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**Significant association of nicotine reinforcement and cue reactivity: A translational study in humans and rats**

Kevin BUTLER<sup>1</sup>, Benoît FORGET<sup>2</sup>, Stephen J. HEISHMAN<sup>3</sup>, Bernard LE FOLL<sup>1,4-9</sup>

1. Translational Addiction Research Laboratory, Centre for Addiction and Mental Health, University of Toronto, ON, Canada.
2. Department of Neuroscience, Pasteur Institute, Paris, France.
3. National Institute on Drug Abuse, Intramural Research Program, Baltimore, United States of America.
4. Acute Care Program, Centre for Addiction and Mental Health, Toronto, ON, Canada.
5. Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada.
6. Department of Family and Community Medicine, University of Toronto, Toronto, ON, Canada.
7. Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada.
8. Department of Psychiatry, Division of Brain and Therapeutics, University of Toronto, Toronto, ON, Canada.
9. Institute of Medical Sciences, University of Toronto, Toronto, ON, Canada.

**Corresponding author:** Dr Bernard Le Foll, Centre for Addiction and Mental Health, 33 Russell Street, Toronto, Ontario, M5S 2S1; Tel: +1 416-535-8501; Email: [Bernard.LeFoll@camh.ca](mailto:Bernard.LeFoll@camh.ca)

**Running Head:** Nicotine reinforcement and cue reactivity

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**Abstract**

Relapse is common amongst smokers attempting to quit and tobacco cue-induced craving is an important relapse mechanism. Preclinical studies commonly use cue-induced reinstatement of nicotine seeking to investigate relapse neurobiology. Previous research suggests dependence severity and nicotine intake history affect smoking resumption and cue-induced reinstatement of nicotine seeking. However, behavioural data may be interpreted in terms of nicotine reinforcement. This translational study investigated whether individual differences in objectively assessed nicotine reinforcement strength were associated with cue-reactivity in both rats and human smokers, which to our knowledge has not been investigated before. Rats (n=16) were trained to self-administer nicotine and were tested on a progressive ratio schedule of nicotine reinforcement, to assess reinforcer strength, and on a test of cue-induced reinstatement of nicotine seeking. Nicotine reinforcement strength was assessed in human smokers (n=104) using a forced choice task (nicotine containing vs denicotinized cigarettes) and self-reported cue-induced craving was assessed following exposure to smoking and neutral cues. Responding for nicotine under progressive ratio was strongly positively correlated with cue-induced reinstatement of nicotine seeking in rats. Nicotine choices in human smokers were significantly associated with cue-induced craving after controlling for dependence severity, years of smoking, and urge to smoke following neutral cues. Findings suggest nicotine reinforcement strength is associated with both types of cue-induced behaviour, implying some translational commonality between cue-induced craving in human smokers and cue-induced reinstatement of nicotine seeking in rats. Findings are discussed in relation to clinical implications and the extent to which reinforcement strength, cue-induced craving and reinstatement of nicotine seeking may index drug 'wanting'.

**Key words:** Cue-induced craving, Cue-induced reinstatement, Cue reactivity, Drug wanting, Nicotine, Nicotine reinforcement, Smoking relapse, Tobacco smokers, Translational research.

## Introduction

Tobacco smoking is a serious global health problem. Estimates suggest there are over 1 billion smokers worldwide and around 8 million deaths annually attributable to tobacco smoking or smoke exposure (World Health Organization, 2019). Smokers often experience multiple quit-relapse cycles (Chaiton et al., 2016) and data from over 40 smoking cessation trials suggests that 12 month abstinence rates are, at best, around 23% even when using available cessation pharmacotherapy (Jackson et al., 2019). Improving our understanding of relapse may lead to more efficacious cessation interventions.

High relapse rates even after lengthy durations of abstinence may be due, at least in part, to the prevalence of tobacco-related cues in the environment and the persistence of cue-reactivity in smokers. Indeed, it is well established that stimuli associated with smoking increase self-reported craving/urge to smoke (Carter & Tiffany, 1999) and importantly, tobacco cue-induced craving predicts smoking behaviour such as time to next cigarette, number of puffs, puff volume, quit duration and perceived quit difficulty (Conklin et al., 2015; Erblich & Montgomery, 2012; Shiffman et al., 2013). Therefore, cue-induced craving may be a key relapse mechanism.

Preclinical assays also provide increased understanding of cue-induced behaviour relevant to tobacco use. For example, cue-induced reinstatement of nicotine seeking is a common assay used to explore the neurobiology of cue-induced relapse (e.g. Khaled, Pushparaj, Di Ciano, Diaz, and Le Foll (2014)) and to assess relapse prevention efficacy of new and existing pharmacological agents and targets (e.g. Forget, Guranda, Gamaledin, Goldberg, and Le Foll (2016); Le Foll et al. (2012)). Briefly, in the cue-induced reinstatement assay, the reintroduction of nicotine-associated cues after extinction of nicotine seeking results in increased responding on a lever previously reinforced by nicotine delivery (Liu et al., 2006).

There is some evidence to suggest that severity of nicotine dependence and nicotine intake history may be important factors in resumption of nicotine seeking behaviour. For instance, smokers with heavier nicotine dependence show greater improvements in mood following their first morning cigarette than those with lower dependence (Adan, Prat, & Sanchez-Turet, 2004). In animal studies, higher compared to lower doses of nicotine are associated with increased resistance to extinction of nicotine seeking (O'Dell et al., 2007) and nicotine infusion rate during self-administration training is positively correlated with responding during extinction of nicotine seeking (Harris, Pentel, & Lesage, 2007). In findings with other drugs of abuse, rats with higher rates of cocaine self-administration are more vulnerable to cue-induced reinstatement of drug seeking (Sutton, Karanian, & Self, 2000). Similarly, the amount of responding for nicotine during self-administration training positively correlated with responding during reinstatement of nicotine seeking (Liu, Caggiula, Palmatier, Donny, & Sved, 2008). In this study, the nicotine reinforcement schedule during self-administration training was gradually increased from fixed ratio (FR)1 to FR5, such that nicotine reinforcement required 1 to 5 active lever presses. These findings have been used to provide evidence that drug history is an important driver of cue-induced smoking resumption (Liu et al., 2008). However, an alternative explanation is that the relative reinforcement strength of nicotine (i.e. how effective a reinforcer individuals find nicotine) may impact vulnerability to cue-induced reinstatement. The reinforcing properties of nicotine underlie addiction to tobacco smoking (Benowitz, 2010). Individuals that find nicotine the most reinforcing may form stronger associations between nicotine and smoking-cues. In turn this could increase craving and relapse in the presence of such cues.

The current study aimed first to replicate the preclinical finding that the amount of responding for nicotine during self-administration training (under FR schedules of reinforcement) correlates with cue-induced reinstatement of nicotine seeking. However, we aimed to extend these findings by establishing, in the same animals, whether there was an association between nicotine reinforcement strength and cue-induced reinstatement of nicotine seeking. To assess reinforcement strength, a progressive ratio (PR) schedule of nicotine reinforcement was used. Under PR schedules of reinforcement there is a systematic increase in the behavioural requirement for reinforcement after each reinforcer delivery. This enables reliable assessment of 'willingness to work' for nicotine, an indicator of reinforcement strength (Jones & Comer, 2013; Killeen, Posadas-Sanchez, Johansen, & Thrailkill, 2009). In addition, we aimed to establish whether preclinical results would translate into human smokers by assessing whether selection of nicotine-containing cigarettes, over denicotinized cigarettes, on a forced choice task (indicative of nicotine reinforcement) was associated with cue-induced craving. To our knowledge, this is the first study to examine the association between objective measures of nicotine reinforcement with cue-reactivity in rats and human smokers. We hypothesised that nicotine reinforcement strength would be associated with cue-induced reinstatement of nicotine seeking in rats and with cue-induced craving in human smokers.

## Methods

### *Pre-clinical study*

#### *Animals*

Experimental procedures were approved by the Centre for Addiction and Mental Health (CAMH) Animal Care Committee and carried out in accordance with the guidelines of the Canadian Council on Animal Care. Sixteen, experimentally naïve, male Long-Evans rats (Charles River, Lachine, PQ, Canada) weighing 250-275 g and housed individually in a temperature-controlled environment on a 12 h reverse light/dark cycle (lights off from 07:00 hours to 19:00 hours) were used. Experiments were conducted during the dark phase. Prior to experimental manipulation, animals were given a minimum of 7 days to habituate to the colony room where they received unlimited access to food and water. After habituation, rats were diet restricted (5 pellets or 20 g daily and free access to water). Food restriction continued until all experiments were completed as this has previously been shown to optimize nicotine self-administration (Corrigall & Coen, 1989; Donny et al., 1998).

#### *Initial training*

Animals were trained for self-administration experiments in chambers (Med Associates, St Albans, VT, USA) located in sound-attenuating boxes and equipped with two levers each with a cue light located above and a house light. For half the animals, the left lever was the active lever and for the other half, the right lever was the active lever. Animals were trained to lever press under the control of a FR1 schedule in which each press on the active lever resulted in delivery of a 45 mg food pellet. Acquisition of lever pressing was trained in 1 h sessions daily for 5 days while the house light was on and with no illumination of cue lights.

#### *Implantation of catheters*

Once food-maintained behaviour was acquired, intravenous catheters were surgically implanted into the jugular vein, exiting between the scapulae. Surgery was performed under anaesthesia induced by xylazine (10 mg/Kg, intraperitoneal) and ketamine hydrochloride (75 mg/Kg, intraperitoneal). Incision sites were infiltrated with the local anaesthetic bupivacaine (0.125%, subcutaneous). Buprenorphine

(0.01 mg/Kg, subcutaneous) was given for post-operative analgesia and a single dose of penicillin (30,000 units, intramuscular) was administered at the completion of surgery. Animals recovered for 1 week before starting drug self-administration sessions.

### *Drugs*

(-)-Nicotine hydrogen tartrate (Sigma-Aldrich, St Louis, MO, USA) was dissolved in saline, pH adjusted to 7.0 ( $\pm 0.2$ ), and filtered through a 0.22 mm syringe filter (Fisher Scientific, Pittsburgh, PA, USA). Nicotine was administered via the intravenous catheter (30  $\mu\text{g}/\text{Kg}/\text{infusion}$  in a volume of 100  $\mu\text{L}/\text{Kg}/\text{infusion}$ ) when response requirements were met. Nicotine dose was based on freebase concentration and was selected to optimize nicotine self-administration (Corrigall & Coen, 1989; Donny et al., 1998).

### *Nicotine self-administration training*

Acquisition of nicotine self-administration behaviour was performed under a fixed-ratio (FR) schedule of reinforcement. Each 60 min session began with illumination of the house light. Completion of the schedule requirement on the active lever (1 to 5 lever presses under FR1 to FR5) resulted in rapid delivery of a nicotine infusion (approximately 1-s delivery time). Each infusion was followed by a 60 second time out period, during which the house light was dimmed and the cue light above the active lever was illuminated. During time out, further presses on the active lever had no programmed consequences and pressing on the inactive lever had no programmed consequences throughout the session. During the first 5 days of self-administration training response requirements were FR1, and this was gradually increased to an FR5 schedule over a further 5 days.

### *Testing under the progressive-ratio schedule*

After training under FR5, animals were switched to a PR schedule where the response requirement increased with each successive nicotine infusion. Response requirement was based on the formula  $5e^{(0.25 \times [\text{infusion number} + 3])} - 5$ , with the first two values replaced by 5 and 10 (modified from Roberts and Bennett (1993)). Thus, response requirements for successive infusions were 5, 10, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208, etc. PR sessions lasted a maximum of 4 h. However, if animals ceased to press the active lever for 30 minutes the session automatically ended, and the last ratio completed was defined as the breakpoint. The animals were allowed 10 days of nicotine self-administration under the PR schedule for stabilization of self-administration before the formal PR test session and all animals reached their breakpoints during the 4 h sessions within this period. Testing for catheter patency was conducted at multiple time points throughout the PR schedule portion of the experiment to ensure data were not included from animals with non-patent catheters. The total number of active lever presses and the breakpoint were collected as indicators of reinforcement strength.

### *Cue-induced reinstatement of nicotine seeking*

Self-administration behaviour was then extinguished by withholding nicotine (no consequences for active lever presses) and its associated cues (house light illuminated but cue lights stayed off) throughout the session until stable extinction was reached. The criterion for stable extinction was fewer than 20 active lever presses per 1-h session over two consecutive days. Once stable extinction was reached the effects of cue-induced reinstatement of nicotine-seeking behaviour was tested. Reinstatement sessions lasting 1 h were conducted under conditions identical to those of self-

administration sessions except that: 1) a single 1 min presentation of the cue light above the active lever while the house light was off was delivered response-independently immediately at the start of the session and 2) responses on the active lever (on an FR5 schedule) resulted in contingent presentation of the cues (light above active lever on and house light off for 60 s) without nicotine availability.

#### *Data analyses*

To assess the associations between both active lever pressing during nicotine self-administration training (total responses during FR1-FR5 schedules) and PR schedule outcome measures (breakpoint and total active lever presses) with cue-induced reinstatement of nicotine seeking, Pearson's correlations were conducted. Data for responding during training and PR outcome measures were log transformed to improve the distribution of the data in order to meet statistical test normality assumptions. Significance threshold was set at  $\alpha = 0.05$  and power calculations indicated that power of 0.6-0.8 was achieved for the correlation effect sizes achieved with 16 animals.

#### *Human study*

##### *Participants*

Data reported were from 104 participants recruited in three studies conducted at two sites (Baltimore, Maryland, USA and Toronto, Ontario, Canada) and analyses with this dataset have previously been reported examining the impact of genetic polymorphisms on nicotine reinforcement and cue-reactivity (Chukwueke et al., 2020). Studies were conducted in accordance with the Declaration of Helsinki (7th revision) and were approved by ethics review boards at the National Institute of Drug Abuse (NIDA) and the Centre for Addiction and Mental Health (CAMH). Participants had to be 18–64 years old, smoke  $\geq 10$  cigarettes per day for at least one year, have a positive urinary cotinine test, and were medically and psychologically healthy (assessed by medical and psychiatric history). Participants were ineligible if they were seeking treatment for nicotine dependence, recently used nicotine replacement products, consumed  $> 15$  alcohol drinks per week, used illicit drugs regularly, were pregnant/nursing, or used medications that would be unsafe during experimental sessions.

##### *Study design*

Full study details are described in Chukwueke et al. (2020). Elements essential for understanding the current analyses are described below. Briefly, participants attended an eligibility assessment where informed consent was obtained followed by collection of demographic and tobacco use related data including the Fagerström Test for Nicotine Dependence (FTND; Heatherton, Kozlowski, Frecker, and Fagerstrom (1991)), medical/psychiatric history and verification of smoker status (positive urine cotinine), illicit drug use (negative urine screen), recent drinking (negative breathe alcohol) and recent smoking (positive breath carbon monoxide). Females were additionally required to provide a negative urine pregnancy test. Eligible participants provided blood for genotyping at one of the study visits (reported in Chukwueke et al. (2020)) and participated in the forced-choice task at a second visit, followed by the cue exposure task at a third visit.

##### *Forced-choice procedure*

Relative reinforcing effects of nicotine are assessed with this task (de Wit & Johanson, 1987; Jones & Comer, 2013; Perkins, Grobe, Weiss, Fonte, & Caggiula, 1996). First participants took 4 puffs of their

preferred-brand cigarette to standardize time from last nicotine exposure. After 30-60 minutes, participants were presented with two identical research cigarettes, one with nicotine and one denicotinized. In four counterbalanced exposure trials separated by 20-30 minute intervals, to avoid nicotine satiation and simulate regular smoking behaviour (approx. 8 puffs every 40 minutes (Hatsukami, Pickens, Svikis, & Hughes, 1988)), participants took 4 puffs of a cigarette in counterbalanced order so that each cigarette was sampled twice. After 20-30 minutes following the last exposure trial, participants completed four choice trials separated by 20-30 minute intervals. In each trial, participants were presented with both nicotine containing and denicotinized cigarettes concurrently and instructed to smoke any combination of 4 puffs from either or both cigarettes. The percentage of nicotine containing cigarette choices was used as an objective indicator of nicotine reinforcement.

#### *Forced-choice cigarettes*

Due to availability, it was not possible to use the same research cigarettes across all participants (from the three studies that make up the sample). Nicotine-containing cigarettes used included Quest<sup>®</sup> 1 cigarettes (Vector Tobacco, 0.6 mg nicotine yield), commercially available Player's brand cigarettes (1.2 mg nicotine yield) and SPECTRUM<sup>®</sup> research cigarettes (RTI international, 0.9 mg nicotine yield). Denicotinized cigarettes used were Quest<sup>®</sup> 3 cigarettes (<0.05 mg nicotine yield) and SPECTRUM<sup>®</sup> research cigarettes (0.03 mg nicotine yield).

#### *Cue exposure task*

Cue-induced craving in response to smoking and neutral cues was assessed with a cue exposure task based on (Weinberger, McKee, & George, 2012). To begin, participants had four puffs of their preferred-brand cigarette to standardize time from last nicotine exposure. Cue exposure started 30-60 minutes after the last puff with the order of smoking and neutral exposure sessions counterbalanced across participants. The smoking cue consisted of a pack of cigarettes, a lighter, and an ash tray. Participants were instructed to light a cigarette from the pack without puffing and hold it for 30-60 seconds before extinguishing the cigarette. The neutral cue was a pack of pencils, a sharpener, and a notepad. Participants were instructed to sharpen a pencil and hold it as if writing for 30-60 seconds. Participants completed a visual analogue scale (VAS) to assess neutral and smoking cue-induced craving. Specifically, participants were asked to rate their 'urge to smoke' prior to cue exposure (baseline) and 15 minutes after cue exposure. Difference scores (urge after exposure – urge at baseline) were computed as an index of cue reactivity for both neutral and smoking cues.

#### *Data analyses*

Descriptive statistics were calculated for demographic data on the whole sample (n=104) except for FTND score due to missing data from one participant. Multiple linear regression tested the association between nicotine reinforcement and tobacco cue-induced craving. Change in tobacco cue-induced urge to smoke score was the dependent variable, percentage of nicotine-containing cigarette puffs on the forced choice task was the independent variable and FTND score, years of smoking and change in urge to smoke score following neutral cues were control variables. These control variables were selected as dependence severity and use history have previously been considered to impact cue-induced behaviour and to control for craving changes that are un-related to smoking cues. Linearity and heteroskedasticity assumptions were checked by inspecting scatter plots for variables and residuals, respectively. Multicollinearity was not a problem of the model, variance inflation factors (VIFs) were all  $\leq 1.05$ . Influential cases were not a problem of the model, Cook's distances were all

below 0.5. Normal distribution of model residuals was checked by inspecting frequency and Q-Q plots and skew and kurtosis values (Kim, 2013). Residuals were not normally distributed but were improved by transforming the most highly skewed variable (percentage of nicotine-containing cigarette puffs) using reflected data that was then log transformed. The significance threshold was set at  $\alpha = 0.05$  and an a priori power calculation indicated a medium effect size and 104 participants would achieve power of 0.89. Our model included data from 102 of the 104 participants as FTND score was missing from one participant and cue-induced urge to smoke scores were missing from another.

## Results

### *Preclinical study*

#### *Nicotine reinforcement associated with cue-induced reinstatement of nicotine seeking*

The mean number of active lever presses made during self-administration training (FR1-FR5), the mean number of active lever presses during the PR schedule, the mean breakpoint and the mean cue-induced reinstatement responses were: 905.88 (SEM:  $\pm 117.41$ ), 878.25 (SEM:  $\pm 189.59$ ), 806.50 (SEM:  $\pm 182.36$ ), and 75.25 (SEM:  $\pm 14.30$ ), respectively. There was a significant positive correlation between the number of active lever responses during nicotine self-administration training (natural log transformed) and the magnitude of cue-induced reinstatement of nicotine seeking ( $r = 0.54$ ,  $p = 0.032$ ; Figure 1). Furthermore, the total number of active lever presses during the PR schedule (natural log transformed) significantly, positively correlated with the number of cue-induced reinstatement responses ( $r = 0.62$ ,  $p = 0.011$ ; Figure 1). Similar findings were found for the correlation between breakpoint (natural log transformed) and cue-induced reinstatement responses ( $r = 0.61$ ,  $p = 0.012$ ).

### *Human study*

#### *Demographics*

Study participants were 54.8% male and 45.2% female, they had a mean age of 41.80 years (SEM:  $\pm 1.09$ ), had been a smoker for a mean of 22.27 years (SEM:  $\pm 1.12$ ) and had a mean FTND score of 5.35 (SEM:  $\pm 0.19$ ).

#### *Nicotine reinforcement associated with tobacco cue-induced craving*

The mean percentage of nicotine (vs. denicotinized) puff choices selected by participants included in the regression model was 73.03% (SEM:  $\pm 2.70$ ) and the mean VAS increase in cue-induced 'urge to smoke' was 10.12 (SEM:  $\pm 1.67$ ). Multiple linear regression was used to investigate the association between percentage of nicotine choices on the forced choice task (reflected and natural log transformed) and tobacco cue induced urge to smoke, controlling for nicotine dependence (FTND score), years of smoking and neutral cue-induced urge to smoke. The full model approached our significance threshold and predicted 5% of the variability in urge to smoke (adjusted  $R^2 = 0.05$ ,  $F(4, 97) = 2.33$ ,  $p = 0.061$ ). More specifically, percentage of nicotine choices (reflected and natural log transformed) was a significant negative predictor of tobacco cue-induced urge to smoke (standardized  $\beta = -0.22$ ,  $p = 0.034$ ) when controlling for these other independent variables. Neither years of smoking nor severity of dependence were significantly associated with urge to smoke in this model (See Table 1).

[INSERT FIGURE 1 AND TABLE 1 NEAR HERE]

## Discussion

Previous research has suggested that nicotine intake history may impact cue-induced reinstatement of nicotine seeking. However, findings may be interpreted in terms of nicotine reinforcement strength. In this translational study we found that responding for nicotine during self-administration training was positively associated with the degree of cue-induced reinstatement of nicotine seeking in rats. In the same animals the propensity to find nicotine an effective reinforcer, assessed via PR schedule of nicotine reinforcement, was associated with the reinstatement of nicotine seeking, such that cues had a greater impact on reinstatement of nicotine seeking in rats that found nicotine a stronger reinforcer. Similarly, in the human study a significant association was found between nicotine reinforcement, assessed by cigarette puff choice, with cue-induced craving, after controlling for FTND score, years of smoking, and urge to smoke following neutral cues. The directionality of this human finding was consistent with the finding in rats (since reflected data were used for puff choice) such that cue-induced craving was greater in smokers that selected more puffs from nicotine containing cigarettes over denicotinized versions. These findings are discussed in terms of assessment of 'drug wanting' and implications for tobacco use disorder treatment.

The finding that responding for nicotine during self-administration training correlates with subsequent cue-induced reinstatement of nicotine seeking behaviour is a replication of previous preclinical findings (Liu et al., 2008). These previous findings suggest that prior history of nicotine intake impacts reinstatement of nicotine seeking in the presence of nicotine-associated cues. However, findings may also be interpreted in terms of nicotine reinforcement. Indeed, we extend these previous findings by showing that there is a larger positive correlation between PR outcome measures and cue-induced reinstatement of nicotine seeking. These results suggest that cue-induced reinstatement of nicotine seeking is greatest in animals that are 'willing to work harder' for nicotine infusions.

In human smokers the number of nicotine-containing cigarette puffs selected in a forced choice task were significantly predictive of tobacco cue-induced craving. FTND score and years of smoking were included as control variables, as severity of dependence and history of nicotine intake may impact smoking resumption and cue-induced behaviour (e.g. Adan et al. (2004); Liu et al. (2008)). Interestingly, these control variables were not themselves significant independent predictors of tobacco cue-induced craving in these smokers. Together with the rat data, our study suggests that there is some degree of translational commonality between reinstatement of cue-induced nicotine seeking in rats and cue-induced craving in human smokers. Other commonalities between these two types of cue-induced behaviour have previously been reported. For example, tobacco cue-induced craving may increase with abstinence duration, even as general, non-cue associated craving and withdrawal symptoms are reducing. This incubation of cue-induced cigarette craving was demonstrated in human smokers across a 35-day abstinence period (Bedi et al., 2011) and may contribute to motivation to smoke and relapse after extended periods of abstinence. There is also evidence of incubation of cue-induced reinstatement of nicotine seeking (Markou, Li, Tse, & Li, 2018) supporting the idea that there are similarities between these different cue-reactivity measures across species.

The findings from rats and from human smokers support the idea that relative reinforcement strength predicts cue-induced behaviour. The PR schedule and the forced choice task may both be laboratory measures that index drug 'wanting', the motivational drive for addictive drug reward as opposed to drug 'liking' or the hedonic aspect of drug reward. It is difficult to dissociate drug 'wanting' and 'liking' with commonly used laboratory tasks, performance is likely to be determined as a function of both

motivational and hedonic value. However, PR schedules have been considered to be more likely to measure motivational 'wanting' rather than hedonic 'liking' because motivation requires action whereas pleasure is experienced more passively (Kissileff & Herzog, 2018). Similarly, forced-choice tasks have been used to index explicit 'wanting' (Finlayson, King, & Blundell, 2007). In addition, the incentive sensitization theory of addiction (Robinson & Berridge, 1993) postulates that addictive drugs and their associated cues are imbued with salience or incentive value that drives drug 'wanting' which is expressed as intense cue-induced craving and promotes continued drug use and relapse. Therefore, the associations found in the current study are consistent with each of the measures (nicotine-reinforcement strength, cue-induced craving and reinstatement of nicotine seeking) indexing drug wanting to varying degrees.

Although we cannot infer causation from correlational data, it is tempting to suggest that the current findings offer some insight into possible intervention personalization. For instance, those individuals who are particularly vulnerable to tobacco cue-induced craving and relapse may benefit most from interventions that are likely to reduce nicotine reinforcement strength such as the nicotinic partial agonist, varenicline. Indeed, varenicline pre-treatment in rats reduces responding for nicotine under a PR schedule (Le Foll et al., 2012), has been shown to antagonise both the primary reinforcing effects of nicotine and the reinforcement-enhancing effect of nicotine on cues (Garcia-Rivas et al., 2019) and reduces cue reactivity (Franklin et al., 2011). Future research could ascertain if varenicline has greatest efficacy among smokers with the largest cue-reactivity. Additionally, the present findings could be taken to suggest that reductions in both nicotine reinforcement and cue reactivity are needed to overcome tobacco use disorder. Targeting of nicotinic acetylcholine receptors affects both mechanisms (as described above with varenicline). Targeting cannabinoid CB1 receptors and dopamine D3 receptors has been suggested as novel therapeutic strategies for smoking cessation (Butler & Le Foll, 2020; Sokoloff & Le Foll, 2017) and there is evidence that these neurobiological systems differentially mediate nicotine reinforcement and cue-reactivity. For instance, cannabinoid CB1 receptor blockade reduces both nicotine reinforcement and cue-induced reinstatement of nicotine seeking (Forget, Coen, & Le Foll, 2009; Schindler et al., 2016). On the other hand, dopamine D3 receptor antagonism blocks cue-induced reinstatement of nicotine seeking in rats (Khaled et al., 2010; Khaled et al., 2014), and our recent genetic study suggests dopamine D3 receptor function may be associated with intensity of tobacco cue-induced craving in human smokers (Chukwueke et al., 2020). However, genetic and pharmacological evidence in humans suggests D3 receptor modulation may not impact nicotine reinforcement (Chukwueke et al., 2020; Lawn et al., 2018). Future research might establish whether strategies attenuating both nicotine reinforcement and cue reactivity are more efficacious than those affecting one mechanism alone.

Limitations of this translational study include that direct drug effects may confound PR performance. However, PR schedules may provide a less confounded measure of reinforcer effectiveness compared to FR schedules (Hodos, 1961). This is because nicotine is infused at the end of each response requirement and because reinforcer effectiveness is not assessed by response rate alone. Another limitation of the PR schedule is that it does not entirely dissociate drug taking history from nicotine reinforcement. Nicotine infusions during the PR schedule contribute to the animals' drug intake history. However, it is compelling to interpret the PR findings in terms of nicotine reinforcement strength given the concordance with the human smoker data. We assessed nicotine reinforcement strength with a forced choice task in human smokers and a PR schedule in rats, although it is possible to use choice procedures and PR schedules in both species. Finding translational commonalities (i.e. significant associations between nicotine reinforcement strength and cue-induced behaviours) despite these procedural differences suggests that observed effects are robust. Nevertheless, future

translational studies would likely benefit, in terms of validity, from closer matching of procedures/outcome measures used across species.

Although male and female smokers were included in the current study, only male rats were studied. Future translational studies should carefully consider matching sex across species particularly where there are known or anticipated sex differences in outcome measures. Finally, the regression model for the cue-induced craving data in human smokers was not a good fit of the data. The full model only approached our significance threshold and described just 5% of the variability in cue-induced craving. Future studies could use an alternative objective measure of nicotine reinforcement in the model to see if this improves its predictive power. Latency to puff could be used instead of percentage of nicotine-containing cigarette choices. There may be more variability in latency data due to less clustering of data at ceiling compared to smokers' puff choices and this could improve model fit.

In conclusion, translational findings suggest that nicotine reinforcement strength is associated with cue-induced reinstatement of nicotine responding in rats and with tobacco cue-induced craving in human smokers. This implies there are translational commonalities between these two cue-induced behaviours. Findings support the idea that, to varying degrees, reinforcement strength, cue-induced craving and reinstatement of drug seeking may all index drug 'wanting'. Additionally, these findings may have implications for the pharmacological treatment of tobacco use disorder.

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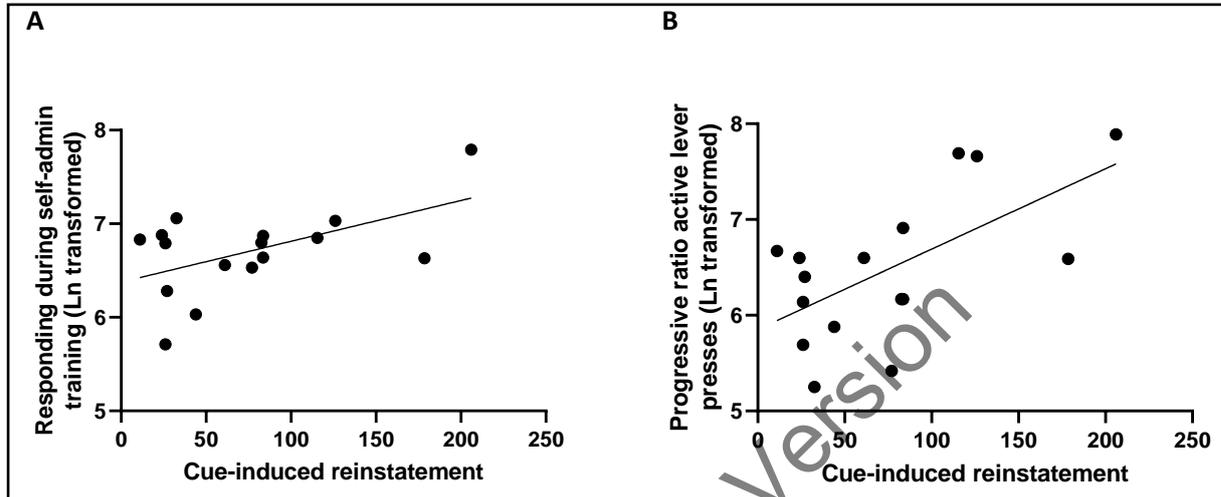
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## Figures and Tables

**Figure 1:** Associations between **(A)** Active lever presses during nicotine self-administration training (FR1-FR5 schedules of reinforcement) and cue-induced reinstatement of nicotine seeking ( $p < 0.05$ ) and **(B)** Active lever presses during the progressive ratio schedule and cue-induced reinstatement of nicotine seeking ( $p < 0.05$ ) in rats.



**Table 1:** Association between forced choice nicotine puffs and cue-induced craving in human smokers.

|  | $\beta$ | t     | Sig.         |
|--|---------|-------|--------------|
| Intercept                                  | -       | 1.86  | 0.067        |
| FTND score                                 | 0.18    | 1.79  | 0.077        |
| Years of smoking                           | -0.14   | -1.39 | 0.169        |
| Urge to smoke (induced by neutral cues)    | 0.02    | 0.16  | 0.871        |
| Nicotine containing cigarette choices (%)* | -0.21   | -2.15 | <b>0.034</b> |

$\beta$  is the standardized regression coefficient, FTND is the Fagerstrom Test for Nicotine Dependence, \* indicates that a log transformation of reflected data was used in the model.