Behavioral Neuroscience

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Online First Publication, March 14, 2019. http://dx.doi.org/10.1037/bne0000311

American Psychological Association

Behavioral Neuroscience

0735-7044/19/$12.00

http://dx.doi.org/10.1037/bne0000311

Effects of Nicotine Exposure and Anxiety on Motivation for Reward and Gambling-Like Cues Under Reward Uncertainty

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Reward uncertainty is a common characteristic of gambling and may powerfully enhance attraction to gambling-related cues, thus promoting maladaptive gambling behaviors in susceptible individuals. The co-occurrence of gambling disorder with tobacco use disorder (60.4%) suggests a common mechanism for their pathology, and comorbid anxiety (41.3%) might further promote the maintenance of these behaviors. However, it is unknown how nicotine or anxiety might contribute to cue and reward attraction, or promote disordered gambling behavior. In the present study, we investigated the effects of nicotine (0.4 mg/kg, SC) on the desire for uncertain rewards and their cues in male and female Sprague–Dawley rats. During an autoshaping task, rats learned to associate a lever + tone cue with the delivery of sucrose pellet rewards under either certain or uncertain (probability and magnitude) reward conditions. Subsequently, we tested the ability of gambling-like cues to serve as a conditioned reinforcer, and to promote motivation for sucrose rewards during a progressive ratio task. Finally, anxiety behavior was measured to examine its interaction with nicotine and uncertainty. Here, we found that nicotine enhanced attraction to the magazine under certain but not uncertain reward conditions, and increased cue-triggered behaviors. Conversely, in the progressive ratio task, exposure to uncertain conditions and nicotine enhanced motivation for reward, compared with certain conditions. These results suggest that nicotine may interact with both certain and uncertain reward conditions to increase cue-triggered behavior and enhance motivation for rewards, providing possible insight into the comorbid relationship between pathological gambling and tobacco use.

Keywords: autoshaping, incentive salience, motivation, nicotine, uncertainty

In the United States, approximately 85% of adults engage in gambling at some point in their lifetime (Cunningham-Williams et al., 2005; Kessler et al., 2008; Shaffer & Hall, 2001). In its most severe form, gambling leads to extreme personal, social, and economic ramifications (Eadington, 2003; Slutske, Piasecki, Blaszczynski, & Martin, 2010), a condition known as gambling disorder (GD). GD is defined by the *Diagnostic and Statistical Manual*, fifth edition (*DSM–5*) as a behavioral addiction characterized by persistent and recurrent betting that is problematic or impairs quality of life (American Psychiatric Association, 2013). It has been approximated that around 2.5% of the general population qualifies for a GD diagnosis (Cunningham-Williams et al., 2005; Kessler et al., 2008; Shaffer, Hall, & Vander Bilt, 1999). Within these clinical reports, data suggest a co-occurrence between the diagnosis of GD, anxiety disorders, and substance abuse (Hellberg, Russell, & Robinson, 2018). For example, treatment-seeking gamblers have a greater incidence of psychiatric disorders, with 41.3% of disordered gamblers reporting the diagnosis of an anxiety disorder (compared with only 10% of the general population). Similarly, up to 63% of pathological gamblers qualify for a substance use disorder (Crockford & el-Guebaly, 1998). Compared with other drugs of abuse, nicotine use disorder is one of the most frequently reported addictions among disordered gamblers (60.4% comorbidity rate), ranking second to alcohol use disorder at 73% (Petry, Stinson, & Grant, 2005). Individuals with nicotine use disorder are seven times more likely to bear the diagnosis of GD compared with nonsmokers. Within this trend, sex differences emerge, with women struggling with tobacco use being 14 times

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Data and ideas from this article have been previously disseminated in the form of a poster presentation at the Society of Neuroscience conference in Washington, DC (2017) and Eastern Psychological Association conference in Boston (2017). All data for the manuscript will be provided by contacting the corresponding author.

Experiments were designed by Trinity I. Russell, Mike J. F. Robinson; data were collected by Trinity I. Russell; data were analyzed and interpreted and the article was written by Trinity I. Russell and Mike J. F. Robinson.

This study was supported by internal funding from Wesleyan University. We thank Charlotte Freeland, Ariel Ben-Ezra, and Samantha Hellberg for their technical assistance. The authors declare no competing financial interests or have any conflicts of interest to disclose that may have influenced the study or presentation of its findings.

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more likely to be diagnosed with GD than nonsmoking women, whereas men with tobacco use disorder are five times more likely to bear the diagnosis of GD compared with nonsmoking men (McGrath & Barrett, 2009; Petry et al., 2005). In addition, both nicotine use and gambling involvement are associated with increased levels of anxiety, a known risk factor for relapse to maladaptive behaviors (Badrick, Kirschbaum, & Kumari, 2007; Kirschbaum, Wüst, & Hellhammer, 1992; Steptoe & Ussher, 2006). Nicotine use and anxiety disorder may converge with gambling disorder to promote and sustain maladaptive behavior. However in humans, the direction of these relationships is almost impossible to determine due to the wide range of factors involved (Hellberg, Russell, & Robinson, 2018). For example, whereas evidence suggests that gambling involvement, even at recreational levels, can predict the onset of anxiety or substance use disorders three years later (Parhami, Mojtabai, Rosenthal, Afifi, & Fong, 2014), research also shows that anxiety disorders often precede the onset of gambling disorder or substance use disorders (Blanco et al., 2015; Kausch, Rugle, & Rowland, 2006; Kessler et al., 2008).

In the specific case of GD and drugs of abuse, numerous studies suggest neurobiological similarities between their effects on reward pathways (Kessler et al., 2008; McGrath & Barrett, 2009; Petry et al., 2005; Smart & Ferris, 1996). Both appear to activate the mesolimbic reward system, which is implicated as a common neural substrate contributing to motivated approach and reward-related behaviors. According to the incentive sensitization theory, repeated exposure to potentially addictive substances, under particular circumstances, causes a persistent hyper-reactivity of the brain circuits associated with the attribution of incentive salience (Robinson, Fischer, Ahuja, Lesser, & Maniates, 2015; Robinson & Berridge, 1993). The repeated activation of these brain circuits is thought to produce a sensitized system, which assigns a pathological degree of incentive salience, *'wanting'*, to rewards and their cues. However, research suggests that there are large individual differences in both human and animals regarding the degree to which these systems are susceptible to incentive sensitization (Bartlett, Hallin, Chapman, & Angrist, 1997; Boileau et al., 2014; Saunders & Robinson, 2011, 2013). Susceptibility to disordered gambling behavior, therefore, might be related to individual differences in incentive motivation for rewards and their cues.

One key feature hypothesized to promote the attribution of incentive salience to gambling cues and rewards is the inability to predict when rewards will be delivered, or how large the rewards will be—a concept known as reward uncertainty. Reward uncertainty appears to be a key component of all gambling games (Costikyan, 2013), and research has shown that reward uncertainty increases mesolimbic dopaminergic activity and can even result in locomotor sensitization and greater drug self-administration (Fiorillo, Tobler, & Schultz, 2003; Mascia et al., 2019; Singer, Scott-Railton, & Vezina, 2012). Modes of gambling that are highly unpredictable and independent on the players' efforts, such as electronic slot machines, contain a greater degree of uncertainty compared with games that are influenced by strategy or practice, such as table games like blackjack or poker. In games of chance, celebratory lights and sounds may be present in win outcomes where the player receives a reward greater than their wager, as well as in loss outcomes when the player receives a reward less than their wager (termed losses disguised as wins) (Dixon, Harrigan, Sandhu, Collins, & Fugelsang, 2010; Griffiths, 1993). Because these cues are salient and capture a players' attention, they may distort perception of events, causing individuals to associate cues with wins despite their potential loss status (Dixon et al., 2010; Spenwyn, Barrett, & Griffiths, 2010). In doing so, cues can become powerful motivators that leverage the effects of uncertainty to promote gambling behavior (Anselme, 2013; Robinson, Anselme, Suchomel, & Berridge, 2015).

Previously, we have shown that uncertainty, in the probability and magnitude of the reward, greatly enhances the attraction to cues, transforming them into even stronger motivational magnets (Anselme, Robinson, & Berridge, 2013; Hellberg, Levit, & Robinson, 2018; Robinson, Anselme, et al., 2015; Robinson, Anselme, Fischer, & Berridge, 2014). Using a Pavlovian autoshaping task, where the brief presentation of a compound cue (lever + tone) predicts either one, two, or three sucrose pellets (magnitude uncertainty) on only 50% of the trials (probability uncertainty), compared with conditions where one pellet is consistently delivered each trial (reward certainty), we have demonstrated that uncertain reward cues are attributed with greater incentive salience leading to greater sign-tracking (Anselme et al., 2013). In particular, animals display greater attentional focus toward the lever cue (sign-tracking), which is often seen as pressing, biting, sniffing, or direct engagement with the lever in some other fashion. This behavior is accompanied by large reductions in their attention (approach, sniffing, biting, etc.) toward the reward delivery dish or magazine (goal-tracking). An increase in sign-tracking behavior and decrease in goal-tracking behavior results in an elevated ratio of lever presses to magazine entries and a higher proportion of sign-trackers in a given population (Anselme et al., 2013; Robinson, Anselme, et al., 2015). This enhancement of incentive salience attribution appears similar to that produced by amphetamine and stress sensitization (Robinson, Anselme, et al., 2015). We have also shown that reward uncertainty recruits and ascribes incentive value to more distal cues (Robinson, Anselme, et al., 2014), that it is greatest in high-anxiety subgroups, and that this attraction is persistent despite the omission of reward (Hellberg, Levit, & Robinson, 2018).

Similarly, previous research suggests that nicotine is particularly effective in establishing or enhancing the incentive-motivational properties of reward-associated conditioned cues (Balfour, Wright, Benwell, & Birrell, 2000; Caggiula et al., 2001). Several studies report that nicotine enhances cue attraction in the form of greater sign-tracking when the brief extension of a retractable lever acts as a conditional stimulus (CS; Stringfield, Palmatier, Boettiger, & Robinson, 2017; Versaggi, King, & Meyer, 2016). However, earlier studies showed that nicotine administration enhances approach behavior to the location of primary reward delivery (the goal) during CS presentation, when the CS consists of an auditory or visual stimulus (Guy & Fletcher, 2013; Olausson, Jentsch, & Taylor, 2003). The distinction in the type of cue-triggered behavior elicited by the presentation of the CS appears to depend on whether the format of the CS is localizable (retractable lever) or diffuse (auditory cue). Whereas the presentation of a localizable cue triggers approach and interaction in the form of sign-tracking, presentation of a diffuse cue elicits goal-tracking. It has been argued that this goal-tracking can be explained by the fact that location cues such as the goal can also be considered as incentives, as these cues are associated with sucrose delivery (Palmatier et al., 2013), and thus become the primary target of cue-triggered ap-

proach. Overall these findings implicate nicotine in the alteration of incentive-motivational processes and cue-elicited behaviors, but highlight a crucial role for the format (localizable vs. diffuse) and sensory modality of the cues (Meyer, Cogan, & Robinson, 2014).

Here, we sought to determine how nicotine exposure and reward uncertainty might contribute to the attribution of incentive salience and motivation for reward cues, which are associated with the development of disordered gambling behavior. Male and female rats were used to investigate the effects of acute nicotine administration on the attribution of incentive salience following exposure to certain and uncertain reward cues, and the reinforcing value of these cues. To measure the attribution of incentive salience, we employed an autoshaping procedure (Hearst & Jenkins, 1974) in which conditional stimuli were paired with the delivery of either certain or uncertain rewards (Anselme et al., 2013). We used a compound CS consisting of a brief extension of an illuminated retractable lever, accompanied by the presentation of an auditory cue to examine the impact of reward uncertainty and nicotine administration to multisensory gambling-like cues. Following autoshaping, we used a conditioned reinforcement task to measure the reinforcing property of either certain or uncertain cues. We also examined the motivation to obtain a previously certain or uncertain sucrose reward in a progressive ratio task. Furthermore, because the literature also suggests comorbidity between nicotine use, GD, and clinical levels of anxiety (el-Guebaly et al., 2006; Kessler et al., 2008; Lorains, Cowlishaw, & Thomas, 2011; Parhami et al., 2014), we sought to determine the effects of nicotine and reward uncertainty on levels of anxiety in rats.

In the present study, we hypothesized that (a) reward uncertainty would enhance attraction to cues, (b) that nicotine would enhance attraction to both the lever and food dish in the presence of a compound (lever + tone) cue, (c) that females would show an increased sensitivity to the effects of nicotine, and (d) that the combination of nicotine and reward uncertainty would be associated with greater motivation and reward seeking.

Method

Subjects and Housing Conditions

Subjects were 80 adult (10–18 weeks old) Sprague–Dawley rats (female, $n = 40$; male, $n = 40$) bred in-house (originally purchased from Harlan) and weaned at postnatal Day 21. Rats were housed by sex in groups of 2–3 animals per cage with ad libitum access to food (LabDiet, Teklad) and water. Cages were stored in climate controlled rooms, and housing rooms were regulated to a 12:12-hr reverse light:dark cycle. Prior to the start of behavioral experiments, animals were handled and habituated for 2–3 days, and were food-restricted to 85% to 90% of initial body weight. All procedures were approved by the Institutional Animal Care and Use Committee at Wesleyan University.

Groups and Conditions

Rats initially underwent a single session on the Elevated Plus Maze to establish baseline anxiety (Figure 1A and 1B). They were

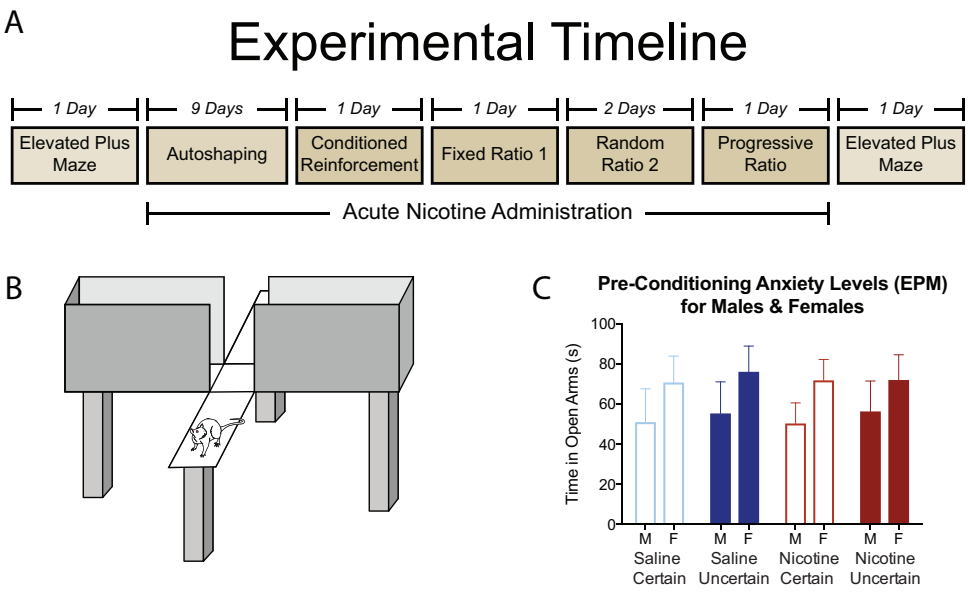

Figure 1. Experimental timeline and the measurement of baseline anxiety levels in rats. (A) Overview of the experimental timeline including procedures and tasks completed throughout the experiment. (B) Schematic of the Elevated Plus Maze used as a behavioral measure for levels of anxiety in rats. Time spent in the open arms of the maze was operationalized as a measure of anxiety, and greater open arm time was associated with decreased levels of anxiety. (C) Rats were assigned to experimental conditions so that average preconditioning anxiety levels were similar across all conditions. The time spent in the open arms showed no difference between groups, although initial levels of anxiety were greater for males (M) than females (F). Data presented are Mean ± *SEM*. See the online article for the color version of this figure.

then divided into four groups (for each group $n = 20$; female = 10, male = 10) according to litter, sex, and baseline anxiety levels, so that anxiety was matched across all groups, and within each sex. The four experimental conditions differed by drug treatment (nicotine or saline) and reward conditions (certain: 100%-1 or uncertain: 50%-1–2–3) during autoshaping:

- Saline Certain, 100%-1 autoshaping reward condition
- Saline Uncertain, 50%-1–2–3 autoshaping reward condition
- Nicotine Certain, 100%-1 autoshaping reward condition
- Nicotine Uncertain, 50%-1–2–3 autoshaping reward condition

Drugs and Injections

All rats, irrespective of group assignment ($n = 80$), received a saline injection (1 ml/kg, SC) 15 min prior to the start of the magazine training session. Starting on the first day of autoshaping, rats received a subcutaneous injection of either saline ($n = 40$) or nicotine ($n = 40$, 0.4 mg/kg) depending on group assignment. Each injection was given 15 min prior to the start of each session. Injections were continued throughout autoshaping, conditioned reinforcement, FR1, RR2, and PR sessions. (−)-Nicotine hydrogen tartrate salt was obtained from Glentham Life Sciences and was dissolved in saline (0.4 mg/kg), and adjusted to a pH of 7. The dose, route of injection, and duration between nicotine pretreatment and behavioral testing was determined based on previous studies assessing Pavlovian paradigms in rats (Palmatier et al., 2013; Palmatier, Kellicut, Brianna Sheppard, Brown, & Robinson, 2014; Stringfield, Boettiger, & Robinson, 2018; Versaggi et al., 2016).

Elevated Plus Maze

Apparatus. The Elevated Plus Maze (EPM) is a known behavioral measure used to index levels of anxiety in rodents (Walf & Frye, 2007). As previously described (Hellberg, Levit, & Robinson, 2018), the apparatus contained two perpendicular platforms that intersected to form a plus-shaped symbol (+). The plus-shaped maze was elevated off the floor by 97 cm and consisted of four arms measuring 40 cm in length and 15 cm in width. The four arms consisted of two closed arms, partially enclosed by dark, nontransparent walls on three sides, and two open arms that were exposed, without walls (Figure 1B). Each arm was located directly across from its matching arm with a 15 × 15 cm square open intersection in the middle, joining each arm of the maze. The apparatus was exposed on top and an infrared video camera (Advidia) placed above the apparatus was used for visualization and recording of behavior for the duration of each session.

EPM Procedure and Scoring. Rats were placed in the center of the maze, with the head and tail facing the open arms. Exploratory behavior was assessed for a period of 5 min under red light conditions. After each trial, rats were returned to their home cage, and the apparatus was cleaned with 90% Versa-Clean Multi-Purpose Cleaner. Videos were manually scored by an investigator blind to the experimental conditions. Time spent and entries into the two closed and two open arms were recorded for each animal's first five minutes on the maze. An arm entry was recorded when all four paws of the animal were located in one arm. The entry ended when all four paws of the animal were no longer in the arm. The duration of all arm entries was summed to determine the total time spent on each of the closed and open arms. The amount of time spent on the two open arms was summed together and used as a measure of anxiety (Hellberg, Levit, & Robinson, 2018). Open arm time inversely correlates with anxiety; thus, the most anxious rats will spend the least amount of time in the open arms (Pellow, Chopin, File, & Briley, 1985; Walf & Frye, 2007).

Pavlovian and Operant Conditioning

Apparatus. All testing was conducted in Med Associates Inc. Modular Test Chambers (25.8 × 32.2 × 33.2 cm) with metal bar floors, two modular front and back walls, and two Plexiglas walls as previously described (Lesser, Arroyo-Ramirez, Mi, & Robinson, 2017). Each chamber was equipped with two retractable levers located on the front wall of the chamber, either side of a recessed magazine dish, which delivered 45 mg sucrose pellets (TestDiet, St. Louis, MO, U.S.A.). A speaker located on the front wall of the chamber, at all times, delivered a 2.9 kHz tone. For the conditioned reinforcement session, the back wall was outfitted with two nose poke holes (one active, one inactive, location counterbalanced), located on either side of a retractable lever. During this time, the food cup on the front wall was covered with a custom metal plate. MedPC® software automatically recorded lever presses, nose pokes, and magazine entries across all sessions. Chambers were placed in sound attenuating cabinets to reduce ambient light and noise. Red LED lights were mounted on the wall inside the cabinet and were turned on during all sessions.

Autoshaping procedure. To examine the attraction for reward-related cues, rats underwent 9 days of Pavlovian autoshaping. The autoshaping procedure consisted of one day of sucrose preexposure, one day of magazine training, and nine days of Pavlovian conditioned approach or autoshaping. Two days prior to the first autoshaping session, rats were given 30–50 sucrose pellets placed in the home cage, to reduce neophobia and habituate the rats to sucrose consumption. One day prior to autoshaping, rats received a magazine training session. During the 30-min training session, rats were habituated to the environment of the testing chamber and received 30 sucrose pellets from the magazine dish. Pellets were delivered on a variable intertrial-interval, averaging 45 seconds (VI-45). Rats then received one autoshaping session per day for a period of 9 days. Each session consisted of 36 trials on a variable intertrial-interval (VI-45), and lasted for approximately 30–35 min. Rats were presented with two levers. One lever, termed the control lever, measured baseline lever attraction, and was available for the duration of the session, and never retracted. Presentation of the second lever served as a CS, which predicted the delivery of sucrose pellets (UCS). The position of the CS and control lever was counterbalanced across subjects. Each CS trial consisted of the presentation of an illuminated lever extended into the chamber accompanied by an 8 second auditory tone. After 8 seconds, the tone silenced, the lever retracted, and sucrose pellets were delivered from an overhead dispenser into the metal magazine dish. Pellets were dispensed according to two reward contingencies: certain (100%-1) and uncertain (50%-1–2–3). In the 100%-1 reward contingency, each CS presentation, during every trial, resulted in the delivery of one sucrose pellet to the magazine dish. In the 50%-1–2–3 reward contingency, half of

the CS presentations (18 trials; order randomized) resulted in the delivery of 0 sucrose pellets, whereas the other half of the CS presentations (18 trials) resulted in the delivery of one, two, or three sucrose pellets, with equal probability. The 50%-1–2–3 reward contingency created uncertainty in the probability and magnitude of reward delivery. However, despite the reward contingency, all rats received 36 pellets and 36 CS presentations by the end of each autoshaping session, and were therefore equally exposed to both the CS and UCS rewards. Throughout each session, responses on both levers and entries into the magazine were recorded but had no programmed consequence.

Sign-tracking and goal-tracking. Although the delivery of reward was independent of behavior, rats typically interact (e.g., sniffing, nibbling, biting, pressing) with the CS lever and magazine dish, and develop two distinct conditioned responses (CR): sign-tracking and goal-tracking. These behaviors may be quantified as a measure of the incentive salience attributed to that cue and reveal individual differences in cue attraction (Robinson, Yager, Cogan, & Saunders, 2014). An animal's response bias toward either cue was determined using the following equation (LP − ME)/(LP + ME) derived from the Pavlovian Conditioned Approach (PCA) index (Meyer et al., 2012), with scores ranging from 1 to −1. Animals with a strong preference for the lever had a response bias between 1 and 0.5 and were classified as sign-trackers, whereas goal-trackers had a response bias between −0.5 and −1. An individual was classified as an intermediate if it directed its responses to both the lever and the food cup, and had a response bias between 0.5 and −0.5. An animal's phenotype was based on responses during the CS presentations of the last day (Day 9) of Pavlovian autoshaping. All animals developed a conditioned response after initial training.

Conditioned reinforcement procedure. After autoshaping, rats completed a 1-day conditioned reinforcement task (30 min) to assess the incentive value of the CS, and to measure to what extent it could act as a reinforcer in the absence of reward. Rats were given the opportunity to work on a Fixed Ratio 1 (FR1) schedule for the presentation for the lever + auditory cue CS. At the start of the session, both nose pokes were illuminated with a muted yellow light. Responses on one nose poke, termed the active nose poke, provided access to the lever + tone CS, which were presented for 3 seconds. The other nose poke, termed the inactive nose poke, had no programmed consequence and served as a control that measured baseline attraction to the nose poke. The position of the active and inactive nose poke was counterbalanced across subjects. Med-PC software recorded active and inactive nose pokes, lever presentations and the number of presses on the lever during the session.

Progressive ratio procedure. Motivation for the sucrose reward was assessed using operant responding and a progressive ratio paradigm. As in autoshaping, the front wall contained two metal levers on either side of the magazine dish. The lever designated as the CS lever during the autoshaping task was assigned as the active lever during operant training. Similarly, the control lever used during autoshaping remained extended yet inactive during operant tasks. The operant training procedure consisted of one day of Fixed Ratio 1 (FR1) training, two days of Random Ratio 2 (RR2), and one day of progressive ratio (PR), each session lasting 30 min. The FR1 reward contingency required rats to execute one lever press for the delivery of one sucrose pellet. Following the FR1 task, rats completed a 2-day RR2 task, which required rats to complete between one and three lever presses to obtain a single sucrose pellet. After RR2, rats completed a 1-day PR task. Progressive ratio assessed rats' willingness to expend effort to obtain a sucrose reward. The number of presses required to obtain a single sucrose pellet increased on an exponential progressive ratio schedule (1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95 . . .) determined by the equation (progressive ratio = $[5e^{(\text{reward number} \times 0.2)}] - 5$) and rounded to the nearest integer (Richardson & Roberts, 1996; Robinson, Warlow, & Berridge, 2014). Med-PC recorded the number of rewards earned and lever presses an animal completed during the task. The highest number of lever presses completed to obtain a single sucrose pellet, was used as a measure of breakpoint.

Statistical Analysis

Data from all tasks were analyzed using one-way/repeated measures ANOVAs or paired *t* tests (IBM SPSS 25 and Graphpad PRISM 6), where appropriate. As stated in the Introduction, our primary intention in this study was to examine the relative contribution of reward condition (certain vs. uncertain) and drug (saline vs. nicotine) on conditioned approach behaviors. As such, after performing factorial analyses across all factors (drug, reward condition, sex), data were analyzed by group using repeated measures ANOVAs. Further analysis between groups was performed using post hoc analyses (Fisher's LSD). Magazine entry data during the intertrial interval was excluded for one animal (Saline Uncertain group) as it was on average more than 3 standard deviations above the average for its group. All analyses were two-tailed and performed at a level of significance of $p < .05$.

Results

Elevated Plus Maze Preconditioning: Initial Anxiety Levels Were Matched Across Groups

All animals were initially exposed to the Elevated Plus Maze (EPM) for 5 min prior to any behavioral procedures. Open arm time was used to assign rats to one of four conditions so that the preconditioning anxiety levels were matched across all groups (n per group = 20; male/female n = 10/10). We confirmed that there was no significant difference in the baseline anxiety levels between the four groups of rats that would later receive exposure to nicotine and uncertainty (Group: $F_{(3,76)} = 0.061$, $p = .980$; Figure 1C). As expected, based on previous findings (Hellberg, Levit, & Robinson, 2018; Lesser et al., 2017) females displayed lower levels of anxiety than males (Sex: $F_{(1,72)} = 4.116$, $p = .046$).

Autoshaping: Nicotine Enhances Magazine Entries During the CS Under Certain Reward Conditions, an Effect Blunted by Reward Uncertainty

To assess attraction for CS cues, rats underwent nine days of an autoshaping procedure that paired a CS lever and tone with the delivery of sucrose pellets, which were presented according to either certain (100%-1 pellet) or uncertain (50%-1–2–3 pellets) reward

conditions. Each day, rats were given an acute injection of either nicotine or saline 15 mins prior to the start of the autoshaping session.

Animals from all four groups were assessed for their attraction to either the lever, measured in the form of lever presses, or the goal, measured as magazine entries during the CS presentation. Across the 9 days of autoshaping all four groups showed a rapid increase in the number of lever presses (Day: $F_{(8,576)} = 129.464$, $p < .001$; Figure 2A). However there was no main effect of nicotine or sex (Drug: $F_{(1,72)} = 1.557$, $p = .216$; Sex: $F_{(1,72)} = 1.141$, $p = .289$), and no differences between the four groups (Group: $F_{(3,76)} = 0.578$, $p = .632$). In contrast, animals showed a progressive decrease in cue-induced magazine entries across sessions (Day: $F_{(8,576)} = 34.855$, $p < .001$; Figure 2B). In particular, nicotine exposure resulted in a greater number of magazine entries (Drug: $F_{(1,72)} = 5.980$, $p = .017$), and slowed the rate at which magazine entries declined over time (Day × Drug: $F_{(8,576)} = 2.442$, $p = .013$). Overall, there was a difference in the level of attraction each group displayed toward the goal during CS presen-

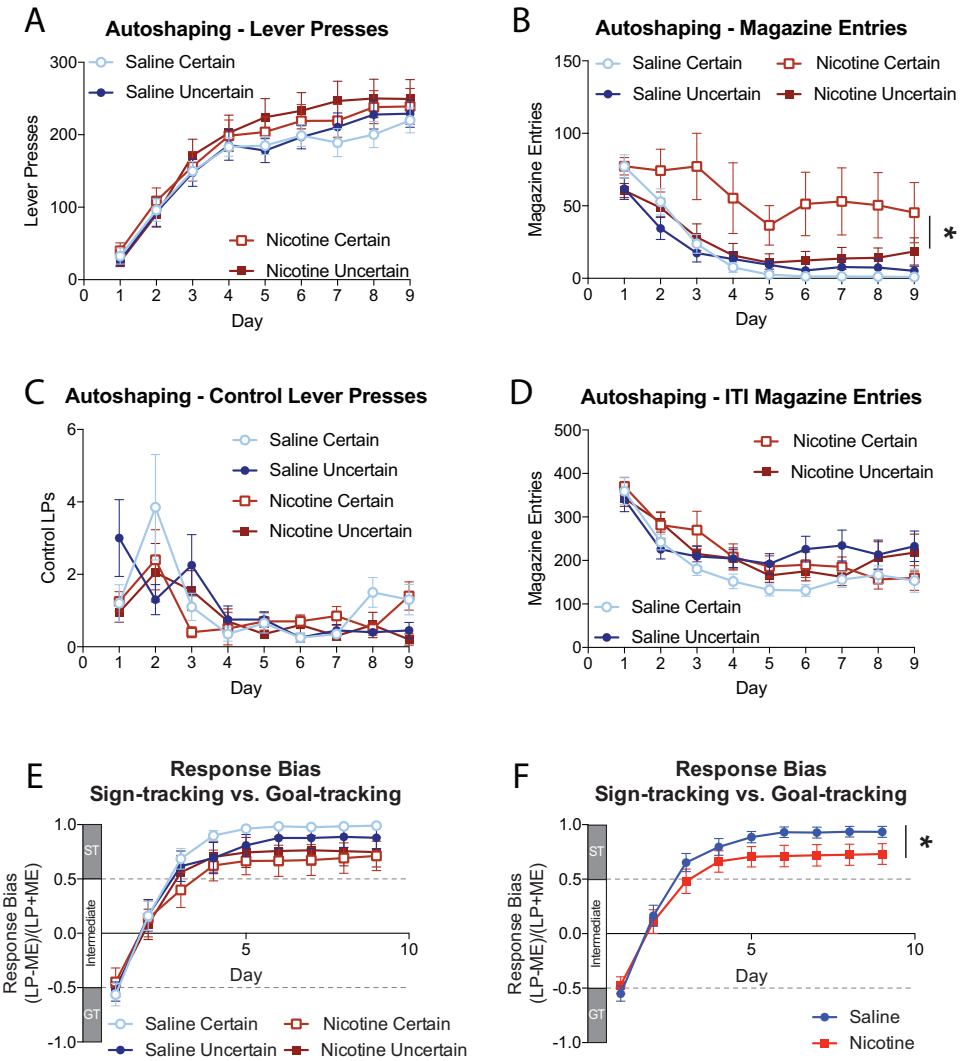

Figure 2. Acquisition of lever pressing and magazine entries throughout autoshaping sessions including behavioral phenotypes (response bias). (A) Lever presses on the active CS across 9 days of autoshaping for animals assigned to each of four reward (certain vs. uncertain) and drug (nicotine vs. saline) conditions, shows no difference across groups. (B) Magazine entries during the CS presentation steadily decreased across 9 days of autoshaping, although animals receiving nicotine under certain conditions maintained higher levels of responding. (C) Responses on the inactive lever were no different across groups and remained low across training days. (D) Neither nicotine nor reward uncertainty had any impact on general magazine entries during the intertrial interval (ITI). (E) Response bias toward the lever (lever presses: LP), reward receptacle (magazine entries: ME), or both (Intermediate) for animals assigned to each of four conditions showed that rats initially began goal-tracking (GT), but predominantly developed a sign-tracking (ST) phenotype across sessions. (F) However, nicotine reduced the rate at which a response bias toward sign-tracking developed across days. Data presented are Mean ± *SEM*. * $p < .05$. See the online article for the color version of this figure.

tations (Group: $F_{(3,76)} = 4.100$, $p = .009$), and how their attraction changed over time (Day × Group: $F_{(24,608)} = 1.615$, $p = .033$). Specifically, animals exposed to nicotine under certain conditions exhibited a greater number of magazine entries across days than all three other conditions (Fisher's LSD: ps < 0.015).

However, the impact of nicotine did not appear to be attributable to any nonspecific effects, because animals across all groups showed no difference in lever presses on the inactive control lever (Drug: $F_{(1,72)} = 1.837$, $p = .180$; Group: $F_{(3,76)} = 0.822$, $p = .486$), which gradually decreased across sessions to less than 1 response on average by Day 9 (Day: $F_{(8,576)} = 7.639$, $p < .001$; Figure 2C). Similarly, magazine entries during the intertrial interval (ITI) progressively decreased across days (Day: $F_{(8,568)} = 38.110$, $p < .001$; Figure 2D), but appeared unaffected by the administration of nicotine (Drug: $F_{(1,72)} = 0.531$, $p = .469$; Group: $F_{(3,76)} = 1.190$, $p = .320$), suggesting that the effects of nicotine reported above were triggered by the presentation of the CS.

Sign-Trackers and Goal-Trackers: Nicotine Attenuates the Degree of Sign-Tracking

Animals were then classified as *sign-trackers* (STs), *goal-trackers* (GTs), or *intermediates* (INTs), based on their lever presses (LP) and magazine entries (ME) during CS presentations on Day 9 of autoshaping, by calculating their response bias (LP − ME)/(LP + ME). STs were defined as animals with a response bias between 1 and 0.5, suggesting a strong preference for interacting with the lever. In contrast, GTs directed the majority of their behavior toward the magazine with a response bias between −0.5 and −1, whereas intermediates possessed a response bias between 0.5 and −0.5. Based on these criteria, 90% of the population were STs ($n = 72$), 6.25% were GTs ($n = 5$), and 3.75% were INTs ($n = 3$). On average, all groups began with a preference toward goal-tracking on the first day of autoshaping. However, across days each group rapidly and similarly developed a predominantly sign-tracking phenotype (Day: $F_{(8,608)} = 183.947$, $p < .001$; Group: $F_{(1,76)} = 0.872$, $p = .459$; Day × Group: $F_{(24,608)} = 1.064$, $p = .381$; Figure 2E). There was no impact of sex, drug, or reward condition on response bias (Fs < 2.015, ps > 0.161), however nicotine did appear to reduce the rate at which sign-tracking developed across days (Day × Drug: $F_{(8,576)} = 2.064$, $p = .037$; Figure 2F). This might be explained by the fact that 80% (four of five) of all goal-trackers and 100% (three of three) of all intermediate animals came from groups exposed to nicotine during training, despite the majority of the population (90%) displaying a sign-tracking phenotype. This suggests that nicotine may have increased the tendency of animals to goal-track.

Nicotine Enhances Cue-Triggered Behavior in Rats Assigned to Certain but not Uncertain Reward Conditions, With Females Displaying Greater Sensitivity to Nicotine

Previous studies of the impact of nicotine on autoshaping and Pavlovian conditioning have shown that nicotine tends to increase magazine entries when animals are presented with only a diffuse auditory or visual cue as a signal of food delivery, but increase sign-tracking and lever presses when the cue is a localizable extended lever (Guy & Fletcher, 2013; Olausson et al., 2003; Stringfield et al., 2018; Versaggi et al., 2016). Because our autoshaping protocol combines the presence of both a physical lever cue and the presentation of an auditory tone and lever light, we examined the impact of nicotine and reward uncertainty on total cue-triggered behaviors, defined as the sum of lever presses and magazine entries (LPs + MEs) across days. This approach ensured that any increases in cue-triggered behavior, whether toward the goal or the sign, would be adequately captured.

Overall, animals demonstrated acquisition of the CS–UCS association by an increase in cue-triggered behaviors across all days (Day: $F_{(8,576)} = 77.784$, $p < .001$). However, animals exposed to chronic administration of nicotine during autoshaping increased their overall amount of cue-triggered behaviors (Drug: $F_{(1,72)} = 11.382$, $p = .001$; Figure 3A), and did so at a higher rate than saline treated animals (Day × Drug: $F_{(8,576)} = 2.878$, $p = .004$). In addition, males tended to perform a slightly greater number of cue-triggered behaviors than females (Sex: $F_{(1,72)} = 4.348$, $p = .041$; Figure 3B), although there was no difference in their rate of acquisition of the task (Day × Sex: $F_{(8,576)} = 0.816$, $p = .589$), suggesting that this may have been attributable to differences in body weight and the males' ability to press on the levers more vigorously. Most notably, there was an overall impact of group on cue-triggered behaviors (Group: $F_{(3,76)} = 4.278$, $p = .008$; Day × Group: $F_{(24,608)} = 1.669$, $p = .024$). Analysis of the impact of nicotine between groups revealed that nicotine increased the number and rate of acquisition of cue-triggered behaviors under conditions of reward certainty (Certain: Saline vs. Nicotine: $F_{(1,38)} = 12.478$, $p = .001$; Day × Group: $F_{(8,304)} = 2.492$, $p = .012$; Figure 3C). However this was not the case for animals exposed to uncertain reward conditions (Uncertain: Saline vs. Nicotine: $F_{(1,38)} = 1.965$, $p = .169$; Day × Group: $F_{(8,304)} = 1.017$, $p = .423$; Figure 3D).

Further analysis by sex highlighted that both males and females exposed to nicotine displayed more cue-triggered behaviors under reward certainty (Certain – Saline vs. Nicotine: Females: $F_{(1,18)} = 8.456$, $p = .009$; Males: $F_{(1,18)} = 6.202$, $p = .023$; Figure 4A and 4C). In females, the effect of nicotine appeared to be present from the beginning of autoshaping (Group – for Days 1–2: $F_{(1,18)} = 4.669$, $p = .044$) and remained constant across all other days and across the course of training (Females: Day × Group: $F_{(8,144)} = 0.52$, $p = .84$). In contrast, in males the effect of nicotine appeared absent initially (Group – for Days 1–2: $F_{(1,18)} = 0.002$, $p = .969$), but grew across days of exposure (Males: Day × Group: $F_{(8,144)} = 3.299$, $p = .002$). This suggests that females were more sensitive to the effects of nicotine by showing an instant change in behavior on the first day.

In comparison, nicotine trended toward increasing cue-triggered behaviors in females exposed to reward uncertainty (Females: $F_{(1,18)} = 3.489$, $p = .078$; Figure 4B), but did not appear to impact males (Uncertain – Saline vs. Nicotine: Males: $F_{(1,18)} = 0.041$, $p = .842$; Figure 4D).

Conditioned Reinforcement: Cues Associated With Reward Uncertainty and Nicotine Also Develop Conditioned Reinforcing Properties, but Females Show More Responding

Rats were tested in a 1-day conditioned reinforcement task after the completion of autoshaping training, to assess whether the lever + tone cue (CS) could act as a conditioned reinforcer, particularly following conditions of reward uncertainty where the CS's predictive value was degraded. During this task, animals

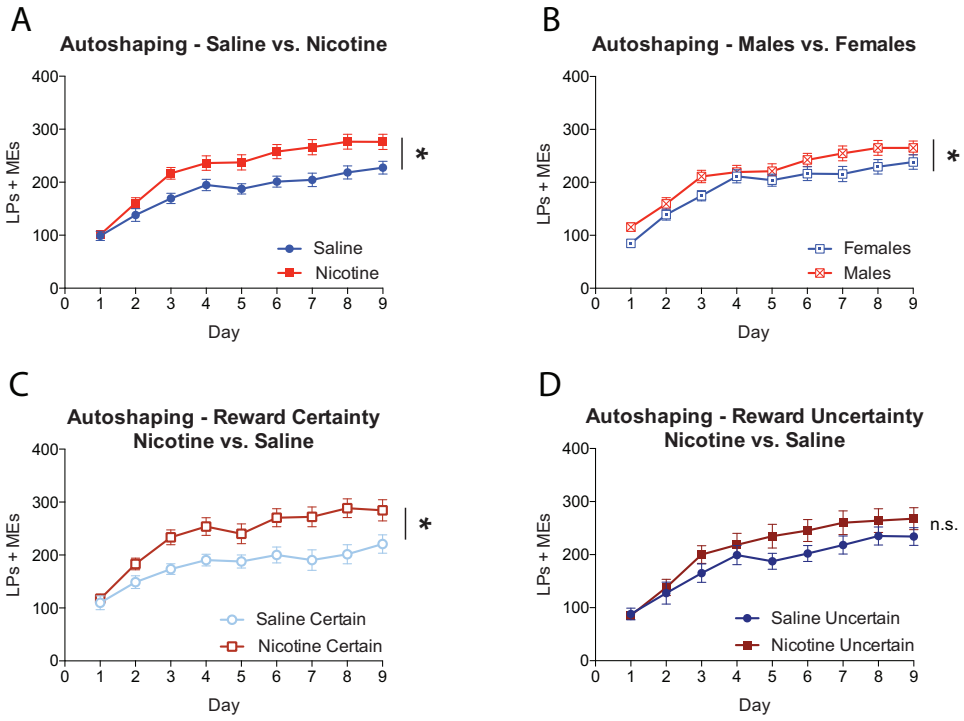

Figure 3. The impact of reward conditions, nicotine exposure, and sex on cue-triggered behaviors. Cue-triggered behaviors were measured as the sum of lever presses and magazine entries (LP + ME) during the CS presentation. (A) Overall, chronic nicotine administration enhanced cue-triggered behaviors across sessions, and (B) males performed a greater number of cue-triggered behaviors in the autoshaping procedure than females but responding increased at the same rate. (C) Rats assigned to the certain autoshaping condition and injected with nicotine performed more cue-triggered behaviors than rats injected with saline. (D) No overall difference was observed in cue-triggered behaviors for animals assigned to the uncertain autoshaping condition and injected with nicotine or saline. Data presented are Mean ± *SEM*. * $p < .05$. See the online article for the color version of this figure.

were able to work to gain brief access to the CS by performing nosepokes (NPs) into an active nosepoke hole, in contrast to an inactive nosepoke hole that had no programmed consequence. Overall, the CS acquired conditioned reinforcing properties across all animals, as they performed significantly greater active than inactive nosepokes (Nosepoke: $F_{(1,72)} = 217.525$, $p < .001$; Figure 5A), and this was the case for each group (Group: $F_{(1,76)} = 0.927$, $p = .432$; For each group: $ts_{(19)} > 5.461$, $ps < 0.001$), irrespective of the CS's prior predictive value. In addition, responses on the active nosepoke were clearly driven by an interest in gaining access to the CS lever, as almost all animals (77 of 80) engaged with the lever in the form of lever presses ($M = 67$ responses), although no single factor affected the number of lever presses on the CS ($Fs < 0.77$, $ps > 0.436$).

However, there was a significant effect of sex on nosepoking behavior. In particular, contrary to what might be expected, females displayed more responding during conditioned reinforcement than males (Sex: $F_{(1,72)} = 9.217$, $p = .003$), including a greater number of responses on the active nosepoke than males (Active Nosepoke: Sex: $F_{(1,79)} = 7.244$, $p = .009$), and there was a trend toward a greater preference in females for the active nose poke (Sex × Nosepoke: $F_{(1,72)} = 3.074$, $p = .084$; Figure 5B). There was, however, no effect of nicotine or reward uncertainty on conditioned reinforcement or any interactions with sex (Drug: $F_{(1,72)} = 1.403$, $p = .24$; Reward Condition: $F_{(1,72)} = 0.024$, $p = .877$; Sex × Drug: $F_{(1,72)} = 0.039$, $p = .845$; Sex × Reward Condition: $F_{(1,72)} = 0.51$, $p = .477$). Overall this would suggest that females ascribed greater conditioned reinforcer and incentive properties to the CS, despite performing significantly lower levels of conditioned approach during autoshaping.

Progressive Ratio: Reward Uncertainty Alone and in Combination With Nicotine Administration Increases Motivation to Obtain Rewards

Results from Pavlovian autoshaping suggest that nicotine enhances cue-triggered behavior under conditions of reward certainty. However, it remains unclear whether nicotine and reward conditions enhance attraction to cues, increase motivation for rewards, or both. Here, the progressive ratio task was used to assess motivation to obtain the sucrose pellet reward under operant conditions. Rats were first trained on an FR1 (1 day) and RR2 (2 days) to acquire operant responding for sucrose pellets. Rats were then required to work for a single sugar pellet on an exponential ratio of responding, and motivation strength was measured as both the number of rewards they attained and the last ratio completed by the animal, also known as the

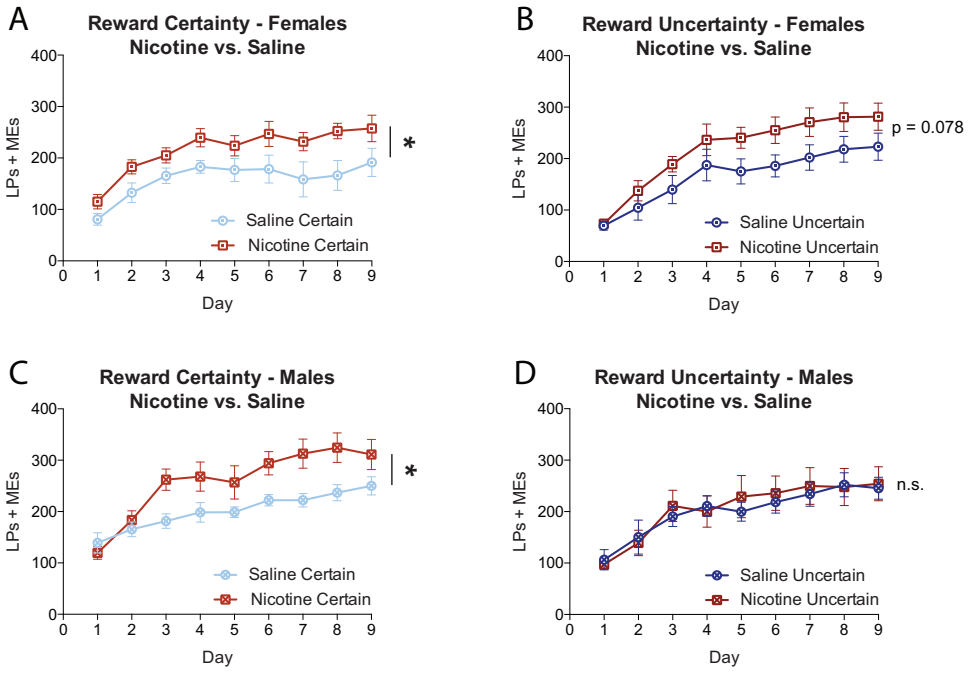

Figure 4. Acquisition and maintenance of cue-triggered behaviors during autoshaping sessions by reward condition, nicotine exposure, and sex. (A) Females exposed to nicotine and reward certainty displayed greater cue-triggered behaviors than their saline counterparts. The effect of nicotine was present from the start of autoshaping and remained throughout training. (B) Exposure to nicotine and reward uncertainty only trended toward increased cue-triggered behaviors in female rats. (C) The enhancing effect of nicotine was initially absent in males assigned to reward certainty but grew to be significant with increased training. (D) No differences attributable to nicotine exposure were observed between males assigned to the uncertain reward condition. Data presented are Mean ± *SEM*. * $p < .05$. See the online article for the color version of this figure.

breakpoint. These two factors (rewards attained and breakpoint) were used as they both capture similar yet slightly different facets of motivation. In particular, because of its exponential nature, the measure of breakpoint exacerbates small differences in the number of trials completed, which are absent when examining rewards attained, due to its linear nature.

During the initial acquisition of operant responding, all groups acquired lever pressing for sucrose reward, but did so to the same level and at the same rate (Trials: Group: $F_{(3,76)} = 0.417$, $p = .742$; Day: $F_{(2,152)} = 26.293$, $p < .001$; Group × Day: $F_{(6,152)} = 0.945$, $p = .465$). However, during exposure to a progressive ratio, analysis of the number of rewards each animal received revealed a significant effect of group (Group: $F_{(3,76)} = 3.742$, $p = .015$; Figure 5C). Further post hoc analyses indicated that the combination of reward uncertainty and nicotine created a significant difference in motivation to attain reward over nicotine administered to rats exposed to certain reward conditions (Fisher's LSD: $p = .002$). Similarly, analysis of the breakpoint suggested a trending effect across groups (Group: $F_{(3,76)} = 2.24$, $p = .09$; Figure 5D), which post hoc analyses showed to be driven by a 35% greater breakpoint in animals exposed to nicotine and uncertainty over those exposed to nicotine and reward certainty (Fisher's LSD: $p = .012$). Finally, it is worth noting that prior exposure to reward uncertainty during autoshaping increased the number of rewards that animals attained (Reward Condition: $F_{(1,72)} = 6.068$, $p = .016$; Figure 5E), and that males also tended to reach higher breakpoints, possibly because of differences in size (Sex: $F_{(1,72)} = 8.675$, $p = .004$; Figure 5F).

Elevated Plus Maze Postconditioning: Neither Nicotine Administration Nor Chronic Exposure to Uncertainty Affected Levels of Anxiety in Rats

Levels of anxiety were once again measured following exposure to autoshaping, conditioned reinforcement, and progressive ratio. To create a sense of novelty and reduce any effect of habituation to the environment on anxiety, the apparatus was relocated to the opposite side of the testing room, and the arms of the maze were rotated 45° about the origin. Unlike during the initial test of anxiety, there was no anxiety difference between males and females (Sex: $F_{(1,72)} = 0.012$, $p = .912$), nor was there an effect of uncertainty, nicotine or between the four experimental groups, when animals were retested following all other behavioral measures (Reward Condition: $F_{(1,72)} = 0.819$, $p = .369$; Drug: $F_{(1,72)} = 0.17$, $p = .681$; Group: $F_{(3,76)} = 0.344$, $p = .794$; Figure 6A).

To assess whether behavioral measures in addition to exposure to reward uncertainty or nicotine had any impact on the development of anxiety-like behavior, we compared final postconditioning anxiety levels to preconditioning levels. We found no overall change in anxiety across time and no effect of reward condition, drug or group on anxiety levels (Pre vs. Post: $F_{(1,72)} = 0.746$, $p = .391$; Reward Condition × Pre/Post: $F_{(1,72)} = 1.463$, $p = .23$;

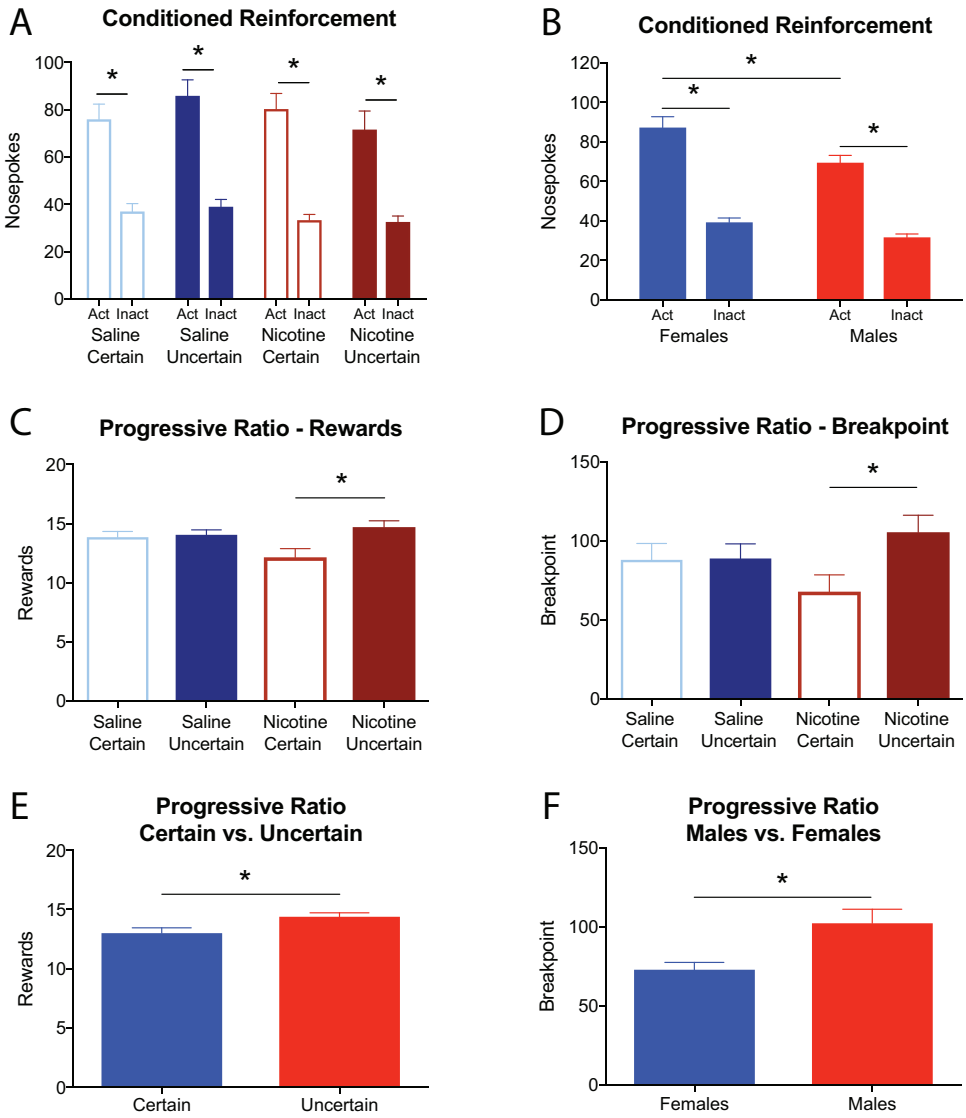

Figure 5. The effect of uncertainty and nicotine exposure on conditioned reinforcement and progressive ratio. (A) Rats across all conditions assigned conditioned reinforcing properties to cues previously paired with reward irrespective of prior reward contingencies or nicotine exposure. They all acquired a new behavior to gain access to these cues over a single session, as seen by significantly greater active than inactive nosepokes. (B) Females displayed overall more responding during the conditioned reinforcement task with a trend toward a greater preoccupation with the active nosepoke than males. (C) Motivation for reward was measured using a progressive ratio task. Here, animals exposed to reward uncertainty and nicotine worked to obtain more rewards than animals exposed to certain conditions and nicotine. (D) Exposure to the combination of reward uncertainty and nicotine also resulted in a greater motivation to obtain rewards shown by a 35% greater breakpoint. (E) Overall, prior exposure to reward uncertainty increased the number of rewards the animal attained, and (F) males typically achieved higher breakpoints compared with females. Data presented are Mean ± *SEM*. * $p < .05$. See the online article for the color version of this figure.

Drug × Pre/Post: $F_{(1,72)} = 0.197$, $p = .659$; Group × Pre/Post: $F_{(1,72)} = 0.551$, $p = .649$; Figure 6B). There was however a trend for sex (Sex × Pre/Post: $F_{(1,72)} = 2.921$, $p = .092$), which suggested that while males showed no change in anxiety between tests (Males: Pre vs. Post: $F_{(1,39)} = 0.328$, $p = .57$), females tended to become more anxious (Females: Pre vs. Post: $F_{(1,39)} = 3.976$, $p = .053$; Figure 6C).

Discussion

Nicotine Enhances Magazine Entries and Cue-Triggered Behavior Under Reward Certainty

Here, we demonstrate the complex interaction between nicotine and reward uncertainty on the attraction to Pavlovian reward-

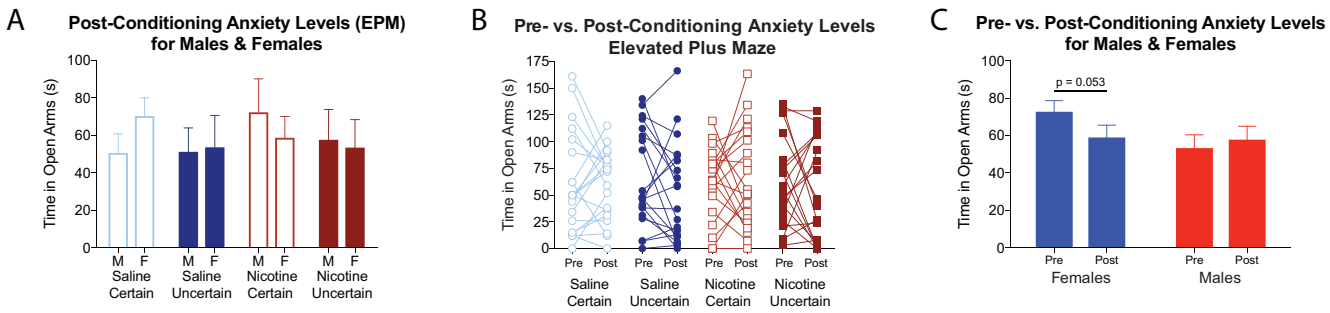

Figure 6. Postconditioning anxiety levels. (A) There was no impact of sex, uncertainty, nicotine, or group assignment on anxiety levels assessed using the elevated plus maze test following conditioning. (B) There was no systematic impact of conditioning and behavioral procedures on anxiety levels when compared with initial preconditioning levels of anxiety regardless of sex, nicotine or uncertainty. (C) However, females trended toward becoming more anxious after conditioning as compared with their initial preconditioning anxiety levels. Data presented are Mean ± *SEM*. See the online article for the color version of this figure.

related cues and reward seeking. Our results show that the administration of nicotine during the autoshaping procedure broadly increases cue-elicited attraction, defined as the combination of lever presses and magazine entries, across conditions. However, this was in large part attributable to an increase in goal-tracking measured by sustained magazine entries under conditions of reward certainty. This resulted in a reduction in the intensity of sign-tracking in nicotine-exposed animals (certain and uncertain), as measured by their response bias. These effects were not attributable to a general increase in overall activity, as nicotine administration had no effect on responses on the inactive lever or magazine entries during the intertrial interval, which is in line with previous reports (Olausson et al., 2003).

Previous studies have shown that nicotine administration during Pavlovian conditioning enhances approach behavior to the location of primary reward delivery (goal-tracking) in the presence of the CS (Guy & Fletcher, 2013; Olausson et al., 2003). However, this effect occurs when the CS consists of an auditory or visual stimulus, which can be diffuse and possibly difficult to locate and approach. In contrast, when the CS consists of a brief lever presentation, several studies report that nicotine enhances sign-tracking rather than goal-tracking responses (Stringfield et al., 2017; Versaggi et al., 2016). In the present studies, the CS was a compound cue consisting of both a lever presentation, lever light, and an auditory stimulus, to provide a multisensory stimulus more akin to that experienced during slot machine gambling using electronic gambling machines. The multisensory component of the cue might explain the increase in magazine entries under certain reward conditions and the overall increase in cue-elicited responses. These results also suggest that in the presence of a compound cue (lever + tone), nicotine may primarily promote an increase in magazine entries over lever responding, as indicated by the Nicotine Certain group.

The effects of nicotine on cue-triggered behavior were most prominent under certain conditions, as it significantly increased the degree of cue-triggered approach to both the lever and food cup for both males and females. However, this effect appeared to be stronger for females. In particular, nicotine increased cue-triggered behavior in females starting on the very first exposure and autoshaping session, and continued throughout the 9 days. In contrast, this was not the case for males, who initially displayed no increase in cue-triggered behavior, but showed a slow progression in response to nicotine starting on Day 3 and continuing across days. A similar trend could be seen under conditions of reward uncertainty in females, where nicotine administration tended to increase cue-triggered behaviors, particularly after the initial acquisition of the task (Days 5–9). In males, however, there was no effect of nicotine under uncertain reward conditions. The greater response to nicotine seen in females under both certain and uncertain conditions suggests a possible heightened sensitivity to nicotine in females, and these results align with previous studies that have highlighted sex differences in response to nicotine and nicotine-associated cues. Notably, female rats have been shown to respond more for and to have greater sensitivity to nicotine infusions than males, an effect that is further heightened by the presence of nicotine-paired cues (Chaudhri et al., 2005). It might therefore be useful for future studies to assess the effect of multiple doses of nicotine on motivation for reward and gambling-like cues in female rats. In addition, evidence suggests that female Sprague–Dawley rats tend to acquire sign-tracking to a cue slightly faster than males (Pitchers et al., 2015). Together, these results could explain why a larger proportion of women tend to engage in slot machine gambling, where cues play a prominent role in the maintenance of gambling behavior (Dow Schüll, 2012; McCarthy et al., 2018).

Surprisingly, nicotine did not appear to enhance cue-triggered behaviors when administered under conditions of reward uncertainty across males and females combined. We also failed to find effects of reward uncertainty alone, in contrast to what has been previously reported (Anselme et al., 2013; Hellberg, Levit, & Robinson, 2018; Robinson, Anselme, et al., 2014). Although the lack of reward uncertainty effects found here is surprising, it is possible that this is attributable to the extremely high proportion and tendency of animals to show sign-tracking behavior, and the extremely low degree of goal-tracking behavior observed in our rats. In particular, previous studies from our group have shown that reward uncertainty tends to increase both lever responding, while also decreasing responding toward the food cup. This typically translates into a large lever press to magazine entry ratio, suggesting a narrowed focus on the lever (Anselme et al., 2013; Hellberg,

Levit, & Robinson, 2018). Here we found that magazine entries were almost nonexistent in animals exposed to reward certainty (Saline Certain Day 9: Mean Magazine Entries = 0.95), making it almost impossible for the presence of reward uncertainty to further reduce behavior aimed toward the food cup. However, when nicotine exposure significantly increased magazine entries under certain reward conditions (Nicotine Certain), the additional exposure to reward uncertainty (Nicotine Uncertain) disrupted that effect, and brought magazine entries down to levels similar to those seen under reward certainty in the absence of nicotine (Saline Certain). This suggests that part of the effect of reward uncertainty may therefore be to draw attention away from the goal and might explain why reward uncertainty has previously been shown to dramatically increase the proportion of sign-trackers in a population (Robinson, Anselme, et al., 2015). Reward uncertainty and nicotine might therefore produce competing mechanisms for increasing the attribution of incentive value. Whereas nicotine might increase approach to the food cup (especially when a diffuse cue such as a tone is presented), reward uncertainty may work to redirect focus toward the lever and away from the food cup, with this latter effect conflicting with the effects of nicotine.

One possible candidate explanation for this competitive effect would be that both treatments increase mesolimbic dopamine activity, but here may do so in different ways. This could be accounted for by differences in the timing of the two treatments, whereby nicotine injections may have broad and long lasting pharmacological effects, whereas reward uncertainty may produce more localized and acute changes that are synchronized with the exposure to cues, resulting in different patterns of dopaminergic activity. Evidence examining the effects of reward uncertainty suggest that it increases dopamine firing in the ventral tegmental area, specifically during the time between the onset of the cue and reward delivery (Fiorillo, 2011; Fiorillo et al., 2003). Similarly, several findings have also reported an impact of nicotine on striatal dopamine activity (Mifsud, Hernandez, & Hoebel, 1989; Nisell, Nomikos, & Svensson, 1994), although most reports only measure dopaminergic activity over periods of minutes rather than seconds. Nonetheless, recent reports show that the administration of dopamine antagonists reduces the enhanced cue attraction produced by nicotine administration in a Pavlovian conditioning task (Palmatier et al., 2014). The idea that both nicotine and reward uncertainty could increase incentive value through activating different patterns of dopaminergic activity could account for our present findings. There is recent evidence to suggest that nicotine receptors on ventral tegmental neurons influence dopaminergic activity and may be involved in uncertainty-induced motivation (Naudé et al., 2016), although the precise patterns of activity these phenomenon generate still remains to be determined. Our current findings suggest that nicotine and reward uncertainty promote competing behavioral expressions of increased incentive attribution. In the present case, the ability of reward uncertainty to reduce magazine entries seems to have been stronger than the tendency for nicotine to increase them. We have previously shown that reward uncertainty produces a behavioral phenotype that is more resistant to changes in conditions that promote goal-tracking, such as exposure to reward omission training, where responses on the lever CS results in omission of reward delivery (Hellberg, Levit, & Robinson, 2018). Nonetheless, sign-tracking and goal-tracking behaviors are seen as competitive, because the increase of one typically results in the decrease of the other (Meyer et al., 2012), and here we found anecdotal evidence that nicotine increased goal-tracking, even in animals exposed to reward uncertainty. In particular, we found that 80% (four of five) of all goal-trackers and 100% (three of three) of all intermediates came from animals exposed to nicotine during training, despite the majority of the population (90%) displaying a sign-tracking phenotype. Specifically, the animals exposed to nicotine and reward certainty consisted of two intermediates and two goal-trackers, whereas joint exposure to nicotine and reward uncertainty still resulted in one intermediate and two goal-trackers. It is therefore possible that exposure to nicotine increased the number of goal-trackers and the rate of goal-tracking, thereby reducing or competing with the ability of reward uncertainty to increase sign-tracking and decrease goal-tracking responses.

It is worth noting that although the animal population in this study consisted of a high proportion of sign-trackers (90%), this is similar to previous studies we have published (Anselme et al., 2013; Hellberg, Levit, & Robinson, 2018; Robinson, Anselme, et al., 2014, 2015), and can possibly be explained in part by several factors, notably the vendor from which the animals initially came from (Fitzpatrick et al., 2013) and the use of a compound (lever + tone) cue. Similarly, the high proportion of sign-trackers could have also been increased by the stress of daily injections during autoshaping. Nonetheless, it is possible, although it seems unlikely, that the observed results regarding the impact of nicotine on behavioral phenotype would be different in a population primarily comprised of goal-trackers.

Further evidence that reward uncertainty and nicotine increase cue-triggered approach through separate and possibly competitive mechanisms comes from studies examining their lasting effect in autoshaping. Whereas previous studies suggest that progressive removal of reward uncertainty does not lead to diminished cue attraction (Robinson, Anselme, et al., 2014), a study by Guy and Fletcher shows that enhanced cue responding disappears when nicotine is no longer administered (Guy & Fletcher, 2014). This latter finding suggests that nicotine administration has the ability to further increase the incentive value of reward-paired cues, possibly imbuing them with the ability to become powerful conditioned reinforcers (Caggiula et al., 2001; Smolka et al., 2006). For example, Caggiula et al. demonstrated that when rats are trained to self-administer intravenous infusions of nicotine paired with two visual stimuli, the cues are capable of maintaining responding in the absence of primary reinforcement from the drug (Caggiula et al., 2001). Another study conducted by Goldberg et al. found that responding for intravenous infusions of nicotine in squirrel monkeys was attenuated by 50% when a light cue that was previously paired with the drug was removed (Goldberg, Spealman, & Goldberg, 1981). This could be particularly problematic in gambling, as nicotine could enable the rewarding lights and sounds present in slot machines to maintain and promote play even during prolonged periods without wins.

Females Display Greater Conditioned Reinforcement for Cues

Cues repeatedly paired with reward delivery can acquire incentive properties, and become wanted independent of the reward they were paired with. Under such conditions, the cue is deemed to

have acquired reinforcing qualities which can be measured through conditioned reinforcement (Olausson, Jentsch, & Taylor, 2004; Robbins, 1978). In gambling, this suggests that games such as those that heavily rely on cues, such as electronic gambling machines, may achieve some of their attraction through the repeated presentation of flashing lights and sounds. For example, players show a strong preference for the presence of winning sounds when playing slot machines, and these sounds have been shown to wrongly inflate their perception of win frequency (Dixon et al., 2013).

Here we report that animals in all conditions acquired a novel behavior to gain access to cues during the conditioned reinforcement task, but no difference in the reinforcing value of cues between any of the conditions was observed. This was even the case under conditions of reward uncertainty, despite the fact that uncertainty degrades the predictive value of a cue, and therefore could be expected to also reduce its ability to act as a conditioned reinforcer. Instead, there was no difference between animals exposed to certain and uncertain reward conditions, which is in line with our previous findings (Hellberg, Levit, & Robinson, 2018). Hence, in games of chance, celebratory lights and sounds can become motivational magnets, even though they do not reliably predict wins. This might be particularly the case in losses disguised as wins, where players bet on multiple lines and win back less than their wager, but are still exposed to win cues during those small wins that are in fact net losses (Dixon, Collins, Harrigan, Graydon, & Fugelsang, 2015).

We also did not find any effect of nicotine on conditioned reinforcement. This is somewhat surprising because previous reports suggest that nicotine enhances responding for reward if animals had been previously exposed to nicotine during initial acquisition of the cue-reward association (Guy & Fletcher, 2013; Olausson et al., 2004; Versaggi et al., 2016). However these effects depend on the treatment given at the time of the test. For example, Guy & Fletcher found no difference in conditioned reinforcement between animals trained on autoshaping with or without nicotine when animals were not given nicotine for the conditioned reinforcement test (all animals developed conditioned reinforcement; Guy & Fletcher, 2014). Instead, the effects of nicotine became apparent only when injected to both groups during the test, including saline animals that were being exposed to nicotine for the first time. This is in contrast to the present study where only animals treated with nicotine during autoshaping received nicotine during the conditioned reinforcement test. It is also possible in the present experiments that nicotine's tendency to increase goal-tracking and the number of goal-trackers mitigated any increase in conditioned reinforcement, because goal-trackers are known to not display conditioned reinforcement (Meyer et al., 2012; Robinson & Flagel, 2009).

In contrast, we did find that females performed more responses overall during conditioned reinforcement. In particular, this effect trended toward selectively more active responses, suggesting a tendency for greater motivation to seek the reward-paired cue. This would further confirm that in females, cues may take on greater incentive value and result in individuals expending more effort to pursue them, a finding which has been shown for smoking-related cues in women (Perkins, Donny, & Caggiula, 1999). In animals, the overall tendency for females to respond at higher levels on conditioned reinforcement than males had been previously shown by Pitchers and colleagues, although their findings suggested that responding indiscriminately increased both active and inactive responses (Pitchers et al., 2015). Here, the finding that females show a tendency to work harder for cues (including lights and sounds) paired with reward, may provide some evidence to explain why women, who predominantly play electronic slot machines, show faster progression of problem gambling behavior (Tavares et al., 2003).

Exposure to the Combination of Nicotine and Reward Uncertainty Enhances Motivation to Obtain Rewards

Gambling is maintained by the persistent motivation to gain rewards despite uncertainty in their availability and magnitude, or the possibility of loss. Cigarette smoking and gambling typically co-occur, and a history of nicotine use is associated with greater gambling and psychiatric symptoms (Petry & Oncken, 2002). Here we found that rats exposed to a combination of nicotine and reward uncertainty achieved a greater number of rewards and a significantly greater breakpoint compared with rats exposed to a combination of nicotine and certainty, or saline regardless of reward probability. Although we also found that prior exposure to reward uncertainty alone increased motivation to attain rewards, this effect seemed to be further exacerbated and driven by the administration of nicotine. Because animals were injected with their respective treatment (nicotine or saline) prior to progressive ratio testing, it is unclear whether these effects are the result of the past history of nicotine or the acute injection on test day, and further studies would be needed to answer this question. However, it should be noted that we previously found that uncertainty combined with prior adolescent exposure to alcohol tended to produce higher breakpoints (26%) than when animals were exposed to alcohol and reward certainty, although these effects did not reach significance (Hellberg, Levit, & Robinson, 2018). So far, our findings suggest that the combination of nicotine and reward certainty increases cue-triggered attraction, whereas the combination of nicotine and uncertainty increases motivation to obtain rewards. The allure of casino gambling games may be maintained by the incongruent effects of nicotine on cue-attraction and motivation for rewards. Attraction to cues associated with certain rewards may maintain illusions that bias attention toward celebratory lights and sounds associated with winning outcomes, which diverts attention from monetary losses. On the other hand, motivation for uncertain rewards might promote persistence and keep players motivated to win large jackpots despite loss.

Females Trend Toward Increased Anxiety After Conditioning

Anxiety is an important factor in the maintenance of behavioral disorders and substance use, such as maladaptive gambling and cigarette smoking. In particular, anxiety has an important relationship with the maintenance of cigarette smoking, as nicotine is commonly used to cope with high levels of anxiety. Jarvik and colleagues showed that moderate to heavy smokers who were allowed to smoke while viewing a stressful anagram experienced lower levels of anxiety (Jarvik, Caskey, Rose, Herskovic, & Sadeghpour, 1989). Deprived smokers that viewed the same anagram had a nonsignificant increase in anxiety. However, the literature on

the role of anxiety in nicotine consumption via cigarette smoking is inconsistent and depends highly on the population studied and the stage of smoking investigated (Morissette, Tull, Gulliver, Kamholz, & Zimering, 2007).

Anxiety also mediates a player's relationship with betting games and the gambling setting. A study by Miu and colleagues using the Iowa Gambling Task to investigate the role of trait-anxiety on decision making found that individuals with high trait-anxiety have impaired decision making abilities and greater physiological responses toward advantageous trials (Miu, Heilman, & Houser, 2008). Another study comparing 14,934 community residents between the ages of 18 and 64 found that respondents with both anxiety and substance or alcohol use disorders were up to five times more likely to bear moderate to severe gambling problems than individuals without these comorbidities (el-Guebaly et al., 2006).

Given the current body of literature that demonstrates the role of anxiety in maladaptive behaviors, and a previous study in our lab that found significant effects in the role of anxiety on attraction to gambling-like cues, we found it important to investigate how our autoshaping procedure affects levels of anxiety in rats. We expected exposure to reward uncertainty to be stressful and therefore associated with heightened levels of anxiety (Morgado et al., 2015). Surprisingly, in this study we found that neither nicotine administration nor chronic exposure to uncertainty affected levels of anxiety in rats. However, we found a trend for higher levels of postconditioning anxiety in females. This trend fits well with the current body of literature that suggests females are more likely to obtain the diagnosis of anxiety, and are more likely to be sensitive to its effects (Boughton & Falenchuk, 2007; McLean, Asnaani, Litz, & Hofmann, 2011).

Conclusion

The goal of the present study was to determine how nicotine and reward uncertainty might affect the attribution of incentive salience for cues and their associated rewards, and possibly contribute to the development of disordered gambling. Previous studies suggest that reward uncertainty sensitizes neural pathways and heightens the degree of incentive salience assigned to reward-paired cues. This promotes craving and the intense preoccupation with rewards seen in disorders of addiction. Here, we found nicotine to enhance incentive salience and cue-triggered approach under certain reward conditions, irrespective of sex. On the other hand, exposure to the combination of nicotine and reward uncertainty enhanced motivation to obtain rewards. This suggests the existence of a dichotomy between the effects of nicotine and reward probability on the attraction to cues versus the motivation to obtain rewards. Therefore, nicotine appears to exert a different effect on motivation for the predictor of reward than it does on motivation for the reward itself. The main effect of nicotine might be its ability to heighten cue-triggered attraction, but to increase motivation for rewards that are unpredictable. We also found an important role for sex, with females acquiring cue-triggered approach more readily in both certain and uncertain conditions when exposed to nicotine. Similarly, responding during conditioned reinforcement was stronger for females, with a tendency toward more active responses, suggesting a greater motivation to seek the reward-paired cue. Although not significant, we found a trend for levels of anxiety to increase for females after the conditioning procedures. Because anxiety is implicated in the maintenance and relapse to maladaptive behaviors, it is important to consider how exposure to the gambling-like setting might affect levels of anxiety or promote maladaptive behavior in players. In all, exposure to nicotine and cues associated with the gambling setting may be particularly salient to females, thus increasing the vulnerability of this population. This is of particular importance, especially when considering the high co-occurrence between cigarette smoking and gambling games, such as slot machines, which are highly unpredictable and rely heavily on cues such as lights and sounds for their reinforcing effects.

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Received August 22, 2018
Revision received February 3, 2019
Accepted February 8, 2019 ■