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Odor-mediated taste learning requires dorsal hippocampus, but not basolateral amygdala activity

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ABSTRACT

Mediated learning is a unique cognitive phenomenon in which mental representations of physically absent stimuli enter into associations with directly-activated representations of physically present stimuli. Three experiments investigated the functional physiology of mediated learning involving the use of odor–taste associations. In Experiments 1a and 1b, basolateral amygdala lesions failed to attenuate mediated taste aversion learning. In Experiment 2, dorsal hippocampus inactivation impaired mediated learning, but left direct learning intact. Considered with past studies, the results implicate the dorsal hippocampus in mediated learning generally, and suggest a limit on the importance of the basolateral amygdala.

1. Introduction

Broadly, studies of associative learning and memory tend to focus on situations in which an innocuous stimulus is directly associated with a harmful or rewarding stimulus (i.e., direct learning). However, learning situations for humans often do not involve direct experience with a biologically-significant unconditioned stimulus (US; e.g., shock, food, etc.) Instead, symbols and surrogates are used to evoke and sometimes fabricate mental representations of real-world biologically significant stimuli. Understanding this cognitive process is important because it is critical for abstract learning, and a potential source of cognitive dysfunction in mental illness (McDannald & Schoenbaum, 2009; McDannald et al., 2011). As such, nonhuman animal models of representation-mediated learning are valuable for exploring the functional neurophysiology that underlies this unique class of learning.

Nonhuman (or nonverbal) animal models of mediated learning require subjects to learn about a stimulus in its absence, based on the activation of a robust mental representation of that stimulus (e.g., Cuevas, Rovee-Collier, & Learmonth, 2006; Dwyer, 1999; Holland, 1981, 1990). In a typical mediated-learning paradigm, a stimulus is paired with an unconditioned stimulus (US) serially or in a simultaneous compound. According to associative-learning theories the subsequent presentation of the CS alone elicits a robust representation of the US, which is the foundation of certain goal-directed forms of conditioned responding. If, during a presentation of the CS alone, a new US is presented that is likely to enter into an association with the absent US, but not the present cue, (i.e., selective associability; Garcia & Koelling, 1966), the absent US can form an association with the new US. This phenomenon has been observed by Holland (1981) as well as Dwyer (1999, 2001) in situations involving taste aversion. In their experiments, auditory and visual stimuli (Holland) or contexts (Dwyer) were initially paired with rewarding tastes. When the CSs were later paired with LiCl-induced illness, they served as surrogates for their associated taste stimuli. These CSs did not form any appreciable first-order association with an illness, but the associatively-activated taste representations did support taste-aversion learning.

The current studies were designed to investigate the brain structures involved in mediated learning of taste aversions. Based on its critical role in the representation of sensory aspects of gustatory rewards in behavioral paradigms (see Holland & Gallagher, 2004) such as responding to reinforcer devaluation (e.g., Hatfield, Han, Conley, Gallagher, & Holland, 1996), the effect of reinforcer-selective Pavlovian cues on instrumental performance (e.g., Corbit & Balleine, 2005), and the differential outcomes effect (e.g., Blundell, Hall, & Killcross, 2001), Dwyer and Killcross (2006) investigated the role of the basolateral amygdala (BLA) in mediated learning. They found that permanent lesions of the BLA attenuated mediated taste-aversion learning. Despite these precedents for involvement of the BLA in representational function in learning, we found no effects of BLA lesions in the following experiments. Thus, we examined the role of another region that has been

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implicated in mediated learning, the dorsal hippocampus (DH). Iordanova, Good, and Honey (2011) found that DH inactivation eliminated mediated fear learning, presumably because the task required hippocampal-dependant episodic-like memory (e.g., Fortin, Wright, & Eichenbaum, 2004). Because of the role of the DH in mnemonic function broadly, and in the formation of associations between discontiguous events specifically (e.g., Wallenstein, Eichenbaum, & Hasselmo, 1998), it is possible that the area is also important for mediated-learning processes that do not necessarily rely on episodic memory.

In our designs, the rats were first trained to drink two odor–taste compounds. The parameters for this treatment closely followed the methods of Saddoris, Holland, and Gallagher (2009) who found that this sort of training allows the odor to produce neural activity in the gustatory cortex that is similar to that produced by the taste itself, which might suggest a robust associatively-activated representation. After the odor–taste pairings, one of the odors was paired with an illness-inducing agent (LiCl). Finally, the animals were given consumption tests with the two tastes to determine whether the associatively-activated representation of the taste associate of that odor was associated with the illness. The basic designs of the experiments are depicted on Table 1.

Experiment 1a was designed to determine whether permanent excitotoxic lesions of the BLA would attenuate mediated learning. Experiment 1b investigated the same question under conditions in which the taste stimuli were more motivationally significant, which might be more likely to engage the BLA (e.g., Blundell, Hall, & Killcross, 2003). Toward this end, food access was restricted as well as water access, and the concentrations of rewarding solutions were increased. Experiment 2 tested the effect of temporarily inactivating the DH during the acquisition of direct and mediated taste aversion learning. Permanent lesions were not used in Experiment 2 because such lesions can augment latent inhibition of taste-aversion learning (e.g., Purves, Bonardi, & Hall, 1995; Reilly, Harley, & Revusky, 1993), which would be a potential problem for our designs because they inevitably involve preexposure of the to-be-conditioned taste cue. This was not a concern in Experiments 1a and 1b because permanent BLA lesions have not disrupted simple taste-aversion learning in outcome-devaluation studies from our laboratory, likely because the BLA lesions are more likely to hinder novel rather than familiar taste-aversion learning (St. Andre & Reilly, 2007). Furthermore, Iordanova et al. (2011) used a similar temporary inactivation manipulation in their study of mediated learning, strengthening the validity of any comparisons between the two findings.

2. Materials and methods

2.1. Subjects

The subjects were male Long-Evans rats (Charles River Laboratories, Raleigh, North Carolina), which weighed 352–493 g immediately before the onset of the experiments. In all experiments, the rats' water access was restricted to a 10-min period in the morning, and a 1-h access period in the evening (separated by 5–6 h). In Experiment 1b, food access was restricted to 2.5 h in the afternoon. These deprivation schedules were maintained for the duration of the experiments, although experimental manipulations replaced the morning access period. All procedures were approved by Johns Hopkins University Animal Care and Use Committee and the facility is accredited by the Association for the assessment and Accreditation of Laboratory Animal Care.

Some of the animals in Experiments 1a and 1b had previously participated in a Pavlovian conditioning experiment that involved exposure to an operant chamber, audiovisual stimuli, and food deprivation (Chang, McDannald, Wheeler, & Holland, 2012). However, they received no exposure to the contexts, odors, tastes, or LiCl used in this experiment.

2.2. Surgical procedures

All surgical procedures were performed with the subjects under isoflurane (Isovet; Mallinckrodt, Mundelein, IL) anesthesia. In Experiments 1a and 1b, BLA lesions were made using NMDA (Sigma–Aldrich, St. Louis, MO) at a concentration of 10.0 mg/ml in phosphate-buffered saline. NMDA was infused at a rate of 0.1 μl/min. Injections were made in two injection sites in each hemisphere, 2.8 mm posterior to bregma, 5.1 mm from the midline, and at 8.7 mm (0.16 μl) and 8.4 mm (0.08 μl) ventral from the skull surface at bregma. These microinjections were made using a 2.0-μl syringe (Hamilton, Reno, NV). During sham surgeries, a needle was placed in the BLA, but no injections were made. Thirteen of the subjects in Experiment 1a and 14 of the subjects in Experiment 1b received BLA lesions. Nine subjects in Experiment 1a and 8 subjects in Experiment 1b received sham lesions.

In Experiment 2, 18 subjects were implanted with guide cannulae (Plastics One, Roanoke, VA) that terminated immediately above the dorsal hippocampus. The cannulae were targeted 3.6 mm posterior to bregma and 2.5 mm lateral to the midline.

2.3. Materials

The subjects remained in their home cages for the experiments, but these cages were transferred to two separate rooms during odor exposure and test to prevent cross-contamination of odor exposure. The cages were placed on a table in both rooms, which were illuminated with fluorescent light. No effort was made to distinguish the rooms from each other, as the intention was that the subjects would associate the tastes with the odors more than the contexts. Sucrose and maltodextrin (M040, Grain Processing Corporation, Muscatine, IA) solutions served as T1 and T2, counterbalanced. In Experiments 1a and 2, both solutions were 8% (w/v), but this concentration was increased to 16% in Experiment 1b. Iso-amyl butyrate (International Flavors & Fragrances Inc., NY, NY) and hexenol, b, gamma (International Flavors & Fragrances Inc., NY, NY) were mixed in water (both 1.25% v/v) and used as O1 and O2, counterbalanced. The odors were presented on saturated filter paper (Pall Corp., Ann Arbor, MI) placed around the base of the drinking spouts, approximately 4 cm from the subjects' snouts when drinking. A 20-ml/kg injection of 0.15-M LiCl (Sigma–Aldrich, St. Louis, MO) was administered i.p. in order to induce illness.

Table 1
Experimental designs.

	Learning	Phase 1	Phase 2	Taste test	Odor test
Experiments 1a and 1b	Mediated	O1T1/O2T2	O1 → LiCl/O2–	T1/T2	O1/O2
Experiment 2	Direct	O1T1/O2T2	Mus → T1 → LiCl/Sal → T2 → LiCl	T1/T2	O1/O2
	Mediated	O1T1/O2T2	Mus→O1 → LiCl/Sal → O2 → LiCl	T1/T2	O1/O2

Note: O1 and O2 = odors; T1 and T2 = nutritive tastes; LiCl = 20-ml/kg i.p. injection of 0.15 M LiCl; Mus and Sal = muscimol and saline infusions, respectively.