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Short Communication

Muscarinic, but not nicotinic, acetylcholine receptor blockade in the ventral tegmental area attenuates cue-induced sucrose-seeking

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HIGHLIGHTS

- We examined cue-induced sucrose-seeking during blockade of VTA AChRs or NMDARs.
- VTA scopolamine infusion attenuated cue-induced sucrose-seeking.
- VTA mecamylamine infusion did not alter cue-induced sucrose-seeking.
- VTA AP5 infusion did not alter cue-induced sucrose-seeking.

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ABSTRACT

The mesolimbic dopamine (DA) system is known to play a role in cue-mediated reward-seeking for natural rewards and drugs of abuse. Specifically, cholinergic and glutamatergic receptors in the ventral tegmental area (VTA) have been shown to regulate cue-induced drug-seeking. However, the potential role of these VTA receptors in regulating cue-induced reward seeking for natural rewards is unknown. Here, we examined whether blockade of VTA acetylcholine receptors (AChRs) and N-methyl-D-aspartate receptors (NMDARs) would alter cue-induced sucrose seeking in male Sprague-Dawley rats. Subjects underwent 10 days of sucrose self-administration training (fixed ratio 1 schedule) followed by 7 days of forced abstinence. On withdrawal day 7, rats received bilateral VTA infusion of vehicle, the muscarinic AChR antagonist scopolamine (2.4 or 24 μg/side), the nicotinic AChR antagonist mecamylamine (3 or 30 μg/side), or the NMDAR antagonist AP-5 (0.1 or 1 μg/side) immediately prior to examination of cue-induced sucrose-seeking. Scopolamine infusion led to robust attenuation, but did not completely block, sucrose-seeking behavior. In contrast, VTA administration of mecamylamine or AP-5 did not alter cue-induced sucrose-seeking. Together, the data suggest that VTA muscarinic AChRs, but not nicotinic AChRs nor NMDARs, facilitate the ability of food-associated cues to drive seeking behavior for a food reward.

Cues associated with natural rewards or drugs of abuse can induce craving, reward-seeking and relapse behavior in humans and in animal models [1–3]. While previous research has revealed a critical role of the mesolimbic dopamine system in cue-induced seeking for multiple drugs of abuse [4–6], there is less understanding of the neurobiological processes that underlie cue-induced food-seeking. Recent work has highlighted the role of dopamine signaling as D1 receptor antagonists, administered systemically or directly to the nucleus accumbens (NAc), attenuate cue-induced sucrose-seeking in rats [1]. D1 receptors are low affinity receptors that are preferentially activated by the high concentration, phasic DA release that results from burst firing of DA neurons [7]. Indeed, the presentation of either food rewards or food-associated cues leads to a time-locked increase in DA burst firing in the ventral tegmental area (VTA) and a time-locked increase in phasic DA release in the NAc [8,9]. Thus, VTA processes that regulate DA activity may also play an important role in cue-induced sucrose-seeking.

Both glutamatergic and cholinergic receptors mechanisms in the VTA regulate DA activity and release. Specifically, activation of NMDA receptors induces burst firing in DA neurons [10]. In addition, the VTA contains two classes of AChRs: the ionotropic, nicotinic AChRs (nAChRs) and the metabotropic, muscarinic AChRs (mAChRs). Multiple nAChR and mAChR subtypes, with specific presynaptic and postsynaptic cellular localization, can powerfully modulate phasic DA activity [11]. Our recent work also revealed

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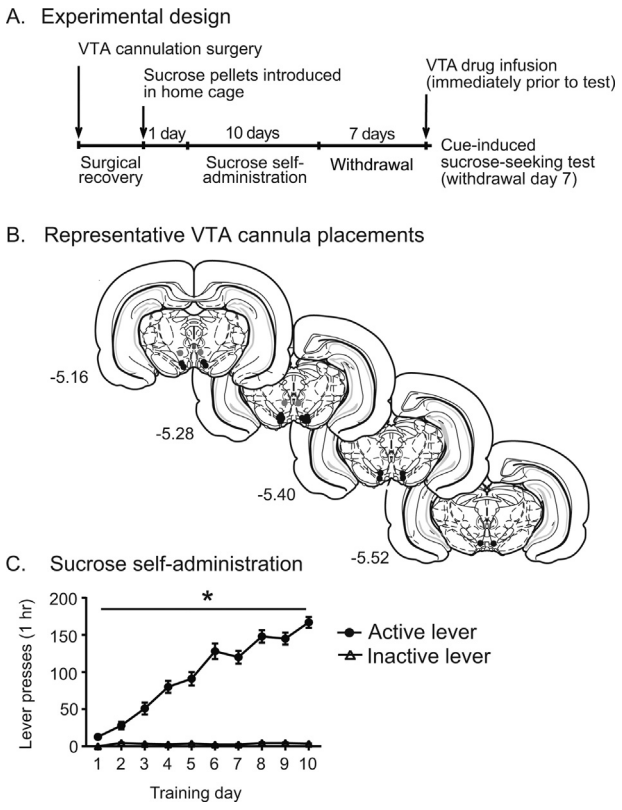

Fig. 1. (A) Experimental timeline. (B) Representative VTA cannula placements. (C) Acquisition of sucrose-self administration behavior (FR 1) over 10 days (main effect of lever and training day, $p < 0.0001$; lever × day interaction, $p < 0.0001$).

that VTA acetylcholine receptors (AChRs) powerfully regulate cue-induced drug-seeking behavior in cocaine-withdrawn subjects [6]. However, the role of VTA AChR mechanisms in cue-induced food-seeking behavior is largely unknown. To date, examinations of VTA cholinergic receptor mechanisms for food reward have mainly focused on free feeding behavior and operant learning [12,13]. In the current study, we sought to determine whether VTA nAChR and mAChR mechanisms mediate cue-induced sucrose-seeking. Given the role of NMDA receptors (NMDARs) in regulating DA responses to cues [14], we also examined the role of VTA NMDARs in cue-induced sucrose-seeking. Our findings suggest that VTA mAChRs, but not nAChRs nor NMDARs, facilitate cue-induced sucrose-seeking during early withdrawal. The understanding gained from this work, together with previous examinations of cue-induced drug-seeking, has important implications for understanding the processes that guide natural reward-seeking and those that guide specific types of drug-seeking behavior. This understanding is important given the emergence of new tobacco products, such as e-cigarettes, which combine sweet flavorants with nicotine delivery.

All experiments were performed in Sprague Dawley rats (250–275 g) acquired from Charles River Laboratories (Wilmington, MA) that were housed on a 12 h light/dark cycle (lights on at 7 am). All experiments were conducted according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Institutional Animal Care and Use Committee (IACUC). After 5–7 day acclimation to the housing facility, rats were anesthetized with ketamine HCl (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p., both from Henry Schein Animal Health, Chicago, IL) and bilateral, 26 gauge cannula were stereotaxically implanted above the VTA (AP −5.3 mm, ML ±0.5 mm, DV −7.0 mm from dura, Fig. 1B). Following 5–7 days of surgical recovery, rats were restricted to 90% free feeding levels for 2–3 days. One day prior to training, 20–30 sucrose pellets (45 mg BioServ pellets, Fisher Scientific, Pittsburgh, PA) were placed into the home cage to introduce the rats to sucrose.

Behavioral data was collected in 3 independently trained cohorts, where vehicle and drug groups were run in parallel on withdrawal day 7 (WD7). All statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA) and all data are expressed as the mean ± standard error of the mean (SEM). Analysis of sucrose self-administration training was performed using a two way, repeated measures ANOVA with lever (active vs. inactive) and training day as factors. "To be" vehicle and "to be" drug groups for the WD7 experiment were assigned in a non-biased manner, with no between group differences in lever presses on training day 10. Analysis of WD7 data was independently performed for each drug using a two way ANOVA with repeated measures, where lever and treatment as factors. For within session analysis of lever presses for each drug experiment (over the 1 h session), lever presses were quantified in 5 min bins and analyzed using a two way repeated measures ANOVA. Additional comparisons between specific drug doses (within each drug experiment) were performed using a post hoc *t*-test with a Bonferroni correction.

Rats were trained for sucrose self-administration in operant chambers (25.4 cm × 30.48 cm × 30.48 cm, Med Associates, St. Albans, VT) on a fixed ratio 1 (FR1) schedule, where active lever depression led to the delivery of a 45 mg sucrose pellet (Fisher Scientific, Pittsburgh, PA) and the simultaneous presentation of a 6 s audio-visual cue (tone + cue light presentation), followed by a 10 s timeout where the lever was retracted. Inactive lever depression had no programmed consequence. Rats received 1 h training sessions over 10 consecutive days and then underwent a 7 day period of forced abstinence, where they had no exposure to sucrose pellets, the operant chamber, or sucrose-associated contextual or discrete cues. Rats that did not acquire stable sucrose self-administration were excluded from the study. Rats included in the study readily acquired operant behavior for sucrose self-administration as revealed by a main effect of lever ($F(1,63) = 327.6$, $p < 0.0001$) and day ($F(9,567) = 57.79$, $p < 0.0001$), and a significant lever × day interaction ($F(9,567) = 62.74$, $p < 0.0001$, Fig. 1C).

VTA infusion of vehicle (0.9% NaCl, saline) or drug (dissolved in saline vehicle) was performed immediately prior to the cue-induced sucrose-seeking test on WD7 using a 25 gauge, 10 μl syringe (Hamilton, Reno, NE) and a micro-infusion pump (Braintree Scientific, Braintree, MA). Saline, mecamylamine (Mec; 3 or 30 μg; Sigma Aldrich, St. Louis, MS, USA), scopolamine (Scop; 2.4 or 24 μg; Sigma Aldrich, St. Louis, MS, USA) or AP-5 (0.1 or 1 μg; Sigma Aldrich, St. Louis, MS, USA) was infused in a 0.5 μL volume over 1 min via a 33 gauge internal cannula (Plastics One, Roanoke, VA) that extended 1 mm beyond the guide cannula to target the VTA (D/V −8.0, Fig. 1B). The internal cannula was left in place for 1 additional min after infusion to allow for complete drug absorption. Immediately after infusion, the extinction test was performed, where active lever depression resulted in delivery of the 6 s audio-visual conditioned stimulus (tone + cue light) in the absence of sucrose pellet delivery. Inactive lever depression again had no programmed consequence. Drug doses were selected based on our previous investigations demonstrating the ability of these doses to alter phasic DA release and behavior [6,15]. Importantly, we previously showed that VTA infusion of 24 μg Scop, 30 μg Mec, or 1 μg AP-5 did not alter locomotor activity [6,16].

Scop infusion on WD7 robustly attenuated cue-induced sucrose-seeking behavior, as revealed by a main effect of treatment ($F(2,20) = 13.03$, $p < 0.0005$) and a lever × treatment interaction ($F(2,20) = 5.67$, $p < 0.01$, Fig. 2A). A test of simple effects further revealed a significant Scop effect at the active lever (Sal vs. 2.4 μg Scop, $p < 0.0001$; Sal vs. 24 μg Scop, $p < 0.0001$), but no effect of Scop