Neurobiology of Learning and Memory 141 (2017) 27–32

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Short communication

Distinct recruitment of basolateral amygdala-medial prefrontal cortex pathways across Pavlovian appetitive conditioning

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ARTICLE INFO

Article history:
Received 9 November 2016
Revised 13 February 2017
Accepted 6 March 2017
Available online 10 March 2017

Keywords:
Associative learning
Acquisition
Basolateral amygdala
Prelimbic cortex
Connections
Reward

ABSTRACT

Associative learning can enable environmental cues to signal food and stimulate feeding, independent of physiological hunger. Two forebrain regions necessary in cue driven feeding, the basolateral area of the amygdala and the medial prefrontal cortex, communicate via extensive, topographically organized connections. The basolateral nucleus (BLA) sends extensive projections to the prelimbic cortex (PL), and our aim here was to determine if this pathway was selectively recruited during cue-food associative learning. The anterior and posterior basolateral nuclei are recruited during different phases of cue-food learning, and thus we examined whether distinct pathways that originate in these nuclei and project to the PL are differently recruited during early and late stages of learning. To accomplish this we used neuroanatomical tract tracing combined with the detection of Fos induction. To identify projecting neurons within the BLA, prior to training, rats received a retrograde tracer, Fluoro-Gold (FG) into the PL. Rats were given either one or ten sessions of tone-food presentations (Paired group) or tone-only presentations (Control group). The Paired group learned the tone-food association quickly and robustly and had greater Fos induction within the anterior and posterior BLA during early and late learning compared to the Control group. Notably, the Paired group had more double-labeled neurons (FG + Fos) during late training compared to the Control group, specifically in the anterior BLA. This demonstrates selective recruitment of the anterior BLA-PL pathway by late cue-food learning. These findings indicate plasticity and specificity in the BLA-PL pathways across cue-food associative learning.

1. Introduction

Cues that signal food can increase the motivation to procure and consume food in the absence of hunger across species (e.g., Birch, McPhee, Sullivan, & Johnson, 1989; Weingarten, 1983; for reviews see Holland & Petrovich, 2005; Petrovich, 2013; Petrovich & Gallagher, 2003). Environmental cues can gain this ability through associative learning, such as during Pavlovian appetitive conditioning. In this preparation, a neutral cue from the environment (conditioned stimulus, CS) is repeatedly followed by food (unconditioned stimulus, US), which innately evokes feeding behaviors (unconditioned response, UR). The CS then becomes the predictor of the US and ultimately drives the same behaviors (conditioned response, CR). These acquired abilities are well established behaviorally; however, much less is known about the neural plasticity, particularly at a circuit level, that underlies cue-food learning.

The amygdala, specifically the basolateral area, is important for appetitive associative learning and subsequent behaviors (Cole, Powell, & Petrovich, 2013; Corbit & Balleine, 2005; for reviews see Crombag, Bossert, Koya, & Shaham, 2008; Everitt, Cardinal, Parkinson, & Robbins, 2003; Gallagher & Schoenbaum, 1999; Holland & Petrovich, 2005; Wassum & Izquierdo, 2015), and its function is conceptualized to involve 'tagging' biologically relevant incoming stimuli and then informing other brain systems via complex and distributed connectional networks (e.g., Swanson & Petrovich, 1998; Weiskrantz, 1956). The amygdala is a heterogeneous structure (Swanson & Petrovich, 1998), and recent work found that distinct nuclei within the basolateral area (containing the lateral, basolateral [BLA] and basomedial nuclei) were differentially recruited during early and late cue-food learning (Cole et al., 2013). Specifically, the anterior basolateral nucleus (BLAa, Swanson, 2004; also known as the magnocellular division based on its morphology, Pitkänen, Savander, & LeDoux, 1997; Savander, Go, LeDoux, & Pitkänen, 1995) was the only amygdalar nucleus that displayed a significant increase in activation (measured with Fos induction) during early learning, which was maintained throughout training. The posterior basolateral nucleus (BLAp, Swanson, 2004; also known as the parvocellular division based on its morphology, Pitkänen et al., 1997; Savander et al., 1995) was recruited during late training along with other amygdalar nuclei that are connected with the BLAa. These results

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http://dx.doi.org/10.1016/j.nlm.2017.03.006
1074-7427/

demonstrate specificity in the recruitment of amygdalar nuclei, and the differential recruitment across early and later learning suggests plasticity within the BLAa and, potentially, with its connectional targets.

The BLA has extensive connections with the medial prefrontal cortex (Hoover & Vertes, 2007; Kita & Kitai, 1990; Reppucci & Petrovich, 2016), which is important for the executive function and control of feeding and other motivated behaviors (Dalley, Cardinal, & Robbins, 2004; O'Doherty, 2011; Swanson & Petrovich, 1998). Specifically, the ventromedial prefrontal cortex, including the prelimbic (PL) and infralimbic (ILA) areas, is critical in appetitive cue learning (Ashwell & Ito, 2014; Baldwin, Holahan, Sadeghian, & Kelley, 2000; Baldwin, Sadeghian, & Kelley, 2002; Burgos-Robles, Bravo-Rivera, & Quirk, 2013; Cole, Hobin, & Petrovich, 2015; Corbit & Balleine, 2003). This area is necessary for feeding driven by learned food cues (Cole, Mayer, & Petrovich, 2015; Petrovich, Ross, Holland, & Gallagher, 2007), can be stimulated to drive food intake (Blasio, Steardo, Sabino, & Cottone, 2014; Land et al., 2014; Mena, Sadeghian, & Baldo, 2011) and alters activity in downstream neural regions mediating feeding behaviors (Mena, Selleck, & Baldo, 2013). Furthermore, disruption of the BLA-mPFC pathway attenuates reward-seeking driven by learned contextual and discrete cues (Fuchs, Eaddy, Su, & Bell, 2007; Mashhoon, Wells, & Kantak, 2010; Stefanik & Kalivas, 2013). Nevertheless, the functional connectivity of the BLA-PL pathways has not been investigated during the acquisition of cue-food associations.

Within the medial prefrontal cortex, the BLA most densely innervates the PL, with topographically distinct pathways originating in the BLAa and BLAp (Hoover & Vertes, 2007; Kita & Kitai, 1990; Reppucci & Petrovich, 2016). The BLAa and BLAp are recruited during different phases of cue-food learning (Cole et al., 2013), suggesting that the BLAa-PL and BLAp-PL pathways may also be differently engaged. The goal of the current study was to determine whether the BLA neurons that send direct projections to the PL are selectively activated during cue-food learning and whether distinct pathways that originate in the BLAa and BLAp are differentially recruited during early and late learning of cue-food associations.

2. Methods

In order to identify BLA-to-PL projecting neurons, rats were iontophoretically injected with the retrograde tracer Fluoro-Gold (FG) into the PL. After recovery, rats received either one training session (early learning; S1) or ten training sessions (late learning; S10) of Pavlovian appetitive conditioning. Each training session included eight presentations of a tone CS that for the Paired condition coterminated with the delivery of two food pellets (US). Rats in the Control group received the CS presentations in the behavioral chambers followed by the US delivery in their home cage at a random interval after each session. The primary measure of learning was the percentage of time rats expressed food cup behavior during the CS. Rats were perfused 90 min after the cessation of S1 or S10 for brain tissue collection. The Control groups did not receive the US on perfusion day. The brain tissue was processed for double-label fluorescence immunohistochemistry for FG and Fos detection (see Supplemental Material for details).

3. Results

3.1. Behavior

During early training (Session 1), the Paired group displayed increasingly more food cup behavior during CSs throughout the session compared to the Control group, signifying learning (Fig. 1A). Repeated measures ANOVA (Training group × CS) found a significant effect of CS ($F_{(1,18)} = 2.713$, $P < 0.05$), but no effect of training group ($F_{(1,18)} = 2.793$, $P > 0.05$), or interaction ($F_{(1,18)} = 1.239$, $P > 0.05$). To assess learning during the session, further analysis compared behavior between the first half and the second half of the session (four CSs each). The Paired group displayed more food cup behavior during the last four CSs compared to their responding during the first four CSs ($P < 0.05$) and compared to the Control group ($P < 0.05$; Fig. 1B). There were no differences between the groups during the first four CSs ($P > 0.05$) or during pre-CS intervals ($P > 0.05$).

Over ten sessions of training, the Paired group showed an increase in food cup behavior during the CSs, while the Control group displayed minimal and non-specific food cup behavior throughout training. Repeated measures ANOVA (Training group × Session) revealed a significant effect of training group ($F_{(1,14)} = 139.018$, $P < 0.0001$), a significant effect of session ($F_{(1,14)} = 6.968$, $P < 0.001$) and a significant interaction across sessions ($F_{(1,14)} = 9.781$, $P < 0.001$). During session 2, the Paired group had higher food cup responding compared to the Control group ($P < 0.05$; Fig. 1C), but similar responding during the pre-CS and CS intervals ($P > 0.05$). Throughout sessions 3–10, the Paired group showed high responding specifically to the CS compared to their pre-CS responding ($P < 0.05$) and compared to the behavior of the Control group during the CS ($P < 0.05$). During the last session of training (session 10), repeated measures ANOVA (Training group - × Time period [CS or pre-CS]) found a significant effect of training group ($F_{(1,14)} = 8.287$, $P < 0.05$), a significant effect of CS vs Pre-CS time period ($F_{(1,14)} = 63.816$, $P < 0.0001$), and a significant interaction ($F_{(1,14)} = 64.858$, $P < 0.0001$). The Paired group showed higher food cup behavior during the CS than the Control group ($P < 0.001$) with no difference in pre-CS behavior between the groups ($P > 0.05$; Fig. 1D).

3.2. Neural analysis

The location and spread of FG injection sites were analyzed throughout the rostro-caudal extent of the prelimbic cortex (PL) based on the Swanson brain atlas (Swanson, 2004). Acceptable injections (see Supplemental Materials) were confined predominantly within the PL (n = 36) and were centered within the mid rostro-caudal extent of the PL (Fig. 2; Levels 6, 7 and 8; +4.2, +3.6, and +3.2 mm from bregma, respectively). The final group numbers were S1 Paired (n = 10), S1 Control (n = 10), S10 Paired (n = 8), and S10 Control (n = 8). Importantly, the total numbers of retrogradely-labeled neurons were similar across groups (Fig. 3B), confirmed by two-way ANOVAs (Training group × Session) in the BLAa (Training group: $F_{(1,32)} = 2.477$, $P > 0.05$; Session: $F_{(1,32)} = 0.585$, $P > 0.05$) and BLAp (Training group: $F_{(1,32)} = 0.542$, $P > 0.05$; Session: $F_{(1,32)} = 0.119$, $P > 0.05$), signifying that any differences found in the number of double-labeled (FG + Fos) neurons are not due to variances in the number of FG-labeled neurons.

Representative images of Fos and FG labeled neurons in the BLAa are shown in Fig. 3A. Fos induction in the BLA neurons was examined during early (session 1; S1) and late (session 10; S10) tone-food conditioning. Within the BLAa, the Paired group had more Fos-positive neurons than the Control group during S1 and S10 (Fig. 3B). The two-way ANOVA (Training group × Session) revealed a significant effect of training group ($F_{(1,32)} = 16.722$, $P < 0.01$), but no effect of session ($F_{(1,32)} = 0.609$, $P > 0.05$), or interaction ($F_{(1,32)} < 0.000$, $P > 0.05$). *Post hoc* analysis confirmed the Paired group had significantly more Fos-positive neurons than the Control group during S1 ($P < 0.01$) and S10 ($P < 0.05$), replicating previous findings using this protocol (Cole et al., 2013).