Subject heading: Neural Circuits

Suggested title: **'Guardians of the memory gate'**

Alternative title: **'Unforgettable VIP experience'**

Alternative title: **'Surprise: your VIP pass'**

Abstract

Unexpected experiences often lead to strong memories. A new study by Krabbe and Paradiso et al. shows that vasoactive intestinal peptide (VIP)-expressing interneurons of the basolateral amygdala control memory strength by gating aversive stimuli scaled by their unexpectedness.

Body

You receive the usual notice: yearly flu shot is under way. You queue in the morning, feel the little sting of the needle, a slight soreness may remain for a few days but the event quickly fades into oblivion. Another day, walking the far end of the campus, you feel a pang of pain: you are bitten by a wasp. The marks are long gone but you are left with a painful memory and you remember to avoid that corner. Unexpected negative events leading to stronger memories has been a common observation. We are starting to understand the neural implementation behind.

Aversive experiences are instructive to associative learning and neural plasticity that underlie the continuous adaptation to an ever-changing environment. The basolateral amygdala (BLA), a cortex-like structure housing both principle neurons (PN) and a variety of interneurons (IN) that convey local inhibition onto other cells, has been known as a major associative area serving such fear-related memories[1]. In a new study, Krabbe and Paradiso et al.[2] dissect a local BLA circuit of PNs and INs that together form a permissive gate for fear memory formation (Fig.1).

The authors focused on a specific type of interneuron expressing VIP. Mice were trained on a fear conditioning paradigm, where an originally neutral tone (conditioned stimulus, CS) was repeatedly paired with a mild footshock (unconditioned stimulus, US). By using deep brain imaging in transgenic mice expressing the calcium indicator GCaMP6s, 76% of VIP-BLA INs were found to exhibit strong activation upon US, demonstrating that the majority of VIP-BLA INs are sensitive to salient aversive stimuli. Importantly, these responses were themselves plastic, since they gradually decreased as the learning progressed.

What may be the local circuit these VIP INs engage in the BLA in threatening situations? In the mid-nineties, a series of seminal anatomical studies demonstrated that many VIP-expressing INs in the hippocampal formation target other INs, predicting these neurons may release PNs from inhibition, thus giving rise to a 'disinhibitory' mechanism[3,4]. Proving this theory *in vivo* required nearly two decades and the advent of optogenetic techniques[5,6]; these and other studies[7] demonstrated that cortical VIP INs target other INs that express somatostatin (SOM) or parvalbumin (PV), which control the distal dendritic inputs and the axonal outputs of PNs, respectively.

To examine whether a similar local circuit exists in the BLA, Krabbe and Paradiso et al. combined patch clamp recordings in acute slices of transgenic mice with photostimuation and showed that VIP-BLA INs mostly target SOM and PV INs in the BLA as well, while those targeted types exert their strongest inhibition on PNs (in accordance with REF[8]). Notably, inhibitory currents elicited by VIP stimulation were slightly stronger in SOM compared to PV INs, consistent with previous findings[3,6,7]. By *in vitro* and *in vivo* electrophysiology recordings, the authors proved that the activation of VIP-BLA INs leads to inhibition of many other INs but activation of a significant, 26% subset of PNs, thus demonstrating a functional disinhibitory microcircuit in the BLA (Fig.1).

Does this VIP-mediated disinhibitory circuit, engaged by fearful or threatening stimuli, contribute to behavioral learning of stimulus-outcome associations? First, by an impressive tour de force of combining calcium imaging and optogenetic manipulations in freely behaving mice holding a head-mounted miniaturized microscope, the authors demonstrated that optogenetic suppression of VIP-BLA INs during fear conditioning indeed elicited a decrease of PN firing upon footshock presentations. Furthermore, the proportion of CS-responsive PNs was significantly lower when VIP-BLA INs were suppressed, indicating that VIP neuronal activity was necessary for PN plasticity. Next, in a key experiment, Krabbe and Paradiso et al. showed that bilateral optogenetic suppression of VIP-BLA INs reduced US-elicited freezing responses during fear learning and eventually resulted in a weaker fear memory tested on the next day, as indicated by reduced freezing in response to the CS. In contrast, optogenetic activation of VIP-BLA INs by and in itself did not generate a fear response. These experiments demonstrated for the first time that VIP-expressing interneurons of the BLA are necessary albeit not sufficient for associative fear-related memory formation. Putting the pieces together, the VIP-mediated disinhibitory microcircuit forms a gate of associative plasticity in the BLA.

Arguably, the most exciting questions about such a gating mechanism are how and by whom the gate is controlled. The authors observed that VIP-BLA activation decreased after repeated footshock presentations, and at the same time, showed increasing responses to predictive cues, suggestive of prediction error coding. After ruling out a simple habituation effect, the authors went on to show that unexpected footshocks not preceded by CS elicit greater VIP-BLA responses, suggesting that the VIP-BLA memory gate is controlled by the unexpectedness of the aversive stimulus.

What may be the source of the expectation signal to the BLA? By using monosynaptic rabies tracing, monosynaptic inputs to the VIP, SOM and PV BLA neurons were revealed. Of these, the basal forebrain might be considered as a good candidate, since rapid expectation signaling was demonstrated both in cholinergic and non-cholinergic basal forebrain neurons[9,10]. However, the ventral pallidum carries reward predictive signals as well[11], while cortical and thalamic sources cannot be ruled out, necessitating further studies.

The paper by Krabbe and Paradiso et al. continues the research line that elevates the status of VIP interneurons from a curious rarity to real VIP: a small unique subset with disproportional influence. While the VIP-mediated disinhibitory circuit has been proposed as a canonical circuit motif of the cortex[12], the present study extends this concept to the BLA. Important open questions remain: which PNs are subject to disinhibitory gating, in what ways are they special, and what other areas do they modulate? Interestingly, a conserved proportion of one in every four-five PNs has been found to be subject of VIP-mediated disinhibition by both Pi et al.[6] and Krabbe and Paradiso et al.; while the former

study demonstrated that tone responsive auditory cortex neurons are overrepresented in the disinhibited auditory PN population, little is known about the ultimate target of the disinhibitory gate.

Is it 'only' associative memory that is gated by the disinhibitory microcircuit? A line of studies suggested that VIP INs may gate information flow to the cortex to control sensory processing and to the hippocampus to support goal-directed spatial learning, modulated by locomotion[13,14]. This foreshadows that VIP-mediated disinhibition may be a more general mechanism for relaying distant sources of control and amplify signals transferred by relatively few long range axons, also providing economically reasonable solutions for efficient processing.

We predict that future studies will aim at a more detailed understanding of the disinhibitory microcircuit. While VIP has been a successful marker for IN-targeting INs, the known heterogeneity of this population remains somewhat overlooked. Indeed, early anatomy studies[4,15] already demonstrated that while a discrete subset of VIPs, likely co-expressing calretinin (CR), are IN-selective, another subset, co-expressing cholecystokinin, are PN-targeting basket cells. Indeed, the authors found a larger than expected VIP-to-PN connectivity. On one hand, this suggests the presence of additional VIP-mediated mechanisms. On the other hand, a more specific circuit element, for instance VIP-CR interneurons, may ultimately be responsible for the gating mechanisms dissected here. Another interesting finding is that VIP interneurons may target other VIPs[8], adding another layer complexity to the network. Future studies will reveal the exact microarchitecture of the canonical disinhibitory gate.

Altogether, these results revealed a VIP-mediated disinhibitory circuit in the BLA, controlling associative learning of unexpected, behaviorally salient aversive events. In the context of former studies, disinhibitory gating by a conserved microcircuit likely applies to multiple brain areas and may support a range of important aspects of neural information processing.

Authors: Panna Hegedüs[1,2], Sergio Martínez-Bellver[1] and Balázs Hangya[1]

Affiliations:

[1]Lendület Laboratory of Systems Neuroscience, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, H-1083, Hungary

[2]János Szentágothai Doctoral School of Neurosciences, Semmelweis University, Budapest, H-1085, Hungary

Correspondence: hangya.balazs@koki.mta.hu

Acknowledgements

We thank Dr. László Acsády for insightful comments on the manuscript. This work was supported by the 'Lendület' Program of the Hungarian Academy of Sciences (LP2015-2/2015), NKFIH KH125294 and the European Research Council Starting Grant no. 715043. BH is a member of the FENS-Kavli Network of Excellence.

References

1. LeDoux, J. E. Emotion circuits in the brain. *Annu. Rev. Neurosci.* **23**, 155–84 (2000).

2. Krabbe, S. *et al.* Adaptive disinhibitory gating by VIP interneurons permits associative learning. *Nat. Neurosci.* https://dx.doi.org/xxx (2019)

3. Acsády, L., Görcs, T. J. & Freund, T. F. Different populations of vasoactive intestinal polypeptide-immunoreactive interneurons are specialized to control pyramidal cells or interneurons in the hippocampus. *Neuroscience* **73**, 317–34 (1996).

4. Hajos, N., Acsady, L. & Freund, T. F. Target selectivity and neurochemical characteristics of VIP-immunoreactive interneurons in the rat dentate gyrus. *Eur. J. Neurosci.* **8**, 1415–31 (1996).

5. Lee, S., Kruglikov, I., Huang, Z. J., Fishell, G. & Rudy, B. A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nat. Neurosci.* **16**, 1662–70 (2013).

6. Pi, H.-J. *et al.* Cortical interneurons that specialize in disinhibitory control. *Nature* **503**, 521–524 (2013).

7. Pfeffer, C. K., Xue, M., He, M., Huang, Z. J. & Scanziani, M. Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. *Nat. Neurosci.* **16**, 1068–76 (2013).

8. Rhomberg, T. *et al.* Vasoactive Intestinal Polypeptide-Immunoreactive Interneurons within Circuits of the Mouse Basolateral Amygdala. *J. Neurosci.* **38**, 6983–7003 (2018).

9. Hangya, B., Ranade, S. P., Lorenc, M. & Kepecs, A. Central Cholinergic Neurons Are Rapidly Recruited by Reinforcement Feedback. *Cell* **162**, (2015).

10. Lin, S.-C. & Nicolelis, M. a L. Neuronal ensemble bursting in the basal forebrain encodes salience irrespective of valence. *Neuron* **59**, 138–49 (2008).

11. Richard, J. M., Ambroggi, F., Janak, P. H. & Fields, H. L. Ventral Pallidum Neurons Encode Incentive Value and Promote Cue-Elicited Instrumental Actions. *Neuron* **90**, 1165–1173 (2016).

12. Hangya, B., Pi, H.-J., Kvitsiani, D., Ranade, S. P. & Kepecs, A. From circuit motifs to computations: mapping the behavioral repertoire of cortical interneurons. *Curr. Opin. Neurobiol.* **26**, 117–124 (2014).

13. Fu, Y. *et al.* A Cortical Circuit for Gain Control by Behavioral State. *Cell* **156**, 1139–1152 (2014).

14. Turi, G. F. *et al.* Vasoactive Intestinal Polypeptide-Expressing Interneurons in the Hippocampus Support Goal-Oriented Spatial Learning. *Neuron* **101**, 1150-1165.e8 (2019).

15. Gulyás, A. I., Hájos, N. & Freund, T. F. Interneurons containing calretinin are specialized to control other interneurons in the rat hippocampus. *J. Neurosci.* **16**, 3397–411 (1996).

Figure

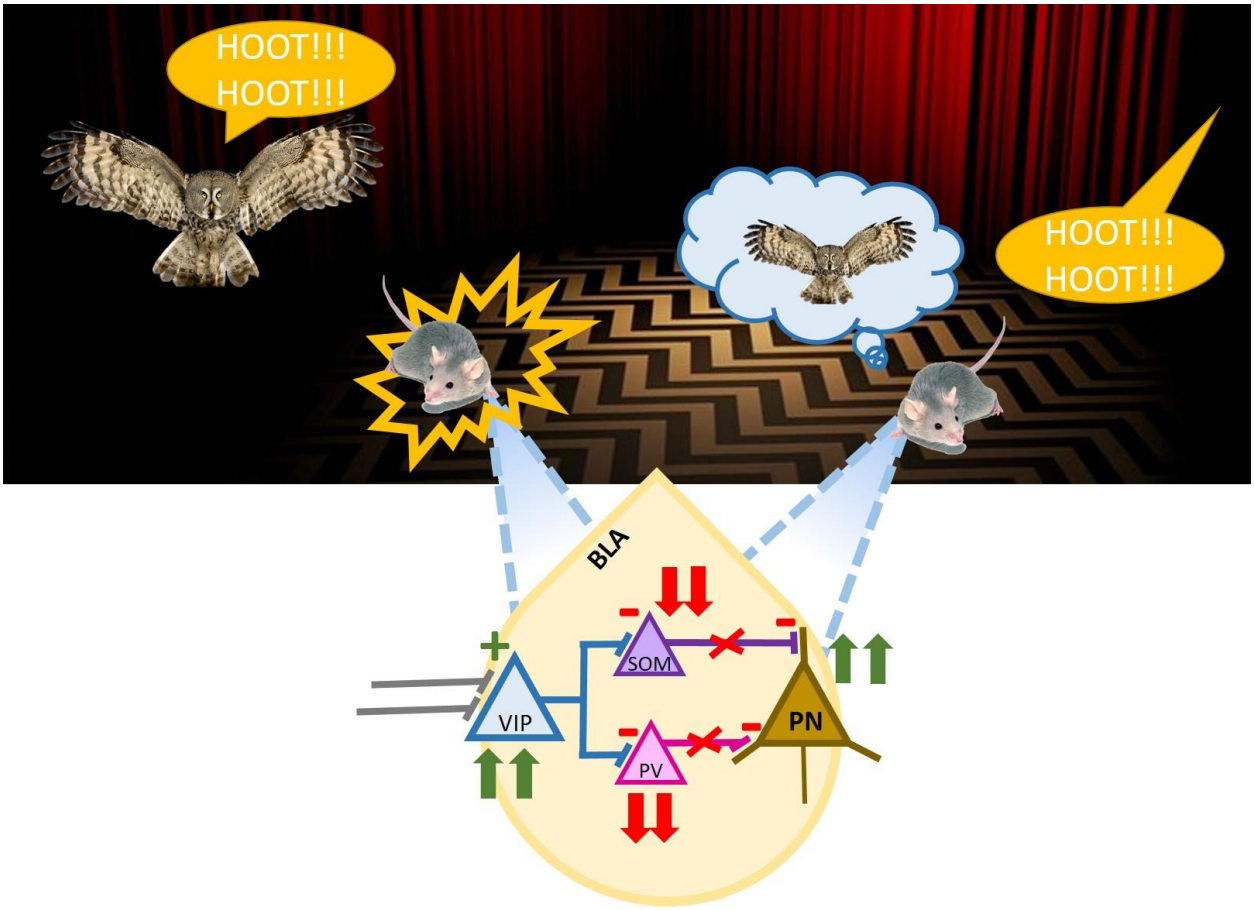
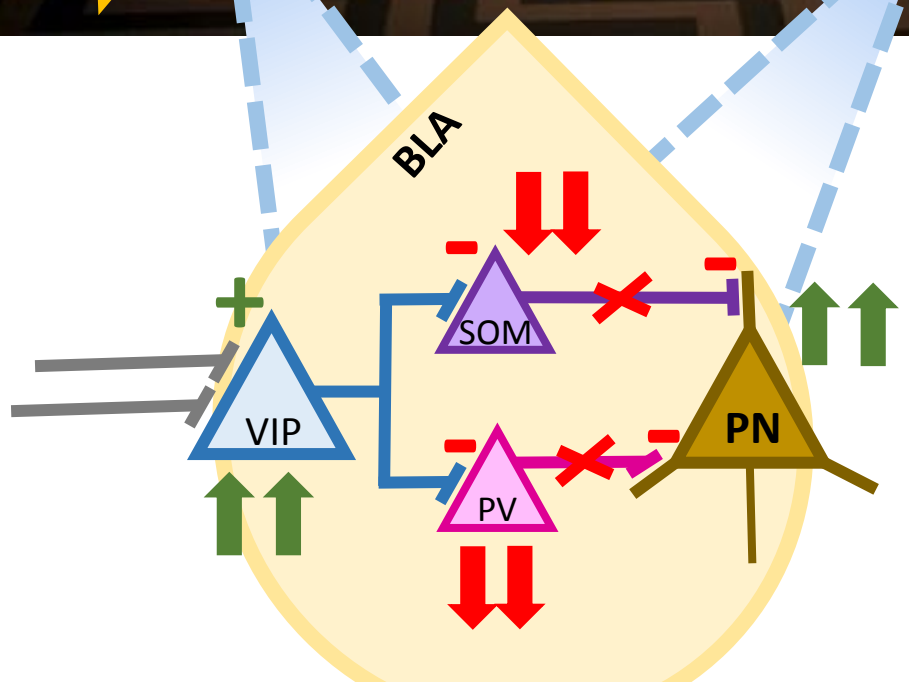


Figure 1: VIP interneurons act as a permissive gate on local BLA circuitry during associative fear learning. An unexpected life-threatening situation (left) can trigger associative memory formation in the basolateral amygdala through disinhibition of principal neurons by a VIP-mediated microcircuit. Once consolidated, these memories may be activated by the VIP disinhibitory system in the presence of environmental clues associated with the original threating experience (right).


HOOT!!!
HOOT!!!
HOOT!!!
HOOT!!!


BLA
SOM
VIP
PV
PN