Research

Inhibition of protein synthesis but not β -adrenergic receptors blocks reconsolidation of a cocaine-associated cue memory

Amber B. Dunbar^{1,2} and Jane R. Taylor^{1,2,3}

¹Department of Psychiatry, Division of Molecular Psychiatry; ²Department of Psychology; ³Interdepartmental Neuroscience Program, Yale University, New Haven, Connecticut 06520, USA

Previously consolidated memories have the potential to enter a state of lability upon memory recall, during which time the memory can be altered before undergoing an additional consolidation-like process and being stored again as a long-term memory. Blocking reconsolidation of aberrant memories has been proposed as a potential treatment for psychiatric disorders including addiction. Here we investigated of the effect of systemically administering the protein synthesis inhibitor cycloheximide or the β -adrenergic antagonist propranolol on reconsolidation. Rats were trained to self-administer cocaine, during which each lever press resulted in the presentation of a cue paired with an intravenous infusion of cocaine. After undergoing lever press extinction to reduce operant responding, the cue memory was reactivated and rats were administered systemic injections of propranolol, cycloheximide, or vehicle. Post-reactivation cycloheximide, but not propranolol, resulted in a reactivation-dependent decrease in cue-induced reinstatement, indicative of reconsolidation blockade by protein synthesis inhibition. The present data indicate that systemically targeting protein synthesis as opposed to the β -adrenergic system may more effectively attenuate the reconsolidation of a drug-related memory and decrease drug-seeking behavior.

Previously consolidated memories have the potential to enter a state of lability upon memory recall, during which time the memory can be altered before undergoing an additional consolidation-like process and being stored again as a long-term memory (e.g., Nader et al. 2000b; Tronson and Taylor 2007). This process, known as memory reconsolidation, is thought to occur as a means of updating memories when new information pertaining to those memories is encountered (Rodriguez-Ortiz and Bermudez-Rattoni 2007; Jones et al. 2012). Manipulations of memory reconsolidation not only can update learned memories with new information but also can strengthen or weaken preexisting memories (Tronson et al. 2006). In fact, interfering with memory reconsolidation has been shown to block memory in a variety of learning paradigms in rodents, including spatial learning (Przybylski et al. 1999; Flint et al. 2007), object recognition (Winters et al. 2009; Balderas et al. 2015), and fear conditioning (Nader et al. 2000a; Tronson et al. 2006). A number of amnesic agents have been identified that effectively block reconsolidation in such paradigms, including NMDAR antagonists (Lee et al. 2006; Winters et al. 2009), β -adrenergic receptor antagonists (Przybylski et al. 1999; Debiec and LeDoux 2004), and protein synthesis inhibitors (Nader et al. 2000a; Morris et al. 2006).

Recently, manipulations of memory reconsolidation have been investigated in the context of appetitive memories, specifically as potential treatments for addiction and relapse-like behavior (e.g., Taylor et al. 2009; Sorg 2012; Torregrossa and Taylor 2013, 2016; Taylor and Torregrossa 2015). More traditional methods of reducing relapse-like behavior in rodents utilize extinction paradigms, in which a cue previously paired with the drug is presented repeatedly in the absence of the drug until the cue no longer

elicits drug-seeking behavior. Because the extinction paradigm induces the learning of a new, inhibitory memory, the original memory still exists within the brain and is subject to renewal and spontaneous recovery, which can lead to relapse-like behavior (Bouton 2004). Reconsolidation blockade, conversely, is thought to directly alter and/or update the original memory, leading to a long-lasting, context-independent change and, thus, to provide potentially superior relapse prevention (Lee et al. 2005; Milekic et al. 2006; Sanchez et al. 2010). Interfering with memory reconsolidation in rodents has been shown to block memories related to a number of drugs, including morphine (Valjent et al. 2006; Taubenfeld et al. 2010), ethanol (Wouda et al. 2010; Schramm et al. 2015), nicotine (Fang et al. 2011; Tedesco et al. 2014), methamphetamine (Zhao et al. 2011b; Yu et al. 2013), amphetamine (Sadler et al. 2007; Contreras et al. 2012), heroin (Hellems et al. 2006; Jian et al. 2014), and cocaine (Milton et al. 2008; Sanchez et al. 2010). Notably, manipulations of memory reconsolidation have also been reported to decrease the strength of drug-related memories in humans (Zhao et al. 2011a; Saladin et al. 2013).

Most investigations of reconsolidation of drug-related memories have utilized a conditioned place preference (CPP) model in rodents, in which the drugs are administered by the experimenter (Prus et al. 2009). Amnesic agents shown to block reconsolidation in neutral and aversive paradigms can also block appetitive memories in the CPP paradigm. For example, both morphine- and cocaine-CPP can be systemically blocked by administration of the β -adrenergic antagonist propranolol (Robinson and

Corresponding author: jane.taylor@yale.edu

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Franklin 2010; Otis et al. 2013) as well as by the protein synthesis inhibitors cycloheximide and anisomycin (Fan et al. 2010; Milekic et al. 2006).

Still unknown is whether systemic injections of propranolol or cycloheximide will block reconsolidation of a cocaine-cue memory that drives reinstatement in rats that have learned to self-administer cocaine. Drug self-administration paradigms may be more ethologically valid models when compared with CPP, since animals are able to control the timing and amount of voluntary drug-intake. Understanding whether drugs that block CPP reconsolidation also block self-administration memories is essential for the translational potential of reconsolidation-based addiction treatments. Of additional importance to the translational benefit of reconsolidation-based treatments is whether the amnesic agent can be administered systemically, as opposed to the majority of rodent studies that administer such agents intracranially in order to examine brain-specific mechanisms. Anisomycin, for example, has been shown to block reconsolidation and cue-induced reinstatement in a rodent model of cocaine self-administration when administered into the medial PFC (Sorg et al. 2015), but the systemic potential of protein synthesis inhibitors to block reconsolidation of self-administration memories has not been reported. Additionally, systemic propranolol has been demonstrated to block reconsolidation of the conditioned-reinforcement value of a cue previously paired with cocaine (Milton et al. 2008), but the ability of post-retrieval propranolol to block cue-induced reinstatement has yet to be examined. As cue-induced reinstatement models relapse-like behavior, assessing the effects of propranolol and cycloheximide on reinstatement will provide insight into the translational potential of such compounds in treating addiction through interfering with reconsolidation.

The present study thus investigates whether systemic administration of cycloheximide or propranolol can block reconsolidation of a cocaine-cue memory and reduce cue-induced reinstatement to cocaine-seeking in rats that have been trained to self-administer intravenous cocaine. Rats underwent self-administration training during which each active lever press resulted in the contingent presentation of a cue paired with a cocaine infusion. Following self-administration, rats underwent lever extinction in order to reduce responding on the active lever so that reinstatement could later be measured. The cue memory was then reactivated by presentations of the cue in the absence of cocaine. Immediately following memory retrieval, animals received systemic injections of the amnesic agent cycloheximide or propranolol and were tested 1 to 3 d later on cue-induced reinstatement. It was hypothesized that rats that received propranolol or cycloheximide would demonstrate decreased responding on reinstatement day, indicative of reduced drug-seeking behavior caused by reconsolidation blockade. In support of the hypothesis, cycloheximide effectively decreased reinstatement in a dose- and reactivation-dependent manner, indicative of reconsolidation blockade, whereas no effect of propranolol on reinstatement or reconsolidation was found.

Results

Effect of low dose (1.0 mg/kg) of cycloheximide on reconsolidation

We first tested whether a low dose of the protein synthesis inhibitor cycloheximide could block reconsolidation. Rats were trained in cocaine self-administration in which each lever press resulted in one infusion of cocaine (0.5 mg/kg) paired with the conditioned stimulus (CS). Lever pressing was subsequently extinguished to reduce responding. Memory reactivation occurred 24 h after the last day of lever extinction and consisted of three non-contingent CS presentations in the absence of any cocaine or levers. Rats received injections of vehicle or cycloheximide (1.0 mg/kg, s.c.) immediately following CS memory reactivation, and cue-induced reinstatement was tested 72 h later (Fig. 1A).

Across the 10 d of cocaine self-administration training, there were no differences in number of cocaine infusions (Fig. 1B), active lever presses, or inactive lever presses between rats that would be injected following memory reactivation with cycloheximide ($N = 8$) or vehicle ($N = 9$; P values > 0.05). Likewise, no differences were found between groups across the 8 d of lever extinction on the number of active (Fig. 1C) or inactive lever presses (P values > 0.05). A main effect of session (last day of extinction versus reinstatement) was obtained on active lever presses ($F_{(1,15)} = 18.16$, $P = 0.001$, $\eta_p^2 = 0.55$), such that rats pressed the active lever more on reinstatement when compared with the last day of extinction, but there was not a significant drug by session interaction on active lever presses (Fig. 1D; $F_{(1,15)} = 1.26$, $P = 0.28$, $\eta_p^2 = 0.08$). No main effects or interaction between session and drug on inactive lever presses was obtained (P values > 0.05). These data indicate that a 1.0 mg/kg dose of cycloheximide is insufficient to reduce reconsolidation or cue-induced reinstatement.

Figure 1. A low dose of cycloheximide (1.0 mg/kg) does not affect reconsolidation or cue-induced reinstatement. (A) Schematic representation of the experimental procedures. (B) Total number of cocaine infusions received across each day of self-administration. (C) Total number of active lever presses during lever extinction. (D) Active lever presses on the last day of extinction and on the cue-induced reinstatement test. ($N_s = 8$ (VEH), 9 (CHX)).

Effect of high dose (2.2 mg/kg) of cycloheximide on reconsolidation

In order to examine whether a higher dose of cycloheximide could block reconsolidation, rats previously trained in cocaine self-administration received injections of vehicle or cycloheximide (2.2 mg/kg, s.c.) immediately following CS memory reactivation and were tested on cue-induced reinstatement 72 h later (Fig. 2A). Across the 10 d of cocaine self-administration acquisition, no differences were found between rats that would be injected following memory reactivation with vehicle ($N = 9$) or cycloheximide ($N = 9$) on number of cocaine infusions (Fig. 2B), active lever presses, or inactive lever presses (P values > 0.05). Likewise, the number of active lever presses (Fig. 2C) and inactive lever presses across the 8 d of extinction did not differ between groups (P values > 0.05).

A significant main effect was found of session (last day of extinction versus reinstatement) on inactive lever presses ($F_{(1,16)} = 6.71$, $P = 0.020$, $\eta_p^2 = 0.30$), such that inactive lever presses increased on reinstatement ($M = 4.89 \pm 0.94$) versus last day of extinction ($M = 2.33 \pm 0.58$). There was also a significant main effect of drug on inactive lever presses during the last day of extinction and reinstatement ($F_{(1,16)} = 5.34$, $P = 0.035$, $\eta_p^2 = 0.25$), such that vehicle-injected rats ($M = 5.00 \pm 0.85$) pressed the inactive lever more than cycloheximide-injected rats ($M = 2.22 \pm 0.85$). Importantly, however, there was no significant interaction between session and drug on inactive lever presses ($P < 0.05$), indicating that the main effects on inactive lever presses were not due to administration of the drug but due to preexisting differences between groups.

A significant main effect of session on active lever presses was also obtained ($F_{(1,16)} = 40.40$, $P < 0.01$, $\eta_p^2 = 0.72$), such that rats pressed the active lever more on reinstatement when compared with the last day of extinction (Fig. 2D). Additionally, a significant

main effect of drug on active lever presses during the last day of extinction and reinstatement was found ($F_{(1,16)} = 8.01$, $P = 0.012$, $\eta_p^2 = 0.33$), such that rats receiving cycloheximide pressed the active lever less than rats receiving vehicle; however, this main effect was qualified by a significant interaction between session and drug (Fig. 2D; $F_{(1,16)} = 9.42$, $P < 0.01$, $\eta_p^2 = 0.37$). Whereas both groups responded equivalently on the active lever on the last day of extinction ($P > 0.05$), on the cue-reinstatement test rats that received post-reactivation cycloheximide had significantly fewer active lever presses than vehicle-injected rats ($F_{(1,16)} = 8.89$, $P < 0.01$, $\eta_p^2 = 0.36$). These data indicate that post-reactivation cycloheximide (2.2 mg/kg) selectively decreases reinstatement to cocaine seeking on the lever previously associated with cocaine through interfering with reconsolidation.

Effect of high dose (2.2 mg/kg) of cycloheximide in the absence of reactivation

To investigate whether the effect of cycloheximide on reinstatement depends upon reactivation and to rule out nonreconsolidation-based mechanisms of cycloheximide's effect, rats received cycloheximide or vehicle treatment following exposure to the novel context without the presence of cocaine-related CSs, and rats were tested 72 h later on cue-induced reinstatement (Fig. 3A). No differences were seen across the 10 d of cocaine self-administration acquisition between nonreactivated rats that would later be injected with vehicle ($N = 8$) or cycloheximide ($N = 8$) on number of cocaine infusions (Fig. 3B), active lever presses, or inactive lever presses (P values > 0.05). Similarly, no between-groups differences in active lever presses (Fig. 3C) or inactive lever presses were found across the 8 d of lever extinction (P values > 0.05).

A significant main effect of session (last day of extinction versus reinstatement) on active lever presses was found ($F_{(1,14)} = 60.05$, $P < 0.01$, $\eta_p^2 = 0.81$), such that responding was higher on reinstatement when compared with the last day of extinction (Fig. 3D). However, no interaction was seen on active lever presses during the last day of extinction and the cue-induced reinstatement test for vehicle- and cycloheximide-injected rats (Fig. 3D; $F_{(1,14)} = 2.82$, $P = 0.12$, $\eta_p^2 = 0.17$). Furthermore, no significant main effect or interaction was found for inactive lever presses on reinstatement and the last day of extinction (P values > 0.05). These data indicate that cycloheximide's effect of decreasing cue-reinstatement requires memory reactivation, a critical component for reconsolidation blockade.

Effect of propranolol (10 mg/kg) on reconsolidation

In order to test whether the β -adrenergic receptor antagonist propranolol blocks memory reconsolidation, propranolol (10 mg/kg) or vehicle was administered immediately following CS memory reactivation, and cue-induced reinstatement was tested 24 h later (Fig. 4A). Across the 8 d of cocaine self-administration, no differences were found between rats that would be injected following

Figure 2. Cycloheximide (2.2 mg/kg) blocks reconsolidation and reduces cue-induced reinstatement. (A) Schematic representation of the experimental procedures. (B) Total number of cocaine infusions received across each day of self-administration. (C) Total number of active lever presses during lever extinction. (D) Active lever presses on the last day of extinction and on the cue-induced reinstatement test. *, Statistically significant ($P < 0.05$). ($N_s = 9$ (VEH), 9 (CHX)).

Figure 3. The effect of cycloheximide (2.2 mg/kg) is dependent upon cue reactivation. (A) Schematic representation of the experimental procedures for rats that did not receive light/tone reactivation. (B) Total number of cocaine infusions received across each day of self-administration. (C) Total number of active lever presses during lever extinction. (D) Active lever presses on the last day of extinction and on the cue-induced reinstatement test. ($N_s = 8$ (VEH), 8 (CHX)).

memory reactivation with vehicle ($N = 9$) or propranolol ($N = 9$) on number of cocaine infusions (Fig. 4B), active lever presses, or inactive lever presses (P values >0.05). Likewise, no differences were found between groups across the 8 d of lever press extinction (Fig. 4C) or inactive lever presses (P values >0.05). A significant main effect of session (last day of extinction versus reinstatement) on active lever presses was found, such that rats pressed the active lever significantly more on reinstatement compared with the last day of extinction (Fig. 4D; $F_{(1,16)} = 57.91$, $P < 0.01$, $\eta_p^2 = 0.78$). A significant main effect of session (last day of extinction versus reinstatement) on inactive lever presses was also found, such that rats pressed the inactive lever significantly more on reinstatement ($M = 6.22 \pm 1.03$) when compared with the last day of extinction ($M = 3.28 \pm 0.68$; $F_{(1,16)} = 17.78$, $P < 0.01$, $\eta_p^2 = 0.53$). However, no interaction between session and drug on active lever presses (Fig. 4D; $F_{(1,16)} = 0.038$, $P = 0.85$, $\eta_p^2 < 0.01$) or inactive lever presses ($F_{(1,16)} = 0.513$, $P = 0.48$, $\eta_p^2 = 0.03$) was obtained. These data indicate that propranolol does not affect reconsolidation of a cocaine-cue memory.

In light of the null findings, no control experiments were performed using propranolol. Additional doses of pro-

pranolol were not tested because nearly all previous studies that have demonstrated an effect of propranolol on the reconsolidation of appetitive as well as aversive behaviors have utilized a 10 mg/kg dose (Przybylski et al. 1999; Debiec and LeDoux 2004; Bernardi et al. 2006; Diergaarde et al. 2006; Robinson and Franklin 2007; Milton et al. 2008; Robinson et al. 2011b; Achterberg et al. 2012; Wei and Li 2014; Schramm et al. 2015). Furthermore, a previous pilot study in our laboratory using a higher dose of propranolol (40 mg/kg) also revealed no propranolol-induced deficits in reinstatement or reconsolidation (data not shown), providing additional evidence that experiments using this higher dose may not be warranted.

Discussion

The results of the present study indicate that post-reactivation injection of cycloheximide dose-dependently (2.2 mg/kg but not 1.0 mg/kg) blocks cue-induced reinstatement. The effect of cycloheximide depends upon retrieval of the drug-related CS, indicating that cycloheximide interferes with memory reconsolidation. In contrast to the original hypothesis, post-reactivation propranolol had no effect on cue-induced reinstatement, indicative of no effect on

Figure 4. Propranolol (10 mg/kg) has no effect on reconsolidation or cue-induced reinstatement. (A) Schematic representation of the experimental procedures. (B) Total number of cocaine infusions received across each day of self-administration. (C) Total number of active lever presses during lever extinction. (D) Active lever presses on the last day of extinction and on the cue-induced reinstatement test. ($N_s = 9$ (VEH), 9 (CHX)).

reconsolidation. Thus, protein synthesis inhibition, but not β -adrenergic inhibition, blocks reconsolidation of a drug-related cue memory in a rodent model of cocaine self-administration.

It is not surprising, however, that propranolol failed to block reconsolidation as measured by cue-induced reinstatement. Previous research indicates that propranolol is not always effective at interfering with reconsolidation in both rodents (Lee and Everitt 2008; Font and Cunningham 2012; Milton et al. 2012; Williams and Harding 2014) and humans (Tollenaar et al. 2009; Bos et al. 2014; Pachas et al. 2015; Spring et al. 2015; Wood et al. 2015), and replications of experiments even within the same laboratory have produced differing results (Kindt et al. 2009; Bos et al. 2014). Some explanations for these inconsistencies include the ability of propranolol to preferentially affect emotional memories over neutral memories (Schwabe et al. 2012a,b), individual differences in participants (Soeter and Kindt 2013), and the mnemonic paradigm under investigation (Muravieva and Alberini 2010; Wei and Li 2014). Furthermore, prior experience with drugs of abuse may engender memories resistant to propranolol blockade (Robinson et al. 2011a; Ortiz et al. 2015), which could explain the present results.

It cannot be ruled out that alterations to the design of the present paradigm might reveal an effect of propranolol on reconsolidation of a cocaine-cue memory. For example, some prior studies have found that repeated reactivation sessions followed by propranolol are required to block reconsolidation (Fricks-Gleason and Marshall 2008; Wouda et al. 2010), yet the present study utilized only a single reactivation session. In addition, it is possible that administering propranolol prior to memory reactivation may induce deficits in reinstatement. However, only drugs administered after memory reactivation can be said to interfere with the restabilization phase of reconsolidation (Milton et al. 2013). Compounds administered prior to reactivation, conversely, may interfere with memory due to an enhancement of memory destabilization or through interfering directly with memory recall (Ben Mamou et al. 2006; Hong et al. 2011). The ability of propranolol to reduce cue-induced reinstatement through either of these alternative processes may be an interesting avenue for future investigation.

Additionally, while it is possible that propranolol may decrease reinstatement at a different dose, nearly all studies demonstrating propranolol's ability to block reconsolidation systemically have used the same dose (10 mg/kg) as was used here in both aversive (Przybylski et al. 1999; Debiec and LeDoux 2004) and appetitive paradigms (Bernardi et al. 2006; Diergaarde et al. 2006; Robinson and Franklin 2007; Milton et al. 2008; Robinson et al. 2011b; Achterberg et al. 2012; Wei and Li 2014; Schramm et al. 2015). Reports of lower effective doses (1 or 5 mg/kg) of propranolol have only been shown to block reconsolidation of drug CPP in stress-exposed mice (Hymel et al. 2014) or of contextual fear memories after very high shock-conditioning sessions in rats (Abrari et al. 2008). Higher doses have not generally been used in reconsolidation studies; however, a 40 mg/kg dose of propranolol given subcutaneously was reported to reduce morphine CPP (Robinson et al. 2011b). The use of a higher dose was not required, as a 10 mg/kg dose also impaired morphine CPP in the same study. Additionally, when we ran a pilot study using this high dose (40 mg/kg), we found no propranolol-induced deficits in reinstatement or reconsolidation (AB Dunbar and JR Taylor, unpubl.). Use of systemic propranolol at these higher doses is also problematic in terms of possible nonspecific mnemonic or molecular consequences. Thus, it is unlikely that the null effect of propranolol seen here is due to dosage, though this hypothesis would need to be experimentally evaluated.

The instrumental behavior of cue-induced reinstatement is modulated by three main Pavlovian processes: conditioned rein-

forcement, conditioned approach, and conditioned motivation (Milton and Everitt 2010). While post-reactivation propranolol has been shown to reduce conditioned reinforcement in a rodent model of cocaine self-administration (Milton et al. 2008), preliminary data from the same laboratory indicate that under the same conditions post-reactivation propranolol may not block cue-induced reinstatement (Milton and Everitt 2009, 2010), which is supported by the present results. Furthermore, while alcohol conditioned reinforcement is blocked by post-reactivation propranolol similarly to cocaine (Milton et al. 2008; Schramm et al. 2015), alcohol conditioned motivation and approach are not (Lee and Everitt 2008; Milton et al. 2012), and the effect of propranolol on cue-induced reinstatement to alcohol-seeking is unclear (Wouda et al. 2010; Williams and Harding 2014). Thus, it is likely that propranolol selectively or preferentially modifies the reconsolidation of conditioned reinforcement. Blocking a conditioned reinforcement memory may not be sufficient to decrease cue-induced reinstatement if conditioned motivation and approach memories are intact. Conversely, protein synthesis inhibitors administered intracranially (anisomycin; Barak et al. 2013; Sorg et al. 2015) and systemically (cycloheximide; present results) do block reinstatement to drug seeking, and intramygdalar protein synthesis inhibition (anisomycin) also blocks conditioned reinforcement (Lee et al. 2005). The role of protein synthesis inhibition in conditioned motivation and approach has yet to be examined. Protein synthesis inhibition may, thus, modulate the memories of different or additional drug-related psychological processes when compared with propranolol, which enables cycloheximide and anisomycin to block reinstatement to drug seeking. Additional research is needed to directly test the ability of propranolol and cycloheximide to interfere with the reconsolidation of different aspects of cocaine-related memories.

The present finding that post-reactivation cycloheximide attenuates cue-induced reinstatement to cocaine seeking is a valuable contribution to the field as it demonstrates that systemic protein synthesis inhibition blocks reconsolidation. The only systemic agents that have previously been found to block reconsolidation as measured by decreased reinstatement are dopamine and NMDAR antagonists (Yan et al. 2014; Exton-McGuinness and Lee 2015). Understanding how reconsolidation can be blocked systemically is essential for improving the translational potential of reconsolidation-based relapse-prevention therapies. Although cycloheximide is not itself suitable for use in humans, future research should investigate the efficacy of other protein synthesis inhibitors with reduced human toxicity, such as antibiotics that target protein synthesis (McCoy et al. 2011; Sutcliffe 2011), at blocking reconsolidation of drug-related memories. The role of protein synthesis inhibition on blocking memory reconsolidation as measured by reduced reinstatement to drug-seeking behavior deserves further investigation as a potential treatment for addiction.

Materials and Methods

Subjects

One hundred male Sprague Dawley rats (250–275 g; Charles River Laboratories) were individually housed on a 12-h light cycle in a temperature and humidity controlled room. All procedures were conducted during the light phase of the cycle. Rats were allowed to acclimate for 7 d prior to the start of the experiment. All procedures were conducted in accordance with the policies of the Yale University Institutional Animal Care and Use Committee and conformed to National Institutes of Health Guidelines on the Care and Use of Laboratory Animals.

Surgery

Animals were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) and injected with carprofen (5 mg/kg, s.c.) and saline (5 mL, s.c.). Rats were implanted with a chronic, indwelling catheter (0.51 × 0.94 mm, Dow Corning) in the right jugular vein. The catheter tubing connected to a cannula (22 gauge, Plastics One) mounted on the back of the animals. The intravenous catheter was flushed with 0.4 mg gentamicin (0.2 mL, Sagent) at surgery and patency was maintained post-surgery by infusion of 0.2 mL of saline containing heparin (35 U/mL, Sagent) and gentamicin (0.08 mg/mL, Hospira) every 2 d. Patency was verified by the infusion of 2 mg of methohexital sodium (0.2 mL, Par). Animals were allowed to recover for 5–7 d before the start of behavioral procedures.

Behavioral apparatus

Behavioral procedures took place in sound-attenuating operant chambers (Med Associates). Context A contained a metal rod floor, two inactive nose ports, an inactive magazine, two retractable levers positioned on the same side of the box, two cue lights positioned directly above the levers, and a fan that provided background noise (65 dB). A metal arm (Med Associates) attached to the operant box held up a spring tether that attached to the back mount on the rats for intravenous cocaine delivery through the catheter. A syringe pump placed outside of the sound-attenuating chamber was connected to the other end of the spring tether by polyethylene tubing (Plastics One) to deliver cocaine infusions. Context B contained an opaque white plastic floor, an illuminated house light, and no fan, levers, nose ports, or magazine. Context B was additionally scented with 1% almond extract.

Behavioral procedures

Rats were restricted to 90% of their free-feeding weight and fed daily to maintain that weight throughout the experiment. Behavioral procedures are similar to those used in previous studies (Sanchez et al. 2010; Wan et al. 2014). Animals underwent acquisition of cocaine self-administration in Context A for 8 d (propranolol experiment) or 10 d (cycloheximide experiments) in daily 1-h sessions. Rats were placed in the operant chamber and secured to the spring tether. Each active lever press resulted in one infusion of cocaine (0.5 mg/kg), followed by a 10-sec time-out. Each cocaine infusion was paired with a CS a 10-sec illumination of the cue light and a simultaneous 10-sec tone (75–80 dB). Inactive lever presses were recorded but had no outcome. Rats were removed from the chambers and returned to their home cage after 60 min. Catheter patency was verified after the last day of self-administration by the infusion of 2 mg of methohexital sodium (0.2 mL, Par). Rats next underwent 8 d of lever extinction training in Context A, in which they were placed in the operant chambers for 1 h per day. No CS presentations or cocaine infusions were available during extinction.

In groups that underwent memory reactivation, the CS-memory retrieval session occurred 24 h following the last day of extinction. Rats were placed in Context B for 6-min total. After a 2-min acclimatization period, the CS was presented three times (1-min intertrial interval), and rats remained in the box for an additional 2 min. In control groups without memory reactivation, rats did not undergo memory retrieval but instead were placed in Context B for 6 min without any CS presentations. Immediately upon removal from the boxes, rats were injected with cycloheximide (s.c., 1.0 or 2.2 mg/kg in 15% DMSO, Sigma), propranolol (i.p., 10 mg/kg in saline, Sigma) or vehicle and returned to their home cages. The drug doses chosen have previously been demonstrated to block reconsolidation in other paradigms for both doses of cycloheximide (Flint et al. 2007; Taubenfeld et al. 2010) and for propranolol (Debiec and LeDoux 2004; Milton et al. 2008).

Seventy-two hours (cycloheximide experiments) or 24 h (propranolol experiment) later, animals were tested on cue-induced reinstatement. Rats were placed in Context A for 1 h. One presentation of the CS was given freely 5 sec after the session

began. For the duration of the session, each active lever press resulted in the contingent presentation of the CS. No cocaine infusions were presented during reinstatement. Inactive lever presses were recorded but had no associated outcome. Rats were removed from the boxes and returned to their home cages.

Statistical analysis

Rats that did not acquire self-administration (<50 total cocaine infusions or <10 infusions on the final day of self-administration) or whose catheters were not patent at the end of self-administration training were excluded from all statistical analyses. All analyses were carried out using SPSS version 23. Acquisition of self-administration was analyzed with repeated-measures analyses of variance (rm-ANOVAs) across the 8 or 10 d of self-administration on number of cocaine infusions, number of active lever presses and number of inactive lever presses. Lever extinction was analyzed with rm-ANOVAs across 8 d on number of active and inactive lever presses. To analyze reinstatement results, rm-ANOVAs across session (last day of extinction versus reinstatement test) were conducted on number of active and inactive lever presses. Following significant rm-ANOVAs, planned comparisons (one-way ANOVAs) were performed on active lever presses between groups during extinction and reinstatement.

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Amber B. Dunbar and Jane R. Taylor

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