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Nicotine-enhanced Pavlovian conditioned approach is resistant to omission of expected outcome

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ABSTRACT

Conditioned stimuli contribute to the resilience of nicotine addiction in that nicotine-associated cues can influence smokers and promote relapse. These stimuli are thought to acquire incentive motivational properties through a Pavlovian mechanism, and this phenomenon can be measured in animals by observing conditioned approach to the conditioned stimulus (sign-tracking) or to the location of unconditioned stimulus delivery (goal-tracking). Goal-tracking is thought to be more flexible than sign-tracking in response to changes in expected outcome. Nicotine exposure can increase the expression of conditioned responses, and we hypothesized that animals exposed to nicotine would also exhibit less flexible conditioned responses after a change in the expected unconditioned stimulus. Adult male rats were exposed to nicotine (0.4mg/kg, s.c.) or saline before Pavlovian conditioned approach training sessions. After training, animals underwent test sessions that reduced (water substitution) or withheld (omission) the unconditioned stimulus (US, 20% sucrose). As expected, nicotine enhanced sign- and goal-tracking. Water substitution moderately and nonspecifically reduced both sign- and goal-tracking in all rats. In contrast, US omission only reduced goal-tracking, with robust effects in saline-exposed rats and smaller effects in nicotine-exposed rats. These data support the hypothesis that both sign-tracking and nicotine exposure confer behavioral inflexibility under US omission.

The influence of drug-associated cues is of particular importance in addiction, as exposure to these cues can precipitate craving and relapse [1]. Cues can develop strong associations with a drug through Pavlovian learning, during which a conditioned stimulus (CS) is repeatedly paired with an unconditioned stimulus (US), such as a drug or natural reward. Over time, the CS can acquire conditioned motivational properties. Multiple drugs of abuse, including nicotine, have been shown to promote the attribution of incentive salience to a CS in animals [24,2,3]. Pavlovian processes are thought to underlie attentional bias to smoking cues in human smokers, measured as excessive allocation of attention to these cues in attentional tasks; such attentional bias often correlates with subjective nicotine craving [4–6]. Thus, developing strategies to reduce the salience of conditioned cues after nicotine exposure could promote smoking cessation.

Pavlovian conditioned approach can be used to measure the incentive properties of a CS in animals. As animals learn the association between CS presentation and US delivery, they begin to exhibit conditioned responses to CS presentation by approaching and interacting with the CS (sign-tracking) or the location of US delivery (goal-tracking). While the expression of any conditioned response indicates the learning of a predictive relationship between the CS and US, sign-tracking is specifically thought to indicate that the CS has become an incentive stimulus [7]. Importantly, the enhanced attribution of salience to a CS can emerge in the absence of drug exposure [8], when drugs are the US [9,10], or after drug exposure outside of training [11–13]. In particular, nicotine exposure enhances both goal-tracking [12,14] and sign-tracking [11,13,14]. Addictive drugs may promote the attribution of incentive properties to a CS, and sign-tracking specifically is linked to other behaviors associated with addiction vulnerability [3,7].

Behavioral studies in this paradigm typically focus on animals pre-classified as sign-trackers or goal-trackers. Sign-trackers may show less behavioral flexibility in response to changes in the previously learned CS-US relationship. While sign-trackers perform similarly to goal-trackers during extinction of instrumental drug self-administration [2,15], they are slower to update their behavior under extinction conditions in a Pavlovian task [16]. Moreover, when animals are trained to exhibit sign-tracking and goal-tracking to separate stimuli, the sign-

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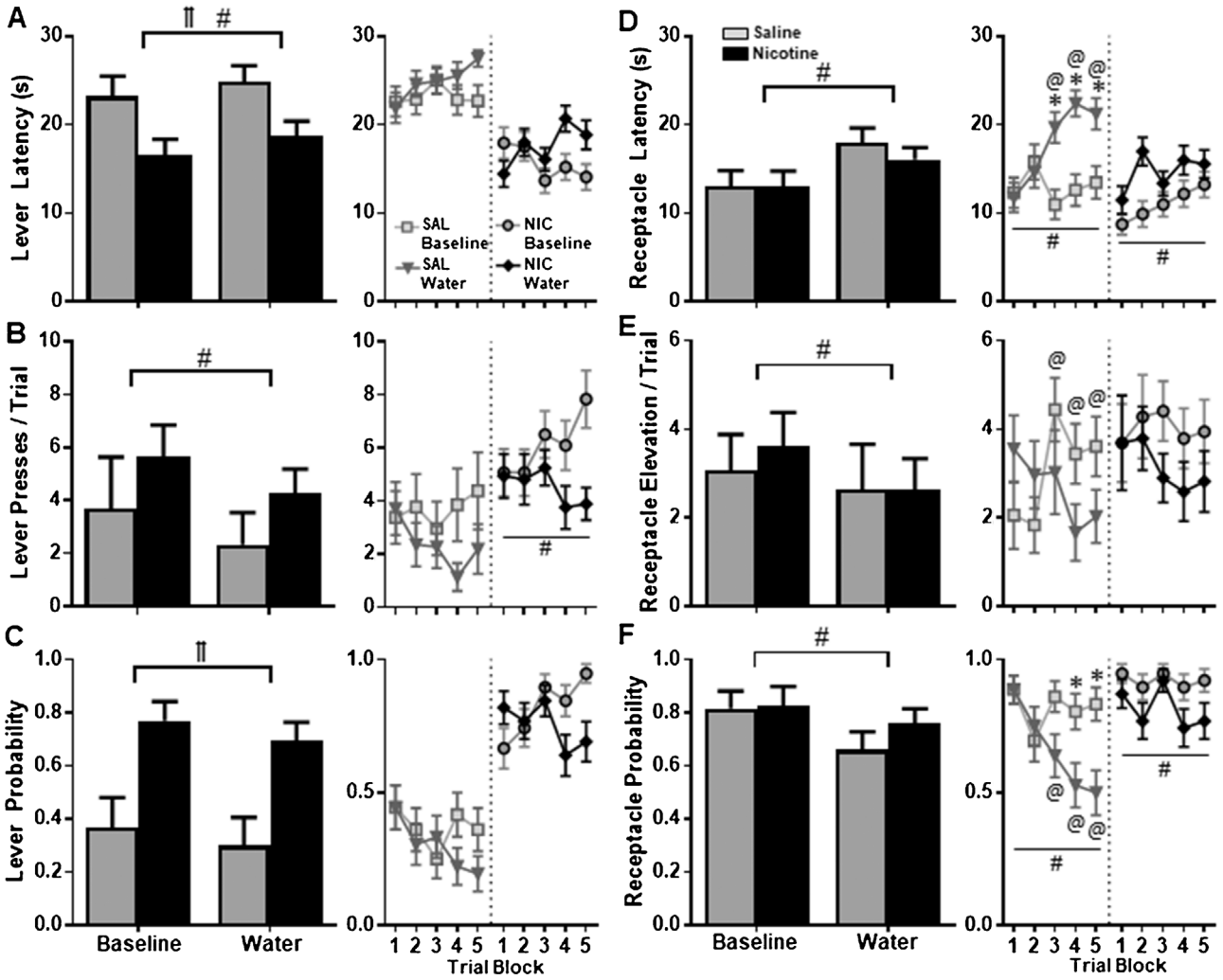

Fig. 1. Water substitution nonspecifically reduced sign- and goal-tracking. Behavioral measures were compared between 'Water' and 'Baseline' days: between groups over the whole session (left, bar graphs), and within groups during each session (right, line graphs, collapsed into 5 blocks of 3 trials each). Measures of sign- and goal-tracking (mean ± SEM) are displayed as latency to press the lever (A), lever presses (B), probability of pressing the lever (C), latency to enter the receptacle (D), receptacle elevation score (E), and probability of entering the receptacle (F).⇑ main effect of group, # main effect of session, * trial block different between sessions, @ different from first block on same session, $p < 0.05$ for all noted analyses.

tracking behavior is more resistant to extinction of the US [17]. Thus, animals classified as sign-trackers appear less flexible in updating their conditioned behavior after a change in the CS-US relationship, and sign-tracking may be less flexible than goal-tracking regardless of the animals' classification. Exposure to drugs such as nicotine enhances the expression of sign-tracking conditioned responses, but the degree to which drugs further reduce the flexibility of conditioned behavior has yet to be established.

To address this knowledge gap, we investigated the extent to which nicotine exposure blunted the flexibility of sign- and goal-tracking after a change in US. Specifically, we evaluated conditioned behavior after delivery of an unexpected and less valuable US (water) and under US omission (single extinction session). We hypothesized that animals exposed to nicotine would be less likely to update conditioned responses after a change in the expected US.

Rats in the present experiments were used in a previous study [14]. Adult male Sprague Dawley rats (225–250 g on arrival) were purchased from Harlan/Envigo (Indianapolis, IN, USA) and pair-housed during initial training, then individually housed after surgery. Animals were provided with food and water *ad libitum* during the entire study. Rats were housed in a vivarium on a 12:12 h light:dark cycle, and experiments occurred during the light cycle. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

Training behavior for this cohort of animals was published previously (Fig. 4 in [14]), during which nicotine exposure enhanced sign- and goal-tracking. Animals were assigned to either a nicotine-exposure group (NIC, n = 12) or a saline-exposed control group (SAL, n = 12). Nicotine hydrogen tartrate salt (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile saline with pH adjusted to 7.0 ± 0.2. Rats received 0.4mg/kg nicotine (s.c., dose calculated from the free base form) or an equivalent volume of saline once/day for two days to habituate them to the injection procedure, then 15 min before each behavioral session. Training was conducted in behavioral chambers (MedAssociates, St. Albans, VT), assembled with a recessed fluid receptacle with photobeam detector, cue light, and retractable lever on one wall of the chamber, and a house light located on the opposite wall. At least 25 daily Pavlovian conditioning sessions were conducted Monday-Friday, and each session included 15 CS−US pairings on a variable interval (VI) 120-second reinforcement schedule. The CS consisted of illumination of the stimulus light and extension of the lever located directly below the light. CS presentations lasted 30 s, and were immediately followed by 0.1 ml of 20% sucrose in the receptacle. Lever deflections and head entries into the receptacle were recorded but had no programmed consequences. After training, all animals were habituated to behavioral