Comment on: **A Class of GABAergic Neurons in the Prefrontal Cortex Sends Long-Range Projections to the Nucleus Accumbens and Elicits Acute Avoidance Behavior**
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Long-range GABAergic neurons in the prefrontal cortex modulate behavior

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ABSTRACT

Cortical glutamatergic projections are extensively studied in behavioral neuroscience, whereas cortical GABAergic projections to downstream structures have been overlooked. A recent study by Lee *et al.* (2014) used optogenetic and electrophysiological techniques to characterize a behavioral role for long-projecting GABAergic neurons in the medial prefrontal cortex. In this Neuro Forum, we discuss the potential implications of this study in several learning and memory models.

It has been a long standing belief that behavior is modulated by cortical control over subcortical structures exclusively through excitatory glutamatergic projections. There has been a general consensus that inhibitory GABAergic neurons in the cortex participate mainly in local microcircuits. For example, GABAergic interneurons in the medial prefrontal cortex (mPFC) compose local microcircuits that shape prefrontal coding of fear expression (Courtin *et al.*, 2014). Generally, models of top-down cortical control involve excitatory projections to downstream regions. In such cases, behavioral regulation depends on whether mPFC fibers selectively target excitatory or inhibitory neurons. This is the case of the mPFC-amygdala model proposed for fear regulation: the prelimbic prefrontal cortex (PL) drives fear by its excitatory projections to excitatory neurons in the basolateral amygdala (BLA); whereas the infralimbic prefrontal cortex (IL) inhibits fear by its excitatory projections to GABAergic intercalated cells (ITCs) in the amygdala (Sotres-Bayon and Quirk, 2010).

Other models involve mPFC projections to distinct subregions that compete for behavioral control. In the reward system, PL excitatory neurons drive reward-seeking through the nucleus accumbens (NAcc) core, whereas IL excitatory neurons inhibit reward seeking through its connections with the NAcc shell (Peters *et al.*, 2009). However, until now, there was no functional evidence of prefrontal inhibitory projections to downstream regions that could directly influence behavior. A recent study by Lee and colleagues elegantly characterized long-projecting GABAergic neurons in the mPFC and tested if these projections to the NAcc can modulate behavior (Lee *et al.*, 2014).

The authors identified cortical GABAergic projections by infusing a viral vector containing channelrhodopsin (AAV-DIO-ChR2-EYFP) into the mPFC of Dlxi12b-Cre mice to selectively target GABAergic neurons. Labeling of GABAergic mPFC fibers was detected at several downstream regions including NAcc and BLA. Using whole-cell recordings in NAcc, the authors found that optogenetic activation of mPFC ChR2-containing terminals in NAcc elicited IPSCs in NAcc neurons. Blocking $GABA_A$, but not glutamate, receptors in NAcc abolished the IPSCs, indicating that these mPFC long-range projections are GABAergic. Previous studies have shown that activation of GABAergic projections from subcortical regions to NAcc elicit aversion. Thus, the authors tested if cortical GABAergic projections could also mediate aversive responses. Indeed, they found that mice refrained from entering a chamber paired with stimulation of GABAergic mPFC fibers in NAcc, suggesting that long-range GABAergic neurons in mPFC can drive aversion through its projections to NAcc.

These findings are timely given that many research groups have investigated how cortical *glutamatergic* projections modulate behavior, overlooking a potential role for cortical *GABAergic* projections. All previous behavioral studies on cortical GABAergic neurons have focused exclusively on local inhibitory circuits. Lee *et al*., (2014) is the first study to demonstrate that cortical GABAergic projections to downstream targets can modulate aversive responses. Going forward, future studies need to characterize the potential role of cortical GABAergic projections in the neuropathology of mental illness.

GABAergic neurons in aversive behavior: cortical versus subcortical projections

Previous studies have demonstrated that subcortical GABAergic projections play a critical role in control of behavior. For example, long-range GABAergic projections from the ventral tegmental area (VTA) can elicit aversion by inhibiting NAcc (Creed *et al.*, 2014). Also, long-range GABAergic projections from the rostromedial tegmentum drive aversion by indirectly inhibiting NAcc (Lammel *et al.*, 2012). Furthermore, the central amygdala (CeA) can drive aversive behavior through long-range GABAergic projections to the midbrain periaqueductal grey matter (Penzo *et al.*, 2014).

Evidently, *subcortical* structures can mediate aversion through GABAergic projections, but it remained untested whether *cortical* GABAergic projection neurons could elicit aversive behavior as well. In this study, Lee et al. (2014) used the real-time place aversion test (RTPA) to demonstrate that indeed cortical GABAergic long-range projections can drive aversion through NAcc. While the authors make an important insight about cortical GABAergic projections and acute aversion, further follow-up studies are necessary to determine the role of these projections in both avoidance and aversive memories. In RTPA, one side of a two-compartment box is paired with a stimulus that triggers an acute aversive response. However, it is important to note that RTPA is different from conditioned place aversion (CPA), in which rodents are brought back to the two-compartment box the following day for an aversive memory test. The RTPA also differs from conditioned avoidance paradigms, in which rodents learn to execute an instrumental response (*e.g*.: shuttling or stepping onto a platform) in order to avoid a signaled footshock. Therefore, further studies using CPA and conditioned avoidance are required to test if activation of mPFC GABAergic projections to NAcc can create an aversive memory, as well as modulate conditioned avoidance responses.

Optogenetic activation can reveal if a region or projection may modulate a specific behavioral response. However, only silencing can determine if a region or projection is necessary to elicit such behavioral response. Therefore, silencing GABAergic mPFC efferents to NAcc is required to test if this projection is essential for aversion coding. On another note, many studies have focused on the antagonistic roles PL and IL have on different behaviors. Although in the present study the authors did not dissociate PL from IL, it is likely that long-range GABAergic neurons in each structure mediate different behaviors.

The findings of Lee *et al*. (2014) emphasize the need to re-evaluate optogenetic projection studies in the cortex, which focus mainly on glutamatergic neurons. As an example, it was previously shown that rodents would self-stimulate glutamatergic projections from BLA to NAcc, but not from mPFC to NAcc (Stuber *et al.*, 2011). Could it be that GABAergic mPFC projections, rather than glutamatergic, would influence hedonic/aversive behaviors through NAcc? Another optogenetic study focused on glutamatergic projections from mPFC to the amygdala in the fear circuit (Cho *et al.*, 2013). Could we be missing a key pathway in the fear circuit by omitting GABAergic mPFC projections to downstream regions? We now know that the field has overlooked a potentially critical mechanism for cortical control of behavior.

GABAergic mPFC neurons mediate aversion: a role in avoidance?

Avoidance is a core symptom of all anxiety disorders, and it can severely decrease a patient's quality of life by interfering with goal attainment. Moreover, avoidance impedes fear extinction by reducing patients' exposure to trauma reminders

within a safe context. Elucidating the mechanisms underlying avoidance will help guide treatments for anxiety disorders. In rodents, pharmacological inactivation of either PL or NAcc impairs the expression of conditioned avoidance, suggesting that PL projections to NAcc mediate expression of conditioned avoidance (Bravo-Rivera *et al.*, 2014). There is evidence that aversion coding from dopaminergic signaling in NAcc is necessary for conditioned avoidance learning (Darvas *et al.*, 2011). Interestingly, Lee *et al*. (2014) showed that stimulating GABAergic mPFC projections to NAcc elicit aversion in RTPA. Although the authors did not test if conditioned avoidance depends on PL GABAergic projections to NAcc, the aversion induced by activating these projections may contribute to the aversion necessary to learn conditioned avoidance(see Figure 1A).

In conditioned avoidance, rodents suppress freezing in order to avoid, and recent studies have shown that IL is necessary for freezing suppression in conditioned avoidance (Moscarello and LeDoux, 2013; Bravo-Rivera *et al.*, 2014). A proposed model of IL-mediated reduction of fear suggests that IL glutamatergic projections activate ITC neurons, which in turn inhibit CeA output that drives freezing (Sotres-Bayon and Quirk, 2010). However, an alternative is that long-range GABAergic neurons in IL decrease fear expression by directly inhibiting BLA neurons that drive freezing through CeA (see Figure 1B).

Cortical long-projecting GABAergic neurons: a role in pathology?

Given that long-projecting GABAergic neurons in the cortex can gate behavior through subcortical structures, there is a need to re-evaluate faulty circuits in psychiatric

disorders. Several studies have suggested that abnormalities in cortical GABAergic neurons are characteristic of several psychiatric disorders including anxiety, drug abuse, schizophrenia and autism. For example, a recent study reported that transgenic mice deficient in cortical GABAergic neurons showed impaired fear extinction and attention (Bissonette *et al.*, 2014). Another study demonstrated that activating GABAergic neurons in PL decrease reward seeking in rodents (Sparta *et al.*, 2014), suggesting that enhancing GABAergic activity in mPFC could be a target for treating addiction. All these studies focused on local GABAergic neurons in the cortex, but they overlooked existing long-projecting GABAergic neurons. Further studies characterizing the role of such cortical projections in behavioral regulation may contribute to the understanding and treatment of many mental illnesses.

Further studies

The study by Lee and colleagues opened opportunities for exciting questions. Future studies could test whether activating mPFC GABAergic projections to NAcc can form an aversive memory. This could be achieved by optogenetically activating these projections in a RTPA paradigm, and testing if rodents avoid the stimulation-paired side on the following day. Also, it could be tested whether silencing mPFC GABAergic projections to NAcc impairs retrieval of a consolidated aversive memory.

Lee *et al*. (2014) did not distinguish between medial prefrontal subregions; evidence suggests that PL promotes fear, whereas IL inhibits fear. Thus, differentiating PL and IL GABAergic projections may reveal opposing roles of these structures in aversion. The present findings may be also relevant for the fear conditioning field, in

which the current model states that IL glutamatergic projections suppress fear by indirectly inhibiting CeA. Future experiments can assess whether optogenetically stimulating IL GABAergic projections suppress fear by directly inhibiting CeA-projecting glutamatergic neurons in BLA. This would suggest another mechanism of IL-mediated fear suppression.

Conclusion

In summary, the study by Lee and colleagues not only characterized long-projecting GABAergic neurons in mPFC, but also showed that these projections can mediate behavior downstream. The cortex is known to be critical for controlling behavior and this study broadens our opportunities to understand the mechanisms underlying behavioral regulation. It also reminds us that technology optimization and cautious examination can change long-standing beliefs in neuroscience. Further studies evaluating the role of these cortical GABAergic projections in aversive and reward circuits will help to understand top-down control of behavior.

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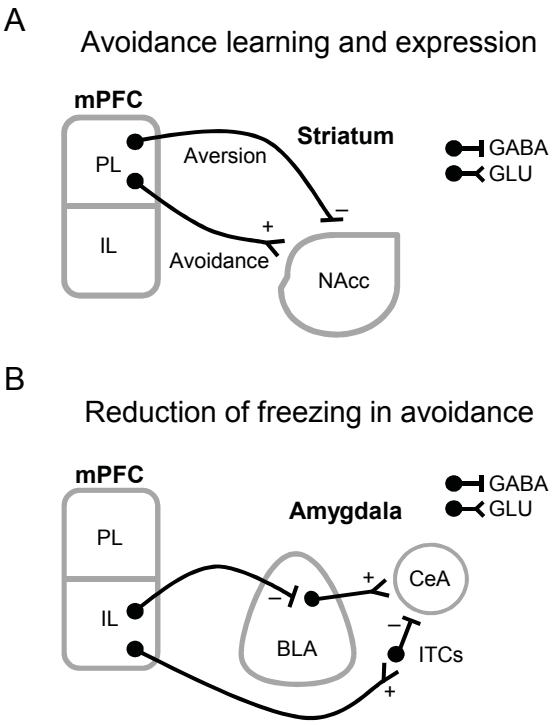
Figure legend

Figure 1. Possible roles of GABAergic projections from mPFC to subcortical structures in conditioned avoidance. **A**. PL GABAergic projections to NAcc could drive aversion necessary for conditioned avoidance learning, whereas PL glutamatergic projections to NAcc could drive expression of conditioned avoidance. **B**. IL glutamatergic projections to ITCs could decrease freezing by inhibiting CeA, whereas IL GABAergic projections to BLA could decrease freezing by directly inhibiting CeA-projecting glutamatergic neurons in BLA. *Legend*: GABA: GABAergic projection, GLU: Glutamatergic projection, mPFC: medial prefrontal cortex, PL: prelimbic cortex, IL: infralimbic cortex, NAcc: nucleus accumbens, BLA: basolateral nucleus of the amygdala, ITCs: intercalated cells of the amygdala, CeA: central nucleus of the amygdala.



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