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Differences in mechanisms underlying reinstatement of cigarette smoke extract- and nicotine-seeking behavior in rats

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Abstract

Despite extensive research, current therapies for smoking cessation are largely ineffective at maintaining abstinence for more than a year. Whereas most preclinical studies use nicotine alone, the goal of the present study was to evaluate whether inclusion of non-nicotine tobacco constituents provides better face validity for the development of new pharmacological therapies for smoking cessation. Here, we trained adult male rats to self-administer nicotine alone or cigarette smoke extract (CSE), which contains nicotine and other aqueous constituents of cigarette smoke. After stable self-administration behavior was established, animals underwent extinction training followed by drug and cue primed reinstatement testing. We show that animals that self-administered CSE had significant reinstatement in all drug and drug + cue stimulus conditions whereas animals that self-administered nicotine only showed significant reinstatement in the drug + cue conditions. AT-1001, an α3β4 nicotinic acetylcholine receptor (nAChR) functional antagonist, attenuated drug + cue-primed reinstatement of both CSE- and nicotine-seeking behavior. However, AT- 1001 was less potent in blocking drug-primed reinstatement in animals that had self-administered CSE than in those that had self-administered nicotine alone. This was the case even when nicotine was used to prime reinstatement in animals that had self-administered CSE, suggesting that prior CSE exposure had altered the functional role of α3β4-containing nAChRs in drug-seeking behavior. These findings confirm the importance of non-nicotine tobacco constituents and α3β4* nAChRs in cue- and nicotine-primed craving. They also suggest that tests using CSE may be more valid models to study tobacco dependence than use of nicotine alone.

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Author Contribution: D.D.R., J.D.B., and F.M.L. were responsible for experimental concept and design; D.D.R., S.J.C., and M.C. performed all experiments; D.D.R. and S.J.C. analyzed data and created figures; D.D.R wrote early drafts of the manuscript; S.J.C. and F.M.L. provided critical revision of the manuscript; J.D.B. and F.M.L. consulted on statistical analysis; J.D.B., N.Z., and F.M.L. edited the manuscript; N.Z. kindly provided AT-1001; F.M.L. is the principal investigator.

Conflict of Interest: AT-1001 was provided by Dr. Nurulain Zaveri, Astraea Therapeutics, Mountain View, CA. The authors declare no conflict of interest.

Keywords

nicotine; tobacco; cigarette smoke constituents; cue reinstatement; drug-primed reinstatement; α3β4 nicotinic acetylcholine receptors

1. Introduction

Tobacco addiction is a chronic relapsing disorder characterized by a compulsive desire to smoke, despite negative consequences and a desire to quit (Koob and Volkow 2010; Lynch and Sofuoglu 2010; Bauzo and Bruijnzeel 2012). Even with behavioral therapies and drug interventions to assist with smoking cessation, over 70% of smokers fail to remain abstinent for more than a year (George and O'Malley 2004). Many cessation therapies are designed to wean smokers from nicotine, the main psychoactive component of cigarette smoke, by maintaining activation of nicotinic acetylcholine receptors (nAChRs); however, most therapeutics are not effective past the initial withdrawal phase (Gómez-Coronado et al. 2018). In humans, a major factor for early relapse is craving (Killen and Fortmann 1997). Reinstatement of drug-seeking induced by drug-associated cues or drug-priming involves, in part, the modulation of nAChRs (O'Connor et al. 2010; Le Foll et al. 2012; Li et al. 2012). A more complete understanding of the neurobiological bases of cue- and drug-induced relapse, as well as the differential involvement of nAChRs, is critical for the identification of novel pharmacological targets and the development of more efficacious smoking cessation medications.

Recent studies have demonstrated the importance of α3β4* nAChRs in tobacco addiction and as a potential new target for smoking cessation (Toll et al. 2012; Costello et al. 2014; Cippitelli et al. 2015; Yuan et al. 2017). Genome-wide association studies reveal that polymorphisms in the gene cluster encoding α3-α5-β4 nAChR subunits are associated with an increased risk for tobacco dependence (Schlaepfer et al. 2008; Berrettini and Doyle 2012; Lassi et al. 2016). Although several animal studies have examined the role of α4β2* and α7 nAChRs in cue- and drug-induced reinstatement, investigation of α3β4* nAChR involvement has been more limited. Recent studies, however, have shown that AT-1001, an α3β4 nAChR antagonist, blocks nicotine self-administration and reinstatement of drug-seeking in rats, without affecting food self-administration (Toll et al. 2012; Costello et al. 2014; Cippitelli et al. 2015; Yuan et al. 2017). Thus, α3β4* nAChRs appear to play an important role in both nicotine reinforcement and relapse to drug seeking behavior.

Most preclinical tests that assess the efficacy of smoking cessation therapies use nicotine alone, although such tests do not readily predict the difficulty smokers experience in maintaining abstinence (Caggiula et al. 2001; Sorge et al. 2009; Rose et al. 2010; Feltenstein et al. 2012; Perry et al. 2014; Swalve et al. 2016). However, recent animal studies have shown that non-nicotine constituents in cigarette smoke may also contribute to tobacco addiction (Belluzzi et al. 2005; Lotfipour et al. 2011; Arnold et al. 2014; Brennan et al. 2014; USDHHS 2014). For instance, other tobacco alkaloids found in cigarette smoke, such as nornicotine, anabasine, anatabine, myosmine, and cotinine, are similar in structure to nicotine and bind to nAChRs, thus likely contributing to tobacco's reinforcing and

dependence-producing effects (Huang and Hsieh 2007; Clemens et al. 2009). Other constituents, like acetaldehyde, the second most commonly found constituent in cigarette smoke (Hoffmann et al. 2001), can stimulate dopamine release in the mesolimbic pathway (Wang et al. 2007), thereby influencing tobacco dependence despite having cellular targets other than nAChRs. In behavioral studies, acetaldehyde is reinforcing on its own and is self-administered intravenously, orally, and into the brain (Myers et al. 1982; Arizzi et al. 2003; Cacace et al. 2012). It also has been shown to enhance the self-administration of nicotine in adolescent animals (Belluzzi et al. 2005), further supporting the role of non-nicotine tobacco constituents in tobacco dependence. Beta-carboline alkaloids, such as harmane and norharmane, are among other constituents present in large concentrations in tobacco smoke (Herraiz 2004), and have been shown to exhibit reinforcement enhancing effects, perhaps through their strong monoamine oxidase (MAO) inhibitory activity (Herraiz and Chaparro 2005). We have shown that norharmane is reinforcing when self-administered alone, and increases reinforced responding for nicotine (Arnold et al. 2014). Together, these findings highlight the contributing role of non-nicotine constituents in tobacco addiction, and suggest that they should be included in screening potential pharmacological cessation therapies.

In order to evaluate the impact of cigarette smoke constituents in models of addiction, we have used an aqueous cigarette smoke extract (CSE) in self-administration tests (Costello et al. 2014; Gellner et al. 2016). We have shown that animals readily self-administer CSE, and show robust reinstatement without the presence of drug-associated cues, unlike reinstatement of nicotine alone which requires the addition of cues (Costello et al. 2014). Further, although AT-1001 reduces self-administration of both CSE and nicotine (Costello et al. 2014), it does not fully block the reinforcing effects of CSE. These findings suggest a differential role for α3β4* nAChRs in the reinforcing effects of nicotine and CSE.

Whereas it has previously been shown that AT-1001 reduces drug- and stress-primed reinstatement of nicotine-seeking behavior (Cippitelli et al. 2015), there has not yet been an assessment of the role of α3β4* nAChRs in drug-primed reinstatement of CSE-seeking behavior. The purpose of the present study was, therefore, to compare drug-primed reinstatement in animals that had self-administered CSE or nicotine, and assess the involvement of α3β4* nAChRs in these behaviors.

2. Materials and Methods

2.1 Animals

Adult male Sprague-Dawley rats (n = 10–14/group; 300–325 g; Charles River Labs, Hollister, CA) arrived at postnatal day 81 and were housed in an AALAC-accredited vivarium on a 12 h light/dark cycle (0700 to 1900 h). All procedures were in compliance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. After two days acclimation to the vivarium, animals were handled for two minutes daily before testing began. All behavioral testing was conducted seven days per week during the light cycle. Animals had dietary restriction to maintain 85% of their free-feeding body weight during food training and 95% during the remainder of the study.

2.2 Drugs

Nicotine hydrogen tartrate (Sigma, St. Louis, MO) was dissolved in sterile saline and adjusted to pH 7.2–7.4. All nicotine doses were calculated as free base. CSE was created daily by bubbling smoke from commercial cigarettes (Camel unfiltered, R.J. Reynolds Co.) through sterile saline, as per our previous methods (Costello et al. 2014; Gellner et al. 2016). Briefly, eight cigarettes were smoked through 35 ml of saline solution (35 ml puffs over 2 sec, repeated every 30 sec) and the final solution was adjusted to pH 7.2–7.4. All CSE doses were defined by the nicotine content in the solution (target concentration = 150 μg/ml) determined by GC-MS after a nicotine extraction (Gellner et al. 2016). Analysis of CSE samples yielded nicotine concentrations of 162.6 ± 13.7 μg/ml. AT-1001 (Astraea Therapeutics, Mountain View, CA) was dissolved in 97% hydroxypropylcellulose (0.5% concentration in water), 2% DMSO and 1% 0.1 M HCl.

2.3 Food training

Animals were trained once per day in a 30 min session to lever press for food pellets (45 mg rodent purified diet; Bio-Serv, Frenchtown, NJ) in lever pressing operant testing chambers (Med Associates, St. Albans, VT) based on (Liechti and Markou 2007; Costello et al. 2014). One wall of the chamber contained two levers, a cue light over each, and a house light. At the beginning of the session, the house light was illuminated and responses at the reinforced (R) lever resulted in reward and illumination of the cue light over that lever. Responses at the non-reinforced (NR) lever had no consequence, but were recorded as a measure of nonspecific activity. The animals started at an FR1TO1 (fixed-ratio 1, 1 sec timeout) schedule of reinforcement, followed by FR1TO10, FR2TO20, and finally FR5TO20, progressing upon earning 50 reinforcers.

2.4 Surgery

After food training, animals were anesthetized with equithesin (0.0035 ml/g body weight) and implanted with indwelling jugular vein catheters based on previously published methods (Belluzzi et al. 2005). During the 2–3 day recovery period, and for the remainder of the study, animals were flushed daily with heparinized saline solution (1 ml of 1000 units/ml heparin into 30 ml of bacteriostatic saline). Catheter patency was verified by infusing 0.1 ml of Propofol (Abbott Laboratories, Chicago, IL) for rapid anesthesia (5–10 sec) after stabilization of self-administration was achieved and again prior to the start of the extinction phase.

2.5 Drug self-administration and extinction

Animals self-administered nicotine or CSE (15 μg/kg/infusion nicotine content) at an FR5 schedule for a 1 hr daily session for a minimum of 10 days, or until they reached stable responding (Reinforced responses (R) within 20% of the mean over the last 3 days; $R \geq 2 \times$ Non-Reinforced (NR) responses; $R \geq 6$). Baseline responding was defined as the average reinforced responses over the last three days of self-administration. After reaching stable responding, extinction-reinstatement testing began. During extinction, animals were placed in the same operant testing chambers; the animals were not connected to the infusion tubing, the house light remained on, and responses on the levers were counted, but had no

consequences. Extinction sessions were 1 hr per day for a minimum of 5 days, or until responding was reduced to 20% of baseline.

2.6 Cue- and drug-induced reinstatement

After reaching extinction criteria, animals were triggered to reinstate drug-seeking behavior using five reinstatement conditions (given in a within-subjects counter-balanced design): cues, CSE-prime alone, nicotine-prime alone, CSE-prime paired with cues, and nicotine-prime paired with cues. Presentation of cues consisted of cue light illumination in the chamber. All drug prime injections contained nicotine (0.15 mg/kg) or CSE with equivalent nicotine content, and were administered intraperitoneally immediately before the reinstatement test. Between reinstatement tests, animals were returned to extinction conditions for a minimum of two days, or until extinction criteria were met. Reinstatement was defined as a significant increase in responding from the last day of extinction.

2.7 α3β4 nAChR blockade of drug- + cue-induced reinstatement

Following self-administration of CSE or nicotine and extinction, another cohort of animals was treated with AT-1001 (0, 0.75, 1.5, and 3 mg/kg; s.c.) 10 min prior to reinstatement testing in a within-subjects Latin-square design, as in Toll et al. (2012). Reinstatement was initiated with a priming dose of CSE or nicotine (0.15 mg/kg, i.p.) in animals that had self-administered CSE, or a priming dose of nicotine (0.15 mg/kg, i.p.) in animals that had previously self-administered nicotine, given immediately before testing. Between reinstatement tests, animals were returned to extinction conditions for a minimum of two days, or until extinction criteria were met. All animals repeated a vehicle dose of AT-1001 at the end of the study to confirm reinstatement was still occurring. Animals that did not pass a reinstatement criterion following the final vehicle dose of 40% or more from the last day of FR5 responding were excluded from the study.

2.8 Data analysis

To normalize data, reinstatement data were analyzed as a percentage of baseline responding, calculated as: (Test day responding/Last day of FR5 responding) x 100. Mean responding was analyzed by a 2-way ANOVA on Self-Administered Drug x Reinstatement Condition or AT-1001 Dose x Drug Prime or Self-Administered Drug x AT-1001 Dose, with repeated measures on Reinstatement Condition or AT-1001 Dose. Significant main effects were analyzed further with 1-way ANOVAs and Bonferroni-corrected paired or unpaired t-tests.

3. Results

3.1 Cue- and drug-induced reinstatement of nicotine or CSE seeking

There were no significant differences in reinforced responses for CSE ($p>0.05$) or nicotine ($p>0.05$) on day 10 of self-administration testing (Figure 1a). Both groups also decreased responding on the reinforced lever to less than 20% of day 10 by the fifth day of extinction testing (n = 13–14/group; Figure 1b).

For the reinstatement test, there were significant main effects of Reinstatement Condition ($F5,125=10.248$; $p<0.001$) and Self-Administered Drug ($F1,25=6.125$; $p=0.02$; Figure 2).

Data were split by self-administered drug to explore the effects of each reinstatement condition (n=13–14/group). Consistent with prior literature (Feltenstein et al. 2012), a priming injection of nicotine reinstated drug-seeking behavior in rats that had previously self-administered nicotine, but only if drug-associated cues were present ($p=0.05$; Figure 2). A priming injection of CSE also reinstated nicotine-seeking behavior in these animals when presented with cues ($p=0.015$), but not without (Figure 2). Cue presentation alone did not produce significant reinstatement in animals that self-administered nicotine (Figure 2).

Animals that had self-administered CSE reinstated responding with priming injections of either CSE or nicotine in the absence of drug-associated cues ($p=0.03$ and 0.03, respectively). Nicotine prime without cues induced significantly higher reinstatement in animals that had self-administered CSE than in those that had self-administered nicotine alone ($p= 0.026$; Figure 2). Addition of cues to the priming drug did not result in further increase of drug seeking behavior in CSE self-administering animals. Cue presentation alone did not significantly reinstate CSE-seeking behavior (Figure 2).

3.3 α3β4 nAChR blockade of reinstatement

In order to test the hypothesis that α3β4* nAChRs mediate reinstatement of responding for CSE and nicotine, the effect of AT-1001 on drug- + cue-primed reinstatement of drug-seeking behavior was examined in a separate cohort of rats. To confirm that the priming drug did not significantly influence reinstatement, animals that self-administered CSE were first analyzed separately from nicotine self-administering animals (Figure 3A). We observed a significant main effect of AT-1001 Dose ($F_{4,80}=34.785$, $p<0.001$), but no significant effect of Drug Prime and no interaction. Since priming drug did not influence reinstatement responses for CSE, both groups were combined for further analysis. Drug + cue prime reinstated responding in vehicle control animals ($p<0.001$), and only the highest dose of AT-1001 significantly attenuated reinstatement (Figure 3A). When CSE self-administering animals were compared to nicotine self-administering animals, there were significant main effects of AT- 1001 Dose ($F_{4,132}=48.174$; $p<0.001$) and Self-Administered Drug ($F_{1,33}=6.960$, $p=0.013$), as well as a significant AT-1001 Dose x Self-Administered Drug interaction ($F_{4,132}=5.479$, $p<0.001$) (Figure 3B). All vehicle controls reinstated when primed with drug + cue ($p<0.001$). AT-1001 dose-dependently attenuated reinstatement in animals that had self-administered nicotine or CSE, although higher doses of AT-1001 were needed for the latter. The 0.75 mg/kg dose of AT-1001 fully blocked reinstatement of drug-seeking in animals that had self-administered nicotine alone, but not in animals that had self-administered CSE ($p=0.001$ vs. extinction; Figure 3B). At this AT-1001 dose, animals that had self-administered CSE reinstated significantly more than animals that had self-administered nicotine ($p=0.005$). At the higher dose, AT-1001 significantly attenuated drug + cue primed reinstatement in both groups.

4. Discussion

The reinstatement procedure is a widely used preclinical paradigm to study smoking “relapse” (Shaham et al. 2003). We have previously shown that animals that self-administered CSE will reinstate responding to stress without associated cues, whereas those

that had previously self-administered nicotine required the addition of cues (Costello et al. 2014). We have now expanded these findings to examine drug-primed reinstatement. Whereas animals that had self-administered nicotine alone required both drug and cues to reinstate responding, those that had self-administered nicotine with the aqueous constituents of tobacco smoke (CSE) significantly reinstated responding to drug prime in the absence of cues. We also show that a α3β4 nAChR antagonist, AT-1001, inhibits drug- + cue-primed reinstatement in animals that had previously self-administered nicotine or CSE. However, AT-1001 is less potent at inhibiting reinstatement in animals that had previously self-administered CSE. These findings suggest that prior self-administration of CSE may alter the role that α3β4 nAChRs play in mediating drug- + cue-primed reinstatement.

Animals that self-administered CSE do not require cues to reinstate drug- seeking behavior

In line with our previous reports (Costello et al., 2014; Gellner et al., 2016), we did not observe differences between nicotine or CSE in baseline self-administration or final extinction behavior. However, drug-seeking behavior after extinction was influenced by the presence of tobacco smoke constituents in CSE. As shown here and by others (Caggiula et al. 2001; Sorge et al. 2009; Swalve et al. 2016), animals that had self-administered nicotine alone required the presentation of drug-associated cues to reinstate after drug priming (Figure 2). This occurred even though cues alone did not significantly reinstate responding to either nicotine or CSE. Although prior studies have found that cues alone do reinstate nicotine seeking behavior, our observed lack of effect may result from methodological differences, such as the lower dose of self-administration drug that we have used in this study. In contrast animals that had self-administered CSE reinstated responding with drug-priming alone without the addition of cues. This is consistent with the conclusions of our previous study that showed that cues were not necessary for full stress-induced reinstatement of drug-seeking behavior in animals that had self-administered CSE (Costello et al., 2014).

Involvement of α3β4 nAChRs

nAChRs have been shown to have important, but differential, roles in mediating drug- or cue-induced reinstatement of nicotine-seeking behavior. The non-selective nAChR antagonist, mecamylamine, blocks cue-induced reinstatement of nicotine-seeking, as well as nicotine-primed reinstatement of conditioned place preference (Biala et al. 2010; Toll et al. 2012; Costello et al. 2014). Furthermore, selective antagonists of α7, α4β2, and α3β4 nAChRs have been shown to block nicotine- or cue-primed reinstatement (O'Connor et al. 2010; Le Foll et al. 2012; Liu 2014; Cippitelli et al. 2015; Wu et al. 2017). The α3β4 nAChR, in particular, represents a promising target for smoking cessation therapy, although research is still limited. In addition to associations between polymorphisms in the CHRNA3/A5/B4 gene cluster and tobacco dependence risk (Schlaepfer et al. 2008; Berrettini and Doyle 2012; Lassi et al. 2016), recent research in animals supports a role for the α3β4* nAChR in nicotine-associated behaviors. Although α3β4 nAChRs have a limited brain distribution compared to other nAChR types, they are predominantly expressed in the medial habenula and interpeduncular nucleus, regions critically involved in nicotine intake and withdrawal (Perry et al. 2002; Salas et al. 2004; Grady et al. 2009; Salas et al. 2010; Fowler et al. 2011; Jackson et al. 2013). Furthermore, AT-1001 blocks nicotine self-

administration and reinstatement of nicotine seeking (Toll et al., 2012; Costello et al., 2014; Cippitelli et al., 2015; Yuan et al., 2017).

Given our current finding that nicotine priming, either alone or paired with cues, can reinstate CSE-seeking behavior, it seems likely that nAChRs are involved in this behavioral response. To test this hypothesis, we administered AT-1001 prior to drug priming and presentation of associated cues. In agreement with published work (Cippitelli et al. 2015), we show that AT-1001 blocks nicotine + cue-primed reinstatement of nicotine-seeking, emphasizing the importance of α3β4* nAChRs in drug-primed craving. AT-1001 also dose-dependently attenuated drug + cue-primed reinstatement in animals that had previously self-administered CSE, but with lower potency than in animals that had self-administered nicotine alone. This was the case whether the priming drug was CSE or nicotine alone, suggesting that the decreased potency of AT-1001 may be due to a diminished contribution of α3β4 nAChRs to mechanisms underlying reinstatement of responding following prior self-administration of CSE. The ability of AT-1001 to attenuate drug-seeking behavior is unlikely to be explained by locomotor depressant effects, as the doses of AT-1001 tested here do not affect spontaneous locomotor activity (Cippitelli et al. 2015; Yuan et al. 2017) or operant responding for food (Costello et al. 2014).

This finding is consistent with our prior observation that AT-1001 does not fully block CSE self-administration, even though it completely blocks that of nicotine alone (Costello et al., 2014). In contrast, we found that the partial α4β2 agonist and full α7 agonist, varenicline, decreased nicotine and CSE self-administration to a similar degree (Costello et al., 2014). We have previously shown that nicotine and CSE administration yield similar plasma and brain levels of nicotine, and that there are no significant differences in CSE and nicotine IC50 values for inhibition of radioligand binding to α4β2, α3β4, α3β2, and α7 nAChRs (Costello et al. 2014). Further studies will therefore be needed to address how CSE exposure alters the functional contribution of α3β4 nAChRs, both to the reinforcing effect of CSE and to drug-primed reinstatement behavior. Since there is no difference in nicotine self-administration dose across animal groups, the differential findings with CSE most likely reflect the actions of other tobacco constituent(s). These may include MAO inhibitors, which are present in CSE and which can create a distinct pattern of neuronal activation from that of nicotine alone (Arnold et al. 2014). Other aqueous constituents that are likely present in CSE include acetaldehyde and other tobacco alkaloids, constituents that have also been shown to have a pharmacologically relevant contribution to the addictive profile of tobacco (Myers et al. 1982; Arizzi et al. 2003; Belluzzi et al. 2005; Clemens et al. 2009). Future studies may examine the role of these individual constituents in modifying sensitivity to nicotine- and cue-induced drug-seeking behavior. Further studies examining the role of other nAChR subtypes in CSE-seeking behaviors are also warranted.

5. Conclusion

We have shown that the inclusion of aqueous cigarette smoke constituents with nicotine alters reinstatement profiles, enhancing seeking behavior after drug re-exposure in the absence of cues. Since nicotine priming alone reinstated responding in animals that had previously self-administered CSE, nicotine is likely the primary constituent in CSE that

mediates drug-primed reinstatement. However, the robust drug-seeking behavior we observed with all reinstatement triggers in animals that had self-administered CSE suggests that non-nicotine aqueous constituents may potentiate nicotine's effects on reinstatement and relapse. Our data also suggest that the role of α3β4* nAChRs in drug-seeking behavior is altered by prior exposure to tobacco smoke constituents. In all, these findings highlight the importance of including whole smoke constituents in preclinical models of tobacco dependence.

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- α3β4 nAChRs are a potential pharmacological target for smoking cessation
- Aqueous tobacco smoke constituents (i.e., CSE) potentiate nicotine reinstatement
- The α3β4 functional antagonist AT-1001 reduces nicotine-seeking behavior
- AT-1001 is less potent at inhibiting CSE reinstatement than nicotine reinstatement

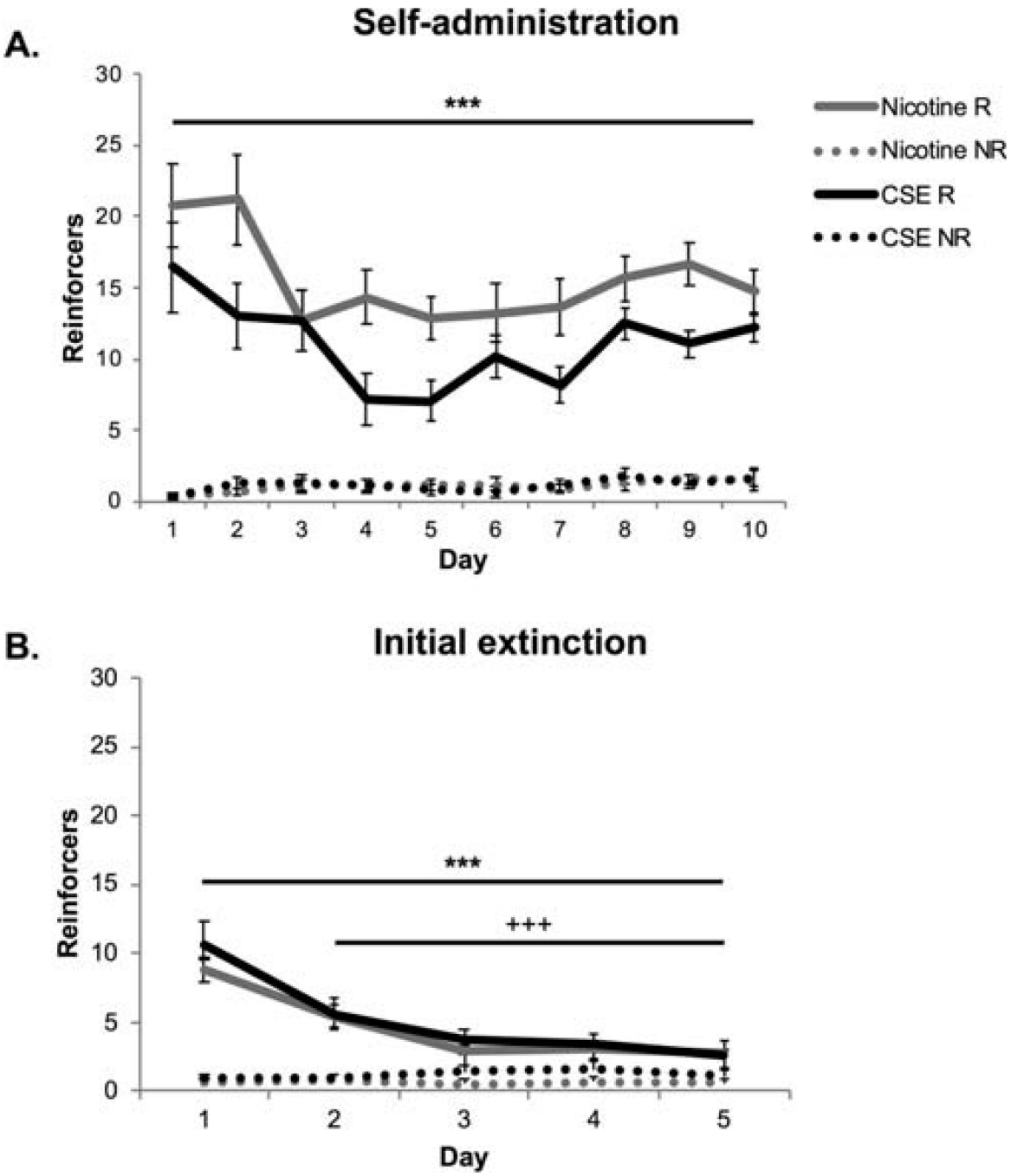

Figure 1. Adult rats self-administer (A) and extinguish (B) nicotine and CSE at similar rates.
Responding is plotted as mean±SEM of FR5 intervals; equivalent to infusions in (A). ***, p≤0.001 Reinforced (R) vs. Non- Reinforced (NR); +++, p≤0.001 vs. Day 1 R responding. n = 13–14 per group.

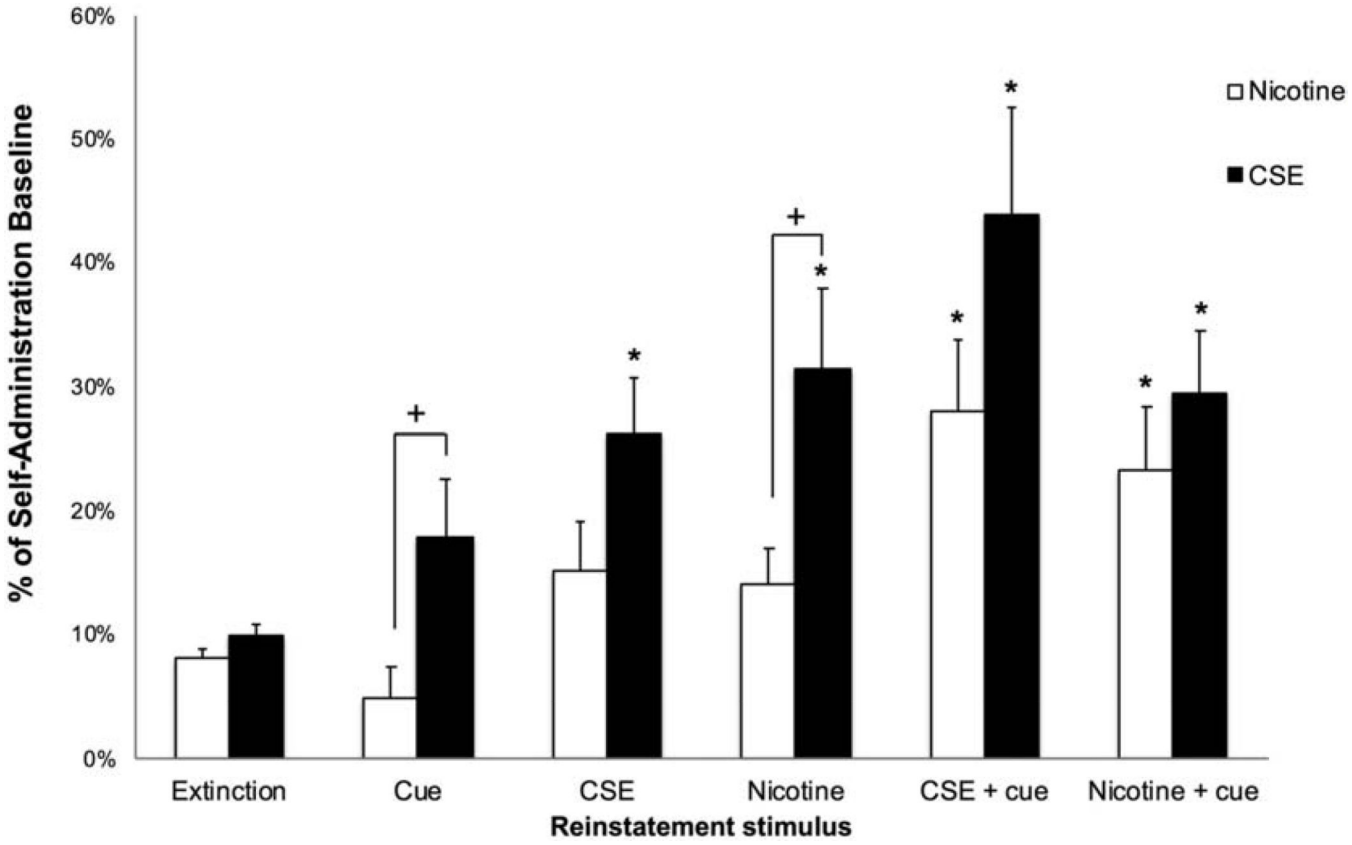

Figure 2. Animals that self-administered CSE show enhanced drug-primed reinstatement than those that self-administered nicotine alone.

Animals that self-administered CSE are sensitized to drug-primed reinstatement compared to those that self-administered nicotine alone. Data plotted as a mean+SEM percent of the last day of self-administration responding. *, p≤0.05, **, p≤0.01 vs. extinction; +, p≤0.05 vs. nicotine. n = 13–14/group.

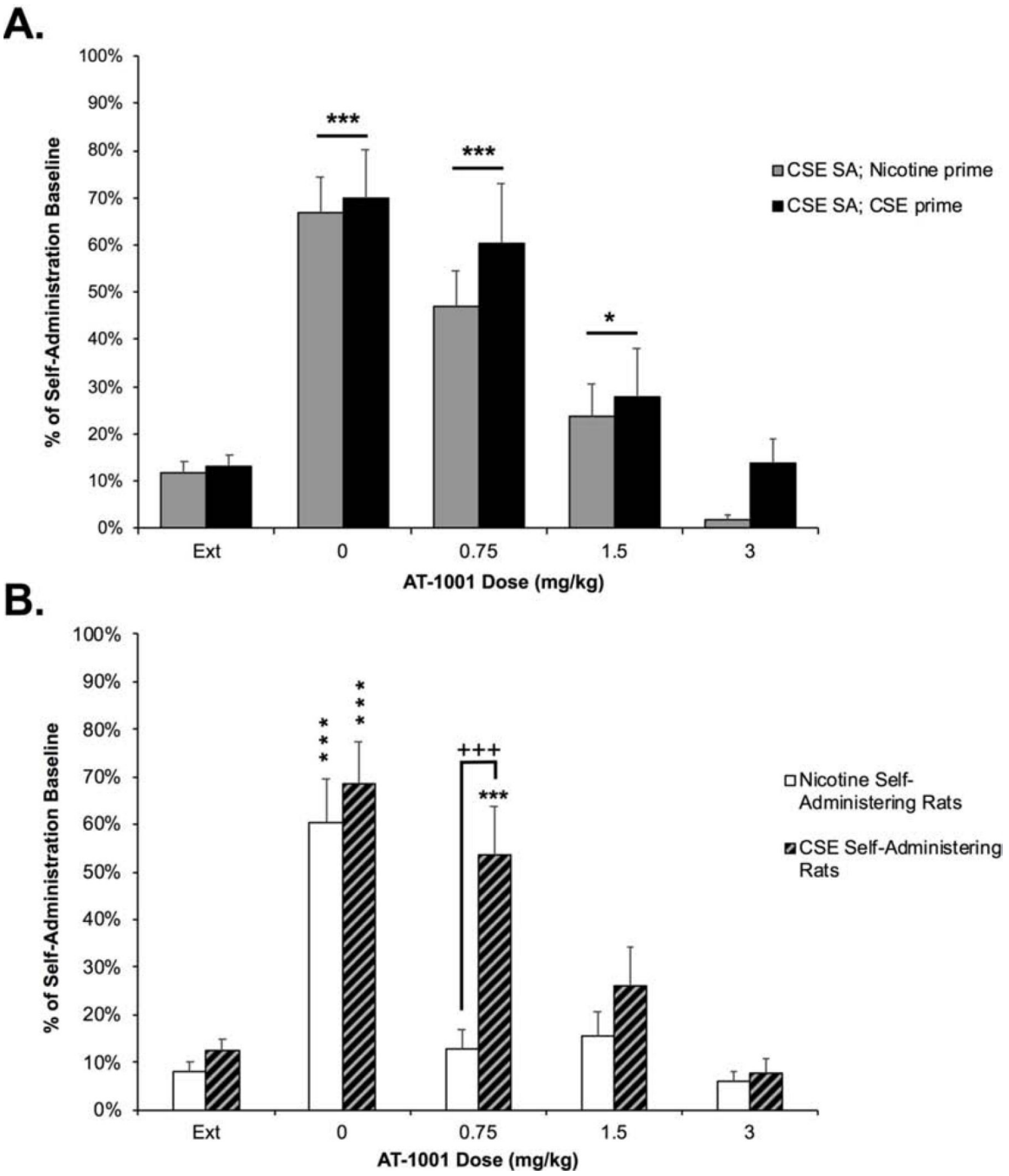

Figure 3. AT-1001 dose-dependently attenuates CSE- and nicotine-primed reinstatement (paired with cues) with higher potency in animals that had previously self-administered nicotine.

(A) Both nicotine and CSE priming reinstates responding in animals that previously self-administered CSE, and only the highest dose of AT-1001 attenuated reinstatement. (B) AT-1001 dose-dependently attenuates CSE- and nicotine-primed reinstatement (paired with cues) in animals that self-administered nicotine or CSE (data collapsed across priming drug). Inhibition of reinstatement is greater in animals that previously self-administered nicotine at the 0.75 mg/kg dose of AT-1001. Data plotted as mean+SEM percent of the last day of self-

administration responding. ***, $p \leq 0.001$; *, $p<0.05$ vs. extinction; +++, $p \leq 0.001$ vs. nicotine. n = 13–23/group.