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Chandelier cells illuminate inhibitory control of prefrontal-amygdala outputs

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Abstract

Inhibitory interneurons comprise a diverse subpopulation of cells that are critical to circuit function. How distinct inhibitory microcircuits control long-range projections remains poorly understood. A recent study by Lu and colleagues (2017) uncovered a unidirectional microcircuit of prefrontal chandelier cells that preferentially innervate and suppress long-range amygdala-projecting pyramidal cells.

Keywords

interneurons; prelimbic; amygdala; microcircuits

Main Text

The neocortex subserves our highest order cognitive abilities including memory, planning, decision-making, behavioral flexibility, and abstract thought. Cortical regions are organized in a laminar fashion and consist primarily (80–90%) of long-range projection or pyramidal cells (PCs) [1]. Distinct long-range projecting PC ensembles function as part of subnetworks that ultimately help control a myriad of behaviors. Approximately 10–20% of cortical neurons are GABAergic interneurons. Interneurons represent a diverse class of cells and have been defined on multiple levels including morphology, physiology, gene expression, connectivity, and development [2]. Understanding how the cortex functions at both the local and long-range level remains a fundamental question in neuroscience.

The advent of new technology and genetic approaches that isolate specific interneuron cell types has now permitted the precise function of interneurons in cortical circuits to be understood. One subtype of interneurons, chandelier cells (ChCs, also referred to as axo-axonic cells), are a unique class of cortical interneuron that selectively innervate the axon initial segment (AIS) of PCs. This positions them to serve a pivotal role in regulating PC

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output to downstream targets. Confirmation of this idea has remained elusive due to poor genetic markers for ChCs. In addition, whether ChCs serve to inhibit or excite PC output is a subject of controversy [3].

In a recent study, Lu and colleagues (2017) performed an elegant set of experiments to understand how ChCs in the prelimbic (PL) subdivision of the medial prefrontal cortex (mPFC) function in cortical circuits at both the local and global level. Specifically, the authors sought to disentangle how ChCs are recruited to influence two separate subnetworks of PCs in PL: the PL-BLA network vs the PL-PL inter-hemispheric network [4].

To selectively target ChCs, the authors used tamoxifen induction in pregnant *Nkx2.1-CreER; Rosa26-loxpSTOPlox-p-TdTomato (Ai14)* mice at embryonic day 17.5 to label a specific subset of layer 2 (L2) ChCs throughout the frontal cortex. They next used two retrograde tracers - cholera toxin subunit B conjugated with two distinct fluorophores - to label and isolate two projection-defined PC subpopulations within PL (contralateral PL→PL vs PL→BLA). Paired whole-cell patch recordings revealed a striking difference between the two subpopulations insofar as L2 ChCs favored innervation of BLA projecting PCs over contralateral PL projecting PCs.

The authors next sought to characterize the inputs that recruit this distinct population of ChCs. To this end, they examined local excitatory inputs within PL that converge onto L2 ChCs. They recorded ChCs synaptic currents after spikes were evoked in either BLA-projecting PCs or contralateral PL projection PCs. Interestingly, they found that a population of contralateral PL projecting cells preferentially innervates ChCs compared to BLA-projecting PCs despite the fact that BLA-projecting PCs were physically closer to ChCs. The authors further confirmed these results using an optogenetic approach and provided compelling evidence that L2 ChCs receive stronger and denser innervation from contralateral PL projecting PCs (i.e., ChCs are recruited by a different population than they themselves innervate creating a unidirectional circuit). Importantly, the authors also demonstrated that this pattern of connectivity does not hold true for parvalbumin (PV) positive interneurons. PV interneurons innervate the perisomatic region of PCs and did not show selective connectivity with a projection-defined subset of PCs in PL. PV interneurons displayed extensive, nonselective reciprocal connections with proximal PCs, differentiating them from ChCs. Thus, the authors demonstrated that ChCs exhibit a largely unidirectional connectivity pattern and therefore likely represent a distinct inhibitory microcircuit that may act locally to control global network function.

The authors next addressed the contribution of ChCs to network function *in vivo* using optogenetic methods and single-unit recordings in awake, behaving mice. After selectively expressing channelrhodopsin (ChR2), a light sensitive ion channel, to depolarize and excite ChCs in the PL, they recorded single-unit activity in PL and field-potentials in the BLA. The results of these experiments were largely consistent with the picture emerging from the *in vitro* studies: L2 ChCs activation *in vivo* suppressed PC firing, and produced a robust stimulation-evoked positive-going (inhibitory) alteration in BLA field-potentials, suggesting that PL ChCs preferentially inhibit BLA projecting PCs.

Overall, this impressive set of experiments provides strong evidence that prefrontal ChCs represent a distinct microcircuit that controls PL output to the BLA. Importantly, this appears to be largely unidirectional insofar as L2 ChCs do not appear to receive reciprocal feedback from the same PL neurons upon which they synapse. In contrast, a distinct subset of contralateral PL projecting PCs (in both the ipsi- and contra-lateral hemispheres) strongly recruit these L2 ChCs which may subsequently act to reduce PL output to the BLA (see Figure 1A). This circuit represents a novel mechanism by which prefrontal cortex networks can exert top-down control over downstream targets, including the amygdala.

However, several important questions remain regarding the functional implications of this newly discovered circuit mechanism. For example, is the revealed role of ChCs unique to the PL, or is it a more general organizing principle relevant to other cortical regions as well? Moreover, and importantly, what is the functional relevance of this particular unidirectional circuit in behavior? The authors propose that this circuit may have a role in fear expression insofar as previous work has implicated PL, the BLA, and reciprocal connections between these regions during heightened fear states [5,6]. Clearly, the circuit revealed by the authors suggests a mechanism that could reduce the activity of BLA projectors in PL to reduce fear expression (Figure 1A). An interesting alternative is that PL PCs recruit ChCs in the neighboring infralimbic (IL) cortex to yield inhibition of BLA projectors in IL, and an increase in fear expression (Figure 1B). Indeed, it has been previously proposed that reciprocal interactions between the PL and IL mediate fear renewal, a high fear state that occurs when an extinguished conditioned stimulus is encountered outside the extinction context [7]. The recruitment of IL ChCs by PL PCs would be expected to inhibit the activity of BLA-projecting IL neurons that normally serve to dampen fear, thereby leading to fear relapse (Figure 1B). Of course, PV interneurons in the prefrontal cortex play a role in fear expression as well [8,9], and it would be interesting to know whether and how PV interneurons and ChCs interact to control PL output.

Defining the roles of interneurons in cortical networks is critical to understanding the role of these networks in regulating behavior. The work by Lu and colleagues (2017) is an important first step in defining the role for ChCs in regulating the activity of prefrontal networks. Ultimately, these findings will drive new research in the field to understand the functional relevance of prefrontal ChCs in behavior and cognition.

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Figure 1. Schematic representation of the unidirectional circuit from Lu et al. 2017

A) Lu and colleagues (2017) described the discovery of a unidirectional circuit by which the PL-PL interhemispheric network controls long-range interactions with the BLA.

Contralateral projecting PL PCs (red triangles) synapse on L2 ChCs (blue circle). These ChCs in turn inhibit BLA-projectors in PL (purple triangles). This circuit may be positioned to decrease fear states. **B)** We propose a hypothetical model in which the bilateral PL-PL network may control IL signaling to the BLA. Contralateral projecting PL PCs (red triangles) may rely on a similar mechanism by which they synapse upon and recruit L2 ChCs in the IL (blue circle) to dampen BLA projectors. This would represent a circuit that may promote high fear states. Dashed lines represent a decrease in projection strength. PL: prelimbic; BLA: basolateral amygdala; L2: layer 2; ChC: chandelier cells; PCs: projection cells (or pyramidal cells); IL: infralimbic.