PAG	++ ²	- ²			

References:

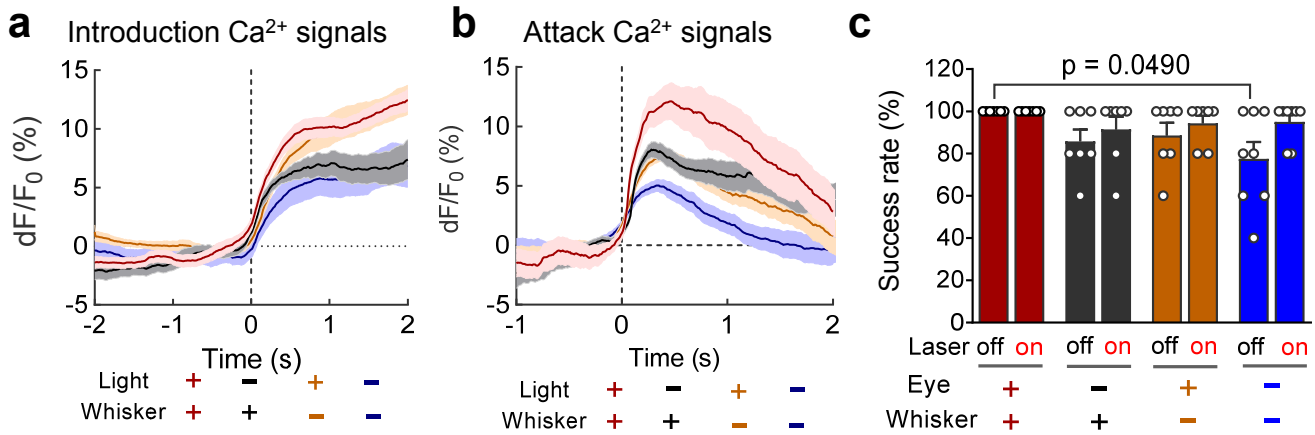
1, Zhang, X. & van den Pol, A. N. Science 356, 853-859 (2017).

2, This manuscript

Supplementary Figure 14. Summary of hunting and feeding phenotypes of the rostromedial ZI.

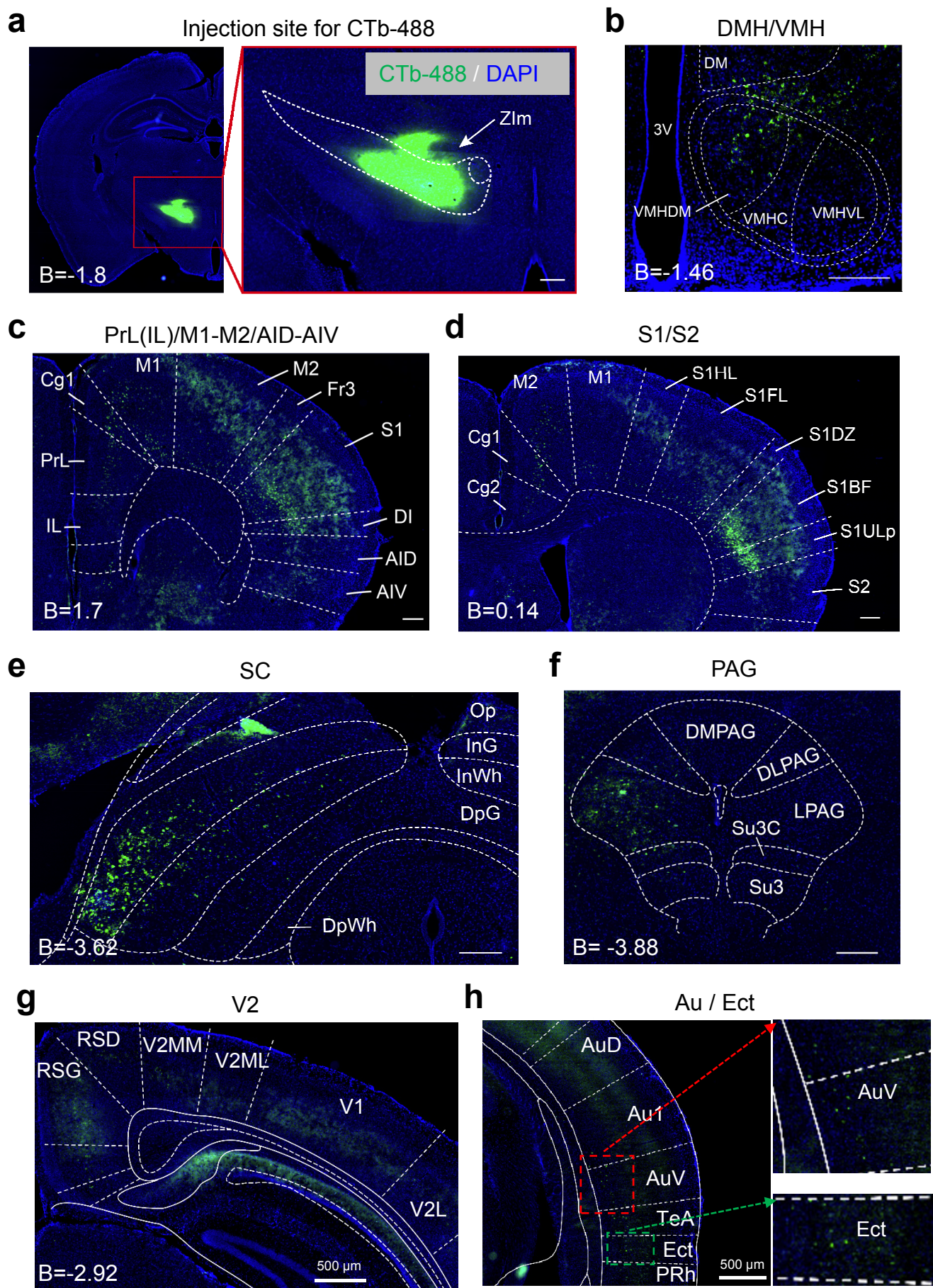
+, ++, positive for indicted responses; -, negative for indicted responses.

B, bregma; Zlr, Zlr, zona incerta, rostral part; Zlm, Zlr, zona incerta, medial part; PAG, periaqueductal gray matter; PVT, paraventricular thalamic nu.



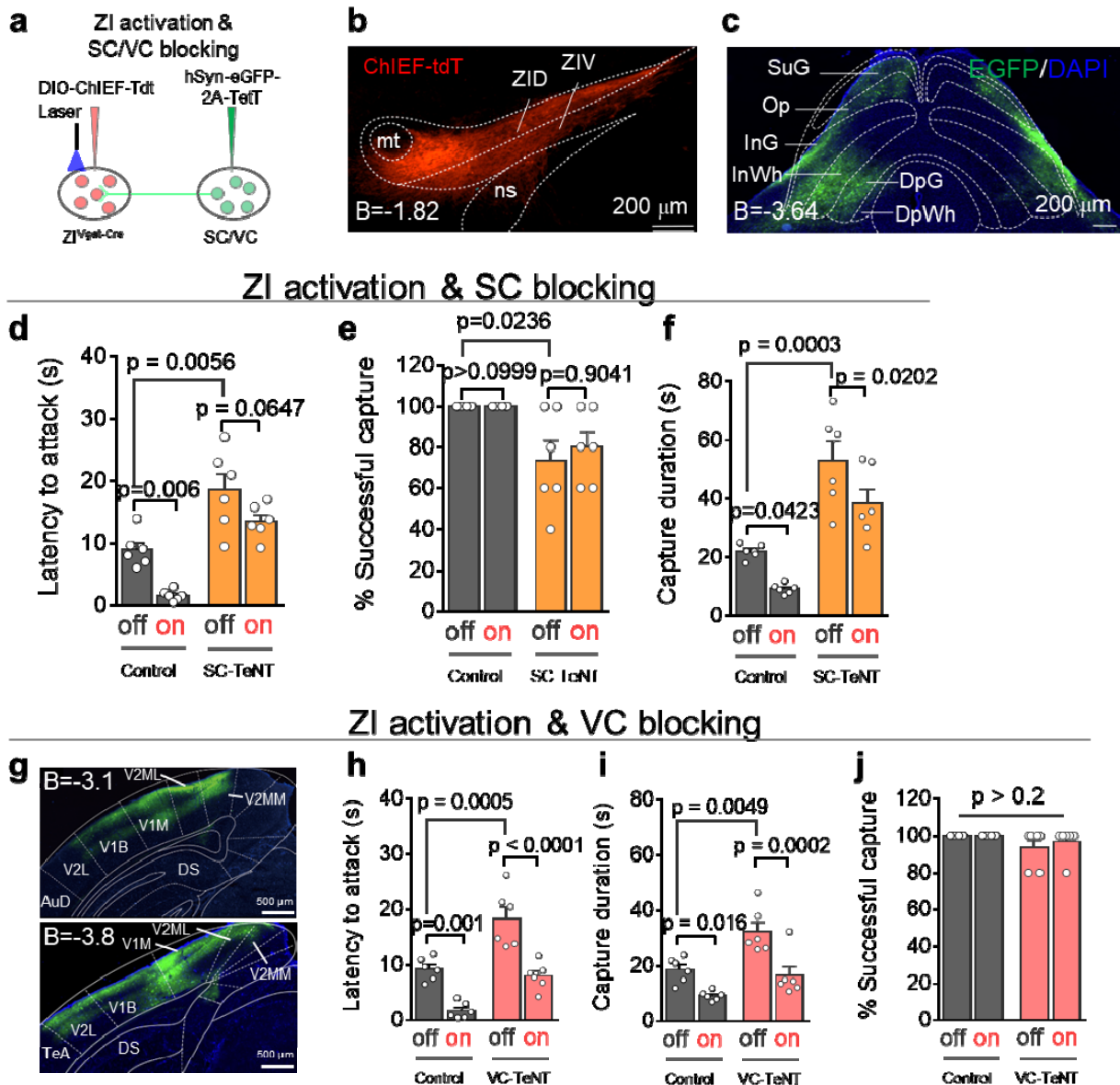
Supplementary Figure 15. Changes of neural activity and behavioral parameters after sensory deprivations, related to Fig. 4.

a, Calcium signal of in Zlm^{Vgat} neurons induced by young crickets, aligned to time point of introducing (floor-touching) ($n = 4$ mice; trial numbers from left to right are 18, 17, 18 and 19 respectively). Light (-): darkness; whisker (-): whisker trimmed. dF/F_0 represents a change in GCaMP6 fluorescence from the mean level of before introduction (-2 to -1 s). **b**, Ca^{2+} transients and average changes induced by first jaw-attack, aligned to the onset of jaw opening ($n = 4$ mice; trial numbers from left to right are 16, 25, 24 and 14 respectively). Light (-): darkness; whisker (-): whisker trimmed. dF/F_0 represents a change in GCaMP6 fluorescence from the mean level of before attacking (-1 to -0.5 s). **c**, Changes in hunting success rate after different sensory deprivations in Zlm^{Vgat} & ChIEF mice. Laser pattern: 473 nm, 10 mW, 20 Hz, 10 ms, 2-min on; eye (-): eyelid sutured; whisker (-): whisker trimmed. Mice numbers from left to right are 7, 7, 7 and 8 respectively. All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on two-way repeated-measures ANOVA with Bonferroni's multiple comparisons. The p value of <0.05 is indicated in **c**.



Supplementary Figure 16. Input areas to the ZIm.

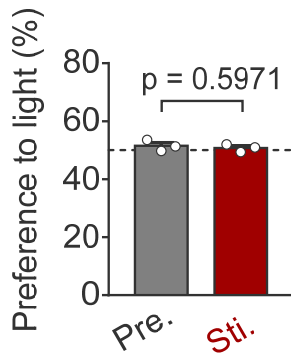
a, The injection of retrograde tracer CTb in the ZIm (n = 3 mice). **b-h**, Input areas labeled by Ctb tracing, which includes the DMH/VMH, the Cg1, the M1/M2, the S1/S2, the SC, the secondary auditory cortex, the ectorhinal cortex (Ect) and the PAG. Scale bars are 200 μ m. Representative images from one mice (n = 3 mice in total). B, bregma; ZIm, zona incerta, medial part; DM, dorsal hypothalamic nucleus; VMH, ventmedial hypothalamic nucleus; VMHDM, ventmedial hypothalamic nucleus, dorsomedial part; VMHVL, ventmedial hypothalamic nucleus, ventrolateral part; VMHC, ventmedial hypothalamic nucleus, central part; 3V, third ventricle; Cg1, cingulate cortex, area 1; Cg2, cingulate cortex, area 2; M1, primary motor cortex; M2, secondary motor cortex; Fr3, frontal cortex, area3; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; DI, dysgranular insular cortex; AID, agranular insular cortex, dorsal part; AIV, agranular insular cortex, ventral part; PrL, prelimbic cortex; IL, infralimbic cortex; S1HL, primary somatosensory cortex, hindlimb region; S1FL, primary somatosensory cortex, forelimb region; S1DZ, primary somatosensory cortex, dysgranular zone; S1BF, primary somatosensory cortex, barrel; S1ULp, primary somatosensory cortex, upper lip; SC, superior colliculus; Op, optic nerve layer superior colliculus; InG, intermediate gray layer superior colliculus; InWh, intermediate white layer superior colliculus; DpG, deep gray superior colliculus; DpWh, deep white superior colliculus; PAG, periaqueductal gray; DMPAG, dorsomedial periaqueductal gray; DLPAG, dorsolateral periaqueductal gray; LPAG, lateral periaqueductal gray; Su3C, supraoculomotor cap; Su3, supraoculomotor periaqueductal gray. AuD, secondary auditory cortex, dorsal part; Au1, primary auditory cortex; AuV, secondary auditory cortex, ventral part; TeA, temporal association cortex; Ect, ectorhinal cortex; PRh, perirhinal cortex; RSG, retrosplenial granular cortex; RSD, retrosplenial dysgranular cortex; V2L, secondary visual cortex, lateral area; V1, primary visual cortex; V2ML, secondary visual cortex, mediolateral part; V2MM, secondary visual cortex, mediomedial part; DS, dorsal subiculum; Or, oriens layer hippocampus; GrO, granule cell layer of the olfactory bulb; GrA, granule cell layer of the accessory olfactory bulb; E/OV, ependymal and subependymal layer/olfactory ventricle; Io, lateral olfactory tract.



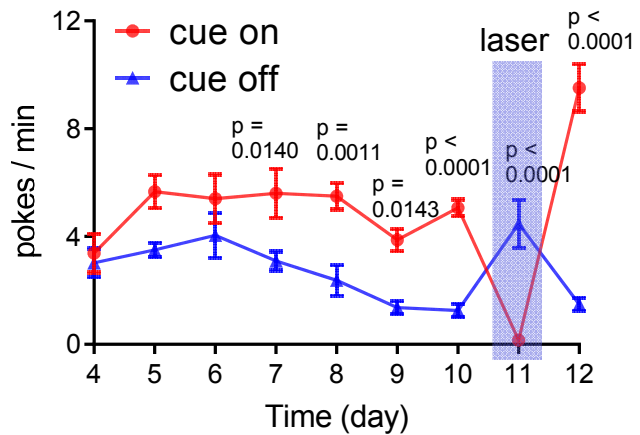
Supplementary Figure 17. Function of the SC and the VC in predatory hunting.

a, The diagram to block SC and VC neurons while optogenetic activation of the ZI^{Vgat} neurons. AAV-hSyn-eGFP-2A-TeNT was injected in the SC and VC and the AAV-DIO-ChIEF-Tdt was injected in the ZI. **b**, The expression of ChIEF-Tdt in the ZI^{Vgat} neurons. **c**, The expression of TeNT in the SC neurons ($n = 6$ mice). **d-f**, Hunting defects after blocking the SC neurons. Optogenetic activation of the ZI^{Vgat} neurons partially restored hunting efficiency by shortening the capture duration, yet did not significantly change the attacking latency (**d**) and hunting success rate (**f**). Laser pattern: 473 nm, 10 ms, 20 Hz, 10 mW, 2-min on. ($n = 6$ mice for control group; $n = 6$ mice for SC-TeNT group). **g**, The expression of TeNT in the VC neurons. **h-i**, Hunting defects after blocking the VC neurons. Optogenetic activation of the ZI^{Vgat} neurons largely restored hunting efficiency by shortening the attack latency (**h**) and capture duration (**i**). **j**, VC blocking did not affect the hunting success rate. ($n = 6$ mice for control group; $n = 6$ mice for VC-TeNT group). All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on ordinary two-way ANOVA with Bonferroni's multiple comparisons. All p values are indicated in the figure. B, bregma; ZI, zona incerta; SC, superior colliculus; VC, visual cortex; ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; mt, mamillothalamic tract; ns, nigrostriatal bundle; SuG, superficial gray superior colliculus; Op, optic nerve layer superior colliculus; InG, intermediate gray layer superior colliculus; InWh, intermediate white layer superior colliculus; DpG, deep gray superior colliculus; DpWh, deep white superior colliculus. AuD, secondary auditory cortex; V2L, secondary visual cortex, lateral area; V1B, primary visual cortex, binocular area; V1M, primary visual cortex, monocular area; V2ML, secondary visual cortex, mediolateral part; V2MM, secondary visual cortex, mediomedial part; DS, dorsal subiculum; TeA, temporal association cortex.

a RTPP after activation of the SC^{Vglut2} & ChR2→ZI projection

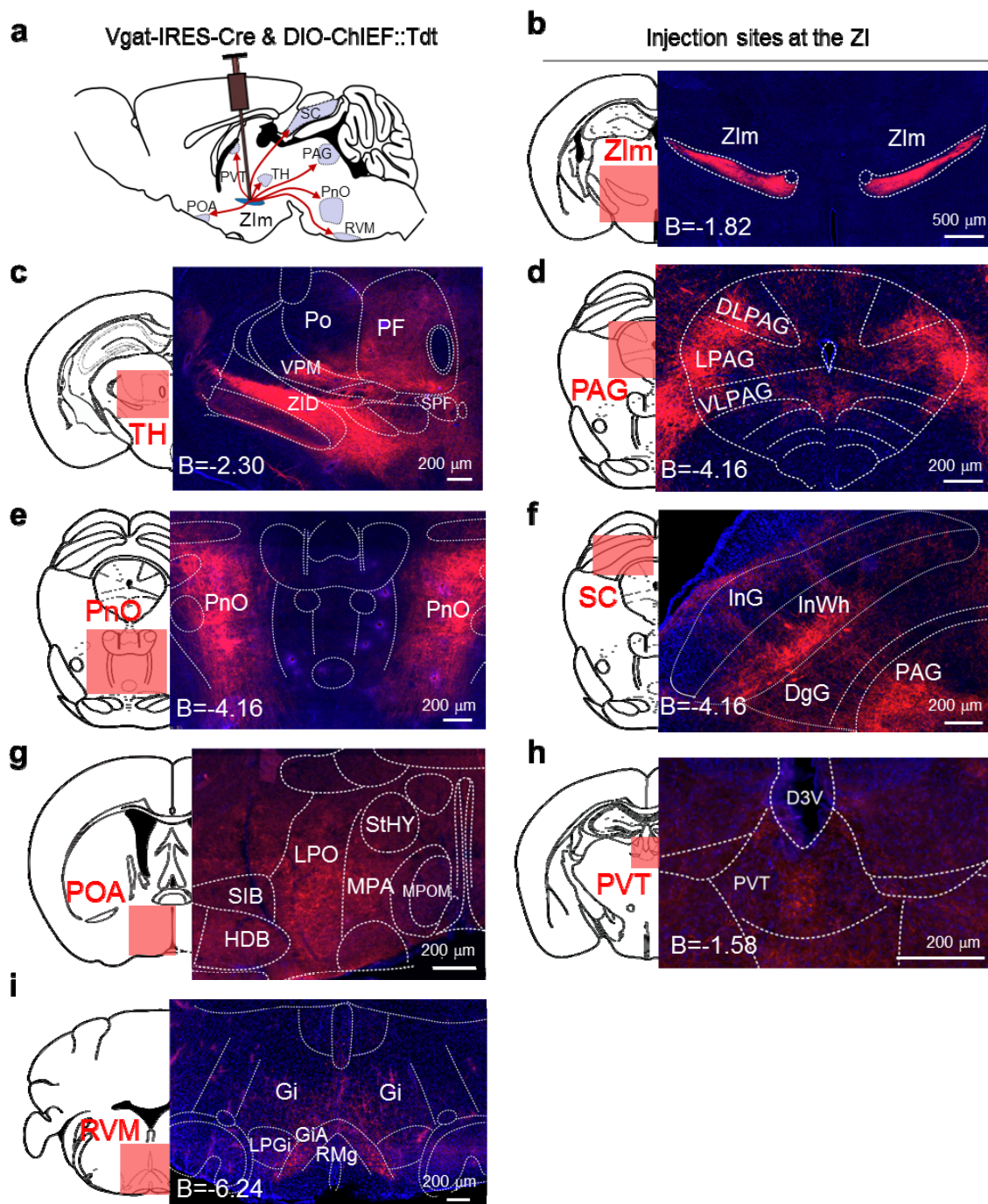


b Cued sucrose-seeking task

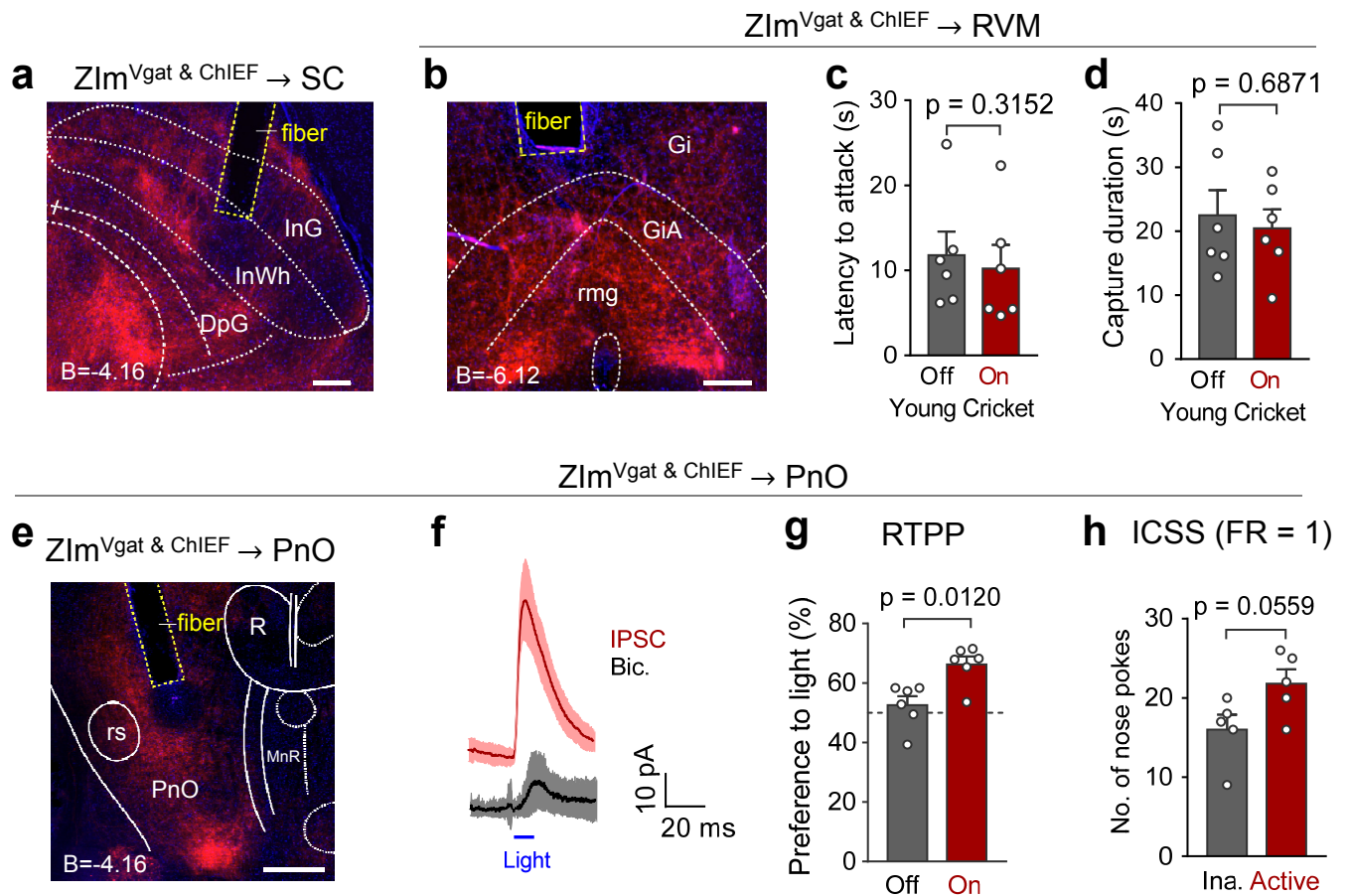


Supplementary Figure 18. Motivational aspects of activation of ZI GABA neurons, related to Fig. 5.

a, Real-time place preference after photoactivation of SC^{Vglut2}-IRES-Cre & DIO-ChR2→ZI projections. Light pattern, 473 nm, 10 Hz, 20 ms, 20 mW. Mice were allowed to explore 5-min in the chamber without laser (Pre. 5 min) and followed by 10-min real-time laser coupling (Sti. 10 min). The laser coupled side was randomly assigned ($n = 3$ mice). **b**, Cued sucrose-seeking task after activation of ZIm GABA neurons. Diet restricted mice (~80 ~ 85 % body weight) were trained to nose poke in order to receive 10% sucrose solution after a house light cue (cue on). Mice were trained for 10 days until average nose poke frequency of cue-on period is twice of cue-off period. On day 11, mice received photostimulation on cue-on period to activate ZIm GABA neurons during the task. Activation of ZIm GABA neurons, did not promote, actually 'interrupted' cued sucrose seeking (which might be due to a competition between sucrose reward and laser reward). Laser pattern: 473 nm, 10 ms, 20 Hz, 10 mW. $n = 5$ mice. All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on ordinary two-way ANOVA with Bonferroni's multiple comparisons. All p values are indicated in the figure.

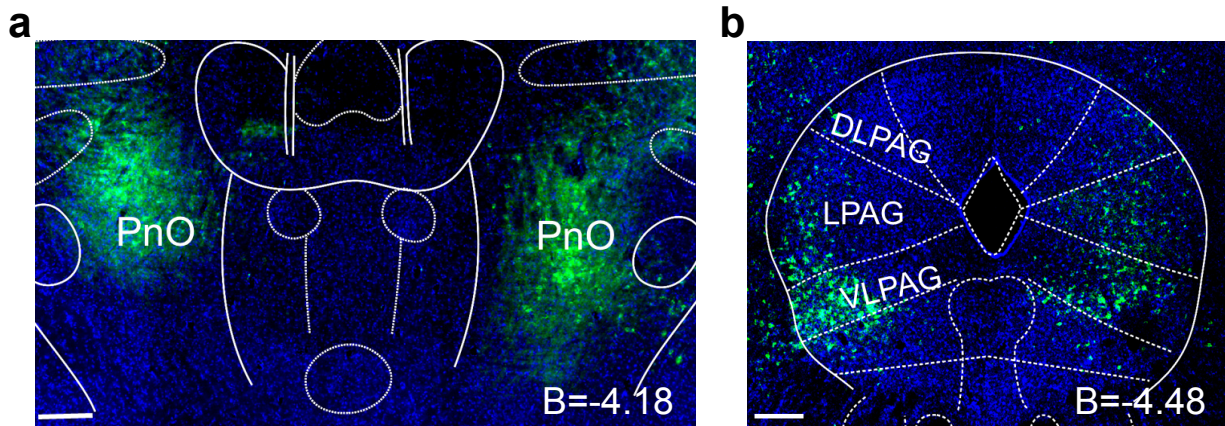


Supplementary Figure 19. Projections of Zlm GABAergic neurons throughout the brain. **a**, Diagram of projection patterns. **b**, Injection sites of AAV-DIO-ChIEF::Tdt in the Zlm. **c-f**, The projection sites with strong terminal fluorescence at indicated regions. **g-i**, The projection sites with moderate terminal fluorescence at indicated regions. **(b-i)** are representative images from one mice ($n = 3$ mice in total). B, bregma; PAG, periaqueductal gray matter; SC, superior colliculus; RVM, rostral ventromedial medulla; PnO, the oral part of pontine reticular nucleus; TH, thalamus; Po, posterior thalamic nuclear group; POA, preoptic nu.; PaF, parafascicular thalamic nucleus; SPF, subparafascicular thalamic nu; VPM, ventral posteromedial thalamic; ZID, zona incerta, dorsal part; DLPAG, dorsolateral periaqueductal gray; DMPAG, dorsomedial periaqueductal gray; LPAG, lateral periaqueductal gray; InG, intermediate gray layer of the superior colliculus; InWh, intermediate white layer of the superior colliculus; DpG, deep gray layer of the superior colliculus; Gi, gigantocellular reticular nucleus; GiA, gigantocellular reticular nucleus, alpha part; LPGi, lateral paragigantocellular nucleus; rmg, raphe magnus nucleus; LPO, lateral preoptic area; StHY, striohypothalamic nucleus; MPA, medial preoptic area; MPOM, medial preoptic nucleus, medial part; SIB, substantia innominata, basal part; HDB, nucleus of the horizontal limb of the diagonal band; D3V, dorsal 3rd ventricle; PVT, paraventricular thalamic nu.



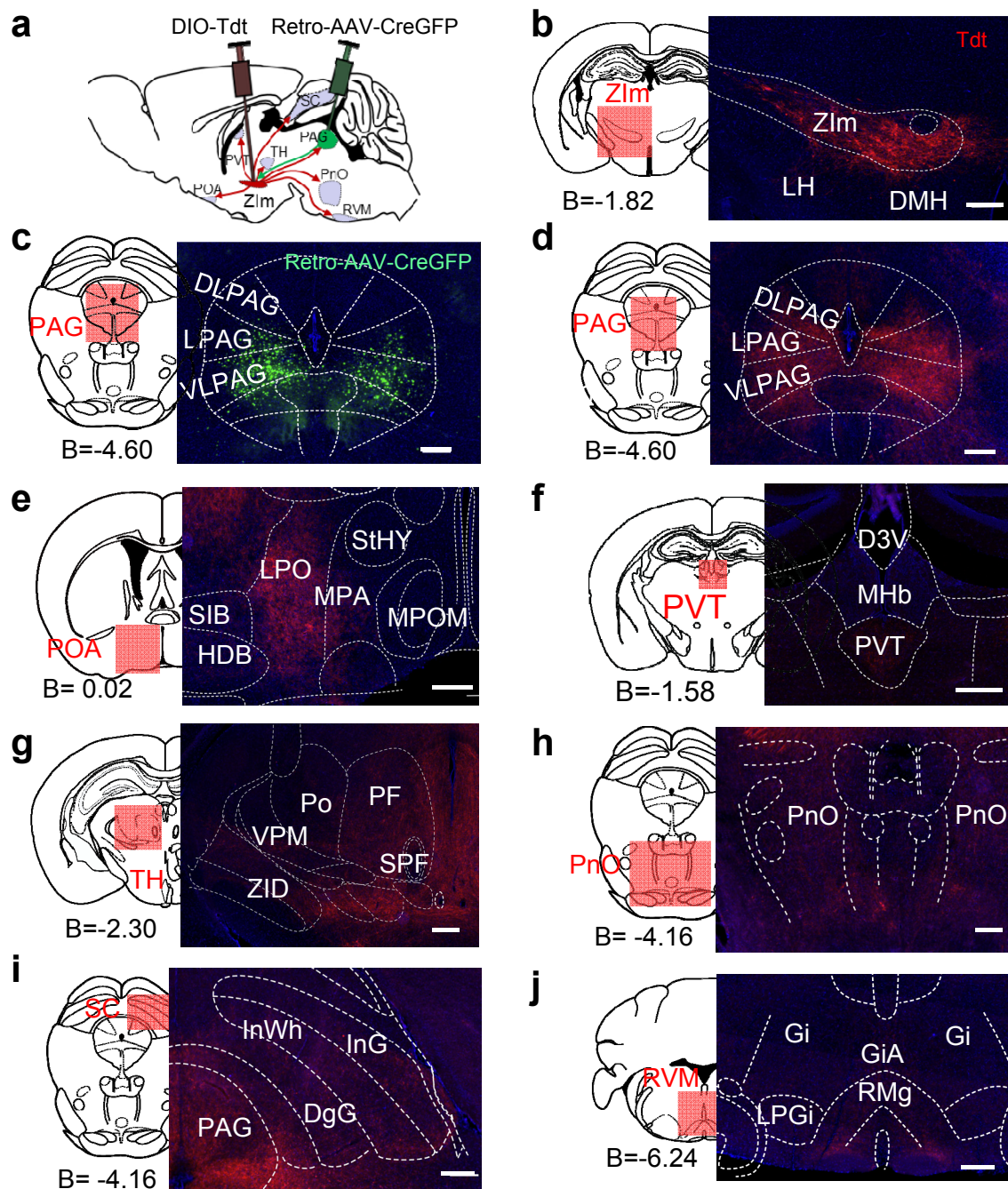
Supplementary Figure 20. Roles of ZIm GABAergic projections in hunting, related to Fig. 6.

a, Terminal fluorescence of ZIm^{Vgat} & ChIEF::Tdt neurons in the SC. The dotted yellow line shows the position of an optical insert used for terminal stimulation ($n = 5$ mice). Scale bar, 200 μ m. **b**, Terminal fluorescence of ZIm^{Vgat} & ChIEF::Tdt neurons in the RVM ($n = 6$ mice). The dotted yellow line shows the position of an optical insert used for terminal stimulation. Scale bar, 200 μ m. **c-d**, Optogenetic activation of ZI→RVM terminals did not affect hunting in terms of attack latency (**c**) and capture duration (**d**) ($n = 6$ mice). **e**, Terminal fluorescence of ZIm^{Vgat} & ChIEF::Tdt neurons in the PnO ($n = 6$ mice). The dotted yellow line shows the position of an optical insert. Scale bar, 500 μ m. **f**, Induction of inhibitory postsynaptic currents (IPSCs) in PnO neurons by light stimulation (blue, 2 mW, 10 ms) of ZIm^{Vgat} & ChIEF neural terminals. IPSCs were blocked by bicuculline (bic.). $n = 23$ traces from 3 mice. **g**, Quantification of the preference to light in the RTPP assay showed a preference to the laser-coupled side ($n = 6$ mice). **h**, Quantification of the nose pokes under an FR = 1 ICSS task where mice poked once to receive a 3-s laser stimulation (473 nm, 10 mW, 20 Hz, 10 ms), revealed no strong motivation associated with PnO terminal photoactivation ($n = 5$ mice). All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on paired two tailed t tests and are indicated in the figure. B, bregma; SC, superior colliculus; RVM, rostral ventromedial medulla; PnO, the oral part of pontine reticular nucleus; InG, intermediate gray layer of the superior colliculus; InWh, intermediate white layer of the superior colliculus; DpG, deep gray layer of the superior colliculus; Gi, gigantocellular reticular nucleus; GiA, gigantocellular reticular nucleus, alpha part; rmg, raphe magnus nucleus; rs, rubrospinal tract; R, red nucleus; MnR, median raphe nucleus.



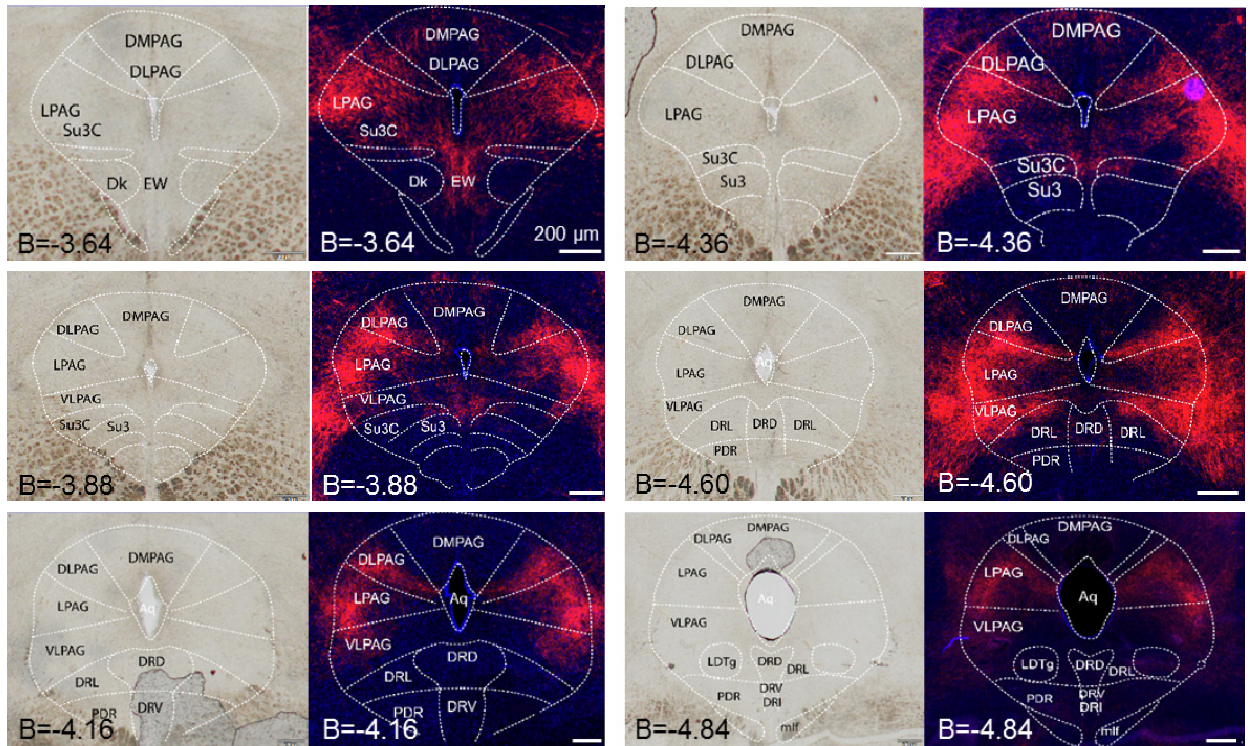
Supplementary Figure 21. h3MD expression in the PnO and the PAG, related to Fig. 6.

a, The expression of hSyn-hM3D in the PnO (n = 5 mice). Scar bar, 200 μ m. **b**, The expression of hSyn-hM3D in the PAG (n = 8 mice). Scar bar, 200 μ m. B, bregma; PnO, the oral part of pontine reticular nucleus; PAG, periaqueductal gray; DLPAG, dorsolateral periaqueductal gray; VLPAG, ventlateral periaqueductal gray; LPAG, lateral periaqueductal gray.

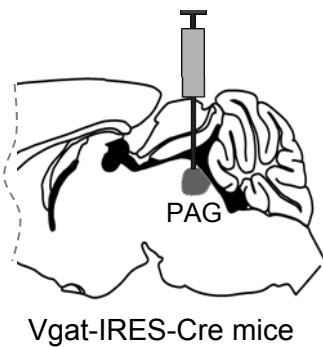
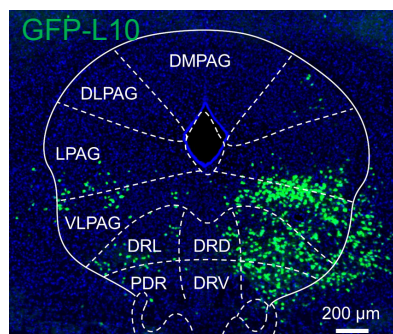
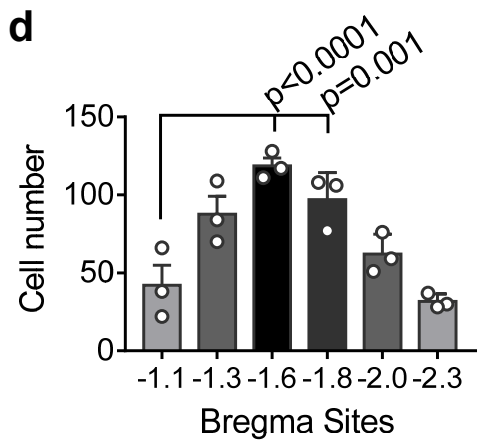
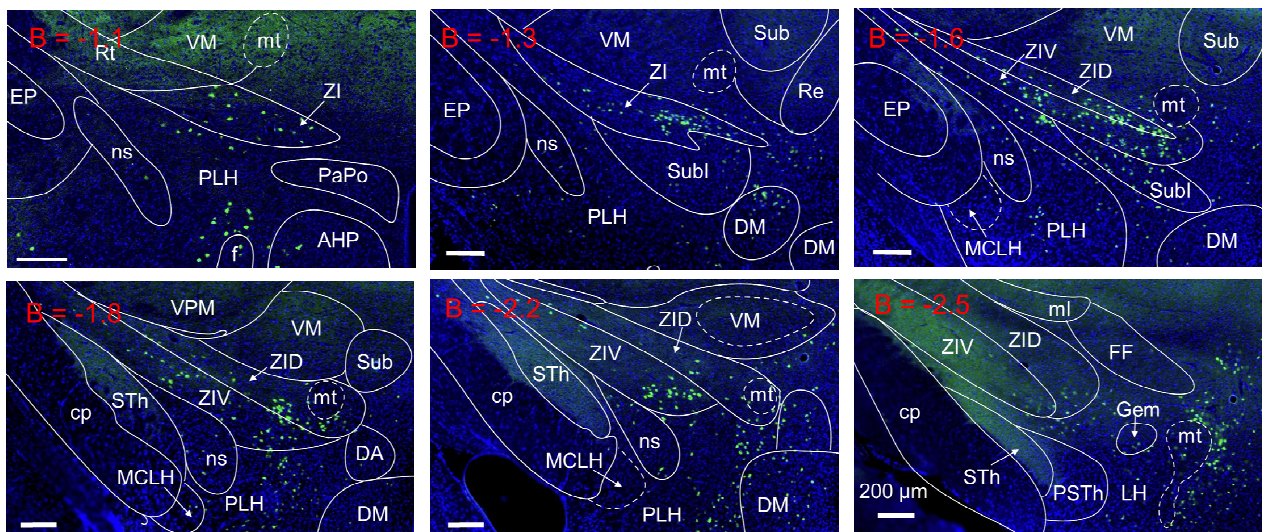


Supplementary Figure 22. Collateral projections of PAG-projected ZIm neurons.

a, Design for retrograde labeling of ZI neurons. Retro-AAV-Cre-GFP was injected in the PAG. The Cre traveled retrogradely to the ZIm to drive DIO-Tdt expression. **b**, Tdt expression in the ZIm. **c**. Cre-GFP expression in the PAG. **d**, Strong terminal fluorescence in the PAG. **e-j**, The projection sites with moderate terminal fluorescence at indicated regions. scale bar, 200 μ m. The representative images from one mice (n = 3 mice in total). B, bregma; PAG, periaqueductal gray matter; SC, superior colliculus; RVM, rostral ventromedial medulla; PnO, the oral part of pontine reticular nucleus; TH, thalamus; Po, posterior thalamic nuclear group; POA, preoptic nu.; PaF, parafascicular thalamic nucleus; SPF, subparafascicular thalamic nu.; VPM, ventral posteromedial thalamic; ZID, zona incerta, dorsal part; DLPAG, dorsolateral periaqueductal gray; DMPAG, dorsomedial periaqueductal gray; LPAG, lateral periaqueductal gray; InG, intermediate gray layer of the superior colliculus; InWh, intermediate white layer of the superior colliculus; DpG, deep gray layer of the superior colliculus; Gi, gigantocellular reticular nucleus; GiA, gigantocellular reticular nucleus, alpha part; LPGi, lateral paragigantocellular nucleus; rmg, raphe magnus nucleus; LPO, lateral preoptic area; StHY, striohypothalamic nucleus; MPA, medial preoptic area; MPOM, medial preoptic nucleus, medial part; SIB, substantia innominata, basal part; HDB, nucleus of the horizontal limb of the diagonal band; D3V, dorsal 3rd ventricle; PVT, paraventricular thalamic nu.

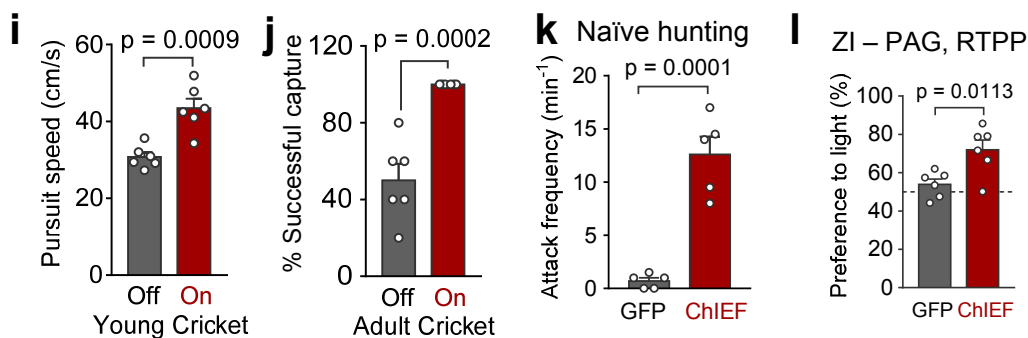
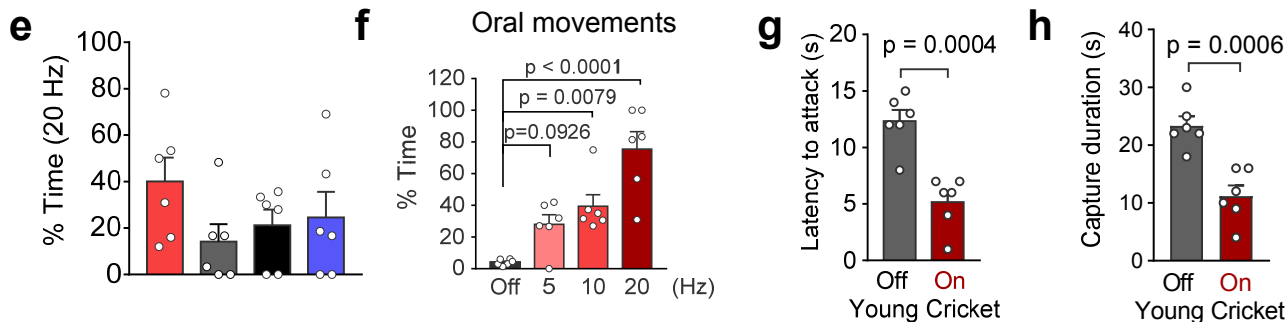
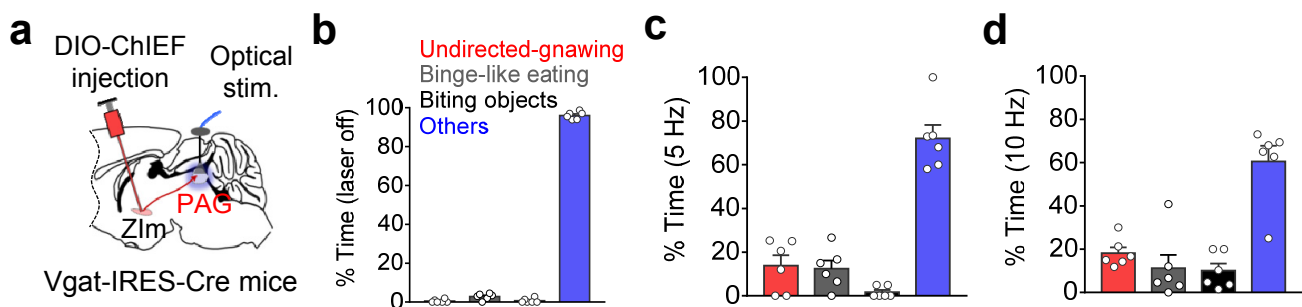
a**b**

Retro-AAV-DIO-GFP-L10

**c****d****e**

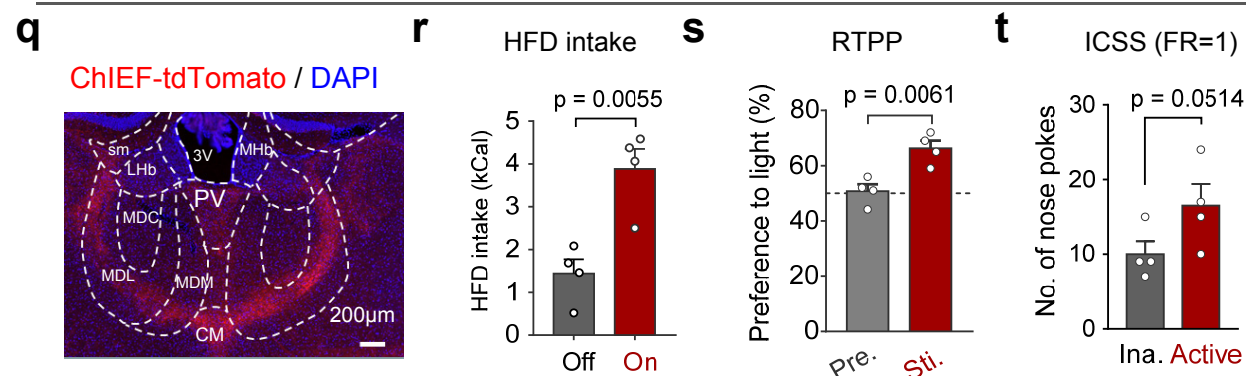
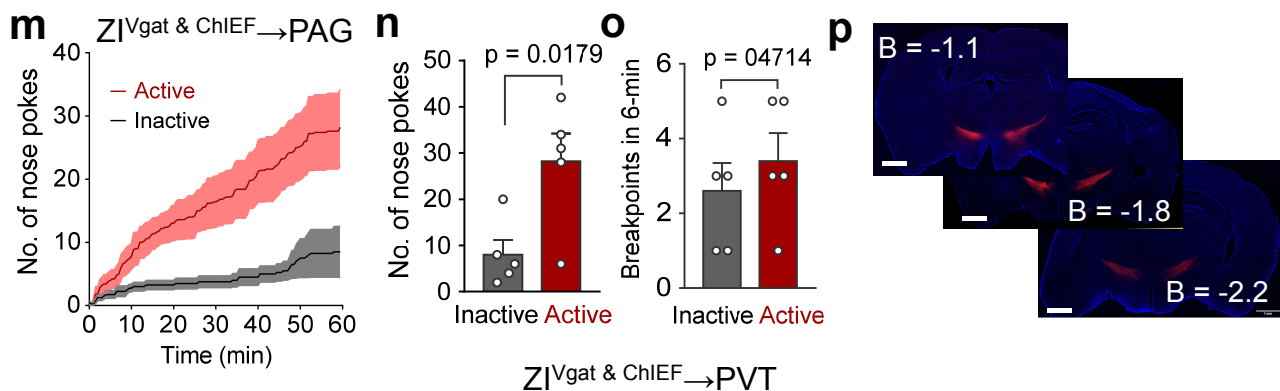
Supplementary Figure 23. The ZIm→PAG projection, related to Fig. 6.

a, The PAG terminal fluorescence of DIO-ChIEF:Tdt in the ZIm^{Vgat} neurons in different bregma sites. Representative images are from one mice (n = 3 mice in total). Scale bars are 200 μ m. **b**, Design for retrograde labeling of ZI neurons. Retro-AAV-DIO-GFP-L10 was injected in the PAG of Vgat-IRES-Cre mice. The Cre traveled retrogradely to the ZIm to drive GFP-L10 expression. **c**, The GFP-L10 expression in the injection site (PAG). **d**, The distribution of GFP⁺ cells in the ZI at different bregma sites. The data are shown as mean $\pm$ s.e.m. The p-values were calculated based on ordinary one-way ANOVA, and are indicated in the figure. **e**, Expression of GFP-L10 at different bregma sites. Scale bar, 200 μ m. (**c** and **e**) are representative images chosen from n = 3 mice. B, bregma; DLPAG, dorsolateral periaqueductal gray; DMPAG, dorsomedial periaqueductal gray; LPAG, lateral periaqueductal gray; VLPAG, ventrolateral periaqueductal gray; Su3C, supraoculomotor cap; Dk, nucleus of Darkschewitsch; EW, Edinger-Westphal nucleus; DRL, dorsal raphe nucleus, lateral part; DRD, dorsal raphe nucleus, dorsal part; PDR, posterodorsal raphe nucleus; DRV, dorsal raphe nucleus, ventral part; DRI, dorsal raphe nucleus, interfascicular part; mlf, medial longitudinal fasciculus; LDTg, laterodorsal tegmental nucleus; mt, mamillothalamic tract; ns, nigrostriatal bundle. VM, ventromedial thalamic nucleus; mt, mamillothalamic tract; ZI, zona incerta; EP, entopeduncular nucleus; vpl, ventral posterolateral thalamic nucleus; ic, internal capsule; ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; Sub, submedial thalamic nucleus; SubI, subincertal nucleus; VPM, ventral posteromedial thalamic nucleus; ns, nigrostriatal bundle; PLH, peduncular part lateral hypothalamic area; VPPC, ventral posterior nucleus of the thalamus, parvocellular part; SPF, subparafascicular thalamic nu; PSTh, parasubthalamic nucleus; cp, cerebral peduncle; DM, dorsomedial hypothalamic nucleus; PH, posterior hypothalamic nucleus.



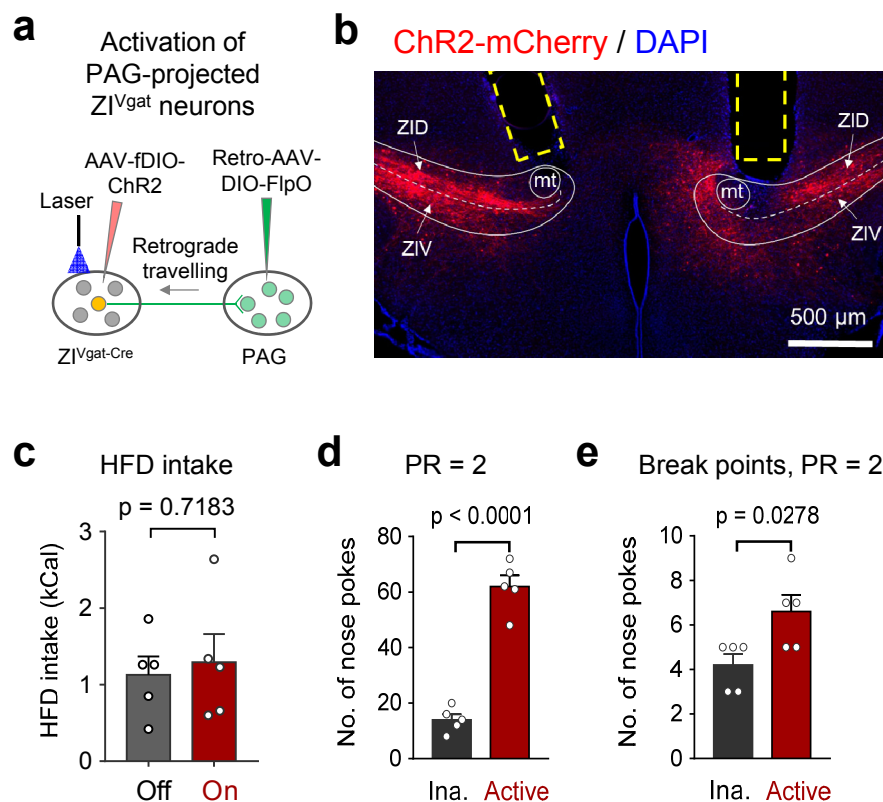
Intracranial self-stimulation (PR = 2)

ZlVgat & ChIEF → PVT



Supplementary Figure 24. Critical role of the $Zl^{Vgat} \rightarrow PAG$ in hunting, related to Fig. 6.

a, Design of PAG terminal photoactivation in Zl^{Vgat} & $ChIEF$ mice. **b-e**, Home-cage behaviors after PAG terminal photoactivation (473 nm, 10 ms, 10 mW, 2-min on followed by 8-min off, total 6 trials) showed an increase in undirected gnawing ($n = 6$ mice). **f**, Summary of the relative time spent on oral movements. **g-i**, Terminal photoactivation in the PAG increased hunting efficiency with young crickets, which shortened attacking latency (**g**) and capture duration (**h**) and increased maximum pursuit speed (**i**). **j**, Terminal photoactivation in the PAG increased hunting success rate with adult crickets. For **b-j**, $n = 6$ mice. **k**, Bite-attack frequency for cricket-naïve mice. Cricket-naïve mice typically could not finish all hunting program in a given short time (2-min). We only quantified bite-attack frequency in 2-min young cricket hunting task. ($n = 5$ mice for both groups). Laser pattern: 473 nm, 10 ms, 20 Hz, 10 mW, 2-min on. **l**, Quantification of the preference to light in the RTPP assay showed a preference to the laser-coupled side ($n = 6$ mice for both groups). **m-n**, Terminal photoactivation in the PAG slightly increased nose-poke numbers in intracranial self-stimulation (PR = 2). **o**, The breakpoints were not significant. ($n = 5$ mice in **m-o**). **p**, Fluorescence of Zl^{Vgat} & $ChIEF::Tdt$ neurons in the ZI of different bregma sites. Scale bar, 1 mm. B, bregma. Representative images are from one mice ($n = 4$ mice in total). **q**, Terminal fluorescence of Zl^{Vgat} & $ChIEF::Tdt$ neurons in the PVT. Scale bar, 200 μ m. The representative image is from one mice ($n = 4$ mice in total). **r**, HFD intake after terminal activation in the PVT. 90-min session (5-min on following 10-min off, total 6 trials). **s**, Quantification of the preference to light in the RTPP assay showed a preference to the laser-coupled side. **t**, Terminal photoactivation in the PVT slightly yet insignificantly increased nose-poke numbers in intracranial self-stimulation (FR = 1). ($n = 4$ mice in **r-t**). All figures except (**a**, **p**, **q**) are shown as mean $\pm$ s.e.m. The p-values were calculated based on two-tailed unpaired t tests except in (**f**) (ordinary one-way ANOVA). All p values are indicated in the figure.



Supplementary Figure 25. PAG-projected ZI GABA neurons promote hunting.

a, Design for retrograde labeling of ZI neurons. Retro-AAV-DIO-FlpO was injected in the PAG. The FlpO traveled retrogradely to the ZIm to drive fDIO-ChR2-mCherry expression in Vgat-IRES-Cre mice. **b**, ChR2-mCherry expression in the ZIm. The representative image is from one mice ($n = 5$ mice in total). **c**, HFD intake after photoactivation of PAG-projected ZI GABA neurons. 90-min session (5-min on following 10-min off, total 6 trials). **d**, Quantification of the nose pokes under PR = 2 task, where nose pokes need to increase by two in order to receive a 3-s laser stimulation. **e**, The breakpoints in the PR = 2 task revealed a strong reinforcing value to photoactivation. For **c-e**, $n = 5$ mice. All data are shown as mean $\pm$ s.e.m. The p-values were calculated based on two-tailed paired t tests. All p values are indicated in the figures. ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; mt, mammillothalamic tract.

Supplementary Table 1. Summary of the overlapped cells in response to different stimuli.

a (related to Fig. 3)

	Flash	Tone	Whisker	Moving spot	Movement
Flash	13				
Tone	6	23			
Whisker	13	21	40		
Moving spot	9	14	20	23	
Movement	10	15	26	16	28

b (related to Supplementary Fig. 8)

	Tone	Object-Whisker	Moving cricket	Movement
Tone	24			
Object-Whisker	8	73		
Moving cricket	7	17	27	
Movement	19	51	14	59

Supplementary Table 2. Summary of statistical analyses

Figure	Sample size (n)	Statistical test	P values
1c	8 mice	one-way repeated-measures ANOVA factor: treatment(laser off, 5 Hz, 10 Hz, 20 Hz) bonferroni's multiple comparisons test	treatment: $F(2.441, 17.09) = 37.2, P < 0.0001$ multiple comparisons: Off vs 5 Hz, $P = 0.0147$ Off vs 10 Hz, $P = 0.0005$ Off vs 20 Hz, $P < 0.0001$
1e	ChIEF, 8 mice GFP, 8 mice	two-way repeated-measures ANOVA factor one: virus (GFP, ChIEF) factor two: laser(laser off, laser on) Bonferroni's multiple comparisons test	virus: $F(1, 14) = 7.954, P = 0.0136$ laser: $F(1, 14) = 4.77, P = 0.0023$ interaction: $F(1, 14) = 12.26, P = 0.0348$ multiple comparisons: GFP: Laser Off vs. GFP: Laser On, $P = 0.5686$ ChIEF: Laser Off vs. ChIEF: Laser On, $P = 0.0004$
1f	ChIEF, 8 mice GFP, 8 mice	two-way repeated-measures ANOVA factor one: virus (GFP, ChIEF) factor two: laser(laser off, laser on) Bonferroni's multiple comparisons test	virus: $F(1, 14) = 9.227, P = 0.0089$ laser: $F(1, 14) = 11, P = 0.0042$ interaction: $F(1, 14) = 18.97, P = 0.0007$ multiple comparisons: GFP: Laser Off vs. GFP: Laser On, $P > 0.9999$ ChIEF: Laser Off vs. ChIEF: Laser On, $P = 0.0002$
1g	ChIEF, 8 mice GFP, 8 mice	two-way repeated-measures ANOVA factor one: virus (GFP, ChIEF) factor two: laser (laser off, laser on) Bonferroni's multiple comparisons test	virus: $F(1, 14) = 6.772, P = 0.0209$ laser: $F(1, 14) = 23.54, P = 0.0003$ interaction: $F(1, 14) = 27.64, P = 0.0001$ multiple comparisons: GFP: Laser Off vs. GFP: Laser On, $P > 0.9999$ ChIEF: Laser Off vs. ChIEF: Laser On, $P < 0.0001$
1h	8 mice	two-way repeated-measures ANOVA factor one: cricket (Young, Adult) factor two: laser (laser off, laser on) Bonferroni's multiple comparisons test	cricket: $F(1, 14) = 26.6, P = 0.0001$ laser: $F(1, 14) = 26.6, P = 0.0001$ interaction: $F(1, 14) = 26.6, P = 0.0001$ multiple comparisons: Young: Laser Off vs. Young: Laser On, $P > 0.9999$ Adult: Laser Off vs. Adult: Laser On, $P < 0.0001$
1i – label *	8 mice	two-way repeated-measures ANOVA factor one: time bin (0 – 30 s, 30 – 60 s, 60 – 90 s, 90 – 120 s) factor two: cricket (young, laser off; young, laser on; adult, laser off; adult, laser on) tukey's multiple comparisons test	time bin: $F(3, 84) = 5.718, P = 0.0013$ cricket: $F(3, 28) = 50.61, P < 0.0001$ interaction: $F(9, 84) = 9.062, P < 0.0001$ 0-30 s: Adult off vs. 0-30 s: Adult on: $P < 0.0001$ 0-30 s: Adult off vs. 0-30 s: Young off: $P < 0.0001$ 0-30 s: Adult off vs. 0-30 s: Young on: $P < 0.0001$
1i – label #	8 mice	one-way ANOVA Uncorrected Fisher's LSD test	$F(3, 28) = 3.07, P = 0.0440$ multiple comparisons: 30 – 60 s vs 0 – 30 s: $P = 0.0737$ 60 – 90 s vs 0 – 30 s: $P = 0.0210$ 90 – 120 s vs 0 – 30 s: $P = 0.0098$
1j	8 mice	two-tailed paired t test	$P = 0.0067$
1k	8 mice	two-tailed paired t test	$P = 0.0004$
1l	6 mice	two-way repeated-measures ANOVA factor one: laser(laser off, laser on) factor two: artificial prey (static, moving) Bonferroni's multiple comparisons test	laser: $F(1, 10) = 567.2, P = 0.0002$ artificial prey : $F(1, 20) = 31.29, P < 0.0001$ Interaction: $F(1, 10) = 34.19, P = 0.0002$ multiple comparisons: laser off, static vs. laser on, static, $P < 0.0001$ laser off, moving vs. laser on, moving, $P < 0.0001$
1m	8 mice	two-tailed paired t test	$P = 0.4314$
1n	8 mice	two-tailed paired t test	$P = 0.0700$
1o	8 mice	two-tailed paired t test	$P = 0.0003$
1p	8 mice	two-tailed paired t test	$P = 0.0002$
2i	GFP: 3 mice KO: 3 mice	two-tailed unpaired t test	$P < 0.0001$

(table continued on the next page)

Supplementary Table 2, continued

Figure	Sample size (n)	Statistical test	P values
2j	GFP : 4 mice KO : 6 mice	two-way repeated-measures ANOVA factor one : virus (GFP, KO) factor two : weeks (0,1,2,3,4,5) Uncorrected Fisher's LSD test	virus: $F(1, 8) = 8.82$, $P = 0.0178$ weeks: $F(5, 40) = 30.17$, $P < 0.0001$ interaction: $F(5, 40) = 5.222$, $P = 0.0009$ multiple comparisons: Week 0: GFP vs. KO. $P = 0.2707$ Week 1: GFP vs. KO. $P = 0.0247$ Week 2: GFP vs. KO. $P = 0.0147$ Week 3: GFP vs. KO. $P = 0.0027$ Week 4: GFP vs. KO. $P = 0.0017$ Week 5: GFP vs. KO. $P = 0.0003$
2j	GFP : 6 mice KO : 6 mice	two-tailed unpaired t test	$P = 0.0126$
2k	GFP : 6 mice KO : 6 mice	two-tailed unpaired t test	$P = 0.0100$
2m	GFP : 6 mice KO : 6 mice	two-tailed unpaired t test	$P = 0.0003$
3i	Excited cells: 28 Insensitive cells: 16	two-way ANOVA factor one: speed (0, 2.5, 7.5, 12.5, 17.5; 22.5) factor two: cells (excited, insensitive) Bonferroni's multiple comparisons test	speed: $F(5, 294) = 10.06$ cells: $F(1, 294) = 92.84$ Interaction: $F(5, 294) = 6.254$ multiple comparisons: 0: $P > 0.9999$; 2.5: $P = 0.2937$; 7.5, 12.5, 17.5 and 22.5: $P < 0.0001$
4g	4 mice	two-way repeated-measures ANOVA factor one: treatment (cricket, HFD, object) factor two: period (baseline, introduction, interaction) Bonferroni's multiple comparisons test	treatment: $F(2, 6) = 34.55$, $P < 0.0005$ period: $F(2, 6) = 957.2$, $P < 0.0001$ Interaction: $F(4, 12) = 20.73$, $P < 0.0001$ multiple comparisons: Cricket: Introduction vs. HFD: Introduction, $P = 0.006$ Cricket: Introduction vs. Object: Introduction, $P = 0.0103$ HFD: Introduction vs. Object: Introduction, $P > 0.9999$ Cricket: Interaction vs. HFD: Interaction, $P < 0.0001$ Cricket: Interaction vs. Object: Interaction, $P < 0.0001$ HFD: Interaction vs. Object: Interaction, $P = 0.4087$
4h	L(+)W(+) : 18 trails L(-)W(+) : 17 trails L(+)W(-) : 18 trails L(-)W(-) : 19 trails (L represents light W represents whisker)	one-way ANOVA factor : condition (L(+)W(+), L(-)W(+), L(+)W(-), L(-)W(-)) Dunnett's multiple comparisons test	$F(3, 68) = 4.957$, $P = 0.0036$ multiple comparisons : L(+)W(+) vs L(-)W(+) : $P = 0.0431$ L(+)W(+) vs L(+)W(-) : $P = 0.9375$ L(+)W(+) vs L(-)W(-) : $P = 0.0046$
4i	L(+)W(+) : 16 trails L(-)W(+) : 25 trails L(+)W(-) : 24 trails L(-)W(-) : 14 trails L represents light W represents whisker)	one-way ANOVA factor : condition (L(+)W(+), L(-)W(+), L(+)W(-), L(-)W(-)) Dunnett's multiple comparisons test	$F(3, 75) = 10.93$, $P < 0.0001$ multiple comparisons : L(+)W(+) vs L(-)W(+) : $P = 0.0023$ L(+)W(+) vs L(+)W(-) : $P = 0.0006$ L(+)W(+) vs L(-)W(-) : $P = 0.0001$
4j	E(+)W(+) : 7 mice E(-)W(+) : 7 mice E(+)W(-) : 7 mice E(-)W(-) : 8 mice E represents light W represents whisker	ordinary two-way ANOVA factor one : treatment (laser off, laser on) factor two : condition (E(+)W(+), E(-)W(+), E(+)W(-), E(-)W(-)) Uncorrected Fisher's LSD test	treatment: $F(1, 50) = 57.07$, $P < 0.0001$ condition : $F(3, 50) = 17.36$, $P < 0.0001$ interaction: $F(3, 50) = 0.9714$, $P = 0.4137$ multiple comparisons : laser off: E(+)W(+) vs. laser off: E(-)W(+), $P = 0.0004$ laser off: E(+)W(+) vs. laser off: E(+)W(-), $P = 0.1699$ laser off: E(+)W(+) vs. laser off: E(-)W(-), $P < 0.0001$ laser on: E(+)W(+) vs. laser on: E(-)W(+), $P = 0.0002$ laser on: E(+)W(+) vs. laser on: E(+)W(-), $P = 0.1918$ laser on: E(+)W(+) vs. laser on: E(-)W(-), $P = 0.0011$ laser off: E(+)W(+) vs. laser on: E(+)W(+), $P = 0.0018$ laser off: E(-)W(+) vs. laser on: E(-)W(+), $P = 0.0033$ laser off: E(-)W(-) vs. laser on: E(-)W(-), $P = 0.0015$
4k	E(+)W(+) : 7 mice E(-)W(+) : 7 mice E(+)W(-) : 7 mice E(-)W(-) : 8 mice	ordinary two-way ANOVA factor one : treatment (laser off, laser on) factor two : condition (E(+)W(+), E(-)W(+), E(+)W(-), E(-)W(-)) Uncorrected Fisher's LSD test	treatment: $F(1, 50) = 67.01$, $P < 0.0001$ condition : $F(3, 50) = 22.98$, $P < 0.0001$ interaction: $F(3, 50) = 2.715$, $P = 0.0546$ multiple comparisons : laser off: E(+)W(+) vs. laser off: E(-)W(+), $P = 0.0004$ laser off: E(+)W(+) vs. laser off: E(+)W(-), $P = 0.0240$ laser off: E(+)W(+) vs. laser off: E(-)W(-), $P < 0.0001$ laser on: E(+)W(+) vs. laser on: E(-)W(+), $P = 0.0002$ laser on: E(+)W(+) vs. laser on: E(+)W(-), $P = 0.0338$ laser on: E(+)W(+) vs. laser on: E(-)W(-), $P = 0.0002$ laser off: E(+)W(+) vs. laser on: E(+)W(+), $P = 0.0019$ laser off: E(-)W(+) vs. laser on: E(-)W(+), $P = 0.0036$ laser off: E(-)W(-) vs. laser on: E(-)W(-), $P = 0.0013$

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Supplementary Table 2, continued

Figure	Sample size (n)	Statistical test	P values
5c	ChIEF : 6 mice GFP : 6 mice	two-tailed unpaired t test	$P < 0.001$
5e	6 mice	two-tailed paired t test	$P = 0.0017$
5h	ChIEF : 6 mice GFP : 6 mice	two-way ANOVA factor one: port (active, inactive) factor two: virus (GFP, ChIEF) tukey's multiple comparisons test	nose port: $F(1, 20) = 71.41, P < 0.0001$ virus: $F(1, 20) = 75, P < 0.0001$ interaction: $F(1, 20) = 71, P < 0.0001$ multiple comparisons: Active: GFP vs. Active: ChIEF, $P = 0.0002$ Active: GFP vs. Inactive: GFP, $P > 0.9999$ Active: ChIEF vs. Inactive: ChIEF, $P = 0.0002$ Active: ChIEF vs. Inactive: GFP, $P = 0.0002$
5j	ChIEF : 6 mice GFP : 6 mice	two-way ANOVA factor one: port (active, inactive) factor two: virus (GFP, ChIEF) tukey's multiple comparisons test	nose port: $F(1, 20) = 25.56, P < 0.0001$ virus: $F(1, 20) = 38.4, P < 0.0001$ interaction: $F(1, 5) = 24.9, P < 0.0001$ multiple comparisons: Active: GFP vs. Active: ChIEF, $P = 0.0012$ Active: GFP vs. Inactive: GFP, $P > 0.9999$ Active: ChIEF vs. Inactive: GFP, $P = 0.0012$ Active: ChIEF vs. Inactive: ChIEF, $P = 0.0021$
5k	ChIEF : 6 mice GFP : 6 mice	two-tailed unpaired t test	$P = 0.0019$
5m	GtACR : 5 mice GFP : 5 mice	two-tailed unpaired t test	$P < 0.0001$
5o	4 mice	two-tailed paired t test	$P = 0.0183$
5r	GtACR : 5 mice GFP : 4 mice	two-way repeated-measures ANOVA factor one: time bin (-5 to 0 s, 0 to 5 s, 5 to 10 s) factor two: virus (GFP, GtACR) Sidak's multiple comparisons test	time bin: $F(2, 14) = 21.15, P < 0.0001$ region: $F(1, 7) = 19.72, P = 0.0030$ interaction: $F(2, 14) = 8.895, P = 0.0032$ multiple comparisons: GFP: -5 to 0 s vs. GtACR: -5 to 0 s, $P = 0.9857$ GFP: 0 to 5 s vs. GtACR: 0 to 5 s, $P < 0.0001$ GFP: 5 to 10 s vs. GtACR: 5 to 10 s, $P = 0.0121$
6b	PAG : 6 mice PnO : 6 mice SC : 5 mice RVM : 6 mice	two-way repeated-measures ANOVA factor one: laser (laser off, laser on) factor two: region (PAG, PnO, SC, RVM) Bonferroni's multiple comparisons test	laser: $F(1, 19) = 135, P < 0.0001$ region: $F(3, 19) = 24.67, P < 0.0001$ interaction: $F(3, 19) = 24.96, P < 0.0001$ multiple comparisons: laser off: PAG vs. laser on: PAG, $P < 0.0001$ laser off: PnO vs. laser on: PnO, $P < 0.0046$ laser off: SC vs. laser on: SC, $P > 0.9999$ laser off: RVM vs. laser on: RVM, $P = 0.0139$
6c	PAG : 6 mice PnO : 6 mice SC : 5 mice RVM : 6 mice	two-way repeated-measures ANOVA factor one: laser (laser off, laser on) factor two: region (PAG, PnO, SC, RVM) Bonferroni's multiple comparisons test	laser: $F(1, 19) = 55.64, P < 0.0001$ region: $F(3, 19) = 24.38, P < 0.0001$ interaction: $F(3, 19) = 23.39, P < 0.0001$ multiple comparisons: laser off: PAG vs. laser on: PAG, $P < 0.0001$ laser off: PnO vs. laser on: PnO, $P < 0.0001$ laser off: SC vs. laser on: SC, $P > 0.9999$ laser off: RVM vs. laser on: RVM, $P > 0.9999$
6d	PAG : 6 mice PnO : 6 mice RVM : 6 mice	two-way repeated-measures ANOVA factor one: laser (laser off, laser on) factor two: region (PAG, PnO, RVM) Bonferroni's multiple comparisons test	laser: $F(1, 15) = 21.55, P = 0.0003$ region: $F(2, 15) = 24.38, P = 0.0003$ interaction: $F(2, 15) = 23.39, P = 0.0003$ multiple comparisons: laser off: PAG vs. laser on: PAG, $P = 0.0074$ laser off: PnO vs. laser on: PnO, $P = 0.0046$ laser off: RVM vs. laser on: RVM, $P > 0.9999$
6f	8 mice	one-way repeated-measures ANOVA factor: treatment: treatment (saline, CNO 1mg/kg, CNO 3mg/kg) bonferroni's multiple comparisons test	treatment: $F(2, 21) = 30.24, P < 0.0001$ multiple comparisons: saline vs CNO 1mg/kg, $P = 0.0001$ saline vs CNO 3mg/kg, $P < 0.0001$
6g	5 mice	two-tailed unpaired t test	$P = 0.5925$
6j	6 mice	two-tailed paired t test	$P = 0.0005$
6k	6 mice	two-tailed unpaired t test	$P = 0.5278$

(table continued on the next page)

Supplementary Table 2, continued

Figure	Sample size (n)	Statistical test	P values
6l	5 mice	two-tailed unpaired t test	$P = 0.0001$
6n	5 mice	two-tailed paired t test	$P = 0.0064$
6o	5 mice	two-tailed paired t test	$P = 0.0065$
6p	5 mice	two-tailed unpaired t test	$P = 0.0019$
6r	3 mice	one-way ANOVA factor : period (Baseline, Latency, Capture, Eating) Uncorrected Fisher's LSD test	$F(3, 8) = 30.45$, $P = 0.0001$ multiple comparisons : Baseline vs Latency : $P = 0.0309$ Baseline vs Capture : $P < 0.0001$ Baseline vs Eating : $P = 0.6218$

Supplementary Table 3. Summary of stereotaxic coordinates

Brain regions		AP (mm)	DV (mm)	ML (mm)	Angle (°)	Application
Zlr		-1.1	-4.4	±0.70	0 (L/R)	glass pipette tip
Zlm		-1.80	-4.40	±1.00	0 (L/R)	glass pipette tip
PAG		-4.60	-2.6	±0.65	0(L/R)	glass pipette tip
SC	a	-3.6	-1.3	±0.60	0(L/R)	glass pipette tip
SC	b	-3.6	-2.2	±1.30	0(L/R)	glass pipette tip
PnO		-4.15	-4.3	±0.70	0(L/R)	glass pipette tip
RVM		-6.35	-5.75	±0.30	0(L/R)	glass pipette tip
VC	a	-2.8	-1.1	±2.40	0(L/R)	glass pipette tip
VC	b	-3.55	-1.1	±2.10	0(L/R)	glass pipette tip
VC	c	-4.25	-1.35	±2.45	0(L/R)	glass pipette tip
Zlr		-1.1	-4.15	+0.70	0(L)	optical fiber tip
		-1.1	-4.25	-1.70	12(R)	
Zlm		-1.80	-4.15	+1.00	0(L)	optical fiber tip
		-1.80	-4.25	-1.80	14(R)	
RVM		-6.35	-5.55	+0.30	0(L)	optical fiber tip
		-6.35	-5.8	-2.00	18(R)	
PAG		-4.60	-2.4	±1.25	14(L/R)	optical fiber tip
PnO		-4.15	-4.05	±1.30	6(L/R)	optical fiber tip
PVT		-1.05	-2.85	±0.05	0(L/R)	optical fiber tip

AP, anteroposterior; DV, dorsoventral; ML, mediolateral

a,b,c represents multiple injection sites so as to cover a large region.

L, left; R, right.