Journal Club

Editor's Note: These short reviews of recent *JNeurosci* articles, written exclusively by students or postdoctoral fellows, summarize the important findings of the paper and provide additional insight and commentary. If the authors of the highlighted article have written a response to the Journal Club, the response can be found by viewing the Journal Club at www.jneurosci.org. For more information on the format, review process, and purpose of Journal Club articles, please see <http://www.jneurosci.org/content/jneurosci-journal-club>.

A New Look at the Role of Mesoamygdaloid Dopamine Neurons in Aversive Conditioning

Daniel F. Cardozo Pinto,^{1*} Lara Taniguchi,^{1†} Zane C. Norville,^{1†} and Matthew B. Pomrenze^{1*}

¹Nancy Pritzker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California 94305

Review of Tang et al.

Identifying and responding to threats is crucial for survival. Accordingly, the nervous systems of organisms as simple as sea slugs have evolved to support the formation of associations between noxious events (unconditioned stimuli [US]) and cues that predict them (conditioned stimuli [CS]): a phenomenon known as fear conditioning (Walters et al., 1979). In mammals, the amygdala has emerged as a critical mediator of this type of learning. Classical studies pairing a tone (CS) with a footshock (US) demonstrated that amygdala-lesioned rodents are impaired in developing a conditioned response, namely, cued freezing (LeDoux et al., 1990). Subsequent experiments showed an increase in CS-evoked neural activity in the BLA during conditioning (Collins and Paré, 2000) and impairment of fear conditioning in animals treated with an NMDA receptor antagonist in the BLA (Miserendino et al., 1990). These studies, and many others, have established synaptic plasticity in the BLA as a fundamental neural mechanism underlying aversive learning.

Plasticity is under powerful neuromodulatory control, and numerous studies have described how modulators, such as serotonin, norepinephrine, and neuropeptides, participate in fear conditioning (Johansen et al., 2011; Knoll et al., 2011; Sengupta and Holmes, 2019). By comparison, the role of ventral tegmental area (VTA) dopamine neurons in fear learning is less well understood, with most reports relying on spatiotemporally imprecise pharmacological approaches and/or pre-dating modern circuit neuroscience technologies. Nevertheless, growing evidence suggests that, in addition to their role in reward processing, VTA dopamine neurons are critically involved in learning from aversive experiences. For example, VTA dopamine neurons projecting to the ventromedial shell of the NAc encode aversive stimuli (de Jong et al., 2019), those projecting to the CeA participate in fear generalization (Jo et al., 2018), and dopaminergic axons in the BLA are excited by appetitive and aversive stimuli alike (Lutas et al., 2019). Despite evidence implicating both VTA dopamine neurons and the amygdala in aversive learning though, the precise role of dopaminergic projections from the VTA to the BLA in this process remained unclear. In a recent issue of *The Journal of Neuroscience*, Tang et al. (2020) used molecular, electrophysiological, and optogenetic techniques to elucidate the contribution of this circuit to classical fear conditioning.

First, Tang et al. (2020) sought to map the VTA-to-BLA pathway, using anterograde and retrograde circuit-tracing methods. Anterograde labeling of VTA

dopamine neurons revealed that, within the amygdala, these neurons primarily innervate the basal nucleus (BA) and the medial portion of the CeA. Retrograde labeling of axons that project to the BA indicated that BA-projecting dopamine neurons were located preferentially in the dorsal VTA.

With this blueprint in hand, Tang et al. (2020) focused on the functional role of VTA dopamine neurons in fear learning. The researchers recorded the electrical activity of eight genetically identified VTA dopamine neurons during fear conditioning and found that these cells display time-locked responses to the footshock US. While these cells did not respond to the auditory CS before it acquired predictive meaning, three-fourths of them acquired CS responses after six CS-US pairings.

Next, the researchers asked whether the VTA dopamine neurons engaged during fear conditioning are the same neurons that project to the BA. Tang et al. (2020) addressed this question by analyzing the colocalization between TdTomato expressed in dopamine neurons, a retrograde label identifying BA-projecting neurons, and the immediate early gene *cfos* used to label cells activated during fear conditioning. Strikingly, they found that the proportion of BA-projecting VTA dopamine neurons that were *cfos*⁺ was ~4 times greater in mice that underwent fear conditioning compared with controls exposed to the CS alone, suggesting that the VTA-to-BA pathway is active during

Received June 9, 2020; revised Aug. 16, 2020; accepted Sep. 2, 2020.

[†]L.T. and Z.C.N. contributed equally to this work.

D.F.C.P. was supported by National Science Foundation Graduate Research Fellowship. Z.C.N. was supported by Vice Provost for Undergraduate Education at Stanford University Major Grant. We thank Drs. Neir Eshel, Elizabeth E. Steinberg, and Robert C. Malenka for comments on the manuscript.

The authors declare no competing financial interests.

Correspondence should be addressed to Daniel F. Cardozo Pinto at dcardozo@stanford.edu or Matthew B. Pomrenze at pomrenze@stanford.edu.

<https://doi.org/10.1523/JNEUROSCI.1483-20.2020>

Copyright © 2020 the authors

the formation of fear memories. To test whether this circuit is causally involved in fear learning, Tang et al. (2020) expressed the inhibitory opsin Archaelhodopsin in VTA dopamine neurons and silenced their somas with light during the presentation of the US. This led to a significant reduction in cued freezing during a fear memory retrieval test. Then, to confirm that this effect was driven specifically by dopamine signaling in the amygdala, the authors repeated this experiment while inhibiting VTA dopamine neurons' axons in the BA or CeA. Silencing dopaminergic input to the BA, but not CeA, reproduced the reduction in conditioned freezing.

Thus, Tang et al. (2020) used modern techniques to demonstrate that dopamine signaling in the BA is crucial for aversive learning. How do the new data fit with our understanding of the mechanisms governing fear conditioning?

One prevailing circuit model of fear conditioning proposes that auditory and somatosensory inputs converge in the LA to drive Hebbian plasticity during learning, and that the LA then orchestrates a conditioned response by activating (either directly or via the BA) the CeA and its downstream targets (Ehrlich et al., 2009). Dopamine's role was thought to be gating this synaptic plasticity in the LA, and indeed exogenously applied dopamine has been shown to trigger a release of feedforward inhibition onto LA principal cells by interneurons expressing D2 receptors (Bissière et al., 2003), as well as an increase in the excitability of LA principal neurons expressing D1 receptors (Kröner et al., 2005). However, the data of Tang et al. (2020) challenge this model. By showing that VTA dopamine axons are sparse in the LA and that inhibiting the dense dopaminergic projection to the BA reduces conditioned freezing, their experiments imply that dopamine may be driving aversive learning by an entirely different mechanism. Interestingly, the BA receives minimal input from the auditory cortex and auditory thalamus (Polepalli et al., 2020), but it is reciprocally connected with limbic areas (Orsini et al., 2011). This suggests that the primary role of endogenous dopamine release in the BLA during fear learning may be to control plasticity between BA neurons and their inputs from the LA, PFC, ventral hippocampus, and/or other limbic structures, rather than between LA neurons and their sensory inputs.

Moreover, these findings suggest that investigating dopaminergic modulation of synaptic function in various BA cell types

will be an important next step for the field. The BA is made up of excitatory principal projection neurons under tight inhibitory control from a diverse set of interneurons. Recently, a stereotyped synaptic architecture comprising two interneuron subtypes has been described where interneurons expressing somatostatin target principal cell dendrites and those expressing parvalbumin synapse on their perisomatic domains (Wolff et al., 2014). Both cell types show reduced firing on shock exposure to disinhibit principal neuron responses to the US (Wolff et al., 2014), but the source of inhibition onto these interneurons is unknown. The data by Tang et al. (2020) reporting that VTA dopamine neurons display time-locked responses to the US, along with a recent article demonstrating dopamine release in the BA after an aversive stimulus (Lutas et al., 2019), suggest that dopamine may be one such teaching signal. This idea is supported by the finding that dopamine inhibits GABA release onto principal cells from D2 receptor-expressing parvalbumin and somatostatin interneurons (Chu et al., 2012). However, the BA also contains additional populations of interneurons that express cholecystokinin or VIP (Krabbe et al., 2018), and it is not known whether these are sensitive to dopamine. Given that VIP interneurons were recently shown to synapse specifically onto other interneurons to disinhibit principal cells during fear conditioning (Krabbe et al., 2019), it is possible that these neurons may be excited by dopamine to amplify its disinhibitory effect on principal cell activity. Taken in this context, work by Tang et al. (2020) suggests that mapping the expression of dopamine receptors onto various interneuron subclasses and characterizing how they each gate principal neuron activity will be critical for understanding how dopamine shapes information flow in this circuit. Thus, the integration of slice electrophysiology with cell type-specific labeling and transcriptomic sequencing will likely yield valuable new insight here.

In agreement with recent work, Tang et al. (2020) also showed that dopamine axons are prevalent in the medial CeA (Jo et al., 2018). Together with evidence that dorsal raphe/periaqueductal gray dopamine neurons project specifically to the lateral CeA (Cardozo Pinto et al., 2019), these data indicate that the amygdala receives complementary innervation from distinct dopaminergic nuclei. Intriguingly, Tang et al. (2020) showed that dopamine

signaling in the BA is important for the acquisition of cue-fear associations, whereas new evidence suggests that dopamine input to the CeA is necessary for their expression (Lin et al., 2020). Characterizing how these parallel input pathways work together to support aversive learning may be another promising future direction to explore.

In light of these advances, a few methodological questions merit discussion. For example, Tang et al. (2020) found that BA-projecting dopamine neurons reside in the dorsal VTA, in contrast to work showing they are concentrated medially (Lammel et al., 2008). One explanation may be that Lammel et al. (2008) injected a retrograde tracer into the posterior BLA, whereas Tang et al. (2020) targeted a more anterior position ventral to the striatum, a region that receives dense dopaminergic input. Thus, distinct populations of VTA dopamine neurons may innervate the anterior and posterior BLA. Alternatively, the possibility that striatum-projecting dopamine neurons were unintentionally labeled along the needle track of the Tang et al. (2020) injections cannot be entirely excluded. Finally, because the inhibitory opsin Archaelhodopsin has been shown to cause a paradoxical increase in spontaneous transmitter release at terminals (Mahn et al., 2016), complementary loss-of-function manipulations of this pathway may be beneficial in the future. Nevertheless, Tang et al. (2020) present compelling evidence that the dopaminergic VTA-to-BA pathway is causally implicated in fear conditioning: an important contribution to our understanding of associative learning and an example of the value of revisiting old questions as new methodologies become available.

References

- Bissière S, Humeau Y, Lüthi A (2003) Dopamine gates LTP induction in lateral amygdala by suppressing feedforward inhibition. *Nat Neurosci* 6:587–592.
- Cardozo Pinto DF, Yang H, Pollak Dorocic I, de Jong JW, Han VJ, Peck JR, Zhu Y, Liu C, Beier KT, Smidt MP, Lammel S (2019) Characterization of transgenic mouse models targeting neuromodulatory systems reveals organizational principles of the dorsal raphe. *Nat Commun* 10:4633.
- Chu HY, Ito W, Li J, Morozov A (2012) Target-specific suppression of GABA release from parvalbumin interneurons in the basolateral amygdala by dopamine. *J Neurosci* 32:14815–14820.
- Collins DR, Paré D (2000) Differential fear conditioning induces reciprocal changes in the

- sensory responses of lateral amygdala neurons to the CS⁺ and CS⁻. *Learn Mem* 7:97–103.
- de Jong JW, Afjei SA, Pollak Dorocic I, Peck JR, Liu C, Kim CK, Tian L, Deisseroth K, Lammel S (2019) A neural circuit mechanism for encoding aversive stimuli in the mesolimbic dopamine system. *Neuron* 101:133–119.
- Ehrlich I, Humeau Y, Grenier F, Ciocchi S, Herry C, Lüthi A (2009) Amygdala inhibitory circuits and the control of fear memory. *Neuron* 62:757–771.
- Jo YS, Heymann G, Zweifel LS (2018) Dopamine neurons reflect the uncertainty in fear generalization. *Neuron* 100:916–925.e3.
- Johansen JP, Cain CK, Ostroff LE, Ledoux JE (2011) Molecular mechanisms of fear learning and memory. *Cell* 147:509–524.
- Knoll AT, Muschamp JW, Sullivan SE, Ferguson D, Dietz DM, Meloni EG, Carroll FI, Nestler EJ, Konradi C, Carlezon WA (2011) Kappa opioid receptor signaling in the basolateral amygdala regulates conditioned fear and anxiety in rats. *Biol Psychiatry* 70:425–433.
- Krabbe S, Gründemann J, Lüthi A (2018) Amygdala inhibitory circuits regulate associative fear conditioning. *Biol Psychiatry* 83:800–809.
- Krabbe S, Paradiso E, d'Aquin S, Bitterman Y, Courtin J, Xu C, Yonehara K, Markovic M, Müller C, Eichlisberger T, Gründemann J, Ferraguti F, Lüthi A (2019) Adaptive disinhibitory gating by VIP interneurons permits associative learning. *Nat Neurosci* 22:1834–1843.
- Kröner S, Rosenkranz JA, Grace AA, Barrionuevo G (2005) Dopamine modulates excitability of basolateral amygdala neurons in vitro. *J Neurophysiol* 93:1598–1610.
- Lammel S, Hetzel A, Häckel O, Jones I, Liss B, Roeper J (2008) Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron* 57:760–773.
- LeDoux JE, Cicchetti P, Xagoraris A, Romanski LM (1990) The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *J Neurosci* 10:1062–1069.
- Lin R, Liang J, Wang R, Yan T, Zhou Y, Liu Y, Feng Q, Sun F, Li Y, Li A, Gong H, Luo M (2020) The raphe dopamine system controls the expression of incentive memory. *Neuron* 106:498–514.e8.
- Lutas A, Kucukdereli H, Alturkistani O, Carty C, Sugden AU, Fernando K, Diaz V, Flores-Maldonado V, Andermann ML (2019) State-specific gating of salient cues by midbrain dopaminergic input to basal amygdala. *Nat Neurosci* 22:1820–1833.
- Mahn M, Prigge M, Ron S, Levy R, Yizhar O (2016) Biophysical constraints of optogenetic inhibition at presynaptic terminals. *Nat Neurosci* 19:554–556.
- Miserendino MJ, Sananes CB, Melia KR, Davis M (1990) Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature* 345:716–718.
- Orsini CA, Kim JH, Knapska E, Maren S (2011) Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. *J Neurosci* 31:17269–17277.
- Polepalli JS, Gooch H, Sha P (2020) Diversity of interneurons in the lateral and basal amygdala. *Sci Learn* 5:1–9.
- Sengupta A, Holmes A (2019) A discrete dorsal raphe to basal amygdala 5-HT circuit calibrates aversive memory. *Neuron* 103:489–417.
- Tang W, Kochubey O, Kintscher M, Schleggenburger R (2020) A VTA to basal amygdala dopamine projection contributes to signal salient somatosensory events during fear learning. *J Neurosci* 40:3969–3980.
- Walters ET, Carew TJ, Kandel ER (1979) Classical conditioning in *Aplysia californica*. *Proc Natl Acad Sci USA* 76:6675–6679.
- Wolff SB, Gründemann J, Tovote P, Krabbe S, Jacobson GA, Müller C, Herry C, Ehrlich I, Friedrich RW, Letzkus JJ, Lüthi A (2014) Amygdala interneuron subtypes control fear learning through disinhibition. *Nature* 509:453–458.