Drug and Alcohol Dependence 203 (2019) 72–82

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Intravenous and oral caffeine self-administration in rats

Curtis A. Bradley, Matthew I. Palmatier*

Department of Psychology, East Tennessee State University, 420 Rogers Stout Hall, P.O. Box 70649, Johnson City, TN, 37614, United States

ARTICLE INFO

Keywords:
Caffeine
Reinforcement
Operant
Self-administration
Oral
Intravenous

ABSTRACT

Caffeine is widely consumed for its psychoactive effects worldwide. No pre-clinical study has established reliable caffeine self-administration, but we found that caffeine can enhance the reinforcing effects of non-drug rewards. The goal of the present studies was to determine if this effect of caffeine could result in reliable caffeine self-administration. In 2 experiments rats could make an operant response for caffeine delivered in conjunction with an oral 'vehicle' including saccharin (0.2% w/v) as a primary reinforcer. In Experiment 1, intravenous (IV) caffeine infusions were delivered in conjunction with oral saccharin for meeting the schedule of reinforcement. In control conditions, oral saccharin alone or presentations of IV caffeine alone served as the reinforcer. In Experiment 2, access to caffeine was provided in an oral vehicle containing water, decaffeinated instant coffee (0.5% w/v), or decaffeinated coffee and saccharin (0.2%). The concentration of oral caffeine was then manipulated across testing sessions. Oral and IV caffeine robustly increased responding for saccharin in a manner that was repeatable, reliable, and systematically related to unit IV dose. However, the relationship between oral caffeine dose and operant behavior was less systematic; the rats appeared to titrate their caffeine intake by reducing the consummatory response (drinking) rather than the appetitive response (lever pressing). These studies establish reliable volitional caffeine self-administration in rats. The reinforcement enhancing effects of caffeine may help to explain widespread caffeine use by humans, who ingest caffeine in complex vehicles with reinforcing properties.

1. Introduction

Caffeine is a widely used psychoactive drug found in beverages, foods, and over-the-counter medications. Caffeine is considered to be a primary reinforcer, meaning that caffeine-containing products are consumed for the psychoactive effects of caffeine and that these effects strengthen the behaviors that lead to caffeine consumption (Griffiths et al., 1989; Griffiths and Woodson, 1988a, 1988b). Caffeine is consumed for its psychoactive effects in pills (e.g., Vivarin®) and beverages such as coffee and energy drinks (Reissig et al., 2009). The reinforcing effects of caffeine are commonly considered to depend on potentiation of mid-brain dopamine systems that mediate reward detection and incentive motivation (Cauli and Morelli, 2005, 2002; Garrett and Griffiths, 1997; Green and Schenk, 2002); these brain systems are also implicated in the reinforcing effects of drugs of abuse such as nicotine (Balfour et al., 2000), alcohol (Weiss et al., 1993), and cocaine (Goeders and Smith, 1983; Kreek, 1996). More specifically, caffeine potentiates postsynaptic dopamine D2R activity in the striatum via antagonism of the adenosine A2AR found in the A2AR-D2 heteromer (Ferré et al., 2016; Volkow et al., 2015). Similar to other abused drugs, caffeinated beverages can induce a dependence syndrome; abstention from caffeine in chronic users can induce anhedonia (malaise), headaches, and irritability (Griffiths et al., 1986).

Although caffeine satisfies many of the heuristics of abuse liability, it fails on a particularly important feature - whether the drug is self-administered by non-human species (Griffiths et al., 1981; Griffiths and Woodson, 1988b). For example, prospective risk assessment of new medications compare therapeutic doses of the drug to doses that maintain self-administration, traditionally in rats, to determine abuse potential (Gauvin et al., 2018) Despite the widespread acceptance that caffeinated beverages are consumed for the psychoactive effects of caffeine and that these effects increase caffeine self-administration in humans (Griffiths et al., 1981), the evidence for non-human animal self-administration of caffeine is equivocal (see Griffiths and Woodson, 1988b for review). For example, intravenous caffeine self-injection has been described as 'erratic' in non-human primates during a drug substitution procedure (Griffiths et al., 1979). Interpretation of these effects is often complicated by the fact that the operant response was previously supported by another abused drug (Griffiths et al., 1979). Similar effects have been observed in rodents with no evidence that caffeine supports a well-defined response (Atkinson and Enslen, 1976), but does produce a large individual variation in self-administration

* Corresponding author at: PO Box 70649, Johnson City, TN, 37614, United States.
E-mail address: palmatier@etsu.edu (M.I. Palmatier).

https://doi.org/10.1016/j.drugalcdep.2019.05.033
Received 29 December 2018; Received in revised form 4 May 2019; Accepted 20 May 2019
Available online 30 July 2019

(Collins et al., 1983). In oral self-administration paradigms, caffeine intake usually occurs at low concentrations that are unlikely to produce a detectable subjective effect and intake of caffeine may be limited by its potent bitter taste (Heppner et al., 1986).

Although there has been limited success in establishing caffeine as a reliable primary reinforcer in non-human subjects, we have had substantial success showing that caffeine serves as a 'reinforcement enhancer' (Sheppard et al., 2012). Caffeine injections robustly increased responding for a visual reinforcer (turning off the houselights for 30-s) as well as a gustatory reinforcer (20% w/v sucrose solution). In those studies, caffeine increased the number of sucrose reinforcers earned under a progressive ratio (PR) reinforcement schedule. The PR schedule measures motivation by increasing the response requirement after each reinforcer is earned – every delivery of sucrose increased the amount of effort required to earn the next sucrose reward. A quitting or 'breaking' point can be established using this schedule – breaking point is the response requirement at which motivation to obtain the sucrose reward was outweighed by the amount of effort required. We established that caffeine increased the motivation to obtain sucrose and that this effect of caffeine was dose-dependent, with moderate doses (12.5 mg/kg) increasing motivation the most and lower (6.25 mg/kg) and higher (25 mg/kg) doses increasing motivation the least. Interestingly, the effect of caffeine tended to decline over daily repeated test sessions, resulting in a downward shift in the dose-response curve, which is consistent with previous studies showing tolerance to the psychomotor stimulant effects of caffeine under a repeated daily dosing protocol (Lau and Falk, 1994).

Caffeine is available in multiple forms (e.g., beverages and pills), however, human consumption of caffeine for its psychoactive effect is dominated by beverages – complex vehicles that often include sugars or sweeteners (e.g., energy drinks) or to which fats, sugars, and sweeteners are commonly added (e.g., coffee, Reissig et al., 2009). The beverage vehicle may be especially important because the psychoactive effects of oral caffeine are not discrete. Although distribution is relatively rapid for an oral drug, peak serum concentrations are observed 20 min after bolus dosing (Lau et al., 1995) and the lifespan of caffeine is long, with peak serum concentrations maintained for at least 4 h following a single PO dose (Lau et al., 1995). The pharmacokinetic profile of caffeine and moderate motivational effects may make it more of a contextual stimulus than a discrete reward. This could reduce the likelihood that the pharmacological effects of the drug alone can be associated with temporally discrete operant responses such as lever pressing or drinking from a sipper tube. However, if caffeine were self-administered with a more discrete stimulus or 'bridge,' and intake of the drug did not surmount the doses at which motivational effects are observed, then caffeine could be 'self-administered' even if somewhat adventitiously.

This rationale prompted us to hypothesize that preclinical models of caffeine self-administration might be more consistent across subjects and repeated tests if the following conditions were met – 1) operant behavior is associated with a discrete non-drug reinforcer; 2) caffeine is self-administered in quantities that are within the range of increased motivational effects (Sheppard et al., 2012), 3) caffeine self-administration is carried out in an intermittent test paradigm (e.g., tests occur every 48 h) and 4) the potent bitter taste of caffeine (Heppner et al., 1986) is mitigated during the self-administration session. In the first two experiments reported here (Experiment 1 and Experiment 2a) caffeine was delivered by intravenous (IV) injection in conjunction with an oral gustatory reinforcer (0.2% saccharin). In the final experiment (Experiment 2b) oral caffeine was provided in solution with 0.2% saccharin and a masking flavor (0.5% decaffeinated coffee). We predicted that response-contingent caffeine administration would strengthen the motivation to obtain these reinforcing non-drug vehicles, thereby resulting in reliable and repeatable increases in operant behavior induced by caffeine (i.e., self-administration).

2. Method

2.1. Subjects

Male rats were housed in a temperature and humidity controlled colony room on a reverse 12:12 h light:dark cycle. Only male rats were used because our preliminary findings were conducted in males, sex differences will be investigated in the future. All manipulations were conducted in the dark part of the cycle. The rats in Experiments 1 and 2 were naïve rats weighing 275–300 g on arrival from Charles River (Portage, MI). Rats had free access to water and food was restricted to approximately 15 g after one week of habituation. Rats were kept between 375–425 g body weight during data collection. All procedures were approved by the Institutional Animal Care and Use Committee at East Tennessee State University.

2.2. Apparatus

Experimental sessions were conducted in standard operant chambers measuring 25 × 31 × 28 (w × l × h) cm and housed inside sound attenuating cubicles (Med Associates, Georgia, VT). Each chamber was equipped with two retractable levers, a sipper tube, stimulus lights, a liquid dipper/receptacle, and a house-light. The retractable levers, liquid dipper/receptacle and stimulus lights were located on one modular wall of each operant chamber. The levers were located 14 cm above the floor on the left and right panels of the modular wall. The dipper and receptacles were located on the center panel of the same wall approximately 2 cm above the floor. Each dipper was fitted with a 0.1 ml cup for liquid reinforcement and each receptacle was equipped with an infrared emitter/detector to monitor head entries. Each chamber was also equipped with a drug delivery system with a syringe pump (Med-Associates, model PHM100 – 10 rpm). MED-PC IV (Med-Associates, Georgia, VT) software was used to control all stimuli and record responses.

2.3. Drugs and solutions

Saccharin (Sigma-Aldrich, St. Louis, MO) was dissolved in tap water (0.2%, w/v) and delivered via liquid dipper cups (0.1 ml/reinforcer). Caffeine anhydrous (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% sterile saline and infused at a volume of 0.2 ml/kg/infusion for intravenous self-administration (Experiment 1). For oral caffeine self-administration (Experiment 2), caffeine was dissolved in tap water with saccharin (0.2%, w/v) and decaffeinated instant coffee (0.5% w/v, Kroger, Johnson City, TN). Instant coffee was used to mask the bitter taste of the caffeine.

2.4. Surgery

Rats in Experiment 1 were anesthetized with isoflurane and implanted with an indwelling jugular vein catheter similar to the procedures used previously (Liu et al., 2008). Subcutaneous ketoprofen injections (3 mg/ml) were used to alleviate pain for three days after surgery. Catheters were flushed daily with sterile saline and Timentin (3.6 mg, bioWorld, Dublin, OH). Operant testing began 7–10 days after surgery to allow full recovery from the procedure.

2.5. Behavioral testing procedures

2.5.1. Shaping

All rats were shaped to associate the activation of the dipper with saccharin solution. During shaping, the liquid dipper was programmed to be 'normally up' (rats could access sucrose in the cup). At varying intervals, the left lever was inserted into the chamber for 15 s and upon retraction was immediately followed by lowering of the liquid dipper (into the saccharin solution) and then raised into the up position after

Table 1
Subjects and group assignments in each experiment.

Experiment	Group	Acquisition Dose	n
1		(mg/kg/infusion)	
		0	10
		0.125	7
	SACC	0.5	9
		1	7
		4	6
		0.125	6
	CAFF	0.5	5
		1	5
		4	6
2		(mg/ml)	
	WATER	0	6
		2.5	6
	SACC	0	7
		2.5	7
	SACC + DECAFF	0	7
		2.5	7

0.5 s. Any responses recorded during the lever presentation resulted in retraction of the lever and 2 activations of the liquid dipper, separated by 5 s. Shaping was operationally defined as earning 60 reinforcers within a 1-hr session, all rats met this criterion within 4 sessions.

2.5.2. Experiment 1

Upon completion of the shaping procedure the rats were randomly assigned to one of two groups separated by the delivery of an oral reinforcer: 0.2% saccharin (SACC) or nothing (CAFF). The SACC group was then randomly assigned to one of five IV caffeine doses (0, 0.125, 0.5, 1, or 4 mg/kg/infusion). Rats in the CAFF group were also assigned to one of the four caffeine dose conditions (CAFF, 0.125–4 mg/kg/infusion). To conserve subjects, there were no rats assigned to respond for nothing (0 mg/kg CAFF). The final number of subjects in each group and dose is listed in Table 1. For rats in the SACC groups, meeting the schedule of reinforcement on the active lever resulted in presentations of 0.2% saccharin with the assigned dose of caffeine. For rats in the CAFF groups, IV infusions of the assigned caffeine dose replaced saccharin (from shaping). At the start of each session both the active and inactive levers were inserted into the chamber. During each session, responses on the active lever were reinforced under a PR schedule of reinforcement, the PR was identical to the schedule used in previous studies (Palmatier et al., 2012) and was calculated with the following formula: $[5e^{(r*0.12)}]$-5 (Richardson and Roberts, 1996), in which r represents the number of reinforcers earned plus one. After each reinforcer a 30-s time-out period was enforced; the house light was extinguished and lever presses were recorded but did not count toward the next ratio requirement. Sessions were conducted 48 h apart to avoid tolerance to the effects of caffeine. All sessions were capped at 60 min, but breaking points operationally defined as 5, 10, or 20 min without earning a reinforcer were calculated during the sessions. Catheter patency was tested at the end of data collection via IV infusions of propofol (0.2 ml). Rats that failed the patency test (no loss of muscle tone after the injection) were excluded from data analysis.

2.5.3. Experiment 2

Experiment 2 investigated whether oral caffeine self-administration was possible in drug-naïve rats. In this experiment we also investigated whether a masking stimulus (coffee flavor provided by decaffeinated coffee) was needed to establish oral caffeine self-administration. First, rats were shaped to respond for 0.2% saccharin on a PR schedule of reinforcement for three days. Upon completion of the shaping procedure, rats were assigned to one of six groups (n = 6–7 per group) that differed by two independent factors: vehicle solution (Water, Saccharin, or Decaff + Saccharin) and drug (0 or 2.5 mg/ml caffeine). Pilot data suggested that the oral route of administration delayed observation of the effects of caffeine on responding for saccharin. Therefore, instead of capping sessions at 60 min, rats were allowed to respond until they reached a 30-min limited hold (e.g., 30 min without earning a reinforcer). At that time, the session ended and the final ratio completed was used as the 'breaking point'. Following acquisition of stable responding at 2.5 mg/ml caffeine, caffeine concentration was manipulated using a within-subjects, Latin square design for the saccharin and decaff + saccharin groups. The caffeine concentrations tested were 2.5, 3.5, 5, and 7.5 mg/ml. Sessions continued at each concentration until responding stabilized – an informal assessment of group- wise responding was used to determine stability (no linear trend across the last 3 days of testing). All other testing procedures were identical to Experiment 1.

2.6. Data analyses

For Experiment 1 the Group and Dose factors were not orthogonal because there was no group responding for no reinforcement (no saccharin and 0 mg/kg/infusion caffeine). Therefore, we used two strategies for omnibus analyses of acquisition data. First, we investigated the effects of IV caffeine infusions on responding for saccharin with a mixed-factors ANOVA included Session (within), Lever (within) and Dose (between) in the SACC groups only. Second, we investigated the effects of oral reinforcement (saccharin or nothing) on responding for IV caffeine infusions with a mixed-factors ANOVA including Session (within), Lever (within) and Group (between), but the 0 mg/kg/infusion SACC group was excluded. A similar strategy was used to analyze reinforcers, but Lever was not included as a factor. For dose-response analyses, each dependent variable (responses or reinforcers) was averaged across the last two days of testing and the same strategy was used. For Experiment 2 responses and reinforcers were analyzed with mixed-factors ANOVA including Session (within), Lever (within), Vehicle (between), and Drug (between) or Concentration (within), as needed. Due to the heteroscedasticity of breakpoint data, reinforcers were analyzed in place of breakpoints to avoid violating the assumption of homogeneity of variance. Second-order contrasts were used to compare groups on individual sessions or across individual doses/concentrations.

3. Results

3.1. Experiment 1

3.1.1. Acquisition

Response-contingent delivery of intravenous caffeine dose-dependently (0.5–1 mg/kg/infusion), and selectively, increased responding on the active lever when delivered with oral saccharin (Fig. 1A–D). This was confirmed with a mixed-factors ANOVA comparing lever presses across caffeine doses in groups receiving the oral saccharin reinforcer. The three way ANOVA revealed significant Lever x Session x Dose interaction [$F(16,136) = 3.3$, $p < .001$]. The Lever x Dose [$F(4,136) = 4.54$, $p < 0.01$] and Session x Dose [$F(16,136) = 2.82$, $p = 0.001$] interactions were also significant. To further probe the interactions, second order contrasts were conducted. To limit the number of comparisons, only active lever responses were used in the contrasts. Caffeine (0.5 mg/kg/infusion) significantly increased active lever responses for the Caffeine + Saccharin group, relative to the Saccharin group and relative to the Caffeine group on Sessions 3–5 ($ps \leq 0.01$). The 1 mg/kg/infusions of caffeine also increased active lever responses Caffeine + Saccharin vs. Saccharin and Caffeine + Saccharin vs. Caffeine), this increase was statistically reliable on Sessions 2–3 ($ps \leq 0.015$), but not on Sessions 4–5 ($ps \geq 0.08$). Active lever responses were also higher in the Caffeine + Saccharin group receiving 0.125 mg/kg/infusion, relative to the Caffeine group, on session 2–5 ($ps < 0.05$). A similar effect (Caffeine + Saccharin vs. Caffeine) was found for rats receiving 4 mg/kg caffeine, but only on session 2 ($p < 0.05$). In

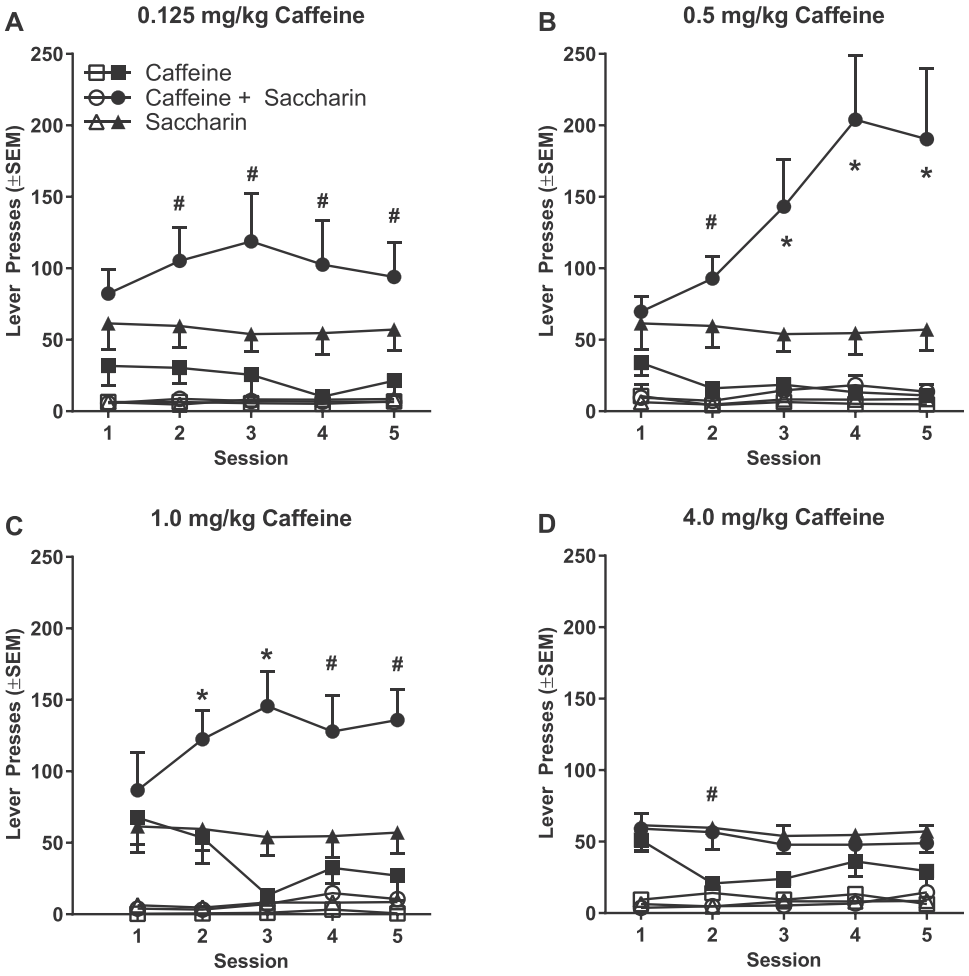

Fig. 1. Average (SEM) lever presses across testing sessions in Experiment 1.
Filled symbols represent active lever responses, open symbols represent inactive lever responses. Each panel represents rats receiving different doses of caffeine, except the Saccharin group (0 mg/kg) which is represented in all four panels for comparison. * indicates significant increase in responses for rats receiving Saccharin + Caffeine, relative to Saccharin group, $p < 0.05$.

addition, caffeine dose-dependently increased the number of reinforcers earned (Fig. 2A–D). The two-way ANOVA revealed a significant main effect of Dose [$F(4,34) = 4.7$, $p < 0.01$] and a Dose x Session interaction [$F(16,136) = 2.6$, $p < 0.01$]. Follow-up simple effects contrasts confirmed that 0.5 and 1 mg/kg caffeine infusions significantly increased the number of reinforcers earned on Sessions 3–5 (Caffeine + Saccharin vs. Saccharin; Caffeine + Saccharin vs. Caffeine, ps < 0.05). The 0.125 mg/kg infusions significantly increased the number of reinforcers earned on Sessions 2–3 ($ps \leq 0.04$), but this trend was not sustained during additional test sessions (Fig. 1A). The highest caffeine dose did not increase or decrease the number of reinforcers earned during testing ($ps \geq 0.29$) (Fig. 1D).

Response-contingent oral saccharin presented with caffeine increased responding on the active lever, relative to caffeine alone (Fig. 1A–D). This was confirmed by four-way ANOVA comparing responding across Dose (0.125–4 mg/kg/infusion), Session (1–5), and Lever (Active vs. Inactive) for each Group (saccharin vs. no oral reinforcer). There was a significant 4-way Session x Lever x Dose x Group interaction [$F(12,172) = 2.03$, $p = 0.024$]. There were also significant 3-way Lever x Session x Group [$F(4,172) = 6.05$, $p < 0.001$] and Lever x Session x Dose [$F(12,172) = 2$, $p = 0.03$] interactions. To explore the 4-way interaction, we conducted second-order contrasts on active lever responses for the Group factor (saccharin vs. no oral reinforcer) within each level of Dose and Session. Saccharin systematically increased responding for rats receiving both reinforcers relative to caffeine infusions alone in the 0.5 (Sessions 2–5, ps < 0.01) and 1 mg/kg groups (Sessions 2–5, $ps \leq 0.03$), but not in the 0.125 mg/kg ($ps \geq 0.07$) or 4 mg/kg ($ps \geq 0.13$) groups. Saccharin also increased the number of reinforcers earned when it was presented with moderate caffeine doses (Fig. 1D–H). There was a significant 3-way Group x Session x Dose interaction [$F(12,172) = 16.5$, $p < 0.001$] as well as significant two-way interactions of Session x Group [$F(4,172) = 7.3$, $p < 0.001$] and Dose x Group [$F(3,43) = 3.83$, $p = 0.02$]. The second order contrasts showed that saccharin increased reinforcers earned on all sessions for rats earning 0.125 mg/kg infusions ($ps \leq 0.03$). Saccharin increased reinforcers earned on Sessions 2–5 for rats earning 0.5 mg/kg (ps < 0.001) and 1 mg/kg ($ps \leq 0.01$) infusions of caffeine. Saccharin only increased the number of reinforcers earned on Session 2 ($p = 0.012$) in the rats earning 4 mg/kg caffeine infusions.

3.1.2. Dose-response relationship

Dose-response curves were generated by averaging the last two days of lever responses (Fig. 3A–B) or reinforcers earned (Fig. 3C) for each subject. The 0.5 and 1 mg/kg/infusion caffeine doses significantly increased responding on the active lever when self-administered with oral saccharin, relative to oral saccharin alone (Fig. 3A). The two-way ANOVA revealed a significant Lever x Dose interaction [$F(4,34) = 5.5$, $p < 0.01$] and both main effects were significant (ps < 0.01). Contrasts confirmed that active lever responses were significantly higher in the 0.5 and 1 mg/kg groups, relative to the 0 mg/kg group ($ps \leq 0.04$).

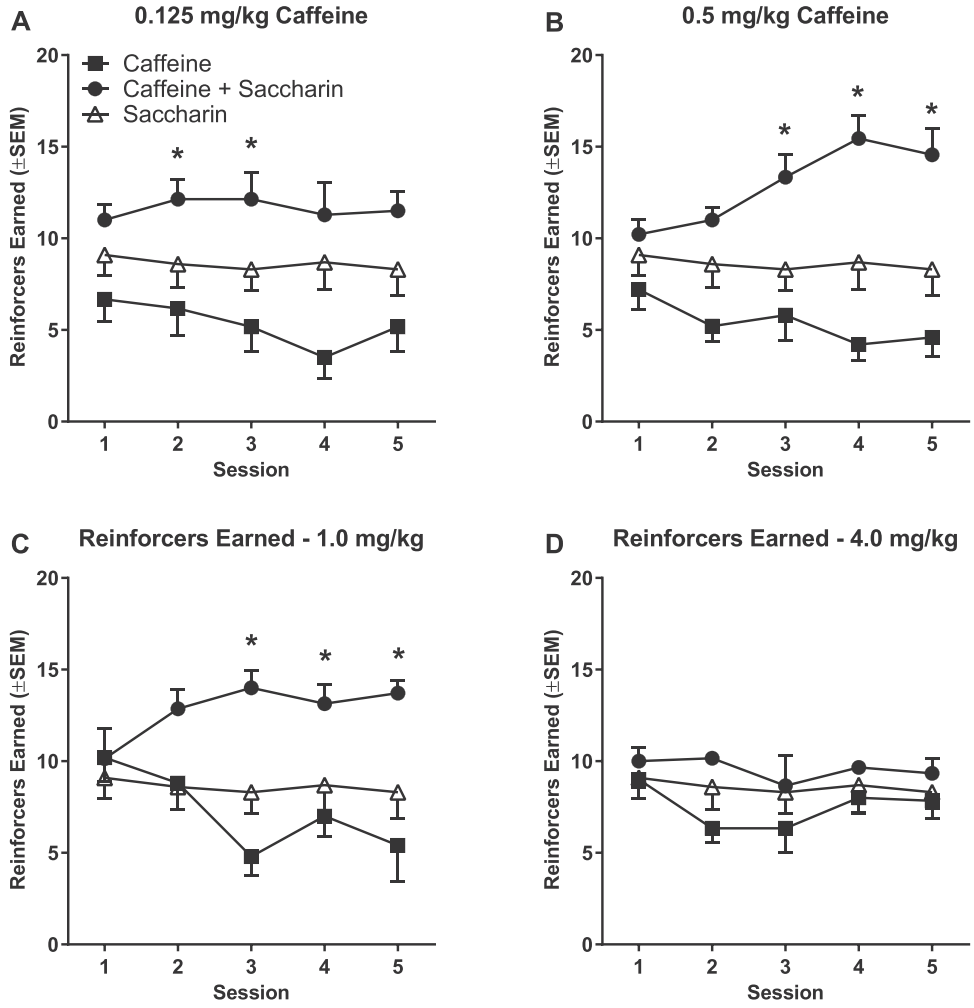

Fig. 2. Average (SEM) reinforcers earned across testing sessions in Experiment 1.
Each panel represents rats receiving different doses of caffeine, except the Saccharin group (0 mg/kg) which is represented in all four panels for comparison. * indicates significant increase in reinforcers earned for rats receiving Saccharin + Caffeine, relative to Saccharin group, $p < 0.05$.

To investigate putative psychomotor stimulant effects of caffeine, similar contrasts were performed on inactive lever responses, but none reached statistical significance (ps ≥ 0.2). Additionally, the 0.5 and 1 mg/kg caffeine doses significantly increased the number of reinforcers earned, relative to saccharin alone (Fig. 3C). The univariate ANOVA on reinforcers earned revealed a significant main effect of Dose [$F(4,39) = 5.2$, $p < 0.001$], and contrasts confirmed that the 0.5 and 1 mg/kg/infusion caffeine doses resulted in more reinforcers earned relative to saccharin alone (0 mg/kg/infusion, ps < 0.01).

Oral saccharin robustly increased active lever responding when it was presented with IV caffeine, relative to IV caffeine alone. The increased responding for caffeine was confirmed by a three-way ANOVA with a significant Lever x Group x Dose interaction [$F(3,43) = 3.4$, $p = 0.03$], as well as significant Lever x Group [$F(1,43) = 27.3$, $p < 0.001$] and Dose x Group [$F(3,43) = 2.96$, $p = 0.04$] interactions. Follow up contrasts were conducted to determine which caffeine doses combined with saccharin altered responding at the active or inactive lever. Saccharin presentation did not increase responding at the inactive lever for any dose (ps ≥ 0.11), but increased responding at the active lever for the 0.125, 0.5, and 1 mg/kg/infusion doses (ps ≤ 0.03). We also found preliminary evidence for a primary reinforcing effect of IV caffeine (Fig. 3B). Contrasts of responding at the active and inactive levers for the caffeine group (e.g., saccharin rats excluded) revealed that rats receiving 1 and 4 mg/kg/infusion made significantly more responses on the active lever, relative to the inactive lever (ps ≤ 0.007). Oral saccharin also robustly increased reinforcers earned when it was presented in conjunction with IV caffeine (Fig. 3C). This was confirmed by the two-way ANOVA with a significant Dose x Group interaction [$F(3,51) = 5.9$, $p = 0.002$]. Contrasts confirmed that oral saccharin increased reinforcers earned for rats the 0.5 and 1.0 mg/kg/infusion doses (ps ≤ 0.02).

3.2. Experiment 2 – oral caffeine self-administration

Caffeine increased operant responding on the active lever but not the inactive lever for rats receiving the vehicles containing saccharin (Fig. 4A–C). Oral caffeine in the water vehicle did not support operant responding relative to controls (Caffeine + Water vs. Water, Fig. 4A). The coffee flavored masking stimulus (Decaff) was not necessary to observe caffeine self-administration as there were no differences between the two groups receiving the saccharin-containing vehicles (Caffeine + Saccharin vs. Caffeine + Decaff + Saccharin, Fig. 4B–C). These findings were confirmed by repeated measures ANOVA with a significant Vehicle x Drug x Lever interaction [$F(2,170) = 11.1$, $p < 0.001$]; there were no main effects or interactions involving Session (ps ≥ 0.12). To further probe the 3-way interaction, we compared the estimated marginal means for active lever responses and inactive lever responses across Drug (0 vs. 2.5 mg/ml) within each of the oral vehicle conditions (water, saccharin, or decaff + saccharin). Caffeine did not increase responding on the active or inactive lever in groups

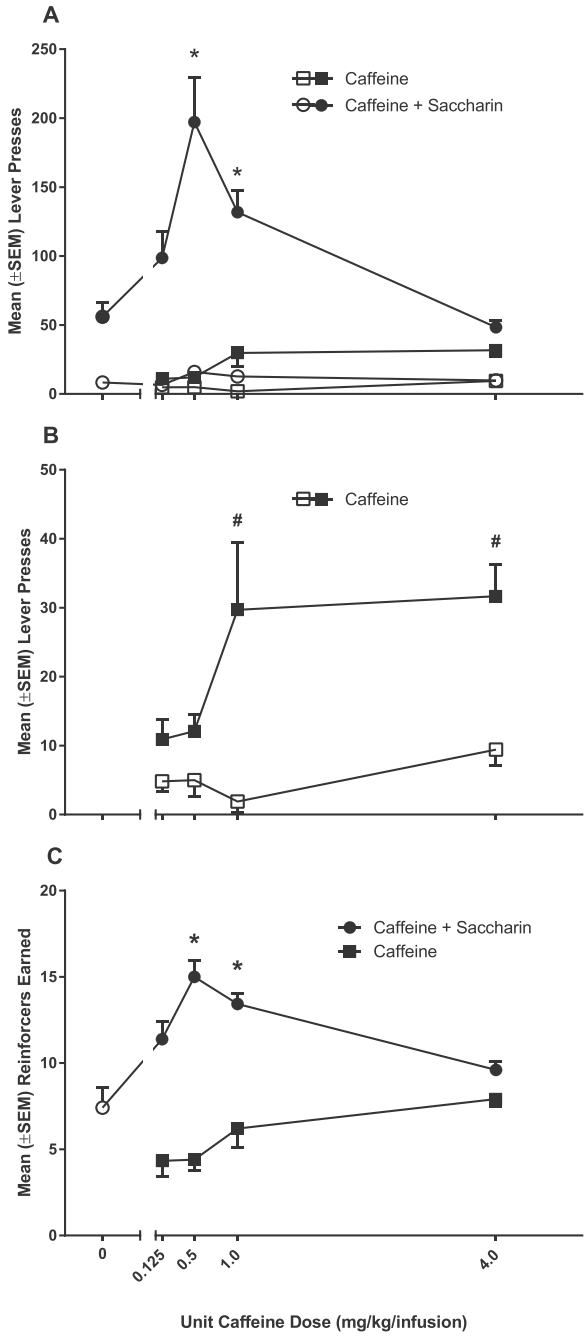

Fig. 3. Average (SEM) responses on the active and inactive levers (Panel A), responses on only the inactive lever scaled to illustrate non-specific effects of caffeine (Panel B), and reinforcers earned (Panel C) in Experiment 1.
In Panels A–B filled symbols represent active lever responses and open symbols represent inactive lever responses. In Panel C open symbols represent saccharin without caffeine, filled symbols represent IV caffeine infusions with saccharin (filled circles) or without saccharin (filled squares). * indicates significant increase in the dependent measure for the Caffeine + Saccharin group, relative to the Saccharin alone group, $p < 0.05$. # indicates significantly more responses on active lever, relative to inactive lever for Caffeine group, $p < 0.05$.

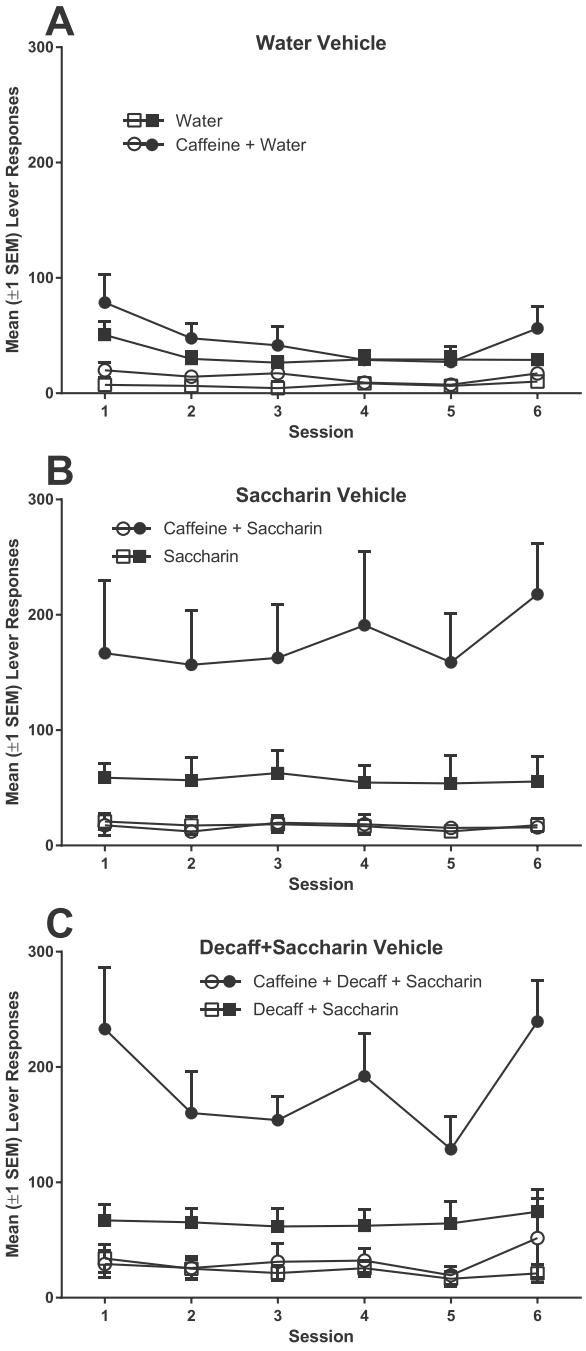

Fig. 4. Average (SEM) active and inactive lever responses for rats receiving oral caffeine in the water (Panel A), saccharin (Panel B) and decaff + saccharin (Panel C) vehicles.
Filled symbols represent active lever responses, open symbols represent inactive lever responses. Squares represent rats responding for the vehicle alone, circles represent rats responding for vehicle with caffeine.

with the water vehicle (ps > 0.05,Caffeine + Water vs. Water, Fig. 4A); thus, we did not find evidence for a primary reinforcing effect of oral caffeine. For groups with the saccharin-containing vehicles (Caffeine + Saccharin vs. Saccharin & Caffeine + Decaff + Saccharin vs. Decaff + Saccharin), caffeine increased responding on the active lever (ps < 0.001), but not the inactive lever (ps ≥ 0.155) across acquisition (Fig. 4B–C); confirming that increased responding is not the result of a generalized psychomotor stimulant effect.

A similar pattern was observed for reinforcers earned (Fig. 5A–C), with the exception that the enhancing effect of caffeine on reinforcers earned emerged across sessions (Fig. 5B). The 3-way ANOVA revealed significant Session x Vehicle [$F(10,170) = 1.97$, $p < 0.05$] and Session x Drug [$F(5,170) = 2.45$, $p < 0.05$] interactions, but the Vehicle x Drug interaction was not significant ($p = 0.13$). This pattern most likely reflects the fact that caffeine only marginally impacted the number of reinforcers earned when it was presented in water (Fig. 5A). There were significant simple main effects of Vehicle, Drug, and Session (Fs ≥ 7.0,

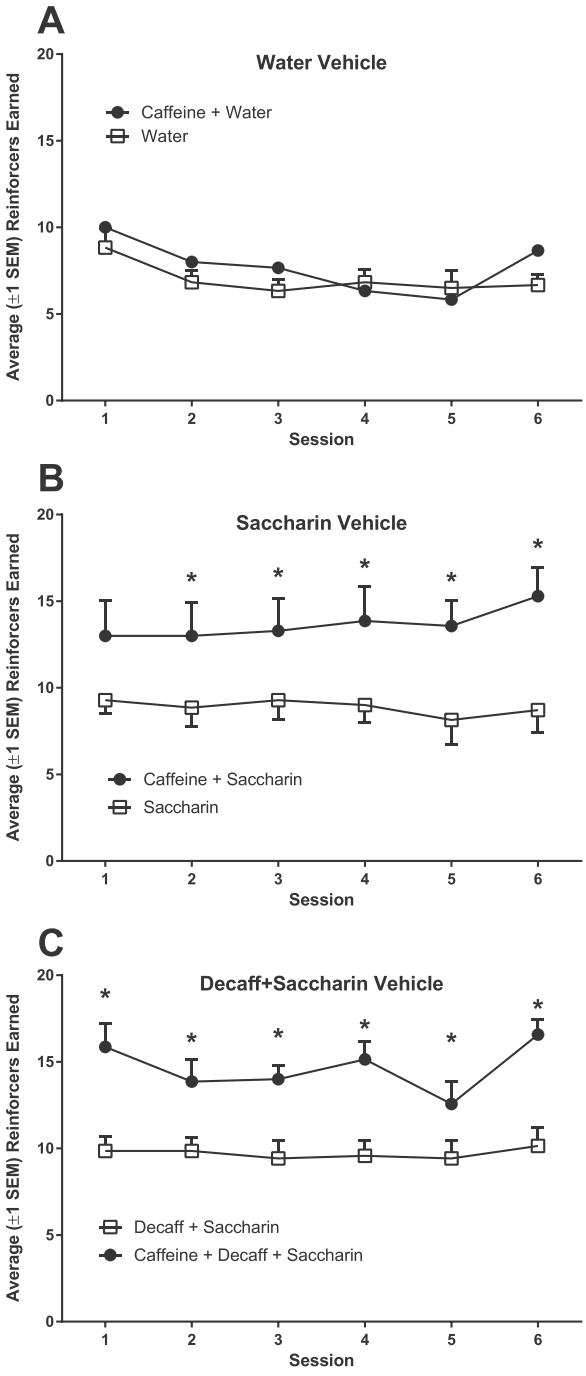

Fig. 5. Average (SEM) reinforcers earned for rats receiving caffeine (2.5 mg/ml) with the oral vehicle containing water (Panel A), 0.2% saccharin (Panel B), or 0.5% decaffeinated coffee with 0.2% saccharin (decaff + saccharin, Panel C). * indicates caffeine increased reinforcers earned relative to vehicle alone, $p < 0.05$.

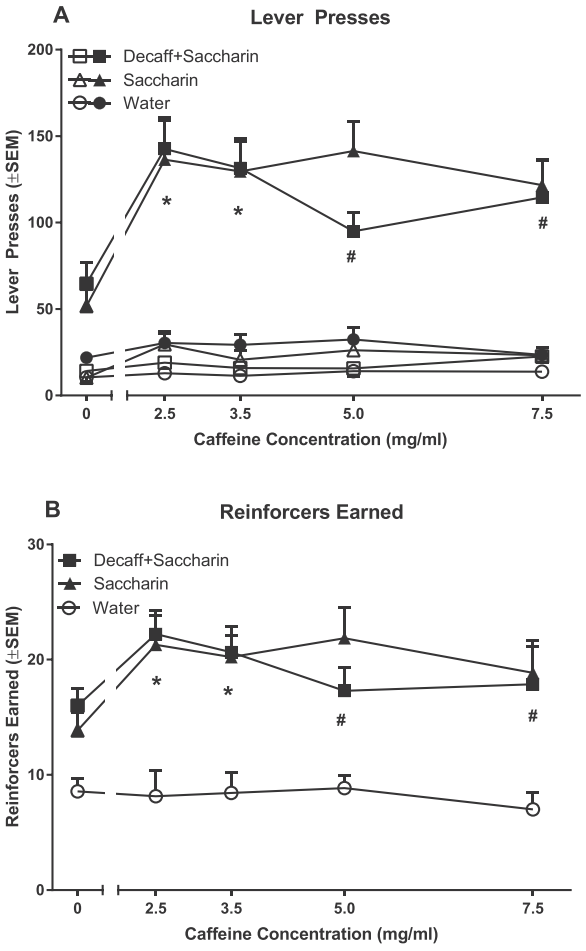

Fig. 6. Average (SEM) lever presses (Panel A) and reinforcers earned (Panel B) across the caffeine concentrations tested.
The 0 dose represents rats receiving vehicle alone, averaged across all test sessions. Caffeine concentrations from 2.5 to 7.5 mg/ml were tested in a within subjects design, minimum 3 days at each test concentration. * indicates caffeine with saccharin or decaff + saccharin increased responses and reinforcers earned relative to vehicle-alone group (0 mg/ml), $p < 0.05$. # indicates that caffeine with saccharin increase responses and reinforcers earned relative to vehicle-alone group.

$ps < 0.001$), and follow-up contrasts confirmed that the water vehicle group earned fewer reinforcers than the saccharin and decaff + saccharin groups (Water vs. Saccharin and Water vs. Decaff + Saccharin, $ps < 0.05$), which did not differ from each other ($p > 0.05$). Second order contrasts probed the interaction by comparing the effect of caffeine on each session within each oral vehicle solution. Caffeine did not alter reinforcers earned on any session for rats receiving the water vehicle (Water vs. Caffeine + Water). However, caffeine did increase reinforcers earned across sessions for rats receiving saccharin on Sessions 2–6 (Caffeine + Saccharin vs. Saccharin, $ps \leq 0.021$, Fig. 5B) and for rats receiving decaff + saccharin on Sessions 1–6 (Caffeine + Decaff + Saccharin vs. Decaff + Saccharin, $ps \leq 0.01$, Fig. 5C).

3.3. Experiment 2 – oral caffeine dose-response tests

Oral caffeine increased responding on the active lever across all concentrations tested when it was presented in saccharin, and for the lowest two concentrations (2.5–3.5 mg/ml) when presented in decaff + saccharin vehicle (Fig. 6A). Oral caffeine did not increase responding at any dose when presented in water. This was confirmed by repeated measures ANOVA with a significant Vehicle x Dose x Drug interaction [$F(6,102) = 2.49$, $p = 0.03$]. The Dose x Drug and Vehicle x Drug interactions were also significant ($ps < 0.05$). To probe the interactions, second order contrasts compared Drug (Caffeine vs. Control) at every level of Dose (each test session) within each Vehicle condition (decaff + saccharin, saccharin, or water). For rats receiving the water vehicle, caffeine did not increase active lever responses at any dose tested (Caffeine + Water vs. Water, $ps \geq 0.55$). For rats tested with saccharin vehicle, caffeine increased active lever responses at every dose tested (Caffeine + Saccharin vs. Saccharin, $ps \leq 0.008$). For rats receiving decaff + saccharin vehicle, caffeine increased active lever responses only at the two lowest concentrations

(Caffeine + Decaff + Saccharin vs. Decaff + Saccharin, ps ≤ 0.002), but not the higher concentrations (ps ≥ 0.08). A separate ANOVA on inactive responses (data not shown) revealed that there were no main effects of Drug, Dose, Vehicle, nor any interactions (ps < 0.05). For reinforcers earned (Fig. 5B), the ANOVA revealed significant main effects of Drug [$F(1,34) = 16.76$, $p < 0.001$] and Vehicle [$F(2,34) = 19.42$, $p < 0.001$] and a Dose x Drug interaction [$F(3,102) = 3.1$, $p = 0.03$]. Second order contrasts showed the same pattern as active lever responses – caffeine increased reinforcers earned at all concentrations in the saccharin vehicle (ps ≤ 0.014), at the two lowest concentrations in the decaff + saccharin vehicle (ps ≤ 0.005), and had no effect on rats with the water vehicle (ps ≥ 0.27).

3.4. Caffeine intake via IV (experiment 1) and oral (experiment 2) routes of administration

To explore the effects of caffeine intake via each route of administration the dose or concentration was transformed to total session intake. For the IV study (Experiment 1) unit dose was multiplied by reinforcers earned. For the oral study (Experiment 2), caffeine intake was computed in mg (R*(mg/ml*Volume)) and divided by average bodyweight (kg) across test sessions; the saccharin and decaff + saccharin vehicle conditions were collapsed into a single "saccharin-containing vehicle" group as reinforcers earned were similar throughout testing. As illustrated in Fig. 7A, oral saccharin significantly increased IV caffeine intake at the three highest unit doses (0.5, 1 and 4 mg/kg/infusion), relative to controls that only received IV caffeine. This was confirmed by a Group x Dose interaction [$F(3,43) = 3.409$, $p = 0.03$], with post-hoc differences between groups at the 0.5, 1, and 4 mg/kg/infusion doses (ps < 0.01). As illustrated in Fig. 7B, saccharin-containing vehicles significantly increased caffeine intake at all concentrations tested. This was confirmed with a Group × Dose interaction [$F(3,57) = 5.203$, $p = 0.003$] and follow-up contrasts which showed significant increases across all caffeine concentrations (ps < 0.05). One potential pitfall of the oral dosing regimen is that the rats did not have to consume all of the liquid in the dipper, they could regulate their caffeine intake at the consummatory link of the operant chain. Although this seems unlikely under the PR schedule of reinforcement, we analyzed the duration of head entries into the liquid dipper receptacle after earning each caffeine reinforcer to explore this possibility (data not shown); a reduction in head entry duration across dose would suggest lower intake. Surprisingly, there was a reduction in head-entry time for rats consuming saccharin-containing vehicles, relative to water (ps ≤ 0.002); however, head entry time did not vary systematically as a function of concentration ($p > 0.05$).

4. Discussion

These experiments are the first to demonstrate reliable and repeatable self-administration of caffeine in rats. In Experiment 1, caffeine self-infusion increased motivation for oral saccharin under a PR schedule, relative to control groups receiving only access to oral saccharin or intravenous caffeine, and the increase in motivation could not be attributed to general increases in activity (i.e., no increase in responding at the inactive lever). The effect of caffeine was selective to dose, moderate unit doses (0.5–1 mg/kg/infusion) increased operant responding, but lower (0.125 mg/kg/inf) and higher (4 mg/kg/infusion) doses did not increase operant responding. Notably, intravenous caffeine alone did increase operant responding at the 1 and 4 mg/kg/infusion unit doses, relative to the inactive lever (Fig. 3B). To our knowledge, these are the first experiments to show caffeine reinforcement and the first to show that caffeine is self-administered by non-humans. They are also the only experiments investigating caffeine reinforcement while taking into account the robust behavioral tolerance to caffeine by separating operant testing sessions by 48 h (Lau and Falk, 1995, 1994). However, the observation of caffeine's reinforcing effects

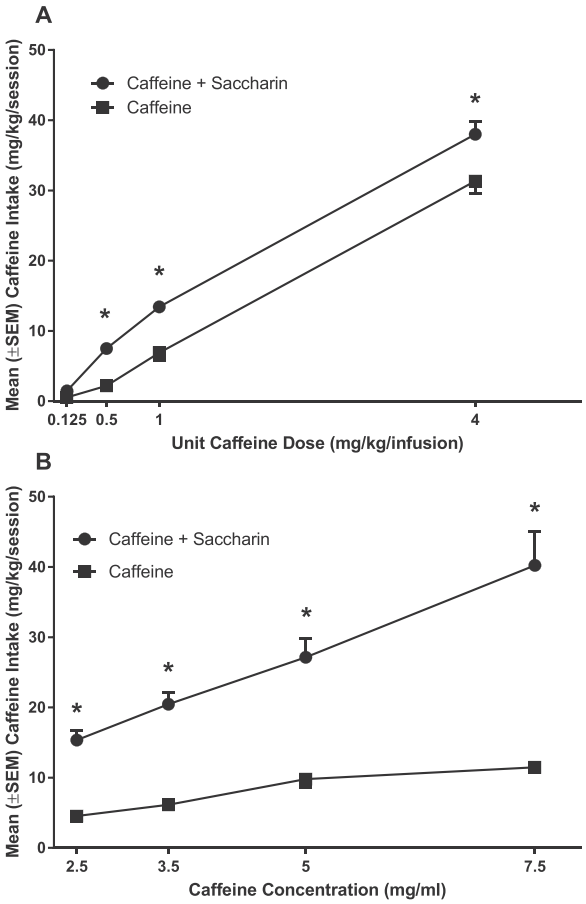

Fig. 7. Average (SEM) caffeine intake for IV self-administration (Experiment 1, Panel A), and oral self- administration (Experiment 2, Panel B) studies. Caffeine doses were tested between subjects in the IV experiment (Panel A) and within subjects in the oral experiment (Panel B). 'Saccharin-Containing Vehicle' refers to combined intake from the decaff + saccharin and saccharin groups self-administering caffeine in Experiment 2. * indicates caffeine with vehicle increased intake of caffeine relative to controls that did not receive saccharin, $p < 0.05$.

occurred after only the 'active' lever was shaped with saccharin, therefore we cannot state unequivocally that IV caffeine has a primary reinforcing effect; future studies are needed to confirm this preliminary finding. Experiment 2 extended the findings of Experiment 1 to an oral caffeine solution and to multiple oral vehicles including either saccharin or saccharin with a masking stimulus (decaffeinated coffee). Experiment 2 showed that the masking stimulus (coffee flavor) was not necessary for observing oral caffeine self-administration – combinations of caffeine with saccharin or caffeine with decaffeinated coffee and saccharin resulted in similar rates of self-administration and both were greater than rats responding for the vehicle alone. Finally, the relationship between oral caffeine concentration and operant responding was flatter and less systematic than the relationship between IV dose and operant responding. These findings raise important questions about the relationship between operant drug self-administration, reinforcement, and the putative interactions of drugs and vehicles that may promote self-administration in the absence of a primary reinforcing drug effect.

The present findings show that, under optimal conditions, caffeine can strengthen operant behaviors that lead to the delivery of caffeine. However, we found limited evidence that caffeine was a primary reinforcer. Instead, we capitalized on the robust reinforcement enhancing effects of caffeine (Sheppard et al., 2012) to establish self-administration. This finding has been mirrored in human studies showing the

novel soft drinks containing caffeine (2 mg/ml) are initially less preferred than caffeine-free beverages but become more preferred after repeated exposure (Temple et al., 2012). This prompts several important questions about how caffeine and other 'reinforcement enhancers' (Donny et al., 2003) increase operant behaviors both in preclinical models and in human drug users. For example, what is the relationship between primary reinforcement, reinforcement enhancing effects, and increases in operant behavior when a drug is self-administered in a complex, non-neutral vehicle? There are several drugs that are commonly used and abused by humans in which self-administration is most prevalent in a salient non-drug vehicle. In addition to caffeine, alcohol (Hargreaves et al., 2011, 2009), nicotine (Caggiula et al., 2009) and delta-9-tetrahydracannabinol (THC; Tanda et al., 2000) are all self-administered by humans in salient non-drug vehicles. Notably, all four of these drugs are difficult to establish as primary reinforcers in non-human animals (Li et al., 1993; Palmatier et al., 2006; Tanda et al., 2000). We have found that nicotine self-administration is increased by inclusion of a reinforcing visual stimulus (Palmatier et al., 2008, 2006). The alcohol self-administration literature is replete with examples of oral ethanol strengthening operant behavior when the alcohol is included in a sweetened solution (Samson et al., 1988) or a beverage vehicle more traditionally self-administered by humans (e.g., beer; Hargreaves et al., 2011). THC lowers the optimal stimulation threshold of intracranial self-stimulation (ICSS; Gardner et al., 1988), facilitates lordosis and sexual receptivity in ovariectomized female rats (Gordon et al., 1978) and hamsters (Turley and Floody, 1981), and has well-documented hyperphagic effects (Jarbe and DiPatrizio, 2005; Williams et al., 1998) which have often been associated with more palatable foods (Foltin et al., 1986; Higgs et al., 2003; Koch and Matthews, 2001). Presumably, any interaction between the 'reinforcement enhancing' effects of these drugs and primary or conditional reinforcing effects of the vehicle would be expected to increase, rather than reduce, self-administration. Further research will be required to determine whether including other non-drug reinforcers in conjunction with each of these drugs is systematically related to their self-administration in humans and non-human animals.

The present studies with caffeine demonstrate that the primary reinforcing effects of the drug are limited and are not needed to establish self-administration. The drug effect appears to strengthen operant behavior merely by increasing the motivation to obtain the sensory primary reinforcer (saccharin) suggesting that caffeine has a moderating effect on reinforced operant behavior – strengthening the relationship between the operant response and the vehicle. Based on the present findings we have hypothesized that the habitual self-administration of caffeine in humans depends on this ability to robustly enhance the motivational value of the caffeine vehicle. However, caffeine alone did have reinforcing effects in Experiment 1 (Fig. 3B), suggesting that preclinical models may be able to detect these primary reinforcing effects by replicating our approach (48 h between tests) and by increasing the sensitivity of testing procedures (e.g., fading saccharin out, rather than switching from oral saccharin to IV caffeine). Regardless, caffeine robustly enhanced responding for an oral reinforcer which is ecologically valid as worldwide is consumption is dominated by beverages, especially coffee and energy drinks, which have spawned subcultures of habitual users (Reissig et al., 2009). Several questions are prompted by this hypothesis. For example, why does caffeine robustly increase behavior elicited by other reinforcers? There are converging answers to this question involving the time-course of the drug effect and its limited role in the mesotelencephalic systems that mediate approach and seeking behaviors.

The pharmacokinetic profile of caffeine conflicts somewhat with the traditional 'primary reinforcement' approach to self-administration, which posits that an association between the operant behavior and the pharmacological effect of the drug is critical to associative learning (Wise, 1987; Wise and Koob, 2014). In this formulation the speed with which the drug reaches the receptors in the central nervous system (CNS) is paramount to drug reinforcement and abuse liability (Bouayad-Gervais et al., 2014). With orally self-administered caffeine, absorption through digestive tissues creates a delay between the operant response (drinking) and the pharmacological effect – making the pharmacological effects more contextual and less discrete. The sensory effects of the beverage, however, are closely related to the operant response, meaning that the beverage may be what is reinforcing and that the drug may be serving a moderating role. Although this explanation may fit the epidemiology of caffeine consumption and the findings from Experiment 2, it may not be the best explanation for why IV caffeine is not self-administered alone in the present studies or in previous research (Atkinson and Enslen, 1976). Assuming that IV caffeine rapidly reaches the brain, it should be strongly associated with the operant response. However, consider that elimination of caffeine is relatively slow, with a half-life as long as 3–4 h in IV (Smith et al., 1999) and oral (Bonati et al., 1984; Latini et al., 1980) routes of administration. Elimination is slowed even further by food deprivation (Lau et al., 1995; Smith et al., 1999). A temporally discrete drug effect, (i.e., rapid onset and short lifespan) would be expected to have a stronger association with a temporally discrete operant response (pressing a lever). For example, when access to a running wheel is used as a reinforcer for pressing a lever, longer periods of access to the running wheel (presumably a stronger reinforcer) have the unexpected effect of reducing operant response rates (Belke, 2013). In addition, relatively brief presentations of a reward-predictive CS (15–30 s) are more disruptive to ongoing operant behavior (e.g., stronger CR) whereas longer CS durations (1, 2, or 3 min) have inconsistent, but less disruptive effects on ongoing behavior (Miczek and Grossman, 1971). In aversive motivational tasks, avoidance responses are acquired more rapidly and more consistently if a temporally discrete 'safety signal' accompanies the avoidance behavior (Bolles and Grossen, 1970; Foree and LoLordo, 1970).

A purely pharmacokinetic interpretation of caffeine effects is incomplete. There are many drugs of abuse with long lifespans that are readily associated with discrete operant responses (e.g., morphine, Goldberg et al., 1971; amphetamine, Yokel and Pickens, 1973) – so reduced associability of the drug effect and the operant is not sufficient to explain the lack of primary reinforcing effect. However, the unique pharmacodynamic effects of caffeine in incentive systems may lend themselves toward a moderating role on appetitive behaviors (Ferré et al., 2016; Volkow et al., 2015). Most drugs of abuse with primary reinforcing effects elicit increases in extracellular dopamine (DA) from neurons in the ventral tegmental area (VTA) that terminate in the nucleus accumbens (NAc, Ostlund et al., 2014; Schultz, 2016; Wise, 1980; Yokel and Wise, 1975). However, adenosine A2a receptors form functional heteroligomers with DA D2 receptors in the striatum (Ferré, 2016; Ferré et al., 2016). Caffeine administration evokes striatal DA release that is less robust than other drugs of abuse (Acquas et al., 2002; Okada et al., 1996; Solinas et al., 2002) and may not evoke phasic DA responses (Volkow et al., 2015). However, caffeine may potentiate the effects of endogenous dopamine binding to postsynaptic D2 receptors by antagonizing the adenosine A2A receptors. This effect of caffeine would be expected to increase the motivational properties of non-caffeine rewards, without necessitating a primary reinforcing role for caffeine. This hypothesis is supported by recent findings from Walker and Kuhn using fast-scan cyclic voltammetry to measure DA transients in the NAc. In that experiment, caffeine alone did not evoke strong DA transients but enhanced the DA transients evoked by cocaine (Walker et al., 2015). In addition, several studies employing the reinstatement model of relapse to drug self-administration have demonstrated that caffeine can precipitate relapse-like behavior after abstinence from cocaine (Schenk et al., 1996; Worley et al., 1994). In that paradigm, caffeine may be enhancing the effect of cocaine-associated cues, increasing their ability to evoke approach or 'drug-seeking' behaviors. The converging evidence suggests that the critical motivational effects of caffeine are in its ability to moderate the valence of salient non-drug

stimuli in the environment. Preliminary data from our laboratory also suggest that caffeine can increase sign tracking (approach to a lever conditioned stimulus) and reduce goal tracking (approach to the location where sucrose will be delivered) in a Pavlovian conditioned approach paradigm. Thus, the interaction between adenosine A2A receptors and DA D2 receptors may enhance the sensitivity of the incentive system to incentive stimuli in a manner that strengthens appetitive behaviors such as operant responding and conditioned approach.

These findings are the first to demonstrate that response-contingent caffeine administration, either oral or IV, can reliably increase a well-defined operant response in non-human subjects. Although there are myriad follow-up and parametric investigations required (reversibility, dose-response, saccharin-fading, etc.), the present studies provide a proof-of-concept that caffeine is self-administered by non-human animals. A pre-clinical model of caffeine self-administration has important public health implications, as both health risks (Kendler et al., 2006; Strain et al., 1994) and benefits (Popat et al., 2011; Zeitlin et al., 2011) are associated with lifetime caffeine use. Acute caffeine use can increase binge-intoxication with alcoholic beverages (Marczinski et al., 2012; O'Brien et al., 2008). Finally, energy drinks often include sucrose and consuming caffeine and sucrose together may promote lipogenesis (Rush et al., 2006). Increased consumption of sugars (Johnson et al., 2009) or artificial sweeteners (Suez et al., 2014) by caffeine could negatively impact health, especially in individuals predisposed to weight-related and cardiovascular disease (Johnson et al., 2009). A reliable preclinical model of volitional caffeine self-administration will facilitate the investigation of both positive and negative health-related effects of caffeine. Finally, the effects of caffeine in this paradigm are related to effects observed with other stimulants (e.g., nicotine) and may prove instructive for analysis of abuse liability of other compounds. Self-administered drugs are very unique stimuli with complex multimodal effects acting over long periods of time that could be serving as conditioned stimuli (Besheer et al., 2004) or discriminative stimuli (Bevins et al., 2006; Murray et al., 2007), in addition to being primary reinforcers. The contextual nature of their effects should not be overlooked.

Role of funding source

Nothing declared.

Contributors

This manuscript includes data that partially fulfilled the doctoral thesis requirements of CAB. MIP was dissertation supervisor. Both authors contributed to experimental design, collection of data, and manuscript preparation.

Declaration of Competing Interest

No conflict declared.

Acknowledgements

We thank CS Bailey, AL Smith, EM Sanders, and SG Malone for their assistance with data collection for these experiments.

References

Acquas, E., Tanda, G., Di Chiara, G., 2002. Differential effects of caffeine on dopamine and acetylcholine transmission in brain areas of drug-naive and caffeine-pretreated rats. Neuropsychopharmacology 27, 182.
Atkinson, J., Enslen, M., 1976. Self-administration of caffeine by the rat. Arzneimittelforschung 26, 2059–2061.
Balfour, D.J., Wright, A.E., Benwell, M.E., Birrell, C.E., 2000. The putative role of extrasynaptic mesolimbic dopamine in the neurobiology of nicotine dependence. Behav. Brain Res. 113, 73–83.
Belke, T.W., 2013. Running and responding reinforced by the opportunity to run: effect of reinforcer duration. J. Exp. Anal. Behav. 67, 337–351. https://doi.org/10.1901/jeab.1997.67-337.
Besheer, J., Palmatier, M.I., Metschke, D.M., Bevins, R.A., 2004. Nicotine as a signal for the presence or absence of sucrose reward: a Pavlovian drug appetitive conditioning preparation in rats. Psychopharmacology (Berl.) 172, 108–117. https://doi.org/10.1007/s00213-003-1621-9.
Bevins, R.A., Wilkinson, J.L., Palmatier, M.I., Siebert, H.L., Wiltgen, S.M., 2006. Characterization of nicotine's ability to serve as a negative feature in a Pavlovian appetitive conditioning task in rats. Psychopharmacology (Berl.) 184, 470–481. https://doi.org/10.1007/s00213-005-0079-3.
Bolles, R.C., Grossen, N.E., 1970. Function of the CS in shuttle-box avoidance learning by rats. J. Comp. Physiol. Psychol. 70, 165–169. https://doi.org/10.1037/h0028412.
Bonati, M., Latini, R., Tognoni, G., Young, J.F., Garattini, S., 1984. Interspecies comparison of in vivo caffeine pharmacokinetics in man, monkey, rabbit, rat and mouse. Drug Metab. Rev. 15, 1355–1383.
Bouayad-Gervais, K., Minogianis, E.-A., Lévesque, D., Samaha, A.-N., 2014. The self-administration of rapidly delivered cocaine promotes increased motivation to take the drug: contributions of prior levels of operant responding and cocaine intake. Psychopharmacology (Berl.) 231, 4241–4252.
Caggiula, A.R., Donny, E.C., Palmatier, M.I., Liu, X., Chaudhri, N., Sved, A.F., 2009. The role of nicotine in smoking: a dual-reinforcement model. Nebr. Symp. Motiv. 55, 91–109.
Cauli, O., Morelli, M., 2005. Caffeine and the dopaminergic system. Behav. Pharmacol. 16, 63–77.
Cauli, O., Morelli, M., 2002. Subchronic caffeine administration sensitizes rats to the motor-activating effects of dopamine D1 and D2 receptor agonists. Psychopharmacology (Berl.) 162, 246–254.
Collins, R.J., Weeks, J.R., Cooper, M.M., Good, P.I., Russell, R.R., 1983. Prediction of abuse liability of drugs using IV self-administration by rats. Psychopharmacology (Berl.) 82, 6–13.
Donny, E.C., Chaudhri, N., Caggiula, A.R., Evans-Martin, F.F., Booth, S., Gharib, M.A., Clements, L.A., Sved, A.F., 2003. Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. Psychopharmacology (Berl.) 169, 68–76.
Ferré, S., 2016. Mechanisms of the psychostimulant effects of caffeine: implications for substance use disorders. Psychopharmacology (Berl.) 233, 1963–1979.
Ferré, S., Bonaventura, J., Tomasi, D., Navarro, G., Moreno, E., Cortés, A., Lluís, C., Casadó, V., Volkow, N.D., 2016. Allosteric mechanisms within the adenosine A2A–dopamine D2 receptor heterotetramer. Neuropharmacology 104, 154–160.
Foltin, R.W., Brady, J.V., Fischman, M.W., 1986. Behavioral analysis of marijuana effects on food intake in humans. Pharmacol. Biochem. Behav. 25, 577–582.
Foree, D.D., LoLordo, V.M., 1970. Signalled and unsignalled free-operant avoidance in the pigeon. J. Exp. Anal. Behav. 13, 283–290. https://doi.org/10.1901/jeab.1970.13-283.
Gardner, E.L., Paredes, W., Smith, D., Donner, A., Milling, C., Cohen, D., Morrison, D., 1988. Facilitation of brain stimulation reward byΔ 9-tetrahydrocannabinol. Psychopharmacology (Berl.) 96, 142–144.
Garrett, B.E., Griffiths, R.R., 1997. The role of dopamine in the behavioral effects of caffeine in animals and humans. Pharmacol. Biochem. Behav. 57, 533–541.
Gauvin, D.V., Zimmermann, Z.J., Baird, T.J., 2018. The gold-standard in preclinical abuse liability testing: it's all relative. J. Pharmacol. Toxicol. Methods 94, 36–53. https://doi.org/10.1016/j.vascn.2018.08.001.
Goeders, N.E., Smith, J.E., 1983. Cortical dopaminergic involvement in cocaine reinforcement. Science 221, 773–775.
Goldberg, S.R., Woods, J.H., Schuster, C.R., 1971. Nalorphine-induced changes in morphine self-administration in rhesus monkeys. J. Pharmacol. Exp. Ther. 176, 464–471.
Gordon, J.H., Bromley, B.L., Gorski, R.A., Zimmermann, E., 1978. Δ9-Tetrahydrocannabinol enhancement of lordosis behavior in estrogen treated female rats. Pharmacol. Biochem. Behav. 8, 593–596.
Green, T.A., Schenk, S., 2002. Dopaminergic mechanism for caffeine-produced cocaine seeking in rats. Neuropsychopharmacology 26, 422–430 https://doi.org/S0893133X01003438 [pii] 10.1016/S0893-133X(01)00343-8.
Griffiths, R.R., Bigelow, G.E., Liebson, I.A., 1989. Reinforcing effects of caffeine in coffee and capsules. J. Exp. Anal. Behav. 52, 127–140. https://doi.org/10.1901/jeab.1989.52-127.
Griffiths, R.R., Bigelow, G.E., Liebson, I.A., 1986. Human coffee drinking: reinforcing and physical dependence producing effects of caffeine. J. Pharmacol. Exp. Ther. 239, 416–425.
Griffiths, R.R., Brady, J.V., Bigelow, G.E., 1981. Predicting the dependence liability of stimulant drugs. NIDA Res. Monogr. 37, 182–196.
Griffiths, R.R., Brady, J.V., Bradford, L.D., 1979. Predicting the abuse liability of drugs with animal drug self-administration procedures: psychomotor stimulants and hallucinogens. In: Thompson, T., Dews, P.B. (Eds.), Advances in Behavioral Pharmacology. Academic Press, Inc, New York, pp. 163–208.
Griffiths, R.R., Woodson, P.P., 1988a. Reinforcing effects of caffeine in humans. J. Pharmacol. Exp. Ther. 246, 21–29.
Griffiths, R.R., Woodson, P.P., 1988b. Reinforcing properties of caffeine: studies in humans and laboratory animals. Pharmacol. Biochem. Behav. 29, 419–427.
Hargreaves, G.A., Monds, L., Gunasekaran, N., Dawson, B., McGregor, I.S., 2009. Intermittent access to beer promotes binge-like drinking in adolescent but not adult Wistar rats. Alcohol 43, 305–314.
Hargreaves, G.A., Wang, E.Y., Lawrence, A.J., McGregor, I.S., 2011. Beer promotes high levels of alcohol intake in adolescent and adult alcohol-preferring rats. Alcohol 45, 485–498.

Heppner, C.C., Kemble, E.D., Cox, W.M., 1986. Effects of food deprivation on caffeine consumption in male and female rats. Pharmacol. Biochem. Behav. 24, 1555–1559.
Higgs, S., Williams, C.M., Kirkham, T.C., 2003. Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after Δ 9-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. Psychopharmacology (Berl.) 165, 370–377.
Jarbe, T.U., DiPatrizio, N.V., 2005. Delta9-THC induced hyperphagia and tolerance assessment: interactions between the CB1 receptor agonist delta9-THC and the CB1 receptor antagonist SR-141716 (rimonabant) in rats. Behav. Pharmacol. 16, 373–380 https://doi.org/00008877-200509000-00009 [pii].
Johnson, R.K., Appel, L.J., Brands, M., Howard, B.V., Lefevre, M., Lustig, R.H., Sacks, F., Steffen, L.M., Wylie-Rosett, J., 2009. Dietary sugars intake and cardiovascular health: a scientific statement from the American Heart Association. Circulation 120, 1011–1020. https://doi.org/10.1161/CIRCULATIONAHA.109.192627.
Kendler, K.S., Myers, J., Gardner, C.O., 2006. Caffeine intake, toxicity and dependence and lifetime risk for psychiatric and substance use disorders: an epidemiologic and co-twin control analysis. Psychol. Med. 36, 1717–1725.
Koch, J.E., Matthews, S.M., 2001. Δ9-Tetrahydrocannabinol stimulates palatable food intake in Lewis rats: effects of peripheral and central administration. Nutr. Neurosci. 4, 179–187.
Kreek, M.J., 1996. Cocaine, dopamine and the endogenous opioid system. J. Addict. Dis. 15, 73–96.
Latini, R., Bonati, M., Marzi, E., Tacconi, M.T., Sadurska, B., Bizzi, A., 1980. Caffeine disposition and effects in young and one-year-old rats. J. Pharm. Pharmacol. 32, 596–599.
Lau, C.E., Falk, J.L., 1995. Dose-dependent surmountability of locomotor activity in caffeine tolerance. Pharmacol. Biochem. Behav. 52, 139–143.
Lau, C.E., Falk, J.L., 1994. Tolerance to oral and IP caffeine: locomotor activity and pharmacokinetics. Pharmacol. Biochem. Behav. 48, 337–344.
Lau, C.E., Ma, F., Falk, J.L., 1995. Oral and IP caffeine pharmacokinetics under a chronic food-limitation condition. Pharmacol. Biochem. Behav. 50, 245–252.
Li, T.-K., Lumeng, L., Doolittle, D.P., 1993. Selective breeding for alcohol preference and associated responses. Behav. Genet. 23, 163–170.
Liu, X., Caggiula, A.R., Palmatier, M.I., Donny, E.C., Sved, A.F., 2008. Cue-induced reinstatement of nicotine-seeking behavior in rats: effect of bupropion, persistence over repeated tests, and its dependence on training dose. Psychopharmacology 196, 365–375.
Marczinski, C.A., Fillmore, M.T., Henges, A.L., Ramsey, M.A., Young, C.R., 2012. Effects of energy drinks mixed with alcohol on information processing, motor coordination and subjective reports of intoxication. Exp. Clin. Psychopharmacol. 20, 129–138.
Miczek, K.A., Grossman, S.P., 1971. Positive conditioned suppression: effects of CS duration. J. Exp. Anal. Behav. 15, 243–247. https://doi.org/10.1901/jeab.1971.15-243.
Murray, J.E., Li, C., Palmatier, M.I., Bevins, R.A., 2007. The interoceptive Pavlovian stimulus effects of caffeine. Pharmacol. Biochem. Behav. 86, 838–846 https://doi.org/S0091-3057(07)00115-3 [pii] 10.1016/j.pbb.2007.03.013.
O'Brien, M.C., McCoy, T.P., Rhodes, S.D., Wagoner, A., Wolfson, M., 2008. Caffeinated cocktails: energy drink consumption, high-risk drinking, and alcohol-related consequences among college students. Acad. Emerg. Med. 15, 453–460 https://doi.org/ACEM085 [pii] 10.1111/j.1553-2712.2008.00085.x.
Okada, M., Mizuno, K., Kaneko, S., 1996. Adenosine A1 and A2 receptors modulate extracellular dopamine levels in rat striatum. Neurosci. Lett. 212, 53–56.
Ostlund, S.B., LeBlanc, K.H., Kosheleff, A.R., Wassum, K.M., Maidment, N.T., 2014. Phasic mesolimbic dopamine signaling encodes the facilitation of incentive motivation produced by repeated cocaine exposure. Neuropsychopharmacology 39, 2441–2449.
Palmatier, M.I., Coddington, S.B., Liu, X., Donny, E.C., Caggiula, A.R., Sved, A.F., 2008. The motivation to obtain nicotine-conditioned reinforcers depends on nicotine dose. Neuropharmacology 55, 1425–1430 https://doi.org/S0028-3908(08)00409-7 [pii] 10.1016/j.neuropharm.2008.09.002.
Palmatier, M.I., Evans-Martin, F.F., Hoffman, A., Caggiula, A.R., Chaudhri, N., Donny, E.C., Liu, X., Booth, S., Gharib, M., Craven, L., Sved, A.F., 2006. Dissociating the primary reinforcing and reinforcement-enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental reinforcers. Psychopharmacology (Berl.) 184, 391–400.
Palmatier, M.I., O'Brien, L.C., Hall, M.J., 2012. The role of conditioning history and reinforcer strength in the reinforcement enhancing effects of nicotine in rats. Psychopharmacology (Berl.) 219, 1119–1131.
Popat, R.A., Van Den Eeden, S.K., Tanner, C.M., Kamel, F., Umbach, D.M., Marder, K., Mayeux, R., Ritz, B., Ross, G.W., Petrovitch, H., 2011. Coffee, ADORA2A, and CYP1A2: the caffeine connection in Parkinson's disease. Eur. J. Neurol. 18, 756–765.
Reissig, C.J., Strain, E.C., Griffiths, R.R., 2009. Caffeinated energy drinks—a growing problem. Drug Alcohol Depend. 99, 1–10.
Richardson, N.R., Roberts, D.C., 1996. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J. Neurosci. Methods 66, 1–11 https://doi.org/0165027095001530 [pii].
Rush, E., Schulz, S., Obolonkin, V., Simmons, D., Plank, L., 2006. Are energy drinks contributing to the obesity epidemic? Asia Pac. J. Clin. Nutr. Southbank 15, 242–244. https://doi.org/10.2254/0964-7058.15.2.0249.
Samson, H.H., Pfeffer, A.O., Tolliver, G.A., 1988. Oral ethanol self-administration in rats: models of alcohol-seeking behavior. Alcohol. Clin. Exp. Res. 12, 591–598.
Schenk, S., Worley, C.M., McNamara, C., Valadez, A., 1996. Acute and repeated exposure to caffeine: effects on reinstatement of extinguished cocaine-taking behavior in rats. Psychopharmacology (Berl.) 126, 17–23.
Schultz, W., 2016. Dopamine reward prediction-error signalling: a two-component response. Nat. Rev. Neurosci. 17, 183.
Sheppard, A.B., Gross, S.C., Pavelka, S.A., Hall, M.J., Palmatier, M.I., 2012. Caffeine increases the motivation to obtain non-drug reinforcers in rats. Drug Alcohol Depend. 124, 216–222. https://doi.org/10.1016/j.drugalcdep.2012.01.008.
Smith, C., Ma, F., Lau, C.E., 1999. Dose independent pharmacokinetics of caffeine after intravenous administration under a chronic food-limited regimen. Drug Metabol. Drug Interact. 15, 83–96.
Solinas, M., Ferre, S., You, Z.-B., Karcz-Kubicha, M., Popoli, P., Goldberg, S.R., 2002. Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. J. Neurosci. 22, 6321–6324.
Strain, E.C., Mumford, G.K., Silverman, K., Griffiths, R.R., 1994. Caffeine dependence syndrome: evidence from case histories and experimental evaluations. JAMA 272, 1043–1048.
Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, G., Thaiss, C.A., Maza, O., Israeli, D., Zmora, N., Gilad, S., Weinberger, A., 2014. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature 514, 181–186.
Tanda, G., Munzar, P., Goldberg, S.R., 2000. Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat. Neurosci. 3, 1073.
Temple, J.L., Ziegler, A.M., Graczyk, A., Bendlin, A., O'Leary, S., Schnittker, Y.S., 2012. Influence of caffeine on the liking of novel-flavored soda in adolescents. Psychopharmacology (Berl.) 223, 37–45. https://doi.org/10.1007/s00213-012-2684-2.
Turley Jr, W.A., Floody, O.R., 1981. Δ-9-Tetrahydrocannabinol stimulates receptive and proceptive sexual behaviors in female hamsters. Pharmacol. Biochem. Behav. 14, 745–747.
Volkow, N.D., Wang, G.J., Logan, J., Alexoff, D., Fowler, J.S., Thanos, P.K., Wong, C., Casado, V., Ferre, S., Tomasi, D., 2015. Caffeine increases striatal dopamine D 2/D 3 receptor availability in the human brain. Transl. Psychiatry 5, e549.
Walker, Q., Pustojovesky, M., Kuhn, C., 2015. Caffeine potentiates cocaine effects on dopamine signaling more in adolescent than adult rat ventral striatum. Poster Session Presented at: Neuroscience 2015. 45th Annual Meeting of the Society for Neuroscience. . Available from https://www.abstractsonline.com/Plan/SSResults.aspx. (Accessed 12 July 2019).
Weiss, F., Lorang, M.T., Bloom, F.E., Koob, G.F., 1993. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J. Pharmacol. Exp. Ther. 267, 250–258.
Williams, C.M., Rogers, P.J., Kirkham, T.C., 1998. Hyperphagia in pre-fed rats following oral Δ9-THC. Physiol. Behav. 65, 343–346.
Wise, R.A., 1987. Intravenous drug self-administration: a special case of positive reinforcement. In: Bozarth, M.A. (Ed.), Methods of Assessing the Reinforcing Properties of Abused Drugs. Springer, New York.
Wise, R.A., 1980. The dopamine synapse and the notion of 'pleasure centers' in the brain. Trends Neurosci. 3, 91–95.
Wise, R.A., Koob, G.F., 2014. The development and maintenance of drug addiction. Neuropsychopharmacology 39, 254.
Worley, C.M., Valadez, A., Schenk, S., 1994. Reinstatement of extinguished cocaine-taking behavior by cocaine and caffeine. Pharmacol. Biochem. Behav. 48, 217–221 https://doi.org/0091-3057(94)90519-3 [pii].
Yokel, R.A., Pickens, R., 1973. Self-administration of optical isomers of amphetamine and methylamphetamine by rats. J. Pharmacol. Exp. Ther. 187, 27–33.
Yokel, R.A., Wise, R.A., 1975. Increased lever pressing for amphetamine after pimozide in rats: implications for a dopamine theory of reward. Science 187, 547–549.
Zeitlin, R., Patel, S., Burgess, S., Arendash, G.W., Echeverria, V., 2011. Caffeine induces beneficial changes in PKA signaling and JNK and ERK activities in the striatum and cortex of Alzheimer's transgenic mice. Brain Res. 1417, 127–136.