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Involvement of the dorsal hippocampus in expression and extinction of cocaine-induced conditioned place preference

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Abstract

A key aspect of substance abuse is that drug taking often occurs in a specific context. As a consequence, exposure to drug-associated contexts can trigger cravings and relapse, even after long periods of abstinence. Although many studies have demonstrated that the hippocampus is critical for developing and retrieving contextual and spatial memories, comparatively little is known about the role of the hippocampus in acquiring and inhibiting memories involving contexts and drugs of abuse. We examined the effects of hippocampal inactivation on expression of cocaine-induced conditioned place preference (CPP) after initial acquisition or extinction of CPP in C57BL/6 mice. During acquisition of CPP, distinct tactile cues were paired with cocaine (20 mg/kg, intraperitoneal, CS+) and different tactile cues were paired with saline (CS-) on alternate days. Groups differed in whether the CS+ and CS- cues were presented in the same large space (one-compartment procedure) or distinct small spaces (two-compartment procedure), as previous findings demonstrate that a two-compartment configuration facilitates acquisition and attenuates extinction of a cocaine-induced CPP. Microinjection of the GABA_A agonist, muscimol, into the dorsal hippocampus impaired (1) retrieval of a place preference after acquisition, (2) extinction of a place preference, and (3) retrieval of extinction. These effects differed depending on the spatial configuration during acquisition or extinction, suggesting that the dorsal hippocampus may differentially modulate drug seeking during retrieval and extinction of CPP.

Keywords

hippocampus; retrieval; extinction; cocaine; muscimol; conditioned place preference

Introduction

Cocaine users often associate environmental cues with their drug-related experiences. Re-exposure to these cues can reactivate drug-related memories, induce cravings, and initiate drug-seeking behavior and relapse. By identifying the neural substrates that underlie changes in drug-seeking behavior, therapies for drug abuse may better target these highly cue-specific learning events. The cocaine-seeking behavior evoked by environmental cues, and the necessity of substrates involved, can be measured in the laboratory using a conditioned place preference (CPP) procedure.

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CPP is a widely used procedure, but in contrast to other Pavlovian preparations, such as fear conditioning, the apparatus and procedures used to generate CPP vary from laboratory to laboratory (Cunningham, Gremel, & Groblewski, 2006; Cunningham, Patel, & Milner, 2006; Cunningham & Zerizaf, 2014; Itzhak, Liddie, & Anderson, 2013; Kramar, Flavia, Barbano, & Medina, 2014; Maze et al., 2010; Meyers, Zavala, Speer, & Neisewander, 2006; Raybuck, McCleery, Cunningham, Wood, & Lattal, 2013; White, Chai, & Hamdani, 2005). One major difference is whether the animal has access to the entire CPP apparatus (one-compartment) or is confined to one side or the other (two-compartment) during training. These procedures differ in several important ways, including the size, visual cues, and relevance of spatial location within the apparatus. Some studies have shown that CPP can be altered with different compartment configurations (Bevins, 2000; Cunningham, Patel, & Milner, 2006; Cunningham & Zerizaf, 2014; Hitchcock, Cunningham, & Lattal, 2014), yet little is known about the neural circuits that underlie these differences (Meyers, Zavala, & Neisewander, 2003; Meyers et al., 2006)

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Hitchcock et al. (2014) found that when a specific side of the apparatus was paired with cocaine in a two-compartment procedure, acquisition was facilitated, but that the same two-compartment procedure impaired extinction. In contrast, a one-compartment procedure impaired acquisition but facilitated extinction. A major way in which these two procedures differ is in whether a spatial location (side of the apparatus) is consistently paired with cocaine; in a two-compartment procedure with consistent spatial cues, the animal always receives drug in the same location, but in a one-compartment procedure, the animal is free to move throughout the entire apparatus.

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The DH is necessary for acquisition, retrieval, and extinction of spatial and contextual memories (Anagnostaras, Maren, & Fanselow, 1999; Gould & Leach, 2014; Riedel et al., 1999). There is also evidence that the DH mediates contextual renewal of reward-seeking behaviors (Fuchs et al., 2005; Neisewander et al., 2000; Todd, 2013) and it may be involved in detecting a mismatch between training and testing contexts (Gill, Mizumori, & Smith, 2011; Jezek, Henriksen, Treves, Moser, & Moser, 2011; Leutgeb et al., 2005). There are fewer studies of how the DH directly modulates different context-drug memories (reviewed in Gould & Leach, 2014), especially in a CPP procedure.

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There is evidence that inactivating the DH before acquisition or before the first preference test impairs expression of CPP (Meyers et al., 2006) and that similar effects occur when the DH is inactivated prior to a test soon or long (30 d) after acquisition (Raybuck & Lattal, 2014). Similar effects of DH inactivation have been found for CPP with other drugs, such as a selective CB1 cannabinoid receptor agonist (Nasehi & Kamali-dolatabadi, 2016). Other studies have focused on the glutamatergic (Tan, 2008) and dopaminergic activity within the DH (Kramar, Flavia, Barbano, Medina, et al., 2014), as well as long-term potentiation in area CA1 (Portugal et al., 2014) that is involved in CPP expression. Thus, although emerging evidence suggests a role for the hippocampus in aspects of CPP acquisition and retrieval, little remains known about how the DH modulates memory during and after extinction of CPP (Sadeghi, Ezzatpanah, & Haghparast, 2016).

The goal of the following experiments was to evaluate the contributions of the DH to extinction of cocaine-induced CPP in different contextual configurations commonly used to assess CPP (i.e., one or two-compartment CPP configurations). We used the GABA_A (gamma-aminobutyric acid, ionotropic) receptor agonist muscimol to inactivate the DH. Muscimol was administered before initial expression (Preference Test 1, Experiment 1), before extinction learning (Extinction, Experiment 2), or before extinction expression (Preference Test 2, Experiment 3) of cocaine-induced CPP under conditions in one- and two-compartment CPP approaches that promote acquisition or extinction.

Methods

Subjects

All experiments used naïve C57BL/6J male mice (Jackson Laboratory, Bar Harbor, ME) that were 8–12 weeks old. Mice (N=79) were housed in standard colony cages, four per cage until separated after surgery, and maintained on a 12-h light/dark cycle (lights on at 6 am) and received ad libitum food and water access. All experiments were conducted during the light phase, were approved by the Oregon Health & Science University Institutional Animal Care and Use Committee, and were performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

CPP Apparatus

CPP was assessed using an unbiased apparatus, consisting of clear acrylic walls (30 cm wide × 15 cm deep × 15 cm high). The apparatus included interchangeable halves (left and right) of two floor types (Grid and Hole), each 15-cm × 15-cm × 15-cm (Cunningham, et al., 2006). Grid floors consisted of 2.3-mm stainless steel rods mounted 6.4-mm apart in an acrylic frame. Hole floors consisted of perforated stainless steel with 6.4-mm round holes on 9.5-mm staggered centers. The CPP chambers were housed in melamine shells (McCarthy Manufacturing, Gresham, OR) with air vents around the side of the chamber, allowing low levels of light to enter each chamber. A camera was mounted to the ceiling in the shell. A clear and removable acrylic barrier divided the CPP chamber into a two-compartment apparatus configuration. The acrylic barrier was removed to create a one-compartment apparatus configuration. Each side of the two-compartment chamber was 15 cm wide; the one-compartment chamber was 30 cm wide.

Drugs

Cocaine Hydrochloride (COC, Sigma-Aldrich, St. Louis, MO) was dissolved in physiological saline (SAL, 0.9% NaCl), for intraperitoneal (IP) injections (10 ml/kg), administered at a dose of 20 mg/kg on conditioned stimulus positive days (CS+). This dose was chosen based on our previous work in mice (Abraham, Neve, & Lattal, 2016a, 2016b). Saline (SAL) was injected (IP) into animals on conditioned stimulus negative days (CS-), matching volume (10 ml/kg) and handling specific effects between COC CS+ and SAL CS- acquisition trials.

Muscimol (Sigma-Aldrich, St. Louis, MO) was dissolved in phosphate buffered saline (PBS) and bilaterally microinjected (0.25 µl/min/side) into the DH. PBS vehicle was microinjected

into control animals in the same volume. In Experiment 1, muscimol was administered in the first cohort of mice at 0.5 ug/side and in the second cohort at 0.25 ug/side (previously demonstrated to be successful in the DH in similar studies: (Nasehi & Kamali-dolatabadi, 2016; Stackman, Cohen, Lora, & Rios, 2016; Yousefi, Farjad, Nasehi, Reza, & Zarrindast, 2013). With no difference on locomotion or preference between drug dose groups after the injection at Test 1 ((One-way ANOVA: 3 drug groups, $p = 0.8$), Cohorts 1 and 2 were pooled for Experiment 1 and 0.5 ug/side was used in both Cohorts 1 and 2 in Experiments 2 and 3. These doses were chosen based on previous findings in our lab and related literature (Matus-Amat, Higgins, Barrientos, et al., 2004; Nasehi & Kamali-dolatabadi, 2016; Raybuck & Lattal, 2011).

Surgical Procedures

Guide cannula (26g) were designed and fabricated (Plastics One, Inc. Roanoke, VA) to target the DH at coordinates A/P -1.7 , M/L ± 1.5 , D/V -2.3 mm from bregma. Mice were anesthetized with isoflurane (2%–5%) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The eyes of mice were lubricated and the scalp was scrubbed with betadine. A small portion of the scalp was cut away, exposing the skull from bregma to lambda. Bilateral holes were drilled through the skull, bilateral cannula were inserted 1.5 mm into the brain, held in place with the stereotax, and Ketac dental cement was added to secure cannula in place and cover the exposed skull. Stainless steel stylets were inserted into the cannula (-2.0 mm into brain) to maintain patency during the 5 d post-surgical recovery period. To infuse vehicle or muscimol solutions, stylets were removed and a microinjector was inserted into the cannula, extending .8 mm beyond the length of the cannula, to a total depth of -2.3 mm from bregma. These targets took into account the loss in depth due to the slight curvature of the brain using bilateral cannula and the thickness of the skull (injector targeted -2.3 to hit a depth between -1.5 and -2.0 mm).

DH Microinjections

A microinfusion pump controlled Hamilton Syringes (10ul, Reno, NV) that were connected to PE20 tubing. Microinjectors connected the syringes and tubing to the DH cannula for DH microinfusions of PBS or muscimol. Mice were able to move freely during the microinjection of PBS or muscimol. Injections occurred 30 min prior to the behavioral session (Test 1 in Experiment 1; Extinction in Experiment 2; Test 2 in Experiment 3) at a rate of 0.25 ul/min for 1 minute. Injectors were left secured in place for an additional 30 seconds after the microinjection.

Experiment 1: Effects of muscimol inactivation prior to a post-acquisition CPP expression test (Test 1)

Cocaine-induced CPP was conducted over consecutive days with one session per day. Twenty-six male C57BL/6 mice received habituation, pretesting, acquisition, and testing phases.

Habituation

On the two habituation days, mice were transported to the experimental room, weighed, and allowed to rest in their home cage for one hour (habituation to room). Each animal was then handled for two minutes (habituation to handling) and returned to their home cage for one hour before being returned to the colony room.

Pretesting

Mice were removed from their cage and placed in the middle of a one-compartment apparatus configuration. One side of the apparatus had a Grid floor and the other side had a Hole floor. The side location of each floor within the apparatus was counterbalanced within groups. To minimize any potential latent inhibition effects the Pretest was 5 min in duration.

Acquisition (A)

Mice were removed from their home cage, given an IP injection of SAL or COC, placed in their assigned acquisition chamber for 15 min, and then returned to their cages. Half of the mice were assigned to one of two floor subgroups (injected with COC and placed on a Grid floor = G+) and half were assigned to the other floor subgroup (injected with COC and placed on a Hole floor = H+). Thus, mice in the G+ subgroup received COC immediately prior to trials on the Grid floor (G+) and SAL immediately prior to trials on the Hole floor (H-). Alternatively, mice in the H+ subgroup received COC immediately prior to trials on the Hole floor (H+) and SAL immediately prior to trials on the Grid floor (G-).

In Experiment 1, mice were assigned to one of two acquisition subgroups (one-compartment acquisition or two-compartment acquisition). Groups of mice that acquired CPP in one compartment were further identified as the A1 group (acquisition in one compartment). In this configuration, the left and right floor types were identical to each other during acquisition trials (either both Grid or both Hole) and mice had access to both sides of the apparatus, with no barrier separating each chamber side. Each floor type was consistently paired with COC or SAL during one-compartment acquisition, with no side cue pairing. Groups of mice that acquired CPP in two compartments were identified as the A2 group (acquisition in two compartments). In this configuration, the left and right floor types were different from each other during all phases of CPP (1 Grid side and 1 Hole side). A clear barrier restricted mice to one floor type and one side of the apparatus per trial. Each side of the chamber was consistently paired with COC or SAL but not both; therefore, each side, as well as each floor, consistently predicted COC or SAL during two-compartment acquisition with consistent side cues. Pretest preference was balanced between subsequent acquisition groups. All mice were trained over a 4 d period with one trial per day (CS+ and CS- trials on alternate days).

Testing

The test sessions were identical to the pretest session, except that the duration of each test was 30 min. Mice received microinjection of muscimol (n=11) or vehicle (n=15) 30-min prior to Test 1. Test 2 occurred 24 hr later with no injections.

Experiment 2: Effects of muscimol inactivation prior to an extinction session

Thirty male C57BL/6 received habituation and pretesting phases identical to Experiment 1.

Acquisition

Acquisition was identical to Experiment 1, except that all mice were trained in the two-compartment apparatus with consistent acquisition cues (A2) over a 4 d period (two CS+ trials, two CS- trials) with one trial per day. Twenty-four hours after the final acquisition session, all mice received a 5-min preference test (Test 1) that was used to assign mice to extinction groups.

Extinction

Extinction began 24 hr after Test 1. During extinction, mice were placed on their previously cocaine-paired (CS+) floor (Grid or Hole) in a one- or two-compartment extinction configuration (E1 or E2, respectively) for one nonreinforced 30-min trial. Mice received a microinjection of muscimol (n=16) or vehicle (n=14) 30-min prior to the extinction session. Twenty-four hours later, mice received a 30-min choice test (Test 2).

Experiment 3: Effects of muscimol inactivation prior to a CPP expression test after extinction (Test 2)

Twenty-three C57BL/6 mice received habituation and pretesting phases identical to Experiments 1 and 2. Each test (Pretest, Test 1, and Test 2) was completed 24 hr after the last Habituation, Acquisition, or Extinction session. The Pretest was 5 min, Test 1 was 5 min (to minimize extinction during the choice testing), and Test 2 was 30 min.

Acquisition (A)

Acquisition was identical to Experiment 2, with all mice were trained in the two-compartment apparatus with consistent cues (A2) over a 4d period (two CS+ trials, two CS- trials) with one trial per day. Twenty-four hours after the final acquisition session, all mice received a preference test (Test 1) that was used to match post-acquisition preference in subsequent extinction groups. Extinction began 24 hr after Test 1.

Extinction (E)

After two-compartment acquisition (A2), mice were assigned to one of two extinction subgroups, one-compartment extinction (E1) or two-compartment extinction (E2). As in Experiment 2, in one-compartment extinction (E1) trials, the left and right floor types were identical (both CS+ floors) and mice had access to both sides of the apparatus, with no barrier separating each chamber side. This resulted in exposure to the previously COC-associated floor (CS+) unrestricted by side. In the two-compartment extinction configuration with consistent cues (E2), mice were exposed to the CS+ floor while being restricted to the CS+ side of the apparatus. The CS- floor was inaccessible on the other side of the apparatus, separated by a clear divider. During extinction mice were removed from their

home cage and placed on their previously cocaine-paired (CS+) floor (Grid or Hole) for 30 min for two consecutive days (one trial per day). Another 30-min choice test (Test 2) occurred 24 hr after the final extinction session. Mice received a microinjection of muscimol (n=11) or vehicle (n=12) 30 min prior to Test 2.

Data Analysis—Dependent variables were locomotion (average distance traveled in cm) and preference (average percent of time spent on the CS+ floor). Locomotor activity and position of each animal in the CPP chamber (left/right side) was recorded by a camera and analyzed by Ethovision software (Noldus, Leesburg, VA). As expected, CPP configuration and drug (cocaine or saline) had effects on the distance traveled during training trials (i.e., acquisition and/or extinction), with one-compartment groups traveling significantly more in a larger area than two-compartment groups confined to a smaller area and cocaine causing more locomotion compared to saline. These effects are expected based on previous reports (e.g., Hitchcock et al., 2014); thus, only distance traveled during testing sessions will be highlighted to demonstrate differences between saline and muscimol groups.

Preference levels and the assignment of CS+ floor (Grid or Hole), CS+ side (left or right in two-compartment groups), microinjection drug (V or M), and order of IP injection (COC or SAL first or second) were counterbalanced between treatment groups. Independent variables of behavioral session (Pretest, Test 1, and Test 2), type of training configuration (one- or two-compartment), and microinjection drug (V or M) were analyzed separately or by repeated measures when applicable.

The magnitude of place preference was determined by the amount of time that each animal spent on the CS+ associated floor during each testing session. This was represented first by comparing the average seconds per minute (sec/min) that each floor subgroup spent on the Grid floor (animals conditioned to associate COC with the Grid floor, G+ subgroup, compared to animals conditioned to associate COC with the Hole floor, G– subgroup). To confirm CPP acquisition (Cunningham, Ferree, & Howard, 2003), the time spent on the Grid floor between conditioning subgroups (G+ and G–) was verified in each experiment (data not shown). No differences emerged by flooring subgroup assignment (G+ versus G–) at Pretest yet reliable differences emerged after conditioning, with no other consistent subgroup differences across all groups and experiments. As a consequence, these values (sec/min) were converted into a second measure of preference, the percent of time that animals spent on their cocaine-paired floor (G+ and H+ subgroups pooled). Preference of each treatment group is presented as a % of time spent on the CS+ floor throughout the paper.

Data were analyzed with Microsoft Office Excel and SPSS software. ANOVAs and planned Bonferroni follow-up tests compared differences at a significance level of 0.05 between behavioral sessions, bins of data within sessions, and by drug and configuration treatment group. Based on previous findings, we examined data in two ways. First, we compared data collected over the first 5 min of each session (0–5 min) to give a common point of comparison between tests and as a measure of initial memory retrieval from the previous session. Second, we compared data collected over the 30 min sessions (0–30 min) as a measure of within test session extinction (over six 5-min bins). All data are reported as the

mean $\pm$ the SEM. A Greenhouse-Geisser correction adjusted the degrees of freedom and p value for any violations of sphericity (Mauchly sphericity test, $p < 0.05$) when applicable.

DH Histology—Representative cannula placements above the cornu ammonis area 1 (CA1) and injector tracks within the DH are shown for each experiment. Placement was confirmed for in the DH by observing gliosis along the cannula and/or infusion tracts. Mouse brains were fixed in 10%-buffered-formalin (4% formaldehyde, 1% methanol stabilizer, Thermo Fisher Scientific Inc.) for at least 48h, sliced on a cryostat (60 μ m), and Nissl stained with cresyl-violet. Figure 1 shows the cannula placement from a representative brain section after cresyl-violet staining.

Results

Experiment 1: Effects of muscimol inactivation prior to a post-acquisition CPP expression test (Test 1)

In Experiment 1 (Figure 2), mice acquired CPP with either a one- or two-compartment procedure (A1 or A2, respectively). We inactivated the DH prior to a CPP test 24 hr after the final acquisition trial to evaluate whether the DH is necessary for expression of CPP. Further, because that test session (Test 1) is an extinction session, we can determine whether the DH is necessary for the development of extinction during that test by testing mice again 24h later (Test 2). An experimental overview and composite of microinjection placements from Experiment 1 are depicted in Figure 2A. There were no differences in DH cannula placement based on treatment in any of the experiments.

Locomotor activity—Mice that were given muscimol were more active than were the mice given PBS vehicle during Tests 1 and 2, primarily driven by results in the first 5 min of each session (Figure 2B). To determine whether locomotion differed during initial retrieval (in the first 5 min of testing) over the three testing sessions, a 3 (test-repeated) $\times$ 2 (drug) $\times$ 2 (configuration) ANOVA revealed a main effect of test ($F(1, 25) = 8.2$, $p = 0.007$), drug ($F(1, 22) = 6.4$, $p = 0.019$), and a reliable interaction of test $\times$ drug ($F(1, 44) = 3.4$, $p = 0.042$). No differences emerged in locomotion between treatment groups at Pretest, but a main effect of drug was confirmed during the first 5 min of Tests 1 and 2 ($F_s(1, 22) > 4.456$, $p_s < 0.046$). Over the 30 min of Tests 1 and 2, there were no reliable main effects of drug on locomotion, suggesting that any muscimol-induced increase in locomotion was transient.

Test preference—All groups showed approximately 50% preference for the CS+ floor during the Pretest (PT in Figures 2C and 2D). We examined effects of DH inactivation during the first 5 min of each test (to measure immediate retrieval) and during the course of the 30-min test (to measure resistance to within-session extinction). As seen in Figure 2C (first 5 min of each test), muscimol administration impaired initial expression of CPP at Test 1 based on the previous acquisition configuration. Correspondingly, Test 2 expression remained similar or rebounded in mice that received muscimol during Test 1. This was revealed by a significant main effect of test and interactions between test $\times$ drug, test $\times$ configuration, and test $\times$ drug $\times$ configuration ($F_s(2, 44) = 21.28, 9.701, 4.083, 3.597$; $p_s 0.001; 0.001, 0.024, 0.036$). We found no effect of drug or configuration at Pretest, but a

main effect of drug ($F(1, 22) = 29.810, p < 0.001$) and a drug $\times$ configuration interaction ($F(1, 22) = 7.274, p = 0.013$) at Test 1, and a main effect of configuration at Test 2 ($F(1, 22) = 8.609, p = 0.008$).

To determine the time course of effects in Tests 1 and Test 2 we examined each 5-min bin of data within and between tests (Figure 2D). A 2 (test-repeated) $\times$ 6 (bin-repeated) $\times$ 2 (drug) $\times$ 2 (configuration) ANOVA confirmed a main effect of configuration ($F(1, 22) = 4.844, p = 0.039$) and an interaction of test $\times$ drug ($F(1, 22) = 13.434, p = 0.001$), with Figure 2D showing an increase in the muscimol groups and a decrease in the vehicle groups at Test 2.

In this first experiment, we trained animals in a one or two-compartment apparatus and found that preference was altered by a DH microinjection and previous apparatus configuration. Muscimol disrupted preference expression in mice trained in a one- or two-compartment procedure, with the largest impairment occurring in the two-compartment treatment group. When tested the next day, that two-compartment muscimol group showed the highest preference, suggesting that impairing hippocampal function during Test 1 interfered with the development of extinction that typically occurs during testing. These findings suggest that the dorsal hippocampus is involved in expression and extinction of CPP following a strong two-compartment acquisition protocol. In Experiment 2, we ask whether the dorsal hippocampus is involved in extinction with different compartment configurations.

Experiment 2: Effects of muscimol inactivation prior to an extinction session

To test whether activity within the DH contributes to either one- (E1) or two- (E2) compartment extinction and subsequent retrieval and extended extinction (during drug-free testing), we inactivated the DH prior to an extinction session. We hypothesized that impairment in DH activity during extinction would lead to maintained preference early at Test 2 (over 0–5 min) and typical extinction rates during the full test (over 30 min). We also hypothesized that if one- and two- compartment configuration activated the DH differently during extinction, then the pattern of CPP expression after extinction may differ between configuration groups at Test 2. An experimental overview and cannula placements for Experiment 2 are shown in Figure 3A.

Locomotor activity—Activity that occurred during extinction and Test 2 is shown in Figure 3B. Activity was generally higher during Test 1 in the one-compartment groups, with highest activity in the first 5 min in the E1 muscimol group. During the first 5 min of extinction, there were reliable main effects of drug ($F(1, 26) = 6.277, p = 0.019$) and configuration ($F(1, 26) = 13.054, p = 0.001$), as well as a reliable interaction between the two ($F(1, 26) = 4.782, p = 0.038$). During the entire 30 min extinction session, there were reliable main effects of configuration ($F(1, 26) = 9.756, p = 0.004$) and bin ($F(1.8, 47.1) = 17.836, p < 0.001$), and an interaction of bin and drug ($F(5, 130) = 3.010, p = 0.013$). There were no differences in activity between treatment groups when we analyzed the entire duration of Test 2, although there was a main effect of bin ($F(3.7, 95.8) = 12.303, p < 0.001$), as activity decreased in all groups through the session.

Test preference—Test preferences for the first 5 min of each session are shown in Figure 3C. There was no effect of drug, but a main effect of session ($F(2, 52) = 53.227, p < 0.001$),

and a session $\times$ configuration interaction ($F(2, 52) = 3.912, p < 0.026$) over the three sessions. A follow-up ANOVA (2 sessions-repeated $\times$ 2 configurations) confirmed that preference changed from Test 1 and Test 2 differently between extinction configuration groups ($F(1, 26) = 6.257, p < 0.019$). In the first 5 min of Test 2 there was a reliable difference between configuration groups ($p = 0.027$), with the one-compartment E1 groups expressing less preference than the two-compartment E2 groups.

As seen in Figure 3D, a main effect of configuration ($F(1, 26) = 4.657, p < 0.040$) and of bin ($F(3.2, 84.0) = 4.530, p = 0.004$) emerged over the 6 bins during Test 2, with no drug effect ($p = 0.190$). Follow-up tests revealed that configuration effects occurred in the first 5 (as noted above) and 10 min of Test 2 ($ps = 0.023$, Figure 5B) with no drug effect in any bin ($ps > 0.11$).

As an additional comparison, we analyzed configuration groups separately for total preference expressed before (Test 1, 5 min) and after (Test 2, 30 min test) extinction. As expected when comparing a short to a long session, there was a main effect of session ($F(1, 13) = 72.78, p < 0.001$) as well as a session by drug effect ($F(1, 13) = 4.824, p = 0.047$) in the one-compartment group. In the two-compartment group there was a session effect ($F(1, 13) = 16.728, p = 0.001$) but no session by drug effect ($F(1, 13) < 1.0, p = 0.998$). This indicated a minor impairment in extinction from Test 1 to the end of Test 2 in the muscimol-treated one-compartment group relative to the vehicle-treated one-compartment group (Figure 5B).

In Experiment 2, we trained animals in a two-compartment apparatus and extinguished their preference in a one- (E1) or two- (E2) compartment apparatus (one trial). We found a slight impairment in mice that received muscimol in the DH prior to one-compartment extinction, suggesting that the hippocampus may be recruited during this type of extinction that leads to a greater loss of CPP in vehicle-treated animals.

Experiment 3: Effects of muscimol inactivation prior to a CPP expression test after extinction (Test 2)

In Experiment 3 (Figure 4), mice acquired a CPP with a two-compartment (A2) procedure. We inactivated the DH prior to a CPP test 24 hr after two extinction trials (one or two compartment) to evaluate whether the DH is necessary for retrieval of CPP extinction (Test 2). We expected that if one- and two-compartment extinction configuration groups activated the DH differently during initial retrieval of CPP extinction, then preference would differ. The experimental overview and depiction of cannula placements are shown in Figure 4A.

Locomotor activity—To test whether activity differed between groups in the initial 5 min of testing, we compared distance traveled over each testing session as a function of drug and configuration treatment (ANOVA: 3 (test-repeated) $\times$ 2 (drug) $\times$ 2 (configuration), Figure 4B). A main effect of test ($F(1.5, 28.4) = 4.765, p = 0.025$) and an interaction between test and drug ($F(2, 38) = 5.316, p = 0.009$) were confirmed, with no main effects of configuration or drug. When we compared distance across the extended 30 min duration of the post-extinction Test 2 (ANOVA: 6 (bin-repeated) $\times$ 2 (drug) $\times$ 2 (configuration)), there was a reliable main effect of bin ($F(3.1, 58.9) = 25.366, p < 0.001$) as extinction occurred, and a bin $\times$ drug interaction ($F(5, 95) = 4.086, p < 0.002$), with no effect of extinction

configuration. There were no effects between treatment groups at Test 1 but a reliable effect of drug emerged at Test 2, such that mice given muscimol at Test 2 reliably increased their distance traveled and vehicle-treated mice did not ($F(1, 19) = 6.713, p = 0.018$).

Test Preference—Expression of CPP did not differ during PT or during the first 5 min of the tests following acquisition (Test 1) or extinction (Test 2, Figure 4C). This was confirmed by a 3 (test-repeated) $\times 2$ (drug) $\times 2$ (configuration) ANOVA, with a reliable effect of test ($F(2, 38) = 44.117, p < 0.001$). After extinction, a decrease in preference occurred in the E1 group, although this did not reach reliable levels ($F(1, 19) = 3.676, p = 0.070$).

We also compared extinction curves over the entire 30-min Test 2 session (6×5 min bins), while muscimol was present in the DH, and confirmed an interaction between test, drug, and configuration ($F(5, 95) = 3.002, p = 0.015$; see Figure 4D). To follow-up the interaction, we compared drug effects at each Test 2 bin within each extinction configuration group, and found a main effect of drug and an interaction between drug and bin in the E1 group ($F(1, 10) = 5.642, p = 0.039$; $F(5, 50) = 2.845, p = 0.025$, respectively) but not in the E2 group ($p > 0.279$).

The findings from Experiment 3 suggest that expression of extinction after a strong behavioral extinction treatment (one-compartment extinction) was impaired by DH inactivation. In contrast, there were no effects of DH inactivation on expression after weak behavioral treatment that does not produce much behavioral extinction (two-compartment extinction).

Discussion

These experiments demonstrate that inactivation of the DH impairs the post-acquisition expression, extinction, and post-extinction expression of a cocaine-induced conditioned place preference. In general, these effects occurred with different apparatus configurations (one- or two-compartment) but were largest in those conditions that led to stronger acquisition (two-compartment) or stronger extinction (one-compartment) in vehicle-treated groups. These findings extend previous work, showing that acquisition and extinction of CPP is sensitive to the configuration of the CPP chamber and that the DH is necessary for retrieval of drug-associated memories (Cunningham, et al., 2006; Cunningham & Zerizet, 2014; Ferbinteanu & McDonald, 2001; Hitchcock, et al., 2014; Meyers et al., 2003, 2006).

Hippocampal inactivation immediately prior to a post-acquisition expression test caused a general impairment in expression of CPP, but this effect was more pronounced in the consistent spatial location condition (two-compartment procedure). Previous work has found that, in general, a two-compartment acquisition procedure leads to stronger CPP compared to a one-compartment procedure (Hitchcock et al., 2014). One difference between these procedures is the role of spatial cues. In the two-compartment procedure, spatial cues consistently predicted the side in which drug was delivered; in the one-compartment procedure, spatial cues were irrelevant. In Experiment 1, inactivating the DH prior to an expression test resulted in a pronounced deficit in mice trained in the two-compartment (spatial) procedure. This suggests that, relative to one-compartment training, two-

compartment training may result in a larger dependence on spatial cues compared to tactile cues, which may in turn require the DH for retrieval.

In Experiment 1, vehicle-treated mice expressed a preference in the absence of cocaine during Test 1 that led to extinction, revealed as low preference at Test 2. However, the two-compartment muscimol-treated mice did not express a preference during Test 1, but then expressed a high preference at Test 2, suggesting that muscimol inactivation of the DH during Test 1 prevented expression of the original memory and the new learning of the extinction contingencies. This finding is consistent with others demonstrating that recall of previous learning is necessary for new extinction (Corcoran, Desmond, Frey, & Stephan, 2005; Holt & Maren, 1999; Meyers, Zavala, Speer, & Neisewander, 2006, but see Peters, et al., 2010).

In contrast to acquisition, which is promoted by two-compartment training, extinction is promoted by one-compartment training, potentially due to the association between extinction and multiple spatial locations within the apparatus (Hitchcock et al., 2014). When the DH was inactivated during the post-acquisition expression test (Test 1 in Experiment 1), extinction was impaired most in the group that received acquisition training in two-compartment. This suggests that in addition to being involved in the expression of CPP, the DH is involved in the learning that occurred during the choice test extinction trial. Finally, when extinction was allowed to occur with normal hippocampal involvement (Test 2, Experiment 3), muscimol injected prior to a post-extinction test disrupted retrieval of extinction in the group that received strong extinction training in one compartment.

In addition to showing that the effects of hippocampal inactivation on expression and extinction of CPP may differ as a function of the configuration of the apparatus, we found that the presence of an effect sometimes differs on when in the test session preference is assessed (i.e., at the beginning or through the entirety of the test session). For example, in Experiment 3 all groups showed similar initial retrieval of extinction in the first 5 min of the post-extinction Test 2, but an effect emerged during the course of that choice test between one-compartment extinction drug groups. This result was similar to the more modest effect that occurred in Experiment 2, as muscimol treatment before one-compartment extinction led to less extinction by the end of Test 2 compared to vehicle treatment before one-compartment extinction. This suggests that the DH is important for within-session extinction in a choice test procedure and may play a role in savings of extinction learning with repeated extinction training (Weidemann & Kehoe, 2003), where subsequent learning can be rapidly recalled or extended after initial training. These findings in general further emphasize that rates of extinction within a test session may reveal different effects from those that occur early in a test session.

It is important to note the consistency of activity effects due to muscimol. In all experiments, activity was reliably higher in muscimol- compared to vehicle-treated mice when the injections occurred immediately prior to testing. Other studies have shown increases, decreases, no change, or have not reported locomotor activity after DH inactivation or lesion (Chee, Menard, & Dringenberg, 2015; Corcoran & Maren, 2001; Douglas, 1967; Fujiwara et al., 2012; Meyers et al., 2003, 2006; Rezayof, Razavi, Haeri-Rohani, Rassouli, & Zarrindast,

2007). The DH is thought to regulate activity and interact with other brain regions, such as the entorhinal cortex (EC), ventral hippocampus (VH), prefrontal cortex (PFC), nucleus accumbens (NA) and amygdala (AMY; Fanselow & Dong, 2010; Gould & Leach, 2014; Hermann, Stark, Blecker, et al., 2017; Khoo, Gibson, Prasad, et al., 2017; Quirk & Mueller, 2008; Strange, Witter, Lein, et al., 2014; Wells et al., 2011; Zelikowsky, Hersman, Chawla, et al., 2014). Inactivation of the DH may inhibit activity in the lateral amygdala (LA), and decrease freezing behavior, especially in a context that was not previously extinguished (Maren & Hobin, 2007). In a similar way, we saw that previous context training and muscimol treatment regulated locomotion and CPP expression, but often in different directions based on the experiment.

Most studies have tested behavior 20 to 60 minutes after intra-DH infusions of muscimol (Corcoran et al., 2005; Matus-Amat et al., 2004; Riaz, Schumacher, Sivagurunathan, Van Der Meer, & Ito, 2017). There is evidence that muscimol binding peaks immediately after injection (samples collected at 0 min post-infusion) with a spread up to ± 1.5 mm in the rostrocaudal direction, that slowly dissipates for approximately 1–2 hours, both in the DH (Corcoran et al., 2005) and similarly in the sensorimotor cortex (Martin & Ghez, 1999). These factors are worth considering for the potential effect on place preference in relation to activity changes. The most common concern with activity is that increases in activity are often co-expressed with decreases in preference and can compete during testing. This makes it less clear whether decreases in preference are due to a conditioned motivational response or an indirect result of increased locomotion (Gremel & Cunningham, 2007).

In our experiments, a DH microinjection of muscimol consistently increased activity but these activity effects corresponded to different effects on preference depending on the experiment. For example, preference expression was decreased (Experiment 1), relatively unchanged (Experiment 2), and increased (Experiment 3), after a muscimol microinjection that consistently increased activity. Similarly, a larger apparatus configuration increased activity during conditioning, with no consistent direction of effects on preference (highest activity – highest preference). The opposite was also found, with no change in activity between drug groups later in testing sessions, preference differences often occurred independently (Experiments 1–3). Therefore, it is unlikely that changes in activity, due to a microinjection or configuration treatment, independently explain the differences demonstrated in preference.

An open question is whether different CPP configurations recruit different circuits and cellular or molecular mechanisms. Our results suggest that the involvement of the DH may change as a function of apparatus configuration, which is supported by other studies. Because the two-compartment training procedure involves a consistent spatial location, it is quite possible that mechanisms that have been identified to represent space in the DH (such as coordinated cell activity between subregions) come more into play than those in the one-compartment procedure, in which spatial cues are explicitly made irrelevant. This could mean that the two-compartment group may recruit other brain regions at training, and then need the DH and other regions (e.g., NA, AMY, ventral tegmental area (Bouton, Westbrook, Corcoran, & Maren, 2006; Crombag, Bossert, Koya, & Shaham, 2008; Dunsmoor, Niv, Daw, & Phelps, 2015) more critically at testing to determine preference in the choice test

configuration. Differences in configuration may also change how the DH is activated and to what degree, potentially tilting the molecular processes that are related.

Perhaps regions that are activated to a greater degree may create more stability in the molecular cascades being initiated. A previous study has shown that spatial and non-spatial water maze learning leads to different expression levels of immediate early genes (IEGs) such as activity-regulated cytoskeleton-associated protein (*arc*) and (*zif*) in the DH, but may or may not be correlated to other regions based on the type of IEG and experience (Guzowski, Setlow, Wagner, & McGaugh, 2001). Other cases have demonstrated that activity-dependent changes can differ based on IEGs, brain region, and changes in behavioral design and context exposure. For example, rats exposed to a previous cocaine self-administration context demonstrate increases in DG *arc* expression, independent of lever presentation or lever activity, whereas CA1 and CA3 *arc* increases only when levers are presented (Hearing, Schochet, See, & McGinty, 2010). This suggests that *arc* induction in the DH may occur during repeated presentations of the same context during exploration of the same space, as context-drug associations are modified, such as during extinction and as consolidation of new contextual and drug information occurs (Camarota et al., 2005; Hearing et al., 2010; Ramirez et al., 2009; Vazdarjanova, 2004). Similar results have been noted in related structures, such as the intercalated cells of the amygdala (Busti et al., 2011) where retrieval and extinction increase phosphorylated alpha Ca^{2+} /calmodulin-dependent protein kinase II (Huang, Chen, Liang, & Hsu, 2014; Meins et al., 2010).

At a theoretical level, the contextual difference between training and testing may identify as a larger mismatch by the DH after two-compartment acquisition training, requiring the DH at post-acquisition testing, but as a small mismatch after one-compartment training, requiring the DH less for expression of preference at testing. This hypothesis is consistent with previous studies (i.e., Meyers, Zavala, & Neisewander, 2003; Meyers, Zavala, Speer, & Neisewander, 2006) and suggests that while both groups may be accessing information in the DH at training and/or testing the two-compartment group may be relying on the DH to a larger degree at testing, and the one-compartment group may be recruiting it more at training. However, salient tactile cues may form stronger associations with the surrounding visuospatial and contextual cues during extinction than during acquisition training and testing. This change towards a context-specific type of learning, from acquisition to extinction, may be facilitated by the one-compartment configuration more than the two-compartment configuration. As extinction is sometimes more resistant to renewal when it occurs in multiple contexts (Balooch et al., 2012; Chelonis, Calton, Hart, & Schachtman, 1999; Gruber & McDonald, 2012), and enhanced with similar training and testing environments, our results suggest that allowing extinction to occur in more than one spatial location within the one-compartment configuration (absolute larger area that has previous associations with both CS+ and CS- floors) enhanced extinction and the reliance on the DH during extinction training and testing. It is also possible that the DH is supporting acquisition and extinction learning and retrieval of learning in different ways (i.e. recalling a contextual memory or navigating in a novel context). This difference in DH involvement may be determined by the configuration used during training.

Together, these results demonstrate hippocampal involvement in cocaine-induced CPP interact with the behavioral conditions that are present at the time of acquisition, extinction, and testing. Our findings that the role of the hippocampus in these different learning processes may change as a function of how the context is configured emphasize the importance of comparing different apparatus configurations in the study of the neurobiology of conditioned place preference. This is especially important in these types of studies because the way that CPP is acquired, extinguished, and tested differs widely among laboratories.

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References

- Abraham AD, Neve KA, Lattal KM. Activation of D1/5 Dopamine Receptors: A Common Mechanism for Enhancing Extinction of Fear and Reward-Seeking Behaviors. *Neuropsychopharmacology*. 2016a; 41(8):2072–2081. <https://doi.org/10.1038/npp.2016.5>. [PubMed: 26763483]
- Abraham AD, Neve KA, Lattal KM. Effects of D1 receptor knockout on fear and reward learning. *Neurobiology of Learning and Memory*. 2016b; 133:265–273. <https://doi.org/10.1016/j.nlm.2016.07.010>. [PubMed: 27423521]
- Anagnostaras SG, Maren S, Fanselow MS. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 1999; 19(3):1106–14. <https://doi.org/http://hdl.handle.net/2027.42/56234>. [PubMed: 9920672]
- Bevins, MTBRA. Conditioned place preference: what does it add to our preclinical understanding of drug reward?. 2000. p. 31-43. <https://doi.org/10.1007/s002130000569>
- Bouton M, Westbrook F, Corcoran K, Maren S. Contextual and Temporal Modulation of Extinction: Behavioral and Biological Mechanisms. *Biological Psychiatry*. 2006; 60(4):352–360. <https://doi.org/10.1016/j.biopsych.2005.12.015>. [PubMed: 16616731]
- Busti D, Geracitano R, Whittle N, Dalezios Y, Ma ko M, Kaufmann W, Ferraguti F. Different Fear States Engage Distinct Networks within the Intercalated Cell Clusters of the Amygdala. *Journal of Neuroscience*. 2011; 31(13):5131–5144. <https://doi.org/10.1523/JNEUROSCI.6100-10.2011>. [PubMed: 21451049]
- Cammarota, M., Bevilaqua, LRM., Barros, DM., Vianna, MRM., Izquierdo, LA., Medina, JH., Izquierdo, I. Retrieval and the extinction of memory. *Cellular and Molecular Neurobiology*. 2005. <https://doi.org/10.1007/s10571-005-4009-z>
- Chee S-SA, Menard JL, Dringenberg HC. The lateral septum as a regulator of hippocampal theta oscillations and defensive behavior in rats. *Journal of Neurophysiology*. 2015; 113(6):1831–1841. <https://doi.org/10.1152/jn.00806.2014>. [PubMed: 25589592]
- Corcoran K, Desmond T, Frey K, Stephan M. Hippocampal Inactivation Disrupts the Acquisition and Contextual Encoding of Fear Extinction. *Journal of Neuroscience*. 2005; 25(39):8978–8987. <https://doi.org/10.1523/JNEUROSCI.2246-05.2005>. [PubMed: 16192388]
- Corcoran K, Maren S. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *The Journal of Neuroscience*. 2001; 21(5):1720–1726. <https://doi.org/10.1523/JNEUROSCI.2246-05.2005>. [PubMed: 11222661]
- Crombag HS, Bossert JM, Koya E, Shaham Y. Review. Context-induced relapse to drug seeking: a review. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 2008; 363:3233–3243. <https://doi.org/10.1098/rstb.2008.0090>. [PubMed: 18640922]
- Cunningham CL, Ferree NK, Howard MA. Apparatus bias and place conditioning with ethanol in mice. *Psychopharmacology*. 2003; 170(4):409–422. <https://doi.org/10.1007/s00213-003-1559-y>. [PubMed: 12955296]

- Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. *Nature Protocols*. 2006; 1(4):1662–1670. <https://doi.org/10.1038/nprot.2006.279>. [PubMed: 17487149]
- Cunningham CL, Patel P, Milner L. Spatial location is critical for conditioning place preference with visual but not tactile stimuli. *Behavioral Neuroscience*. 2006; 120(5):1115–32. <https://doi.org/10.1037/0735-7044.120.5.1115>. [PubMed: 17014262]
- Cunningham CL, Zerizel CL. Effects of combining tactile with visual and spatial cues in conditioned place preference. *Pharmacology, Biochemistry and Behavior*. 2014; 124:443–450. <https://doi.org/10.1016/j.pbb.2014.07.012>.
- Douglas R. The hippocampus and behavior. *Psychol Bull*. 1967; (67):416–442. [PubMed: 4859959]
- Dunsmoor JE, Niv Y, Daw N, Phelps EA. Rethinking Extinction. *Neuron*. 2015; 88(1):47–63. <https://doi.org/10.1016/j.neuron.2015.09.028>. [PubMed: 26447572]
- Fanselow MS, Dong H-W. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron*. 2010; 65(1):7–19. <https://doi.org/10.1016/j.neuron.2009.11.031>. [PubMed: 20152109]
- Ferbinteanu J, McDonald RJ. Dorsal / Ventral Hippocampus, Fornix, and Conditioned Place Preference. *Hippocampus*. 2001; 11(2):187–200. <https://doi.org/10.1002/hipo.1036>. [PubMed: 11345125]
- Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, See RE. The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*. 2005; 30(2):296–309. <https://doi.org/10.1038/sj.npp.1300579>. [PubMed: 15483559]
- Fujiwara H, Sawa K, Takahashi M, Lauwereyns J, Tsukada M, Aihara T. Context and the renewal of conditioned taste aversion: The role of rat dorsal hippocampus examined by electrolytic lesion. *Cognitive Neurodynamics*. 2012; 6(5):399–407. <https://doi.org/10.1007/s11571-012-9208-y>. [PubMed: 24082961]
- Gill PR, Mizumori SJY, Smith DM. Hippocampal episode fields develop with learning. *Hippocampus*. 2011; 21(11):1240–1249. <https://doi.org/10.1002/hipo.20832>. [PubMed: 20665593]
- Gould TJ, Leach PT. Cellular, molecular, and genetic substrates underlying the impact of nicotine on learning. *Neurobiology of Learning and Memory*. 2014; 107:108–32. <https://doi.org/10.1016/j.nlm.2013.08.004>. [PubMed: 23973448]
- Gremel CM, Cunningham CL. Role of test activity in ethanol-induced disruption of place preference expression in mice. *Psychopharmacology*. 2007; 191(2):195–202. <https://doi.org/10.1007/s00213-006-0651-5>. [PubMed: 17216157]
- Guzowski JF, Setlow B, Wagner EK, McGaugh JL. Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2001; 21(14):5089–5098. <https://doi.org/10.1523/JNEUROSCI.2114-01.2001> [pii]. [PubMed: 11438584]
- Hearing MC, Schochet TL, See RE, McGinty JF. Context-driven cocaine-seeking in abstinent rats increases activity-regulated gene expression in the basolateral amygdala and dorsal hippocampus differentially following short and long periods of abstinence. *Neuroscience*. 2010; 170(2):570–9. <https://doi.org/10.1016/j.neuroscience.2010.07.027>. [PubMed: 20654701]
- Hermann A., Stark R., Blecker CR., Milad MR., Merz CJ. Brain structural connectivity and context-dependent extinction memory; *Hippocampus*. 2017 Apr. p. 1–22. <https://doi.org/10.1002/hipo.22738>
- Hitchcock LN, Cunningham CL, Lattal KM. Cue configuration effects in acquisition and extinction of a cocaine-induced place preference. *Behavioral Neuroscience*. 2014; 128(2):217–27. <https://doi.org/10.1037/a0036287>. [PubMed: 24773441]
- Holt W, Maren S. Muscimol inactivation of the dorsal hippocampus impairs contextual retrieval of fear memory. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 1999; 19(20):9054–9062. <https://doi.org/10.1523/JNEUROSCI.1051-99.2000>. [PubMed: 10516322]
- Huang C-C, Chen C-C, Liang Y-C, Hsu K-S. Long-term potentiation at excitatory synaptic inputs to the intercalated cell masses of the amygdala. *The International Journal of*

- Neuropsychopharmacology. 2014; 17(8):1233–1242. <https://doi.org/10.1017/S1461145714000133>. [PubMed: 24556032]
- Itzhak Y, Liddie S, Anderson KL. Sodium butyrate-induced histone acetylation strengthens the expression of cocaine-associated contextual memory. *Neurobiology of Learning and Memory*. 2013; 102:34–42. <https://doi.org/10.1016/j.nlm.2013.03.007>. [PubMed: 23567105]
- Jezek K, Henriksen EJ, Treves A, Moser EI, Moser M-B. Theta-paced flickering between place-cell maps in the hippocampus. *Nature*. 2011; 478(7368):246–249. <https://doi.org/10.1038/nature10439>. [PubMed: 21964339]
- Khoos SYS, Gibson GD, Prasad AA, McNally GP. How contexts promote and prevent relapse to drug seeking. *Genes, Brain and Behavior*. 2017; 16(1):185–204. <https://doi.org/10.1111/gbb.12328>.
- Kramar CP, Flavia Barbano M, Medina JH, Barbano MF, Medina JH, Flavia Barbano M, Medina JH. Dopamine D1/D5 receptors in the dorsal hippocampus are required for the acquisition and expression of a single trial cocaine-associated memory. *Neurobiology of Learning and Memory*. 2014; 116:172–180. <https://doi.org/10.1016/j.nlm.2014.10.004>. [PubMed: 25452086]
- Leutgeb JK, Leutgeb S, Treves A, Meyer R, Barnes CA, McNaughton BL, Moser EI. Progressive transformation of hippocampal neuronal representations in “morphed” environments. *Neuron*. 2005; 48(2):345–348. <https://doi.org/10.1016/j.neuron.2005.09.007>. [PubMed: 16242413]
- Maren S, Hobin JA. Hippocampal regulation of context-dependent neuronal activity in the lateral amygdala. *Learning & Memory*. 2007; 14(4):318–324. <https://doi.org/10.1101/lm.477007>. [PubMed: 17522021]
- Martin JH, Ghez C. Pharmacological inactivation in the analysis of the central control of movement. *Journal of Neuroscience Methods*. 1999; 86(2):145–159. [https://doi.org/10.1016/S0165-0270\(98\)00163-0](https://doi.org/10.1016/S0165-0270(98)00163-0). [PubMed: 10065983]
- Matus-Amat P, Higgins EA, Barrientos RM, Rudy JW. The Role of the Dorsal Hippocampus in the Acquisition and Retrieval of Context Memory Representations. *Journal of Neuroscience*. 2004; 24(10):2431–2439. <https://doi.org/10.1523/JNEUROSCI.1598-03.2004>. [PubMed: 15014118]
- Maze I, Covington HE, Dietz DM, LaPlant Q, Renthall W, Russo SJ, Nestler EJ. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science (New York, N.Y.)*. 2010; 327(5962):213–216. <https://doi.org/10.1126/science.1179438>.
- Meins M, Herry C, Müller C, Ciocchi S, Moreno E, Lüthi A, Monard D. Impaired fear extinction in mice lacking protease nexin-1. *European Journal of Neuroscience*. 2010; 31(11):2033–2042. <https://doi.org/10.1111/j.1460-9568.2010.07221.x>. [PubMed: 20529116]
- Meyers, Ra, Zavala, AR., Neisewander, JL. Dorsal, but not ventral, hippocampal lesions disrupt cocaine place conditioning. *Neuroreport*. 2003; 14(16):2127–2131. <https://doi.org/10.1097/00001756-200311140-00023>. [PubMed: 14600510]
- Meyers, Ra, Zavala, AR., Speer, CM., Neisewander, JL. Dorsal hippocampus inhibition disrupts acquisition and expression, but not consolidation, of cocaine conditioned place preference. *Behavioral Neuroscience*. 2006; 120(2):401–12. <https://doi.org/10.1037/0735-7044.120.2.401>. [PubMed: 16719704]
- Nasehi M, Kamali-dolatabadi L. Physiology & Behavior Possible involvement of the CA1 GABA A receptors upon acquisition and expression of the ACPA-induced place preference in mice. *Physiology & Behavior*. 2016; 161:155–165. <https://doi.org/10.1016/j.physbeh.2016.04.021>. [PubMed: 27090228]
- Neisewander JL, Baker DA, Fuchs Ra, Tran-Nguyen LTL, Palmer A, Marshall JF. Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2000; 20(2):798–805. <https://doi.org/10.1038/466194a>. [PubMed: 10632609]
- Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ. Induction of Fear Extinction with Hippocampal-Infralimbic BDNF. *Science*. 2010; 328(5983):1288–1290. <https://doi.org/10.1126/science.1186909>. [PubMed: 20522777]
- Portugal GS, Al-Hasani R, Fakira AK, Gonzalez-Romero JL, Melyan Z, McCall JG, Moron JA. Hippocampal Long-Term Potentiation Is Disrupted during Expression and Extinction But Is Restored after Reinstatement of Morphine Place Preference. *Journal of Neuroscience*. 2014; 34(2):527–538. <https://doi.org/10.1523/JNEUROSCI.2838-13.2014>. [PubMed: 24403152]

- Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*. 2008; 33(1):56–72. <https://doi.org/10.1038/sj.npp.1301555>. [PubMed: 17882236]
- Ramirez DR, Bell GH, Lasseter HC, Xie X, Traina SA, Fuchs RA. Dorsal hippocampal regulation of memory reconsolidation processes that facilitate drug context-induced cocaine-seeking behavior in rats. *The European Journal of Neuroscience*. 2009; 30(5):901–12. <https://doi.org/10.1111/j.1460-9568.2009.06889.x>. [PubMed: 19712098]
- Raybuck JD, Lattal KM. Double dissociation of amygdala and hippocampal contributions to trace and delay fear conditioning. *PLoS ONE*. 2011; 6(1):8–12. <https://doi.org/10.1371/journal.pone.0015982>.
- Raybuck JD, Lattal KM. Differential effects of dorsal hippocampal inactivation on expression of recent and remote drug and fear memory. *Neuroscience letters*. 2014; 569:1–5. [PubMed: 24686177]
- Raybuck JD, McCleery EJ, Cunningham CL, Wood MA, Lattal KM. The histone deacetylase inhibitor sodium butyrate modulates acquisition and extinction of cocaine-induced conditioned place preference. *Pharmacology, Biochemistry, and Behavior*. 2013; 106:109–16. <https://doi.org/10.1016/j.pbb.2013.02.009>.
- Rezayof A, Razavi S, Haeri-Rohani A, Rassouli Y, Zarrindast MR. GABAA receptors of hippocampal CA1 regions are involved in the acquisition and expression of morphine-induced place preference. *European Neuropsychopharmacology*. 2007; 17(1):24–31. <https://doi.org/10.1016/j.euroneuro.2006.02.003>. [PubMed: 16624534]
- Riaz S, Schumacher A, Sivagurunathan S, Van Der Meer M, Ito R. Ventral, but not dorsal, hippocampus inactivation impairs reward memory expression and retrieval in contexts defined by proximal cues. *Hippocampus*. 2017; 27(7):1–49. <https://doi.org/10.1002/hipo.22734>.
- Riedel G, Micheau J, Lam AG, Roloff EL, Martin SJ, Bridge H, ... Morris RG. Reversible neural inactivation reveals hippocampal participation in several memory processes. *Nature Neuroscience*. 1999; 2(10):898–905. <https://doi.org/10.1038/13202>. [PubMed: 10491611]
- Sadeghi B, Ezzatpanah S, Haghighparast A. Effects of dorsal hippocampal orexin-2 receptor antagonism on the acquisition, expression, and extinction of morphine-induced place preference in rats. *Psychopharmacology*. 2016; 233(12):2329–2341. <https://doi.org/10.1007/s00213-016-4280-3>. [PubMed: 27048158]
- Stackman RW, Cohen SJ, Lora JC, Rios LM. Temporary inactivation reveals that the CA1 region of the mouse dorsal hippocampus plays an equivalent role in the retrieval of long-term object memory and spatial memory. *Neurobiology of Learning and Memory*. 2016; 133:118–128. <https://doi.org/10.1016/j.nlm.2016.06.016>. [PubMed: 27330015]
- Strange BA, Witter MP, Lein ES, Moser EI. Functional organization of the hippocampal longitudinal axis. *Nature Publishing Group*. 2014; 15(10):655–669. <https://doi.org/10.1038/nrn3785>.
- Tan SE. Roles of hippocampal NMDA receptors and nucleus accumbens D1 receptors in the amphetamine-produced conditioned place preference in rats. *Brain Res Bull*. 2008; 77(6):412–419. <https://doi.org/10.1016/j.brainresbull.2008.09.007>. [PubMed: 18929625]
- Todd TP. Mechanisms of renewal after the extinction of instrumental behavior. *Journal of Experimental Psychology. Animal Behavior Processes*. 2013; 39(3):193–207. <https://doi.org/10.1037/a0032236>. [PubMed: 23627796]
- Vazdarjanova A. Differences in Hippocampal Neuronal Population Responses to Modifications of an Environmental Context: Evidence for Distinct, Yet Complementary, Functions of CA3 and CA1 Ensembles. *Journal of Neuroscience*. 2004; 24(29):6489–6496. <https://doi.org/10.1523/JNEUROSCI.0350-04.2004>. [PubMed: 15269259]
- Weidemann G, Kehoe EJ. Savings in classical conditioning in the rabbit as a function of extended extinction. *Learn Behav*. 2003; 31(1):49–68. <https://doi.org/10.3758/BF03195970>. [PubMed: 18450069]
- Wells AM, Lasseter HC, Xie X, Cowhey KE, Reitinger AM, Fuchs RA. Interaction between the basolateral amygdala and dorsal hippocampus is critical for cocaine memory reconsolidation and subsequent drug context-induced cocaine-seeking behavior in rats. *Learning & Memory (Cold Spring Harbor, N.Y.)*. 2011; 18(11):693–702. <https://doi.org/10.1101/lm.227311>.

- White NM, Chai SC, Hamdani S. Learning the morphine conditioned cue preference: Cue configuration determines effects of lesions. *Pharmacology Biochemistry and Behavior*. 2005; 81(4):786–796. <https://doi.org/10.1016/j.pbb.2005.06.002>.
- Yousefi B, Farjad M, Nasehi M, Reza M, Zarrindast MR. Involvement of the CA1 GABAA receptors in ACPA-induced impairment of spatial and non-spatial novelty detection in mice. *Neurobiology of Learning and Memory*. 2013; 100:32–40. <https://doi.org/10.1016/j.nlm.2012.12.001>. [PubMed: 23231965]
- Zelikowsky M, Hersman S, Chawla MK, Barnes CA, Fanselow MS. Neuronal Ensembles in Amygdala, Hippocampus, and Prefrontal Cortex Track Differential Components of Contextual Fear. *Journal of Neuroscience*. 2014; 34(25) Retrieved from <http://www.jneurosci.org/content/34/25/8462.long>.

Figure 1. Representative dorsal hippocampal (DH) cannulation
Mouse section (60um) after microinjection and staining with cresyl-violet.

Figure 2. Effects of intra-DH muscimol prior to a post-acquisition CPP Test (Experiment 1)
(A) Overview of the design of Experiment 1. Mice received a pretest (PT) and acquisition (ACQ) trials in one (A1) or two (A2) compartments. Test 1 (T1) occurred following muscimol (M) or vehicle (V) injections indicated by the arrow. Test 2 (T2) occurred 24 hr later. Cannula placement for all groups is also shown. **(B)** Effects of muscimol or vehicle on locomotion during PT and 5-min blocks of T1 and T2. **(C)** Cocaine-induced CPP represented as the percent of time spent on the cocaine paired (CS+) floor during the 5-min PT, the first 5 min of T1, and the first 5 min of T2. **(D)** CPP during the 5-min PT, and during each 5-min block of T1 and T2. Group sizes were A1V (N=7); A1M (N=7), A2V (N=8), A2M (N=4). Error bars indicate the standard error of the mean ($\pm$ SEM).

Figure 3. Effects of intra-DH muscimol prior to extinction of CPP (Experiment 2)

(A) Overview of the design of Experiment 2. Mice received a pretest (PT), acquisition (ACQ) trials in two compartments, a post-acquisition test (T1), extinction (EXT) in one (E1) or two (E2) compartments, and a post-extinction test (T2). Extinction occurred following muscimol (M) or vehicle (V) injections indicated by the arrow. Cannula placement for all groups is also shown. (B) Effects of muscimol or vehicle on locomotion during PT, T1, EXT, and T2 in 5-min blocks. (C) Cocaine-induced CPP represented as the percent of time spent on the cocaine paired (CS+) floor during the 5-min PT, the 5-min T1, and the first 5 min of T2. (D) CPP during the 5-min PT, the 5-min T1, and during each 5-min block of T2. Group sizes were E1V (N=7); E1M (N=8), E2V (N=7), E2M (N=8). Error bars indicate the standard error of the mean ($\pm$ SEM).

Figure 4. Effects of intra-DH muscimol prior to a post-extinction CPP Test (Experiment 3)

(A) Overview of the design of Experiment 3. Mice received a pretest (PT), acquisition (ACQ) trials in two compartments, a post-acquisition test (T1), extinction (EXT) in one (E1) or two (E2) compartments, and a post-extinction test (T2). T2 occurred following muscimol (M) or vehicle (V) injections indicated by the arrow. Cannula placement for all groups is also shown. (B) Effects of muscimol or vehicle on locomotion during PT, T1, and T2 in 5-min blocks. (C) Cocaine-induced CPP represented as the percent of time spent on the cocaine paired (CS+) floor during the 5-min PT, the 5-min T1, and the first 5 min of T2. (D) CPP during the 5-min PT, the 5-min T1, and during each 5-min block of T2. Group sizes were E1V (N=6); E1M (N=6), E2V (N=6), E2M (N=5). Error bars indicate the standard error of the mean ($\pm$ SEM).