### Title

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# Self-administration of nicotine and cigarette smoke extract in adolescent and adult rats

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## Abstract

Although smoking initiation typically occurs during adolescence, most preclinical studies of tobacco use involve adult animals. Furthermore, their focus is largely on nicotine alone, even though cigarette smoke contains thousands of constituents. The present study therefore aimed to determine whether aqueous constituents in cigarette smoke affect acquisition of nicotine self-administration during adolescence in rats. Adolescent and adult male rats, aged postnatal day (P) 25 and 85, respectively, were food trained on a fixed ratio 1 (FR1) schedule, then allowed to self-administer one of 5 doses of nicotine (0, 3.75, 7.5, 15, or 30 μg/kg) or aqueous cigarette smoke extract (CSE) with equivalent nicotine content. Three progressively more difficult schedules of reinforcement, FR1, FR2, and FR5, were used. Both adolescent and adult rats acquired self-administration of nicotine and CSE. Nicotine and CSE similarly increased non-reinforced responding in adolescents, leading to enhanced overall drug intake as compared to adults. When data were corrected for age-dependent alterations in non-reinforced responding, adolescents responded more for low doses of nicotine and CSE than adults at the FR1 reinforcement schedule. No differences in adolescent responding for the two drugs were seen at this schedule, whereas adults had fewer responses for CSE than for nicotine. However, when the reinforcement schedule was increased to FR5, animals dose-dependently self-administered both nicotine and CSE, but no drug or age differences were observed. These data suggest that non-nicotine tobacco smoke constituents do not influence the reinforcing effect of nicotine in adolescents.

### Keywords

Adolescence; Cigarette smoke; E-cigarettes; Nicotine; Self-administration; Tobacco

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**Conflict of interest**

None

**Author Contributions**

CAG and FML designed the research; CAG performed the research and analyzed the data; JDB and FML consulted on data analysis; and CAG and FML wrote the article. All authors critically reviewed content and approved final version for publication.

## 1. Introduction

Tobacco use is the leading preventable cause of death worldwide, killing more than 6 million people a year (World Health Organization, 2015). In the United States, 1 of every 5 deaths is attributed to cigarette smoking (Center for Disease Control, 2014). Smoking is an adolescent-onset disorder, with almost 90% of smokers trying their first cigarette by the age of 18 (Center for Disease Control, 2014). Although current rates of conventional cigarette use have markedly declined, the use of electronic nicotine delivery systems (e-cigarettes) among school-age children has tripled in the last year (Arrazola, 2015). E-cigarettes, which are marketed as safer alternatives and smoking cessation aids, may actually increase the likelihood of continuing and increasing tobacco use among adolescents (Dutra and Glantz, 2014).

Adolescence is characterized as a period of development when individuals demonstrate risk-taking and novelty seeking behaviors (Spear, 2000). Both clinical (Chen and Millar, 1998; Everett et al., 1999) and preclinical (Belluzzi et al., 2004; Brielmaier et al., 2008; Vastola et al., 2002) studies have found adolescents to be more sensitive to the rewarding properties of nicotine. Adolescent rats have been shown to acquire nicotine self-administration more readily, and to take more nicotine, than adults (Chen et al., 2007; Levin et al., 2007, 2003). In conditioned place preference, rats in early adolescence display enhanced sensitivity to the rewarding effects (Belluzzi et al., 2004; Brielmaier et al., 2008; Vastola et al., 2002), and reduced sensitivity to the aversive effects of nicotine (Shram et al., 2006; Torres et al., 2008; Wilmouth and Spear, 2004).

Cigarette smoke contains more than 7,000 constituents; hundreds of which are harmful, and about 60 are known to cause cancer (National Toxicology Program, 2014). However, animal models of tobacco dependence have traditionally examined only the effects of nicotine (Donny et al., 1995), the main psychoactive component of tobacco (Stolerman and Jarvis, 1995). Some studies have begun to look at the non-nicotine constituents found in cigarette smoke to understand how they may affect nicotine self-administration. Biologically active components such as monoamine oxidase inhibitors have been shown to increase nicotine self-administration (Arnold et al., 2014; Guillem et al., 2005; Villégier et al., 2007, 2006). Acetaldehyde, a combustion product of tobacco, also enhances nicotine self-administration in adolescent, but not adult, rats (Belluzzi et al., 2005). Although these findings show that single constituents interact with nicotine, they exclude most tobacco smoke constituents and ignore the possible interactions that may occur between them. In order to study these interactions, we have created a model in which the behavioral effects of aqueous cigarette smoke extract (CSE) are examined. Previous work by our group has shown that CSE is more potent than nicotine alone in adult male rats during the acquisition and maintenance phases of self-administration, and yields sensitized reinstatement to stressors (Costello et al., 2014).

Using a modified method from Costello et al. (2014), in order to assess the influence of age, we have now compared the acquisition of self-administration of nicotine or CSE at varying doses in adolescent and adult male rats. Since initiation of smoking typically occurs during adolescence, it is important to study this period of development in animal models of tobacco dependence.

## 2. Materials and Methods

### 2.1 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 by bubbling smoke from commercial cigarettes (Camel unfiltered, RJ Reynolds) through sterile saline, using a method described in Costello et al., 2014. Briefly, eight cigarettes were smoked through 35 ml of saline solution (35 ml puffs over 2 s, repeated every 30 s) and the final solution was adjusted to pH 7.2–7.4. The CSE solution was prepared fresh each day immediately before experimental testing in order to minimize differences resulting from differential stability of the constituents. All CSE doses were defined by the solutions nicotine content, which was analyzed by an outside facility (UCSF Clinical Pharmacology Laboratory).

### 2.2 Subjects

Male Sprague–Dawley rats were obtained from Charles River at postnatal (P) days 17 and 81. Adolescent rats remained with dam until weaning (P21). Animals, both adolescents and adults, were group-housed throughout the experiment. All rats were maintained on a 12-h light/dark cycle (lights on at 07:00 am) with food and water available *ad libitum*. No more than one animal per litter per experimental group was used to avoid potential confounds. All experimental procedures were in compliance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

Rats were minimally food-restricted beginning two days prior to operant conditioning to promote exploration of the operant chamber and aid in acquisition of the operant task. Adolescent and adult rats were fed 15–25 or 20–25 g of food, respectively, to maintain normal growth during self-administration testing. Food was given 15 min after each experimental session, and any remaining chow was removed an hour before the following day test session. Food maintenance continued until the end of the experiment. Growth curves for both adolescents and adults followed normal trajectories (data not shown).

### 2.3 Behavioral Studies

**2.3.1 Apparatus—**Animals were tested in plexiglass operant chambers (Med Associates, St Albans, VT), equipped with two levers. Responses at the reinforced (R) lever resulted in illumination of a cue light over the lever and activation of an externally mounted syringe pump that infused drug. During the infusion (5.6 s yielding 100 μl of solution) and timeout period (20 s) the cue light remained illuminated and the house light was turned off. Responses on the non-reinforced (NR) lever were recorded but had no consequences.

**2.3.2 Food Training—**Adolescent and adult rats, aged P25 and 85, respectively, were first trained to lever-press for food pellets (45 mg rodent purified diet; Bio-Serv, Frenchtown, NJ) under a fixed ratio 1 schedule with a 1 second timeout period (FR1TO1), followed by FR1TO10, and completed with FR1TO20. Rats progressed to the next timeout period when they earned at least 35 or 50 reinforcers (adolescents and adults, respectively) in the daily 30-minute session.

**2.3.3 Surgery—**Following successful acquisition of food responding, rats were anesthetized with equithesin (0.0035 ml/g body weight) and implanted with indwelling jugular vein catheters (Belluzzi et al 2005). During the 3-day recovery period, catheters were flushed daily with a heparinized saline solution to maintain patency. The day before initiation of self-administration, and at intervals thereafter, catheter patency was verified for rapid (5–10 s) anesthesia by infusing propofol (5 mg/kg, i.v.). Patency was tested at the end of each schedule and only animals showing rapid anesthesia were included in analyses.

**2.3.4 Self-Administration—**After recovery, adolescents and adults, aged P37 and 97, respectively, were allowed to self-administer a single dose of nicotine or CSE (0, 3.75, 7.5, 15, or 30 μg/kg/infusion nicotine content). Rats self-administered nicotine or CSE for 7 days at the FR1TO20 schedule, before transitioning to the FR2TO20 schedule for 2 days, and finishing with 3 days at the FR5TO20 schedule during daily 1-hour sessions.

### 2.4 Statistical Analyses

The average of the last 3 days of self-administration at the FR1 schedule (Day 5–7) and the FR5 schedule (Day 10–12) were analyzed separately with a four-way ANOVA on Age × Drug × Dose × Lever with repeated measures on Lever. Any significant main effects or interactions were further analyzed by three- or two-way ANOVAs with Dunnett's, Bonferroni-corrected paired (levers) or unpaired (drug) t-test post hoc comparisons. Drug intake, calculated as the number of infusions per session multiplied by the dose of drug self-administered, was analyzed with a three-way ANOVA on Age × Drug × Dose. Any significant main effects were further analyzed by two-way ANOVAs with Bonferroni-corrected unpaired t-test post hoc comparisons. Non-reinforced (NR) responding data was analyzed with a three-way ANOVA on Age × Drug × Dose. Any significant main effects were further analyzed by a two-way ANOVA with Dunnett's or Bonferroni-corrected unpaired t-test post hoc comparisons. Corrected reinforced responding data was analyzed with a three-way ANOVA on Age × Drug × Dose. Any significant main effects were further analyzed by two-way ANOVAs with Bonferroni-corrected unpaired t-test post hoc comparisons.

## 3. Results

At the FR1 schedule of reinforcement, main effects of Levers [$F(1,165)=352.285$, $p<0.0001$], Age [$F(1,165)=109.535$, $p<0.0001$], Drug [$F(1,165)=5.113$, $p<0.05$], and Dose [$F(4,165)=11.050$, $p<0.0001$] were found. Significant Levers*Age [$F(1,165)=25.194$, $p<0.0001$], Levers*Age*Dose [$F(4,165)=193.978$, $p=0.005$], and Age*Dose [$F(4,165)=6.926$, $p<0.0001$] interactions were also found. Given the significant main effect of age and its interaction with multiple factors, adolescents and adults were analyzed separately to further assess these effects (Figure 1). Adolescents showed significant effects of Levers [$F(1,81)=167.009$, $p<0.0001$], Dose [$F(4,81)=8.646$, $p<0.0001$] and Levers*Dose [$F(4,81)=6.571$, $p<0.0001$], but not Drug, indicating that the non-nicotine constituents did not enhance acquisition of self-administration behavior at this schedule (Figure 1a). Adolescents preferred the reinforced to the non-reinforced lever at all doses, including 0 ($p<0.05$ vs non-reinforced). Adolescents exhibited an inverted U dose-response curve for

reinforced responding, with higher responses as compared to saline at the three lowest drug doses (Figure 1a). Non-reinforced responding at all drug doses was significantly higher than for saline.

At the FR1 schedule of reinforcement, adults showed significant effects of Levers [$F(1,84)=273.606$, $p<0.0001$], Drug [$F(1,84)=10.594$, $p<0.01$], and Dose [$F(4,84)=3.252$, $p<0.05$]. Significant Levers*Drug [$F(1,84)=14.837$, $p<0.0001$], Levers*Dose [$F(4,84)=78.017$, $p=0.003$], Levers*Drug*Dose [$F(4,84)=69.320$, $p=0.005$], and Drug*Dose [$F(4,84)=3.827$, $p=0.007$] were also found. Adults preferred the reinforced to the non-reinforced lever at all doses, including 0 ($p<0.05$ vs non-reinforced). Whereas CSE exhibited a flat dose-response curve, there was enhanced reinforced responding for nicotine at the 7.5 dose as compared to saline ($p<0.05$ vs 0 dose). Animals responding for nicotine had significantly higher reinforced responding at the 7.5 and 30 μg/kg doses than for CSE with equivalent nicotine content ($p<0.05$ vs CSE; Figure 1b).

When the schedule of reinforcement was increased to FR5, significant main effects of Levers [$F(1,165)=192.43$, $p<0.0001$], Age [$F(1,165)=31.903$, $p<0.0001$], and Dose [$F(4,165)=12.667$, p<l0.0001] were found. Significant Age*Dose [$F(4,165)=2.742$, $p=0.030$] and Drug*Dose interactions [$F(4,165)=3.013$, $p=0.020$] were found. Given the significant main effect of age and its interaction with dose, adolescents and adults were analyzed separately to further assess these effects. Adolescents showed significant effects of Levers [$F(1,81)=100.335$, $p<0.0001$], Dose [$F(4,81)=8.456$, $p<0.0001$] and Levers*Dose [$F(4,81)=3.962$, $p=0.005$], but not Drug, indicating that the non-nicotine constituents did not enhance self-administration behavior in adolescents. At this schedule, adolescent rats showed a preference for the reinforced lever at all doses, including 0 ($p<0.05$). In addition, adolescents showed enhanced reinforced and non-reinforced responding at the 3 highest doses compared to saline ($p<0.001$–$0.0001$, Figure 2a).

At FR5 in adults there were significant effects of Levers [$F(1,84)=92.011$, $p<0.0001$] and Dose [$F(4,84)=4.232$, $p=0.004$], with Lever*Dose [$F(4,84)=4.078$, $p=0.005$] and Lever*Drug interactions [$F(1,84)=4.527$, $p=0.036$]. As with adolescents, adults showed a preference for the reinforced lever at all doses, including 0 ($p<0.05$). Although there was a significant Lever*Drug interaction, post-hoc analysis showed no significant differences in self-administration of the two drugs at any dose. However, reinforced responding was significantly higher than saline for CSE at the 15 μg/kg nicotine content dose, and for nicotine at the 30 μg/kg dose ($p<0.05$ vs 0 dose, Figure 2b).

Drug intake is shown in Figure 3. At the FR1 schedule (Figure 3a), there were main effects of Age [$F(1,165)=127.428$, $p<0.0001$], Drug [$F(1,165)=9.664$, $p<0.01$], and Dose [$F(4,165)=94.434$, $p<0.0001$]. When data were split by Age, adolescents showed main effects of Dose [$F(4,81)=51.385$, $p<0.0001$] but not Drug, indicating that adolescents take similar amounts of CSE and nicotine. Adolescents showed higher nicotine intake than adults at all drug doses ($p<0.05$). Adults displayed main effects of Drug [$F(1,84)=31.087$, $p<0.0001$] and Dose [$F(4,84)=70727$, $p<0.0001$], and had higher nicotine intake compared to CSE at the 7.5 and 30 μg/kg doses ($p<0.05$, $p<0.01$).

For drug intake at the FR5 schedule (Figure 3b), main effects of Age [F (1,165)=13.761, p<0.0001], Drug [F(1,165)=4.960, p=0.027], and Dose [F(4,165)=65.152, p<0.0001] were found. When data were split by Age, adolescents showed significant effects of Dose [F(4,81)=41.470, p<0.0001] with a Drug*Dose interaction [F(4,81)=3.838, p=0.007]. Post hoc analysis revealed that adolescents had higher nicotine intake as compared to CSE at the 30 μg/kg dose (p<0.05). Adults showed significant effects of Drug [F(1,84)=3.946, p=0.050] and Dose [F(4,81)=23.968, p<0.0001], but further analysis did not reveal any significant drug differences.

To examine if the increase in drug intake during adolescence at the FR1 schedule was due to non-specific activity alone, non-reinforced responding was analyzed separately (Figure 4). At the FR1 schedule, there were main effects of Age [F(1,175)=73.496, p<0.0001] and Dose [F(4,175)=5.069, p<0.01], but not Drug. Adolescents, but not adults, showed a drug-related increase in non-reinforced responding during the FR1 schedule (Figure 4a). Non-reinforced lever pressing on the FR5 schedule of reinforcement also showed main effects of Age [F(1,175)=53.235, p<0.0001] and Dose [F(4,175)=6.878, p<0.0001], but not Drug. Again, adolescents, but not adults, showed a drug-related increase in non-specific activity (Figure 4b).

To correct for differences in non-reinforced responding and allow for an accurate age comparison, non-reinforced responding was subtracted from reinforced responding for each animal (Figure 5). At the FR1 schedule (Figure 5a), main effects of Age [F(1,165)=25.194, p<0.0001], Drug [F(1,165)=4.580, p<0.05], and Dose [F(4,165)=8.882, p<0.0001] were found. An Age comparison showed that adolescent responding for drug was significantly higher than that of adults at the 3.75 dose (p<0.001). Adolescents also showed main effects of Dose [F(4,81)=6.571, p<0.0001] but not Drug, indicating that adolescents self-administer nicotine and CSE equally at all doses. Adults had main effects of Drug [F(1,84)=14.837, p<0.0001] and Dose [F(4,84)=4.470, p<0.001], with post hoc analysis showing significantly lower adult responding for CSE than for nicotine at the three highest doses (p<0.05). At the FR5 schedule of reinforcement, main effects of Dose [F(4,165)=7.420, p<0.0001] but not Age [F(1,165)=2.351, p=0.127] or Drug [F(1,165)=3.204, p=0.075] were found. Thus, adolescent and adult male rats show similar self-administration behavior on the FR5 schedule of reinforcement when corrected for non-reinforced responding (Figure 5b).

## 4. Discussion

The present study focused on understanding whether aqueous constituents of tobacco smoke influence acquisition of nicotine self-administration in adolescent and adult male rats. As has been shown previously in adults (Costello et al., 2014), we now demonstrate that adolescents also acquire self-administration of CSE. Nicotine and CSE similarly increased non-reinforced responding in adolescents at both FR1 and FR5 schedules of reinforcement, leading to enhanced overall drug intake as compared to adults. When data were corrected for age-dependent alterations in non-reinforced responding, adolescents were found to be more sensitive to low doses of nicotine and CSE than were adults at the low, FR1 reinforcement schedule. There were no differences in adolescent responding for CSE or nicotine at this schedule, whereas adults had fewer responses for CSE than for nicotine at equivalent doses.

When the task was made harder by increasing to a FR5 reinforcement schedule, animals' dose-dependently self-administered both nicotine and CSE, but no drug or age differences were observed.

### 4.1 Methodological issues

Traditionally, rat self-administration studies have examined the effects of nicotine alone in adults (Corrigall and Coen, 1989; Donny et al., 1995). However, more recent studies have begun to examine the effects of the non-nicotine constituents present in cigarette smoke. Individual constituents, such as minor alkaloids, monoamine oxidase inhibitors and acetaldehyde, have been shown to enhance nicotine self-administration (Arnold et al., 2014; Belluzzi et al., 2005; Guillem et al., 2005; Hall et al., 2014; Villégier et al., 2006). However, these do not examine the combined effects of tobacco smoke constituents. Smoke extracts have been shown to contain many combustion products that are not present in tobacco extracts (Bates et al., 1999; Brennan et al., 2014; Seeman et al., 2002) and potentially provide a better model of tobacco dependence.

To investigate the combined effects of tobacco smoke constituents our lab has created CSE as a drug model for behavioral studies. As previously mentioned by Costello et al. (2014), CSE does have limitations in that the exact composition is unknown and the non-aqueous components of cigarette smoke are not included. Although CSE does have limitations, it still provides a novel tool for investigating the combined effects of aqueous tobacco smoke constituents. We have previously shown that adult male rats will self-administer aqueous CSE, and that this was more potent than equivalent doses of nicotine alone (Costello et al., 2014). Using a method modified from that of Costello et al. (2014), to assess the influence of age, we did not find CSE to be more potent than nicotine in adults; indeed, at a low reinforcement schedule it was not self-administered more than saline. At the FR5 schedule used previously by our group (Costello et al., 2014), we found both CSE and nicotine to be self-administered by adults but with no significant differences between drug groups.

This discrepancy may reflect major methodological differences between the two studies, with experimental modifications being introduced in the current study to accommodate the needs of adolescent rats. In our earlier study, two experimental approaches were used, both of which were different from those used here. The first was to conduct drug acquisition training at an FR1 schedule using nose pokes and no prior food training. This approach was determined to be unsuitable for use in adolescents because of their high non-reinforced responding on nose pokes. The second was to food train on levers to an FR5, not FR1, schedule, and then use the same training dose of drug for all animals to reach stable responding before performing a within-subjects dose response analysis. In the present study, prolonged food training at FR1 was necessary for adolescents, and was not extended to FR5 because of constraints in the duration of this developmental stage. Instead, all animals were food trained to FR1 then switched to different doses of nicotine or CSE in a between-subjects design, similar to that employed by (Donny et al., 1998). Following stable responding at FR1, animals were then escalated to drug responding at FR5, an approach that worked for both ages.

Previous studies employing different methods for self-administration of nicotine, nose pokes versus lever presses, have also produced differing results (Belluzzi et al., 2005; Chen et al., 2007). For example, Chen et al. (2007), using lever presses, found different results than that of a study that employed nose pokes (Belluzzi et al., 2005) for self-administration of nicotine. Consistent with our results, these studies demonstrate that a natural behavior (nose pokes) versus a novel behavior (lever presses) may elicit divergent responding for drug.

### 4.2 Age-differences in drug sensitivity

We have previously shown that nicotine stimulates locomotor activity in adolescent rats, while reducing it in adults (Cao et al., 2010). Consistent with this observation, both nicotine and CSE increased non-reinforced responding, a measure of activity, in adolescents but not adults. This hyperactivity resulted in substantially higher nicotine intake in the younger animals that self-administered either nicotine or CSE. When this higher activity level was corrected for, by subtracting non-reinforced lever presses, adolescent rats worked harder than adults for the lowest dose of drug (3.75 μg/kg/infusion nicotine content) on the FR1 reinforcement schedule. Whereas adolescent rats self-administered similar amounts of CSE and nicotine on this schedule, adult rats self-administered more nicotine than CSE at the higher doses. However, both age and drug differences were eliminated when the task was made harder by increasing the reinforcement schedule to FR5. One interpretation of the FR5 data is that the adolescents are older during the FR5 schedule, and may behave more like adults at this older age. However, the behavior is learned during adolescence. Therefore, we believe that the lack of age effects at FR5 are due to the FR schedule being more difficult and that the differences observed at FR1 were not robust and should be interpreted with care. Our findings are consistent with other studies that have shown age differences in responding for low doses of nicotine at differing schedules of reinforcement (Schassburger et al., 2016; Shram et al., 2008). However, in contrast to these other studies, we have found adolescent rats to be more sensitive to the reinforcing effect of low doses of drug at the FR1 schedule.

### 4.3 Clinical implications

Our current findings demonstrate that nicotine with and without tobacco smoke constituents is reinforcing to male adolescent rats. This finding is important given recent epidemiological observations of a switch in teenagers' initial preference from smoking conventional cigarettes to e-cigarettes (Arrazola, 2015). Our preclinical data are consistent with clinical observations that suggest that nicotine delivered through e-cigarettes is reinforcing (Dutra and Glantz, 2014). It should be noted that initial acquisition, as measured here, is only one measure by which the addictive properties of nicotine alone can be compared with cigarette smoke. Other measures, including withdrawal and craving or reinstatement, may show significant differences in the effects of nicotine alone or with other tobacco smoke constituents. A recent study has noted that passive exposure to the smoke of e-cigarettes resulted in lower precipitated withdrawal in mice than exposure to smoke from conventional cigarettes (Ponzoni et al., 2015). Having established a self-administration model in adolescent rats, we can in future determine whether extinction and reinstatement are differentially impacted by presence of tobacco smoke constituents in CSE.

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## Highlights

- Adolescent rats acquire self-administration of cigarette smoke extract (CSE).
- Adolescents displayed increased non-reinforced responding.
- Hyperactivity in adolescents resulted in enhanced drug intake.
- Adolescents display enhanced sensitivity to low doses of drug.
- Non-nicotine constituents don’t enhance nicotine self-administration in adolescents

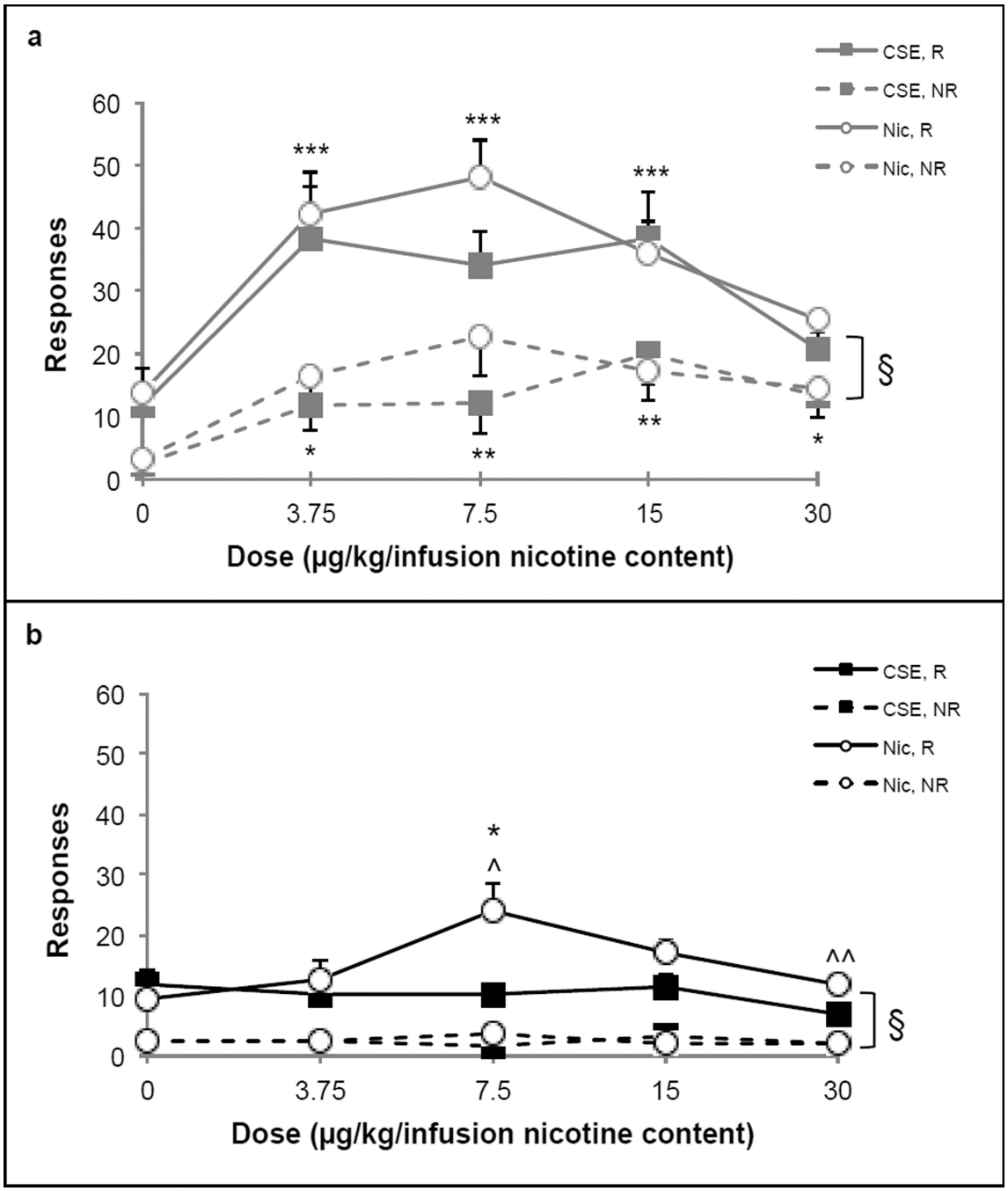


**Fig. 1.**

Self-administration of CSE and nicotine in (**a**) adolescent and (**b**) adult male rats. Data shown are an average of the last three days in which animals self-administered at the FR1 schedule. Both adolescents and adults preferred the reinforced lever at all doses (§$p<0.05$). Significantly different from saline, *$p<0.05$, **$p<0.01$, ***$p<0.0001$; significantly different from CSE, ^$p<0.05$, ^^$p<0.01$.n = 8–12 per group

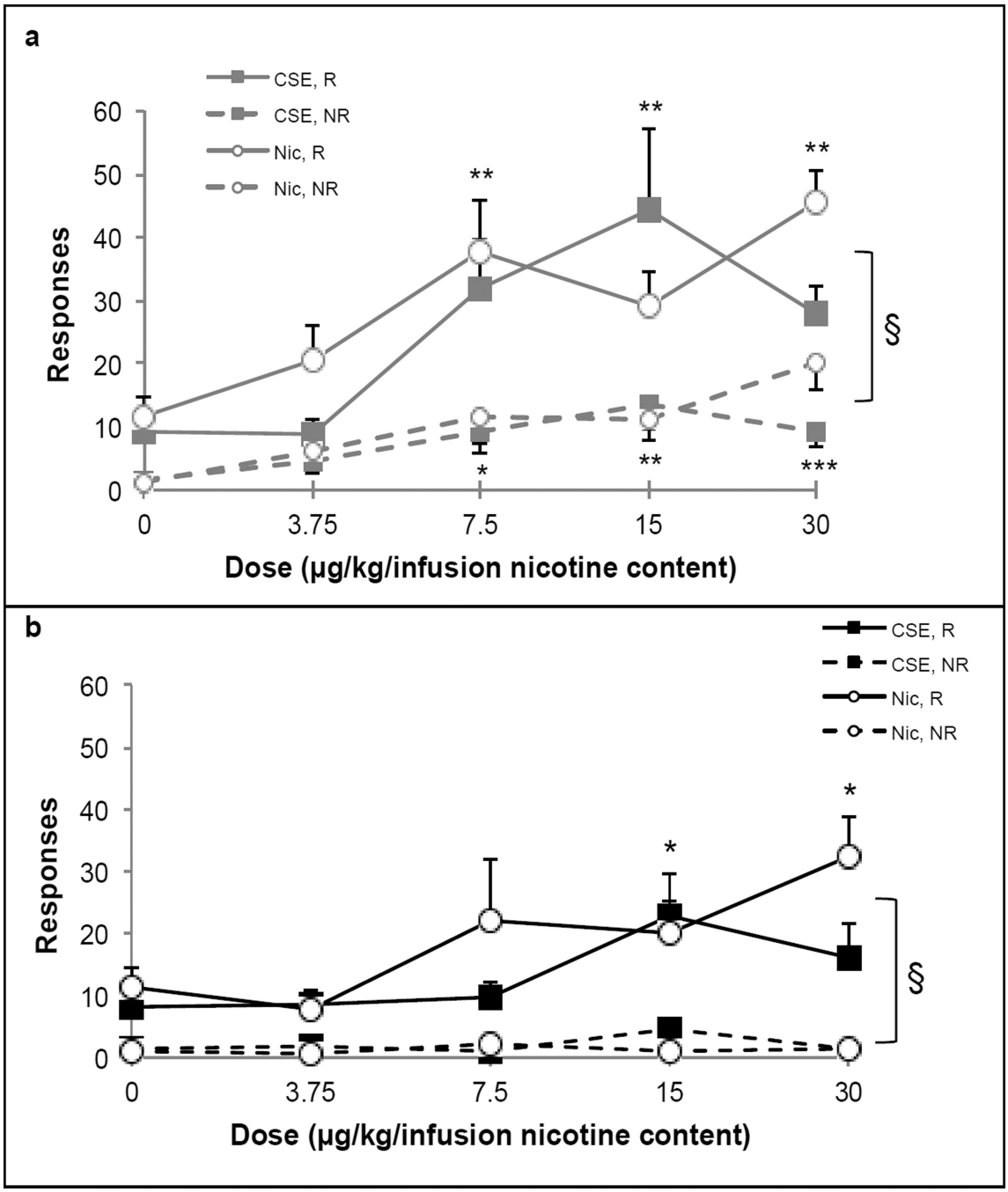


**Fig. 2.**

Self-administration of CSE and nicotine in (**a**) adolescent and (**b**) adult male rats. Data shown are an average of the three days in which animals self-administered at the FR5 schedule. Both adolescents and adults preferred the reinforced lever at all doses (§$p<0.05$). Significantly different from saline, *$p<0.05$, **$p<0.01$, ***$p<0.0001$. n = 8–12 per group

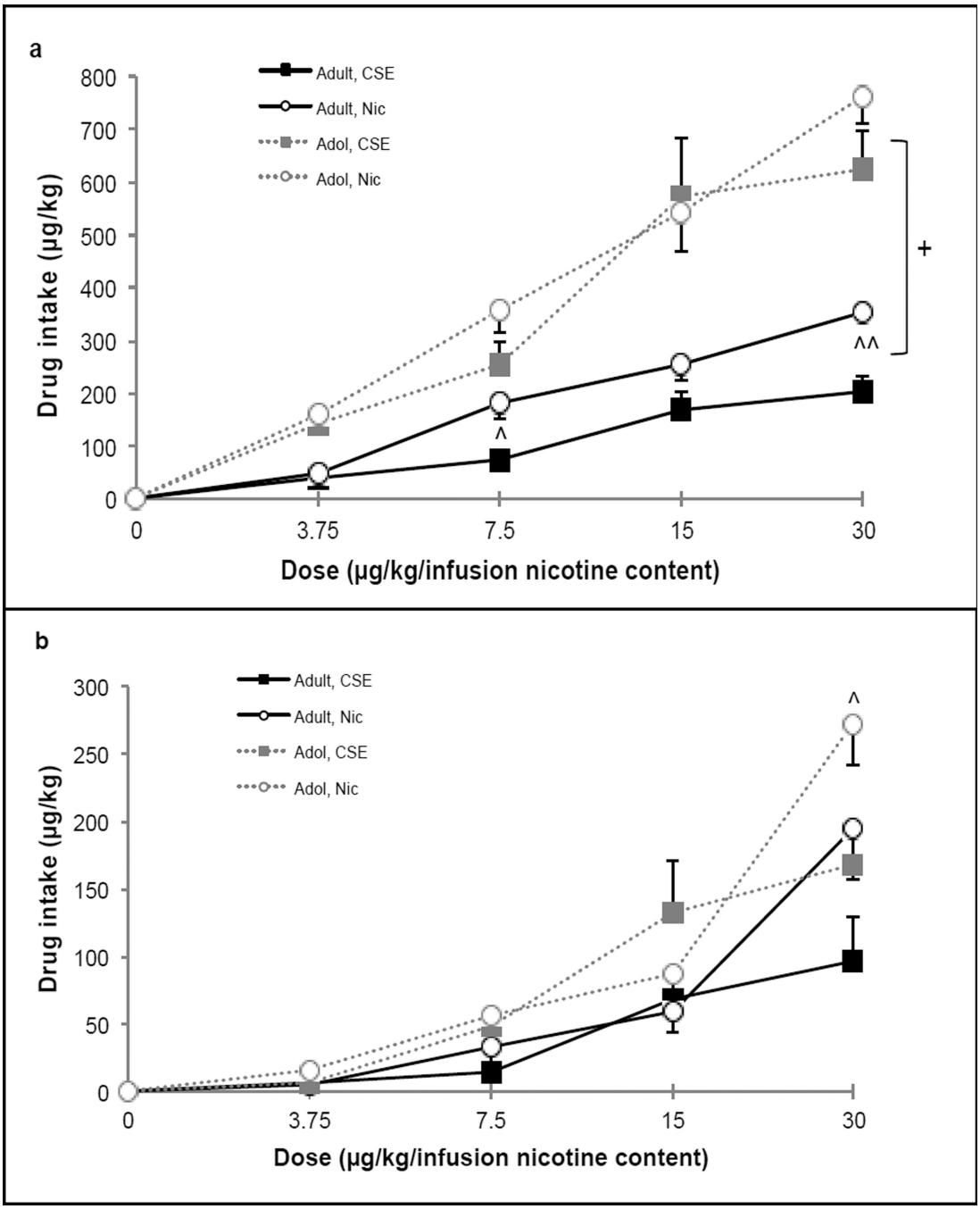


**Fig. 3.**

Adolescent and adult nicotine intake at (**a**) FR1 and (**b**) FR5 schedules. Intake is calculated as the number of infusions per session multiplied by the self-administered dose. Adolescents significantly different from adults, +$p<0.05$; nicotine significantly different from CSE, ^^$p<0.01$, ^$p<0.05$. n = 8–12 per group

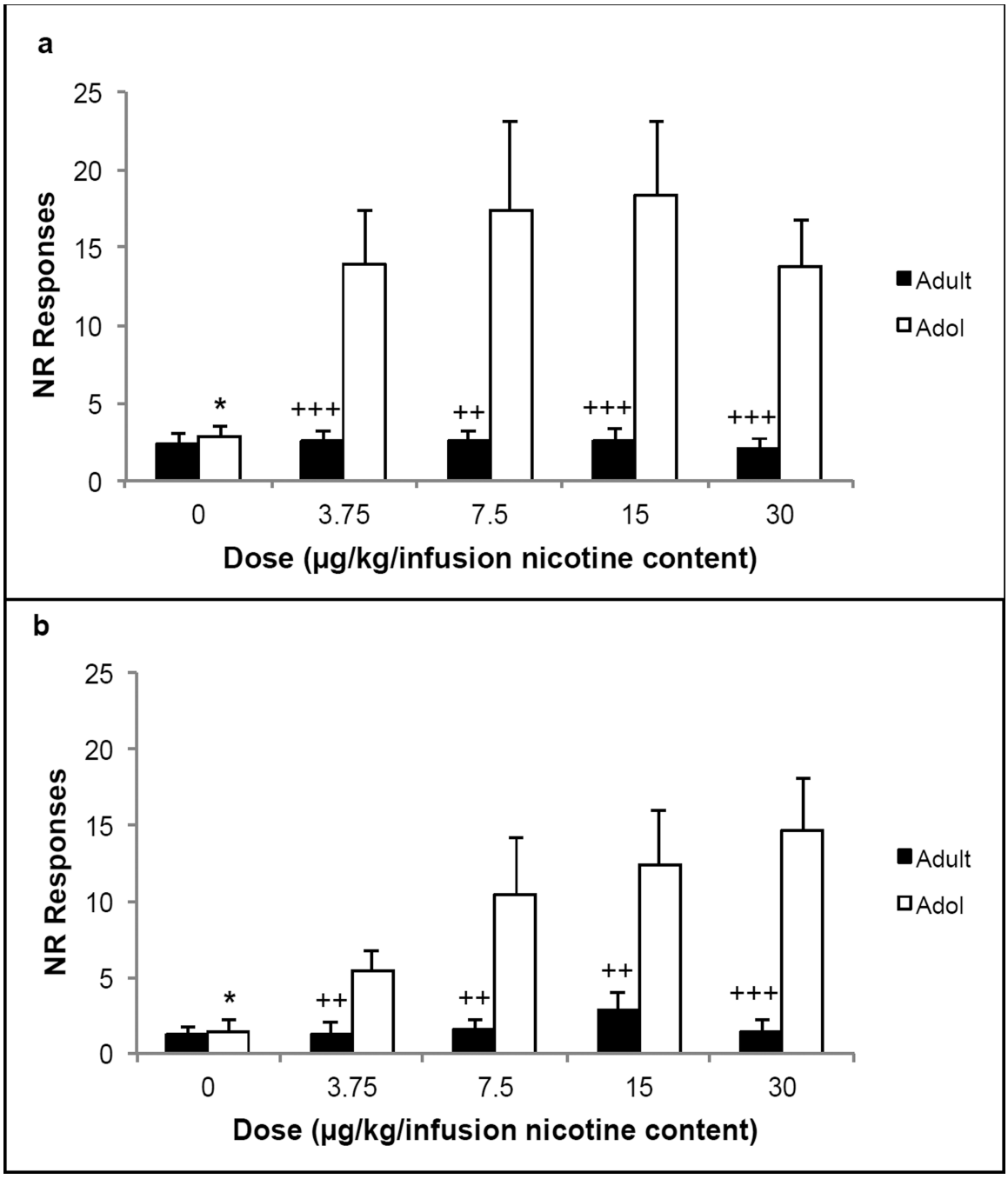


**Fig. 4.**

Adolescent rats show drug-induced increases in non-reinforced responding at the (**a**) FR1 and the (**b**) FR5 schedule of reinforcement. *p<0.05 vs all other doses; +++p<0.0001, ++p<0.01 vs adults. n=8–12 per group

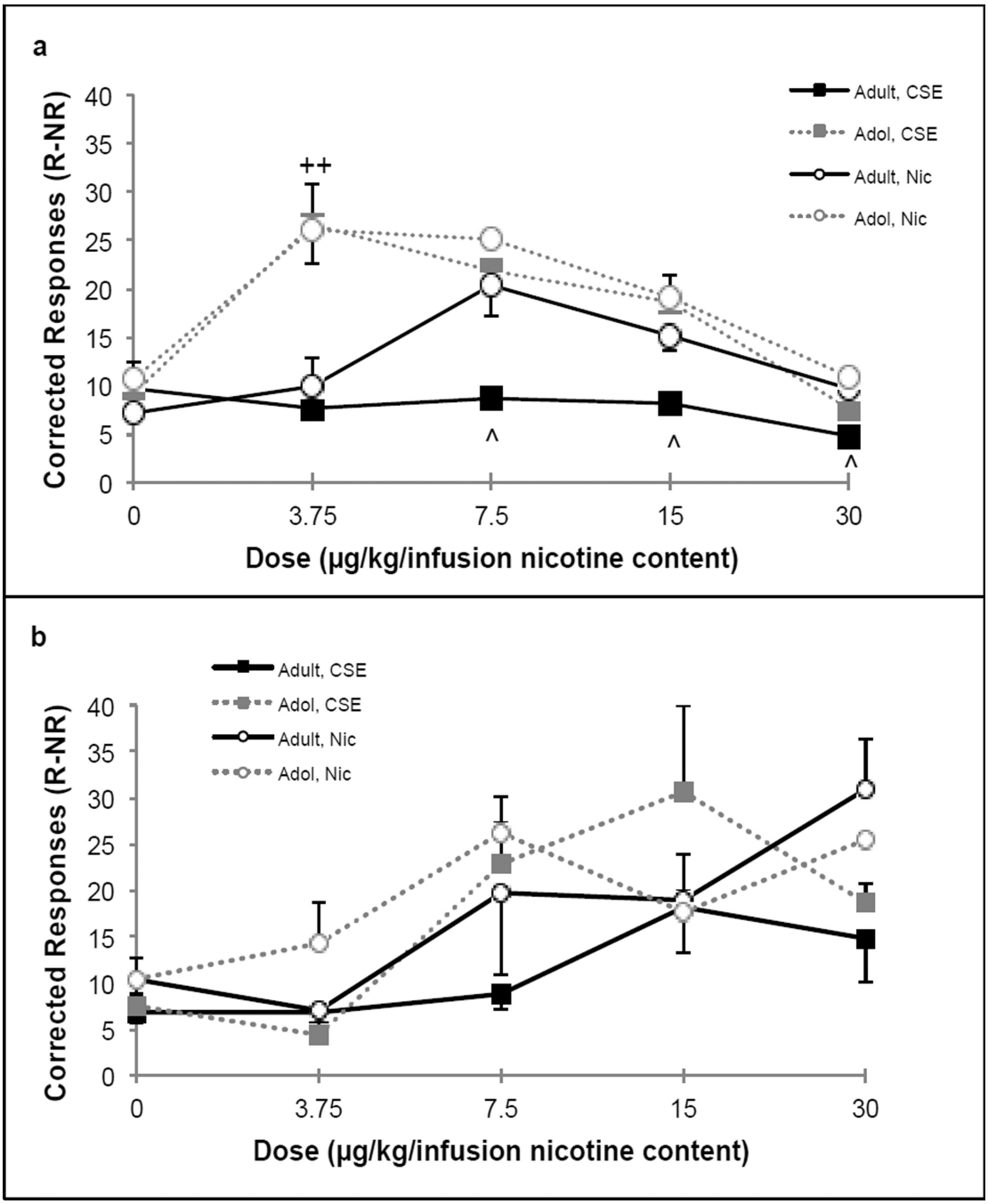


**Fig. 5.**

Reinforced responding corrected for differences in non-reinforced responding. (**a**) Adolescents self-administer more drug than adults on the FR1 schedule (++p<0.001). Adults self-administer more nicotine than CSE at the three highest doses (^p<0.05). (**b**) Adolescent and adult rats behave similarly on the FR5 schedule. n=8–12/group