Effects of dose on acquisition and persistence of a new response for a remifentanil-associated stimulus

Stephen H. Robertson and Emily M. Jutkiewicz

Previous research demonstrated that a remifentanil-associated stimulus facilitated the acquisition of a previously unlearned response; however, it is unclear how long a remifentanil-associated stimulus maintains conditioned reinforcing properties under conditions of daily testing. To address this gap, we exposed adult male rats to response-independent stimulus presentations and deliveries of remifentanil (1.0, 3.2, or 10.0 μg/kg/infusion). Rats either received the stimulus presentations and remifentanil deliveries together (Paired Pavlovian conditioning) or according to separate clocks (Random control group). In the sessions following Pavlovian conditioning, we allowed rats to emit nose-poke responses for the presentation of the stimulus alone and measured the extent to which the stimulus facilitated and maintained a previously unlearned response. We tested responding for the stimulus presentations across 28 daily sessions to assess the Pavlovian extinction (degradation of the drug-stimulus association) of the conditioned reinforcing properties of the remifentanil-associated stimulus. We observed the highest and most persistent levels of responding in rats with a Paired Pavlovian conditioning history at 3.2 and 10.0 μg/kg/infusion. In addition, we included analyses of the variability in responding for each group, which revealed individual differences in the susceptibility of the remifentanil-associated stimulus acting as a conditioned reinforcer. These findings demonstrate that a remifentanil-associated stimulus has the ability to sustain drug-seeking behavior and underscores the importance of Pavlovian conditioning in promoting drug abuse. *Behavioural Pharmacology* 31: 207–215 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc.

Department of Pharmacology, University of Michigan, Ann Arbor, Michigan, USA

Correspondence to Emily M. Jutkiewicz, PhD, Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109, USA
E-mail: ejutkiew@umich.edu

Introduction

Stimuli that have been repeatedly paired with the self-administration of a psychoactive drug take on conditioned reinforcing properties, which can then play a role in maintaining drug use and abuse (Di Ciano and Everitt, 2004; Everitt and Robbins, 2005; Stewart *et al.*, 1984). The observation that drug-associated stimuli have the ability to function as conditioned reinforcers has been documented in non-human animals across procedures, such as second-order schedules (Whitelaw *et al.*, 1996; Everitt and Robbins, 2000; Ito *et al.*, 2002; Vanderschuren *et al.*, 2005) and procedures in which a response-contingent drug-associated stimulus only is presented during extinction (instrumental extinction; Schuster and Woods, 1968; Weiss *et al.*, 2001). In humans, exposure to drug-associated cues in a laboratory setting has been shown to lead to increased drug-seeking (Hogarth *et al.*, 2008) and drug-taking (Hogarth *et al.*, 2010) as well as increased dopamine D_2 activity in the dorsal striatum (Volkow *et al.*, 2006). Due to the ability of drug-associated stimuli to function as conditioned reinforcers, they are thought to play a critical role in relapse (Carroll and Comer, 1996).

The new response acquisition procedure has been proposed as a particularly rigorous procedure for evaluating conditioned reinforcement (Davis and Smith, 1976; Bertz and Woods, 2013; Bertz *et al.*, 2015; Bertz *et al.*, 2016). The new response acquisition procedure is divided into two phases. In phase 1, a Pavlovian conditioning procedure is used to establish an association between the presentation of a stimulus (e.g. illumination of a light) and drug delivery, such that rats receive response-independent intravenous drug infusions that are delivered concurrently with the presentation of a stimulus (Paired Pavlovian conditioning). The control group (Random control group) receives the same number of drug deliveries and stimulus presentations, but they are scheduled according to two independent clocks (i.e. not explicitly unpaired). This serves as an appropriate control group because the contingency between the two events is removed, but animals in the control group receive the same number of drug infusions and stimulus presentations as the experimental group (Rescorla, 1967). In phase 2, manipulanda are placed in the operant chamber (e.g. nose-pokes) and rats are allowed to respond for presentations of the stimulus alone to test the extent to which the stimulus functions

as a conditioned reinforcer for a previously unlearned response. This procedure meets the criteria outlined by Williams (1994) that behavior is under the control of conditioned reinforcement because it is maintained by a previously arbitrary stimulus that has gained reinforcing properties due to the relation between that stimulus and a primary (drug) reinforcer.

Using this procedure, Bertz and Woods (2013) investigated the extent to which rats would respond for a remifentanil- (a short-acting mu-opioid receptor agonist) associated stimulus. Generally, they found that rats that received remifentanil infusions paired with the presentation of the stimulus made a higher number of responses to the active nose-poke than rats that received random remifentanil infusions and stimulus presentations and made a higher number of responses on the active nose-poke relative to the inactive nose-poke. This study demonstrated that remifentanil-associated stimuli functioned as a conditioned reinforcer that promoted the acquisition and maintenance of a previously unlearned response; however, the extent to which responding will persist, as well as how persistence in responding interacts with the training dose of remifentanil, is unknown.

Some researchers have investigated persistence of the conditioned reinforcing properties of a drug-associated stimulus. For instance, drug-paired stimuli have been shown to maintain conditioned reinforcing properties for 14 (Di Ciano *et al.*, 2008) to 16 (Di Ciano *et al.*, 2007) days under conditions of daily testing and up to 59 days when tested intermittently (Di Ciano and Everitt, 2004). However, these studies did not continue to test responding for a drug-associated stimulus until drug-seeking reduced to control levels. As such, it remains unclear the extent to which the drug-associated stimulus retains its value as a conditioned reinforcer.

In the current study, we assessed persistence of responding for a remifentanil-associated stimulus across 28 daily acquisition sessions. Further, we examined individual differences in the in conditioned reinforcing properties of the remifentanil-associated stimuli, which may begin to inform an understanding of individual susceptibility to drug abuse.

Methods
Subjects
Male Sprague–Dawley rats (n = 48) from Envigo (Haslett, Michigan, USA) were housed in a temperature- (21–23°C) and humidity-controlled colony on a 12 h light/dark cycle with lights on at 0700. All animals were provided with food (LabDiet, 5L0D) and water *ad libitum*. The experimental procedures were approved by the University of Michigan Committee on the Care and Use of Animals.

Surgery
Animals were implanted with an indwelling catheter placed in the femoral vein for intravenous (i.v.) infusions of remifentanil. Animals were first anesthetized via *i.p.* injections of ketamine (90 mg/kg) and xylazine (10 mg/kg). Carprofen (5 mg/kg) was administered via a subcutaneous injection before surgery and 24 h after surgery. After making an incision approximately 1 cm from the leg, the femoral vein was isolated and a catheter (Micro-Renathane Tubing, MRE-040; Braintree Scientific Inc., Braintree, Massachusetts, USA) was inserted into the vein. The catheter was led subcutaneously to a mesh backplate (PlasticsOne, Roanoke, Virginia, USA, 8I313000BM14) that was sutured between the scapulae. The backplate had a 22-gauge stainless steel tube for externalization. Catheters were maintained daily via flushing with 0.5 ml of heparinized saline (50 USP/ml).

Apparatus
Six Med Associates standard operant chambers (Med Associates Inc., St. Albans, Vermont, USA) housed in sound-attenuating cubicles were used. For Pavlovian conditioning, a house light, which was situated (18.5 cm above the grid floor) on the left side of the chamber and a speaker, which was situated (16 cm above the grid floor) on the right wall of the chamber. The speaker was used to play a white noise stimulus using the multipurpose sound generator (ENV-230). Each speaker was calibrated to play white noise at 80 dB using the Med Associates sound pressure level measurement package (ANL-929A-PC). No response manipulandum were present in the chamber during the Pavlovian conditioning phase. For drug infusions, a 10 ml syringe was attached to tubing and placed into a variable infusion rate syringe pump (Med Associates, PHM 107) that was used to deliver remifentanil. The tubing was attached to a 22 G metal tube on a swivel attached to a drug delivery arm. A plastic tubing tether was run from the swivel and secured to the externalized stainless-steel tube on a rat's backplate. The tether was covered by a metal spring. White noise was produced using a sound generator (Med Associates, ENV 230).

For the Acquisition phase, two nose-poke manipulanda (ENV-114BM) were added to the right wall of the chamber below the speaker, 7 cm above the grid floor and approximately 9 cm apart. Each nose-poke was illuminated by a single LED light throughout the sessions in Acquisition phase. One nose-poke was active and the other nose-poke was used as an inactive control. All responses made on both nose-pokes were recorded.

Procedure
Pavlovian conditioning
For the Pavlovian conditioning phase, we assigned rats to one of three dosing conditions: 1.0, 3.2, or 10.0 µg/kg/infusion. These doses were selected based on the findings of Bertz *et al.* (2016). Within each dosing condition, rats were assigned to Paired Pavlovian (n = 8 per dose) or Random control (n = 8 per dose) conditioning. For

the Paired Pavlovian conditioning group, an infusion of remifentanil paired with the stimulus presentation (white noise + house light illumination) was delivered according to a variable time (VT) 3-min schedule, under which the remifentanil-stimulus pairings occurred on average once every 3 mins (range 0–6 min). For the Random control group, animals received infusions of remifentanil and presentations of white noise and the houselight illumination that were delivered according to two independently operating VT 3-min schedules; however, the drug infusion and stimulus presentations were not explicitly paired. The VT 3-min schedule was selected to allow sufficient time to metabolize remifentanil between drug infusions (Bertz and Woods, 2013). Drug infusions and stimulus presentations lasted approximately 2.0 ± 0.5 s, depending on the rat's body weight. All rats received 20 infusions of remifentanil and 20 stimulus presentations per day for 5 days (100 total pairings). Each session lasted approximately 60 min ($\pm$5 min).

Instrumental acquisition
We determined the duration of the Instrumental Acquisition phase by assessing the level of active responding emitted by rats in the Paired Pavlovian conditioning group vs. the Random control group. We found that after 28 Acquisition sessions, there were no differences in the level of active responding across the conditioning groups. During Instrumental Acquisition, rats were attached to the tether (but the syringe was filled with saline and the pump did not operate during the session) and placed into the operant chamber. At the start of the Acquisition session, the nose-poke lights were illuminated and rats could emit nose-pokes for presentations of white noise and the houselight illumination only. The first response was programmed to produce the white noise + house light illumination. Following the first active response, responses produced stimulus presentations according to a random ratio (RR) 2 schedule of reinforcement. According to a RR 2 schedule, each response has a 50% chance of being reinforced. A RR 2 schedule was selected based on the findings of Bertz and Woods (2013). Each session lasted 60 min. Sessions were run 6–7 days per week for 28 days.

Drugs
Remifentanil (Ultiva brand; GlaxoSmithKline, Uxbridge, Middlesex, UK) was obtained from the University of Michigan hospital pharmacy and dissolved into sterile physiological saline.

Data analysis
In order to test omnibus differences between active and inactive responding as a function of conditioning history (Paired Pavlovian vs. Random control) and remifentanil dose (1.0, 3.2, or 10.0 µg/kg/infusion), data were analyzed using a mixed analysis of variance (ANOVA) with day and response type (active vs. inactive) as within-subject variables and dose of remifentanil and conditioning history were between-subjects variables. To assess differences as a function of conditioning history in active responding across the 28-day acquisition phase, we computed a series of independent t-tests for Paired vs. Random conditioning history for each dose. Next, we calculated preference scores by subtracting the number of inactive responses from the number of active responses for each day of acquisition. To better understand and visualize the magnitude of difference between preference scores of rats conditioned with Paired Pavlovian vs. Random control conditioning within each dose, we computed a Cohen's d effect size ($d = M_2 - M_1 / SD_{pooled}$) for each day of testing. Next, we assessed patterns of total active vs. inactive responding using a mixed ANOVA with response type as the repeated measure and conditioning history and dose as the between-subjects variable. Post-hoc tests were used to compare active vs. inactive responding within each dosing and conditioning group. For all independent t-tests and post-hoc tests, we adjusted for inflated type I error rate following multiple pairwise comparisons using a Bonferroni adjustment.

Results
We first analyzed the levels of responding as a function of conditioning history, dose of remifentanil, response type (active vs. inactive) and day of testing (Fig. 1). Rats that received 1.0 µg/kg/infusion showed similar levels of active and inactive responding, regardless of assignment to Paired Pavlovian conditioning or Random control group. Rats that received Paired Pavlovian conditioning using 3.2 or 10.0 µg/kg/infusion showed a greater level of active responding relative to inactive responding than rats assigned to the Random control group. The observation that rats that received Paired Pavlovian conditioning tended to emit more active responses than rats in the Random control procedure was supported by a main effect of conditioning that approached significance, $F(1, 42) = 3.08$, $P = 0.09$, $\eta_p^2 = 0.07$. Levels of active and inactive responding tended to decrease across days, which was supported by a main effect of day, $F(7.63, 320.56) = 21.08$, $P < 0.001$, $\eta_p^2 = 0.33$. Levels of active responding tended to be higher than levels of inactive responding, which was supported by a main effect of response type, $F(1, 42) = 69.30$, $P < 0.001$, $\eta_p^2 = 0.62$. There was no main effect of dose ($P = 0.89$). The level of responding depended on day of testing, response type, and conditioning, which was supported by a significant day × conditioning interaction, $F(7.63, 320.56) = 2.74$, $P = 0.007$, $\eta_p^2 = 0.06$, a significant response type × conditioning interaction, $F(1, 42) = 5.13$, $P = 0.03$, $\eta_p^2 = 0.11$, a day × response type interaction that approached significance, $F(9.56, 401.77) = 1.84$, $P = 0.06$, $\eta_p^2 = 0.04$, and a significant day × response type × conditioning interaction, $F(9.56, 401.77) = 2.08$, $P = 0.03$, $\eta_p^2 = 0.05$. These results further suggest that animals that received Paired Pavlovian conditioning tended to emit

Fig. 1
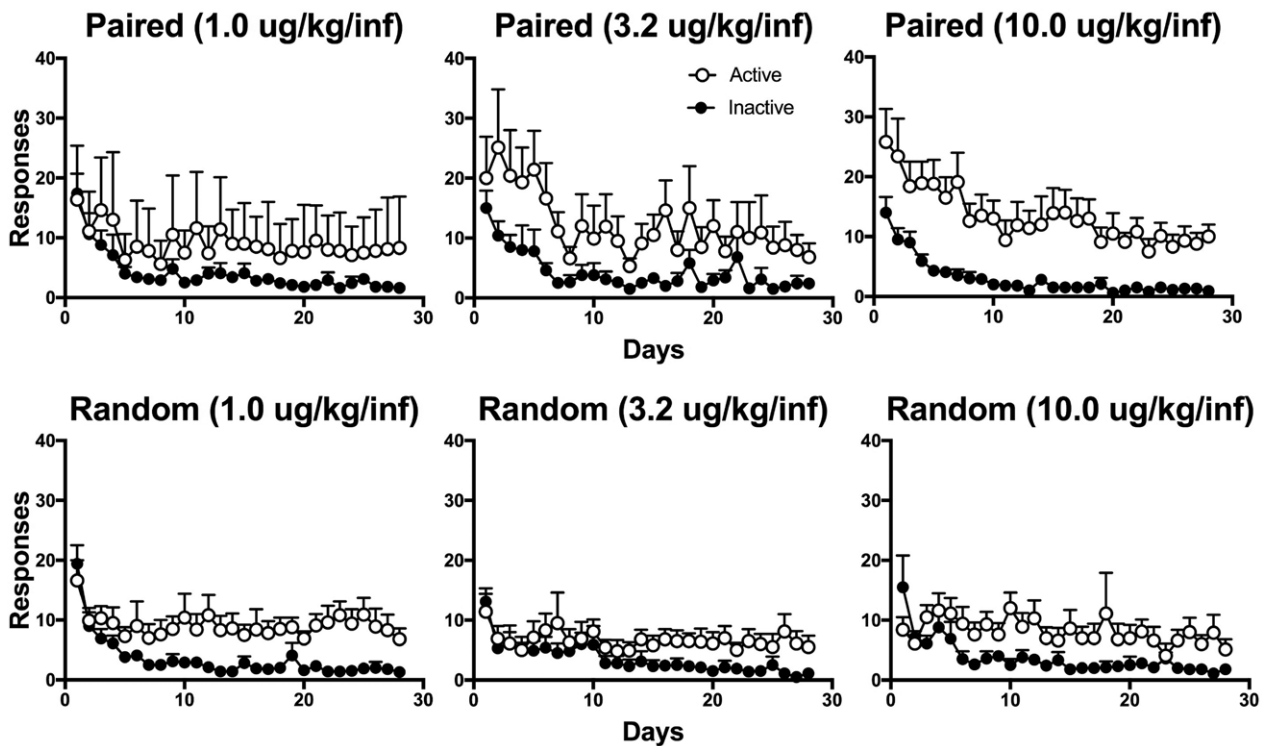
Shows average (±SEM) active (open circles) and inactive (filled circles) responses made as a function of Paired Pavlovian conditioning (top panel) or Random control procedure (bottom panel) for animals that received 1.0 µg/kg/infusion (left panel), 3.2 µg/kg/infusion (center panel), and 10.0 µg/kg/infusion (right panel) of remifentanil.

Fig. 2
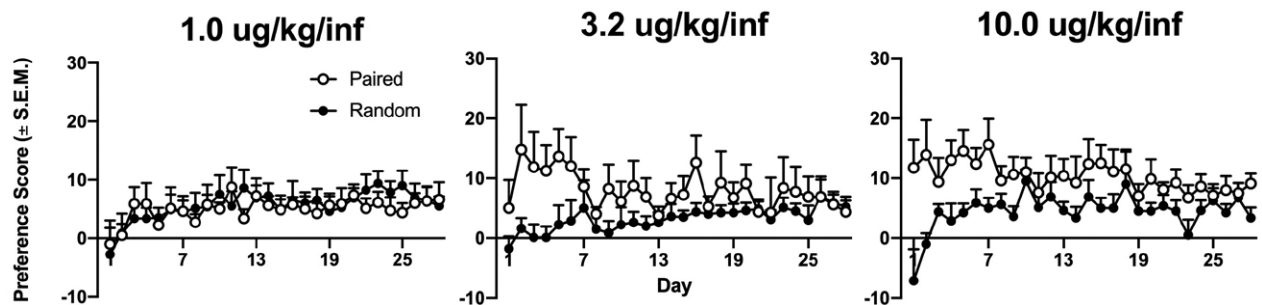
Shows average (±SEM) preference scores (active − inactive responses) for rats that received Paired Pavlovian conditioning vs. Random control procedure using 1.0 µg/kg/infusion (left panel), 3.2 µg/kg/infusion (center panel), and 10.0 µg/kg/infusion (right panel).

more active responses relative to inactive responses than rats in the Random control group. We found no additional interaction effects (P's > 0.16). Because we found effects of conditioning but not dose, we further probed these interactions by computing a series of independent t-tests to compare active responding between animals assigned to the Paired vs. Random control groups on each day of testing within each dose; however, none of the pair-wise comparisons reached statistical significance.

Next, we calculated preference scores by subtracting the number of inactive responses from active responses for each rat, which allowed us to account for both the number of active and inactive responses in a single value. We plotted the average preference score for each day within each dosing and conditioning group (Fig. 2). In order to further probe persistence, we calculated Cohen's d effect sizes (using the preference scores) for each day of the Acquisition phase (Fig. 3). Evaluating

Fig. 3
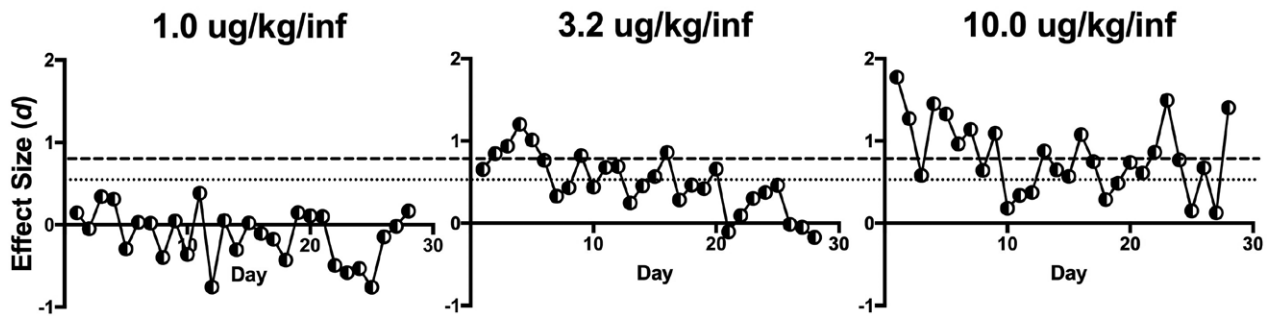
Shows Cohen's d ($M_2 - M_1/SD_{pooled}$) effect sizes that compare the magnitude of difference in preference scores in animals assigned to Paired Pavlovian conditioning vs. the Random control group for each day of testing. The dashed line refers to the conventional cutoff for a large effect size and the dotted line refers to the conventional cutoff for a medium effect size.

effect sizes enabled us to assess the magnitude of difference between preference scores as a function of conditioning history across the acquisition phase. There are two horizontal lines on the graphs to mark the conventional cutoffs for a large effect size (0.80, dashed line) and a medium effect size (0.50, dotted line). Using this data transformation, we found that animals conditioned with 1.0 µg/kg/infusion had no medium or large effect sizes. For animals conditioned with 3.2 µg/kg/infusion, we observed medium and large effect sizes for out to 20 days of testing. For animals conditioned with 10 µg/kg/infusion, we observed medium and large effect sizes out to 28 days of testing. As such, when persistence is quantified in this manner, we observed medium to large magnitude effects in active responding between animals with a Paired Pavlovian vs. Random control conditioning history that persisted for 20–28 days following conditioning for animals conditioned with 3.2 or 10 µg/kg/infusion, respectively.

We further probed the data to better understand patterns of active and inactive responding for individual animals. First, we calculated the total number of active and inactive responses emitted across the acquisition phase for each animal. The histogram (Fig. 4) shows the distribution of total active and inactive responses emitted across the 28 days of the acquisition phase for both conditioning groups at each dose of remifentanil. The bars show data for individual rats (rat identification numbers are plotted along the x-axis) and are rank-ordered according to the number of active responses emitted (highest to lowest). Plotting total active vs. inactive responses for individual animals enabled a clear observation of the variability between subjects within each dosing and conditioning group, such that some animals showed high levels of active responding, whereas other rats showed low levels of active responding.

We calculated the average of the total number of responses for each dosing and conditioning group

(Fig. 5). To characterize patterns in total active and inactive responding as a function of conditioning history and dose of remifentanil, we conducted a mixed ANOVA that analyzed the effects of conditioning history, dose, and response type on the total number of responses emitted. In general, rats tended to emit a higher number of active than inactive responses, regardless of condition, which was supported by a main effect of response type, $F(1, 42) = 65.87$, $P < 0.001$, $\eta_p^2 = 0.61$. In addition, we found an interaction between response type and conditioning history, $F(1, 42) = 4.46$, $P = 0.04$, $\eta_p^2 = 0.10$, supporting the observation that rats with a Paired Pavlovian conditioning history showed a higher number of total active responses (M = 320.63, SD = 203.69) than rats with an Random control history (M = 222.21, SD = 120.69). Rats that experienced Paired Pavlovian (M = 110.0, SD = 56.83) or Random control (M = 98.58, SD = 40.70) conditioning showed a similar level of inactive responding.

We investigated the extent to which rats emitted more active than inactive responses within each dose and conditioning group via a series of t-tests (adjusted critical cutoff: $P = 0.05/6 = 0.008$). For animals with a history of Paired Pavlovian conditioning at 1.0 µg/kg/infusion, $t(7) = 3.40$, $P = <0.012$, $d = 1.20$, 3.2 µg/kg/infusion, $t(7) = 2.86$, $P = 0.024$, $d = 1.01$, or 10.0 µg/kg/infusion, $t(7) = 4.78$, $P = 0.002$, $d = 1.69$, rats showed a higher level of total active vs. total inactive responding. Surprisingly, from the Random control group with 1.0 µg/kg/infusion, $t(7) = 3.18$, $P = 0.015$, $d = 1.13$, 3.2 µg/kg/infusion, $t(7) = 2.67$, $P = 0.032$, $d = 0.77$, or 10.0 µg/kg/infusion, $t(7) = 4.33$, $P = 0.003$, $d = 1.53$, also showed a higher level of total active vs. total inactive responding.

Discussion
We found that animals that had a Paired Pavlovian conditioning history showed a higher level of active responding relative to animals assigned to the Random control group, in particular for animals conditioned with 3.2 and 10.0 µg/kg/infusion of remifentanil. These findings are

Fig. 4
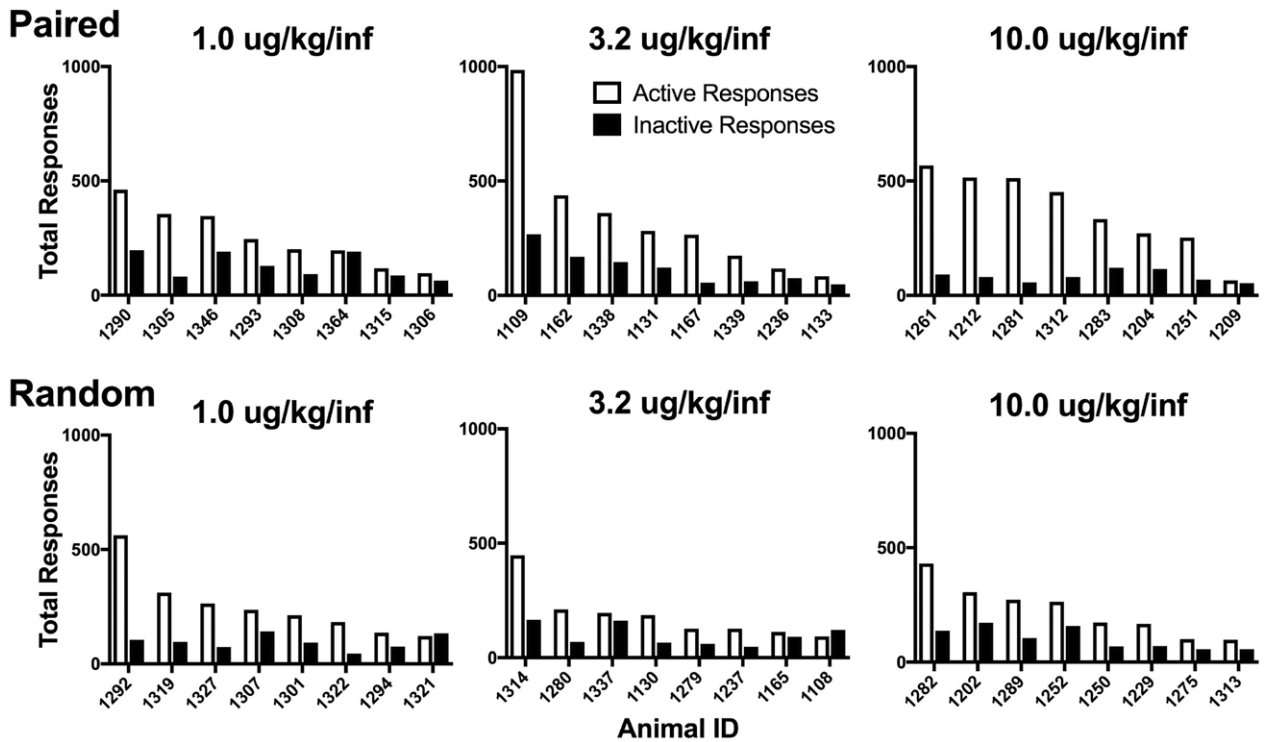
Shows total number of active (open bars) and inactive (filled bars) responses made as a function of Paired Pavlovian conditioning (top panel) or the Random control procedure (bottom panel) using 1.0 µg/kg/infusion (left panel), 3.2 µg/kg/infusion (center panel), or 10.0 µg/kg/infusion (right panel) of remifentanil. The bars are rank-ordered according to the active number of responses emitted. The x-axis shows identification numbers for individual rats.

Fig. 5
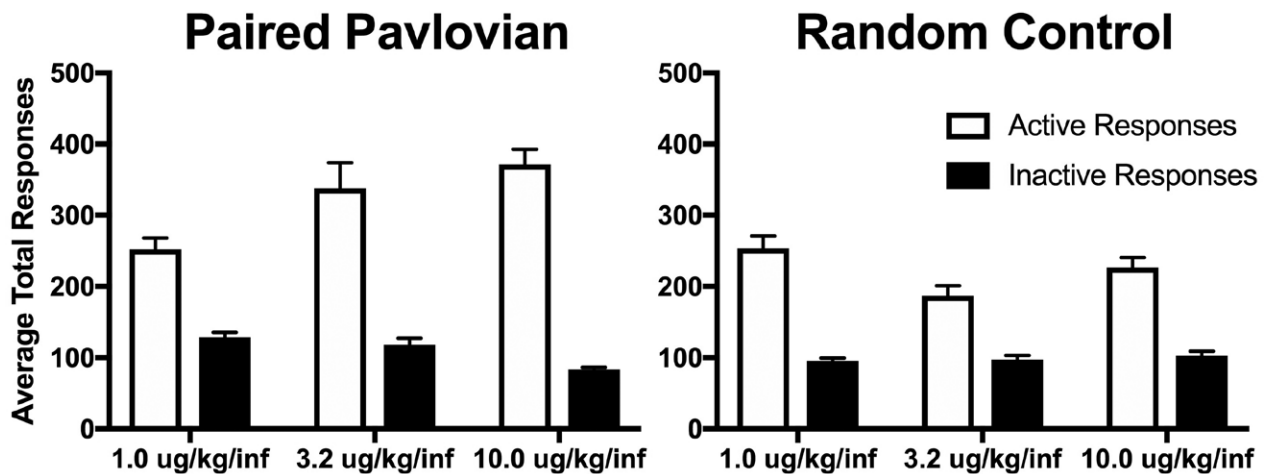
Shows the average (±SEM) of the total number of active (open bars) and inactive (filled bars) for rats that experienced Paired Pavlovian conditioning (left panel) or the Random control procedure (right panel).

consistent with Bertz and colleagues (2013; 2015; 2016) and indicate that a Paired Pavlovian conditioned history engendered a higher level of conditioned reinforcing properties. We extended the findings of Bertz and colleagues by characterizing persistence and by using an effect size analysis. We found meaningful differences in

active responding that persisted for up to 20 or 28 days of acquisition following conditioning with 3.2 or 10.0 µg/kg/infusion, respectively. In addition, we sought to better understand patterns of responding by individual animals by examining the total number of active and inactive responses. Generally, we found that regardless of conditioning history or training dose of remifentanil, rats showed a higher number of total active responses than inactive responses, which suggests that the stimulus obtained some conditioned reinforcing properties in all groups.

These data should be interpreted with a consideration in mind that we did not include a control condition in which saline was used (instead of remifentanil) during the Pavlovian conditioning phase. We determined the range of doses of remifentanil based on the findings of Bertz *et al.* (2016), who did include a saline control. They found that pairing saline infusions with stimulus presentations did not engender the stimulus with conditioned reinforcing properties. In addition, we replicated the dose-dependent patterns of responding observed by Bertz *et al.* (2016), with minimal differences in active and inactive responses between Paired Pavlovian and Random control groups at 1.0 µg/kg/infusion. Therefore, we are confident that the results reported in the current study reflect the ability of a remifentanil-associated stimulus to act as a conditioned reinforcer.

Visual inspection (Fig. 1) of the data revealed that the average level of active responding rarely approximated the average level of inactive responding. One interpretation of this observation is that the discrimination between active and inactive responding was typically facilitated rapidly for most rats. For rats that underwent Paired Pavlovian conditioning at 3.2 and 10.0 µg/kg/infusion, the early and persistent separation between active and inactive responding supports an interpretation that the remifentanil-associated stimuli took on robust conditioned reinforcing properties. For animals assigned to the Random control procedure, it appears that the stimuli alone may be reinforcing or that the remifentanil-associated stimulus potentially took on weak conditioned reinforcing properties, which is discussed in detail below.

We found that rats assigned to the Random control group showed some evidence of conditioning due to the greater total number of active than inactive responses. We included the Random control group following Rescorla (1967), who argued that the most appropriate control group in a Pavlovian conditioning study is one in which the contingency between the unconditioned and initially arbitrary stimulus is suspended. The lower levels of active responding observed in rats that were assigned to the Random control group support the conclusions that the conditioned reinforcing properties of the remifentanil-associated stimulus were much weaker than in rats assigned to received Paired Pavlovian conditioning. It is

possible that these weak conditioned reinforcing properties developed due to incidental pairings or the temporal contiguity between the remifentanil infusion and stimulus presentation. For rats assigned to the Random control group, approximately 10–20 out of 100 stimulus presentations and remifentanil deliveries incidentally occurred together. Previous research has shown that one session of Paired Pavlovian conditioning (20 total pairings) is insufficient to establish a remifentanil-associated stimulus as a conditioned reinforcer (Bertz and Woods, 2013). As such, it is unlikely that these incidental pairings produced significant conditioned reinforcing properties. However, given that, in this experiment, the incidental pairings occurred across multiple sessions, they may have resulted in weak conditioned reinforcing properties. It may be possible to further reduce the conditioned reinforcing properties of the stimulus in animals assigned to the Random control group by increasing the VT schedule, which should better control for the temporal contiguity between the stimulus presentations and remifentanil infusion. That is, selecting a VT schedule in which all possible intervals are greater than 5–6 half-lives of the drug would allow for a greater proportion of the stimulus deliveries to occur in the absence of remifentanil, thereby limiting the predictive utility of the stimulus. It is noteworthy that remifentanil was selected due to its short half-life and that encountering this problem was unexpected, which underscores the importance of considering this factor in future research.

We found individual differences in the extent to which the light + tone stimulus functioned as a conditioned reinforcer within each dosing and conditioning group. These findings suggest that there may be individual variability in the susceptibility to conditioned reinforcing properties of remifentanil, which has been documented previously by Yager *et al.* (2015). Specifically, these researchers found that animals that were classified as sign-trackers (animals that show approach behavior to a conditioned stimulus, such as a stimulus light, that is associated with a primary reinforcer) showed a significantly higher number of nose-pokes for a remifentanil-associated stimulus relative to goal-trackers (animals that show approach behavior to the location of the delivery, such as a food pellet receptacle, of the primary reinforcer). These findings suggest that the conditioned reinforcing properties of the remifentanil-associated stimulus was mediated by an animal's tendency to sign- or goal-track. Although we did not classify animals as sign-trackers or goal-trackers in the current study, it is possible that this phenomenon contributed to the individual variability in the ability of the remifentanil-associated stimulus to act as a conditioned reinforcer.

Similar to Di Ciano and Everitt (2004), we found that responding to an opioid-associated cue was characterized by persistent responding. We also found a number of differences between the current study and Di Ciano

and Everitt (2004) in terms of magnitude of response and duration of persistence. Di Ciano and Everitt (2004) found that following conditioning with cocaine or heroin, rats emitted a greater level of active responding than in the current study and that the persistence of the response lasted up to 59 days in Di Ciano and Everitt, whereas in the current study it lasted only for a few weeks. Differences in the days tested during acquisition (intermittent vs. daily), drugs used for conditioning (heroin or cocaine vs. remifentanil), the contingent vs. noncontingent nature of the drug-stimulus pairings, the rate of the drug-stimulus pairings, and the total number of drug-stimulus pairings could have led to these discrepancies. Systematic characterization of the contribution of these variables to the conditioned reinforcing properties of the drug-associated stimulus would elucidate the basic behavioral processes that determine conditioned reinforcer strength.

It is noteworthy to mention that our interest in considering the persistence of the conditioned reinforcing properties of a remifentanil-associated stimulus was inspired by work in behavioral momentum theory (Nevin, 2002). Behavioral momentum theory has been used by researchers to characterize factors that contribute to the tendency of an animal to continue to engage in an operant despite some disruption (e.g. instrumental extinction) and has relevance to the understanding of perpetuation of drug abuse and relapse (Podlesnik and Shahan, 2010). The new response acquisition procedure provides a robust demonstration of the role of Pavlovian conditioning in drug-seeking behavior. Similarly, behavioral momentum, in part, depends on Pavlovian contingencies (Podlesnik and Shahan, 2010). Behavioral momentum theory is an especially useful framework because researchers have relied on a strong tradition of quantitative analysis to better understand factors that influence resistance-to-change (Nevin et al., 2017). We did not include any of those quantitative analyses in the current study because (to our knowledge) information on rates of responding during training, as well as extinction, are required to run the analysis. Given that the procedure in the current study used response-independent infusions during the Pavlovian conditioning phase of the study, we were unable to directly apply these quantitative models to our data set. Future work that seeks to develop quantitative models that can be applied to data generated using the new response acquisition procedure is warranted.

Researchers have used a variety of procedures to examine of the role of Pavlovian conditioning in drug-seeking and relapse, such as second-order schedules (Whitelaw et al., 1996; Everitt and Robbins, 2000; Ito et al., 2002; Vanderschuren et al., 2005), extinction procedures (instrumental extinction; Schuster and Woods, 1968; Weiss et al., 2001), and Pavlovian-to-Instrumental transfer procedures (LeBlanc et al., 2012). Although each of these procedures are useful in characterizing behavioral and neural factors that mediate the ability of drug-associated stimuli to contribute to drug abuse and relapse, the new response acquisition procedure offers unique strengths. First, the new response acquisition procedure allows researchers to tightly control experimental events. For instance, given that the Pavlovian conditioning phase uses response-independent drug-stimulus pairings, it is possible to precisely manipulate the rate of drug-stimulus pairings. Second, because the novel response has never directly produced the primary (drug) reinforcer, it allows researchers to isolate the conditioned reinforcing effects of a stimulus. That is, in other procedures (e.g. second-order schedules, extinction), examination of the role of drug-associated stimuli can be confounded with the association between a response and a primary reinforcer. In addition, in these procedures, it can be unclear if the stimulus has acquired conditioned reinforcing properties, discriminative stimulus properties, or both. Third, new response acquisition procedure offers a time-effective tool to study the conditioned reinforcing properties of drug-associated stimuli, such that an entire experiment can be completed in as few as 7 days (Bertz and Woods, 2013). The new response acquisition procedure offers a powerful tool to characterize factors that contribute to the conditioned reinforcing properties of a drug-associated stimulus and is a useful procedure to investigate the neurobiological underpinnings that mediate conditioned reinforcement.

Conclusion
In summary, we replicated the findings of Bertz and colleagues (2013; 2015; 2016), such that we demonstrated that 3.2 and 10 µg/kg/infusion of remifentanil led to similar conditioned reinforcing properties of a remifentanil-associated stimulus, and we extended the work by demonstrating that responding persists at a meaningful level for around three weeks. Further, we found that responding in the new response acquisition procedure is associated with variability within each dosing and conditioning group – which may suggest individual variation in the susceptibility to the conditioned reinforcing properties of a drug-associated stimulus. Such individual variation suggests that additional behavioral and neurobiological variables may mediate conditioned reinforcing properties of a drug-associated stimulus. These analyses represent a preliminary attempt to better understand variability in conditioned reinforcing properties in the context of the new response acquisition procedure and should help to create a foundation for future research in which we seek to understand pharmacological and neurobiological manipulations that modify conditioned reinforcing properties. Ultimately, this information will help inform effective treatment approaches for drug dependence that focus on preventing relapse.

Acknowledgements

Conflicts of interest
There are no conflicts of interest.

References

Bertz JW, Chen J, Woods JH (2015). Effects of pramipexole on the acquisition of responding with opioid-conditioned reinforcement in the rat. *Psychopharmacology (Berl)* **232**:209–221.

Bertz JW, Jackson EL, Barron DR, Woods JH (2016). Effects of sex and remifentanil dose on rats' acquisition of responding for a remifentanil-conditioned reinforcer. *Behav Pharmacol* **27**:137–147.

Bertz JW, Woods JH (2013). Acquisition of responding with a remifentanil-associated conditioned reinforcer in the rat. *Psychopharmacology (Berl)* **229**:235–243.

Carroll ME, Comer SD (1996). Animal models of relapse. *Experimental and Clinical Psychopharmacology* **4**:11–18.

Carroll ME, Meisch RA (1984). Increased drug-reinforced behavior due to food deprivation. *Adv Behav Pharmacol* **4**:47–88.

Di Ciano P, Everitt BJ (2004). Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behaviour. *Neuropharmacology* **47** (Suppl 1):202–213.

Di Ciano P, Benham-Hermetz J, Fogg AP, Osborne GE (2007). Role of the prelimbic cortex in the acquisition, re-acquisition or persistence of responding for a drug-paired conditioned reinforcer. *Neuroscience* **150**:291–298.

Di Ciano P, Robbins TW, Everitt BJ (2008). Differential effects of nucleus accumbens core, shell, or dorsal striatal inactivations on the persistence, reacquisition, or reinstatement of responding for a drug-paired conditioned reinforcer. *Neuropsychopharmacology* **33**:1413–1425.

Davis WM, Smith SG (1976). Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. *Pavlov J Biol Sci* **11**:222–236.

Everitt BJ, Robbins TW (2000). Second-order schedules of drug reinforcement in rats and monkeys: measurement of reinforcing efficacy and drug-seeking behaviour. *Psychopharmacology (Berl)* **153**:17–30.

Everitt BJ, Robbins TW (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* **8**:1481–1489.

Hogarth L, Dickinson A, Duka T (2010). The associative basis of cue-elicited drug taking in humans. *Psychopharmacology (Berl)* **208**:337–351.

Hogarth L, Dickinson A, Janowski M, Nikitina A, Duka T (2008). The role of attentional bias in mediating human drug-seeking behavior. *Psychopharmacology* **201**:29–41.

Ito R, Dalley JW, Robbins TW, Everitt BJ (2002). Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. *J Neurosci* **22**:6247–6253.

Leblanc KH, Ostlund SB, Maidment NT (2012). Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behav Neurosci* **126**:681–689.

Nevin JA (2002). Measuring behavioral momentum. *Behav Processes* **57**:187–198.

Nevin JA, Craig AR, Cunningham PJ, Podlesnik CA, Shahan TA, Sweeney MM (2017). Quantitative models of persistence and relapse from the perspective of behavioral momentum theory: fits and misfits. *Behav Processes* **141**:92–99.

Podlesnik CA, Shahan TA (2010). Extinction, relapse, and behavioral momentum. *Behav Processes* **84**:400–411.

Rescorla RA (1967). Pavlovian conditioning and its proper control procedures. *Psychol Rev* **74**:71–80.

Schuster CR, Woods JH (1968). The conditioned reinforcing effects of stimuli associated with morphine reinforcement. *The International Journal of the Addictions* **3**:223–230.

Stewart J, de Wit H, Eikelboom R (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychol Rev* **91**:251–268.

Vanderschuren LJMJ, Di Ciano P, Everitt BJ (2005). Involvement of the dorsal striatum in cue-controlled cocaine seeking. *J Neurosci* **25**:8665–8670.

Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, *et al.* (2006). Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *J Neurosci* **26**:6583–6588.

Weiss F, Martin-Fardon R, Ciccocioppo R, Kerr TM, Smith DL, Ben-Shahar O (2001). Enduring resistance to extinction of cocaine-seeking behavior induced by drug-related cues. *Neuropsychopharmacology* **25**:361–371.

Whitelaw RB, Markou A, Robbins TW, Everitt BJ (1996). Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaine-seeking behavior under a second-order schedule of reinforcement. *Psychopharmacology* **127**:213–224.

Williams BA (1994). Conditioned reinforcement: experimental and theoretical issues. *The Behavioral Analyst* **17**:261–285.

Yager LM, Pitchers KK, Flagel SB, Robinson TE (2015). Individual variation in the motivational and neurobiological effects of an opioid cue. *Neuropsychopharmacology* **40**:1269–1277.