The Impact of Acetylcholine on Basolateral Amygdala Macrocircuits

Evelyne K. Tantry
(evelyne.tantry@rice.edu)
Department of Neuroscience,
Rice University
6100 Main St, Houston, TX 77005
USA

Joshua Ortiz-Guzman
(joshua.ortiz@bcm.edu)
Department of Developmental Biology,
Baylor College of Medicine
1 Baylor Plaza, Houston, TX 77030
USA

Benjamin R. Arenkiel
(arenkiel@bcm.edu)
Department of Neuroscience,
Baylor College of Medicine
1 Baylor Plaza, Houston, TX 77030
USA

Abstract

Neural circuits governing food intake have been widely studied. However, our current understanding hinges on a binary hypothalamic neuronal model that fails to address more adaptive feeding behaviors underpinning variable environmental conditions. Previous work in our lab posits an extra-hypothalamic circuit involving the cholinergic-rich diagonal band of Broca (DBB) and the valence encoding basolateral amygdala (BLA). To further analyze this circuit, we use a projection defined approach to characterize the cellular composition of the BLA. We used a stereotactic frame for bilateral injections of channelrhodopsin and tdTomato containing viruses into the DBB, and the nucleus accumbens (NAc) or the lateral hypothalamic area (LHA), respectively. The latter regions were chosen because of their established involvement in feeding. We then determined projection profiles of BLA cells using channelrhodopsin assisted circuit mapping (CRACM) and optogenetics, and found that neurons projecting to the LHA exclusively possess fast-acting nicotinic synapses, whereas neurons expressing slow-acting muscarinic synapses project exclusively to the NAc. The contrasting nature these receptors indicate there to be more dynamic neural regions involved in orchestrating complex feeding behaviors.

Keywords: feeding behavior; valence; electrophysiology; optogenetics; neural circuits

Background

Human behavior is dependent on energy supplied by metabolic systems that maintain caloric homeostasis. Vital to this system is feeding behavior, which when disrupted, can lead to disorders such as anorexia and hyperphagia. Despite the fundamental importance of feeding, we lack a basic understanding of underlying mechanisms implicated in feeding behaviors.

Previous studies have demonstrated that food intake is largely governed by “stop” and “go” signals from the Hypothalamus (Jennings, J.H., et al., 2013). The diagonal band of Broca (DBB), basolateral amygdala (BLA), nucleus accumbens (NAc), and lateral hypothalamic area (LHA) are regions that have emerged as extra-hypothalamic regions in the brain that may regulate food intake. The DBB is a cholinergic center of the brain involved in the regulation and release of acetylcholine. Classically, the DBB is known to govern attention and motivation, but when cholinergic signaling in the DBB is abolished, mice display a hyperphagic phenotype and become obese (Herman et al., 2016). This same study looks at projections from the DBB to various nodes, one cholinergic specific projection being the BLA, a key regulator of positive and negative valence stimuli (Beyeler, A., et al., 2018). Although these tracings implicate connectivity, it was unknown how they were connected. Therefore, through viral tracing and electrophysiological testing, it was discovered that the DBB and BLA were anatomically and functionally connected.

Using terminal field stimulation of the cholinergic fibers from the DBB to the BLA via ChR2 using an optic fiber in awake animals, we found a proportional decrease in food intake, thus implicating the role of the BLA in appetite. This discovery led to our current study of BLA projection-defined cells using viral constructs to expand our current understanding of the BLA and its involvement with feeding.

Connectivity between the basolateral amygdala and basal forebrain regions

Little is known about the genetic diversity of BLA neurons; thus, we aim to classify cells using a projection defined approach. Viral tracing experiments have implicated a number of regions to which the BLA projects, among which include the LHA and the NAc. Previous work established the LHA as a modulator of hunger and satiation, while the nucleus accumbens mediates the hedonic impact of food rewards (Castro, D.C., et al., 2015). These regions work in tandem to mediate affective and salient motivational functions. Therefore, we postulate that the LHA and the NAc may be involved in the feeding systems of the DBB and the BLA.

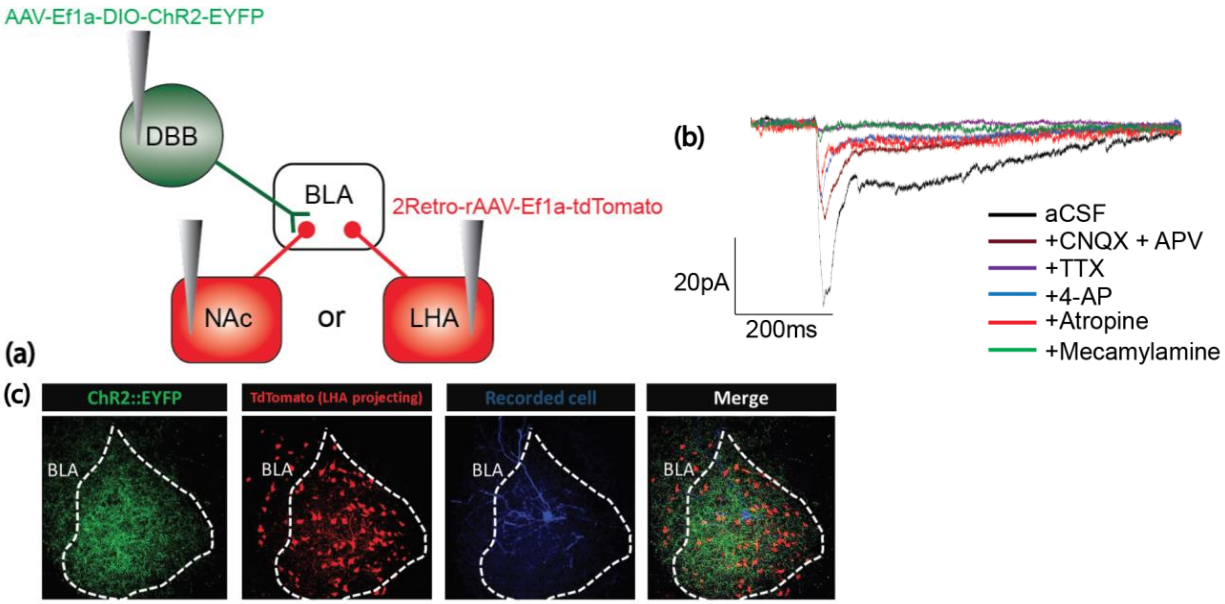

Figure 1: (a) Diagram of the dual stereotactic injections performed on the proposed BLA microcircuit. (b) Representative whole cell membrane potential recording of a BLA cell projecting to the NAc. Data indicative of monosynaptic muscarinic synaptic event. (c) Representative coronal image of recorded neuron. ChR-expressing DBB presynaptic neuron is green. Virally infected neuron expressing tdTomato is red. The Biocytin filled recorded neuron is blue.

Using a *ChAT-Cre*/+ mouse model, we performed dual stereotactic injections into the cholinergic DBB and the LHA or NAc to identify projections from the BLA. We injected 2Retro rAAV-Ef1a-tdtomato into either the NAc cohort or the LHA cohort which infects neuron terminals to express a red fluorescent protein that retrogradely identifies neurons projecting to the NAc or the LHA. This viral approach enables the determination of anatomical connectivity between the BLA→LHA or BLA→NAc. Secondly, we injected rAAV-ef1a-DIO-ChR2::EYFP into the DBB of both groups as seen in *Figure 1a*. This second viral injection allows us to manipulate neurons in a cell-type dependent manner. This virus encodes channelrhodopsin (ChR), a membrane-bound light-activated cation channel. ChR facilitates spatial and temporal control of neural activity by activating cation exchange to induce neuronal firing using blue (473nm) light to regulate cholinergic fibers originating from the DBB.

Functional and monosynaptic connectivity determination

Channelrhodopsin assisted circuit mapping (CRACM) determines the functional connection of neurons by expression of ChR in presynaptic neurons and observing the effect of ChR2 activation on fluorescently marked postsynaptic neurons when stimulated by blue (473nm) light using brain slices and patch clamping (Petreanu, L., et al., 2007).

The dual injected ChR2 and tdTomato mice were sac'd and the brains were sliced into 40µm thick slices. These slices were then bathed in artificial cerebrospinal fluid (aCSF) to obtain a baseline for cell specific action potentials. This bath was then replaced with CNQX (AMPA receptor) and APV (NMDA receptor) that effectively block GABAergic and glutamatergic receptors, respectively. The remaining membrane potential we observe (*Figure 1b, brown)* indicates the unblocked synaptic activity due to acetylcholine. The cells are then inundated with tetrodotoxin (TTX), which effectively blocks all voltage gated sodium channels, and 4-AP, which blocks all voltage gated potassium channels, and promotes conditions to enable cholinergic signaling. Therefore, the observation of a membrane potential in 4-AP strongly implicates the BLA→LHA or BLA→NAc projecting cells are monosynaptically connected to the DBB.

To parse out the cholinergic identity of this circuit, we systematically blocked muscarinic and nicotinic receptors of the cholinergic neurons using atropine and mecamylamine, and observed the response of the circuit within the NAc and LHA cohorts. The addition of atropine within the NAc cohort silenced membrane activity, suggesting a muscarinic identity. Conversely, the addition of mecamylamine to the LHA cohort ceased membrane activity, suggesting a nicotinic profile.

Evaluation of whole-cell recordings within LHA projecting BLA cells revealed that 33% of the recorded neurons received fast nicotinic inputs from cholinergic

DBB cells. These data indicate projection-specific receptor profiles within BLA cellular populations, in which nicotinic receptors project to the LHA, and muscarinic receptors project to the NAc.

These results reveal different cholinergic receptors in cells based on their projection profiles. Despite both being acetylcholine receptors, muscarinic and nicotinic receptors possess unique structures that afford them differing mechanisms (Purves, R.D., 1976). The slow-acting nature of muscarinic receptors in neurons projecting to the NAc suggests a mechanism for the gradual decrease in hedonic food rewards as appetite is satiated. The fast-acting effects of nicotinic receptors may explain the near immediate decision in evaluating food palatability. Overall, the implications of receptor specific cells in the BLA offers a glimpse at the breadth of molecular and circuit regulation involved in controlling appetite and feeding behaviors.

Acknowledgments

J.O.G. conceived and designed the study. E.K.T. and J.O.G. performed the experiments, analyzed the data and interpreted results. This work was supported by a Klingenstein-Simons Fellowship, NIH-NINDS, and the McNair Foundation to B.R.A.

References

Beyeler, A., et al., (2018). *Organization of Valence Encoding and Projection-Defined Neurons in the Basolateral Amygdala. Cell Reports,* 22, 905-918. Doi: 10.1016/j.celrep.2017.12.097

Castro, D.C., Cole, S.L., Berridge, K.C., (2015). *Lateral hypothalamus, and ventral pallidum roles in eating and hunger: interactions between homeostatic and reward circuitry. Font. Syst. Neurosci.*, https://doi.org/10.3389/fnsys.2015.00090

Herman, A.M., et al., (2016). *A cholingergic basal forebrain feeding circuit modulates appetite suppression. Nature*, 538 (7624), 253-256. Doi:10.1038/nature19789

Jennings, J.H., et al., (2013). *The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. Science.* 341:1517–1521. https://doi.org/10.1126/science.1241812

Petreanu, L., Huber, D., Sobczyk A., Svoboda., K., (2007). *Channelrhodopsin-2–assisted circuit mapping of long-range callosal projections. Nature Neuroscience*, 10, pages 663–668

Purves, R. D. (1976). *Function of muscarinic and nicotinic acetylcholine receptors*. *Nature*, 261(5556), 149-151. doi:10.1038/261149a0