

Minireview

Neural and Genetic Basis of Evasion, Approach and Predation

Seahyung Park^{1,2}, Jia Ryou^{1,2}, and Daesoo Kim^{1,*}

¹Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Korea, ²These authors contributed equally to this work.

*Correspondence: daesoo@kaist.ac.kr
<https://doi.org/10.14348/molcells.2022.2032>
www.molcells.org

Evasion, approach and predation are examples of innate behaviour that are fundamental for the survival of animals. Uniting these behaviours is the assessment of threat, which is required to select between these options. Far from being comprehensive, we give a broad review over recent studies utilising optic techniques that have identified neural circuits and genetic identities underlying these behaviours.

Keywords: approach, evasion, genetic, neural, predation

INTRODUCTION

Risk cannot be destroyed, only transformed. As animals perceive danger, they can engage in flight to avoid potential harm; but this comes at the expense of foregoing exploration, foraging and pursuit, which bears the risk of not being able to locate resources, conspecifics and assessing the environment. Conversely, when potential threat is perceived to be sufficiently low, predation represents a state where animals pursue nutrition at the risk of exposing themselves to other predators and bodily harm. Failure to accurately gauge danger and select appropriate behavioural outputs can severely impact an animal's ability to survive. Thus, neural circuits that are able to assess the scale of danger and ultimately engage appropriate behavioural output are fundamental for the survival of animals.

PHYSIOLOGY AND GENETIC MARKERS FOR EVASION NEURONS

During states of perceived danger, animals may engage in evasive behaviours for survival; commonly manifested as flight in rodents.

Ventral tegmental area (VTA) Gad2 neurons have been shown to mediate flight in response to a looming stimulus and their photoactivation is sufficient to invoke flight in the absence of a stimulus (Zhou et al., 2019). VTA^{Gad2} neurons in turn have been shown to project to the central amygdala (CeA), lateral hypothalamus (LH), and periaqueductal grey (PAG), although whether activation of these projections is sufficient to induce flight has yet to be shown.

Potentially upstream of the VTA, optogenetic activation of paraventricular hypothalamus (PVH) Sim1 neuron projections to the ventral midbrain (vMB) also elicits flight (Mangieri et al., 2019) and the induction of Fos in non-dopaminergic vMB neuron, which raises the possibility that VTA^{Gad2} neurons may be a target of PVH^{Sim1} neurons. Although PVH neurons and more specifically PVH^{CRH} are responsive to looming threats, their suppression in response to appetitive stimuli suggests that they may mediate negative valence as opposed to assessing danger *per se* (Kim et al., 2019).

More anterior to the PVH, MPA^{vGlut2}-PAG projections have been shown to mediate certain evasive behaviours, such as jumping (Zhang et al., 2021). Results during an open-field test suggest that it may be driven by a state of severe anx-

Received 17 November, 2021; revised 10 January, 2022; accepted 11 January, 2022; published online 24 February, 2022

eISSN: 0219-1032

©The Korean Society for Molecular and Cellular Biology.

©This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/3.0/>.

iousness.

Ventromedial hypothalamic (VMH) SF1 neurons have also been implicated in evasive and freezing behaviours (Kunwar et al., 2015). VMH^{SF1}-anterior hypothalamic nucleus and VMH^{SF1}-dorsolateral PAG (dIPAG) projections have been shown to induce jumping and freezing respectively (Wang et al., 2015). Activation of VMH^{SF1} neurons was also capable of modulating autonomic responses such as heart rate, which scaled with the intensity of photostimulation (Viskaitis et al., 2017); hinting towards a population coding mechanism within the VMH^{SF1} neuron population. A separate study used fibre photometry to show that dorsomedial (dmVMH) SF1 neurons in particular are physiologically responsive to various threatening stimuli including rats and ultrasonic sweeps, although notably were not responsive to looming stimuli thus suggesting that their responses to threatening stimuli are context or modality-specific (Kennedy et al., 2020).

Another PAG projection in the hypothalamus is the LH^{vGluT2} to ventrolateral PAG (vIPAG) and lateral PAG (lIPAG) projection, photoactivation of which leads to evasive behaviour against a moving food dish (Li et al., 2018). Within the PAG, vIPAG^{Gad2} neurons have been shown to receive excitatory input from dl/IPAG^{vGluT2} and inhibitory inputs CeA^{Gad1} neurons. Optogenetic activation of either vIPAG^{Gad2} or dl/IPAG^{vGluT2} neurons induces flight (Tovote et al., 2016). This is mediated in part by inhibiting the freezing response evoked by local vIPAG^{vGluT2} neurons. These findings raises the possibility that LH^{vGluT2} neurons may excite vIPAG^{Gad2} neurons directly, or indirectly through dl/IPAG^{vGluT2} neurons, although this has yet to be tested. Interestingly a separate study recapitulated the flight caused by activation of dl/IPAG^{vGluT2} neurons, but then further showed that inhibition of these neurons during presentation of a looming stimulus can instead cause freezing (Evans et al., 2018), thus highlighting again the antagonistic relationship between flight and freezing.

Finally, a pontine projection towards the IPAG capable of driving flight, is the dorsal lateral parabrachial nucleus (dLPBN) to IPAG projection. Its photoactivation is capable of evoking running and jumping in rodents in response to various noxious stimuli (Chiang et al., 2020). Although this raises the possibility that this projection merely signals pain, it was shown instead to mediate analgesia, thus representing a higher-order response to danger by engaging both evasive motor behaviour and descending inhibition to aid survival.

Overall, various circuits are involved in shaping evasive behaviours. Interestingly, these circuits have been shown to interact with neural populations that can induce freezing, suggesting a mechanism of evaluating the magnitude of threat presented in order to select flight over freezing.

PHYSIOLOGY AND GENETIC MARKERS FOR APPROACH NEURONS

As animals face lower levels of perceived threat, they may begin to engage in exploratory or approach behaviour, which are required for finding prey, investigating objects and assessing the environment. Although ethologically relevant for foraging, mate-seeking and familiarisation with the environment, there is a relative lack of data regarding the neural

circuits that mediate such behaviour. However, some data exists regarding neural populations that mediate approach towards objects in particular.

Photoactivation of medial preoptic area (MPA) CaMKII α neuron projections to the IPAG has been shown to elicit robust approach and pursuit behaviour towards objects, to an extent where activation of the circuit can be used to guide the movement of mice in a remote, closed-loop manner (Park et al., 2018). Notably, the activity MPA^{CaMKII α} neurons also increases during interactions with objects, even at the expense of social interactions, thus representing a physiologically relevant role of these neurons in approaching behaviour towards non-social targets.

However, photoactivation of a GABAergic MPA projection, the MPA^{Vgat}-PAG projections results in increased movement in an open field test and preference to the stimulated side in a real time place preference (RTPP) test (Zhang et al., 2021). Although this could be interpreted as an anxiolytic function, the inability of this projection to create reinforcement during a conditioned place preference test suggests that the increased preference seen in an RTPP test may simply be due to an increase in exploratory behaviour (Ryoo et al., 2021). Photoactivation of this projection is also incapable of affecting object interaction, suggesting a possible separation between object-directed exploration and innate exploration (non-homeostatic driven exploration).

More recently, optogenetic stimulation of zona incerta (ZI) Gad2 neuron projections to the IPAG have been shown to enhance investigation towards novel conspecifics and objects, and was recapitulated by photostimulation of a more discrete subpopulation of GABAergic ZI neurons, namely ZI^{Tac1} neurons (Ahmadlou et al., 2021). Moreover, fibre photometry and inhibition experiments showed these neurons are active during deep investigation with objects and is necessary for such deep investigation. However, whether this novelty-seeking behaviour results in increased exploration in the absence of objects and whether it is ethologically relevant for behaviours such as foraging remain open questions.

PHYSIOLOGY AND GENETIC MARKERS FOR PREDATION NEURONS

Threat-aversion is low during predatory behaviour, as animals potentially expose themselves to being predated on. Thus many of the same nodes involved in evasive behaviours could be expected to also be involved in predation by scaling down threat aversion.

Indeed, the CeA is well known for mediating evasive and freezing behaviours and has been implicated in hunting through CeA^{Vgat}-vIPAG^{vGluT2} projections, which have been shown to mediate the pursuit and capture of prey. A parallel CeA^{Vgat} to parvocellular reticular formation (PCrT) Vgat neuron projection mediates the mandibular movements required for killing of prey (Han et al., 2017). Notably, optogenetic activation of this CeA^{Vgat} population had no change on food intake or conspecific aggression, thus indicating specificity towards hunting prey.

Although photoactivation of MPA^{CaMKII α} -IPAG projections can drive hunting-like behaviour both towards prey and ob-

jects such as pursuit and biting, while not affecting conspecific interactions (Park et al., 2018), it does not conclude with consumption suggesting that the pursuit and capture shown may be an extension of investigatory behaviour rather than hunger-driven.

Optogenetic activation of LH^{Vgat}-IPAG projections drives hunting of prey while imparting negative valence, but also leads to aggressive behaviour towards conspecifics and inedible objects (Li et al., 2018), suggesting that this pathway may extend to general aggression. However, activation of LH^{Gad2} neurons, a partially separate GABAergic population in the LH, does not result in consumption of prey after capture or increased aggression toward conspecifics and moreover were associated with positive valence in an RTPP test (Rossier et al.,

2021). Activation of medial ZI (ZIm) Vgat neuron projections to the vIPAG increased hunting rate while imparting positive valence (Zhao et al., 2019), independently of the increase in food intake normally seen from broadly activating ZI^{Vgat} neurons. The ZI was shown to receive prey-related sensory information through the superior colliculus (SC), activation of which can also lead to predatory behaviour (Shang et al., 2019). Since activation of ZI^{Vgat} was also shown to be able to evoke voracious feeding (Zhang and van den Pol, 2017), it is plausible that the hunting behaviour evoked by the ZI could be hunger-driven. However, another study showed that ZIm^{Gad2} neurons are associated with broad investigatory behaviour rather than hunting *per se* (Ahmadlou et al., 2021), which raises the possibility that the hunting evoked from ac-

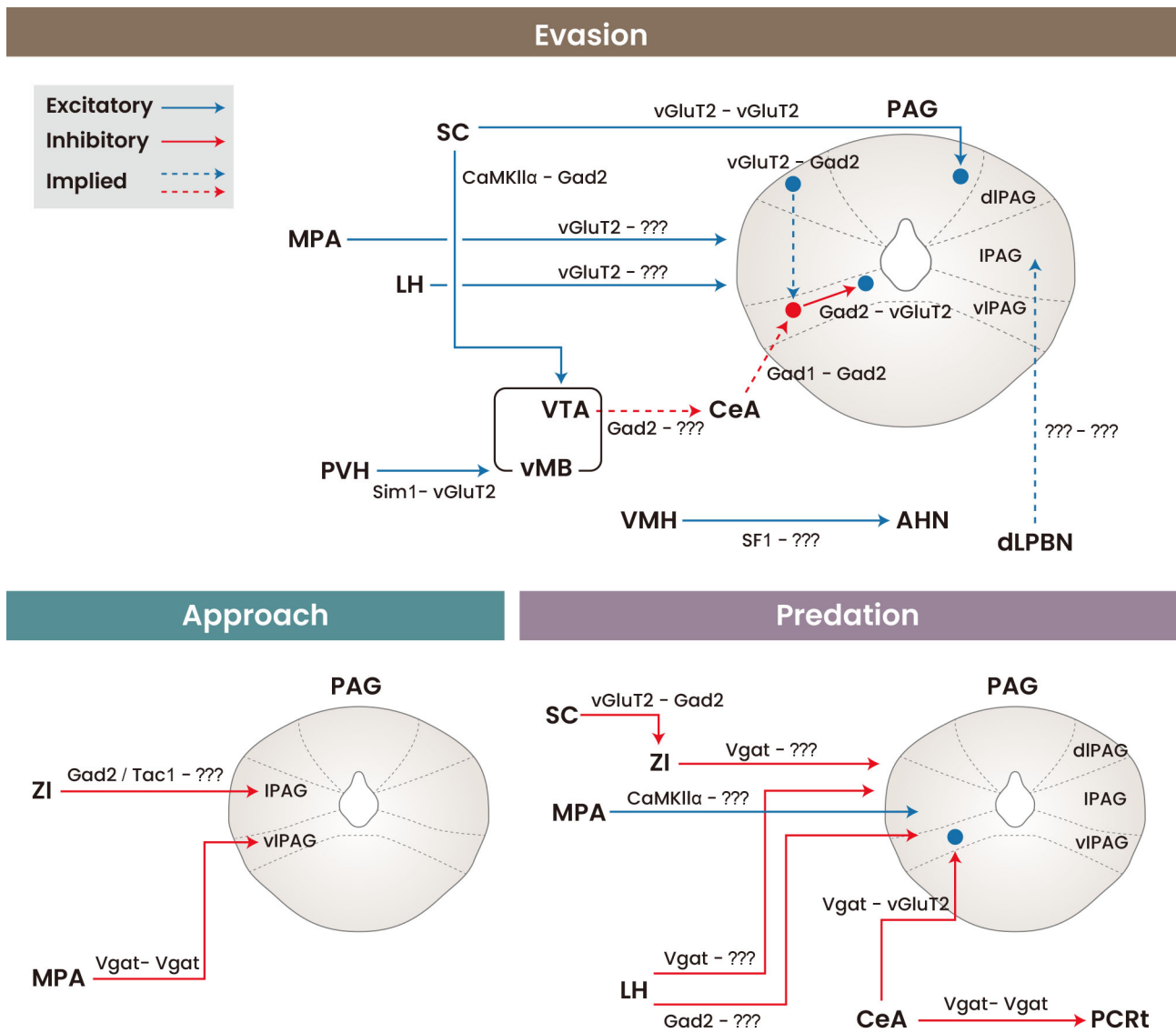


Fig. 1. Schematic summary of neural circuits that evoke evasion, approach and predation. Projection data based on terminal photostimulation experiments. Implied projections have supporting data but their behavioural outputs remain to be elucidated with terminal photostimulation experiments.

tivation of Zl^{Vgat} neurons may be an extension of investigatory behaviour, although it has yet to be shown whether Zl^{Gad2} and Zl^{Vgat} neurons form majorly overlapping populations. Thus, the existence of multiple hunting circuits and their differing valences suggests that the primary motivation for hunting across these circuits may be different; which may range from hunger-driven, investigatory, generalised aggression or scaling down of threat-aversiveness.

THREAT ASSESSMENT AND DECISION IN THE PAG

The convergence of many of the circuits mentioned above at the PAG suggests that the PAG may act to generally integrate and evaluate various types of danger (McNaughton and Corr, 2018).

For instance, the capability of the PAG to drive either freezing or flight, through $vIPAG^{vGluT2}$ and $dIPAG^{vGluT2}$ neurons respectively (Tovote et al., 2016) and the ability of the latter to inhibit the former population, suggests that the PAG can drive diverse types of behaviour in response to differing degrees of perceived risk. Mild threats may elicit freezing, whereas more serious threats may require flight. Low perceived danger may facilitate exploration or pursuit which then pinnacles during predation. Findings such as the direct inhibition of $vIPAG^{vGluT2}$ by CeA^{Vgat} neurons (Han et al., 2017) can be incorporated in this framework by positing that they may act to dampen the freezing response to facilitate predation. Conversely activation of $LH^{vGluT2-vIPAG}$ projections may increase the perception of threat, which may underlie the evasive behaviour in response to an otherwise innocuous stimulus (Li et al., 2018). Evidence in support of a rate-coding mechanism of the PAG in the assessment of risk was shown through optogenetic activation of $dIPAG^{CaMKII\alpha}$ neurons, which evoke freezing at lower frequencies and flight at higher frequencies (Deng et al., 2016). However, population coding mechanisms may also play a part, since endoscopic calcium imaging of $dIPAG$ neurons showed distinct neural populations that are responsive to either higher or lower threatening situations (Reis et al., 2021).

CONCLUSIONS AND FUTURE PERSPECTIVES

Although evasion, approach and predation represent outwardly very different behaviours, their tendency to all converge in the PAG suggests that they are all fundamentally behavioural outputs in response to a perceived level or absence of risk (Fig. 1). Most circuit-based studies of the PAG have identified particular behavioural outputs, but the exact type of information being conveyed by these upstream neural populations to drive the observed behaviour remains to be elucidated. In many studies, inputs from the SC have been implicated in conveying visual signals (Evans et al., 2018; Shang et al., 2019; Zhou et al., 2019), which might be expected given the bias of tectal nuclei to mediate more lower-level sensory-motor reactions. This naturally raises the question as to how other sensory modalities are conveyed to the PAG and whether these connections are specific for innate behaviours. Aside from sensory signals, other relevant inputs include the motivational signals that may drive or inhibit

evasion, approach or predation. Certain motivational drives may be able to modulate risk-aversion (e.g., hunger causing animals to forage or hunt despite the presence of a threat) and naturally raises the questions as to whether this occurs by lowering the perception of danger and/or by inhibiting evasive behaviours. How potentially competing motivational and sensory signals are processed in the PAG to calibrate risk and ultimately drive appropriate behavioural output remains an important question. Such research may require deeper understanding of the micro-circuitry within the PAG and its dynamics under different levels of threat. These types of experiments are challenging for regions such as the $vIPAG$, as fibre-based techniques may involve a certain degree of damage to the $dIPAG$ above it, which may hamper its interactions with the $vIPAG$. Evidence for an ensemble-coding mechanism in the PAG also naturally raises the question as to whether these neural clusters are distinct in terms of their anatomical connections or neuropeptide identities. Finally, there is also sparse research using more modern neuroscience techniques regarding neural circuits that mediate innate exploration and novelty-seeking which are more risk-tolerant behaviours. This may in part be due to difficulties in distinguishing between curiosity-driven exploration and other types of goal-directed exploration, which may require the development of new behavioural models. Together, such future studies will help our understanding of how risk is assessed within the PAG.

ACKNOWLEDGMENTS

This work was supported by Samsung Science and Technology Foundation under Project Number SSTF-BA1301-53, and KAIST Global Singularity Research Program. S.P. was supported by National Research Foundation of Korea (NRF-2021R111A1A01060418).

AUTHOR CONTRIBUTIONS

S.P. and J.R. wrote the manuscript. D.K. oversaw the manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

ORCID

Seahyung Park <https://orcid.org/0000-0002-3021-6158>
Jia Ryoo <https://orcid.org/0000-0003-4440-590X>
Daesoo Kim <https://orcid.org/0000-0001-9915-1463>

REFERENCES

- Ahmadlou, M., Houba, J.H., van Vierbergen, J.F., Giannouli, M., Gimenez, G.A., van Weeghel, C., Darbanfouladi, M., Shirazi, M.Y., Dziubek, J., Kacem, M., et al. (2021). A cell type-specific cortico-subcortical brain circuit for investigatory and novelty-seeking behavior. *Science* 372, eabe9681.
- Chiang, M.C., Nguyen, E.K., Canto-Bustos, M., Papale, A.E., Oswald, A.M.M., and Ross, S.E. (2020). Divergent neural pathways emanating from the lateral parabrachial nucleus mediate distinct components of the pain response. *Neuron* 106, 927-939.e5.
- Deng, H., Xiao, X., and Wang, Z. (2016). Periaqueductal gray neuronal activities underlie different aspects of defensive behaviors. *J. Neurosci.* 36, 7580-7588.
- Evans, D.A., Stempel, A.V., Vale, R., Ruehle, S., Lefler, Y., and Branco, T.

- (2018). A synaptic threshold mechanism for computing escape decisions. *Nature* 558, 590-594.
- Han, W., Tellez, L.A., Rangel, M.J., Jr., Motta, S.C., Zhang, X., Perez, I.O., Canteras, N.S., Shammah-Lagnado, S.J., van den Pol, A.N., and de Araujo, I.E. (2017). Integrated control of predatory hunting by the central nucleus of the amygdala. *Cell* 168, 311-324.e18.
- Kennedy, A., Kunwar, P.S., Li, L.Y., Stagkourakis, S., Wagenaar, D.A., and Anderson, D.J. (2020). Stimulus-specific hypothalamic encoding of a persistent defensive state. *Nature* 586, 730-734.
- Kim, J., Lee, S., Fang, Y.Y., Shin, A., Park, S., Hashikawa, K., Bhat, S., Kim, D., Sohn, J.W., Lin, D., et al. (2019). Rapid, biphasic CRF neuronal responses encode positive and negative valence. *Nat. Neurosci.* 22, 576-585.
- Kunwar, P.S., Zelikowsky, M., Remedios, R., Cai, H., Yilmaz, M., Meister, M., and Anderson, D.J. (2015). Ventromedial hypothalamic neurons control a defensive emotion state. *Elife* 4, e06633.
- Li, Y., Zeng, J., Zhang, J., Yue, C., Zhong, W., Liu, Z., Feng, Q., and Luo, M. (2018). Hypothalamic circuits for predation and evasion. *Neuron* 97, 911-924.e5.
- Mangieri, L.R., Jiang, Z., Lu, Y., Xu, Y., Cassidy, R.M., Justice, N., Xu, Y., Arenkiel, B.R., and Tong, Q. (2019). Defensive behaviors driven by a hypothalamic-ventral midbrain circuit. *eNeuro* 6, ENEURO.0156-19.2019.
- McNaughton, N. and Corr, P.J. (2018). Survival circuits and risk assessment. *Curr. Opin. Behav. Sci.* 24, 14-20.
- Park, S.G., Jeong, Y.C., Kim, D.G., Lee, M.H., Shin, A., Park, G., Ryoo, J., Hong, J., Bae, S., Kim, C.H., et al. (2018). Medial preoptic circuit induces hunting-like actions to target objects and prey. *Nat. Neurosci.* 21, 364-372.
- Reis, F.M., Lee, J.Y., Maesta-Pereira, S., Schuette, P.J., Chakerian, M., Liu, J., La-Vu, M.Q., Tobias, B.C., Ikebara, J.M., Kihara, A.H., et al. (2021). Dorsal periaqueductal gray ensembles represent approach and avoidance states. *Elife* 10, e64934.
- Rossier, D., La Franca, V., Salemi, T., Natale, S., and Gross, C.T. (2021). A neural circuit for competing approach and defense underlying prey capture. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2013411118.
- Ryoo, J., Park, S., and Kim, D. (2021). An inhibitory medial preoptic circuit mediates innate exploration. *Front. Neurosci.* 15, 716147.
- Shang, C., Liu, A., Li, D., Xie, Z., Chen, Z., Huang, M., Li, Y., Wang, Y., Shen, W.L., and Cao, P. (2019). A subcortical excitatory circuit for sensory-triggered predatory hunting in mice. *Nat. Neurosci.* 22, 909-920.
- Tovote, P., Esposito, M.S., Botta, P., Chaudun, F., Fadok, J.P., Markovic, M., Wolff, S.B., Ramakrishnan, C., Fenno, L., Deisseroth, K., et al. (2016). Midbrain circuits for defensive behaviour. *Nature* 534, 206-212.
- Viskaitis, P., Irvine, E.E., Smith, M.A., Choudhury, A.I., Alvarez-Curto, E., Glegola, J.A., Hardy, D.G., Pedroni, S.M., Pessoa, M.R.P., Fernando, A.B., et al. (2017). Modulation of SF1 neuron activity coordinately regulates both feeding behavior and associated emotional states. *Cell Rep.* 21, 3559-3572.
- Wang, L., Chen, I.Z., and Lin, D. (2015). Collateral pathways from the ventromedial hypothalamus mediate defensive behaviors. *Neuron* 85, 1344-1358.
- Zhang, G.W., Shen, L., Tao, C., Jung, A.H., Peng, B., Li, Z., Zhang, L.I., and Tao, H.W. (2021). Medial preoptic area antagonistically mediates stress-induced anxiety and parental behavior. *Nat. Neurosci.* 24, 516-528.
- Zhang, X. and van den Pol, A.N. (2017). Rapid binge-like eating and body weight gain driven by zona incerta GABA neuron activation. *Science* 356, 853-859.
- Zhao, Z.D., Chen, Z., Xiang, X., Hu, M., Xie, H., Jia, X., Cai, F., Cui, Y., Chen, Z., Qian, L., et al. (2019). Zona incerta GABAergic neurons integrate prey-related sensory signals and induce an appetitive drive to promote hunting. *Nat. Neurosci.* 22, 921-932.
- Zhou, Z., Liu, X., Chen, S., Zhang, Z., Liu, Y., Montardy, Q., Tang, Y., Wei, P., Liu, N., Li, L., et al. (2019). A VTA GABAergic neural circuit mediates visually evoked innate defensive responses. *Neuron* 103, 473-488.e6.