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Neural Coding of a Appetitive Food Experiences in the Amygdala

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ABSTRACT

Real-life experiences involve the consumption of various foods, yet it is unclear how the brain distinguishes and categorizes such food experiences. Despite the crucial roles of the basolateral amygdala (BLA) in appetitive behavior and emotion, how BLA pyramidal cells and interneurons encode food experiences has not yet been well characterized. Here we employ large-scale tetrode recording techniques to investigate the coding properties of pyramidal neurons vs. fast-spiking interneurons in the BLA as mice freely consumed a variety of foods, such as biscuits, rice, milk and water. We found that putative pyramidal cells conformed to the power-of-two-based permutation logic, as postulated by the *Theory of Connectivity*, to generate specific-to-general neural clique-coding patterns. Many pyramidal cells exhibited firing increases specific to a given food type, while some other pyramidal cells increased firings to various combinations of multiple foods. In contrast, fast-spiking interneurons can increase or decrease firings to given food types, and were more broadly tuned to various food experiences. We further show that a subset of pyramidal cells exhibited rapid desensitization to repeated eating of the same food, correlated with rapid behavioral habituation. Finally, we provide the intuitive visualization of BLA ensemble activation patterns using the dimensionality-reduction classification method to decode real-time appetitive stimulus identity on a moment-to-moment, single trial basis. Elucidation of the neural coding patterns in the BLA provides a key insight into how the brain's emotion and memory circuits performs the computational operation of pattern discrimination and categorization of natural food experiences.

1. Introduction

Eating foods is one of the most basic experiences conserved across all animal species (Baxter and Byrne, 2006; Craig, 1918; Everitt, Cardinal, Parkinson, and Robbins, 2003), and its dysregulation in neural circuits processing feeding behaviors can be detrimental to health, leading to obesity and diabetes (Morton, Cummings, Baskin, Barsh, and Schwartz, 2006; Morton et al., 2006; Nishijo, Uwano, Tamura, and Ono, 1998; Nectow et al., 2017). Eating foods can produce rich emotions and vivid memories, which often consist of multiple factors, such as visual and olfactory attractiveness, texture, food palatability and social factors (Berridge, 1996; Berthoud, 2004; Guven-Ozkan and Davis, 2014; Hsu, Hahn, Konanur, Noble, Suarez, Thai, Nakamoto, and Kanoski, 2015; Kadohisa, Rolls, and Verhagen, 2005a; Peng, et al., 2015; Scott, 2005; Simon, de Araujo, Gutierrez, and Nicolelis, 2006; Zhang et al., 2003), as well as prior experiences (Adaikkan and Rosenblum, 2015; Carballo-Marquez, Vale-Martinez, Guillazo-Blanch, and Marti-Nicolovius, 2009; Cui, Lindl, Mei, Zhang, and Tsien, 2005; Rampon, et al., 2000). Substantial progress has been made toward the better understanding of the receptors and primary sensory responses to simple odors and tastants (Fontanini, Grossman, Figueroa, and Katz, 2009; Hallem and Carlson, 2006; Scott, 2005; Simon et al., 2006; Zhang et al., 2003) as well as motivational aspects of food-seeking behaviors regulated by reward pathways (e.g., dopamine circuits) (Berridge, 2007; Dayan and Balleine, 2002; Hommel et al., 2006; Palmiter, 2008; Szczypka, et al., 1999; Wise, 2006) and energy homeostatic pathways (e.g., the lateral hypothalamus, arcuate nucleus, etc.) (Carter, Soden, Zweifel, and Palmiter, 2013; de Araujo, et al., 2006; Ko et al., 2015; Stanley, Urstadt, Charles, and Kee, 2011; Tschop, Smiley, and Heiman, 2000; Wu, Clark, and Palmiter, 2012; Nectow et al., 2017).

The amygdala – such as the basolateral amygdala (BLA) – is well

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known for its roles in regulating emotion, especially using fear learning and memory models (Cahill, 2000; Davis, 1992; Kluver and Bucy, 1997; LeDoux, 2000; Saunders, 2000). However, using head-restrained animals or the force-feeding of simple tastants (intra-orally via a tube or pipette), researchers have reported that cells in the amygdala or other parts of the brain circuits responded to simple tastants such as NaCl, citric acid or sucrose solutions as well as to other ingestive features related to temperature, texture, palatability, etc. (Fontanini et al., 2009; Hallem and Carlson, 2006; Kadohisa, Verhagen, and Rolls, 2005b; Nishijo et al., 1998; Scott, 2005; Simon et al., 2006; Zhang et al., 2003). These experiments support the notion that the amygdala, especially the BLA, represents a key site for processing appetitive behaviors. Lesion and pharmacological inhibition experiments showed the amygdala is required to generate both specific and general behavioral responses to appetitive experiences (Murray, Gaffan, and Flint, 1996; Petrovich and Gallagher, 2003; Wise, 2006; Wu et al., 2012).

Given the fact that real-life experience typically involves the consumption of various foods over the course of a given meal, it is surprising that the question of how the amygdala achieves discrimination, categorization and generalization of various food experiences has not been well investigated. In the present study, we employed large-scale *in vivo* tetrode recording techniques to examine how the BLA responded as the mice consumed biscuits, rice, milk and/or water during the self-initiated, freely behaving state. Given the existence of distinct cell types in BLA, we further examined the question of whether/how distinct cell types, such as excitatory pyramidal cells vs. inhibitory interneurons, differ in their responses to natural food experiences. Moreover, we investigated whether BLA cells would respond during rapid behavioral habituation over the course of consuming the same food item. Finally, by combining large-scale recording techniques with dimensionality-reduction methods, we provided the intuitive visualization of real-time BLA neural ensemble traces for various food experiences on a moment-to-moment basis.

2. Methods

2.1. Ethics statement

All animal work described in the study was carried out in accordance with the guidelines established by the National Institutes of Health regarding the care and use of animals for experimental procedures and the protocols approved by the Institutional Animal Care and Use Committee at the Medical College of Georgia at Augusta University (Approval AUP number: BR10-12-392).

2.2. Construction of tetrode headstages and animal surgery

We employed adjustable 128-channel or 64-channel tetrode microdrives to target the BLA bilaterally (Lin, et al., 2005; Lin, Chen, Xie, Zaia, Zhang, and Tsien, 2006b). Tetrodes and headstages were constructed using the procedures as we have previously described (Lin et al., 2006b). To construct tetrodes, four wires (Fe-Ni-Cr, Stablohm 675, 13-μm diameter or 90% platinum, 10% iridium, 17-μm diameter, California Fine Wire Company, Grover Beach, CA) were twisted together using a manual turning device and soldered with a low-intensity heat source (variable temperature heat gun 8977020, Milwaukee, Brookfield, WI) for six seconds. The impedances of the tetrodes were measured with an electrode impedance tester (Model IMP-1, Bak Electronics, Umatilla, FL) to detect any faulty connections, and our tetrodes were typically around 0.5 MΩ. The insulation was removed by moving the tips of the free ends of the tetrodes over an open flame for approximately one second. The tetrodes were then placed into appropriate polyimide tubes. Only tetrodes, but not the surrounding polyimide tubes, were inserted into the brain tissue, thereby minimizing the tissue damage.

Adult male wild-type mice (5–7 months old) were housed in a large bucket inside the chronic recording rooms a week prior to surgery. During this period, the animals were also handled daily to minimize stress from human social interaction. On the day of the surgery, the animal was given an intraperitoneal injection of 60 mg/kg ketamine (Bedford Laboratories, Bedford, OH) and 4 mg/kg Domitor (Pfizer, New York City, NY) prior to the surgery. The head of the animal was secured in a stereotaxic apparatus, and an ocular lubricant was used to cover the eyes. The hair above the surgery sites was removed, and Betadine solution was applied to the surface of the scalp. An incision was then made along the midline of the skull. Hydrogen peroxide was placed onto the surface of the skull so that bregma could be visualized. The correct positions for implantation were then measured and marked. Three mice were implanted with 128-channel tetrodes (64-channel bilaterally), two mice were implanted with 64-channel on the left side. For fixing the microdrive headstage, four holes for screws (B002SG89S4, Amazon, Seattle, WA) were drilled on the opposing side of the skull and, subsequently, the screws were placed in these holes with reference wires being secured to two of the head screws. Stereotaxic coordinates used for targeting the BLA are as follows: 1.7 mm posterior to bregma, 3.5 mm lateral, −4.0 mm ventral to the brain surface. Craniotomies for the tetrode arrays were then drilled, and the dura mater was carefully removed. Afterwards, the electrodes were inserted slightly above the BLA. The microdrive was secured to the skull with dental cement, and the reference wires from the connector-pin arrays were soldered such that there would be a continuous circuit between the ground wires from the head screws and those from the connector-pin arrays. Finally, the copper mesh was used to surround the entire headstage to aid in protection and to reduce noise during recordings. The animals were then awakened with an injection of 2.5 mg/kg Antisedan. The animal was allowed to recover post-surgery for three to five days before recordings. Then, the electrode bundles targeting the BLA were slowly advanced over several days in small daily increments.

2.3. Behavioral paradigm and in vivo recording

Prior to the recording experiments, mice were placed on a food-deprivation schedule to reduce their weight to 80–85% of their baseline weight. They were fed with mouse chow in their home cages in the morning each day (limited to 2 g/mouse/day). Water was available at all times in the home cages.

For desensitization-based alternative food preference test, the mice were habituated to three different foods - namely, KOKUHO premium quality rice (~20 mg), rodent diet (Harlan Laboratories, ~20 mg per pellet) and a milk droplet (25 μl, made from instant nonfat dry powder at 25 g in 50 ml water, Stop & Shop) in the cages overnight for three days. On the preference test day, each subject mouse was fed seven rice pellets first. Then, each mouse was given the rice as well as another different food, rodent diet or milk droplets. The food preference test was quantified by computing the total consumption number of rice pellets vs. another different food in the first 14 consumptions. All data were presented as mean ± s.e.m. Differences were considered significant if p value was < 0.05.

For tetrode-recording experiments, we first recorded the BLA neural activity in freely-behaving mice in the home cage for at least 30 min as a baseline. Three different types of foods (20 mg rice, 20 mg rodent biscuit pellet, and 25 μl milk droplets) were delivered to the small petri dish located in the home recording chamber. Each food (pellet or droplet) was delivered with a 15–30 s time interval after consumption within a given food session. Mice typically consumed the food within 10–20 s. The time intervals between the different food sessions were 5–10 min. The order of delivery was randomized. In a small set of recording experiments, a water bottle was also available and the number of drinking bouts by these mice was monitored. The recordings were continued for an additional 30 min after all the appetitive experiments were completed. The experiments were videotaped by a camera placed