Neuroscience 286 (2015) 187–202

APPETITIVE ASSOCIATIVE LEARNING RECRUITS A DISTINCT NETWORK WITH CORTICAL, STRIATAL, AND HYPOTHALAMIC REGIONS

S. COLE, M. P. HOBIN AND G. D. PETROVICH *

Department of Psychology, Boston College, 140 Commonwealth Avenue, Chestnut Hill, MA 02467-3807, USA

Abstract—The amygdala, prefrontal cortex, striatum and other connected forebrain areas are important for reward-associated learning and subsequent behaviors. How these structurally and functionally dissociable regions are recruited during initial learning, however, is unclear. Recently, we showed amygdalar nuclei were differentially recruited across different stages of cue-food associations in a Pavlovian conditioning paradigm. Here, we systematically examined Fos induction in the forebrain, including areas associated with the amygdala, during early (day 1) and late (day 10) training sessions of cue-food conditioning. During training, rats in the conditioned group received tone-food pairings, while controls received presentations of the tone alone in the conditioning chamber followed by food delivery in their home cage. We found that a small subset of telencephalic and hypothalamic regions were differentially recruited during the early and late stages of training, suggesting evidence of learning-induced plasticity. Initial tone-food pairings recruited solely the amygdala, while late tone-food pairings came to induce Fos in distinct areas within the medial and lateral prefrontal cortex, the dorsal striatum, and the hypothalamus (lateral hypothalamus and paraventricular nucleus). Furthermore, within the perifornical lateral hypothalamus, tone-food pairings selectively recruited neurons that produce the orexigenic neuropeptide orexin/hypocretin. These data show a functional map of the forebrain areas recruited by appetitive associative learning and dependent on experience. These selectively activated regions include interconnected prefrontal, striatal, and hypothalamic regions that form a discrete but distributed network that is well placed to simultaneously inform cortical (cognitive) processing and behavioral (motivational) control during cue-food learning.

Key words: appetitive conditioning, Fos, prefrontal cortex, lateral hypothalamus, orexin, dorsal striatum.

INTRODUCTION

Appetitive associative learning is important in the control of motivated behaviors essential for survival, including feeding. Through associative learning, neutral cues from the environment can become signals for food and gain the ability to powerfully control feeding behavior. Associative food cues can alter multifaceted aspects of feeding behavior. Learned cues prepare the animal physiologically to ingest the food (i.e., salivation and insulin changes), increase approach and action toward the food, and increase consumption of the food even in the absence of hunger (Pavlov, 1927; Estes, 1948; Woods and Kuskosky, 1976; Weingarten, 1983).

Within the laboratory the most often used form of appetitive associative learning is Pavlovian conditioning (Pavlov, 1927), where an initially neutral signal from the environment such as a tone (conditioned stimulus, CS) is repeatedly paired with reward delivery (unconditioned stimulus, US). Through this training the CS acquires the ability to act as a food signal and subsequently influence the motivation for food seeking and consumption. This conditioning forms the basis of many paradigms examining facets of appetitive learning, reward, and ingestive behavior, including cue-potentiated feeding, Pavlovian-instrumental transfer (PIT), and other forms of incentive learning, (for review see Holland and Petrovich, 2005). Even though this basic associative learning plays a critical role in guiding motivated appetitive behaviors, the neural substrates underlying the CS-food learning are largely unknown. Identifying the learning network and critical changes during CS-food association is important in revealing the neural mechanisms and plasticity underlying

*Corresponding author. Tel: +1-617-552-0416; fax: +1-617-552-0523.
E-mail address: gorica.petrovich@bc.edu (G. D. Petrovich).

Abbreviations: ABC, avidin biotin complex; ACAd, anterior cingulate area, dorsal part; ACB, nucleus accumbens; *ACBc, nucleus accumbens, core; *ACBshD, nucleus accumbens, shell, dorsal part; *ACBshV, nucleus accumbens, shell, ventral part; AId, agranular insular area, dorsal part; ARH, arcuate hypothalamic nucleus; BLAa, basolateral amygdalar nucleus, anterior part; CP, caudoputamen; CS, conditioned stimulus; *DLS, dorsolateral striatum; DMH, dorsomedial hypothalamic nucleus; *DMS, dorsomedial striatum; ILA, infralimbic area; IR, immunoreactive; KPBS, potassium phosphate-buffered saline; LHA, lateral hypothalamic area; *LHAl, lateral hypothalamic area, lateral region; *LHApf, lateral hypothalamic area, perifornical region; *LHAv, lateral hypothalamic area, ventral region; NHS, normal horse serum; ORBl, orbital area, lateral part; *pDS, posterior dorsal striatum; PIT, Pavlovian-instrumental transfer; PL, prelimbic area; PVH, paraventricular hypothalamic nucleus; PVHdp, paraventricular hypothalamic nucleus, dorsal parvicellular part; PVHmpd, paraventricular hypothalamic nucleus, medial parvicellular part, dorsal zone; PVHmpv, paraventricular hypothalamic nucleus, medial parvicellular part, ventral zone; PVHpml, paraventricular hypothalamic nucleus, posterior magnocellular part, lateral zone; PVHpv, paraventricular hypothalamic nucleus, periventricular part; *PVTa, paraventricular thalamic nucleus, anterior part; *PVTp, paraventricular thalamic nucleus, posterior part; SUBv, subiculum, ventral part; US, unconditioned stimulus; *Asterisks, denote nomenclature that differs from Swanson (2004).

http://dx.doi.org/10.1016/j.neuroscience.2014.11.026
0306-4522/

this form of learning and its numerous behavioral sequelae.

We recently began to study the neural substrates underlying Pavlovian appetitive conditioning, focusing on the amygdala, and using induction of the immediate early gene *c-fos* protein (Fos) as a marker of activation. We compared amygdalar recruitment during early and late training sessions. Rats received identical tone-food training sessions for either 1 or 10 days, which therefore allowed us to directly assess differences due to the amount of learning (see Fig. 1A). We found that distinct basolateral and central amygdalar nuclei were differentially activated across training (Cole et al., 2013), suggesting evidence of learning-induced plasticity. These amygdalar nuclei operate through dissociable circuitries that could convey information about the CS-food association to a distributed network, and ultimately mediate different aspects of learning and the subsequent control of behavior. How that connectional network is functionally recruited during learning is unknown. Therefore, here we extended this earlier analysis and examined 27 additional telencephalic and hypothalamic regions. These regions included both areas connected with the critical amygdalar nuclei previously identified, and areas important for learning and ingestive behavior. The aim of this study was to generate a functional map of forebrain areas underlying appetitive associative learning.

EXPERIMENTAL PROCEDURES

Animals

Forty-seven experimentally naïve, male Long-Evans rats (276–300 g) obtained from Charles River Laboratories (Portage, MI, USA) were used. The animals were individually housed with *ad libitum* access to food and water except when otherwise noted. The colony room was maintained at 21 °C on a 12-h light/dark cycle (lights on 06:00) and all behavioral testing was conducted during the light phase of the cycle. Rats were given 1 week to acclimate to the colony room during which time they were handled and weighed daily. The housing and testing procedures were in accordance with the National Institute of Health *Guidelines for Care and Use of Laboratory Animals* and approved by the Boston College Institutional Animal Care and Use Committee.

Apparatus and behavioral procedures

Behavioral training was described in detail previously (Cole et al., 2013), and was conducted in a set of behavioral chambers (Coulbourn Instruments, Allentown, PA, USA) located in a room different than the housing room. Each chamber was located in a sound- and light-attenuating cubicle that was equipped with a ventilation fan (55 dB) and video camera attached to a recording system in an adjacent room to record each training session (Coulbourn Instruments, Allentown, PA, USA). The CS was a 10-s, 75-dB, 2-kHz tone, and the US consisted of two food pellets (formula 5TUL, 45 mg: Test Diets, Richmond, IN, USA) delivered to the food-cup of each chamber, unless otherwise noted.

Rats were gradually reduced to 85% of their *ad libitum* weight, and remained food-restricted throughout behavioral training. All animals initially received 2 days of habituation to the behavioral chambers for 32 min each. Following the second habituation session all animals received 1 g of the food US in their home cage to familiarize them with the pellets.

Experimental design is shown in Fig. 1A and described next. Behavioral training sessions were 32 min long and consisted of eight presentations of a tone CS followed immediately by delivery of the food US into the food-cup (Paired group). The inter-trial intervals between CS presentations in each training session were random (range 110–326 s) and varied across days. Control groups received identical sessions in the behavioral chambers, but with eight presentations of the CS only, followed after a random interval ranging from 30 to 270 min by US (16 pellets) delivery in their home cage. One of the control groups was sacrificed after the session in the chamber (Tone group; thus they did not receive the US on the final day), and the other after food consumption (Food group). For the groups that received US delivery in the home cage during training (Tone and Food), the 16 pellets were delivered at once onto the bedding. All rats in all groups consumed all the pellets given.

To examine Fos induction early in training half of the animals were perfused and the brains collected after a single training session (early training). Animals in the conditioning group (early Paired: $n = 8$) and one of the control groups (early Tone: $n = 8$) were perfused 120 min following the beginning of the training session. The second control group (early Food: $n = 8$) was perfused 105 min following US presentation in the home cage to coincide perfusion time with the middle of the training session for the Paired and Tone groups.

To examine how neuronal activation patterns changed as a result of learning, the other half of the animals received 10 days of training (late training). Late training animals (late Paired: $n = 7$, late Tone: $n = 8$, late Food: $n = 8$) were perfused at the same time points following training/stimulus presentation as early training animals. Thus, both early and late training groups received identical training sessions, the only difference was how many sessions they received.

The control groups in this study (groups Tone and Food) were designed for Fos imaging, and as such aid interpretation of differential Fos induction. A potential alternative control group would be an unpaired group, which would receive explicitly unpaired presentations of the tone and food in the training context. However, such training would result in the animals learning a context-food association (rather than a cue-food association) and so it would not allow for differential detection of learning-driven activation, which was the main purpose of our study. Instead, the inclusion of the Tone and Food controls used here allowed identification of a critical subsystem that was selectively recruited during tone-food learning, and allows numerous alternate interpretations of the results to be ruled out (see Discussion).