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Research report

Nucleus accumbens dopamine and *mu*-opioid receptors modulate the reinstatement of food-seeking behavior by food-associated cues

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ABSTRACT

The high attrition rates for dietary interventions aimed at promoting a healthier body mass may be caused, at least in part, by constant exposure to environmental stimuli that are associated with palatable foods. In both humans and animals, conditioned stimuli (CSs) that signal reward availability reliably reinstate food- and drug-seeking behaviors. The nucleus accumbens (NAcc) is critically involved in the cue-evoked reinstatement of food-seeking, but the role of individual neurotransmitter systems within the NAcc remains to be determined. These experiments tested the effects of intra-accumbal pharmacological manipulations of dopamine (DA) D_1 and D_2 receptors, *mu*-opioid receptors, or serotonin (5-HT) receptors on cue-evoked relapse to food-seeking. Rats were trained to lever press for sucrose pellets and the concurrent presentation of a light-tone CS. Once training was complete, lever-pressing was extinguished in the absence of either sucrose or CS presentation. Once each rat had reached extinction criterion, they received two reinstatement sessions in which lever pressing was renewed by response-contingent presentation of the CS. Prior to each reinstatement test, rats received NAcc microinfusions of saline or the selective D_1 receptor antagonist SCH 23390, the D_2 receptor antagonist raclopride, the *mu*-opioid receptor agonist [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO), or 5-HT hydrogen maleate. Compared to saline test days, intra-accumbens infusions of SCH 23390 (1 μg/0.5 μL), raclopride (1 μg/0.5 μL), or DAMGO (0.25 μg/0.5 μL) effectively blocked the cue-evoked reinstatement of food-seeking. In contrast, stimulation of serotonin (5-HT) receptors by 5-HT hydrogen maleate (5 μg/0.5 μL) had no effect on cue-induced reinstatement. These novel data support roles for NAcc DA D_1, D_2, and *mu*-opioid receptors in the cue-evoked reinstatement of food seeking.

1. Introduction

One of the most salient issues in the present health media is the rising rate of obesity. Currently, it is estimated that at least 34% of adults in the United States are obese (i.e., BMI ≥ 30). This rate is disconcerting due to the fact that obesity is linked to a plethora of health problems, including cardiovascular disease, type II diabetes, sleep apnea, and infertility [1]. Unfortunately, dietary changes aimed at a lower and healthier body mass are notoriously difficult to maintain. Many weight loss interventions fail to produce body weight reductions that last longer than 4–5 years [2]. For instance, only 2% of women in a longitudinal study were able to maintain weight loss achieved during young adulthood (i.e., age 18–30) until the age of 50 [3]. One potential factor influencing the failure to maintain weight loss may be high rates of dietary relapse. Considering the consistent bombardment of food-associated cues in modern society (e.g., food commercials, fast-food signs, etc.), dietary maintenance may be undermined by exposure to a plethora of stimuli associated with palatable food intake.

Numerous human and animal studies have indicated that cues associated with reward availability have incentive properties and can effectively reinstate food and drug-seeking behaviors [4–9]. Reward-associated conditioned stimuli (CS) can activate mnemonic circuitry and evoke motivated approach behaviors based upon the learned incentive value of the previously experienced reward [10–12]. Evidence in support of this contention is present in studies of both drug and food-related cravings. Human neuroimaging studies have demonstrated that cocaine users have enhanced glucose metabolism in memory circuits (e.g., prefrontal cortices, temporal lobe) related to self-reports of cocaine craving following exposure to cocaine cues versus neutral cues [13]. Based upon this and other evidence, several theories of drug addiction emphasize a role for learned incentives in drug craving [11,14].

Abbreviations: CS, conditioned stimulus; NAcc, nucleus accumbens; DA, dopamine; 5-HT, serotonin; DAMGO, [D-Ala2, N-MePhe4, Gly-ol]-enkephalin; VR, variable ratio; FI, fixed-interval; PIT, Pavlovian-Instrumental Transfer.

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Along similar lines, chronic dieters show an enhanced desire to seek out and consume specifically those foods associated with a particular CS following its presentation (e.g., enhanced desire to specifically consume pizza when exposed to the smell of freshly baked pizza), while craving for non-cued foods (e.g., fresh-baked cookies) is unaltered. This evidence indicates that food-associated cues do not merely increase the motivation to consume any available food, and that incentive learning is critically involved in food craving [15]. Given the powerful role for conditioned stimuli in directing appetitive behaviors, it is of interest in the midst of the present obesity epidemic to determine the neural substrates that are involved in driving the relapse to food-seeking behavior by associated conditioned stimuli.

Prior research has firmly established a role for mesolimbic reward circuitry, including the nucleus accumbens (NAcc), in the reinstatement of both food and drug-seeking behaviors [5,6,16]. Anatomically, the NAcc is well-positioned to transfer relevant cued reward associations into motivated behavioral output. Neural structures involved in encoding and retrieving the affective mnemonic trace of a CS, such as the hippocampus, prefrontal cortex, and amygdala, all have converging inputs to the NAcc [17,18]. In turn, the NAcc projects to motor output structures in the basal ganglia, such as the ventral pallidum [19]. Recent evidence suggests that the NAcc plays a critical role in directing behavior based upon the learned incentive value of environmental cues. For instance, pharmacological inactivation of the NAcc disrupts lever pressing behavior in the presence of discriminant stimuli associated with natural reinforcer availability [9]. Furthermore, a recent study by Floresco et al. [5] demonstrated that inactivation of the NAcc core attenuated the reinstatement of lever-pressing in extinguished rats when the lever presses resulted in the presentation of cues that had previously been associated with food reinforcement. In contrast, similar inactivation of the NAcc shell invigorated instrumental responding in the reinstatement condition.

This evidence strongly suggests that the NAcc is critical for directing the seeking of natural rewards in the presence of discrete cues that predict reinforcer availability. However, it remains to be determined which neurotransmitter systems within NAcc are specifically involved in the cue-evoked reinstatement of food-seeking. Multiple neurotransmitters within the ventral striatum are known to modulate the appetitive and consummatory phases of food and drug-motivated behaviors. For instance, antagonism of NAcc D_1 or D_2 receptors reliably attenuates drug-seeking [16,20] and reduces the effort that rats will expend to obtain a preferred food [9,21]. Also, rodent models of motivated behaviors indicate that *mu*-opioid receptor stimulation within the NAcc robustly increases the intake of preferred diets [22,23] and is involved in precipitating cocaine and alcohol craving and relapse [24,25]. Drugs that impact serotonergic function affect food intake when given systemically, and recent reports have shown that individual serotonin receptors within the NAcc modulate the intake of standard rat chow under deprived conditions, as well as food intake in response to palatable diet presentation in sated rats [26,27]. Thus, any or all of these neurotransmitters within the NAcc may play a role in modulating the reinstatement of food-seeking behavior in the presence of food-associated CSs.

In these experiments, we examined the effects of manipulating NAcc dopaminergic receptors, *mu*-opioid receptors, or serotonin receptors on cue-induced reinstatement of food seeking behavior. Individual groups of food restricted rats were trained across seven days to lever press for sucrose pellets, which were delivered concurrently with the presentation of a light-tone stimulus. Once training was complete, rats received daily extinction sessions, during which lever presses resulted in neither sucrose delivery nor the light/tone CS. Once lever-pressing behavior had lowered to extinction criterion, rats were given two reinstatement sessions, during which lever pressing elicited the presentation of the light/tone CS. Across the two reinstatement sessions, individual groups of rats received NAcc injections of either saline or the D_1 receptor antagonist SCH 23390 (Experiment 1), the D_2 receptor antagonist raclopride (Experiment 2), the *mu*-opioid receptor agonist DAMGO (Experiment 3), or the serotonin receptor agonist 5-HT hydrogen maleate (Experiment 4). The effects of each drug treatment on cue-induced reinstatement of lever-pressing behavior were then assessed (Fig. 1).

2. Materials and methods

2.1. Subjects and housing

Male Sprague–Dawley rats (Harlan, Madison, WI) were dually housed in transparent polycarbonate cages with wire covers in a temperature and light controlled vivarium (21 °C, 12-h light-dark cycle, lights on/off- 7 am/7 pm). In order to minimize stress, animals were handled daily upon arrival. Behavioral testing was conducted during the light phase. All experiments were conducted in accordance with NIH animal care guidelines and were approved by the Wake Forest University Animal Care and Use Committee.

2.2. Surgery

After the animals for each experiment were acclimated to the housing environment for a period of one week, rats underwent cannula placement surgery. The rats were anesthetized with a Ketamine–Xylazine cocktail (90 mg/kg–9 mg/kg) and standard aseptic surgical procedures were followed to implant stainless steel indwelling guide cannulas (23 gauge) bilaterally above the medial NAcc, near the transitional zone between the shell and core (flat skull surgery, A-P: 1.7 mm anterior to bregma, M-L: ±1.4 mm, D-V: −7.5 mm from the top of skull). The cannulas were affixed to the skull with self-curing dental acrylic adhered to three stainless steel jeweler's screws. Wire stylets were placed in the guide cannulas to prevent occlusion.

2.3. Apparatus

Four standard operant chambers (Med-Associates, St. Albans, VT, USA) were utilized for the present experiments. Each was enclosed in a sound-attenuating chamber equipped with ventilation fans. The chambers were fitted with a house light and two retractable levers on each side of the central food receptacle. Two identical 100-mA stimulus lights were located just above each lever. A programmable speaker, positioned on the wall opposite to the food receptacle, presented auditory stimuli.

2.4. Cue-evoked reinstatement of food-seeking paradigm

Rats were allowed to recover from surgery for a period of one week before they were gradually food restricted until reaching approximately 90% of their *ad libitum* body weight. Two days prior to the beginning of magazine training, rats were habituated to the sucrose pellets (45 mg; BioServ) by supplementing their daily food ration with 2 g of the pellets.

The following reinstatement procedure was adapted from the experimental protocol utilized by Floresco et al. [5]. All experimental procedures were conducted during the beginning of the light phase. Magazine training consisted of two days (Days 1 and 2) of 30 min random time 60 s training sessions in which sucrose pellet delivery was presented at an average of 60 s between deliveries, in the absence of any levers or stimulus presentations. The day following the magazine training period (i.e., Day 3), both levers were inserted into the chambers for operant training and rats received 20 min training sessions where lever presses on the active lever resulted in the presentation of a combined CS (light/tone)–US (sucrose pellet). An inactive lever with no programmed consequences was presented on the opposite side of the food receptacle. Left/right positioning of the active lever was counterbalanced across animals in all experimental groups. Active lever presses on the first operant training day were reinforced on a fixed-ratio 1 schedule in which each lever press resulted in the presentation of a 5 s light-tone cue (the light above the stimulus lever was illuminated, in conjunction with the presentation of a 80 dB, 3 kHz tone). Exactly 0.5 s following the onset of the CS cue, a sucrose pellet primary reinforcer was delivered to the magazine. On the fourth day of training, rats were further acclimated to the procedure on a fixed-ratio 2 schedule in which every two lever presses resulted in the same cue-reward presentation. Active lever presses during the cue presentation were recorded, but not reinforced nor counted toward ratio requirements. Days 5–9 consisted of a variable-ratio (VR) 5 reinforcement schedule superimposed upon a fixed-interval (FI) 20 schedule of reinforcement. This VR-5, FI-20 schedule resulted in the first CS-pellet delivery on an average of 5 active lever presses. After this initial reinforcer was earned, a 20 s time out period initiated in which lever presses resulted in no consequences. Following this rest interval, the VR-5 schedule resumed until another reinforcer was earned and the schedule repeated.