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Research report

Dissociable hippocampal and amygdalar D1-like receptor contribution to discriminated Pavlovian conditioned approach learning

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HIGHLIGHTS

- Dopamine D1 receptor (D1R) activation is critically involved in reward learning.
- D1R involvement in three discrete brain sites was interrogated.
- Discriminated Pavlovian approach was impaired following D1R inactivation.
- D1R inactivation produced three differential behavioral profiles, dependent on site.
- Latent inhibition may have been blocked by D1R blockade.

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ABSTRACT

Pavlovian conditioning is an elementary form of reward-related behavioral adaptation. The mesolimbic dopamine system is widely considered to mediate critical aspects of reward-related learning. For example, initial acquisition of positively-reinforced operant behavior requires dopamine (DA) D1 receptor (D1R) activation in the basolateral amygdala (BLA), central nucleus of the amygdala (CeA), and the ventral subiculum (vSUB). However, the role of D1R activation in these areas on appetitive, non-drug-related, Pavlovian learning is not currently known. In separate experiments, microinfusions of the D1-like receptor antagonist SCH-23390 (3.0 nmol/0.5 μL per side) into the amygdala and subiculum preceded discriminated Pavlovian conditioned approach (dPCA) training sessions. D1-like antagonism in all three structures impaired the acquisition of discriminated approach, but had no effect on performance after conditioning was asymptotic. Moreover, dissociable effects of D1-like antagonism in the three structures on components of discriminated responding were obtained. Lastly, the lack of latent inhibition in drug-treated groups may elucidate the role of D1-like in reward-related Pavlovian conditioning. The present data suggest a role for the D1 receptors in the amygdala and hippocampus in learning the significance of conditional stimuli, but not in the expression of conditional responses.

1. Introduction

When a predictive relationship (i.e., contingency) exists between an environmental stimulus (e.g., a sound) and a biologically-relevant stimulus (e.g., food or pain), the presentation of that environmental event alone can come to elicit a characteristic response [69]. For example, by turning on a metronome (i.e., a conditional stimulus, or "CS") and then presenting food powder (i.e., an unconditional stimulus, or "US") to a dog's tongue, Pavlov demonstrated that the sound of the metronome alone could produce salivation [65]. Pavlovian, or classical conditioning, is an elementary form of behavioral adaptation that enables an animal to more effectively anticipate important events in a world filled with competitors and predators. However, Pavlovian conditioning can also produce maladaptive responses like the elicitation of withdrawal symptoms following exposure to environmental stimuli (CS)—drug (US) contingencies. This learning can be long-lasting, for exposure to drug-conditioned cues can elicit withdrawal or cravings even after long periods of drug abstinence, thus contributing heavily to the compulsive and seeming intractable nature of addiction [60,77]. Thus, a greater understanding of the neurobiology of appetitive Pavlovian conditioning is likely to yield significant information for addressing problems like drug abuse and relapse.

A crucial role for the mesolimbic dopamine system in appetitive Pavlovian conditioning has been demonstrated in many experiments. Electrophysiological studies in primates have shown that mid-brain dopamine (DA) neurons in the ventral tegmental area (VTA) fire to stimuli that predict upcoming rewards [75]. Micro-

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dialysis studies confirm elevated DA in the amygdala following appetitive Pavlovian conditioning [38], a key target of mesolimbic DA system. Pharmacological studies have also begun to determine the post-synaptic mechanisms of DA's actions in Pavlovian conditioning, implicating a crucial role for DA D1 receptors in Pavlovian conditioned approach (PCA) following systemic blockade [20,28]. Local antagonism of D3 receptors in the central nucleus of the amygdala (CeA) have also been shown to produce deficits in PCA. Further, D1-LIKE antagonism in the amygdala during drug-cue conditioning attenuates the ability of that cue to reinstate drug-seeking behavior in a self-administration, re-instatement paradigm [9]. Another target of the mesolimbic DA system, the ventral subiculum (vSUB), is thought to compete with the amygdala over control of plasticity-related inputs to the Nucleus Accumbens, a site critical for the expression of PCA [11,63]. Combined these data strongly suggest a crucial role for dopamine and dopamine receptor activation in appetitive Pavlovian conditioning, in a distributed network.

Dopamine is also hypothesized to serve crucial modulatory functions in neural and synaptic plasticity, which are widely considered to be critical in the cellular/molecular processes instantiating learning. Long-term enhancement of synaptic strength occurs when DA D1R activation is temporally coordinated with NMDAR activation [46,82]. D1R antagonists block NMDA-dependent LTP in striatal slices [50], while LTP in hippocampal-PFC synapses, in vivo, depends on co-activation of D1R and NMDAR [35,36,47,48]. More recent data suggests that D1R, via PKA activation, phosphorylates ERK, thereby reconfiguring networks involved in learning and drug use [49,80]. ERK phosphorylates CREB [24,51], a transcription factor thought to be an evolutionarily conserved modulator of memory processes [58,78]. It is presumed that a more thorough understanding of brain reward learning systems will have immense potential for extensively improving outcomes through the development and discovery of new therapeutics to restore reward-related neurobiological pathways gone awry.

However, while substantial data exist on the role of dopamine and dopamine receptors in Pavlovian conditioning, significant omissions also exist. Several of the aforementioned studies used systemic D1R blockade [20,28] or pharmacological agents that also blocked D2 receptors [25], which leaves open questions of receptor and region specificity. Lesions of discrete subnuclei of the amygdala produce dissociable effects on learning and performance of Pavlovian conditioned behavior [12,16,39,41–43], but they also remain silent on the question of receptor specificity and do not address the possibility that other mechanisms may compensate for or recover function of the lesioned area. In addition, D1-like activation in the amygdala has not been studied in the context of non-drug-related appetitive Pavlovian learning, although the amygdala's role in aversive conditioning has been well-characterized [32,34,53,57]. D1-LIKE antagonism in both the BLA and CeA blocks appetitive operant conditioning, suggesting a role in appetitive, as well as aversive, learning processes [3]. D1-like antagonism in the ventral subiculum (vSUB) also blocks appetitive operant learning [4], although D1-like activation may have been involved in Pavlovian conditioning processes that modulated operant responding. The present experiments, therefore, explored the role of D1-like activation in the BLA, CeA, and vSUB during appetitive Pavlovian conditioning using a discriminated Pavlovian conditioning approach (dPCA) paradigm.

2. Method

2.1. Subjects

Male Sprague-Dawley rats (Harlan, Madison, WI) were housed in pairs in polyethylene cages in colony room with a 12:12 h light/dark cycle. They were approximately 90 days old at the start of experimentation and weighed approximately 300 g each. Each experiment started with 16 rats (8 per group); final n's are presented in the results. All rats were weighed and handled daily and provided with food and water ad libitum prior to surgery. Following recovery from surgery, each rat was reduced to 85% of their ad libitum weight. During food restriction, and prior the start of testing, rats were given approximately 3 g of sucrose pellets in their home cages per day; the 85% weight was maintained for the remainder of the experiment. Care of the rats was in accordance with University of Wisconsin-Madison animal care committee guidelines.

2.2. Apparatus

Sessions were conducted in 8 identical commercially constructed conditioning chambers (Coulbourn Instruments, Allentown, PA) enclosed in sound attenuating, ventilated chests. Fans provided some masking noise continuously throughout the session. Two retractable levers, approximately 6 cm apart, were located on the right-side wall (these levers were never projected into the chamber during the present experiments). Spaced equally between the two levers was a feeder trough into which 45 mg Bio-Serv® sucrose pellets could be delivered. The feeder trough was equipped with a photo sensor, such that the number and timing of nose pokes into the tray could be recorded. Above the feeder trough were a row of three stimulus lights (red, yellow, and green; not used in the present experiments) and a 28-V houselight. Experimental events were arranged and recorded via a personal computer in the same room as the chambers, running Graphic State Notation (Coulbourn Instruments, Allentown, PA).

2.3. Surgery

Rats were anesthetized with a ketamine/xylazine mixture (100/10 mg/kg) and placed in a standard stereotaxic surgery device (incisor bar at −3.8 mm; flat skull). Indwelling stainless cannulae (23 gauge) were implanted bilaterally and secured to the skull with stainless steel screws and dental cement. Cannulae were aimed 2.5 mm above the injection targets: the central nucleus of the amygdala (CeA), or the basolateral amygdala (BLA), or the ventral subiculum (vSUB). Stainless steel stylets prevented occlusion of the cannulae. Coordinates (flat skull, in mm) were: CeA, −2.0 AP from bregma, ±4.0 LM from midline, and −5.7 DV from the skull surface; BLA, −2.8 AP, ±4.8 LM, and −5.8 DV; vSUB, −6.0 AP, ±4.3 LM, and −6.2 DV.

2.4. Drugs and microinfusions

The selective D_1 antagonist, R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH-23390), was dissolved in isotonic sterile saline. A dose of 1 μg (3 nmol) SCH-23390 or vehicle (saline) was administered via bilateral intracerebral microinfusions in a volume of 0.5 μl, each side. After removing the stylets, injectors (30 gauge) were inserted 2.5 mm below the tips of the guide cannulae to the site of the infusion (−8.2 mm DV for CeA, −8.3 mm DV for BLA, and −8.7 mm DV for vSUB). A Harvard Apparatus pump, set at a rate of 0.32 μl/min, infused drug or vehicle for 1 min 33 s, followed by 1 min of diffusion time. The injectors were removed and the stylets replaced. Rats were immediately placed in the conditioning chambers after microinfusions. The volume of injectate was based on previous studies in our lab [3]. Quantitative autoradiography studies have demonstrated that injections of 0.33 μl of SCH-23390 takes at least 20 min to diffuse outside of the CeA [15].