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## A drug-vs-food “choice” self-administration procedure in rats to investigate pharmacological and environmental mechanisms of substance use disorders

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### Abstract

**Background:** Preclinical drug self-administration procedures are commonly used to investigate expression, mechanisms, and treatment of substance use disorders.

**New Method:** The aims were to back-translate an intravenous drug-vs-food choice procedure primarily utilized in monkeys to male and female rats and to develop a surgical method for sustained intravenous catheter patency suitable for long-term drug-choice studies.

**Results:** The surgical protocol resulted in a median intravenous jugular catheter patency in male and female rats of 126 days (range: 25–365 days). Drug-vs-food choice was established with opioids (fentanyl and heroin), psychostimulants (cocaine, methamphetamine, and amphetamine), and an opioid/psychostimulant mixture (fentanyl+methamphetamine). The average time from catheter implantation to stable choice behavior across all drugs was 27 sessions (range: 16–44 sessions). Choice behavior stabilized more quickly for cocaine and fentanyl than for other drugs. Manipulations of both environmental variables (e.g., response requirement or food reinforcer magnitude) and pharmacological variables (e.g., extended access drug self-administration or continuous buprenorphine treatment via osmotic pump) significantly shifted opioid-vs-food choice consistent with previous monkey studies.

**Comparison with existing methods:** Duration of intravenous catheter patency in rats was suitable for long-term, within-subject drug choice studies. Effects of environmental and pharmacological manipulations in rats confirmed and extended previous results from monkeys.

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Conflict of Interests

The authors report no potential or perceived conflicts of interest.

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**Conclusions:** The concordance of behavioral results between rats and monkeys using the present drug-vs-food choice procedure supports its utility to improve our basic understanding of the expression and mechanisms of substance use disorders towards to development of more effective therapeutics.

### Keywords

drug self-administration; choice; fentanyl; heroin; cocaine; methamphetamine

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## 1. Introduction

Substance Use Disorder (SUD) occurs in a commodity-rich and complex environment that includes access to both drugs of abuse (e.g., methamphetamine, heroin, alcohol) and nondrug reinforcers (e.g., social interaction and food). Because SUD is a pathology of behavior for which there are no accepted biomarkers, this psychiatric disorder is currently diagnosed based on behavioral criteria outlined in Diagnostic and Statistical Manual of Mental Health Disorders (Association, 2013). Thus, both preclinical and clinical research related to the expression, mechanisms, and treatment of SUD requires the inclusion of behavioral endpoints. Although there are numerous behaviors that can be examined in preclinical and clinical SUD research, drug self-administration procedures, in particular, allow for the direct measurement of volitional drug-taking behavior in both nonhumans and humans. Drug self-administration procedures that assess choice between a drug and a non-drug reinforcer offer additional interpretative power for at least two reasons. First, choice procedures model the concurrent availability of multiple reinforcers available to human drug users and permit assessment of variables that promote or retard drug choice at the expense of more adaptive behaviors maintained by nondrug reinforcers. Second, choice procedures provide dependent variables for both behavioral allocation (often expressed as % drug choice) and behavioral rate (often expressed as rate of responding or reinforcement). These two variables facilitate interpretation of effects produced by experimental manipulations, such that selective changes in drug reinforcement are indicated by shifts in drug choice, whereas nonselective changes in general motor competence or motivation are indicated by changes in behavioral rate.

For both rodents (Weeks, 1962) and nonhuman primates (Thompson and Schuster, 1964), the earliest intravenous (IV) drug self-administration experiments used simple “single-operant” schedules of reinforcement in which drug injection was the only reinforcer available and rate of responding or reinforcement was the only dependent measure. The technical demands of IV catheter maintenance limit catheter patency longevity and thus constrain the types of behaviors that can be trained and tested during the period of reliable catheter life. This technical constraint was overcome first in nonhuman primates, and the use of choice procedures emerged early and continue today as a sustained if muted theme (Aigner and Balster, 1978; Findley et al., 1972; Foltin et al., 2015; Gasior et al., 2004; Griffiths et al., 1975; Iglauer and Woods, 1974; Maguire et al., 2013; Nader and Woolverton, 1991; Negus, 2003). Despite the well-documented value of nonhuman primates in drug abuse research (Banks et al., 2017; Weerts et al., 2007), the translation of drug self-administration procedures from humans or monkeys to rodents may facilitate improved

understanding of SUD towards the development of safer and more effective treatment strategies. Furthermore, the rapid expansion of molecular neuroscience tools affords unique research opportunities to investigators to combine sophisticated behavioral and neuroscience techniques in rodents that are currently either unavailable or not easily accessible to nonhuman primates.

Therefore, the aims of the present study were to 1) translate and 2) validate an IV drug-vs-food choice procedure originally developed for nonhuman primates to rats (Banks and Blough, 2015; Negus, 2006; Negus, 2003). The drug-vs-food choice procedure described in this manuscript varies from a published drug-vs-food choice procedure in rats that is also based on and originally developed for nonhuman primates (Thomsen et al., 2013; Thomsen et al., 2008). The Thomsen (2008) procedure used a chained schedule and an observing lever response link to initiate the concurrent “choice” schedule link; whereas the present study eliminated the chained schedule and observing response in an effort to shorten training time and align more with the nonhuman primate studies reference above. Towards that goal, a surgical procedure for IV jugular catheter implantation was developed and refined in an attempt to improve longevity in catheter patency modified from previously published techniques (Lenoir et al., 2013a; Thomsen and Caine, 2005) and our experience with venous catheter implantations in nonhuman primates. A total of five drugs of abuse: fentanyl, heroin, cocaine, methamphetamine, and amphetamine were trained in the drug-vs-food choice procedure to determine the broad applicability of the procedure across two pharmacological classes. Once drug-vs-food choice was established, environmental variables (e.g., food reinforcer magnitude, response requirement) and pharmacological variables (continuous buprenorphine treatment) were manipulated to validate translation to rats of published results in nonhuman primates (Nader and Woolverton, 1992; Nader and Woolverton, 1991; Negus, 2006; Negus, 2003). Furthermore, a final experiment determined behavioral interactions between fentanyl and methamphetamine reinforcement because of the rising clinical incidence of polysubstance opioid and psychostimulant abuse (Al-Tayyib et al., 2017; Karilsa et al., 2019; LaRue et al., 2019). For this experiment, a multi-modal drug self-administration procedure was used that included a 2h drug-vs-food choice session component (to assess behavioral allocation) and a 12h extended access drug self-administration component (to allow for increased drug intake) to model aspects of a similar multi-modal drug self-administration procedure in rhesus monkeys (Banks and Negus, 2010; Negus, 2006) and in rats studies with fentanyl alone (Townsend et al., 2019a).

## 2. Methods

### 2.1 Subjects

Sprague-Dawley female and male rats were acquired at weights of 240–260g (~12 weeks) and 290–310g (~11 weeks), respectively from a commercial supplier (Envigo, Frederick, MD, USA). Final sample sizes are reported for each experiment and ranged from 6 to 12. Rats were singly housed in a temperature and humidity-controlled vivarium to protect the vascular access port. Lights in the vivarium were programmed to a 12-h light/dark cycle (lights off at 6pm). Water and food (Tekland Rat Diet, Envigo) were provided ad-libitum in the home cage. Animal maintenance and research were conducted in accordance with the

2011 Guidelines of the National Institutes of Health Committee on Laboratory Animal Resources. Both research and enrichment protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

## 2.2 Intravenous catheter implantation

Rats were aseptically implanted with custom-made jugular catheters and commercially purchased vascular access ports (VABR1B/22, Instech Laboratories, Plymouth Meeting, PA). A list of the surgical instruments and catheter materials is shown in Supplemental Table 1, and Supplemental Figures 1 and 2 show the surgical instruments and method for the custom-made catheters. Prior to surgery, the rat was weighed and the cuffed end of the catheter was trimmed with a scalpel blade so that the distance from catheter tip to behind the first cuff equaled 1 cm for each 100 g of rat weight, with a maximal length of 4 cm. Rats were anesthetized with 2–3% isoflurane in oxygen, and the right neck and back areas were shaved and prepped for surgery with povidone and alcohol three times. An initial incision of ~0.75 cm in length was made directly above the visibly pulsating jugular vein, with the depth minimally sufficient to expose the muscle layer. Forceps were used to separate muscle fibers until the right jugular vein was visible. Subsequently, smaller forceps removed the fascia layer surrounding the vein, allowing for two 10 cm lengths of synthetic nonabsorbable suture (e.g., 21275126, Patterson Vet, Greeley, CO, USA) to be placed underneath a one cm length of vein. The ends of suture #1 were secured with hemostats and placed onto the ribcage (sterile drape placed between rat and hemostats), with the “v” of this suture loosely underlying the end of the vein segment furthest from the rat’s head. The ends of suture #2 were similarly secured with hemostats, which were placed just beyond the nose of the rat. The “v” of suture #2 was made taught underneath the end of the vein segment closest to the rat’s head, allowing for leverage to puncture the top exposed surface of the vein (20 g needle) and insert the custom-made polyurethane catheter (BTPU-040, Instech Laboratories). The distal end of the catheter away from the cuffs was connected to a 5 ml, saline-filled syringe with a luer stub. This saline-filled catheter had two 1-mm cuffs (BTSIL-047, Instech Laboratories) based on our nonhuman primate catheter design and similar to a previously published rat catheter design (Lenoir et al., 2013a). The weight-adjusted portion of the catheter and the cuff closest to the catheter tip was inserted into the vein toward the heart (i.e., toward the rat’s tail) with forceps and Dumont tweezers, such that only the cuff furthest from the catheter tip remained outside of the vein. Venous access was verified by filling the catheter with blood by gently pulling the plunger on the 5 ml syringe, followed by a 0.5 ml saline flush. Next, hemostats were removed from Suture #1 and a knot was placed snugly around the vein and between the two cuffs without occluding the catheter. A knot was also tied around the uncatheterized portion of the vein with suture #2 to prevent unnecessary bleeding. An additional length of suture #3 was used to place a knot behind the cuff most distal from the catheter tip (i.e., the cuff *not* inserted into the vein), anchoring the catheter to nearby muscle tissue. Next, an incision (2.5 cm in length, minimally sufficient to expose the muscle layer) was made in the shaved skin of the back and 1 cm posterior to the scapulae. A subcutaneous pocket beneath the incision was opened using curved hemostats to accommodate the eventual implantation of the vascular access port (e.g., Model VABR1B/22, Instech Laboratories). Next, the unsecured end of the catheter was subcutaneously routed to the incision site using curved hemostats, clamped

with non-serrated hemostats, trimmed such that approximately 5 cm of catheter material was exposed from the mid-scapular incision, and connected to the vascular access port with suture. As the vascular access port was subcutaneously inserted, excess catheter material (~5 cm) was looped under the skin to function as a “shock absorber” and protect against undesirable tension on the catheter, with the loop placed near the scapulae. Skin incisions were closed with suture material (e.g. SN-5698G, Covidien, Dublin, Ireland), and the catheter was flushed with 0.1 ml of gentamicin (4 mg/ml) followed by 0.1 ml of heparinized saline (30 U/ml). Subjects were administered ketoprofen (5 mg/kg, SC) immediately following surgery and 24h post-operatively. Rats were allowed to recover for five days prior to initiating self-administration training.

### 2.3 Apparatus and catheter maintenance

Modular operant chambers (ENV-007CT or ENV-008CT, Med Associates, St. Albans, VT) located in sound-attenuating cubicles (ENV-017M, ENV-018MD-W, or ENV-018MD, Med Associates) were equipped with two retractable levers (ENV-112CM, Med Associates) on the same side of the operant chamber, a set of three LED lights (ENV-222M, Med Associates) (red, yellow, green) mounted above each lever, and a retractable “dipper” (ENV-202M-S, Med Associates) cup (0.1 ml) located between the levers for presenting liquid food (0–100% v/v vanilla-flavored Ensure® in tap water, Abbott Laboratories, Chicago, IL). Corncob bedding was present in waste pans located under the stainless-steel rod floor of the operant chamber. IV drug infusions were delivered by activation of a syringe pump (PHM-100, Med Associates) located inside the sound-attenuating cubicle. Infusions were delivered through co-extruded 22-gauge PE/PVC tubing (BTCOEX-22, Instech Laboratories) attached via a 22-gauge luer stubs (LS22, Instech Laboratories). This tubing then connects to the top of a fluid swivel (375/22PS, Instech Laboratories) mounted on drug delivery arm (PHM-110-SAI, Med Associates) above the operant chamber. The bottom of the fluid swivel was connected to a 1-channel magnetic tether (VABR1TH/22, Instech Laboratories) that passed through a hole in the operant chamber roof and terminated in a magnetic injector that was inserted into the vascular access port. Behavioral sessions were operated by custom programs written for Med-PC IV (Med Associates), and the drug-vs-food choice Med-PC IV program used in our laboratory can be found in Supplemental Materials. After each behavioral session, catheters were flushed with 0.1 ml of gentamicin (4 mg/ml) followed by 0.1 ml of heparinized saline (30 U/ml). Catheter patency was verified at the end of each experiment by instantaneous muscle tone loss following IV methohexital (1.6 mg) administration.

### 2.4 Drug-vs-food choice training

At least five days were allowed following internal jugular catheterization surgery to ensure the vascular access port surgical site had healed enough to support the tension of the tether. Rats were trained on the terminal 2-h drug-vs-food choice procedure using a series of steps described below. First, rats are trained to respond for IV drug infusions under an initial fixed-ratio (FR)1 / 20-s time out schedule of reinforcement during daily 2-h behavioral sessions. Each session began with a non-contingent drug infusion followed by a 60-s time out. The response period was signaled by extension of only the right lever and illumination of the right green stimulus light. Following each response requirement completion, the lever

retracted, the green light was extinguished, and an IV drug infusion was administered. Initial drug self-administration training doses were: fentanyl (3.2  $\mu\text{g}/\text{kg}/\text{infusion}$ ), heroin (0.032  $\text{mg}/\text{kg}/\text{infusion}$ ), cocaine (0.32  $\text{mg}/\text{kg}/\text{infusion}$ ), methamphetamine (0.1  $\text{mg}/\text{kg}/\text{infusion}$ ), and amphetamine (0.1  $\text{mg}/\text{kg}/\text{infusion}$ ). Initial drug doses were based on our published (Townsend et al., 2019b) and unpublished rat self-administration data from dose-effect functions under an FR5 schedule of reinforcement. Selected doses were the first half-log dose on the descending limb of the dose-effect function following the peak dose. This schedule of reinforcement remained until the number of injections earned was >10 injections per 2-h session for at least three consecutive days. Once rates of drug self-administration were stable, the FR requirement was steadily increased over consecutive days from FR1 to FR2 and FR3 until the terminal FR5 schedule was reached. Rats remained on the FR5 schedule for at least five days. Second, rats were trained to respond on the opposite lever for 0.1 mls of diluted liquid food (either 18% or 32% vanilla-flavored Ensure, Abbot Laboratories, Chicago, IL) presentations using a switchable liquid dipper for rats (Model ENV-202M-S, Med Associates) fitted with a 0.1 ml cup (ENV-202C-10). Two different concentrations were used because Abbott changed the Ensure formulation/taste of Ensure in 2019 which resulted in 18% no longer maintaining reliable operant responding. As in Step 1, the response requirement was initially FR1 and those conditions were implemented for at least one day. If high rates of behavior were observed on the food-associated lever (>100 responses), then the FR was increased to the terminal FR5 schedule for at least one day.

Third, once both drug and food-maintained responding had been established separately, rats were then trained on the drug-vs-food choice procedure with exposure to the terminal drug-choice conditions and no intermediary steps. This was similar to our training of a within-session drug-vs-food choice in rhesus monkeys (Banks and Blough, 2015; Negus, 2006; Negus, 2003). The terminal drug-choice session consisted of five 20-min response components each preceded by a 4-min “sample” component. Each sample component started with a non-contingent infusion of the unit drug dose available during the subsequent response component followed by a 2-min time out. Next, a 5-s presentation of liquid food was programmed followed by a 2-min time out. Following this second time out, the response component would begin. During each response component, both levers were extended, a red stimulus light above the left lever was illuminated to signal liquid food availability and a green stimulus light above the right lever was illuminated to signal IV drug availability. Response-requirement (FR5) completion on the left lever resulted in a 5-s presentation of liquid food, whereas response-requirement (FR5) completion on the right lever resulted in infusion of the IV drug dose available for that component. Responding on one lever reset the ratio requirement for the other lever. The liquid-food concentration was held constant across components. A different drug dose was available during each of the five successive response components (e.g., 0, 0.32, 1.0, 3.2, and 10  $\mu\text{g}/\text{kg}/\text{infusion}$  fentanyl during components 1–5, respectively). Drug dose was varied by changing the infusion duration (300g rat; 0, 0.5, 1.56, 5, and 15.6-s of pump activation during components 1–5, respectively) and visually signaled by the frequency of the flashing of the right green light above the drug-associated lever in 3-s cycles (component 1: off; component 2: on for 0.1 s and off for 2.9 s; component 3: on for 0.3 s and off for 2.7 s; component 4: on for 1 s and off for 2 s; component 5: on) similar to our rhesus-monkey drug-vs-food choice studies. During each response component, rats



could complete up to 10 total ratio requirements between the food- and drug-associated levers. Each ratio requirement completion initiated a 20-s time out, the retraction of both levers, and extinction of the red and green stimulus lights. If all 10 ratio requirements were completed before 20 min had elapsed, then both levers retracted, and stimulus lights were extinguished for the remainder of that response component. Drug-vs-food choice behavior was considered stable when the smallest drug dose maintaining 80% drug choice did not change 0.5 log units over three consecutive days. In instances where rats did not allocate 80% of behavior towards drug injections during any response component, interventions were imposed for 1–2 experimental sessions to promote responding on the drug-associated lever. These interventions included increasing the response requirement for the food reinforcer (e.g., FR30) or substituting water for liquid food as the alternative reinforcer. Using the rats included in Table 1 as an example, these training interventions were successfully implemented in 6/12 fentanyl rats, 5/12 methamphetamine rats, 5/12 heroin rats, and 3/9 amphetamine rats. Training interventions were not required for the cocaine or the 1:54 fentanyl/methamphetamine rats included in Table 1.

## 2.5 Environmental manipulations

Three types of environmental manipulations were performed under conditions of fentanyl- or heroin-vs.-food choice. In experiment #1, the role of discriminative and consequent stimuli in the drug-vs-food choice procedure were examined in six rats (4 females and 2 males). Three different manipulations were conducted across consecutive weeks: 1) removal of non-contingent fentanyl infusions before each response component, 2) removal of response-contingent fentanyl infusions during the response component, and 3) removal of the visual discriminative stimulus on the fentanyl-associated lever. Each experimental week consisted of a baseline fentanyl-vs-food choice session on Monday and one of the experimental manipulations described above for behavioral sessions conducted Tuesday-Friday. The order of these three experimental manipulations was counterbalanced across different rats and the liquid-food concentration was 18%. Data are presented as the average of each 4-day experimental manipulation and the average of all baseline Monday behavioral sessions before each weekly experimental manipulation.

For experiment #2, the effects of manipulating the liquid-food concentration were examined on fentanyl-vs-food choice in 12 rats (6 males and 6 females) and on heroin-vs-food choice in eight rats (3 males and 5 females). Rats were initially trained on the fentanyl-vs-food and heroin-vs-food choice procedure with 18% liquid food. Subsequently, the effects of decreasing (i.e., 1.8%) or increasing (i.e., 100%) the liquid food concentration on opioid-vs-food choice were examined. Each liquid food concentration manipulation was implemented for two days, with data collected on the second day used for subsequent analyses. Rats were then returned to initial liquid food (i.e., 18%) conditions for at least three days and until choice behavior was stable and returned to pre-test levels. Liquid-food concentration manipulations were counterbalanced across rats.

In experiment #3, the effects of manipulating the response requirement on the heroin or food-associated lever were examined in six rats (3 males and 3 females). The liquid-food concentration was 18% for these experiments. For heroin FR (FRheroin) manipulations, the

FR on the food associated-lever was held constant at FR5 while the FR on the heroin-associated lever was varied from FR 1 to FR 100. For food FR (FR<sub>food</sub>) manipulations, the FR on the drug associated-lever was held constant at FR5 while the FR on the food-associated lever was manipulated from FR 1 to FR 30. Each manipulation was implemented for three consecutive days and data presented are an average of the 3-day period. After each manipulation, baseline FR5: FR5 choice behavior was reestablished in each rat. Baseline FR values were reinstated for at least three days and until baseline heroin choice stabilized at pre-test levels.

## 2.6 Pharmacological manipulations

The effects of two different pharmacological manipulations were determined on drug-vs-food choice. For experiment #4, continuous 0.01 – 0.32 mg/kg/h buprenorphine treatment effects on heroin-vs-food choice were determined in six rats (3 females and 3 males). The liquid-food concentration was 18% for these heroin-choice studies. Continuous buprenorphine treatment was achieved with osmotic minipumps (Model 2001 and 2ML1, Alzet, Cupertino, CA, USA) aseptically implanted into a subcutaneous space on the right or left lower flank. Model 2001 pumps delivered vehicle (15% ethanol, 20% DMSO, and 65% sterile water) and the lower buprenorphine doses (0.01 and 0.032 mg/kg/h). Model 2ML1 pumps delivered the higher buprenorphine doses (0.1 mg/kg/h and 0.32 mg/kg/h). Osmotic pumps were implanted on Friday afternoons, and daily heroin-vs-food choice sessions were conducted the following Monday-Thursday. Pumps were aseptically removed on Friday. Data presented are an average of the last three days of pump implantation. All rats received all buprenorphine doses except for one male rat that received only the three smallest buprenorphine doses. After pump removal, heroin-vs-food choice sessions were conducted for at least one week and continued until choice behavior was stable and returned to pre-test conditions. This series of buprenorphine treatment and non-treatment conditions continued until vehicle and all buprenorphine doses were tested. In general, buprenorphine doses were tested in a counterbalanced order, with the exception that 0.32 mg/kg/h was tested as the final dose in all rats because baseline heroin-vs-food choice behavior took several weeks to recover following 0.1 mg/kg/h buprenorphine treatment.

Experiment #5 determined fentanyl and methamphetamine interactions using a multi-modal drug self-administration procedure that retained daily 2-h drug-vs-food choice sessions and added a daily 12-h extended-access drug self-administration session. This is a procedure we have used previously to evaluate effects of exposure to and withdrawal from extended fentanyl access on fentanyl-vs.-food choice (Townsend et al., 2019a). Eleven rats (6 males and 5 females) were trained to self-administer a 1:54 fixed-proportion fentanyl/methamphetamine mixture based on the relative potency (ED<sub>50</sub> value) of each drug alone in the drug-vs-food choice procedure using the same methods described above. Once fentanyl/methamphetamine-vs-food choice dose-effect functions were stable as described above with 18% liquid food, 12-h extended access fentanyl/methamphetamine self-administration sessions were scheduled to begin at 6pm on Sunday-Thursday each week and continue until 6am the next morning. During this extended access component, 1:54 µg/kg/infusion fentanyl/methamphetamine was available under an FR5 / 20-s time out schedule of reinforcement. Fentanyl/methamphetamine choice sessions were conducted from 2–4pm



Monday-Friday, and thus began 8 h after each extended-access session. This sequence of 12-h extended access and 2-h choice behavior sessions was continued for 14 days. In addition, rats were observed 5-min before each fentanyl/methamphetamine-vs-food choice session and scored for the presence of six somatic opioid withdrawal behavioral signs over 30 seconds (Cobuzzi and Riley, 2011; Stephens and Riley, 2009). The behaviors scored were tremor, teeth chatter, eye twitch, mastication, yawn, wet dog shake, piloerection, ptosis, and presence of diarrhea on the rat. Using this behavioral scoring system, the maximum value for any behavior was 1 and a maximum score was 9.

## 2.7 Data analysis

The primary dependent measures for each component of the drug choice session were 1) percent drug choice, defined as  $\{(\text{number of ratio requirements, or 'choices', completed on the drug-associated lever} \div \text{total number of choices completed on both the drug- and food-associated levers}) \times 100\}$ , and 2) reinforcement rate defined as total number of choices completed. Additional dependent measures were percent session drug choice, defined as  $\{(\text{number of drug choices completed for the entire session} \div \text{total number of drug and food choices completed for the entire session}) \times 100\}$  and total, food, and drug choices completed during the entire 2-h session. For the extended access drug self-administration sessions, the primary dependent measure was the number of injections earned. Data were analyzed using one-way or two-way repeated-measures ANOVA with self-administered drug dose and experimental manipulation as the main factors, and the Geisser-Greenhouse correction was utilized when appropriate for within-subject analyses (Prism 8, GraphPad, La Jolla, CA, USA). Somatic opioid withdrawal signs were analyzed using the non-parametric Friedman test and Dunn's multiple-comparisons post-hoc test because this dependent measure violates parametric statistic assumptions. Significant main effects or interactions were followed by post-hoc tests appropriate for the pre-planned comparisons and corrected for multiple comparisons. Significance was established *a priori* at the 95% confidence level ( $p < 0.05$ ).

## 2.8 Dose Addition analysis

The  $ED_{50}$  values for fentanyl and methamphetamine alone were defined as the dose that produced 50% choice and were calculated using non-linear regression (Prism 8, GraphPad). For the 1:54 fentanyl/methamphetamine mixture, the  $ED_{50}$  value was defined as the dose of each drug in the mixture that produced 50% choice. In addition, a related quantity,  $Z_{\text{mix}}$ , was also calculated as the total drug dose (i.e., dose fentanyl + dose methamphetamine) that produced 50% choice. Drug interactions were assessed using both graphical and statistical approaches to dose-addition analysis (Tallarida, 2000; Wessinger, 1986; Woolverton, 1987) as described previously (Negus, 2005; Stevenson et al., 2003). Graphically, mean  $ED_{50}$  values for fentanyl choice alone or as part of the mixture were plotted as a function of  $ED_{50}$  values for methamphetamine choice alone or as part of the mixture. This data presentation is known as an isobologram, and the line that connects the data points for each drug alone shows predicted data for drug mixtures assuming additivity of drug effects. Points that fall below the line are suggestive of super-additivity or synergism and points that fall above the line are suggestive of sub-additivity or antagonism. Statistical evaluation of fentanyl/methamphetamine interactions was accomplished by comparing the experimentally determined  $ED_{50}$  value for the fentanyl/methamphetamine mixture ( $Z_{\text{mix}}$ ) with the predicted

additive ED<sub>50</sub> value ( $Z_{\text{add}}$ ) as described by Tallarida (2000).  $Z_{\text{mix}}$  was determined empirically as described above.  $Z_{\text{add}}$  was calculated from the equation  $Z_{\text{add}}=fA+(1-f)B$ , where A was the ED<sub>50</sub> for fentanyl alone, B was the ED<sub>50</sub> for methamphetamine alone, and  $f$  was the fractional multiplier of A in the computation of the additive total dose. Any choice of  $f$  is related to the proportion of drug A ( $\rho A$ ) in a mixture according to the equation  $\rho A = fA/Z_{\text{add}}$ . This study tested a mixture that yielded a value of  $f=0.5$ .  $Z_{\text{mix}}$  and  $Z_{\text{add}}$  values were determined to be different if the 95% confidence limits were non-overlapping (i.e.,  $p<0.05$ ).

## 2.9 Drugs

Fentanyl HCl, (–)-Cocaine HCl, and heroin HCl were provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD, USA). (+)-Amphetamine hemisulfate and (+)-methamphetamine HCl were purchased from a commercial supplier (Millipore Sigma, St. Louis MO, USA). Buprenorphine HCl was also purchased from a commercial supplier (Spectrum Chemicals, Gardena, CA, USA) and dissolved in 15% ethanol, 20% DMSO (Fisher Scientific, Waltham, MA, USA), and 65% sterile water for injection. All other drugs were dissolved in bacteriostatic sterile saline. All solutions were passed through a 0.22-micron sterile filter (Model #SLGV033RS, Millipore Sigma) before IV or subcutaneous (SC) administration.

## 3. Results

### 3.1 Catheter patency longevity

The records from the first 105 rats implanted with their first jugular catheters in the laboratory by three different lab members were retrospectively surveyed for catheter patency longevity. The median first catheter patency was 126 days, the mode was 144 days, and the range was 25 to 365 days. For the experiments reported in this manuscript, a second catheter implantation surgery was not necessary. However, in some of our other published studies, when the first catheter failed, a second catheter was successfully implanted into the left internal jugular vein and attached to the exist vascular access port to complete the experiments.

### 3.2 Drug-vs-food choice training

Figure 1 shows drug-vs-food choice dose-effect functions in male and female rats with two mu-opioid receptor agonists (A: fentanyl, C: heroin) and three monoamine transporter ligands (E: cocaine, G: methamphetamine, I: amphetamine). Table 1 shows the average number of sessions on drug only training, food only training, and drug-food choice training until stability for each drug. The liquid-food concentration was 18% liquid food for fentanyl and methamphetamine, 18% or 32% for heroin, and 32% only for cocaine and amphetamine. The median number of sessions until choice behavior was stable was 26 sessions, the mode was 25 sessions, and the range was 16 to 44 sessions. There were significant differences between the different drugs on the number of choice training sessions until stability ( $F_{5,79} = 11.2$ ,  $p<0.0001$ ). For example, training of cocaine choice was significantly faster than training heroin, methamphetamine, and amphetamine choice. Training of fentanyl and 1:54 fentanyl/methamphetamine choice occurred significantly faster than training heroin and amphetamine choice. There were also significant differences in the number of total training

sessions ( $F_{5,79} = 6.0, p < 0.0001$ ), with cocaine, fentanyl, and 1:54 fentanyl/methamphetamine being significantly faster than heroin. Cocaine and 1:54 fentanyl/methamphetamine were also significantly faster than amphetamine. There were no significant differences between the number of drug-only and food-only training sessions between the five different self-administered drugs. Furthermore, there were no sex differences observed during any of the training conditions. Infrequently, rats would experience health issues that precluded further self-administration training before stable choice behavior was established. In the present studies, the number of rats that successfully completed the experiments out of the total number of rats that were successfully trained on drug-vs-food choice were: cocaine (10/10), fentanyl (12/15), methamphetamine (12/14), amphetamine (9/10), heroin (12/15), and fentanyl/methamphetamine (10/10). Moreover, with the exception of heroin, we have been able to establish stable drug choice behavior in all rats that have started the training procedure described in this manuscript. For heroin, there have been 10 out of 47 rats that have failed to acquire stable heroin-vs.-food choice behavior.

### 3.3 Environmental manipulations on drug-vs-food choice

Figure 2 shows effects of removing the visual discriminative stimuli, the non-contingent fentanyl infusion, or the contingent fentanyl infusions on choice between fentanyl and 18% liquid food. Selective removal of either the non-contingent fentanyl infusions before the response component or removal of the contingent fentanyl infusions during the response component significantly decreased behavioral allocation to the fentanyl-associated lever (fentanyl dose/component:  $F_{1,1,5,6} = 59.5, p = 0.0003$ ; manipulation:  $F_{1,4,7,2} = 16.5, p = 0.003$ ; interaction:  $F_{3,2,13} = 6.5, p = 0.006$ ). Selective removal of fentanyl-associated visual stimuli had no effect on percent fentanyl choice. Selective removal of either the non-contingent fentanyl infusions before the response component or removal of the contingent fentanyl infusions during the response component also significantly increased the number of choices completed in the last two choice-session components (fentanyl dose/component:  $F_{1,8,8,9} = 28.2, p = 0.0002$ ; manipulation:  $F_{1,6,8} = 13.9, p = 0.003$ ; interaction:  $F_{2,6,10,9} = 17.7, p = 0.0002$ ).

Figure 3 shows effects of manipulating the liquid-food concentration on both fentanyl-vs-food choice (Panels A and C) and heroin-vs-food choice (Panels B and D). Decreasing the liquid-food concentration significantly decreased food choice and produced a reciprocal increase in fentanyl choice, whereas increasing the food concentration significantly increased food choice and decreased fentanyl choice (fentanyl dose:  $F_{1,3,14,7} = 104.4, p < 0.0001$ ; food concentration:  $F_{2,0,21,4} = 51.1, p < 0.0001$ ; interaction:  $F_{3,1,34,1} = 5.0, p = 0.005$ ). Decreasing the liquid-food concentration also resulted in a decrease in the number of choices completed per component during early components when rats primarily chose food (fentanyl dose:  $F_{1,7,18,6} = 219.6, p < 0.0001$ ; food concentration:  $F_{1,2,13,3} = 12.6, p = 0.003$ ). Because there was no significant fentanyl dose  $\times$  liquid-food concentration interaction, the results defaulted to a one-way repeated-measures ANOVA for food concentration collapsed across fentanyl doses, and choices completed during availability of 1.8% liquid food was significantly lower compared to 100% liquid food (food concentration:  $F_{1,7,5,1} = 11.6, p = 0.014$ ). For the heroin-vs-food choice studies, there was a significant main

effect of heroin dose ( $F_{1,9,13} = 52.8, p < 0.0001$ ) and food concentration ( $F_{1,9,13,3} = 10.3, p = 0.002$ ) on choice behavior, but no significant heroin dose  $\times$  food concentration interaction. Thus, the statistical analysis defaulted to a one-way repeated-measures ANOVA for liquid-food concentration manipulations collapsed across heroin doses. There was a significant main effect of food concentration ( $F_{1,5,4,5} = 8.0, p = 0.037$ ), and heroin choice tended to decrease as liquid-food concentration increased; however, no food concentration was significantly different from any other food concentration after correcting for multiple comparisons upon post-hoc analysis. Manipulating the food concentration did significantly decrease the number of choices completed during early session components (e.g., 0.0032 and 0.01 mg/kg/infusion heroin) when 1.8% liquid food was available and significantly increased the number choices completed during the 0.1 mg/kg/infusion heroin component when 100% liquid food was available (food concentration:  $F_{1,7,2} = 15.6, p = 0.005$ ; interaction:  $F_{2,14,2} = 9, p = 0.003$ ).

Figure 4 shows effects of manipulating the response requirement on either the heroin- or food-associated lever on behavioral allocation between IV heroin infusions and 18% liquid food. Panels A and B show the effects of manipulating the FR response requirement on the heroin-associated lever (FR<sub>heroin</sub>) while holding the FR requirement on the food-associated lever constant at FR 5. Increasing FR<sub>heroin</sub> produced systematic rightward/downward shifts in the heroin choice dose-effect function (FR<sub>heroin</sub>:  $F_{2,9,58,4} = 42.4, p < 0.0001$ ; interaction:  $F_{12,80} = 9.4, p < 0.0001$ ). Increasing the FR<sub>heroin</sub> to 10 and 30 significantly decreased 0.032 mg/kg/infusion heroin choice, and increasing the FR<sub>heroin</sub> to 100 significantly decreased both 0.032 and 0.1 mg/kg/infusion heroin choice. The number of choices completed per component increased with increasing FR<sub>heroin</sub> values as rats reallocated their responding away from heroin and toward food (FR<sub>heroin</sub>:  $F_{2,3,45,4} = 29.6, p < 0.0001$ ; interaction:  $F_{12,80} = 18.3, p < 0.0001$ ). Increasing the FR<sub>heroin</sub> to 100 resulted in a significant increase in the number of choices completed per component during 0.1 mg/kg/infusion heroin availability. Panels C and D show the effects of manipulating the FR response requirement on the food-associated lever (FR<sub>food</sub>) while holding the FR requirement on the heroin-associated lever constant at FR 5. Increasing the FR<sub>food</sub> produced a leftward/upward shift in the heroin choice dose-effect function (FR<sub>food</sub>:  $F_{2,6,51,1} = 37.4, p < 0.0001$ ; interaction:  $F_{9,60} = 4.9, p < 0.0001$ ). Increasing the FR<sub>food</sub> to 30 significantly increased 0.032 and 0.01 mg/kg/infusion heroin choice. The number of choices completed per component significantly decreased with increasing FR<sub>food</sub> (FR<sub>food</sub>:  $F_{1,5,29} = 20.9, p < 0.0001$ ; interaction:  $F_{9,60} = 2.8, p = 0.008$ ). Increasing the FR<sub>food</sub> to 30 significantly decreased the number of choices completed during components when unit doses of 0 and 0.0032 mg/kg/infusion heroin were available.

### 3.4 Pharmacological manipulations on drug-vs-food choice

Figure 5 shows effects of continuous 7-day vehicle and buprenorphine treatment on heroin-vs-18% food choice. Relative to vehicle treatment, buprenorphine treatment significantly decreased heroin-vs-food choice and produced a rightward/downward shift in the heroin choice-dose effect curve (heroin dose:  $F_{1,5,7,4} = 38.7, p = 0.0002$ ; buprenorphine dose:  $F_{2,7,13,5} = 13.5, p = 0.0003$ ). Because there was no significant heroin dose  $\times$  buprenorphine dose interaction, the statistical analysis defaulted to a one-way repeated-measures ANOVA

collapsed across heroin doses, and 0.032 and 0.32 mg/kg/h buprenorphine doses significantly decreased heroin choice compared to vehicle (buprenorphine dose:  $F_{1,5,4,6} = 14.6$ ,  $p=0.012$ ). Panel B shows that 0.1 and 0.32 mg/kg/h buprenorphine treatment significantly increased the number of choices completed per component during 0.1 mg/kg/infusion unit heroin dose availability as subjects reallocated their responding away from heroin and toward food (buprenorphine dose:  $F_{2,5,12,5} = 11$ ,  $p=0.001$ ; heroin dose:  $F_{1,3,6,7} = 29.2$ ,  $p=0.0008$ ; interaction:  $F_{2,9,13,5} = 7.1$ ,  $p=0.005$ ).

Figure 6 shows 1:54 fentanyl/methamphetamine-vs-food (18%) choice dose-effect functions for percent drug mixture choice (Panel A), choices per component (Panel B), and the isobologram (Panel C). Table 2 shows  $ED_{50}$  choice values for fentanyl alone (plotted from Figure 1), methamphetamine alone (plotted from Figure 1), and each drug in the fentanyl/methamphetamine mixture. The  $ED_{50}$  choice values for fentanyl and methamphetamine as part of the 1:54 fentanyl/methamphetamine mixture were both significantly smaller compared to each respective drug alone based on non-overlapping confidence limits. Furthermore,  $Z_{mix}$  (mean: 34.2; 95% confidence limits: 27.2, 41.2) was significantly lower than  $Z_{add}$  (mean: 57.5; 95% confidence limits: 50.6, 64.5) based on non-overlapping confidence limits indicating a synergistic interaction on drug choice.

Figure 7 shows effects of extended access 1:54  $\mu\text{g}/\text{kg}/\text{infusion}$  fentanyl/methamphetamine mixture self-administration on subsequent fentanyl/methamphetamine mixture-vs-food choice. During this 2-week study, weekday 2-h drug-vs-food choice sessions (2–4pm) were preceded by 12-h extended-access drug self-administration sessions (6pm–6am). Thus, under these conditions, extended-access sessions provided opportunities for increased drug intake, and choice sessions began 8 h after conclusion of the preceding extended-access session. Somatic opioid withdrawal signs were assessed immediately before each choice session. Fentanyl/methamphetamine intake during the extended access session was stable over the experimental period and did not significantly change over time (Panel A). Panel B shows somatic opioid withdrawal signs across the experimental period. Relative to Day 1 (i.e., Pre-extended access), opioid withdrawal scores were significantly increased Days 4–6, 9, and 10–14 (Panel B: Friedman statistic = 50.2,  $p<0.0001$ ). These opioid withdrawal signs were associated with significantly increased fentanyl/methamphetamine choice on Days 2–6, but not Days 9–13, compared to pre-extended access (Panel C: extended access:  $F_{1,6,13,8} = 4.3$ ,  $p=0.041$ ). Extended access fentanyl/methamphetamine self-administration also significantly decreased both total and food choices completed without significantly altering fentanyl/methamphetamine choices (Panel D: dependent measure:  $F_{1,1,10,2} = 16.8$ ,  $p=0.002$ ; extended access week:  $F_{1,3,12} = 30.9$ ,  $p<0.0001$ ; interaction:  $F_{1,9,16} = 8.8$ ,  $p=0.003$ ).

#### 4. Discussion

This manuscript described an IV jugular catheter surgical protocol and training method for translation into rats a drug-vs-food choice procedure originally developed for nonhuman primates. Building on previous studies using a similar cocaine-vs.-food choice procedure in rats (Thomsen et al., 2013; Thomsen et al., 2008), there were four main findings for the present study. First, the IV jugular catheter surgical protocol described resulted in a slightly longer first catheter patency duration than previously published methods (Thomsen and

Caine, 2005). Second, drug-vs-food choice was established in male and female rats for not only cocaine, but also for the other monoamine transporter ligands methamphetamine and amphetamine and for the opioids fentanyl and heroin. All five drugs of abuse alone and an opioid/psychostimulant mixture maintained dose-dependent increase in choice over an alternative nondrug food reinforcer, consistent with previous results in nonhuman primates (Banks and Blough, 2015; Griffiths et al., 1981; Townsend et al., 2020a; Woolverton and Balster, 1979). Third, opioid-vs-food choice in rats was sensitive to environmental manipulations, such as alternative reinforcer magnitude and response requirement, consistent with and extending previous results from cocaine-vs-food choice studies in nonhuman primates (Foltin et al., 2015; Johnson et al., 2016; Nader and Woolverton, 1992; Negus, 2003) and rats (Cantin et al., 2010; Kerstetter et al., 2012; Thomsen et al., 2013). Lastly, these results illustrate use of opioid-vs.-food choice procedures in rats to examine effects of pharmacological manipulations that have been examined previously in rhesus monkeys and that include effects of chronic treatment with candidate medications (Negus, 2006; Townsend et al., 2020a) and effects of opioid-stimulant interactions (Negus, 2005). Overall, the results presented in this manuscript provide an empirical foundation supporting the feasibility of drug-vs.-food choice procedures in rats and the utility of these procedures to address questions that heretofore have been examined primarily in nonhuman primates. Utilization of these methods could improve our understanding of the expression and mechanisms of different substance use disorders towards the development of safer and more effective therapeutic strategies.

#### 4.1 Establishing drug-vs-food choice in rats

The training method described in this manuscript was effective in establishing drug-vs-food choice behavior in male and female rats with both opioids (i.e., fentanyl and heroin) and monoamine transporter ligands (i.e., cocaine, methamphetamine, and amphetamine). Although there were differences in the number of sessions until stable operant choice behavior was established between the different drugs, all five drugs and an opioid/stimulant mixture could be established as reinforcers under the current drug-vs-food choice procedure. Furthermore, all five drugs maintained dose-dependent increases in choice behavior over an alternative nondrug reinforcer consistent with published results in rats (Beckmann et al., 2019; Chow and Beckmann, 2020; Thomsen et al., 2008), nonhuman primates (Findley et al., 1972; Maguire et al., 2013; Nader and Woolverton, 1992; Negus, 2006; Negus, 2003; Townsend et al., 2020a), and humans (Comer et al., 1998; Foltin et al., 2015; Hart et al., 2000; Heishman et al., 2000; Lile et al., 2020). However, the present results may appear to be inconsistent with other published drug-choice studies in rats using discrete-trial procedures, wherein preference of the drug reinforcer is observed only in a relatively small subset of the experimental subjects (Augier et al., 2018; Canchy et al., 2020; Cantin et al., 2010; Caprioli et al., 2015; Lenoir et al., 2013b; Lenoir et al., 2007; Tunstall et al., 2014; Venniro et al., 2018). We do not interpret our results as inconsistent with these published drug choice studies in rats, but rather a robust demonstration of the effectiveness of alternative nondrug reinforcers in decreasing the potency of abuse drugs to function as reinforcers in a choice context. As has been consistently demonstrated in these discrete trial choice procedures, experimental manipulations that decrease the relative reinforcing effectiveness of these nondrug reinforcers results in orderly behavioral reallocation towards



drug and away from the nondrug alternative reinforcer (Canchy et al., 2020; Cantin et al., 2010; Vandaele et al., 2016; Venniro et al., 2018). Thus, we hypothesize that these differences in drug-choice behavior merely reflect differences in individual laboratory research methods and interests as opposed to fundamentally different behavioral phenotypes.

The environmental context has consistently been shown to impact operant drug self-administration behavior since the initial study by Weeks (1962). For example, recent studies have argued that the presence or absence of drug at the time that operant behavior occurs influences subsequent behavioral allocation between drug and nondrug reinforcers (Canchy et al., 2020; Freese et al., 2018; Vandaele et al., 2016). In the present experiments, the non-contingent fentanyl injection functioning as a discriminative stimulus before the response component was an important determinant of subsequent behavioral allocation between fentanyl and food compared to the visual discriminative stimuli. This result was perhaps not surprising given the poor visual acuity of albino rats compared to nonhuman primates. Nonetheless, the present results were consistent with previous results with cocaine as the discriminative or consequent stimulus (Thomsen et al., 2013). Thus, the present results manipulating the non-contingent or contingent fentanyl infusions are somewhat consistent with this interpretation suggesting the presence of drug in the subject (and at a sufficient concentration) can influence subsequent drug-vs-food choice behavior. Furthermore, this non-contingent drug infusion did not appear to significantly alter food-maintained responding as demonstrated by the results in the heroin FR100 experiment where exclusive food choice was reported and no robust decrease in operant responding. Moreover, the present fentanyl results are consistent with similar experiments examining cocaine-vs-food choice behavior in rats (Beckmann et al., 2019; Chow and Beckmann, 2020; Thomsen et al., 2013) and drug-vs-food choice in monkeys (Banks et al., 2011; Gasior et al., 2004; Negus, 2003; Townsend et al., 2019c). Overall, the present results suggest the non-contingent drug infusion in the present drug-vs-food choice procedure was the primary discriminative stimulus, more so than the stimulus lights, that facilitated subsequent drug-choice behavior. To the degree that this non-contingent drug infusion is a limitation of the current procedure, a future direction to refine the drug-vs-food choice procedure would be to remove the non-contingent drug infusion serving as the discriminative stimulus.

## 4.2 Environmental manipulations of choice behavior

Manipulating either the liquid-food concentration or the response requirement on the food- or opioid-associated lever resulted in systematic changes in behavioral allocation between IV opioid infusions and food. The present results are consistent with the published drug-choice literature in rats (Chow and Beckmann, 2020), monkeys (Cantin et al., 2010; Nader and Woolverton, 1991; Negus, 2003; Thomsen et al., 2013), and humans (Comer et al., 1998; Heishman et al., 2000; Lile et al., 2016). Collectively, the present results and the literature cited above are consistent with the view that drug self-administration is value-based and sensitive to environmental contingencies (Epstein et al., 2018; Pickard, 2020; Venniro et al., 2020). However, implementing these contingencies in our natural environment such as the cost or magnitude of nondrug reinforcers is easier said than done compared to controlled laboratory environments.

### 4.3 Pharmacological manipulations of choice behavior

One focus of preclinical drug self-administration procedures is the evaluation of candidate medications for substance use disorders. Preclinical drug self-administration procedures have good translational concordance in being *sensitive* to currently approved substance use disorder medications (Czoty et al., 2016; Haney and Spealman, 2008; Mello and Negus, 1996; Venniro et al., 2020). Moreover, preclinical drug-choice procedures have emerged as being both *sensitive* to clinically utilized medications and *selective* against novel therapeutics that have failed in either human laboratory drug self-administration studies or clinical trials (Negus and Banks, 2020; Townsend et al., 2020b; Venniro et al., 2020). In the present study, buprenorphine maintenance-induced decreases in heroin-vs-food choice in rats was consistent with nonhuman primate opioid-vs-food choice studies (Negus, 2006; Townsend et al., 2020a) and human laboratory drug-choice studies and clinical trials (Comer et al., 2005; Greenwald et al., 2002; Mello and Mendelson, 1980; Nasser et al., 2016). The present buprenorphine results were also generally consistent with acute and chronic buprenorphine treatments on opioid self-administration in rats (Bossert et al., 2020; Hammerslag et al., 2020). Overall, the present results and the extant literature cited above support the utility of preclinical drug-vs-food choice procedures in evaluating substance use disorder medications.

### 4.4 Utility of drug choice procedures for drug interactions and polysubstance abuse

Concurrent abuse of an opioid and a psychostimulant has been and continues to be a common form of polydrug abuse. Drug choice procedures may have utility in investigating drug interactions on reinforcement endpoints because the primary dependent measure (i.e. choice) is less sensitive to disruptions in operant behavior that result from reinforcement-independent drug effects (e.g. motor competence) (Banks and Negus, 2012). Using dose-addition analysis, the present results suggest that a fixed-proportion of fentanyl and methamphetamine interacted synergistically in a drug-vs-food choice procedure in nonopioid-dependent rats. Previous results in nonopioid-dependent rhesus monkeys reported that heroin and cocaine interacted in an additive manner in a drug-vs-food choice context (Negus, 2005). Whether the synergistic interaction between fentanyl and methamphetamine in the present study reflects differences in the mechanism of action of the stimulant (i.e., cocaine as a monoamine transporter inhibitor vs. methamphetamine as a monoamine transporter substrate), species differences, or the relative proportion of fentanyl and methamphetamine in the drug mixture, remains to be empirically determined. Nonetheless, the present results provide empirical evidence for the utility of drug-choice procedures to investigate the expression, mechanisms, and potential candidate medications for polysubstance abuse.

Fentanyl and methamphetamine interactions were also examined in the context of extended access drug self-administration. The length of the drug self-administration session has been an established independent variable in behavioral pharmacology research (Ahmed and Koob, 1998; Ahmed et al., 2000; Aigner and Balster, 1978; Deneau et al., 1969; Johanson, 1975). For example, previous research in both rhesus monkeys (Griffiths et al., 1975; Negus, 2006) and rats (Lenoir et al., 2013b; Townsend et al., 2019a) has demonstrated that extended access opioid self-administration conditions may produce opioid dependence and

withdrawal-associated increases in opioid-vs-food choice. In contrast, extended access cocaine self-administration did not significantly impact cocaine-vs-food choice in monkeys (Banks and Negus, 2010) or rats (Lenoir et al., 2007). The fentanyl/methamphetamine mixture in the present study was self-administered to sufficient levels during extended access conditions to produce opioid dependence, expression of spontaneous somatic withdrawal signs, and withdrawal-associated increases in fentanyl/methamphetamine choice despite no time-dependent increase in drug intake or “escalation.” Previous studies examining time-dependent changes in drug intake for either fentanyl or methamphetamine alone demonstrated that a similar fentanyl dose (1.25 µg/kg/infusion) failed to show “escalated” intake (Wade et al., 2015), whereas a similar methamphetamine dose (50 µg/kg/infusion) did show “escalated” methamphetamine intake (Kitamura et al., 2006). The present fentanyl/methamphetamine results also suggest that “escalated” drug intake during extended access self-administration sessions was not necessary for enhancing drug-vs-food choice during periods of drug withdrawal. Moreover, these fentanyl and methamphetamine interaction results highlight one potential method of evaluating the effects of different drug access conditions on measures of drug reinforcement that are less sensitive to reinforcement-independent rate-disrupting effects of the self-administered drug or drug mixture.

## 5. Conclusions

The training protocols and experiments described in this manuscript demonstrate the broad applicability of preclinical drug-vs-food choice procedures in rats to address translational research questions related to the expression, mechanisms, and treatment of substance use disorders. Drug-vs-food choice in rats was found to be sensitive to environmental manipulations that were consistent with results in both nonhuman primates and human laboratory studies. Opioid-vs-food choice was also sensitive to pharmacological treatments known to be effective in reducing opioid self-administration in both nonhuman primates and humans. Drug-choice procedures may also have utility in improving our mechanistic understanding of drug mixture interactions on reinforcement-related endpoints. In summary, our hope is this manuscript will serve as a useful resource for scientists interested in incorporating drug choice procedures into their research programs.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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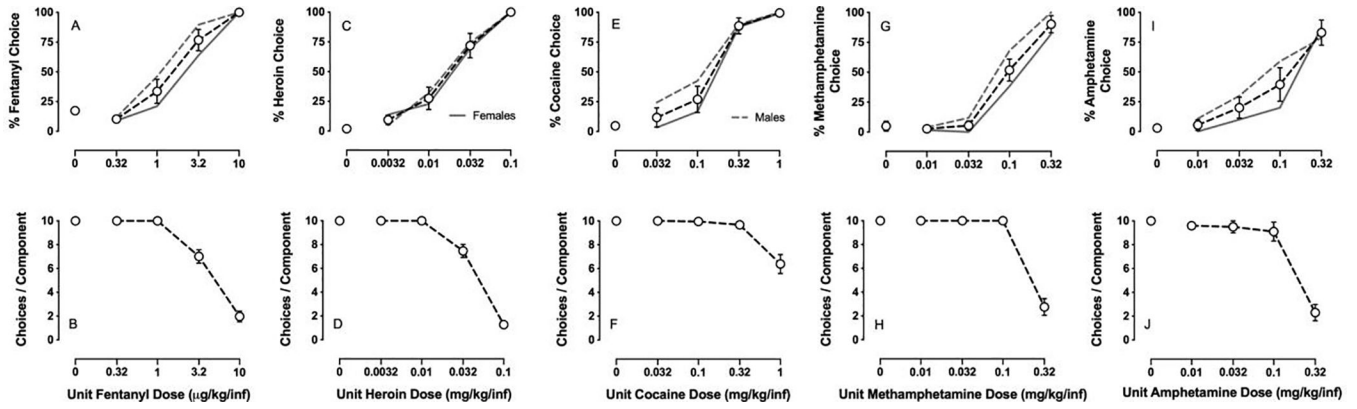
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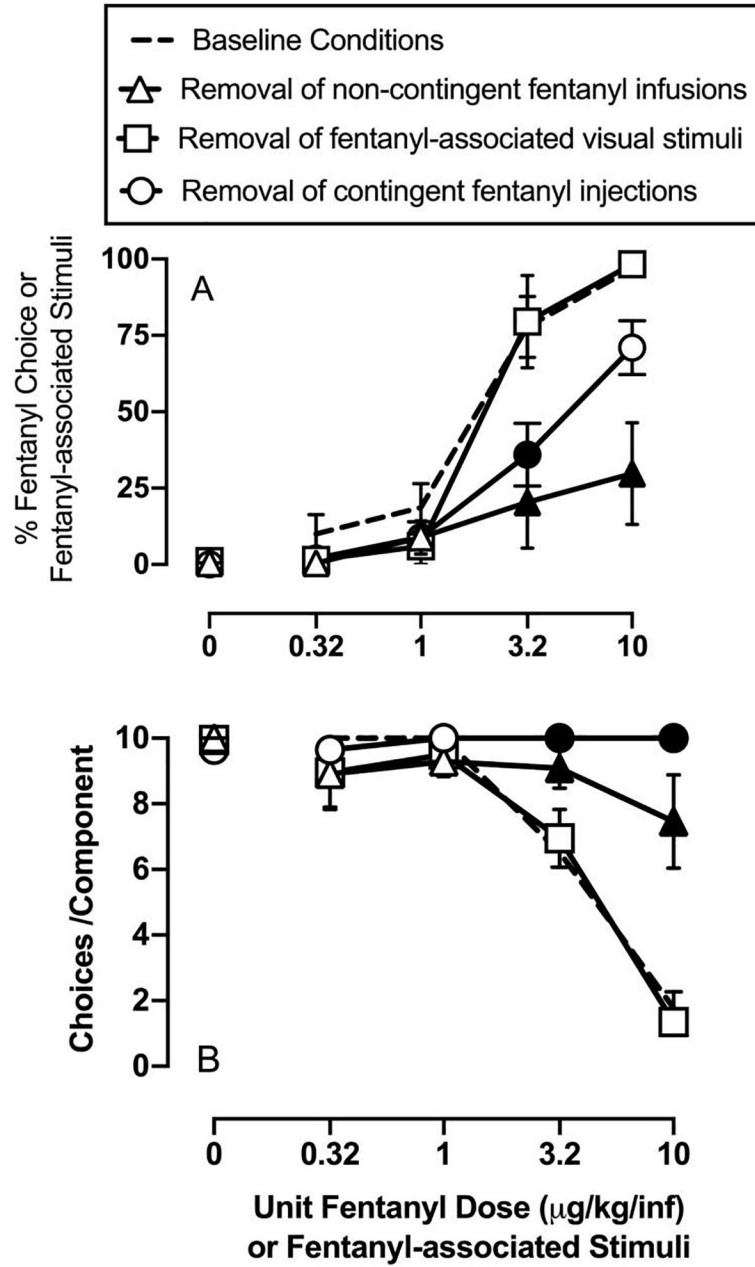
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**Highlights**

- Surgical technique resulted in median first catheter life of 18 weeks
- Intravenous drug-vs-food choice could be trained in rats within ~27 sessions
- Opioid, stimulant, and opioid+stimulant drug-vs-food choice was established
- Consistency between monkey and rat drug choice results supports translatability

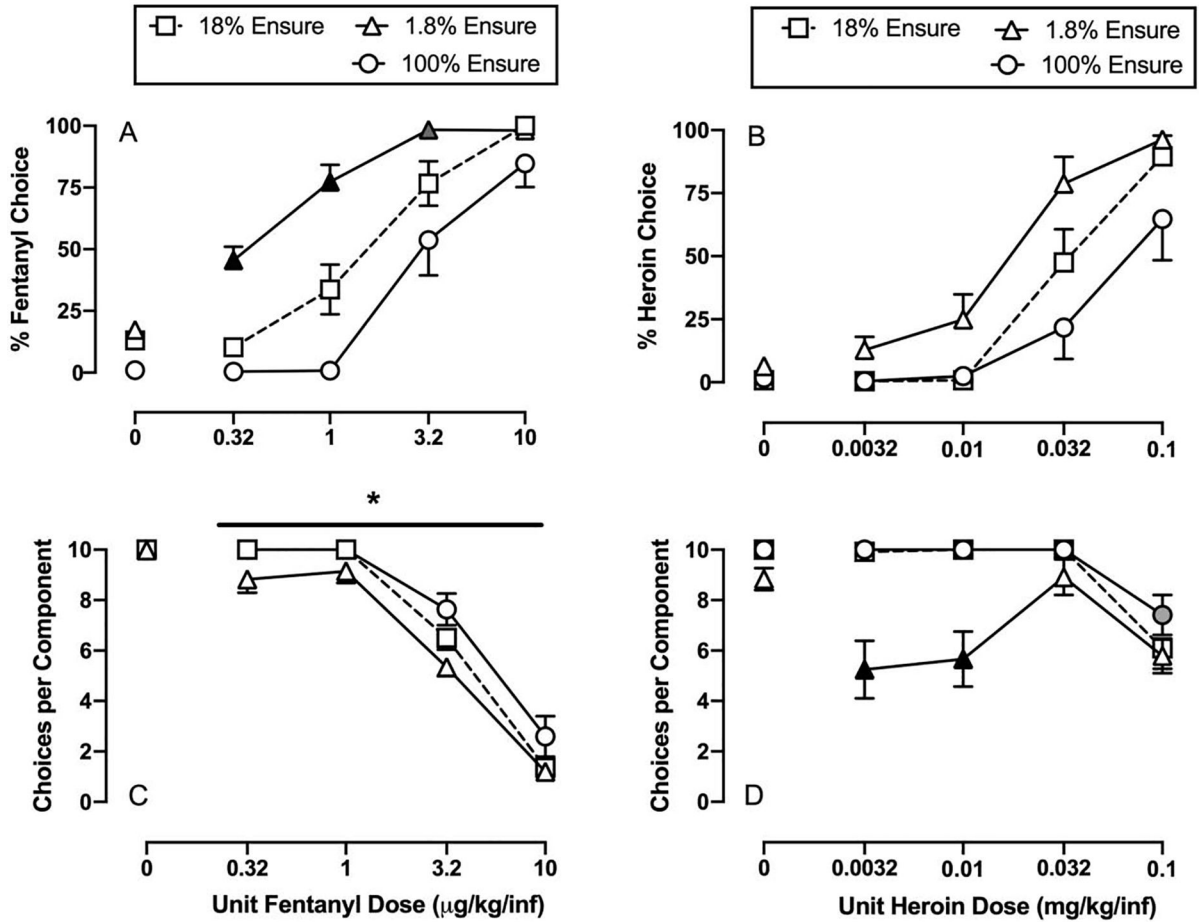


**Figure 1:** Demonstration of intravenous (IV) drug-vs-food choice with (A, B) fentanyl, (C, D) heroin, (E, F) cocaine, (G, H) methamphetamine, and (I, J) amphetamine in male and female rats. Top panels show percent drug choice as a function of unit drug dose in either micrograms or milligrams per kilogram per infusion. Bottom panels show the number of choices completed per component as a function of the unit dose drug available as the alternative to liquid food (18% for fentanyl and methamphetamine; 18 and 32% for heroin; 32% for cocaine and amphetamine). All points represent the mean  $\pm$  s.e.m. of the follow group sizes: fentanyl (12 rats); heroin (12 rats); cocaine (10 rats); methamphetamine (12 rats); amphetamine (9 rats). Dashed gray line shows mean data from males whereas solid gray line shows mean data from females.

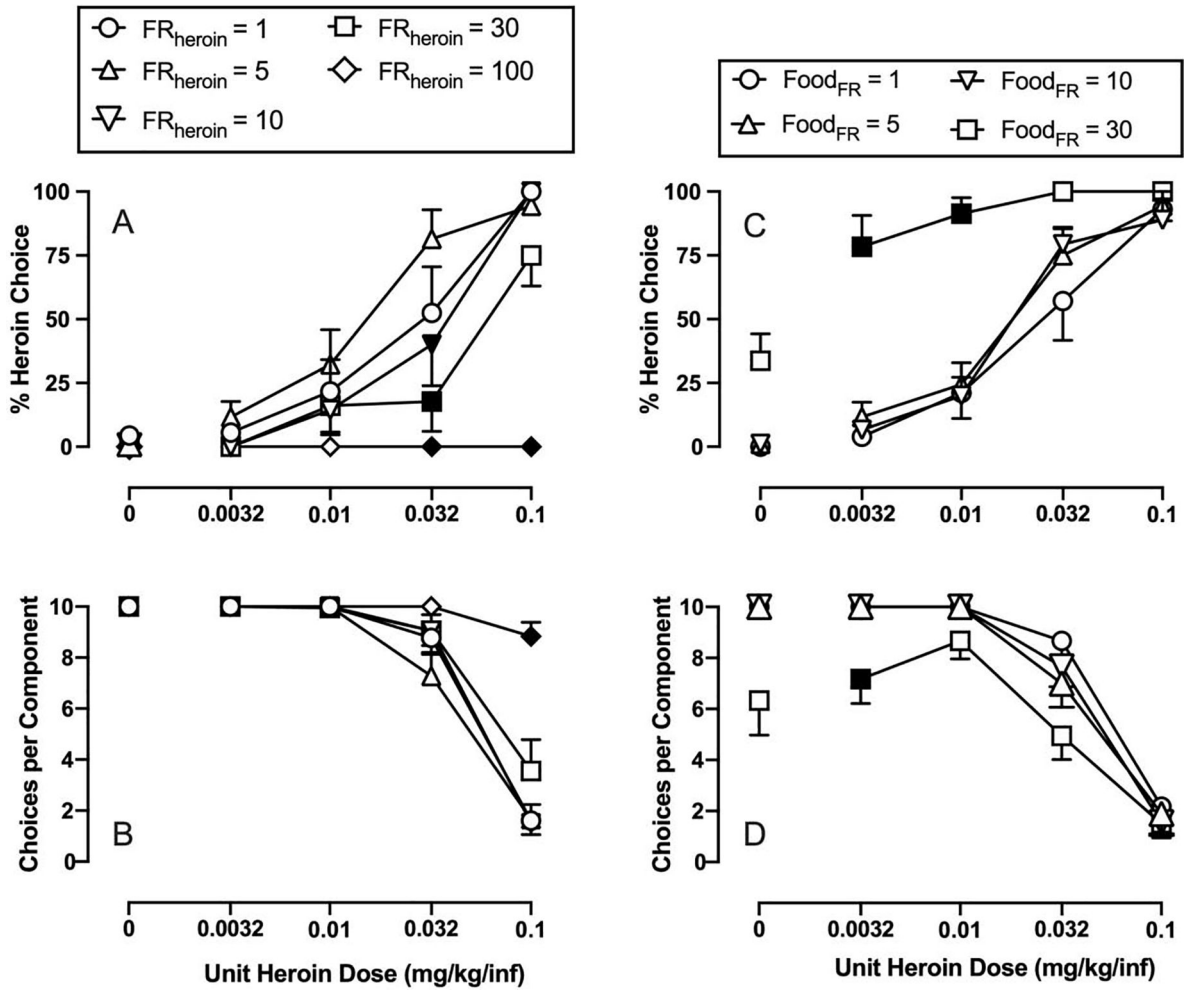


**Figure 2:**

Effects of discriminative- and consequent-stimulus manipulations on fentanyl-vs-food choice in male and female rats. Top panel shows percent choice of fentanyl or fentanyl-associated stimuli as a function of unit fentanyl dose in micrograms per kilogram per infusion. Bottom panels show the number of choices completed per component as a function of the unit fentanyl dose or fentanyl-associated stimuli available as the alternative to 18% liquid food. All points represent the mean  $\pm$  s.e.m. of 6 rats. Filled symbols denote statistical significance ( $p < 0.05$ ) compared to baseline conditions.

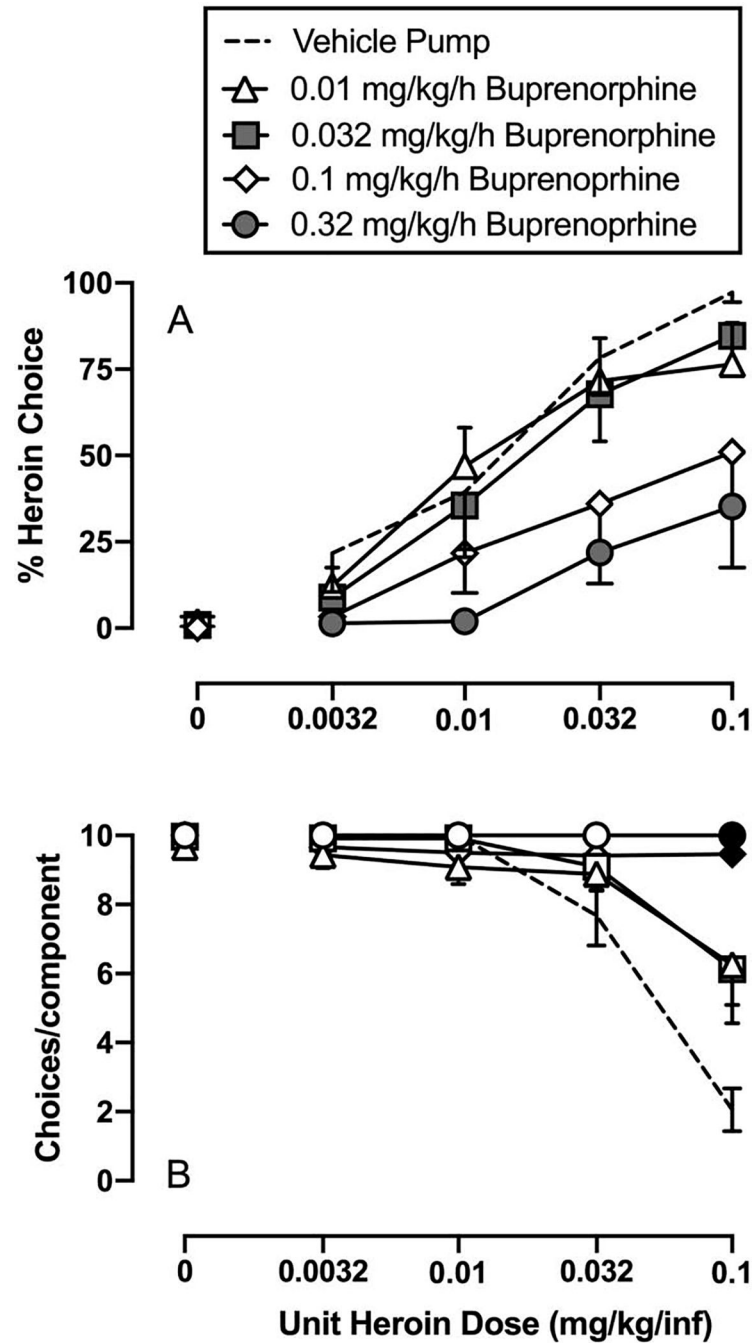


**Figure 3:** Food reinforcer-magnitude manipulations on fentanyl-vs-food choice (Panels A and C) and heroin-vs-food choice (Panels B and D) in male and female rats. Top panels show percent drug choice as a function of unit drug dose in either micrograms or milligrams per kilogram per infusion. Bottom panels show the number of choices completed per component as a function of the unit dose drug available as the alternative to liquid food (1.8–100%). All points represent the mean ± s.e.m. of 12 rats in the fentanyl experiments and 8 rats in the heroin experiments. Black filled symbols denote statistical significance ( $p < 0.05$ ) compared to all other liquid food concentrations and gray filled symbols denote statistical significance compared to 18% liquid food only. Asterisk denotes statistical significance between 1.8% and 100% liquid food regardless of unit fentanyl dose.

**Figure 4:**

Response-requirement manipulations on either the heroin-associated lever (Panels A and B) or food-associated lever (Panels C and D) on heroin-vs-food choice in male and female rats. Top panels show percent heroin choice as a function of unit heroin dose in milligrams per kilogram per infusion. Bottom panels show the number of choices completed per component as a function of the unit heroin drug dose available as the alternative to 18% liquid food. All points represent the mean  $\pm$  s.e.m. of 6 rats. Filled symbols denote statistical significance ( $p < 0.05$ ) compared to baseline conditions (i.e. FR5 for both liquid food and heroin).





**Figure 5:** Effects of 7-day continuous 0.01–0.32 mg/kg/h buprenorphine treatment on heroin-vs-food choice in male and female rats. Top panels show percent heroin choice as a function of unit heroin dose in milligrams per kilogram per infusion. Bottom panels show the number of choices completed per component as a function of the unit heroin drug available as the alternative to 18% liquid food. All points represent the mean  $\pm$  s.e.m. of 6 rats, except the 0.32 mg/kg/h buprenorphine dose, which is 5 rats. Gray filled symbols in Panel A denote statistical significance ( $p < 0.05$ ) compared to vehicle pump conditions regardless of unit

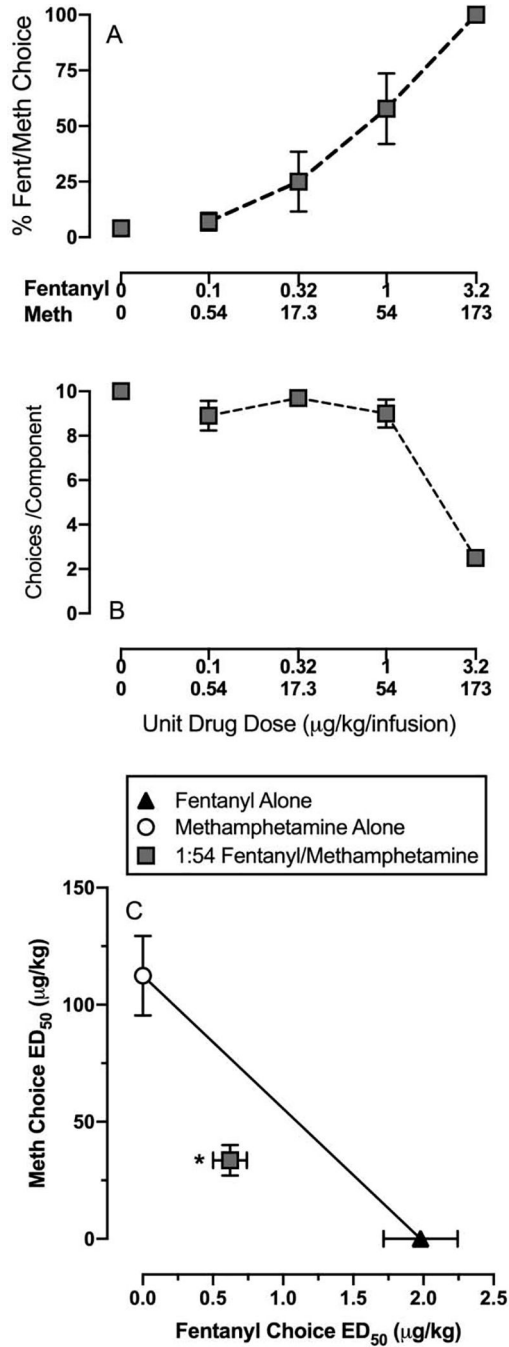
heroin dose, whereas black filled symbols in Panel B denote statistical significance compared to vehicle pump conditions within a unit heroin dose.

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**Figure 6:**

Interactions between fentanyl and methamphetamine in a drug-vs-food choice procedure in nonopioid-dependent male and female rats. Top panel (A) shows percent drug choice for a 1:54 fentanyl/methamphetamine mixture, which corresponds to a 1:1 ED<sub>50</sub> choice mixture based on the potency of each drug alone. Middle panel (B) shows the number of choices completed per component as a function of the unit drug-mixture dose available as the alternative to 18% liquid food. Bottom panel (C) shows an isobologram for interactions between fentanyl and methamphetamine on drug choice. All points represent the mean ±

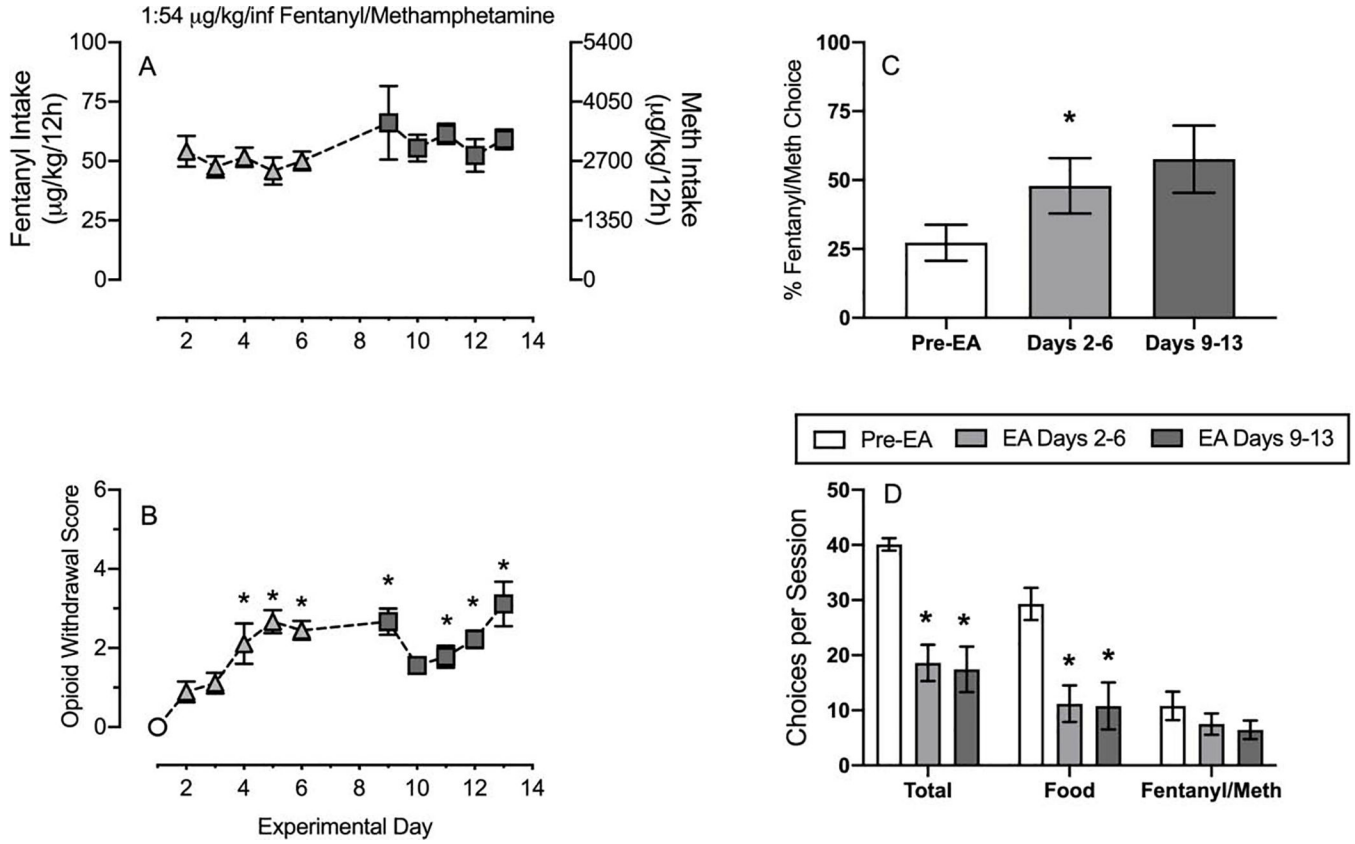
s.e.m. of 10 rats. Asterisk denotes statistically significant difference from predicted additivity.

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**Figure 7:**

Effects of 12h overnight extended access (EA) 1:54 fentanyl/methamphetamine self-administration on subsequent fentanyl/methamphetamine-vs-food choice in male and female rats. Panel A shows fentanyl (left ordinate) and methamphetamine (right ordinate) intake over the 10 EA sessions. Panel B shows opioid withdrawal scores 5-min before each fentanyl/methamphetamine choice session as a function of experimental period. Panel C shows percent session fentanyl/methamphetamine choice pre-EA and after one week (Days 2–6) and two weeks (Days 9–13) of EA fentanyl/methamphetamine self-administration. Panel D shows total session choices, total food choices, and total fentanyl/methamphetamine choices as a function of experimental period. All points represent the mean  $\pm$  s.e.m. of 10 rats. Asterisks denote statistical significance ( $p < 0.05$ ) compared to pre-extended access conditions.

**Table 1:**

Group mean ( $\pm$  SD) number of sessions under each of the training conditions until drug-vs-food choice in male and female rats was stable for each of the self-administered drugs listed.

Self-administered Drug	Drug Only Sessions	Food Only Sessions	Choice Sessions	Total Sessions
Cocaine (n=10)	12 ( $\pm$ 4)	4 ( $\pm$ 1)	5 ( $\pm$ 0)	21 ( $\pm$ 5)
Fentanyl (n=12)	12 ( $\pm$ 4)	4 ( $\pm$ 1)	9 ( $\pm$ 5)	25 ( $\pm$ 5)
Methamphetamine (n=12)	12 ( $\pm$ 4)	3 ( $\pm$ 0)	11 ( $\pm$ 5) *	26 ( $\pm$ 8)
Amphetamine (n=9)	13 ( $\pm$ 2)	3 ( $\pm$ 1)	15 ( $\pm$ 6) *,#	30 ( $\pm$ 6) *
Heroin (n=12)	12 ( $\pm$ 4)	4 ( $\pm$ 3)	16 ( $\pm$ 6) *,#	32 ( $\pm$ 6) *,#
1:54 Fentanyl/Methamphetamine (n=10)	8 ( $\pm$ 2)	4 ( $\pm$ 2)	6 ( $\pm$ 2)	17 ( $\pm$ 6)

The sample size for each of the different self-administered drugs is shown.

\* denote statistical significance ( $p < 0.05$ ) compared to cocaine or 1:54 fentanyl/methamphetamine and

# denotes statistical significance compared to fentanyl.



**Table 2:**

ED<sub>50</sub> choice values in µg/kg/infusion (95% confidence limits) for fentanyl alone, methamphetamine alone, and a 1:54 fentanyl/methamphetamine mixture in male and female rats.

	Fentanyl ED <sub>50</sub>	Methamphetamine ED <sub>50</sub>
Fentanyl Alone (n=12)	2.0 (1.5 – 2.6)	–
Methamphetamine Alone (n=12)	–	112.4 (83 – 152.3)
1:54 Fentanyl/Methamphetamine (baseline; n=10)	0.6 (0.4 – 0.9)	33.6 (22.7 – 49.7)

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